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Blow-Fill-Seal Aseptic Processing

Introduction

Blow-Fill-Seal (BFS) technology was developed in the early 1960s and was initially used for filling many liquid product categories, for example, nonsterile medical devices, foods, and cosmetics. The technology has been developed to an extent that today BFS systems are used to aseptically produce sterile pharmaceutical products such as respiratory solutions, ophthalmics, and wound-care products throughout the world.

The BFS technology is an advanced aseptic processing technique which allows plastic containers to be formed by means of molded extruded polymer granules, filled, and sealed in one continuous process. This differs from conventional aseptic processing where container formation, preparation, and sterilization, and container filling and closure are all separate processes.

Because of the level of automation of the entire process, little human intervention is required during manufacture compared to traditional aseptic filling and it is considered an advanced aseptic filling process. It is therefore possible to achieve very high levels of sterility confidence with a properly configured BFS machine designed to fill aseptically.

Outline of the BFS Process

The pharmaceutical BFS process combines the formation of plastic containers by blow/vacuum-molding extruded pharmaceutical-grade polymers with an aseptic solution filling system. Polymer granules are continuously fed to a machine hopper through an adiabatic screw extruder. Within the extruder the polymer is subjected to high temperature (usually above 160°C) and pressure (up to 350 bar or 10^5 Pa) and melts. It is then extruded through a die-and-pin set forming an open-ended tube of molten polymer known as a parison. The parison is supported by sterile air (parison support air) which is fed into its center through a sterilizing-grade air filter with oil-free compressed air. The parison is held in position by a clamp, which on some machines also serves to seal the parison bottom. A mold set consisting of two halves then moves over to the parison and closes around it. Molding is facilitated by vacuum slots in the mold. The molded plastic is severed from the continuously extruding parison by a hot knife, and is shuttled within the mold set to the filling position.

The filling mandrels are comprised of a set of filling tips which are held within a protective air shower; this is a small area within the filling machine which is typically fed with sterile filtered air. When the molds are beneath the air shower, the filling tips are lowered into the neck of the partially formed container and the containers are filled. The mandrels return to the protective air shower, and the containers are sealed

by a second mold set (head mold) which forms the neck and closure of the BFS containers.

The entire cycle takes only a few seconds, and therefore the open container is only minimally exposed to the surrounding clean room/air shower environment. The mold then opens and the filled containers surrounded by excess polymer are released. Excess plastic is removed (typically on line by means of a mold-specific cropping tool).

Liquid product is fed to the BFS machine from a holding tank or vessel. The pathway is sterilized in place prior to receiving product, and product is sterilized by means of in-line sterilizing-grade filters. There is usually more than one stage of sterile filtration required on the product pathway.

Filling Environment

Aseptic BFS machines are housed within classified clean areas of a minimum specification of class M5.5 (Federal Standard 209E) for 0.5-μ particles and greater (or equivalent) at rest. The most recent BFS machines are also capable of operating with significantly lower particle levels. The localized filling environment or air shower is of a higher classification, meeting the specification of class M3.5 (FS 209E) for 0.5-μ particles and greater.

Total particle levels should meet the required specifications and be measured, with the machine at rest, at defined intervals by means of a laser particle counter (or other suitable instrument) to demonstrate continued compliance.

Levels of viable contamination are, however, of importance in operation. Microbiological monitoring for viable contaminants should be carried out to coincide with routine manufacture at normal levels of dynamic activity. As with traditional aseptic filling, viable contamination within the clean area should be controlled by means of an effective routine cleaning and disinfection program and the adoption of appropriate clean room behavior and practices by trained personnel. The BFS technology has the advantage of being able to operate without the continuous presence of personnel within the clean area. However, operators need to enter the area to start up the machinery and to attend the machine as necessary to make routine adjustments. Within the European forum it is required that the clean room garments be worn to enter the class M5.5 (FS 209E) clean room are of a standard appropriate for a higher (M3.5) classification clean room.

A routine microbiological environmental monitoring program should be established and documented based on historical and operational data to demonstrate continued compliance with specifications and to monitor trends. A typical monitoring regime within the clean room would include quantitative air and surface monitoring. Semiquantitative air monitoring by the use of settle plates can also be useful in producing data associated with a longer period of time in operation (up to 4 hr exposure). Recommended limits for viable contaminants (not specific to BFS processes) in clean rooms are quoted in various guidelines, including the current USP and EC directive 91/356 (*MCA rules and Guidance for Pharmaceutical Manufacturers and Distributors 1997*, Annex 1, see Table 1). Alert and action levels should be clearly defined based upon both operational data and published recommendations.

TABLE 1 EU and USP Guidelines for Clean Room Microbial Limits

Classification	Air, cfu^a/m^3	Settle plates, cfu^a/4 hr	Surface samples, cfu^a/5 mm-dia plate
EC grade C^b	100	50	25
USP grade M5.5	20	not specified	10 (floors)
			5 (other surfaces)

^{a} cfu = colony-forming units.
^{b} EC grade C is the closest classification to Federal Standard grade M5.5 (FS209E).
In both cases, the limits specified are guidelines only and not regulatory requirements.

Consideration should also be given to monitoring the localized filling zone (air shower). Although access to this area is prohibited (and extremely dangerous) during operation, some monitoring for viable and nonviable contaminants may be possible at rest (for example, at the end of a product batch). It may also be feasible to install a remote means of obtaining samples during operation.

Contamination of the BFS Container from the Environment

As already stated, for aseptic BFS, the container is filled in a localized air shower provided with sterile filtered air. However, there is a short period of time between container formation and filling, when the open container is transferred from the parison formation position to the filling position and exposed to the clean room environment. During this shuttling period, there is a possibility for contaminants from the room environment to enter the container. The air used to form the parison (parison support air) is typically sterile filtered air. If this is not the case, it is also possible for nonsterile air to enter the parison during parison formation.

It has been demonstrated [1] during a simple practical experiment that broth-filled units (totalling over 44,000) manufactured over several days in a highly contaminated environment remained sterile. The environment was contaminated by means of high levels of personnel activity in order to generate contaminants in keeping with those generated under normal conditions (albeit at grossly elevated levels).

During a more controlled study carried out within an environment artificially contaminated with high levels of individual nebulized spores of *Bacillus subtilis* [2], a level of contamination within the environment was achieved which led to the contamination of broth-filled units. The results were extrapolated to suggest a contamination rate of 1 unit in 4×10^6 with a surrounding environmental contamination of 1 cfu/m^3.

Routes of air-borne contamination into BFS containers have been investigated during a study using sulfur hexafluoride (SF$_6$) tracer gas [3]. During this experiment, the tracer gas was released into a clean room, housing an aseptic BFS machine, at a known concentration. Levels of the tracer gas were measured within subsequently filled BFS units. The study concluded that the container was effectively protected by the localized air shower. Although not necessarily representative of deposition of microbial contaminants, there was also conclusive evidence of some room air within

the BFS containers. The control of environmental contamination within the clean room is therefore important.

Extensive process simulation (broth fill) results for BFS effectively demonstrate that high levels of sterility confidence can be obtained with a properly configured and validated machine. However, in order to maintain high levels of sterility assurance, it is important that levels of microbial contamination are controlled within the filling environment.

Contamination from Product Components

As with traditional aseptic filling, in order to comply with pharmaceutical GMP, it is important to minimize contamination at all stages of manufacture. Raw materials should be of a high quality and tested for microbial contamination. Water used for product manufacture should be of low bioburden and high purity (preferably water-for-injection quality, although this requirement is dependent upon the nature of the product being manufactured).

A program of bioburden testing for each product batch at various stages of manufacture should be established and documented. It is dependent upon the manufacturing process, but should as a minimum include bioburden analysis of bulk solutions prior to any sterile filtration. The maximum life of the bulk solution in a nonsterile environment (generally within a mixing tank) should be limited to prevent increase in bioburden beyond an acceptable level. Bioburden testing at this stage should be carried out on samples taken at the end of the holding period to give ''worst case'' data.

Considerable machine downtime is required with BFS technology associated with activities such as clean in place (CIP) and steam in place (SIP) to prepare a machine for manufacture. Initial machine adjustments are necessary for integral and visually acceptable units of the correct fill volume to be consistently produced. It therefore can be advantageous to fill larger product batches once this is achieved. In order to facilitate this with respect to maintaining a low bioburden throughout all stages of liquid processing, it is a common practice to have a sterilized storage vessel into which bulk product is sterile filtered. This sterilized bulk solution can be used to feed the filling machine without escalation of microbial levels. Further stages of sterile filtration are required on the filling machine closer to the point of fill. A facility for sampling products during the course of the filling stage prior to further filtration can be incorporated. This will give data to confirm the low/zero bioburden of the product prior to the final stages of filtration, during the course of a larger batch.

The BFS container is produced from high-grade virgin polymer granules. Studies have been carried out to investigate the lethality of the extrusion process with respect to container sterilization, the most recent of which is discussed below under Validation. The bioburden of polymer granules can be tested to establish base-line data. Virgin polymer granules, if handled and stored correctly, should be of very low bioburden.

Equipment

In order to produce sterile pharmaceutical products with a high degree of sterility confidence, it is of key importance that the equipment is operated by experienced and trained personnel with a full understanding of both the technology and aseptic processing. Operator intervention during machine operation is limited due to the nature of the technology. However, BFS machines are complex and some operator activity is required from time to time during normal manufacture. There must be clearly documented rules to clarify which activities are prohibited during batch manufacture and which are permitted. For example, if a fault occurs which requires immediate corrective actions involving the sterile product pathway, or within the direct vicinity of the filling zone, these would typically be prohibited leading to termination of the product batch. Activities such as parison and fill volume adjustments are part of the normal operation of the machinery and are permitted. There should be a proceduralized means of documenting these activities, however routine they may be.

Interventions should be categorized according to their potential for affecting the product being manufactured, and only those with no risk to product sterility should be permitted during operations.

As with all machinery, to maintain effective operation with the minimum of operator activity, BFS machines must be properly maintained. A documented preventative maintenance program should be in place, specifying appropriate frequencies for all machine components and associated systems and services. Maintenance activities should ensure that moving parts are sufficiently (but not overly) lubricated, and that excess lubricants are removed at regular intervals to maintain the cleanliness of the machine. Abrasion between moving parts, particularly hoses and flexible pipework, can be a problem with BFS machines and can cause undesirable particle generation and leaks leading to unplanned maintenance and downtime. Moving parts should be inspected at regular intervals to prevent abrasion and to check for wear and tear. Regular seal changes with reconciliation of new and old seals should be included.

Coolant systems are an integral part of container formation and serve to cool the molds and, if applicable, the parison clamp assembly. Coolant, although not in direct contact with product pathways, is in close proximity to the containers, and maintenance should be carried out to prevent leakage. Coolant systems are prone to microbiological contamination and should be routinely treated to keep the bioburden under control. They should be regularly sampled and tested for bioburden to ensure continuous compliance to a predefined specification.

Validation

The BFS machinery and associated equipment for aseptic manufacture should be constructed in such a way that the product pathways are of hygienic design with hygienic valves and minimal joints to facilitate cleaning and sterilizing in place.

Clean in Place (CIP)

As for all machinery involved in aseptic manufacture, CIP is necessary for all equipment in contact with the product. This would typically include a bulk mixing tank, transfer lines, and the BFS machine itself, and also a holding vessel with associated transfer lines. The CIP validation should be carried out to establish routine CIP practices which clean the manufacturing equipment in such a way that the products manufactured would be free of contamination and that safety, identity, quality, and purity of the drug would be within requirements. The CIP procedures should be established by cleaning validation following the manufacture of ''worst case'' products (i.e., those which are most difficult to reduce to acceptable levels due to their solubility or activity). Means of measuring CIP efficacy include analysis of swabs taken directly from product contact machine parts and analysis of rinse waters. When establishing areas for swabbing, account needs to be taken of the specific equipment design, and areas that are potentially most problematic should be selected for analysis (e.g., filter housings or areas which may cause product hold-up).

Steam in Place (SIP)

Aseptic BFS machines are subject to steam-in-place sterilization following standard CIP cycles. The SIP cycles are routinely measured by thermocouples located in fixed positions along the product pathway. Validation of SIP cycles should be carried out to demonstrate that consistent sterilization temperatures are achieved throughout the equipment to prove that the system can be effectively sterilized. Validation should also identify suitable positions for routine use, or justify the fixed probe positions already in place. The SIP validation is generally carried out with the help of additional thermocouples and should include the use of biological indicators (appropriate for moist heat sterilization). Test locations should include areas which may be prone to air or condensate entrapment. An accurate engineering line drawing of the system to aid identification of suitable test locations and document test locations selected should be available.

Qualification of Aseptic Filling

The standard, and most appropriate method for the qualification of aseptic filling is by means of a broth fill (or media fill). Units of liquid microbiological growth media (usually a full-strength general purpose medium such as Tryptone Soy Broth) are filled and incubated. Following an appropriate incubation period, the units are inspected for contamination. In this way, an indication of the level of contamination during the filling process can be evaluated.

There is no appropriate defined sterility confidence level which can be translated directly into acceptance criteria for broth fill contamination for BFS processes. The most commonly recognized acceptance criterion is a sterility assurance level (SAL) of 10^{-3}, although modern aseptic filling techniques such as BFS can achieve a higher SAL. This should be reflected by broth fill results and acceptance criteria for this advanced technology.

Broth fills should be a major part of the operational qualification of a new BFS machine to demonstrate aseptic processing capability prior to product manufacture (typically three successful consecutive broth fills are required) and should be carried out at defined intervals thereafter.

Broth fills should be carried out under conditions that are representative of those during normal operation. A deviation from routine processes should only be in the direction of presenting a higher rather than a lower challenge to the process. Due to the level of automation of BFS technology, it is extremely difficult to take ''extra care'' in order to reduce the chance of container contamination during a broth fill, and results are therefore not as operator dependent as other less automated aseptic manufacturing processes.

For a new facility, some background environmental monitoring data are desirable. It is important that environmental monitoring data are obtained during the course of broth fill batches to demonstrate a normal level of environmental contamination. The validity of broth fills carried out in an environment of consistently lower contamination levels than those obtained during routine batch manufacture could be questioned.

Batch manufacture, storage, and transfer should be carried out in accordance with routine procedures involving the same operators. The machine should be cleaned and sterilized by the usual procedures, although if an overkill sterilization cycle is used routinely, a partial sterilization (although still meeting standard parameters) may be chosen as ''worst case.''

Broth filled BFS units should meet all of the necessary product acceptance criteria such as fill volume, wall thickness, container integrity, and appearance. The operator activity required at the start of a product batch is arguably more intrusive than at any other stage of manufacture. Product units routinely produced at the start of a batch are usually discarded because of fill volume, appearance, or other deficiencies as the machine setup is adjusted. However, it is a good practice during a broth fill to retain and incubate all start-up units (except those that leak) to demonstrate that start-up activities have not affected product sterility. Such units should be separated from the subsequent units meeting the acceptance criteria and labelled accordingly.

It can also be useful to retain and incubate reject units filled during the course of a broth fill batch (again, excluding those that leak) for additional information. Again, these should be separated from acceptable units and labelled accordingly. Although such units would be rejected during normal production, microbial contamination found in such units may indicate a problem which requires attention.

During the course of a broth fill, operator activity is as necessary as with routine manufacture. However, additional activities can be carried out to cover all permissible activities in order to provide evidence that product sterility is not affected. Such interventions should be planned and documented for each batch.

Frequency and size of broth fills must be clearly defined. The size of fill is usually based upon the statistical probability of detecting an acceptably low incidence of microbial contamination. Tables have been published to this effect [4], but the BFS operator has to decide both size and frequency of broth fills based upon their specific facility, routine product batch sizes, and operation. For high speed BFS machines used for filling routine product batches in excess of 100,000 units, broth fill batches larger than traditional aseptic filling lines are both feasible and appropriate.

TABLE 2   Broth Fill Data[a]

Units	Company					
	A	B	C	D	E	F
Total number filled	6,462,570	222,900	2,697,496	1,534,626	1,042,254	31,600
Total number of non-sterile detected	3	0	0	0	0	0
Contamination rate, %	0.0005	0	0	0	0	0

[a] Not included are positive units detected which were assigned a definitive cause, unrelated to the BFS process as practiced during routine product manufacture.

The internal surfaces of broth filled units should be fully wetted to ensure capture of any contaminants within the broth. This is commonly achieved by agitation or inversion of the units before or during the incubation period.

Incubation time and temperature should be adjusted so that macroscopic microbial growth of a wide range of common isolates will be detected. This should be routinely demonstrated by including positive control units inoculated with a low level of compendial microorganisms. Additional testing is desirable to demonstrate that the incubation time and temperature selected promote the growth of isolates obtained from machine operating environments. Incubation of 14 days at 25–32°C is recommended by the Pharmaceutical BFS Operators Association.

Broth fill data from various BFS users were studied in a survey carried out in 1998 by the Pharmaceutical BFS Operators Association. These results, together with some more recent data are shown in Table 2.

Some of the media fills carried out were full production batch volumes with hundreds of thousands of units filled in a single batch. In addition to the data in Table 2, a run of over 1,500,000 units is also recorded with the detection of a single contaminated unit.

It is clearly impractical to produce a very high number of broth filled units on a routine basis, but if unpreserved products are manufactured, it is good practice to fill broth directly following product batches with no further machine flushing or sterilization.

Given the high performance demonstrated during media fills, acceptance criteria should be based upon realistic goals. During broth fills of standard size, any incidence of contamination among the units filled should be investigated. In the absence of a definite cause, even at very low levels of contamination, consideration should be given to machine recommissioning. This procedure should also be carried out if modifications to a filling machine have been made which may have an effect on process capability (e.g., changes to the sterile product pathway or air shower).

BFS Containers

The BFS container is formed as an integral part of the process from medical-grade virgin polymer granules. A recent study of the lethality of the extrusion process chal-

lenged with a high bioburden of spores is presented in Ref. 5. The spores of the test organism *Bacillus subtilis* var. *niger* were selected as they are known to be resistant to dry heat. The same strain was the organism of choice for biological indicators used in dry heat sterilization processes. A series of broth fills were carried out using polymer batches inoculated with various levels of spores between 2×10 and 2×10^5 spores per gram. The broth-filled units were incubated in line with the company's routine broth-fill procedure (25–32°C for 14 days). Spore contamination of units was observed with batches of polymer inoculated with high spore levels. The experiment demonstrated a relationship between polymer contamination and product contamination which was dependent upon both the level of contamination in the polymer and the resistance of the contaminant (in terms of D value) to dry heat sterilization. The study also demonstrated spore inactivation on granules with strong evidence of lethality associated with the extrusion process.

Routine bioburden testing of virgin pharmaceutical-grade polymer granules tends to give very low or zero counts per gram of polymer tested, with contaminants generally much more heat labile than *Bacillus subtilis* spores. The study detailed was carried out using a BFS machine adjusted to extrude at the lower end of the operating temperature range for extrusion. Thus there is evidence that the extrusion process renders the contaminants unavailable, with sufficient bioburden reduction and inactivation for it to be appropriate for aseptic formation of BFS containers. This is further endorsed by routine broth-fill data.

The BFS containers are closed within the automated process by the head mold set forming around the top of the severed section of parison following filling. The integrity of the container and closure is generally tested by a manual or automated method of leak detection performed outside of the filling environment following removal of excess plastic (deflashing) from the filled product units.

In order to minimize the number of leaking units, it is important that the mold sets are correctly aligned. Even very slight misalignment may potentially lead to the production of units with very slight leaks which may be difficult to detect by routine methods. Correct molding is therefore of key importance and can usually be simply checked by careful and experienced visual examination of units.

The integrity of the container can be tested effectively by a bacterial challenge. Using this method, sterile broth-filled units are submerged for a period of time (e.g., 24 hr) within a buffered solution containing a high level bacterial challenge (there are no regulations or guidelines that specify which organism to use, but it would seem logical to use a factory isolate or a relatively small organism such as a *Pseudomonas spp*). Units are then removed, incubated, and checked for growth of the challenge organism. An absence of growth shows an integral unit and closure. This method is extremely sensitive, and although it is not a test which is practical to perform on a routine basis, it can be occasionally a useful tool.

Filtration

Hydrophilic and hydrophobic sterilizing-grade filters are used throughout the BFS process for the sterilization of product and air, respectively. Filters used should be

purchased from an approved supplier and should be certified as meeting the regulatory requirements for sterilizing-grade filters. This means, by definition, that the filters exhibit full bacterial retention when subjected to an aqueous challenge of *Brevundimonas diminuta* (ATCC 19146) at a minimum concentration of 1×10^7 cfu/cm^2 of filter surface area.

Hydrophobic filters do not come directly into contact with the product, and therefore the standard bacterial retention test alone is generally sufficient validation. However, as hydrophilic filters are in direct contact with the product, additional validation is necessary for each product type to demonstrate that the filters selected for product sterilization do not affect the safety, identity, strength, quality, or purity of the drug product. Qualification of hydrophilic filters is also necessary to demonstrate that the specific product type in conjunction with a bacterial challenge does not affect the filter efficacy. Validation of filters by means of bacterial retention tests requires special equipment and is often arranged between the filter manufacturer and the BFS operator.

Summary

Aseptic pharmaceutical BFS technology for the manufacture of sterile liquid products has demonstrated high levels of sterility assurance when correctly operated and configured. The technology is continually improving as more expertise is developed. However, an understanding of the possibilities of container contaminations and the implementation of systems operating to minimize are important to maintain the high standards achievable with this technology.

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DEBORAH J. JONES

Compounding, Contemporary

Introduction

Pharmacists are unique professionals, well-trained in the natural, physical, and medical sciences and sensitized to the potential tragedy that may result from a single mistake that may occur in the daily practice of their profession. The demonstrated expertise, demeanor, and manner in which pharmacists have practiced over the years has resulted in a continued rating of pharmacists as the most respected and trusted individuals in society. This is because of the reputation of pharmacists being available in the local community to interact with patients, provide needed medications, and work with patients to regain or maintain a certain standard or quality of health, and just being there in time of need.

Pharmacy is a complex mixture of different practices and practice sites. No longer is pharmacy simply community pharmacy or hospital pharmacy. It is a diverse profession and offers many opportunities for those willing to look around, find their niche, and practice pharmacy to meet the needs of their own community of patients. Pharmaceutical compounding is an area that is rapidly growing and providing needed products and services to patients and healthcare practitioners. Most compounding pharmacists appear to be very interested and excited about their practices. In fact, many pharmacists intimately involved in pharmaceutical care have now realized the importance of providing individualized patient care through the preparation of patient-specific products. Compounding is a professional prerogative that pharmacists have performed since the beginning of their profession. Even today, the definitions of pharmacy include the ''preparation of drugs'' [1–3]:

> Pharmacy is the art or practice of preparing and preserving drugs, and of compounding and dispensing medicines according to the prescriptions of physicians.

Compounding has always been a basic part of pharmacy practice; the drugs, dosage forms, and equipment and techniques used are the variables. Pharmacists possess knowledge and skills that are unique and not duplicated by any other profession. Pharmacy activities to individualize patient therapy include compounding and clinical functions; either activity in the absence of the other results in placing pharmacy in a disadvantaged position. It is important to utilize a pharmacist's expertise to adjust dosage quantities, frequencies, and even dosage forms for enhanced compliance. All pharmacists should understand the options presented by compounding.

Pharmaceutical compounding is dramatically increasing due to the impact of home health care, unavailable drug products (especially pediatric formulations), orphan drugs, veterinary compounding, and biotechnology-derived drug products. Newly evolving dosage forms and therapeutic approaches suggest that compounding of pharmaceuticals and related products specifically for individual patients will become even more common in pharmacy practice.

A pharmacy compounder may be defined as a pharmacist who actively promotes and provides prescription compounding services for the express purpose of attracting this type of prescription to his or her practice. One of the responsibilities of a compounder requires that the pharmacist become actively engaged in the clinical assessment of a patient to assist the prescriber in determining the customized patient-specific formula to be extemporaneously compounded. In addition, this responsibility requires the pharmacist to interact with prescribers and the patient as the customized formulation and dosage form are determined. The use of clinical skills and physician–patient interaction have been identified in previous studies as intrinsic factors that enhance a pharmacists job satisfaction [4–7]. Therefore, a compounder utilizing clinical skills and interacting with prescribers and patients is usually predisposed to a higher job satisfaction than would be a pharmacist whose responsibilities may not require such activities [8].

Pharmaceutical compounding requires training in mathematics, science, and technology more than some of the other practices of pharmacy. It has been stated [9] that:

> The sciences are what support pharmacy's expertise in drug distribution and drug use. Recent history leads one to question whether we in the profession, and some in pharmaceutical education, recognize and appreciate the contribution that the pharmaceutical sciences have made and continue to make to the pharmacy profession and health care. The pharmaceutical sciences are what make us unique. They provide us the special value that we bring to the bedside. No other health professional is capable of bringing to the pharmacotherapeutic decision-making table such concepts as pH, particle size, partition coefficient, protein binding, structure-activity relationships, economics, and epidemiology. The pharmaceutical sciences, combined with pharmacy's infrastructure including pharmaceutical education, are what make the pharmacist an indispensable participant on the health care team.

What area of pharmacy practice has the opportunity of using the scientific education and training as much as pharmacists involved in individualizing patient care through extemporaneous compounding? The pharmaceutical sciences, especially chemistry and pharmaceutics, serve as the foundation for pharmacists' ability to formulate specific dosage forms to meet patients' needs.

Definition of Compounding

Compounding has been defined by the National Association of Boards of Pharmacy [10] as follows:

> Compounding means the preparation, mixing, assembling, packaging, or labeling of a drug or device (i) as the result of a practitioner's prescription drug order or initiative based on the pharmacist/patient/prescriber relationship in the course of professional practice, or (ii) for the purpose of, as an incident to research, teaching, or chemical analysis and not for sale or dispensing. Compounding also includes the preparation of drugs and devices in anticipation of prescription drug orders based on routine, regularly observed patterns.

Compounding may hold different meanings to different pharmacists. It may mean the preparation of oral liquids, topicals, suppositories, the conversion of one dose or

dosage form into another, the preparation of select dosage forms from bulk chemicals, of intravenous admixtures, parenteral nutrition solutions, pediatric dosage forms from adult dosage forms, or of radioactive isotopes, or the preparation of cassettes, syringes, and other devices for the administration of drugs in the home setting.

Types of Compounding

Ambulatory Care Compounding

If the patient is able to walk, he or she is considered mobile or ambulatory, that is, not bedridden. Consequently, most pharmacists are involved in ambulatory care and most ambulatory patients are outpatients. The term actually can also be applied to homecare patients and even institutionalized patients that are mobile. A general characteristic of an ambulatory patient is that he or she is generally responsible for obtaining the required medication, storing it, preparing it (if necessary), and taking it [11]. It seems almost incongruous that as healthcare professionals become more aware that patients are ''individuals,'' respond as individuals, and must be treated as individuals, some healthcare providers appear to be grouping patients into categories for treatment and categories for reimbursement from third party or categories for determining levels of care in managed care organizations, and using categorized fixed-dose products provided by pharmaceutical manufacturers that are available because the marketing demand is sufficiently high to justify their manufacture and production. Since when does the availability, or lack of availability, of a specific commercially available product dictate the therapy of a patient?

Pharmacists have an opportunity to extend their activities in patient care as the emphasis continues to shift from inpatient care to ambulatory care. The latter situation generally encourages a team approach to health improvement, prevention, health maintenance, risk assessment, early detection, management, curative therapy, and rehabilitation [12]. Ambulatory care offers a variety of opportunities for individualizing patient care through pharmaceutical compounding. In fact, it is the area where most compounding pharmacists practice.

The tasks of pharmacists in ambulatory care patients can include, among others, (1) dispensing, (2) compounding, (3) counseling, (4) minimizing medication errors, (5) compliance enhancement, (6) therapeutic drug monitoring, and (7) minimizing expenditures [11–13]. Most reimbursement for ambulatory patients originates in the dispensing or compounding process. Little financial consideration is given to counseling, minimizing medication errors, compliance enhancement, therapeutic monitoring, and minimizing expenditures; however, these activities are important and should be included. Due to the unique nature of compounded medications, counseling is an absolute must for these patients.

From the above discussion of the activities of an ambulatory-care pharmacist, it should be evident that extemporaneous compounding can be vitally important in ambulatory patient care.

Institutional Pharmacy Compounding

The ever-present responsibility of the health care industry is to provide the best available care for the patient, using the best means to do so, and providing that care in

a conducive environment. This requires cooperation on the part of the institutional administration, the medical staff, and the employees (nurses and pharmacists, in particular with regard to medication usage) and must involve the patient. One of the effective means by which institutions, and therefore institutional pharmacies, can meet these challenges is to consider expanding extemporaneous compounding services within the institutional pharmacy. Pharmaceutical care and pharmaceutical compounding can provide cost savings to the institution while providing needed options to the physician through problem-solving approaches and stimulating the institutional pharmacist through new challenges that allow the expression of both skills and art.

Institutional pharmacists have always been actively involved in compounding, or producing medications for the patient. Daily intravenous (iv) therapy is provided through compounding of medications. Antibiotic ''piggybacks,'' total parenteral nutrition (tpn) solutions, iv additives, and many others are daily calculated, compounded, dispensed, and generally administered by the nursing staff. The preparation of pediatric dosage forms has also been an area of extensive activity in some institutions.

Members of the institutional staff are familiar with the current literature and are generally aware of innovative thought and practices by their peers. As physicians become aware of the skill, availability, and importance of pharmaceutical compounding and that they can literally have almost any medication they need, in the form and strength they need for a specific situation, they generally request it more often. As the institutional pharmacy staff demonstrates expertise and problem-solving skills, the medical staff consistently calls upon them [14].

In the consideration of meeting patient-specific needs, the institutional pharmacist must look at various modalities as potential solutions. When traditional institutional processes and procedures are not meeting the patient's need, extemporaneous compounding should be a consideration. Improving outcomes and getting patients well and out of the institution as quickly as possible should be the end goal. Individualized dosage forms, dosage strengths, and alternative routes of administration can often help attain these goals. Improving outcomes will assist the medical staff in allowing them to spend their time dealing with new problems as hospital pharmacy meets the challenge of past problems. Nursing and pharmacy will have an enhanced opportunity to interact and use the skills they have developed, and provide opportunities for pharmacy to have more patient involvement and job satisfaction.

Veterinary Compounding

The first symposium on veterinary compounding in September 1993 [15] offered a significant forum for discussion by experts and provided a pivotal point in the history of veterinary compounding. The meeting was important because it assembled an impressive group of experts on veterinary compounding, who explained and defined the roles of the veterinarian and the pharmacist.

The interest of the Food and Drug Administration (FDA) in compounding by and for veterinarians dates back to the early 1990s. The avowed purpose of the symposium in 1993 was to provide a forum for a comprehensive, public debate in response to the American Veterinary Medical Association (AVMA) position on compounding prior to the issuance of the FDA *Compliance Policy Guide* on veterinary compound-

ing. Numerous speakers presented views on compounding by veterinarians and for veterinarians by pharmacists. Topics such as conflicts of interest, lack of compounding training by veterinarians, the ''new drug'' issue, and bioequivalency standards were discussed in detail.

There are many reasons why veterinary compounding is necessary; for example, with multiple species ranging from very small to extremely large it would be impossible to practice effective medicine without compounded products! Do we simply refuse to treat exotic species or very small animals? Do we abandon oncology in veterinary medicine?

A more specific area of need is the lack of an ideal anesthetic drug, which has led veterinarians to devise anesthetic combinations inducing good-quality anesthesia, with minimal risk to the patient. Compounding is essential for safe and effective veterinary anesthetic practice. Veterinarians need to administer anesthetic drugs to a wide variety of animals with a wide variety of temperaments in settings that are less than ideal. Veterinarians are called upon to anesthetize elephants, gorillas, tigers, ostriches, sharks, horses, cows, and poisonous snakes, among others. Other reasons for veterinary compounding include:

- The necessity for multiple injections in the absence of a compounded product,
- Rapid changes in management and disease problems in veterinary medicine,
- Problems associated with the treatment of large numbers of animals with several drugs within a short period of time.
- Cost-prohibitive factors associated with the very large volume of some large-volume parenterals required for animals, and
- The need for previously prepared antidotes for cases of animal poisoning.

Unique aspects of veterinary compounding compared to compounding for human patients include the potential impact on human health of compounded veterinary products in food animals, and variability in animal response and size.

The ideas expressed at the meeting are summarized as follows:

1. Veterinarians have a definite need for drug compounding.
2. Drug compounding is necessary in all areas of veterinary medicine.
3. The compounding of poisoning antidotes, such as sodium nitrite, sodium thiosulfate, methylene blue, or calcium EDTA, was considered necessary.

Compounding will continue to be practiced in the future for the reasons discussed above to fill therapeutic needs in veterinary medicine, as well as in medicine for human patients. Difficulties and costs associated with the veterinary drug-approval process will make compounding necessary to fill therapeutic needs not being met by the introduction of therapeutic agents. An increasing interdependence is developing between the veterinarian and the pharmacist, resulting in higher standards of veterinary care [15].

Nuclear Pharmacy Compounding

Nuclear pharmacy is a specialty practice which has been defined as a patient-oriented service that embodies the scientific knowledge and professional judgment required

to improve and promote health through assurance of the safe and efficacious use of radioactive drugs for diagnosis and therapy [16]. Radioactive drugs, commonly referred to as radiopharmaceuticals, are a special class of drugs that are regulated by the FDA. They are unique in that they contain an unstable nuclide (radioactive nuclide) as part of the compound designed to localize an organ or tissue. Since these pharmaceuticals are radioactive, the Nuclear Regulatory Commission or a similar state agency is involved in regulatory matters relevant to radiopharmaceuticals.

Most radioactive nuclides employed in radiopharmaceuticals have a short half-life. This is beneficial to the patient as the total number of radioactive atoms given to the patient to produce an image is small when the half-life of the radioactive nuclide is short, as compared to longer half-life radioactive nuclides. Fewer total atoms reduce the radiation dose to the patient and thus the risk from a nuclear medicine procedure. However, the short half-life of the radioactive nuclide results in a short shelf-life for the radiopharmaceutical. As a result, most radiopharmaceuticals are compounded on a daily basis. The most common radioactive nuclide used for this purpose is technetium-99m (Tc-99m) with a half-life of 6 hr, emiting only gamma radiation with an energy almost ideal for detection.

A nuclear pharmacist is expert at preparing (compounding) radiopharmaceuticals with Tc-99m sodium pertechnetate and a reagent kit. The kit consists of multidose vials containing the compound to be ''labeled'' with Tc-99m in order to create the radiopharmaceutical. The contents within the vial are sterile and pyrogen-free as is the Tc-99m sodium pertechnetate. Most radiopharmaceuticals are administered intravenously and the nuclear pharmacist must be expert at maintaining aseptic conditions during compounding.

The most common setting for the preparation radiopharmaceuticals by a nuclear pharmacist is a commercially centralized nuclear pharmacy. Radiopharmaceuticals are prepared early in the morning (2–3 AM) and unit doses delivered quickly to hospitals in the region.

Today there are several hundred commercial centralized nuclear pharmacies providing a significant fraction of radiopharmaceuticals used in nuclear medicine procedures. What started as limited service in large medical centers and universities by a few pharmacists with education beyond the bachelor of pharmacy degree has grown to extensive services provided by several hundred professional-degree pharmacists. This is truly a remarkable change in a time period of 20–25 years, due to dedicated entrepreneurs aiming to make a difference in patient care through quality products and pharmaceutical care.

History of Compounding

The heritage of pharmacy, spanning over 5000 years, has centered around the provision of pharmaceutical products for patients. Pharmacists are the only health professional that possess the knowledge and skill required to compound and prepare medications to meet the unique needs of patients. The responsibility to extemporaneously compound safe, effective prescription products for patients who require special care is fundamental to the pharmacy profession.

In the 19th century the art of compounding continued, but did give way, however grudgingly, to new technology. It has been estimated that a ''broad knowledge of

compounding'' was still essential for 80% of the prescriptions dispensed in the 1920s. Although pharmacists increasingly relied on chemicals purchased from the manufacturer to make up prescriptions, there still remained much to be done secundum artem [1].

The pharmaceutical industry began to take over the production of most medications used by the medical profession. In many ways this has provided superior service, new methods, and a vast array of innovative products that could not have been provided in a one-on-one situation. Research and development have been the hallmarks of the pharmaceutical manufacturers. However, the very nature of providing millions of doses of a product requires that the dosage forms (capsule, tablet, suppository) and doses (individual strengths of each dose) be limited and results in a one-sided approach to therapy. It is simply not economical for a pharmaceutical company to produce a product in 50 different conceivable doses, or 15 different dosage forms, to meet the needs of the entire range of patients receiving therapy. Windows of activity are determined which meet most patient needs, but the very nature of the process is not able to meet all patient needs.

Furthermore some individuals and their health care needs do not fall in the ''windows'' or ''categories'' of theoretical dosage strength and forms, and large-scale manufacturers cannot cost-effectively tailor-make a medication for a small group of patients and meet the ever-changing needs of a given patient or institution. The skills of the pharmacists in practicing their art of compounding fills in this gap to meet individualized needs. By this assessment the pharmacist may, through understanding of the principles of compounding and recognition of the skill level in working secundum artem, recommend that therapy be provided that is not provided by the pharmaceutical industry, but is individualized for a specific patient's needs at a specific time.

Pharmaceutical compounding is increasing due to the impact of home health care, unavailable drug products, orphan drugs, veterinary compounding, and biotechnology-derived products. Newly evolving dosage forms and therapeutic approaches suggest that compounding of pharmaceuticals and related products specifically for individual patients will become more common in pharmacy practice. Compounding pharmacy is unique as it allows the professional to use a scientific and technical background to a fuller extent than some other types of practices. Compounding pharmacists develop a special and unique relationship with the patients they serve. They work hand in hand with physicians to solve problems not addressed by commercially available dosage forms.

Technical and Other Considerations for Compounding

The following questions have to be considered: Is the product commercially available in the exact dosage form, strength and packaging? Is the prescription rational, including ingredients, use, dose, and method of administration? Are the physical, chemical, and therapeutic properties appropriate? Will the compounded preparation satisfy the intent of the prescribing physician for the needs of the patient? Is there an alternative dosage form, route of administration, etc., by which the patient could benefit? Can the ingredient identity, quality, and purity be assured? Does the pharmacist have the required training and expertise to prepare the prescription? Are the proper equipment, supplies, chemicals, and drugs available? Is there documentation on the use, prepa-

ration, stability, administration, and storage of the preparation? Can the pharmacist perform the necessary calculations to prepare the product? Can the pharmacist project a reasonable and rational ''beyond-use'' date for the prescription? Can the pharmacist perform some basic quality control to check the preparation prior to dispensing (capsule weight variation, pH, visual observations)?

Guidelines and Regulations for Compounding

The three documents of special importance in providing regulations and guidelines for pharmaceutical compounding include the *Food, Drug and Modernization Act of 1997*, the *United States Pharmacopeia/National Formulary* (chapters ⟨795⟩ Pharmacy Compounding and ⟨1206⟩ Sterile Drug Products for Home Use, and other portions of the *USP/NF*), and the National Association of Boards of Pharmacy *Good Compounding Practices Applicable to State Licensed Pharmacies*.

The Food, Drug and Modernization Act of 1997 (FDAMA97)

The reason for the inclusion of the compounding provisions in FDAMA97 is that the FDA considered every compounded prescription as a new drug subject to the new drug requirements (IND, NDA, etc.). In order to be exempt from these requirements, compounded prescriptions must meet the following conditions.

The drug product must be compounded for an individual patient based on the unsolicited receipt of a valid prescription order or a notation on the order, approved by the prescribing practitioner, that a compounded product is necessary for the identified patient. The drug product must also meet the requirements of this Act, and the compounding must be done by a licensed pharmacist or physician in a state-licensed pharmacy or a federal facility. It must be written on a prescription order for an individual patient by a licensed physician or other licensed practitioner authorized by state law to prescribe drugs. It can also be compounded by a licensed pharmacist or licensed physician in limited quantities before the valid prescription order for the individual patient is received. The anticipatory compounding must be based on a history of the licensed pharmacist or licensed physician receiving valid prescription orders for the compounding of the drug product, the orders being generated solely within an established relationship between the licensed pharmacist or physician and the individual patient for whom the prescription order is provided.

A drug product may be compounded if the licensed pharmacist or licensed physician compounds the drug product using bulk drug substances, as defined in FDA regulations that comply with the standards of an applicable United States Pharmacopeia or National Formulary monograph, if a monograph exists, and the United States Pharmacopeia chapter on pharmacy compounding. If such a monograph does not exist, the drug substances must be components of drugs approved by the FDA, or appear on a list developed by the FDA through regulations. These drug substances must be manufactured by an establishment that is registered with the FDA and must be accompanied by valid certificates of analysis for each bulk drug substance. The pharmacist or physician cannot compound a drug product that appears on a list pub-

lished by the FDA in the *Federal Register* of drug products that have been withdrawn or removed from the market because these products or their components have been found to be unsafe or not effective. The pharmacist or physician cannot compound regularly or in inordinate amounts (as defined by the FDA) any drug products that are essentially copies of commercially available drug products. A copy of a commercially available drug product does not include a drug product in which a change was made for an identified individual patient, as determined by the prescribing practitioner.

A drug product may be compounded only if such a product is not identified by FDA regulations as a drug product that presents demonstrable difficulties for compounding that reasonably demonstrate an adverse effect on the safety and effectiveness of that drug product. Such a drug product is compounded in a state that has been entered into a memorandum or understanding with the FDA which addresses the distribution of inordinate amounts of compounded drug products interstate and provides for appropriate investigation by a state agency of complaints relating to compounded drug products distributed outside such state. Alternatively, the drug product has not been entered into the memorandum of understanding, and the licensed pharmacist, pharmacy, or physician distributes (or causes to be distributed) compounded drug products out of the state in which they are compounded in quantities that do not exceed 5% of the total prescription orders dispensed or distributed by such pharmacy or physician. The FDA shall, in consultation with the National Association of Boards of Pharmacy, develop a standard memorandum of understanding for use by the states.

The FDA shall convene and consult an advisory committee on compounding that includes representatives from the National Association of Boards of Pharmacy, the United States Pharmacopeia, pharmacy, physician, and consumer organizations, and other selected experts.

The FDA, in consultation with the United States Pharmacopeial Convention, Inc., shall promulgate regulations identifying drug substances that may be used in compounding for which a monograph does not exist or which are not components of drug products approved by the FDA.

As used in the Act, the term compounding does not include mixing, reconstituting, or other such acts that are performed in accordance with directions contained in approved labeling provided by the products manufacturer and other manufacturer directions consistent with that labeling.

United States Pharmacopeia

Following are summaries of the lengthy chapters ⟨795⟩ and ⟨1206⟩ in the USP/NF:

Chapter ⟨795⟩ Pharmacy Compounding [18] is divided into (1) Compounding environment, (2) Stability of compounded preparations, (3) Ingredient selection and calculations, (4) Checklist for acceptable strength, quality, and purity, (5) Compounded dosage forms, (6) Compounding process, (7) Compounding records and documents, (8) Quality control, and (9) Patient counseling. The compounding environment section describes guidelines for the facilities and equipment that should be available, calibrated, maintained, and used in a compounding pharmacy. The stability

section provides guidelines for ''beyond-use'' dates to be placed on compounded preparations. The checklist for the USP/NF hallmarks of standards of acceptable strength, quality, and purity is presented in a series of questions to be answered. Examples of compounded dosage forms are discussed along with some precautionary statements as appropriate. A step-by-step presentation on the compounding process is outlined to ensure uniformity of activities in preparing each preparation. Documentation is described for the formulation record, the compounding record, and the material safety data sheets (MSDS) files that should be maintained. The chapter ends with various aspects for patient counseling involving the proper use, storage, and evidence of instability of the compounded preparation.

Chapter ⟨1206⟩ Sterile Drug Products for Home Use covers the areas of (1) Responsibility of the dispensing pharmacist, (2) Risk levels, (3) Validation, (4) Low-risk operations, (5) High-risk operations, (6) Environmental quality and control, finished product release, checks, and tests, (7) Storage and expiration dating, (8) Maintaining product quality and control after it leaves the pharmacy, (9) Patient or caregiver training, and (10) Patient monitoring and complaint system. The compounding pharmacist dispensing home-use sterile drug products is responsible for ensuring that the product has been prepared, labeled, controlled, stored, dispensed, and distributed properly. Low-risk and high-risk levels of sterile products compounding are defined with examples of each. Validation of the sterilization and aseptic processing procedures are described as related to personnel, facilities, equipment, and processes. Low-risk and high-risk operations are described along with the validations required for each. Environmental quality and control procedures for the work area and personnel, along with suggested Standard Operating Procedures (SOPs) and an example of an environmental monitoring program, are described. Tests and procedures for the finished product are described with the guidelines that only those products that are free from defects and meeting all quality specifications will be distributed. Guidelines are discussed for the preparation, storage, and beyond-use dating after the preparation leaves the pharmacy. At this time, the caregiver or patient should receive training to ensure understanding and compliance with the storage, handling, and administration of the preparations. The various aspects of the training program are outlined in Chapter ⟨1206⟩. Also included are the recommendations for written policies and procedures for monitoring of patients employing home-use sterile drug products, and the handling and reporting of adverse events. It is evident in this chapter that the responsibility of the compounding pharmacist ranges from the activities involved in the compounding of the product through its proper storage, distribution, use, and disposal.

National Association of Boards of Pharmacy

The following is a summary of the Good Compounding Practices (GCPs) of the National Association of Boards of Pharmacy:

Subpart A, General Provisions and Definitions. It sets forth the minimum current good compounding practices for the preparation of drug products by state-licensed pharmacies for dispensing and/or administration to humans or animals and includes definitions and requirements, as follows:

Based on the existence of a pharmacist–patient–prescriber relationship and the presentation of a valid prescription, pharmacists may compound, in reasonable quantities, drug products that are commercially available in the marketplace. For the compounding process, pharmacists shall receive, store, or use drug substances that have been made in an FDA-approved facility and/or drug components that meet official compendial requirements. If neither of these requirements can be met, pharmacists shall use their professional judgment to procure alternatives.

Pharmacists may compound drugs in very limited quantities prior to receiving a valid prescription based on a history of receiving valid prescriptions. Pharmacists shall not offer compounded drug products to other state-licensed persons or commercial entities for subsequent resale, except in the course of professional practice for a prescriber to administer to an individual patient.

Subpart B, Organization and Personnel. It discusses the responsibilities and authority of the compounding pharmacist and other individuals involved in the compounding process.

Subpart C, Drug Compounding Facilities. These are discussed further in the next section of this article. It also discusses bulk drugs and materials as well as the compounding of sterile products, radiopharmaceuticals, and products requiring special precaution such as the handling of penicillins.

Subpart D, Equipment. The equipment used in the compounding of drug products shall be of appropriate design, adequate size, and suitably located to facilitate operations, cleaning, and maintenance.

Subpart E, Control of Components, Drug Product Containers, and Closures. Components, drug product containers, and closures used in the compounding of drugs shall be handled and stored in a manner to prevent contamination.

Subpart F, Drug Compounding Controls. This explains the written procedures for the compounding of drug products that must be performed in order to assure that the finished products have the identity, strength, quality, and purity they purport or are represented to possess. It also discusses some of the final quality control procedures, including, but not limited to, the following (as appropriate):

- Capsule weight variation,
- Adequacy of mixing to assure uniformity and homogeneity, and
- Clarity, completeness, and pH of solutions.

Appropriate procedures, designed to prevent microbiological contamination of compounded drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation of any sterilization process.

Subpart G, Labeling Control of Excess Products. If a quantity of a compounded drug product is prepared in excess of that to be initially dispensed in accordance with Subpart A, the excess product shall be labeled or documented with the complete list of ingredients (components), the preparation date, and the assigned expiration date based upon professional judgment, appropriate testing, or published data.

Subpart H, Records and Reports. Any procedures or other records required to be maintained in compliance with these Good Compounding Practices shall be retained

for the same period of time as required by the state for the retention of prescription files.

Facilities, Equipment, and Supplies

Compounding Facility

For traditional compounding a separate area is recommended, rather than simply cleaning off a small area of the dispensing counter. The compounding pharmacist needs a clean, neat, well-lighted, quiet working area. If aseptic compounding is considered, a ''clean room'' with a laminar airflow hood should be used, dependent also upon the individual state board of pharmacy requirements. The actual facility to be used depends upon the level of compounding to be done.

Adequate lighting and ventilation shall be provided in all drug compounding areas. Potable water shall be supplied under continuous positive pressure in a plumbing system free of defects that could contribute contamination to any compounded drug product. Adequate washing facilities, easily accessible to the compounding area(s) of the pharmacy, shall be provided. These facilities shall include, but not be limited to, hot and cold water, soap or detergent, and air-driers or single-use towels. The area(s) used for the compounding of drugs shall be maintained in a clean and sanitary condition. Trash shall be held and disposed of in a timely and sanitary manner.

Compounding Equipment

Much of the equipment used today in compounding has changed. Electronic balances are more often used than torsion balances, micropipets are commonplace, and ultra-freezers are sometimes required in addition to standard refrigerator–freezers. This area is constantly changing and the compounding pharmacist should be aware of the available technology to prepare accurate and effective prescriptions. Becoming acquainted with the local representative of a laboratory supply company is very helpful.

The equipment needed is determined by the type and extent of the services chosen to provide. Hospitals already utilize laminar flow hoods for aseptic compounding of sterile solutions. The same hoods can be used to compound other sterile products such as eye drops. A balance, preferably electronic, is essential. Ointment slabs (pill tiles), along with spatulas of different types and materials, should be on hand. A few mortars and pestles (both of glass, ceramic, and/or plastic) should be obtained and some glassware. It may not be necessary to buy a roomful of equipment, but one should purchase what is needed to start the service, and build it up as the service grows and expands to different arenas.

Equipment used in the compounding of drug products shall be of suitable composition so that surfaces that contact components, in-process materials, or drug products shall not be reactive, additive, or absorptive affecting the safety, identity, strength, quality, or purity of the drug product.

Equipment and utensils used for compounding shall be cleaned and sanitized immediately prior to use to prevent contamination. Cleaning, sterilization, and mainte-

nance procedures of the equipment, utensils, and containers and closures as set forth in the NABP Model Rules for Sterile Pharmaceuticals must be followed.

Previously cleaned equipment and utensils used for compounding drugs must be protected from contamination prior to use. Immediately prior to the initiation of compounding operations, they must be inspected by the pharmacist and determined to be suitable for use.

Automatic, mechanical, or electronic equipment, or other types of equipment or related systems that perform a function satisfactorily may be used in the compounding of drug products. If such equipment is used, it shall be routinely inspected, calibrated (if necessary), or checked to assure proper performance.

Compounding Supplies

Pharmacists have been using chemicals and other materials for prescription compounding throughout history. In the past, these chemicals and materials have been made from natural products, raw materials, and household ingredients. Today compounding pharmacists use chemicals from various sources, depending upon their availability.

The ⟨795⟩ *Pharmacy Compounding* monograph in the *USP 24/NF 19* can be summarized as follows [18]:

A USP- or NF-grade drug substance is the preferred source of ingredients for compounding all drug preparations. If that is not available, the use of another high-quality source, such as analytical reagent (AR) or certified American Chemical Society (ACS) grade, is an option for professional judgment. If the substance is not an official preparation or substance, additional information, such as a certificate of analysis, needs to be obtained by the pharmacist to ensure its suitability.

A manufactured drug product may be a source of active ingredient. Only manufactured drugs from containers labeled with a batch control number and a future expiration date are acceptable as a potential source of active ingredients. When compounding with manufactured drug products, the pharmacist must consider all ingredients present in the drug product relative to the intended use of the compounded preparation.

In summary, it is the responsibility of the pharmacist to select the ''most appropriate'' quality of chemical for compounding, beginning with the USP/NF as the first choice and, if this is not available, descending the list of purity grades using professional judgment and discretion. A certificate of analysis for the chemicals should be obtained and kept on file in the pharmacy for these selected chemicals [17,18].

Stability of Compounded Preparations

Stability is the extent to which a product retains, within specified limits, and throughout its period of storage and use, the same properties and characteristics that it possessed at the time of its preparation. Chemical stability is important for selecting

storage conditions (temperature, light, humidity) and the proper container for dispensing (glass vs. plastic, clear vs. amber or opaque, cap liners), and for anticipating interactions when mixing drugs and dosage forms.

Factors Affecting Stability

An important component of compounding is the consideration of factors influencing the stability of the final preparation. These factors include pH, temperature, solvent, light, air (oxygen, carbon dioxide, moisture), humidity, particle size, ionic strength, dielectric constant, polymorphism, crystallization, vaporization, and adsorption.

Types and Examples of Stability

The USP/NF defines five types of stability, three of which are described here.

- Physical stability is concerned with the original physical properties of the preparation, including appearance, palatability, uniformity, dissolution, and suspendibility.
- Chemical stability implies that each active ingredient retains its chemical integrity and labeled potency, within specified limits.
- Microbiological stability implies that sterility or resistance to microbial growth is retained according to the specified requirements. Antimicrobial agents that are present will retain their effectiveness within specified limits.

Beyond-Use Dating

Whereas commercially manufactured products are required to possess an ''expiration date,'' compounded products are assigned a ''beyond-use'' date. There are numerous sources of information that can be utilized for determining an appropriate ''beyond-use'' date, such as chemical companies, manufacturers literature, laboratory data, journals, and published texts on the subject. Generally, most pharmacists prepare and dispense small quantities of compounded products, recommend storage at room, cool, or cold temperatures, and use a conservative ''beyond-use'' date.

The guidelines published in the *USP 24/NF 19* Chapter ⟨795⟩, *Pharmacy Compounding* [18] state that:

> In the absence of stability information that is applicable to a specific drug and preparation, the following maximum beyond-use dates are recommended for nonsterile compounded drug precautions that are packaged in tight, light-resistant containers and stored at controlled room temperature unless otherwise indicated.
>
> For nonaqueous liquids and solid formulations (where the manufactured drug product is the source of active ingredient), the beyond-use date is not later than 25% of the time remaining until the product's expiration date or 6 months, whichever is earlier.
>
> A USP or NF substance is the source of active ingredient. The beyond-use date is not later than 6 months.

For water-containing formulations (prepared from ingredients in solid form), the beyond-use date is not later than 14 days when stored at cold temperatures.

For all other formulations, the beyond-use date is not later than the intended duration of therapy or 30 days, whichever is earlier. These beyond-use date limits may be exceeded when there is supporting valid scientific stability information that is directly applicable to the specific preparation (i.e., the *same* drug concentration range, pH, excipients, vehicle, water content, etc.).

Quality Control

Physical Tests

Pharmacists can perform a number of physical quality control tests to ensure the uniformity and accuracy of many small-scale compounded preparations, including individual dosage unit weights, average individual dosage unit weights, total product weight, pH, and physical observations such as appearance, taste, and smell.

Physical Observations

Physical observations can include color, clarity, uniform distribution, hardening, brittleness, softening, discoloration, expansion and distortion, caking, odor, precipitation, discoloration, haziness, gas formation, clarity, breaking, creaming, difficulty in resuspending, consistency, grittiness, dryness, shrinkage, water evaporation, shriveling, and the presence of oil stains on packaging.

Sterile Products Testing

If appropriate, because of the number of sterile preparations compounded, products may be tested for sterility and the effectiveness of incorporated preservatives, if present. This may be done either at the pharmacy or in a contract laboratory.

Especially if sterile preparations are made from nonsterile bulk materials, pyrogen testing should be done at the pharmacy or in a contract laboratory.

Contract Analytical Laboratories

Contract analytical testing can be utilized for purity, potency, sterility, and pyrogenicity testing. The frequency of testing is generally related to the volume and frequency of product preparation.

Flavors, Sweeteners, and Colors

Preparation of an Esthetic Product

Flavoring, sweetening, and coloring are important to enhance patient compliance when medications are orally administered. These can often be adjusted to meet the preferences of the patient.

Flavoring

Pharmacists must be familiar with the four primary tastes (sweet, sour, salty, and bitter) and be aware of the correlations between select chemical properties and taste and odor. Using this information, they can use a number of approaches to prepare an acceptable product to minimize the bad taste of drugs, including blending, overshadowing, physical methods (insoluble compounds, emulsification, effervescence, viscosity), chemical methods (adsorption, complexation), and physiological techniques (cooling due to negative heat of solution and the anesthetic action of some ingredients). Flavor intensifiers can also be used, including citrus enhancers such as citric, maleic, or tartaric acids.

Sweetening

A number of different types of sweeteners, caloric and noncaloric, are available for use, depending upon the specific prescription and patient. Some sweeteners have aftertastes that must also be considered. Today, patients also can have their choice of natural or synthetic sweeteners.

Coloring

Coloring is not always necessary, but may be of value in certain medications. For example, oral liquids are generally colored using a dye to match the flavor of the medication. Pharmacists must be aware of the different oil, alcohol, and water solubilities of the dyes they use.

Preservation, Sterilization, and Depyrogenation

Preservation

Some compounded preparations are naturally preserved, as in the case of certain syrups and elixirs; others require the addition of a preservative. Preservatives are commonly added to products to minimize microbial growth, as in the case of oral liquids, topicals, and multi-dose parenterals. A preservative is selected based upon its characteristics, including concentration, pH, taste, odor, and solubility.

In some situations, it is advisable to have a preservative effectiveness test conducted on a preparation that may require routine compounding or extended storage for some reason.

Sterilization

Compounding pharmacists routinely use in-process sterilization, such as sterile filtration, or terminal sterilization, such as autoclaving or dry-heat sterilization. In some situations, combinations of these methods may be used along with chemical sterilization.

Depyrogenation methods used by compounding pharmacists include dry heat and rinsing with Sterile Water for Injection, USP.

Compounding Pharmaceutical Solids

Powders and Granules

Powders are thorough mixtures of dry, finely divided drugs and excipients that are intended for internal or external use. Granules are dosage forms that consist of particles ranging in size from about 4 to 10 mesh (2000–4750 μm). Both powders and granules are easy to use and to compound. An example formula of a currently compounded powder includes the following:

Rx: Misoprostol and lidocaine hydrochloride powder

Misoprostol	600 μg
Lidocaine hydrochloride	500 mg
Polyox WSR-301	2.45 g
Methocel E4M Premium	22.05 g

Capsules

Capsules are dosage forms in which unit doses of powder, semisolid, or liquid drugs are enclosed within a hard or a soft envelope, or shell. Examples of currently compounded capsules are given in Table 1 with the contents as powders, in oil, or in a semisolid-filled capsule, and modified strength capsules.

Tablets

Tablets are solid dosage forms that are compressed or prepared by a sintering process, including sublingual, buccal, chewable, effervescent, and compressed tablets. Some of these can be easily compounded; an example of a tablet triturate is as follows:

Rx: Sodium fluoride 2.2 mg tablet triturates (#100)

Sodium fluoride	220 mg
Sucrose, powdered	1.15 g
Lactose, hydrous	4.63 g

Lozenges and Troches

Lozenges and troches are solid preparations designed to dissolve or disintegrate slowly in the mouth; their base is usually flavored and sweetened. Examples of compounded troches include anesthetic (lidocaine), hormonal (testosterone), analgesic (ketamine), and antifungal (nystatin) preparations (Table 2).

Suppositories

Suppositories are solid dosage forms that are used to administer medicine through the rectum, vagina, or urethra. They are of different sizes or shapes, depending upon

TABLE 1   Compounded Capsules

Ingredients	Quantity
Rx: *Dextromethorphan hydrobromide 30 mg and morphine sulfate 10 mg capsules (#100)*	
Dextromethorphan hydrobromide	3 g
Morphine sulfate	1 g
Lactose	35.5 g
Capsule size #1	#100
Rx: *Triple estrogen 2.5 mg slow-release capsules (#100)*	
Estriol	200 mg
Estrone	25 mg
Estradiol	25 mg
Methocel E4M Premium	10 g
Lactose	23.75 g
Capsule size #1	#100
Rx: *Triple estrogen 2.5 mg in oil capsules (#100)*	
Estriol	200 mg
Estrone	25 mg
Estradiol	25 mg
Peanut oil	20 mL (18.38 g)
Capsules size #1	#100
Rx: *Triple estrogen 2.5 mg semisolid-filled, hard-gelatin capsules (#100)*	
Estriol	200 mg
Estrone	25 mg
Estradiol	25 mg
Polyethylene glycol 1450	20 g
Polyethylene glycol 3350	20 g
Capsules size #1	#100

the body orifice for administration. Examples of compounded suppositories include antinauseant combinations (lorazepam, diphenhydramine, haloperidol, and metoclopramide), and analgesic (morphine), antifungal, and hemorrhoidal (lidocaine, tannic acid) preparations (Table 3).

Sticks

Sticks are a convenient form of administering topical medications and come in different sizes and shapes. They are transportable and can be easily compounded. Examples of compounded sticks include those containing antivirals for herpes and emollients and sunscreens for environmental exposure (Table 4).

Compounding Pharmaceutical Liquids

Solutions

Solutions are liquid preparations containing one or more drug substances that are molecularly dispersed in a suitable solvent or a mixture of mutually miscible solvents.

TABLE 2 Compounded Troches

Ingredients	Quantity
Rx: Testosterone 10 mg troches (#24)	
Testosterone	240 mg
Aspartame	500 mg
Silica gel	480 mg
Acacia	360 mg
Flavor	qs
Polyethylene glycol 1450	23 g
Rx: Pediatric chewable gummy gels	
Active drug	qs
Bentonite	500 mg
Aspartame	550 mg
Acacia powder	500 mg
Citric acid monohydrate	700 mg
Flavor	qs
Gummy gel base	26.6 g
Rx: Gummy gel base	
Gelatin	43.4 g
Glycerin	155 mL
Purified water	21.6 mL
Rx: Nystatin popsicles (#10)	
Nystatin powder	2,500,000 units
Sorbitol 70% solution	20 mL
Simple syrup	50 mL
Flavor	qs
Purified water qs	300 mL
Rx: Tetracaine 20 mg lollipops (#30)	
Tetracaine HCl	600 mg
Lemon essence	0.5 mL
Green food color	2 mL
Sucrose	150 g
Potassium bitartrate	500 mg
Purified water	55 mL

Solutions are used topically as well as internally. Examples of compounded liquids include topicals (wart solutions), oral syrups and elixirs, nasal solutions, otic solutions, iontophoretic solutions (dexamethasone sodium phosphate), and many others (Table 5).

Suspensions

Suspensions are two-phase systems consisting of a finely divided solid dispersed in a liquid, solid, or a gas (Table 6). They are appropriate when the drug to be incorporated is not sufficiently soluble in an ordinary solvent or cosolvent system. They are used orally and topically. Examples of compounded suspensions include pediatric oral liquids where a commercial pediatric dosage form is not available. Commercial tablets and capsules are formulated into a vehicle and can be individually flavored to the patient's preference.

TABLE 3   Compounded Suppositories

Ingredients	Quantity						
Rx: *Fluconazole 200 mg vaginal suppositories (#1)*							
Fluconazole	200 mg						
Polyethylene glycol base	qs						
Rx: *Morphine sulfate slow-release suppositories (#1)*							
Morphine sulfate	50 mg						
Alginic acid	25%						
Witepsol H-15 qs							
Rx: *Gralla type antiemetic suppositories[a] (#1)*							
Ingredient	#1	#2	#3	#4	#5	#6	#7
Metoclopramide HCl	10	—	20	10	40	20	20
Haloperidol	0.5	5	—	—	1	1	—
Diphenhydramine HCl	—	25	25	—	25	25	25
Dexamethasone	—	—	10	—	10	10	5
Lorazepam	0.5	2	—	—	1	—	—
Diazepam	—	—	—	5	—	—	—
Hydroxyzine HCl	25	—	—	—	—	—	—
Promethazine HCl	—	—	—	25	—	—	—
Benztropine mesylate	—	—	—	1	—	—	
Silicon dioxide	20	30	20	15	—	—	—
Fatty acid base qs	2 g	—	2 g	2 g	2 g	2 g	2 g
Polyethylene glycol base qs	—	2 g	—	—	—	—	—

[a] Quantities given in milligrams unless otherwise noted.

TABLE 4   Compounded Sticks

Ingredients	Quantity
Rx: *Fluorouracil 5% topical stick[a]*	
Fluorouracil	5 g
Polyethylene glycol 3350	27 g
Polyethylene glycol 300	68 g
Rx: *Acyclovir stick with sunscreen[b]*	
Acyclovir 200 mg capsules	#5
Para-aminobenzoic acid	150 mg
Silica gel, micronized	120 mg
Polyethylene glycol 3350	6.5 g
Polyethylene glycol 300	15 mL

[a] Twenty 5-g tubes.
[b] Five 5-g tubes.

TABLE 5 Compounded Solutions (100 mL)

Ingredients	Quantity
Rx: *Dexamethasone and lidocaine solution for ionto-phoresis (100 mL)*	
Dexamethasone sodium phosphate	200 mg
Lidocaine hydrochloride	1 g
Sterile water for injection	100 mL
Rx: *Buprenorphine hydrochloride 150 µg/100 µL nasal spray (100 mL)*	
Buprenorphine hydrochloride	150 mg
Glycerin	5 mL
Methylparaben	200 mg
0.9% Sodium chloride injection	95 mL

Emulsions

Emulsions are heterogeneous systems (Table 7) consisting of at least one immiscible liquid that is intimately dispersed in another liquid in the form of droplets or globules, whose diameters generally exceed 0.1 µm. There are also thermodynamically unstable mixtures of two essentially immiscible liquids and an emulsifying agent that helps hold them together. Compounded emulsions are used for topical and oral applications. Topical emulsions include creams and even liposomal preparations. This category includes the pluronic–lecithin organogels, which are penetration-enhancing gels.

Compounding Pharmaceutical Semisolids

Ointments and Pastes

Ointments are semisolid preparations to be applied externally to the skin or mucous membranes; they soften or melt at room temperature. Pastes are thick, stiff ointments

TABLE 6 Compounded Solutions

Ingredients	Quantity
Rx: *Indomethacin 4% topical spray (100 mL)*	
Indomethacin	4 g
Hydroxypropyl cellulose	200 mg
Sodium lauryl sulfate	100 mg
Purified water	10 mL
Alcohol, 95% qs	100 mL
Rx: *Testosterone 10 mg/0.1 mL sublingual drops (10 mL)*	
Testosterone	1 g
Saccharin	100 mg
Silica gel	200 mg
Flavor	qs
Almond oil qs	10 mL

TABLE 7   Compounded Emulsions

Ingredients	Quantity
Rx: *Emulsion base (100 mL)*	
Mineral oil, heavy	25 mL
Isopropyl myristate	25 mL
Polysorbate 80	7 mL
Methylparaben	200 mg
Propylparaben	100 mg
Purified water qs	100 mL
Rx: *Ketamine 10% in pluronic-lecithin organogel (100 mL)*	
Ketamine hydrochloride	10 g
Isopropyl palmitate : soy lecithin 1 : 1	20 g
Plutonic® F127 20% gel qs	100 mL

that ordinarily do not flow at body temperature and thus protect and coat the areas to which they are applied. Examples of compounded ointments and pastes are given below.

Rx: Testosterone–menthol eutectic ointment (2% testosterone) (100 g)
Testosterone–menthol eutectic mixture	6.33 g
Hydrophilic petrolatum	93.67 g

The testosterone–menthol eutectic mixture can be prepared by mixing 31.6 g of testosterone with 68.4 menthol, using sufficient methyl alcohol to dissolve both and allowing the alcohol to evaporate to dryness.

Rx: Aluminum acetate paste (100 g)
Aluminum acetate solution	17 mL
Anhydrous lanolin	34 g
Lassar's plain zinc paste	49 g

Creams

Creams are opaque, soft solids or thick liquids intended for external application. Creams may contain medications dissolved or suspended in water-soluble or vanishing cream bases and can be either water-in-oil or oil-in-water. An example is given below.

Rx: Progesterone, micronized (5 g)
Glycerin	qs
Hydrophilic ointment	95 g

Commercial oil-in-water vehicles can be used and the quantity of progesterone is variable.

TABLE 8 Compounded Gels

Ingredients	Quantity
Rx: Piroxicam 0.5% alc. Gel (100 mL)	
Piroxicam 20-mg capsules[a]	#25
Hydroxypropyl cellulose	1.75 g
Isopropyl alcohol, 70%	98.25 mL
Propylene glycol	4.1 mL
Polysorbate 80	1.7 mL
Rx: Ketoprofen, cyclobenzaprine, and	
lidocaine PLO gel (100 mL)	
Ketoprofen	10 g
Cyclobenzaprine hydrochloride	1 g
Lidocaine	5 g
Propylene glycol	10 mL
Sorbic acid	200 mg
Lecithin : isopropyl palmitate solution	20 g
Plutonic® F-127 20% gel qs	100 mL

[a] Piroxicam powder can also be used.

Gels

Gels are semisolid systems consisting of suspensions made up of small inorganic particles or large organic molecules interpenetrated by a liquid. Some gels are clear, others are turbid because their ingredients may or not be completely molecularly dispersed or they may be aggregates, which disperse light. Examples of compounded gels (Table 8) include topical, oral cavity, and even rectally administered preparations.

Compounding Sterile Preparations

Ophthalmics

Ophthalmic preparations in the form of solutions, suspensions, and ointments, are sterile, free from foreign particles, and prepared especially for instillation into the eye. Examples of compounded ophthalmics include drugs such as acetylcysteine, acyclovir, alteplase, amikacin, amphotericin B, ascorbic acid, bacitracin, calcium gluconate, penicillins, cephalosporins, aminoglycosides, cromolyn, cyclosporin, deferoxamine, anti-inflammatory corticosteroids, chelating agent, oncology agents, and numerous others prepared as both solutions and ointments, an example is given below.

Rx: Acetylcysteine 1.5% Ophthalmic solution
Acetylcysteine   15 g
Sterile water for injection   qs   100 mL
Sodium hydroxide to pH of 6–7.5

TABLE 9   Compounded Inhalation Solutions

Ingredients	Quantity
Rx: *Morphine sulfate 0.25% inhalation solution*	
Morphine sulfate	250 mg
Citric acid, hydrous	100 mg
Sterile water for injection, qs	100 mL
Rx: *Albuterol sulfate and ipratropium bromide*	
inhalation solution	
Albuterol sulfate	100 mg
Ipratropium bromide	16.7 mg
Benzalkonium chloride 50% solution	0.003 mL
Sodium chloride injection, 0.9%, qs	100 mL

Inhalation Solutions

Inhalation solutions are designed to deliver a drug into the respiratory tree of a patient for a local or systemic effect. Examples of compounded inhalation solutions (Table 9) include individually and in combinations of albuterol, cromolyn, morphine sulfate, corticosteroids, ipratropium, metaproterenol, terbutaline, and others.

Parenterals

Parenterals are administered to the body by injection. They must be sterile, nonpyrogenic, and particulate-free. Examples of compounded parenterals include high-dose analgesics for patient controlled analgesia (morphine sulfate 50 mg/mL), antiemetic injections, fentanyl and bupivacaine injections for ambulatory pump reservoirs, oncology combinations, and others (Table 10).

Compounding with Biotechnology Preparations

Biotechnology preparations are developed using the techniques of engineering and technology with living organisms. Biotechnology presents compounding pharmacists with a unique and new source of therapeutic agents that may require their special expertise.

Biotechnology products differ in their method of preparation and potential problems they present in their formulation. Pharmacists involved in compounding with biologically active proteins are interested in their stabilization, formulation, and delivery. Most of the current biotechnology products are proteins, but soon some may be smaller peptide-like molecules.

Physicochemical Considerations

In working with biotechnology-derived drugs, the compounding pharmacist must be cognizant of both the active drug constituent and the total drug delivery system or

TABLE 10 Compounded Parenterals

Ingredients		Quantity
Rx: Antinauseant injection (132.5 mL)		
Reglan® *(5 mg/mL)*	30 mL	150 mg
Ativan® (2 mg/mL)	0.5 mL	1 mg
Mannitol 25%	50 mL	12.5 mg
Compazine (5 mg/mL)	2 mL	10 mg
Dextrose injection 5%	50 mL	
Rx: Fentanyl citrate–bupivacaine hydrochloride–epinephrine hydrochloride injection (100 mL)		
Fentanyl citrate 50 µg/mL	2.5 mL	
Bupivacaine hydrochloride, 0.5%	8.75 mL	
Epinephrine hydrochloride, 1:100,000	6.9 mL	
Sodium chloride injection 0.9%	81.85 mL	
Rx: Parenteral nutrition solution (1200 mL)		
Dextrose injection 50%	500 mL	
Amino acids 8.5% with electrolytes	500 mL	
Lipids 10%	200 mL	
Calcium gluconate	1 g	
Magnesium sulfate	2 g	
Trace elements	1 unit	
M.V.I.®-12[a]	1 unit	

[a] Multiple Vitamin Injection.

carrier. Proteins being generally very potent, are used in low concentrations. Pharmacists must be aware of the vehicle, buffer, and stabilizer requirements for these preparations, including the use of surfactants, amino acids, polyhydric alcohols, fatty acids, proteins, antioxidants, reducing agents, and metal ions. Chelating agents, preservatives, pH control, and tonicity adjusting agents must be considered.

Quality Control and Stability

The factors involved in handling proteins are important to retain a drug's biologic activity up to the time when it is administered to the patient. Proteins are inherently unstable and their degradation profiles can be very complex. Compounding pharmacists may be involved in the selection of an appropriate vehicle for drug delivery, individualizing dosages, administering drugs through novel delivery systems, preparing drugs for delivery through these systems, monitoring their efficacy, and counseling patients on their use. An example of a compounded prescription using a biotechnology-derived product is given below.

Rx: Tissue plasminogen activator 25 µg/100 µL ophthalmic solution

Tissue plasminogen activator	20 mg vial
Sodium chloride injection 0.9%	60 mL
Sterile water for injection	20 mL

Veterinary Preparations

Veterinary compounding can be considered when:

- No effective FDA-approved products are available,
- The available dosage forms are inappropriate,
- Multiple and concurrent disease states are present,
- An additive therapeutic effect could be obtained from simultaneous administration of two or more products or to minimize side effects,
- Economic realities would preclude treatment with the approved product, and
- Compounding would encourage compliance of dosage and therapeutic regimens.

Most FDA-approved veterinary drugs are for certain species, either food-producing or for a large target population (household pets). If veterinarian pharmaceutical companies do not perceive a sufficiently large market for a product, they will not seek FDA approval. This has left a large vacuum, or a potential market, that can be filled by compounding pharmacists. There are no FDA-approved products for exotic species due to the limited market and there are only few FDA-approved products for some of the more common species. Veterinarians need patient-specific products which pharmacists know how to prepare. Consequently, a team approach has been developed to the benefit of the veterinarian, pharmacist, and the animals.

Questions that often come up include the following: What is the overall goal of the treatment of this animal? Are there any commercially available products that can be used? What are the regulatory concerns? Is this a food- or a milk-producing animal? Will there be a residue problem? What do we know about the physical and chemical compatibility of these drugs? What is the stability of these drugs before, during, and after the compounding process? What are the pharmacokinetics of the active ingredients? Will personnel be at risk by handling the drug during compounding or while using the compounding form?

Examples of some veterinarian preparations are given in Table 11.

TABLE 11   Compounded Veterinarian Preparations

Ingredients	Quantity
Rx: *Acetylcysteine, gentamicin, and atropine* *ophthalmic solution (15 mL)*	
Acetylcysteine	720 mg
Gentamicin[a]	36 mg
Atropine sulfate	36 mg
Sterile water qs	15 mL
Rx: *Methylpyrazole injection (20 mL)*	
4-Methylpyrazole	1 g
Polyethylene glycol 400	9 mL
Bacteriostatic water for injection qs	20 mL

[a] As gentamicin sulfate.

Summary

Pharmacy compounding is providing pharmacists with a unique opportunity to practice their time-honored profession. It will become an even more important part of pharmacy practice in the future, including those involved in community, hospital, nursing home, home health care, veterinary, and specialty practices. Pharmaceutical compounding is a practice where the clinical expertise of pharmacists can be merged with the scientific expertise of pharmacists to make pharmaceutical care a reality.

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Drug Delivery—Oral, Colon-Specific

Introduction

Oral ingestion has historically been the most convenient and commonly employed method for drug delivery. For sustained as well as for controlled-release systems, the oral route of administration has received the most attention. This is due to greater flexibility in dosage form design for the oral than for the parenteral route. Patient acceptance of oral administration of drugs is high. It is a relatively safe route of drug administration compared with most parenteral forms, and the constraints of sterility and potential damage at the site of administration are minimal.

Colon-specific drug delivery systems offer several potential therapeutic advantages. In a number of colonic diseases like colorectal cancer, Crohn's disease, spastic colon, and others, it has been shown that local is more effective than systemic delivery [1]. Colonic drug delivery can be achieved by oral or by rectal administration. Rectal delivery forms (suppositories and enemas) are not always effective since a high variability is observed in the distribution of drugs administered by this route. Suppositories are effective in the rectum due to the confined spread, whereas enema solutions can only be applied topically to treat diseases of the sigmoid and the descending colon. Therefore, the oral route is preferred. Absorption and degradation of the active ingredient in the upper part of the gastrointestinal tract is the major obstacle with the delivery of drugs by the oral route and must be overcome for successful colonic drug delivery.

Drugs for which the colon is a potential absorption site (e.g., peptides and proteins) can be delivered to this region for subsequent systemic absorption. The digestive enzymes of the gastrointestinal tract generally degrade these agents. However, these enzymes are present in significantly lower amounts in the colon as compared to the upper portion of the gastrointestinal tract [2].

Colon-specific drug delivery has been attempted in a number of ways that mainly seek to exploit the changes in the physiological parameters along the gastrointestinal tract [1,3]. These approaches include the use of prodrugs, pH-sensitive polymers, bacterial-degradable polymers, hydrogels and matrices, and multicoating time-dependent delivery systems. The use of these strategies to target drug delivery to the colon will be discussed here in detail.

Anatomical, Physiological, and Biochemical Characteristics of the Colon

In terms of size and complexity, the human colon falls between that of carnivores, such as the ferret which have no discernable junction between ileum and colon, and herbivores like the rat, which has a voluminous cecum. The cecum, colon ascendens, colon transversale, colon descendens, and rectosigmoid colon make up the colon. It

is about 1.5 m long with the transverse colon being the largest and the most mobile part (Fig. 1, [4]). Unlike the small intestine, the colon does not have any villi. However, due to the presence of plicae semilunares, which are crescentic folds, the intestinal surface of the colon is increased to approximately 1,300 cm^2 [3]. The physiology of the proximal and distal colon differs in several respects that can have an effect on drug absorption at each site. The physical properties of the luminal content of the colon also change, from liquid in the cecum to semisolid in the distal colon.

The colon serves four major functions:

- Creation of a suitable environment for the growth of colonic microorganisms, such as *Bacteroids*, *Eubacterium*, and *Enterobacteriaceae*, among others;
- Storage reservoir of fecal contents;
- Expulsion of the contents of the colon at a suitable time; and
- Absorption of water and Na$^+$ from the lumen, concentrating the fecal content, and secretion of K$^+$ and HCO$_3^-$ [5]. The active secretion of K$^+$ is stimulated by mineralocorticoids.

The following factors influence absorption from the colon:

1. Physical characteristics of the drug (pKa, degree of ionization)
2. Colonic residence time as dictated by gastrointestinal tract motility
3. Degradation by bacterial enzymes and by-products
4. Selective and nonselective binding to mucus
5. Local physiological action of the drug
6. Disease state
7. Use of chemical absorption enhancers, enzyme inhibitors or bioadhesives

Colon-specific drug delivery is primarily dependent on two physiological factors, the pH and the transit time.

The pH of the gastrointestinal tract is subject to both inter- and intrasubject variations. Diet, diseased state, and food intake influence the pH of the gastrointestinal fluid. The change in pH along the gastrointestinal tract has been used as a means for targeted colon drug delivery [6]. This can be achieved by applying coatings that are intact at the low pH of the stomach but which dissolve at neutral pH. The right, mid,
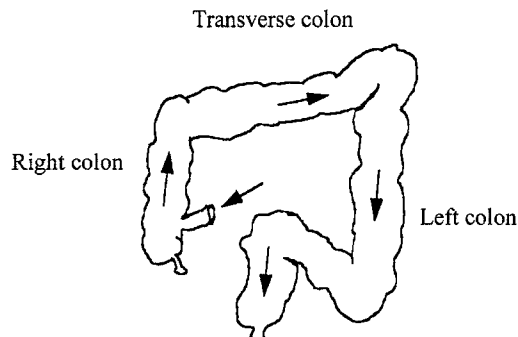
FIG. 1.   Right, transverse, and left colon (adapted from Ref. 4).

and left colon have pH values of approximately 6.4, 6.6, and 7.0, respectively [7]. The pH of the colon is often lower than the pH of the small intestine, which can be as high as 8 or 9 [7]. In vitro experiments simulating the pH of the different regions of the gastrointestinal tract suggest that drug release from delivery systems that are triggered by a change in pH might already occur in the small intestine [8]. However, other studies have concluded that the pH can be used for drug release in the colon [6]. These contradictions may be a result of nonstandardized in vitro methods as well as be due to the difference in drug properties and preparation methods of drug delivery systems. Interspecies variability in pH is a major concern when developing and testing colon-specific delivery systems in animals and applying the information to humans [4].

Drug delivery to the colon by the oral route depends on gastric emptying and the small-bowel transit time. Drugs taken before meals usually pass out of the stomach within 1 hr, but can take up to 10 hr if taken after a meal. The transit time in the small intestine is relatively constant, ranging between 3 and 4 hr, regardless of various conditions such as physical state, size of dosage form, or presence of food in the stomach [9].

A major determinant of the absorption of a compound from the colon is the residence in any particular segment of the colon. The time taken for food to pass through the colon accounts for most of the time the food is present in the gut. In normal subjects this is about 78 hr for expulsion of 50% ingested markers but may vary from 18 to 144 hr. Steady-state measurements of mean transit time after ingestion of markers for several days gave a mean transit time of 54.2 hr [10].

When the dosage forms reach the colon, transit depends on size. Small particles pass through the colon (236 min for 3 mm tablets and 238 min for 6 mm tablets on study day 1; 226 min for 9 mm tablets and 229 min for 6 mm tablets on study day 2; 218 min. for 12 mm tablets and 219 min for 6 mm tablets on study day 3) more slowly than the larger units [11], but the density and size of larger single units had no real effect on colonic transit. It has been shown that pellets move faster than tablets through the ascending colon and therefore may have a more favorable action than tablets with respect to colonic drug absorption [11].

Colonic transit time is only slightly affected by food, but is reduced under stress. Studies have shown that drugs, which act on the parasympathetic or sympathetic nervous system, affect the propulsive motor activity, thereby influencing the colonic transit time [12]. Although not significantly affected by most diseases [13], the transit time is shorter in patients who complain of diarrhea and longer in patients with constipation.

Permeability and Metabolic Characteristics

Drug Permeability in the Colon

Drug absorption in the colon is restricted by a number of barriers. The bacterial flora, which is significantly higher in the distal colon, can affect drug bioavailability [14]. The mucus present at the epithelial surface of the colon acts as a physical barrier to uptake due to specific and nonspecific drug binding. Drug binding to the colonic lumen may facilitate enzymatic or environmental degradation by increasing the resi-

dence time of the drug. Cephalosporins, penicillins, and aminoglycosides are some of the small molecule drugs that bind to the negatively charged mucus [15]. This binding can prevent drugs from reaching the epithelial surface as well. Some molecules, like carbachol and serotonin, that stimulate mucus secretion can therefore reduce their own absorption as well as the absorption of any coadministered drug [16]. The removal of this mucus barrier with mucolytic agents can result in increased absorption. Schipper et al. showed that chitosans had a pronounced effect on the permeability of mucus-free Caco-2 layers and enhanced the permeation of atenolol 10–15-fold [17]. The binding of chitosan to the epithelial surface and subsequent absorption enhancing effects were significantly reduced in mucus-covered HT-29-H cells. Alterations of this mucus layer, however, have been associated with pathological conditions.

The single most formidable barrier to the uptake of drugs occurs at the level of the epithelium [18]. The lipid bilayer of the individual colonocytes and the occluding junctional complex (OJC) between these cells provide a physical barrier to drug absorption. The OJC structures found at the apical neck of the cells in the gastrointestinal tract limit paracellular transport of water and small molecules (less than 350 Da) in a cation-selective fashion [19]. The movement of water and ions via the paracellular route is driven by transcellular ionic gradients, with the proximal colon appearing to be slightly more conducive to such activity than the distal colon [20].

Carrier-mediated absorption in the colon is not extensive and usually relates to the metabolic events in the residual bacteria [21]. Drugs that pass from the apical to the basolateral side of this epithelial layer can do so by the transcellular route (i.e., by passing directly through the colonocytes) or by the paracellular route (i.e., by passing through adjacent colonocytes). Mathematical models describing the transport of small drug molecules across these barriers have been developed [22]. The movement of peptides is predominantly dependent upon hydrogen bonding [23]. Compared with peptides, proteins experience a greater thermodynamic barrier for passing through cell membranes and are more vulnerable to denaturation at the interface.

Drug molecules that have traversed the physical and enzymatic barriers of the colonic mucosa may enter the blood-capillary bed or the lymphatic sinuses. Intact drug that reaches the venous capillaries from the submucosa is transported to the liver via the hepatic-portal system where they may undergo significant metabolism. On the other hand, uptake into the lymphatic sinuses of the colon results in direct delivery into the systemic circulation that causes less metabolic breakdown of the absorbed drug [3].

Drug Metabolism in the Colon

Metabolic reactions in the liver and the small intestine are well documented [24]. However, only sparse information is available on drug metabolism in the colon. Drug metabolism in the colon can be brought about by the host enzymes in the epithelial cells or by the microbial enzymes in the gut flora. Metabolic activities in the wall of the colon can be attributed to the cytochrome P450, esterases, amidases, and various transferases [25]. Reductive drug metabolism does not appear to be important at this site.

TABLE 1 Drug Metabolizing Enzymes in the Human Colon that Catalyze Reductive Reactions

Enzymes	Microorganism	Metabolic Reaction Catalyzed
Nitroreductase	*E. coli, Bacteroids*	Reduce aromatic and heterocyclic nitrocompounds
Azoreductase	*Clostridia, Lactobacili, E. coli*	Reductive cleavage of azocompounds
N-oxide reductase, sulfoxide reductase	*E. coli*	Reduce N-oxides and sulfoxides
Hydrogenase	*Clostridia, Lactobacili*	Reduce carbonyl groups and aliphatic double bonds

The colonic mucosa resembles the small intestinal mucosa with respect to the spectrum of metabolizing enzymes [26]. However, the total metabolic capacity of the colonic wall is inferior, since the mucosal mass in the lower part of the intestine is several times smaller than in the upper part. But this may be more than offset by the high metabolic capacity found in the gut flora in the large intestine.

The gastrointestinal tract contains a variety of microorganisms that participate in the metabolism of ingested material (Tables 1 and 2) [27]. The upper part contains only a small number of mainly gram-positive bacteria, whereas the colon contains a vast amount of largely anaerobic bacteria, with *Bacteriods*, *Bifidobacterium*, and *Eubacterium* being the most common species found [28]. Gram-positive cocci like *Clostridium enterococci* and various species of *Enterobacteriaceae* are also present [29]. The growth of the bacteria is regulated by gastric acids, peristaltic activity, and microbial interaction including bacterial metabolic by-products. Administration of antibiotics as well as the onset of disease and age can affect the metabolic activity of the intestinal microflora [30].

Redox reactions and hydrolysis are the predominant metabolic conversions triggered by the intestinal microflora. The main reductive enzymes produced by the intestinal microflora are nitroreductase, deaminase, urea dehydroxylase, and azoreductase; the hydrolytic enzymes are β-glucoronidase, β-xylosidase, β-galactosidase, and α-L-arabinosidase. Studies conducted by Macfarlane and co-workers have shown that proteolysis can also happen in the colon [31]. More recent findings by this group indicate that bacterial fermentation of proteins in humans could account for 17% of

TABLE 2 Drug Metabolizing Enzymes in the Colon that Catalyze Hydrolytic Reactions

Enzymes	Microorganism	Metabolic Reaction Catalyzed
Esterases and amidases	*E. coli, P. vulgaris, B. subtilis, B. mycoides*	Cleavage of esters or amidases of carboxylic acids
Glucosidase	*Clostridia, Eubacteria*	Cleavage of β-glycosidases of alcohols and phenols
Glucuronidase	*E. coli, A. aerogenes*	Cleavage of β-glucuronidases of alcohols and phenols
Sulfatase	*Eubacteria, Clostridia, Streptococci*	Cleavage of O-sulfates and sulfamates

the short chain fatty acids in the cecum and for 38% in the sigmoid and the rectum [32].

The unique enzymatic features of the microbial flora present in the colon makes it conductive for drug targeting. Active metabolites intended for local therapy can be liberated from poorly absorbed parent molecules by microbial processes. Advantage can also be taken for release of drugs from delivery systems, disintegrating only by the action of microbial enzymes, as a means for targeted delivery. This approach is suitable for drug molecules that are unstable in the upper part of the gastrointestinal tract, but can be absorbed from the lower part.

Characteristics of Drugs that Favor Colonic Delivery

The colon is a less hostile environment than the stomach and the small intestine [33]. It has a longer retention time and is responsive to agents that cause an increase in the absorption of poorly absorbed drugs [34]. Drugs that benefit from colon targeting include those for the treatment of inflammatory bowel disease and irritable bowel syndrome. Drugs metabolized in the upper gastrointestinal tract would also be candidates for colon targeting. Drugs like theophylline, nifedipine, ibuprofen, diclofenac, metoprolol, brompheniramine, pseudoephedrine, denitrate, isosorbide, oxprenolol, low molecular weight peptides, and peptide-like drugs have been shown to be effectively absorbed from the colon [35].

The permeability of the colonic epithelium may not be sufficient for achieving a transport rate required for therapeutic activity. This hurdle may be overcome, at least in part, by using the penetration enhancers listed below [36].

1. Nonsteroidal anti-inflammatory agents (NSAIDS) (e.g., indomethacin and salicylates)
2. Calcium ion chelating agents (e.g., EDTA and citrate)
3. Surfactants (e.g., polyoxyethylene lauryl ether (BL-9EX) and saponin)
4. Bile salts (e.g., taurocholate and glycocholate)
5. Fatty acids (e.g., sodium caprate, sodium caprylate, sodium laurate, and sodium oleate)
6. Mixed micelles (e.g., monoolein taurocholate, oleic acid taurocholate, oleic acid–polyoxyethylene hydrogenated castor oil (HCO 60), and oleic acid glycocholate)
7. Other agents (e.g., acylcarnitine, azone (1-dodecylazacycloheptan-2-one), dicarboxylic acids, and enamine)

Comparison of their rate of onset and recovery of a treated mucosa has been made [37]. Fatty acids have strong and fast reactivity and allow for a fast recovery of the barrier function. Bile salts and salicylates are moderate, fast-acting agents with fast barrier-function recovery. Strong surfactants and chelators have strong or moderate reactivity and a slow recovery of barrier function. Solvents like dimethylsulfoxide and ethanol have moderate reactivity and act primarily as agents to improve drug miscibility in an aqueous environment. The enhancers listed above are also effective in the small intestine [22]. Enhancers that are more colon specific include ethylacetoacetate, which must be first metabolically transformed to enamine [38].

Several chemical enhancers, such as sodium ethylenediaminetetraacetate and sodium taurocholate, oleic acid [37], polyoxyethylated nonionic surfactants, citric acid, and dihydroxy bile salts open the paracellular route, presumably by disruption of the intracellular OJC function [37]. The use of nitric oxide is another approach [39]. Moreover, manipulation of the cyclooxygenase pathway activities to trigger the release of compounds like substance P may provide another means for altering colonic permeability [40].

Molecules like sodium caprate, sodium caprylate, and sodium salicylate can enhance transcellular uptake of poorly permeable compounds through the colonic mucosa in low concentrations [41,42]. Some of these absorption enhancers can cause local irritation [43]. It has been suggested that these enhancers might function to denature membrane proteins and/or modify lipid–protein interactions as a means of inducing drug uptake [37]. Mixed micelles appear to produce only limited disordering of surface mucosal cells, possibly by reducing the damaging effect of surfactants and somehow augmenting their enhancing activity. Absorption enhancements by surfactants, fatty acids, and mixed micelles may be due to improved solubility and/or stability of the drug being transported.

Stable molecules (e.g., dextran, polyethylene glycol) have been used in studies to address the size of the transport window that can be opened in the colon. Muranishi and Takada [44] have demonstrated that, upon transport enhancement, low molecular weight drugs are directed twice as often to a transcellular route than the paracellular route, whereas molecules of approximately 20 kDa are directed almost equally through transcellular and paracellular routes. Enhanced transport with caprate, laurate, and mixed micelles suggest opening of colonic pores of 1.4–1.6 nm (14–16 Å) and enhancement with tuarocholate or caprylate suggests the formation of 1.1–1.2 nm (11–12 Å) pores [37].

Chemical enhancers have been demonstrated to produce transport windows in colonic epithelia large enough for the passage of many bacterial toxins. Patients suffering from inflammatory bowel diseases and colitis typically have increased colonic permeability [45] due to bacterial toxins, both entertoxins and cytotoxins that increase capillary permeability. Increased colon permeability associated with a diseased state may be useful in treatment where improvement of the condition might reduce mucosal permeability and naturally reduce drug transport.

Strategies for Colon-Specific Drug Delivery

Attempts have been made to achieve colon-specific delivery of drugs. These include prodrugs and enteric coated polymers that are sensitive to degradation by bacterial enzymes, and matrices and hydrogels susceptible to degradation by bacterial enzymes.

Prodrugs

The prodrug sulfasalazine (SAS), used in the treatment of Crohn's disease, ulcerative colitis, and rheumatoid arthritis, is the earliest example of targeted drug delivery in

the colon (Figs. 2 and 3) [1]. Only 12% of the drug is absorbed in the small intestine following oral delivery. When SAS reaches the colon after oral administration, the diazoreductase of the colon bacteria cleaves the azo bond, releasing 5-aminosalicylic acid and sulfapyridine into the colonic lumen [46]. Osalazine (Dipentum), which consists of two molecules of 5-aminosalicylic acid linked by an azo bond [47], was developed to deliver 5-aminosalicylic acid directly to the colon. Balsalazide is another prodrug that is reputed to benefit from a less toxic carrier molecule than that in SAS. Balsalazide is 5-aminosalicylic acid linked by a diazo moiety to 4-aminobenzoyl-β-alanine [48].

In rats, a comparable total release of 5-aminosalicylic acid has been observed from SAS and 5-aminosalicylic acid azo-linked to a polymeric prodrug consisting of polysulfonamidoethylene as the carrier molecule (Polyasa) [49]. Yet, the latter was more effective in reducing inflammation in the guinea pig ulcerative colitis model. The release of 5-aminosalicylic acid from polymeric prodrugs was dependent upon the structure of the polymeric backbone [50]. The drawback of coupling 5-aminosalicylic acid to a polymeric backbone is the large amount of the drug that has to be taken orally.

A second approach that has been attempted involves prominent bacterial enzymes such as glycosidases and polysaccharides [51]. Prodrugs were developed by coupling glucose or galactose to steroids like dexamethasone, prednisolone, hydrocortisone, and fludrocortisone via a β-glycosidic bond. Taken orally, these polar prodrugs undergo minimal absorption in the small intestine. In the colon, the bacterial glycosidases cleave the polar moiety and release the steroid. Corticosteroids have been delivered in this manner to treat disorders of the large intestine. The side effects of corticosteroids can be minimized in this manner because of the minimal absorption of the drug in the small intestine as well as dose reduction. The oral delivery of the
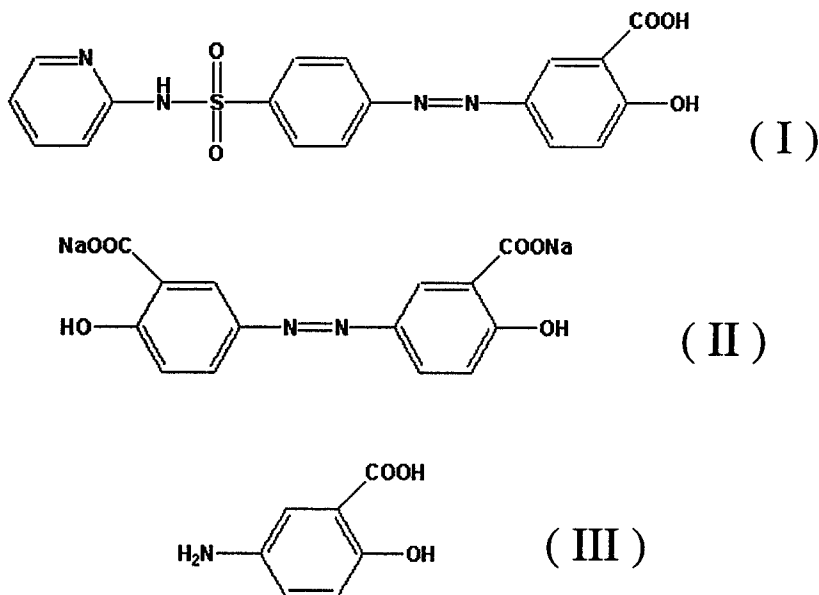
FIG. 2. Chemical structures of sulphasalazine (I), olsalazine (II), and 5-ASA (III) (adapted from Ref. 1).
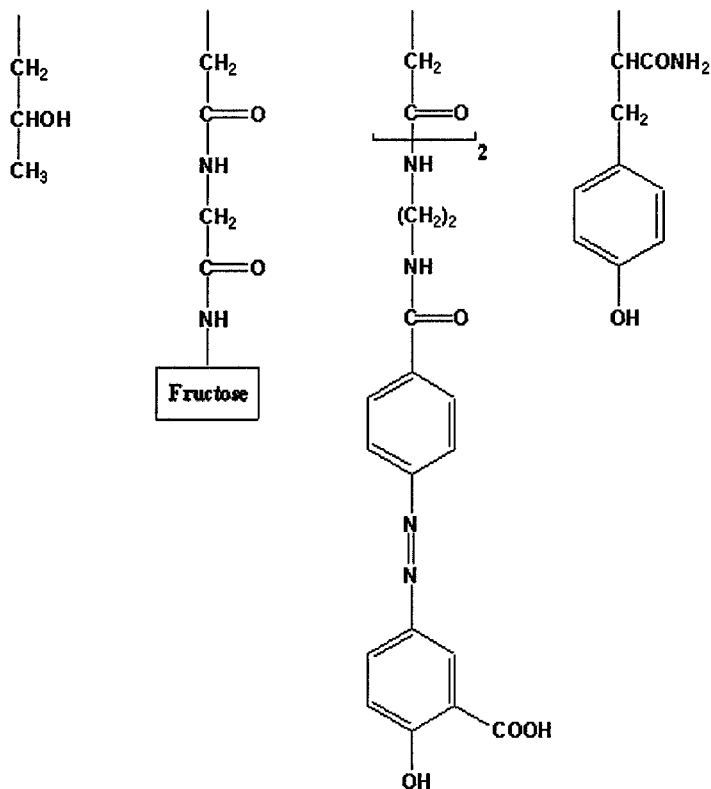
FIG. 3. The polymeric prodrug of 5-ASA (adapted from Ref. 1).

prodrug form of dexamethasone produced the same therapeutic effect as the parent drug in guinea pig models. However, the dose was lower with the prodrug [52]. This approach has been extended to the development of glycoside prodrugs of budesonide and menthol and with dexamethasone poly(L-aspartate) [53].

The prodrug methodology has been used with naproxen conjugated to dextran by an ester linkage [54]. The release of naproxen was 17 times higher in the cecum and in the colon homogenates of the pig than in the control medium or homogenates of the small intestine. The dextran prodrugs of methylprednisolone and dexamethasone have also been developed [55]. In both cases, most of the drugs were released in the contents of the large intestine, and only minor chemical hydrolysis occurred in the upper gastrointestinal tract.

Recent developments involve utilization of cyclodextrins that are absorbed only in minor quantities in the small intestine but are fermented by the colonic bacteria. In a recent study [56,57], the use of biphenyl acetic acid conjugates of β-cylcodextrins was described. The ester conjugate released the drug preferentially when incubated with rat cecal contents, and almost no release was observed on incubation with the contents of the stomach or the small intestine.

Thus, it can be seen that prodrug-based approaches are being utilized extensively to deliver drugs in increased amounts to the colon. Consistency of conversion from

the prodrug to the active ingredients in vivo is of concern as colonic bacterial populations can be modulated by diet, health, and a variety of antibiotics [29].

Polymers

Enteric Coated

Drugs can be delivered locally and selectively to the colon by taking advantage of the difference in the pH of the different regions of the gastrointestinal tract. The pH is low in the stomach but increases in the small and the large intestines. For the purpose of targeting drugs to the colon, an outer enteric coating (for example, of cellulose acetate phthalate) can be used to protect the drug in the low stomach pH. In the small intestine (pH about 7.5), the enteric coating dissolves, exposing a polymeric coating, typically of ethylcellulose with microcrystalline cellulose and plasticizers. The polymeric coating is designed to stay intact in the small intestine until the dosage form reaches the colon. In the colon, bacteria are expected to digest the microcrystalline cellulose to allow for the disintegration of the polymeric coating around the drug. Such a scenario has been confirmed in a study by Hirayama et al. [56], in which all the compressed tablets with dual coating remained intact in the small intestine and 85% of which disintegrated in the colon.

   The problems with this approach is that the intestinal pH may not be stable, since it is affected by diet, disease, and presence of fatty acids, carbon dioxide, and other fermentation products. Moreover, there is considerable difference in inter- and intra-individual gastrointestinal tract pH, which causes a major problem in reproducible drug delivery to the large intestine [58].

   Spherical pellets containing 5% triamcinolone acetonide were prepared by Villar-Lopez and co-workers [59] by extrusion/spheronization following formulation with microcrystalline cellulose and/or a hydrophilic excipient like lactose, sodium carboxymethylcellulose, or β-cyclodextrin. Their suitability for coating, with a view toward colonic drug delivery, was assessed in terms of their size, sphericity, and dissolution test response. The best results were afforded by a 5:90:5 composition of microcrystalline cellulose, β-cyclodextrin, and triamcinolone acetonide, prepared by complexation of triamcinolone acetonide with β-cyclodextrin prior to the addition of microcrystalline cellulose.

   Commercial formulations of 5-aminosalicylic acid use enteric coatings of pH-sensitive methacrylic resins called Eudragit (Fig. 4). Both water-soluble and water-insoluble forms of Eudragit have been tested for colon targeting. Eudragit L dissolves at a pH above 5.6 and is used for enteric coating, whereas Eudragit S, which dissolves at a pH above 7.0 (due to the presence of higher amounts of esterified groups in relation to carboxylic groups) is used for colon targeting. Studies have revealed that Eudragit S exhibits poor site specificity [60].

   In a study performed by Khan et al. [61], lactose placebo tablets were coated with different combinations of Eudragit L and Eudragit S. The Eudragit L–Eudragit S combinations (w/w) studied were 1:0, 4:1, 3:2, 1:1, 2:3, 1:4, 1:5, and 0:1. The disintegration data obtained from the placebo tablets demonstrate that the disintegration rate of the tablets is dependent on the polymer combination used to coat the tablets, the pH of the disintegration media, and the coating level of the tablets.

$R_1 = CH_3;\ H$

$R_2 = CH_3;\ CH_3CH_2^-$

$R_3 = $ -COOH (Eudragit® L and S)
-COOCH$_2$CH$_2$N$^+$(CH$_3$)$_3$Cl$^-$ (Eudragit® RL and RS)
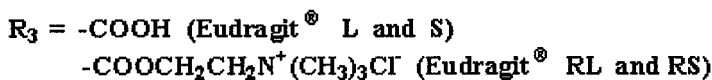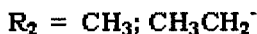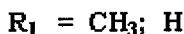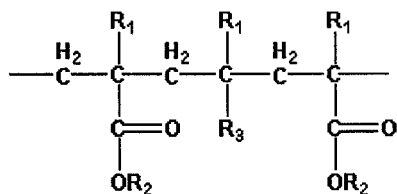
FIG. 4. Chemical structures of Eudragit (adapted from Ref. 1).

It has been shown that polymers with free phthalic acid groups dissolve much faster and at a lower pH than those esterified with acrylic or methacrylic groups. The presence of plasticizer and the nature of the salts in the dissolution medium influence the dissolution rate [62].

In a recent study by Peeters and Kinget [63], the free carboxylic groups of Eudragit S were partially methylated. The product was found to dissolve in water at a pH slightly higher than that of the original polymer. The effectiveness of this product as a colon-specific coating material had been established with human volunteers by in vivo scintigraphic studies [64].

In a study by Gazzaniga and co-workers [65], an oral dosage form was developed consisting of a core with two polymeric layers. The outer layer, an enteric coating, dissolved at a pH above 5. The inner layer, made up of hydroxypropyl methylcellulose, acted as a retarding agent to delay drug release for a predetermined period of time. The thickness of the inner layer determined the lag time. This system was found to release the drug in the colon of the rat between the 5th and the 10th hour.

A pulsed system, called Time-Clock System, has been developed. It comprises a solid dosage form coated with a hydrophobic surfactant layer to which a water-soluble polymer is attached to improve adhesion to the core [66]. The thickness of the outer layer determines the time required to disperse in an aqueous environment. Following the dispersion of the outer layer, the core becomes available for dispersion. An advantage is that common pharmaceutical excipients can be used to manufacture this system. Studies performed on human volunteers showed that the lag time was not affected by gastric residence time. Furthermore, the dispersion of the hydrophobic film was not influenced by the presence of intestinal digestive enzymes or by the mechanical action of the stomach.

Another system, based on the same principle as the Time-Clock System, is called the Time-Controlled-Explosion drug delivery system [67]. It contains a four-layered spherical structure, with a core containing the drug and a swelling agent, and a water-insoluble polymer membrane made of ethylcellulose. This system is characterized by rapid drug release with a programmed lag time. On contact with water through

the polymeric membrane, the swelling agent expands and finally explodes, leading to the release of the contained drug. Drug release is not affected by pH, but the lag time is a function of the thickness of the outer polymeric membrane. A similar approach based on ethylene–vinyl acetate polymers was tested for release of the drug isosorbide-5-nitrate [68].

Ishibashi and co-workers [69] have recently developed a colon-targeted delivery capsule based on pH-sensitivity and time-release principles (Fig. 5). The system contains an organic acid which acts as a pH-adjusting agent and is filled in a hard gelatin capsule together with the drug substance. This capsule is coated with a three-layered film consisting of an acid-soluble layer, a hydrophilic layer, and an enteric layer. After ingestion, these layers prevent drug release until the environmental pH inside the capsule decreases by dissolution of the organic acid, upon which the enclosed drug is quickly released. Therefore, the onset time of drug release is controlled by the thickness of the acid-soluble layer. In fact, capsule disintegration and hence drug release does not start until 5 hr after gastric emptying, regardless of whether the formulation is administered to fasted or fed subjects.

Recently, Yoshikawa et al. [70] reported a new in vitro dissolution test, called the rotating beads method, for drugs formulated in pressure-controlled colon delivery capsules. This dissolution method was applied to acetaminophen sustained-release tablets and two other drugs having low solubility in the colon, tegafur and 5-ASA. There was good correlation between the in vitro dissolution rates and the in vivo absorption rates.

In the development of the aforementioned time-dependent systems, care has to be taken to ensure a homogeneous coating. If the coat is not homogenous, its rigidity will be affected, possibly leading to undesirable infiltration of the aqueous medium and, in turn, undesired alteration of the lag time before which the drug is supposed to be released.

Polymers Sensitive to Degradation by Bacterial Enzymes

Drugs can be administered locally and selectively to the colon if they are enclosed in a dosage form, such as a capsule, coated with an azo-aromatic cross-linked polymer
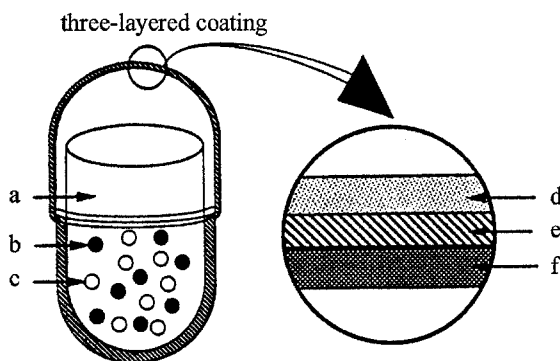
FIG. 5.   Design of the colon-targeted delivery capsule: (a) gelatin capsule; (b) active ingredient; (c) organic acid; (d) enteric layer; (e) hydrophilic layer; and (f) acid-soluble layer (adapted from Ref. 69).

subject to cleavage by azo-reductases of the colonic microflora. This method of coating a drug with biodegradable material for colon targeting can be used to administer a large amount of the drug. Moreover, the rate of drug release is dependent on the activity of the bacterial enzymes in the colon, rather than that of the host.

A system was developed by Saffran and co-workers [68] in which insulin or vasopressin was encapsulated in gelatin coated with an impermeable polymer. The coat, prepared with azo-functional cross-linking agents based on divinylazobenzene, was resistant to degradation in the stomach and the small intestine. However, problems were encountered because of variability in absorption, which may be due to intra- and intersubject differences in microbial degradation of the coating that may not be hydrophilic enough. Indeed, Kimura et al. [72] noted that only polymers with a sufficient degree of hydrophilicity could be degraded within an acceptable period of time. However, there is a possibility of premature drug release if the polymeric coating is too hydrophilic. The impact of the spacer length of the incorporated azo agent appears to be of limited importance.

A popular theory with azo materials is that their degradation products are always aromatic amines, like azo dyes. Ueda and co-workers observed that the azo bonds in segmented polyurethenes were reduced to hydrazo intermediates after incubation with human feces, since no decrease in the molecular weight was observed [73]. It was then theorized that drug release from pellets coated with these azo polymers was due to both a conformational change and a breakdown of the film structure. Other studies also concluded that the polymers were reduced to hydrazo intermediates or were completely degraded to aromatic amines depending upon their hydrophilic/hydrophobic nature.

There has been no definitive conclusion regarding the toxicity of azo polymers, although it is known that azo dyes contain several potential carcinogens. In order to avoid possible azo-related toxicity issues, other biodegradable natural substances, capable of forming coatings that degrade in the colon have been studied. Common problems encountered with these materials are poor film-forming capability and excessive water solubility. Therefore, efforts are currently being made to mix the natural materials with synthetic polymers to obtain a film-forming mixture or to derivatize them to reduce their water solubility.

Natural polysaccharides like pectin, xylan, and guar gum are not digested in the human stomach or small intestine, but are degraded in the colon by the resident bacteria. Recent studies conducted with 5-ASA [74] and indomethacin [75] confirmed that selective delivery of these drugs to the colon can be achieved using guar gum as carrier, since it would protect the drugs from being released in the physiological environment of the stomach and the small intestine. The polysaccharides under active investigation for colon-specific drug delivery include pectin and its salts, chrondroitin sulfate, amylose, and inulin.

Veervort and Kinget [76] demonstrated that the incorporation of inulin in Eudragit films resulted in increased permeability with an increase in incubation time in the degradation medium (Fig. 6) [78]. After 8, 16, and 24 hr of incubation, the permeability coefficient increased by a factor of 6, 20, and 70, respectively. However, because of manufacturing problems due to the high methoxy pectin content, film coatings were developed consisting of ethylcellulose and pectin. Wakerly and co-workers produced [77] film coatings with ethylcellulose and pectin. In vitro degradation studies indicated that release was controlled by the ratio of ethylcellulose to pectin.
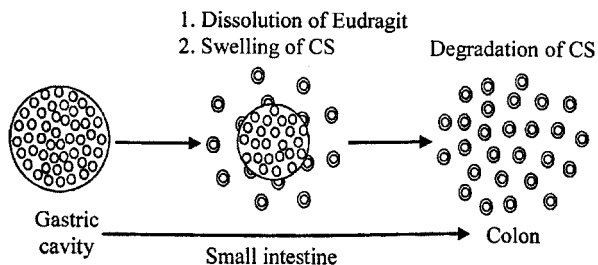
FIG. 6. Scheme of possible drug release from Eudragit microencapsulated chitosan microspheres (adapted from Ref. 78).

Lorenzo-Lamosa and co-workers prepared a system in which chitosan microcores were entrapped within acrylic microspheres of Eudragit L-100 and Eudragit S-100, forming a multireservoir system [78]. This system was designed to combine the specific biodegradability enforced by colonic bacteria with pH-dependent release of the drug sodium diclofenac and tested in in vitro systems. A continuous release for 8–12 hr was obtained at the pH in which the Eudragit coats were soluble. The authors have proposed a combined mechanism of release, comprising dissolution of the Eudragit coating, swelling of the chitosan microspheres, dissolution of the drug, and its further diffusion through the chitosan gel cores (Fig. 6) [78].

Recent studies conducted by Tozaki et al. [79] with 5-ASA containing chitosan capsules revealed that the drug concentration in the colon was higher than that afforded by a suspension of the drug. Ramdas et al. used the bioadhesiveness of polyacrylic acid, alginate, and chitosan in formulations with drugs like 5-fluorouracil and insulin to bypass the acidity of the stomach and release loaded drug for long periods into the intestine [80]. Chitosan succinate and chitosan phthalate have been used successfully as potential matrices for the oral colon-specific delivery of sodium diclofenac [81].

Amylose, another natural polysaccharide, prepared under appropriate conditions, is not only able to produce films, but is also found to be resistant to the action of pancreatic α-amylase while remaining vulnerable to the colonic flora [82]. However, incorporation of ethylcellulose was necessary to prevent premature drug release through simple diffusion [83]. In vitro release of 5-aminosalicylic acid from pellets coated with a mixture of amylose–ethylcellulose in a ratio of 1:4 was complete after 4 hr in a colonic fermenter. By contrast, it took more than 24 hr to release only 20% of the drug under conditions that mimic that of the stomach and of the small intestine.

A suspension of natural polygalactomannans in polymethacrylate solution applied to a degradable coating around the drug core delayed the drug release in the small intestine, but was degraded by bacterial enzymes in the colon [83]. This formed the basis for studying the usefulness of guar gum-containing polygalactomannans as carrier for colonic drug delivery. Matrix tablets of dexamethasone were evaluated for colon-specific drug delivery with preparations containing guar gum [84]. In a gamma-scintigraphic study, guar gum, in the form of matrix tablets, was evaluated for its performance in healthy human volunteers using technetium-99m-DTPA as tracer [85]. It was observed that the matrix tablets entered the colon intact and released the bulk of the tracer in the colon by virtue of the enzymatic action of the colonic bacteria.

Studies conducted by Bauer and Kusselhut have demonstrated that lauryl dextran esters can form films and have been found to release tablets containing theophylline selectively in the colon [86]. Theophylline tablets were coated with a dispersion of 4% lauroyl dextran in a study performed by Hirsch and co-workers [87]. Theophylline dissolution was monitored for 4 hr in a buffer of pH 5.5, after which the passage to the cecum was simulated by the addition of dextranase. Almost linear dissolution was observed during the first 4 hr. The rate of release was inversely proportional to the amount of ester applied on the coating. After the addition of dextranase, the coatings were degraded leading to the complete release of the drug in less than 2 hr, following the addition of the enzyme. The results of these studies, where natural biodegradable polymers have been derivatized using acceptable reactants, is promising as far as colon-specific drug delivery is concerned.

Matrix and Hydrogels Susceptible to Degradation by Bacterial Enzymes

In this design, the active ingredient, the degradable polymer, and other additives are compressed to form a monolithic or a multiparticulate solid dosage form. The drug is embedded in the matrix core of the degradable polymer. Biodegradable matrix systems of cross-linked chondroitin sulfate with different levels of cross-linking have been tried for the delivery of indomethacin. A direct relationship was found between the degree of cross-linking of the polymer and the amount of drug released in rat cecal content [88].

Rubinstein and Rudai [89] observed that highly compressed matrices based on pectin in the form of plain tablets or compression coated tablets were able to retain indomethacin in simulated gastric and intestinal juice before being degraded in a medium that contained pectin-degrading enzymes. In vitro experiments showed that methoxyl pectin, when added as a compression coat, was capable of protecting a core tablet under conditions mimicking mouth-to-colon transit, and was susceptible to enzymatic attack in the colon. A higher degree of methoxylation of pectin reduced the susceptibility toward degradation by colonic bacterial enzymes, whereas the presence of calcium increased it [90].

The problem with the above mentioned monolithic unit system is that it tends to be detained at the ileocecal junction, leading to drug loss prior to entry in the colon. To circumvent this problem, multiparticulate dosage forms were devised, which passed freely through the ileocecal junction. In a recent study, a multiparticulate system, based upon amidated pectin, was tested [91]. Coating the amidated pectin beads with chitosan significantly reduced the release of sulfamethoxazole and indomethacin in simulated gastric and intestinal juice compared to beads without coating.

Macleod et al. [92] have studied the potential of pectin–chitosan–hydroxypropyl methylcellulose films for colonic drug delivery [92]. The results showed that in all cases the tablets were able to pass through the stomach and small intestine intact. The tablets started to break up once they were in the colon, due to degradation of the coat by colonic bacteria.

Kopecek and co-workers (Fig. 7) [93] have developed novel types of hydrogel capsules, based on acrylic acid, *N, N*-dimethylacrylamide, and *N-tert*-butylacrylamide cross-linked with 4,4′-di(methacryloylamino)azobenzene [94]. These hydrogels did not swell significantly in the stomach. However, in transit through the small intestine,
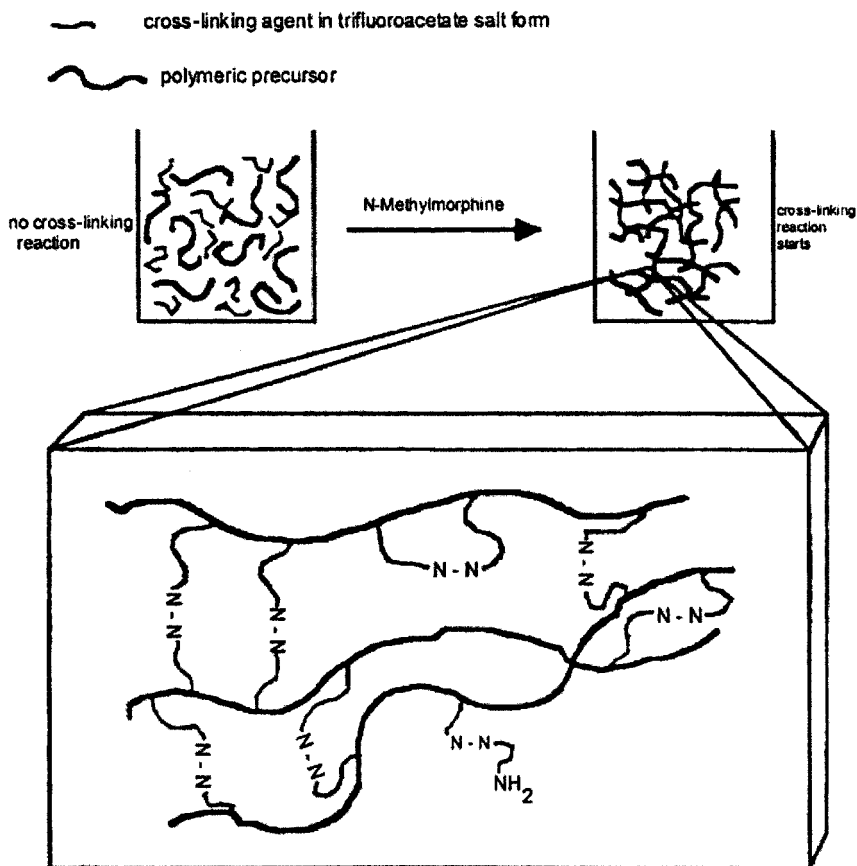
FIG. 7. Schematic representation of the synthesis of hydrogels by cross-linking polymeric precursors (adapted from Ref. 93).

swelling increased due to higher pH. In the colon, the degree of swelling reached a threshold when the cross-links became accessible to bacterial azoreductases, which in turn caused the breakdown of the hydrogel and release of the drug. The rate of degradation was found to be directly related to the equilibrium degree of swelling of the hydrogels and inversely proportional to the cross-linking density. Hydrogels prepared by cross-linking polymerization but having the same polymer composition and cross-link structure followed predominantly a bulk-degradation-like process. By contrast, hydrogels prepared by cross-linking polymeric precursors or by a polymer–polymer reaction followed predominantly a surface-erosion process at a low degree of cross-linking and a bulk-degradation-like process at a higher degree of cross-linking [95]. In a comparative study to determine the degradation rate of the azo functionality present in a soluble azo polymer and a hydrogel, the degradation rate was 125 times lower than that of the soluble azo dye.

Dextran hydrogels, cross-linked by diisocyanate, released drug only in the distal part of the colon, where the conditions for absorption are not as conducive as in the proximal part [96]. The pH-sensitive dextran hydrogels were prepared by activation

of dextran (T-70) with 4-nitrophenyl chloroformate, followed by conjugation of the activated dextran with 4-aminobutyric acid and cross-linking with 1,10-diaminode-cane [97]. The release rate of bovine serum albumin from this system was further enhanced by the addition of dextranase in buffer solutions. However, in a study where Hirsh et al. [98] investigated lauroyl dextran and cross-linked galactomannam as microbiologically degradable film-coating materials for site-specific drug delivery to the colon, the ideal zero-order dissolution before and quick degradation after enzyme addition was not realized. On the other hand, hydrogels made by copolymerization of 2-hydroxyethyl methacrylate with 4-methacryloyloxyazobenzene led to the release of aniline when the hydrogel was degraded by the colonic bacterial enzymes [99]. Another study reported the biodegradable properties of guar gum cross-linked with borax [100]. This system was found to be capable of releasing drugs in the proximal part of the colon.

Hydrogels based on natural products are more acceptable from the standpoint of toxicity-related issues and are therefore preferable to azo-based polymers. However, chemical derivatization, if performed without proper understanding, can lead to modi-fications of the hydrogels to products that do not degrade readily in the colon, as it is possible that the new structures are not recognized by the colonic bacterial enzymes for degradation. Moreover, bulk degradation is preferred to surface erosion as it leads to a more rapid rate of drug release. The one major drawback of employing hydrogels and matrix systems is that only a limited amount of drug can be incorporated. Thus, when a large amount of drug is required at the target site in the colon, this may not be the most suitable carrier.

Conclusion

A great deal of research has been conducted in the past two decades on delivery systems for targeting drug release in the colon. This is clearly a region of the gastroin-testinal tract worthy of the attention, as it does not have the hostile environment responsible for drug degradation in the stomach and the small intestine. Local therapy of colon pathologies and reduced drug availability due to degradation of the active ingredients by digestive or mucosal enzymes can benefit from colonic delivery. How-ever, the large intra- and intersubject variations in gastrointestinal pH makes the de-livery systems based on pH-dependent polymers less suitable for targeted drug deliv-ery to the colon. Therefore, systems that rely solely on the prevalent conditions of the colon, for example, those that depend upon degradation by colonic bacteria for drug release, are promising. However, care should be taken in designing such systems as there are concerns regarding toxicity with the use of azo-based polymeric delivery systems. The natural polymers, on the other hand, may not have sufficient film-form-ing capacity and may be less prone to degradation in the stomach, following chemical derivatization. A major problem in comparing different delivery systems to the colon is that the degradation studies are carried out under different experimental conditions. Moreover, despite promising results in animal studies, none of the polymeric systems have yet been tested clinically. Clearly, much work remains to be done to satisfacto-rily answer the concerns that have surfaced about colon-specific drug delivery.

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The European Agency for the Evaluation of Medicinal Products (EMEA)

Introduction

History of the European Union

After World War II, the idea of a United States of Europe was promulgated, and in 1957 the Treaty of Rome was signed establishing the European Economic Community (EEC) between six countries: Germany, Belgium, France, Italy, Luxembourg, and the Netherlands. In 1973, Ireland, the United Kingdom, and Denmark joined the EEC. In 1974, heads of state and government decided that a European Parliament be elected by direct universal suffrage and also decided to meet regularly as the European Council (EC) to deal with community affairs and political cooperation. Greece joined the EC in 1981 and Spain and Portugal in 1986. In 1992, the 12 foreign affairs ministers signed the Maastricht Treaty establishing the European Union (EU), including the four areas of labor, capital, goods, and services. Finally, Austria, Finland, and Sweden joined the EU in 1995. The Treaty of Amsterdam, which came into force on 1 May 1999, led to further institutional changes such as that no draft text can become law without the formal agreement of both the European Parliament and the Council. Thus the European Union now consists of 15 member states each of which has its own national government and legislative bodies.

The European Community has three important powers:

- It adopts European laws which apply in the 15 member countries (directives and regulations).
- It controls a budget to finance certain programs carried out in its member states.
- It signs international agreements on cooperation and trade.

All these decisions are taken by common institutions situated in Brussels, Strasbourg, and Luxembourg.

Since 1974, the European Council has brought together the heads of state and government of the 15 member states and the president of the European Commission to set key guidelines and political goals and to arbitrate conflicts for which agreements had not been found within the European Union Council of Ministers. Each member country presides over the Council for a six-month period.

European Union Institutions and Legislative Instruments

The European Commission, representing the Community's interests, draws up common projects and, after a decision has been taken by the EU Council of Ministers, supervises their proper implementation. It is directed by 20 commissioners and is assisted in its work by a permanent staff of 17,000 most of whom are based in Brussels. It is independent of the governments but is subject to control of the European Parliament. It implements common policies and negotiates international agreements.

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It may bring an action before the European Court of Justice should community laws not be respected by the member states. It is here, within the Pharmaceuticals and Cosmetics Unit of the Directorate-General for Enterprise (formerly the Directorate-General for Industry, DG III), that European legislation on medicines is drawn up and implemented.

The European Parliament is made up of 626 deputies who are elected by direct universal suffrage every five years. It examines all proposals for European directives and regulations, which it may accept, modify, or refuse. It supervises the work of the European Commission, which it can dismiss with a motion of censure and it votes the annual community budget.

The European Union Council of Ministers meets to adopt proposed European directives and regulations in light of the advice given by the European parliament. The ministers meet depending on the subject that the Council is dealing with and according to their areas of competency. Thus, the ministers of health of the 15 member states are present at a Council meeting dealing with questions of drug regulation or health. The country presiding over the European Council also holds the presidency of the EU Council of Ministers. Because of the principle of subsidiarity, community legislation is only introduced with regard to points of common interest and in order to further the aim of a balanced and dynamic Europe.

The Council of Ministers can adopt several types of legislation, which are more or less restrictive:

- Regulations are binding and directly applicable to all citizens.
- Directives are binding on all citizens but indirectly after they have been incorporated into the laws of each country.
- Decisions are binding and directly applicable but only to the institutions, bodies, businesses, or citizens specifically named.
- Recommendations, advisory opinions, and resolutions are consultative or guidance texts addressed to the states.

Legislation for Pharmaceutical and Veterinary Products

The foundation of European pharmaceutical legislation is Directive 65/65/EEC [1], which, when it was promulgated in 1965, applied only to the initial six member states. In this directive, the definition of a medicinal product is given and the data required to obtain approval are described. This original directive has been continually updated, amended, and supplemented with subsequent legislation, but remains the basis of pharmaceutical legislation.

Ten years later, three directives sought to further promote public health and the free movement of medicinal products within the community. Directive 75/318/EEC [2] set analytical, pharmacotoxicological, and clinical standards for testing proprietary medicinal products, Directive 75/319/EEC [3] established the Committee for Proprietary Medicinal Products (CPMP) and its partial mutual recognition procedure, whereas Directive 75/320/EEC [4] established a Pharmaceutical Committee to examine problems in implementing the pharmaceutical directives.

In the years that followed, cooperation between national health authorities at European Union level was further encouraged. Directives 83/570/EEC [5] and 87/22/

EEC [6] established the multistate procedure and the ''concertation'' procedure. These procedures provided a mechanism for the exchange of information about all aspects of product licensing between member states and made it easier for national licensing authorities to recognize each other's decisions. In the concertation procedure, the Committee for Proprietary Medicinal Products (CPMP) is charged with passing on the approvability of an application, which, however, is not binding on the member states' national authorities. The multistate procedure is based on the principle of recognition of an approval in one member state by the national health authorities in other member states.

The European Agency for the Evaluation of Medicinal Products (EMEA) was established by Council Regulation (EEC) No. 2309/93 [7] of 22 July 1993, and London was chosen as its seat by decision of the Council on 29 October 1993. It began operation on 1 February 1995. Regulation 2309/93 also established the legal basis for a single community-wide centralized procedure for the approval of medicinal and veterinary products.

Simultaneously, Directive 93/39/EEC [8] amended directives 65/65/EEC [1], 75/318/EEC [2], and 75/319/EEC [3] to establish the decentralized procedure (commonly known as the mutual recognition procedure).

The EMEA

Mission

The mission of the EMEA is to contribute to the protection and promotion of public and animal health by:

- Mobilizing scientific resources throughout the European Union to provide high quality evaluation of medicinal products, to advise on research and development programs, and to provide useful and clear information to users and health professionals;
- Developing efficient and transparent procedures to allow timely access by users to innovative medicines through a single European marketing authorization; and
- Controlling the safety of medicines for humans and animals, in particular through a pharmacovigilance network and the establishment of safe limits for residues in food-producing animals.

Structure

The European system is based on cooperation between the national health authorities of the member states and the EMEA. The latter acts as a focal point of a network which coordinates the scientific resources made available by the member states. This partnership between the EMEA, national health authorities, and the European Union institutions is crucial to the functioning of the European authorization procedures.

A management board supervises the EMEA, while its scientific activities are largely carried out through its two scientific committees and their working parties. The board and the scientific committees and their working parties are supported by the EMEA secretariat, headed by an executive director.

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The management board is made up of two representatives from each member state, the European Parliament, and the European Commission. Representatives of Iceland and Norway, who are members of the European Economic Area (EEA) but not of the EU, also attend board meetings. On 1 January 2000, these countries formally joined the EMEA. The board appoints the executive director and approves the budget and work program each year.

The principal scientific bodies of the EMEA are the Committee for Proprietary Medicinal Products (CPMP) and the Committee for Veterinary Medicinal Products (CVMP). These committees have two members from each member state as well as from Norway and Iceland which are appointed to give independent scientific advice to the EMEA.

The EMEA secretariat comprises four units: administration, evaluation of medicines for human use, technical coordination, and evaluation of medicines for veterinary use.

The administration is responsible for carrying out administrative and financial functions to ensure that the secretariat and staff are able to perform their statutory tasks under satisfactory conditions. It has two subsections for personnel, budget, and facilities and for accounting.

The unit for the evaluation of medicines for human use is responsible for the:

- Management and follow-up of marketing authorization applications under the centralized procedure,
- Postmarketing maintenance of authorized medicinal products,
- Management of Community referrals and arbitrations arising from the mutual recognition procedure, and
- Support to European and international harmonization activities of the CPMP and its working parties.

This unit has three subdivisions or sectors: for regulatory affairs and pharmacovigilance, for biotechnology and biologicals, and for new chemical substances.

The unit for the evaluation of medicines for veterinary use is responsible for the:

- Management and follow-up of marketing authorization applications under the centralized procedure,
- Management of applications for the establishment of maximum limits for residues of veterinary medicinal products that may be permitted in foodstuffs of animal origin,
- Postmarketing maintenance of authorized medicinal products,
- Management of Community referrals and arbitrations arising from the mutual recognition procedure, and
- Support to European and international harmonization activities of the CVMP and its working parties.

This unit has two sectors: for CVMP and veterinary procedures, and for the safety of veterinary medicines.

The technical coordination unit is responsible for providing logistical support to both human and veterinary medicines evaluation activities as well as a number of general services to the EMEA, including document management, conference services, and information technology support. It has four sectors: for inspections, for document management and publishing, for conference services, and for information technology. The sector for inspections coordinates the work of inspectors, the implementation of mutual recognition agreements, and the monitoring of medicines authorized in the Community. It provides the secretariat of the quality working party and coordinates the agency's quality management program.

Scientific Committees

The Committee for Proprietary Medicinal Products (CPMP) and the Committee for Veterinary Medicinal Products (CVMP) have the responsibility to facilitate the adoption of scientific decisions between member states with regard to the authorization of medicinal products with the scientific criteria of quality, safety, and efficacy.

Within the frame of the EMEA, members of the CPMP and CVMP act independently of their nominating member state. The scientific committees are aided by a network of about 2300 European experts, nominated by the appropriate national authorities of the member states on the basis of proven experience in the assessment of medicinal products. Experts may serve on working parties or expert groups of the CPMP or CVMP.

The scientific committee decides the appointment of rapporteurs and corapporteurs, who are the members of the CPMP or CVMP taking the lead in reviewing a dossier. The committees are required to ensure that all members are willing to undertake the role of rapporteur or corapporteur.

Compensation is provided to national appropriate authorities for the services provided by committee members or European experts at the specific request of the agency.

The CPMP and the Evaluation of Medicinal Products for Human Use

The CPMP members act as rapporteurs or corapporteurs for centralized procedures, and the CPMP gives an opinion on the approvability or otherwise of such applications. The EMEA is intimately involved in the management of this procedure, including the preparation of the CPMP opinion in all 11 official EU languages. Quality management standards have been implemented for the preparation of scientific advice and opinions and a tracking system throughout the life cycle of centrally authorized products has been developed. Postauthorization, variations, and extensions to the license may be submitted and rapporteurs play a major role with these maintenance activities. There is also ongoing activity with regard to the reporting of adverse drug reaction (ADR), periodic safety update reports (PSURs), and other follow-up measures. Rapporteurs and corapporteurs are particularly involved in urgent safety restriction procedures.

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Pharmaceutical sponsors may seek advice from the CPMP concerning their development programs. The CPMP has established a scientific advice review group to strengthen and widen CPMP input and to guarantee the availability of proper expertise. A standard operating procedure for dispensing scientific advice by the CPMP for innovator medicinal products has been adopted.

Working Parties

There are four CPMP working parties and four CVMP working parties as well as a joint CPMP/CVMP quality working party. There is also an EMEA working party on herbal medicinal products. The CPMP working parties are concerned with biotechnology, efficacy, safety, and pharmacovigilance. The CVMP working parties are concerned with the safety of residues, immunological veterinary products, veterinary pharmacovigilance, and efficacy. These working parties produce position papers, points to consider, notes for guidance, and joint CPMP/CVMP/ICH guidelines which provide up-to-date scientific opinion regarding matters of current interest to all member states and pharmaceutical and veterinary manufacturers.

Biotechnology Working Party

This working party considers the aspects of the manufacture and control of biotechnological and biological medicinal products and is also involved in the provision of scientific advice. Workshops on the application of assays for markers of transmissible spongiform encephalopathies (TSE) and on the potential risk of transmitting new variant Creutzfeld-Jakob disease (nv-CJD) through plasma-derived medicinal products have recently been held.

Efficacy Working Party

Clinical trial methodology and guidelines for special disease-related therapeutic fields are discussed. In cooperation with other working parties, guidance for modified-release oral and transdermal dosage forms, pharmacokinetics, and clinical investigation of new vaccines, gene therapy, and cell-cultured influenza vaccines has been given.

Pharmacovigilance Working Party

This working party considers safety-related issues at the request of the CPMP and national authorities, resulting in the harmonization of the summary of product characteristics and package leaflets of marketed products. Regular videoconferences are held with the U.S. Food and Drug Administration (FDA) to discuss issues of mutual interest. A pilot project has been started for the electronic transmission of individual case safety reports with a restricted number of participants from national authorities and marketing authorization holders.

Safety Working Party

Preclinical and safety issues are discussed in cooperation with the biotechnology working party. A guide with regard to the quality and preclinical and clinical aspects of gene transfer products has recently been produced.

Ad Hoc and Other Groups

Ad hoc groups for excipient, lipodystrophy, and antiretroviral medicinal products have been formed. A multidisciplinary group has been set up to evaluate medicinal products containing thiomersal with a view to limiting exposure to mercury and organomercurial compounds.

Cooperation with Competent Authorities

European Monitoring Center for Drugs and Drug Addiction (EMCDDA)

The EMEA has supported the development of guidelines for risk assessment of new synthetic drugs causing drug addiction.

International Conference on Harmonization (ICH and VICH)

The EMEA, as one of the six partners in the ICH process, is intimately involved in the production and update of ICH guidelines. The unit for the evaluation of medicinal products for human use supports the steering committee, the EU topic leaders, the CPMP, and the various working parties in the preparation and review and administration of ICH guidelines. Similarly, since the establishment in 1996 of VICH, the unit for evaluation of veterinary medicinal products supports the steering committee and the CVMP as well as the various working parties in this initiative.

Central and Eastern Europe

A number of central and eastern European countries (CEECs) are candidates for EU admission. The candidates are Bulgaria, the Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, Slovakia, and Slovenia as well as Cyprus. In order to help pharmaceutical registration authorities in these countries to prepare for EU membership a collaborative agreement of drug regulatory authorities of European Union associated countries (CADREAC) has been concluded. In addition, a simplified procedure for the recognition of centrally authorized medicinal products by the national authorities of central and eastern European countries has been established. The procedure is optional and is initiated at the request of the marketing authorization holder in the EU.

In addition, under the auspices of a pharmaceutical Pan-European Regulatory Forum (PERF), established by the European Commission, the EMEA administers and provides executive assistance to CEEC and EU regulators in the conduct of working groups and training sessions in order to facilitate the adoption of common techni-

cal requirements. Topics include the implementation of community legislation, pharmacovigilance, and the assessment of dossiers for marketing authorization based on quality, safety, and efficacy.

Meanwhile, to help eliminate technical barriers to pharmaceutical trade with the CEECs, protocols to permit mutual recognition of good manufacturing practice compliance for medicinal products are being negotiated.

The CVMP and the Evaluation of Medicinal Products for Veterinary Use

The CVMP operates in a similar fashion to the CPMP and is heavily involved in the review of centralized procedures for veterinary products. It has developed a broad range of new guidelines to assist applicants in topics related to research and development for which no guidance existed previously.

The unit for the evaluation of medicinal products for veterinary use has also been involved in the PERF initiative as well as other activities related to implementation of community legislation and the quality of medicinal products.

When the EMEA began operations in January 1995, maximum residue limits (MRLs) had to be established for more than 600 substances. The assessment of these products has been completed before the January 2000 deadline.

Veterinary Working Parties

Similarly to the CPMP working parties, the working parties for efficacy, safety of residues, immunologicals, and pharmacovigilance develop guidelines for the testing and reporting requirements of studies for veterinary products.

Institutional Partners

The major contact within the services of the European Commission is the pharmaceuticals and cosmetics unit of the Directorate-General for Enterprise but information is also continually exchanged with the Directorate-General for Health and Consumer Protection. Other contacts include the Directorate-General for Research and the Joint Research Center.

European Technical Office for Medicinal Products (ETOMEP)

The European Commission Joint Research Center has established a technical office at the EMEA responsible for the management of a telecommunications network and other computer technologies to facilitate the dissemination of information on medicinal products. It also manages the EMEA Internet website. A new mechanism for the secure exchange of documents through the Internet has been put in place to facilitate, among other things, the transmission of individual safety reports within the pilot project on pharmacovigilance between EMEA, national authorities, and the pharmaceutical industry.

The EudraNet (European Union drug regulatory authorities' network) is an Internet working service provided to EU medicinal regulatory authorities in collabora-

tion with the European Commission Directorate-General for Enterprise. Part of the EudraNet is accessible to industry and the general public.

Joint Interpreting and Conference Service (JICS)

The Joint Interpreting and Conference Service of the European Commission serves the institutions of the European Union as well as the decentralized agencies and bodies located in EU member states. A JICS representative is based at the EMEA to coordinate translation and conference needs. A glossary of specialized, technical EMEA terms to assist interpreters at meetings is being developed.

The European Department for the Quality of Medicines (EDQM)

In 1964, the European Pharmacopoeia (EP) was founded by Belgium, France, Germany, Italy, Luxembourg, The Netherlands, Switzerland, and the U.K. under a Council of Europe Convention, to help standardize their national pharmacopoeias. The European Pharmacopoeia now has 26 signatories (15 member states, the European Community, and 10 other European countries). Its monographs have the force of law, replacing the old national pharmacopoeias. Directive 75/318/EEC requires EU pharmaceutical manufacturers to use these monographs when compiling marketing authorization applications. The EMEA participates in the work of the European Pharmacopoeia Commission as part of the EU delegation.

The European Network of Official Medicines Control Laboratories (OMCL) is a joint project between the EU and the Council of Europe to allow the coordination of laboratory controls between EU and EFTA states. In 1999, a contract was signed between the EMEA and the EDQM to organize sampling and testing of centrally authorized medicinal products by the OMCL network.

European Approval Procedures

There are two European procedures for obtaining a marketing authorization in more than one EU member country, the centralized procedure and the decentralized or mutual recognition procedure.

The Centralized Procedure

The centralized procedure must be used for biotechnology products and may be used for so-called high technology products as well as for new active pharmaceutical ingredients, that is, products that have never before been approved for marketing. The centralized procedure is promulgated in Council Regulation (EEC) N° 2309/93 [7] and Directive 93/41/EEC [9].

In the centralized procedure, one license to market the drug in all of the EU is issued, and in principle there is only one evaluation of the dossier. In fact, both a rapporteur and corapporteur are appointed and each assesses the dossier with its own

team. The rapporteur and corapporteur are members of the CPMP who are assigned to a particular dossier by the CPMP. Each member is obliged to act as rapporteur or corapporteur.

Before submission of the dossier, the sponsor company contacts the CPMP or CVMP to announce its intention to submit a registration and to request the appointment of a rapporteur. If, as is usually the case, the sponsor has had contact with national health authorities, they may request that a particular CPMP or CVMP member be appointed as rapporteur. The CPMP/CVMP is not obliged to follow this request, but in many cases either the rapporteur or the corapporteur is the CPMP/CVMP member requested.

After submission, the rapporteur and corapporteur have 120 days for their review and to write a draft assessment report. The two assessments are then discussed by the parties and a list of outstanding issues are sent to the sponsor at which point the clock is stopped. After the answers have been received, the rapporteur has a further 30 days to finalize the assessment report, which is sent to the CPMP or CVMP. The CPMP/CVMP members also receive a copy of Part I of the dossier and may request the full dossier. After a total of 210 days, the CPMP or CVMP delivers an opinion, favorable or unfavorable.

If the opinion is favorable, the second stage of the procedure, the decision-making process, begins. During this period, the Commission makes sure that the marketing authorization complies with community law and finalizes the decision for all the member states. Should the CPMP decision be unfavorable, the sponsor may appeal and a second CPMP opinion must be prepared within 60 days

The agency forwards its opinion to the pharmaceutical unit of the commission in all eleven community languages together with the summary of product characteristics, the particulars of the manufacturing authorization holder responsible for batch release and of the manufacturer of the active substance as well as the labeling and package leaflet. The Commission has 30 days to prepare a draft decision. During this period, various Commission directorates-general are consulted and are able to give their opinion.

The draft decision is then sent to the Standing Committee on Medicinal Products or the Standing Committee on Veterinary Products for their opinions. Should there be detailed opposition from a member state to the draft decision, the standing committee can refer it back to the CPMP if the opposition is scientific. If the matter is nonscientific, the council decision is made on the basis of a qualified majority, where each member state has a different number of votes depending on its size and importance and the majority of votes must be in favor. If within a further 30 days there is no opposition, the draft decision is forwarded to the Commission secretariat-general for adoption, enabling the Commissioner for enterprise and the information society to issue the final decision which is published in the *Official Journal of the European Communities*.

Decentralized Procedure

The decentralized procedure is made on the basis of mutual recognition. Council Directive 93/39/EEC [8] has been implemented in all member states in accordance

with Directives 65/65/EEC [1] and 75/319/EEC [3]. The sponsor makes a submission to the national health authority of one member state, with a request to assess the dossier for mutual recognition. Within 210 days, the so-called reference member state (RMS) must approve the application, prepare an assessment report, and agree to a summary of product characteristics (SPC). The clock may be stopped to obtain further information during this time.

The mutual recognition submission can then be made to any number of the other member states and the RMS sends a copy of the assessment report to the concerned member states (CMS). Within 90 days, member states must raise serious objections, and if there are none, each CMS issues a national marketing authorization with an identical SPC.

To facilitate the mutual recognition procedure, a mutual recognition facilitation group (MRFG) and a veterinary mutual recognition facilitation group have been set up, although this was not foreseen in the original directive. These groups meet one day before each CPMP/CVMP meeting. The objections raised are discussed and the RMS tries to reach agreement on the approvability of the dossier and the most appropriate labeling. If necessary, further sessions with the sponsor can be held to finalize labeling details.

Should no agreement be reached within the MRFG/VMRFG, the matter is sent to the CPMP/CVMP for an opinion. Thereafter the procedure is similar to that of the centralized procedure, the end result being a commission decision after which national licenses must be issued within 30 days.

Referrals and Arbitration

A sponsor company or a national authority may make referrals to the EMEA under Article 10 of Directive 75/319/EEC, in order to harmonize the summary of product characteristics in all member states for products previously approved under national legislation.

Similarly, where there are public health concerns as a result of pharmacovigilance data, nationally authorized products or products authorized by the mutual recognition procedure may be referred under Articles 12 or 15 of Directive 75/319/EEC. The CPMP/CVMP gives an opinion on variation, suspension, or withdrawal of the marketing authorization in such cases.

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5. *OJ* Nº L 332, 28.11.1983, pp. 001–0002.
6. *OJ* Nº L 15, 17.1.1997, pp. 0038–0041.
7. *OJ* Nº L 214, 24.8.1993, pp. 0001–0002.

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8. *OJ* Nº L 214, 24.8.1993, pp. 0022–0030.
9. *OJ* Nº L 214, 24.8.1993, p. 0040.

Useful Internet Sites

European Union

http://europa.eu.int
http://europa.eu.int/eur-lex

Pharmaceuticals in the EU

http://pharmacos.eudra.org
http://www.eudra.org
http://www.eudra.org/emea.html
http://perf.eudra.org

National Agencies

http://heads.medagencies.org
http://hevra.org

International Council on Harmonization

http://www.ifpma.org/ich1.html
http://vich.eudra.org

European Pharmacopoeia

http://www.pheur.org

DAVID JACOBS

Harmonization—Compendia

Introduction

The USP and NF standards of strength, quality, purity, and packaging and labeling have been recognized by the U.S. Federal Food, Drug, and Cosmetic Act and Amendments since 1906. These requirements are enforced by the Food and Drug Administration (FDA), a party in the harmonization of requirements for drugs. (Throughout this article, the abbreviation USP, when used alone, signifies the United States Pharmacopeial Convention, Inc. The abbreviation USP in italics and followed by Roman numerals signifies a particular revision of the United States Pharmacopeia. The abbreviation USP-NF signifies the United States Pharmacopeia-National Formulary, two books in a single binding.)

Although originally founded as an organization to standardize medicines in the United States, USP and its products and services are now known and utilized throughout the world. In today's transitional and global economy for pharmaceuticals, the USP has a strong international presence and influence. Economic forces are driving major trading parties to affiliate in order to reduce trade barriers. Integral to this process is harmonization of requirements, regulations, and standards governing the approval and marketing of drugs, devices, etc., by governments.

Mission

The mission of the USP is to promote public health through establishing and disseminating legally recognized standards of quality and information for the use of medicines and related articles by health care professionals, patients, and consumers.

This mission is not limited to the United States. Almost from its beginning in 1820, USP has been aware of and part of international initiatives affecting pharmacopeial standards and their use by governments and professional organizations to control drug quality. That early commitment to internationalism has now grown to formal, on-going projects of harmonization with the pharmacopeias of Europe and Japan and agreements with pharmacopeias of Argentina, Brazil, and Mexico. International programs in drug information exist with a number of multinational organizations, foreign governments, and professional groups.

Harmonization is wanted strongly for the role of pharmacopeias in product registration exercises. That is, product developments can proceed without the later repeating of studies or testing to support registration in other than the original region. The primary beneficiaries thus are international companies. But harmonization has an independent value in facilitating international commerce in existing products, especially excipients.

Globalization

Twenty years ago the vast majority of all drug substances and finished dosage forms used in the United States were prepared in United States. For various reasons before

globalization, United States lost synthetic chemical operations due to stringent environmental requirements. In addition, some manufacturing was lost because of tax benefits offered by other governments to pharmaceutical manufacturers to relocate to their countries. Neither of these factors lead to globalization. Globalization may be the single most important historic trend at this time. The entire structure of international commerce and the allocation of capital and expertise are features of globalization. In this regard the pharmaceutical industry is not particularly different from any other industries. Today's development of drugs or biological products may occur in United States, Europe, or a combination of nations and no one nation can be pointed to as to the innovator of a particular drug. Furthermore international companies would prefer to market the minimum number of formulations worldwide. However, product registration of formulations is a very complex area and the pharmacopoeias are one part of the fact that different formulations may require different test methods. The end point of harmonization must be monographs that are acceptable to the registration authorities in different regions. Differences in pharmacopeial standards could be seized upon to create technical barriers to trade.

Alternative methods are possible: the three regional pharmacopeias (United States, European, and Japanese) allow an individual laboratory able to do the official method to validate an alternative method of analysis. The latter is chosen usually for speed, convenience, or expense but also to incorporate an existing database when a new or revised pharmacopeial method is adopted. Under those provisions, a laboratory can validate a method from another pharmacopeia, thereby avoiding duplication of routine work. In all three cases, only the official method could be used in compliance or contest. One point of harmonization is to avoid even the more remote instances of duplicative testing in addition to international product registration.

Both the national and international communities should support pharmacopeial harmonization. There should be support both for harmonization of excipients and for harmonization of common general tests and assays. In doing so, meaningful standards should be preferred, not the lowest common denominator or the most stringent. This statement at the *International Conference on Harmonization 1* was entirely consistent with the concept of forward harmonization.

Major support for pharmacopeial harmonization would come from increased cooperation and contribution to pharmacopoeias on all the non-harmonization work. Harmonization takes away scarce resources from pharmacopoeias, and there are other constituencies of the pharmacopoeias to be served. This is an obvious consequence of the fact that pharmacopeial standards apply to products already in the marketplace, both brand and generic.

Harmonization has three essential values. The first is the facilitation of international commerce. The second is facilitation of product registration processes in multiple nations. The third is to reduce duplicative testing costs. Facilitation of registration is for any new molecular entity a one-time event for each country, whereas the facilitation of international commerce and reduction of duplicative testing remains throughout the lifetime of the product.

Pharmacopeial harmonization is challenging. Differences exist because of the different histories of the pharmacopoeias. There are many factors. The most obvious are content, language, legalities, speed, and the audiences for the standards. United States Pharmacopeia applies also to the practice of pharmacy, both in a com-

munity and a hospital, and thus, the standards set are appropriate to those environments.

The opposite of harmony is disharmony. An example of disharmony is the need to repeat tests using rabbits for pyrogen where testing for bacterial endotoxin is otherwise prescribed. This represents the most extreme disharmony of methods. But there is even a greater disharmony, and that would be to reach different conclusions as to pass/fail the specimen. In this case, the quality control professionals must make a judgment as to whether or not this material can be sold in one or more regions. Functionally equivalent to harmonization is the absence of disharmony. Because of a difference in policy, pharmacopeias may not differ on adoption of a test at all. If certain tests are considered necessary by one pharmacopeia in order to protect the consumer, it is appropriate for that pharmacopeia to adopt the test without reference to any other region.

Reference Standards

Most discussions of harmonization revolve around excipients or general tests and assays. But the performance of even a harmonized method using different reference standards is not an optimal situation. In fact, harmonization of reference standards preceded many of the harmonization efforts of the last 10 years. Pharmacopeias and the World Health Organization have in the past shared bulk materials to create their individual reference standards. Where a drug exists in a highly purified crystalline state, the difference in pharmacopeial reference standards is administrative and legal in that no difference in results in laboratories is to be seen. This is not the case with mixtures such as an antibiotic reference standard which may be established based on different microbiological assays. Hormones were part of the very earliest reference standard programs.

Biotechnology-derived products have led to renewed interest in establishing reference standards based on the same bulk of material. Thus a single formulation, assay, and reference standard may be the fact worldwide. This situation can become complex such as with insulin where both biotechnology-derived insulin and animal-source insulin are in the marketplace at the same time.

History of Harmonization

One of the earliest references to USP's commitment to international harmonization may be found in the historical introduction to the 3rd revision [1] of the U.S. Pharmacopeia (1851): ''The new Dublin and London Pharmacopoeias were compared with our own, with a view of introducing uniformity wherever more important considerations did not seem to forbid the requisite modifications. . . .'' Note that uniformity for its own sake was not the sine qua non.

Awareness of the Committee of Revision in 1851 of the importance of keeping the Pharmacopeia up-to-date may have been enhanced because of the enactment of the Drug Import Act in 1848, which mandated that drugs imported into the United States had to meet the standards of the country of origin and had to comply with the

standards of the USP or one of the major European pharmacopeias. The U.S. Customs Service established laboratories at major port cities and analyzed the imported drugs according to the declared standard. Ties to European medicine remained strong through Americans traveling to foreign study.

Harmonization of pharmacopeial standards as a practical matter began at the International Congresses of Pharmacy between 1865 and 1910 [2], but the first formal attempt can be traced to 1902. Both USP President Horatio C. Wood, M.D., and Frederick M. Power, Ph.D., an American chemist of the Wellcome Chemical Research Laboratories of London, were appointed by the U.S. Secretary of State as delegates to represent the United States government at the *International Conference for the Unification of the Formulae for Heroic Medicines*, a conference of 19 countries from Europe and North America [3]. The second conference occurred in 1918. The 3rd in 1925 was attended by 31 countries from all continents except Asia and Australia. They drafted a new International Convention, which came in force in 1929. It revised the 1902 agreements on 77 ''heroic'' medicines and introduced the concept of maximum dose. It also requested that the League of Nations create a permanent secretariat of pharmacopeias [4]. Andrew G. DuMez, Ph.D., represented the USP, and was officially appointed by the U.S. Public Health Service to represent the United States at this conference [4,5]. An expert committee of the League of Nations planned a third conference for 1938, but it was never convened because of World War II [2].

Other attempts to exchange information among committees of revision of pharmacopeias were attempted through the International Congresses of Pharmacy. Joseph B. Remington, Ph.D., Chairman of the USP Committee of Revision, attending the 1913 conference in The Hague as a delegate of the American Pharmaceutical Association, introduced a resolution to establish an International Bureau of Information to provide information to pharmacopeial revision committees in every country and to operate a testing laboratory. Remington was named to a committee to implement the plan as put forth by Prof. Alexander T. Schirch of Berne, Switzerland [6].

Latin America

Seeking to establish dialogues with Central and South America, the USP in 1905 responded to a request for a Spanish edition of the Pharmacopeia by contracting with Dr. Jose Guillermo Diaz, Dean and Professor of the College of Pharmacy of the University of Havana, Cuba. Support for this project may have come from a resolution adopted by the Second International Sanitary Convention of the American Republics in 1905, which read in part [3]: ''Resolved, that a translation of this United States Pharmacopoeia into the Spanish language would prove of great benefit to the medical profession and pharmacists in each of the republics represented in this Convention.''

The Spanish edition of *USP VIII* was published in 1908. It was adopted by Cuba, Puerto Rico, and the Philippines. Addressing the Convention of 1910, Joaquin Bernardo Calvo, representing Costa Rica, stated [3]:''. . . offering to our physicians and pharmacists who do not speak English the Pharmacopoeia of the United States translated into Spanish; it is one of the most useful works of its kind, if not the most useful, among those published up to the present date.'' Spanish editions continued

to be published through *USP XV* in 1955. The Spanish edition of *USP XI* was adopted as the official pharmacopeia first by Costa Rica.

In his report to the Convention of 1960, however, Secretary Adley B. Nichols stated [7]: ''The distribution picture of the *USP* in Spanish has not been satisfactory for some time, and this is especially the case with *USP XV*. In no country is there a marked demand for the translation. Apparently the English language is sufficiently widely known today to permit the use of the readily available English edition.'' Perhaps the best summary of why the USP produced a Spanish edition of the pharmacopeia can be found in the words of Dr. Charles H. LaWall, Chairman of the Committee of Revision: ''The publication of the Spanish edition can never be considered financially advantageous to the Convention, but it should be continued as a patriotic duty and in recognition of the in-use of the book in the Spanish-speaking American countries.''

The USP was again published in Spanish in 1995, with semi-annual supplements since. This was now possible through ''machine translation.'' The situation today is mixed, but the English version maintains its importance in Latin America.

Modern Forums for Harmonization of Drug Quality Standards

Forums for harmonization emerged immediately after the USP Open Conference in May of 1989 in Williamsburg, Virginia [8]. It was concluded there that a thrust should be made toward harmonizing excipients and, possibly, test methods. This position was laid before two international meetings in 1989. The first, in Strasbourg, France, in June of that year, celebrated the 25th anniversary of the European Pharmacopoeia [9]. The second, in September in Tokyo, Japan, sponsored by the Pharmaceutical Manufacturers Associations of Tokyo and Osaka, focused on a theme of drug quality and the role of the pharmacopeias in the year 2000 [10]. At both of these well-attended conferences representatives of the industry spoke of the need for harmonization of standards among the pharmacopeias representing the major drug discovery and drug manufacturing areas of the world, the United States, Europe, and Japan, to facilitate international commerce in pharmaceuticals.

The areas of pharmacopeial standards most frequently cited as in need of harmonization at those meetings were pharmaceutical excipients and analytical tests and assays. Excipients posed the greatest barrier to commerce as a result of a patchwork of standards in the U.S., European, and Japanese pharmacopeias for a small universe of substances and many natural products of animal, mineral, and vegetable origin that are shared throughout the world. Standards for these common substances reflect cultural, scientific, and temporal differences in how and when these standards were established and last revised. Similarly, differences in tests and assays among the three compendia frequently resulted in situations where meeting the standards of a pharmacopeia in one sector would not predict meeting the standards for that same substance in another, or testing for the same parameter by another method, often resulting in extra expenditures for capital equipment for laboratories, as well as extra

time and resources for conducting the tests and in maintaining trained analysts for different procedures.

Pharmacopeial Discussion Group

Founded in Tokyo in September 1989 as the Quadripartite Group, the Pharmacopeial Discussion Group (PDG) was originally composed of representatives of the British, European, Japanese, and U.S. Pharmacopeias. The current group includes members from the European, Japanese, and United States Pharmacopeias. At its first meeting at USP headquarters in Rockville, MD, in March 1990, important agreements were reached:

- To meet twice yearly in a small group consisting of the senior staff executive and scientific officers of each pharmacopeia,
- To implement the concept of forward harmonization which was agreed in Tokyo [10]. Forward harmonization has three characteristics: a preference for the selection of methods that would be acceptable well into the future; retaining of any standard meaningful to an individual pharmacopeia; and unilateral progress not inhibited by trying to have every new advance occur simultaneously in every pharmacopeia,
- Two additional concepts of harmonization include prospective—to avoid conflicts among pharmacopeial standards before they occur, and retrospective—to resolve conflicts among existing pharmacopeial standards,
- To solicit advice from the pharmaceutical industry and government regulatory agencies on candidate articles for harmonization and their relative priorities, and
- To convene open international pharmacopeial conferences.

Articulating the three concepts for harmonization was particularly important. Prospective and retrospective clarify the distinction between work required to avoid conflict when establishing standards for pharmacopeial articles for which standards do not exist, or where few standards exist among the pharmacopeias, from work required to reconcile differences among well-established standards for articles that may have been in the pharmacopeias for considerable time. Prospective harmonization was inaugurated for biotechnology-derived products. Retrospective harmonization focused on pharmaceutical excipients and analytical tests and methods. Forward harmonization expresses a philosophy and environment for harmonization consistent with advances in pharmaceutical analysis.

Establishing a process for harmonization required recognizing that each pharmacopeia is a sovereign entity. Each pharmacopeia has certain authorities and obligations derived from the legislation or treaty that created it and that harmonization processes must recognize. Complicating the process was the realization that, different from anything that had been attempted before, a forward-moving process was being devised involving the revision systems of three pharmacopeias, each having evolved in different cultures and histories over periods ranging now from 30 to 180 years, resulting in profound differences in pharmacopeial policies.

Importantly, this was a voluntary effort; there was no legislative or treaty mandate to harmonize. No organization can compel harmonization. Further complicating the process are the differences among times to appearance of a first monograph, revision of publication schedules, public notice and comment opportunities, and updating pro-

visions. The ideal system would allow closely concurrent, if not simultaneous, actions by each pharmacopeia. The realities of level of funding, publication, and acceptance procedures, however, fall short of ideal. As a matter of fact, harmonization takes resources away from all other pharmacopeial programs since no specified support is received.

The first attempt at soliciting advice for pharmacopeial priorities was the joint issuance of a letter in May 1990 by the USP in English, by the EP in English and French, and by the JP in Japanese [11]. It went to the regulatory agencies and pharmaceutical industry associations in the countries and regions served by each of these pharmacopeias. Reflecting the sentiments of the speakers at the 1989 conferences, the letter was devoted to pharmaceutical excipients and asked only three questions.

1. Which excipients have been a source of problem or delay?
2. Has it been necessary to repeat stability or bioavailability studies because of differences in standards for excipients?
3. Which are candidates for the top 10 excipients for harmonization?

It also asked respondents to identify the specifications, tests, and assays in the monographs for these excipients that are most important to be harmonized.

Responses were returned from individual pharmaceutical companies through their industry associations and the regulatory agencies in the respective sectors. The complete response was compiled by the USP, and a list of the top 10 excipients was developed as the focal point for initial harmonization efforts, after review by each pharmacopeia. The idea of a lead, subsequently called the coordinating pharmacopeia, was adopted that would take the leadership for the revision of the monographs for the excipients for which it had volunteered. The initial list of assignments included: Magnesium Stearate, Microcrystalline Cellulose, Lactose, Starch, Cellulose derivatives, Sucrose, Povidone, Stearic Acid, Calcium Phosphate, and Polyethylene Glycol [12].

The three pharmacopoeias have periodicals in which the respective publics are informed of any proposed changes or additional standards [13]. Standards do not fall out of synchronization through ignorance because the other two pharmacopoeias are familiar with upcoming harmonization-related text in each other's periodicals. The industry, by and large, subscribes to all three periodicals and therefore should be expected to be kept abreast of developments in harmonization. The disconnect arises out of the working speeds and legal procedures of each.

Prospective harmonization is particularly successful when dealing with biotechnology-derived products [14,15] because only a few manufacturers are involved. There should be no reason for the pharmacopoeias to arrive at different standards proposed for any particular medicine. This is in stark contrast to the situation where many manufacturers of drugs are no longer covered by patent protection. There is no possibility of harmonization of the some 4000 monographs for individual substances and preparations.

The United States Pharmacopeia must pay strict attention to the legal situation in United States. ''Lock-out specifications'' are not admissible which would keep somebody out of the business of pharmaceuticals. The other two pharmacopoeias, confronted with the same situation, in due course reach the same conclusion but not

at the speed that USP would necessarily demonstrate in avoiding any possibility of lock-out specifications. In the presence of a valid medical or pharmaceutical reason for specifications that lock out competitors, the pharmacopeia will set such standards.

The expression to ''essentially harmonize'' is frequently heard. To essentially harmonize is the practical limit of what is possible. It is necessary to rate harmonization on a scale from zero to 100% harmonized. But in passing judgment an old expression pertains: ''Where you sit is where you stand.'' The USP's scale for harmonization takes the point of view of a laboratory supervisor who schedules work, training, and capital goods purchases. This would seem to be the most practical point of view. To assign a quantitative characterization, judgments have to be made as to the significance of differences. A completely harmonized requirement would use the same method and establish the same limits. Another, slightly less harmonized requirement would use the same method, but two pharmacopoeias would have different limits where one set of limits is nested within the other. If those limits are not nested, there is a degree of disharmony. Summaries of the overall state of excipients harmony, both from the PDG list and of 200 excipients that appear in the NF and elsewhere, have appeared in the Pharmacopeial Forum [16].

The reason a scale for harmonization is necessary is the fact that each pharmacopeial monograph may contain some ten requirements, and individual monographs will be harmonized on perhaps seven or eight and perhaps even all ten of those requirements. When eight requirements out of ten are harmonized, the monograph is reported as an 80% harmonized [16].

The goal of harmonization is to bring the policies, standards, monograph specifications, analytical methods, and acceptance criteria of pharmacopoeias into agreement. Such unity may, however, not always be achievable. Where unity cannot be achieved, harmonization means agreement based upon objective comparability and a clear statement of any differences. The goal, therefore, is harmony, not unison.

Interpharmacopeial Open Conferences

Presented together by the British, European, Japanese, and U.S. Pharmacopeias, the first Interpharmacopeial Open Conference on Standards for Excipients was convened in Orlando, FL, from January 30–February 1, 1991 (Table 1). Attended by 165 participants, representation included 11 countries, 59 pharmaceutical or excipient manufacturers or suppliers, three regulatory agencies (FDA, EEC, and HPB), and seven pharmacopeias (the presenters and the French, Italian, and Spanish Pharmacopeias) [17]. In preparation for this conference, USP convened open meetings on Magnesium Stearate and Lactose attended by almost every major manufacturer from Europe and the United States.

The conference endorsed the goals of the pharmacopeias to improve and harmonize standards for existing excipients, to focus on testing methods, and address specifications after test methods had been agreed upon, and to develop functionality tests, including particle size, surface area, and density.

Implementation of the recommendations led to a change in configuration of the membership in the pharmacopeial harmonization process. Whereas the British Pharmacopoeia had been an independent member of the Quadripartite Group from its inception, implementation of harmonization of standards and tests for excipient was

TABLE 1 Interpharmacopeial Open Conferences

Conference	Location	Date
Joint Pharmacopeial Open Conference on International Harmonization of Excipient Standards	Orlando, FL	January–February 1991
Interpharmacopeial Open Conference on Harmonization of Biotechnology-derived Products Standards	Verona	April 1993
Second Joint Pharmacopeial Open Conference on International Harmonization of Excipient Standards	St. Petersburg, FL	January–February 1994
Joint Pharmacopeial Open Conference on Sterility and Preservatives	Barcelona	February 1996
International Harmonization of General Monographs on Dosage Forms and Pharmacotechnological Test Methods	Seville	October 1998

recognized as a regional matter under the aegis of the European Pharmacopoeia, and the independent membership of the British Pharmacopoeia ended. The resulting group of the U.S., Japanese, and European Pharmacopeias, known as the Pharmacopeial Discussion Group (PDG), continued its efforts in tripartite configuration.

A second Joint Pharmacopoeial Open Conference on International Harmonization of Excipients was held in St. Petersburg, FL, in January 1994 [18]. Major progress was achieved and the principle established that conferences were the key component of harmonization. Progress on pharmacopeial harmonization was reported at the International Conference on Harmonization in Brussels in November 1991 (see below); the activity of the PDG to fulfill its goals is high. Several tangible and important milestones have been reached. First and most important is the recognition by the Japanese Pharmacopoeia of its need to develop a vehicle for public notice and comment for pharmacopeial revision and the necessity of such a vehicle to the communication process. Recognizing that need, the JP reached a decision to publish the *Japanese Pharmacopoeial Forum* (JPF) on a quarterly basis. The first edition in January 1992 [11] included proposals for the revision of the Magnesium Stearate and Lactose monographs. Notable is also the fact that matters in JPF relating to international harmonization are printed in English whereas domestic revision matters appear in Japanese.

A second milestone was a letter by the PDG in May 1992 asking for further candidates, beyond excipients. Responses to that inquiry focused primarily on tests and assays. Replies were ranked by order of priority. The priority of excipients was expanded to the top 25, based upon further analysis of responses. The lists of combined assignments and priorities for pharmacopeial harmonization appeared in the forum publications of the pharmacopeias [12,13].

The Japanese Pharmacopoeia took another step toward harmonization by announcing that it would implement an annual supplement program to update the JP between editions, beginning with an October 1993 supplement to *JP XII* [11].

Refinement of the process of pharmacopeial harmonization is continuing (see Ap-

pendix I representing the process as of December 1999). Accommodating the revision processes, time requirements, and publication schedules of three revision systems and nine publications proved not to be feasible as initially envisaged. It requires continual adjustment as issues reach stages in revision that had not been foreseen or did not fit within existing systems. Also complicating progress was the fact that although USP in 1980 established the first expert group that focused on excipients (Subcommittee on Pharmaceutic Ingredients), the other pharmacopeias did not have task groups readily at hand devoted specifically to excipients.

Experience gained by harmonizing the first of the excipients, Lactose and Magnesium Stearate showed that because so many parties are affected and many expert groups must be convened, forward, retrospective harmonization is intrinsically a lengthy process.

Tests and Standards

Impurities in Excipients

Limit tests have a long standing in pharmacopeias. For some, heavy metals for example, the sensitivity of the method was the basis for the standard. Modern limits in the USP-NF are toxicity based. There is divergence in harmonization because of toxicity-based rather than method-based standards. The modern basis avoids the exclusion of safe products from the marketplace, whereas the older approach could lead to lock-out specifications known as technical barriers to trade.

Biotechnology-Related Standards

Implementation of prospective harmonization began formally with a conference on standards for biotechnology-derived products in 1993. This conference was attended by about 150 scientists and regulators from 20 countries. With participation by experts on pharmacopeial revision bodies from each of the three pharmacopeias, the conference produced a series of recommendations relating to informational chapters, general chapters on tests and assays, and group and individual monographs for selected biotechnology-derived human drugs and biologics [14].

The introduction of biotechnology-derived products presents a decisive moment [15]. Here is an emerging technology and an opportunity to avoid conflicting standards through the commitment of the pharmacopeias to the process of harmonization for pharmacopeial standards. Included in this opportunity are the practical values obtained from common reference standard materials. The complexity of the technology, in concert with medical conditions and clinical environments, and the desire for instant globalization of return on investment call for facilitation and avoidance of ambiguities by uniform standards worldwide.

Biotechnology-derived drugs are of mutual interest to USP and the ICH-Q (International Conference on Harmonization-Quality). The relationship is uncertain between compendial standards and the development and approval of biotechnology-derived drugs by regulatory agencies. The USP contains many general chapters (i.e., ''horizontal standards'' such as stability, injections, bacterial endotoxins, etc.), which are cited extensively by the biotechnology industry, as well as an informational chap-

ter on biotechnology-derived products that explains terminology and facilitates communication.

Status of Interpharmacopeial Harmonization

Supplements to *USP24-NF19* contain updates of an informational chapter by this name. It lists all of the projects undertaken by PDG. Tables 2 and 3 list the projects as of Jan, 1, 2000, and identify the coordinating pharmacopeia. However, the official status of each project is not reported since this changes with each supplement.

Because revision programs run on different schedules, one should compare only the current texts of two or more pharmacopeias whenever possible divergence of mandatory requirements is an issue. The official pharmacopeial texts should be compared.

Harmonization proposals do not have official status. The work of the PDG is finished at Stage 5B. The progress of the harmonization projects can and should be verified in the most recent number of *Pharmacopeial Forum, Japanese Pharmacopoeial Forum*, and *Pharmeuropa*. For standards in force in *USP24-NF19*, see also the latest Supplement or Interim Revision Announcement.

Equivalence

Tests and assays of EP and JP elaborated by the PDG procedure are considered as equivalent to USP-NF, except as noted in Chapter ⟨1196⟩ *Status of Interpharmacopeial Harmonization* found in Supplements to *USP24-NF19*. Since the legal status of each may not be at the same stage, a precautionary check should be made to support any plans or actions.

Equivalence is attributed to monographs, tests, or assays that have arrived at Stage 5B. The nature and reason for divergences are expected to be described in the three pharmacopeial periodicals. Only those stated exceptions are considered nonequivalent.

International Conference on Harmonization

Founded in 1990, the International Conference on Harmonization (ICH) is comprised of the pharmacopeial manufacturers associations in Europe (EFPIA), Japan (JPMA), the United States (PMA), and the drug regulatory agencies in Europe (EEC), Japan (MHW), and the United States (FDA), with the International Federation of Pharmaceutical Manufacturers Association (IFPMA) serving as secretariat. Pharmacopeias are not members of the ICH, where membership is reserved for three PMAs and three regulatory agencies. Invited observers include Canada, WHO, and the European Free Trade Association (EFTA).

With expert working groups in the areas of drug efficacy, drug safety, and drug quality, the ICH is the foremost opportunity for harmonization among the leading drug regulatory and manufacturing groups in the world. The ICH Expert Working Group on Drug Quality (EWG-Q) includes the topic Q4, ''Pharmacopoeias.''

TABLE 2   Excipient Harmonization[a]

Excipient	Coordinating Pharmacopoeia
Alcohol	EP
Benzyl alcohol	EP
Dehydrated alcohol	EP
Calcium disodium edetate	JP
Calcium phosphate, dibasic	JP
Calcium phosphate, dibasic (anhydrous)	JP
Carboxymethylcellulose, calcium	USP
Carboxymethylcellulose, sodium	USP
Carboxymethylcellulose, sodium (cross-linked)	USP
Cellulose (microcrystalline)	USP
Cellulose (powdered)	USP
Cellulose acetate	USP
Cellulose acetate phthalate	USP
Citric acid (anhydrous)	EP
Citric acid (monohydrate)	EP
Crospovidone	EP
Ethylcellulose	EP
Hydroxyethylcellulose	EP
Hydroxypropyl cellulose	USP
Hydroxypropyl cellulose (low-substituted)	USP
Hydroxypropyl methylcellulose	JP
Hydroxypropyl methylcellulose phthalate	USP
Lactose (anhydrous)	USP
Lactose (monohydrate)	USP
Magnesium stearate	USP
Methylcellulose	JP
Methyl *p*-hydroxybenzoate	EP
Petrolatum	USP
White petrolatum	USP
Polyethylene glycol	USP
Polysorbate 80	EP
Povidone	JP
Saccharin, calcium[b]	USP
Saccharin (free)	USP
Saccharin, sodium	USP
Silicon dioxide	JP
Silicon dioxide (colloidal)	JP
Sodium chloride	EP
Sodium starch glycolate	USP
Starch, corn (maize)	USP
Starch, potato	EP
Starch, rice	EP
Starch, wheat	EP
Stearic acid	EP
Sucrose	EP
Talc	EP
Titanium dioxide	JP
Ethyl *p*-hydroxybenzoate	EP
Propyl *p*-hydroxybenzoate	EP
Butyl *p*-hydroxybenzoate	EP
Glycerol	USP

[a] The PDG affirmed that harmonization was not to be undertaken in view of the different drinking waters standards elaborated by various governments. See the monograph for Purified Water.

[b] The JP declines to participate in harmonization, so the USP and EP will harmonize bilaterally.

TABLE 3 Status of General Tests and Assays

Tests and Assays	Coordinating Pharmacopeia	ICH-Q6 Lists
Dissolution[a]	EP/USP	*
Disintegration	EP/USP	*
Dose uniformity[b]	JP/USP	*
Color and clarity	EP	*
Extractable volume	EP	*
Heavy metals	USP	*
Particulate matter	EP	*
Residue on ignition, sulfated ash	JP	*
Sterility	EP	*
Bacterial endotoxin	JP	*
Microbial contamination	EP	*
Preservative effectiveness[c]	EP	*
Particle size distribution estimation by analytical sieving	USP	*
Inhalations	EP	
Bulk density and tapped density	EP	
Optical microscopy, powder fineness	USP	
Powder flowability	USP	
Specific surface area	EP	
Tablet friability	USP	

[a] Apparatuses 1, 2, and 4 are harmonized. Not all apparatuses appear in other pharmacopeias and decision rules are harmonized. Selection of media is not harmonized, and may not be a valid subject in view of population and formulation differences.
[b] Includes content uniformity and weight variation.
[c] In view of the two-test nature of an otherwise highly similar procedure used in different modes in the United States and Europe, this cannot be harmonized on test times and decision values.

The pharmacopeias have worked with the ICH process to facilitate the international environment of pharmaceutical research and product registration. On the other hand, the additional situation for compendia is that the standards which they have published now apply to all of the already marketed products. In that case a company has testing history and product history in their quality control departments. These are the most conservative elements within the pharmaceutical industry as is necessary to their task. Quality control departments are reluctant to change methods when they feel that their products are properly represented by the current of tests. Therefore, a tension is created between trying to develop harmonized standards, which facilitate one area of activity in the world of pharmaceuticals, and not disturbing a satisfactory marketplace. A vast amount of progress has been made in the harmonization of pharmacopeial methods.

At the first biennial meeting of the International Conference on Harmonization in Brussels in November 1991, the U.S., Japanese, and European Pharmacopeias presented papers relating to progress being made in harmonization of pharmacopeial standards for excipients [19]. Other topics, such as stability, validation, impurities,

and biotechnology, were established. The pharmacopeias are involved in all these issues. In fact, USP general chapters served as background for harmonization for some of these topics.

The ICH Steering Committee responded favorably to a request by USP for observer status, recognizing it as a nongovernment, nonindustry body with official status under U.S. statutes. Now each of the three pharmacopeias can participate in EWG-Q activities and in ICH biennial meetings as independent bodies.

Stability is a key quality concern that is addressed in various ways by pharmacopeial standards. It was also the first subject for which the quality working group (EWG-Q) developed a guideline. USP has redefined the concept of Controlled Room Temperature in terms of a mean kinetic temperature of 25°C, which is identical with the long-term storage temperature promoted by ICH. Furthermore, ICH has advised against including recommended storage label statements that could conflict with U.S. or other regions' practices. Thus, the USP standards and the ICH Guideline agree on this overarching concern for the stability of pharmaceuticals. The USP actively participated in achieving this desirable outcome.

Validation of analytical procedures is intrinsic to both new drug approval and compendial revision. The USP had already established an informational chapter, ⟨1225⟩ *Validation of Compendial Methods*, before the international harmonization effort began. It was a joint effort among PhRMA, FDA, and USP. It was useful in the work of the EWG-Q in preparing a document on validation of analytical procedures that concentrated on the submission of a new drug to the reviewing authorities. Later, in response to demands of users, the ICH document was expanded to more readily meet the scope of USP ⟨1225⟩. Differences were resolved, thereby securing the harmonized situation. The emergent ICH document and the USP chapter are harmonized in breadth and detail in such a way that the vocabularies of validation and the underlying analytical strategies are in concert.

A recommendation by the PDG to establish a harmonized procedure for stability to light was taken up by the EWG with Japanese participants responsible for a first draft of a guideline. A harmonized procedure emerged, again for the purpose of new drug approval.

Impurities are of many kinds, and therefore the issues for harmonization are numerous. Everyone concerned agrees that toxic impurities must be controlled at very low levels, and that the analytical difficulties in measuring low levels limit choice. It follows that harmonization of methods is straightforward, once the objectives of analysis are laid out. Both USP and ICH limit measurement to impurities at or above 0.1%, which is of practical significance in everyday commerce in bulk pharmaceutical chemicals. In addition, the ICH guideline refers to the pharmacopeial limits on toxic impurities such as heavy metals. Thus, on all critical issues there is no conflict between the ICH guideline for new chemical entities and established USP impurity policies, which apply to hundreds of drugs already on the market. The USP further identifies as *Signal impurities* those that are distinctly informative regarding the purification or decomposition of a drug substance. The remaining impurities are considered in the nonspecific categories of *Ordinary impurities* and a labeling requirement for *Other impurities*; these are limited to 2% total. The ICH sets no such ceiling limit. These policies do not require disclosure of proprietary synthesis or purification details, yet accomplish the necessary task of limiting bias, thereby assuring meaning-

fulness of tests and assays. However, the ICH guidelines demand identification of each impurity and of individual limits, requiring detailed lot-to-lot bookkeeping on all impurities. Impurities in excess of 0.1% are to be ''qualified'' (i.e., toxicity considered), and USP policy requires notification to USP of any known toxic impurities.

For some years USP had a requirement on *Organic volatile impurities.* When the European Pharmacopoeia was adopting a similar requirement, it was clear that toxicologists on different sides of the ocean would come to different conclusions as to appropriate limits on solvents. At the request of the pharmacopoeias, this topic was taken up by the ICH Expert Working Group on Drug Quality. The effort was successful and a guideline on residual solvents emerged. Relative to the existing USP limits it was necessary to revise some upward and some downward, but none to any great extent. This new effort was 15 years older than the initial exercise. However, the problems presented by benzene have not been resolved.

Specifications Documents

The ICH-EWG-Q produced two guidelines, Q6A and Q6B, dealing with specifications to support a new drug registration. Biotechnology-related specifications are treated by Q6B, and all others previously subject to EWG-Q guidelines are treated by Q6A. Pharmacopeial methods are intrinsic to these guidelines.

The ICH-EWG-Q developed a list of 12 general chapters (tests and assays) that were deemed critical to new product registration and urged the PDG to concentrate on prompt harmonization (Table 3), The chapter *Antimicrobial Preservatives Effectiveness* was dropped in 1999 when it became clear that it could not be harmonized because of differences in the essence of utilization of the same microbiological procedure. Of the eleven, all but two (*Microbial Limits of Non-sterile Articles* and *Dose Uniformity*) were harmonized with regard to scientific content by the end of 1999, only the necessary publication sequences were unfinished.

The Q6A and Q6B documents were preceded by the North American Conference on Specifications. In all cases, no method selection, scope of application, or overall policy (dissolution, impurities, particulate matter, etc.) is at odds with USP, a remarkable condition in view of the breadth of the topics covered. The main difference is the proportion of active ingredient in a formulation that triggers choice of determination of content or of weight to establish *Uniformity of dosage units.*

Concordance

In the future, harmonization could be accelerated by reference to laboratory data, without trying to achieve harmonized texts, tests, or assays. That is, to establish that concordance exists between two different pharmacopeial tests or assays which establish the same attribute of an article (e.g., water by titration vs. loss on drying). Inherent in this allowance is the assumption that concordant methods can be shown to yield comparable outcomes with regard to acceptable identification, strength, quality, purity, bioavailability, or labeling in the context of the monograph of a recognized article. In the event of a dispute, however, only the result obtained by the procedure given in the appropriate pharmacopeia is conclusive.

This concordance rests on the probable presumption of Good Manufacturing Practices environments in production and control, now a reasonable presumption in today's international environment.

The use of concordant methods does not necessarily require identical reagents, procedures, or measurements. Official procedures of pharmacopeias per se require no validation, but validation of the applicability of such to each preparation (formulation) is to be presumed (e.g., the presence of interfering ingredients).

Concordance would facilitate international commerce in official articles by allowing the reduction of duplicate testing or delay in national product registration or approval proceedings. No provision of the USP General Notices is abrogated and the allowance therein for alternative methods is not foreclosed by a monograph citation to the concordance.

As stated in the General Notices, an article is recognized in USP-NF when a monograph for the article is published in it. Each monograph contains standards that define an acceptable article and describes tests and assays and other specifications designed to demonstrate that the article is acceptable. Monographs and their interpretation are subject to the provisions of the General Notices and general chapters. The tests and assays required in a single monograph are intertwined. Therefore, the monograph may further state any necessary variation from the designated general chapters or the General Notices. That is, pharmacopeial monographs define interdependent attributes, each drawn in light of other monograph requirements and the General Notices, and must be viewed in total. Consequently, allowances for concordance would only be utilized when supported by an initial verification through duplicate testing of the same specimens and evidence that both methods were validated.

Appendix—Working Procedures of the Pharmacopeial Discussion Group (PDG)

Stage 1. Identification

PDG identifies subjects to be harmonized and nominates a coordinating pharmacopoeia for each subject.

Stage 2. Investigation

The coordinating pharmacopoeia for a subject to be harmonized collects the information on the existing specifications in the three pharmacopoeias, on the grades of products marketed, and on the potential analytical methods. For new products or new methodologies, existing information in the scientific literature or from manufacturers is collected and analyzed. The coordinating pharmacopoeia prepares a draft monograph or chapter, accompanied by a report giving the rationale for the proposal with validation data where appropriate and available. Stage 2 ends with the proposal draft, which is mentioned in this procedure as the Stage 3 draft. The Stage 3 draft, accompanied by supporting comments or data that explain the reasons for each test method or limit proposed, is sent by the coordinating pharmacopoeia to the secretariats of the other two pharmacopoeias.

Stage 3. Proposal

The three pharmacopoeias publish the Stage 3 draft in the next available issue of their forums. The Stage 3 draft is published in the forum of each pharmacopoeia in the style provided by the coordinating pharmacopoeia. If necessary, questions are addressed to the readers of the forums when specific issues require their advice, information, or data. In *Pharmeuropa* and the *Japanese Pharmacopoeial Forum*, the Stage 3 draft is published in a specific section entitled International Harmonization. In the *Pharmacopeial Forum (USP)*, the Stage 3 draft is published in the Pharmacopeial Previews section. The draft is published in its entirety. The corresponding secretariats may have to add information needed for the understanding of implementation of the texts (e.g., the description of an analytical method or of reagents that did not exist in the pharmacopoeia). Comments by readers of the forum resulting from this preliminary survey are to be sent to their respective pharmacopoeial secretariat preferably within 4 months of publication in the Forum. The period for public review and comment should, however, not exceed 6 months. Each pharmacopoeia analyses the comments received and submits its consolidated comments to the coordinating pharmacopoeia within 2 months of the end of the public review and comment period. The coordinating pharmacopoeia reviews the comments received and prepares a harmonized document (Stage 4 draft) accompanied by a commentary discussing comments received regarding the previous text and providing reasons for action taken in response to those comments. The Stage 4 draft together with the commentary is sent to the secretariats of the other pharmacopoeias (end of Stage 3).

Stage 4. Official Inquiry

The Stage 4 draft is published in the Forum of each pharmacopoeia. The style may be adapted to that of the pharmacopoeia concerned. In *Pharmeuropa* and the *Japanese Pharmacopoeial Forum*, the Stage 4 draft together with the commentary is published in a specific section entitled International Harmonization. In the *Pharmacopeial Forum*, the Stage 4 draft is published in the In-Process Revision section. Comments regarding this draft are to be sent by readers of the Forum to their respective Pharmacopoeial secretariat, preferably within 4 months and at most within 6 months of publication in the Forum. Each pharmacopoeia analyses the information received and submits its consolidated comments to the coordinating pharmacopoeia within 2 months of the end of the review and comment period. The coordinating pharmacopoeia reviews the comments received and prepares a draft harmonized document (Stage 5A draft) accompanied by a commentary discussing comments received regarding the previous text and providing reasons for action taken in response to those comments. The Stage 5A draft together with the commentary is sent to the secretariats of the other two PDG members (end of Stage 4).

Stage 5. Consensus

A. Provisional

The stage 5A draft is reviewed and commented on by the other two pharmacopoeias within 4 months of receipt. The three pharmacopoeias shall do their utmost to

reach full agreement already at this stage with a view to reaching a final consensus. If the consensus is reached, a Stage 5B draft is developed. In those rare instances where consensus is not reached or where novel, unanticipated serious issues are identified by any of the parties and need to be considered, depending on the complexity and gravity of the issues, more than one mechanism for resolution may be adopted. This includes a call by the coordinating pharmacopoeia for a meeting of experts from the three pharmacopoeias to search for a consensus. This group prepares a modified 5A document (5A-2) to be published in the three Forums for public comments. These are then reviewed by the group of experts that finalizes the consensus document (5B).

B. Final

When consensus has been reached, the Stage 5B draft (consensus document) is sent by the coordinating pharmacopoeia to the other pharmacopoeias for final sign-off.

Note: The last two stages of the implementation of the ''harmonized'' chapters and monographs take place individually according to the procedures established by each pharmacopoeial organization.

Stage 6. Adoption

The document is submitted for adoption to the organization responsible for each pharmacopoeia. Each pharmacopoeia incorporates the harmonized draft according to its procedure. If necessary, the Stage 5B draft can be adopted with specific amendments identified as such corresponding to a general policy in the territory of the pharmacopeia in question. The monographs may therefore be harmonized, but not identical in every respect. Adopted texts are published by the three pharmacopoeias in the Supplements or, where applicable, in a new edition/revision. If a consensus has not been reached at Stage 5A, the pharmacopoeias prepare together an article on divergences to be published by all three pharmacopoeias in the respective Forums.

Stage 7. Date of implementation

The pharmacopoeias will inform each other of the date of implementation in the particular region.

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Inhalation, Dry Powder

Introduction

Drug delivery to the lung has historically aimed at controlling local respiratory disease where the central airways may be as suitable a target for drug deposition as the deeper lung. With little exception, currently marketed inhalation products provide therapy for asthma, chronic obstructive pulmonary disease, and bronchitis. More recently, however, there has been significant interest in using the lung, and in particular the deep-lung alveolar surface, as a portal to the systemic circulation for drugs not readily administered orally. Therapeutic targets have therefore broadened substantially as pulmonary delivery is recognized for its potential to provide a noninvasive alternative to injection. New delivery systems capable of delivering drug in particles or droplets small enough to reach the peripheral or deep lung are under intense development to meet these new therapeutic targets.

Fine particle powders can be produced by various methods, such as micronization or spray drying. The physicochemical nature of these fine particles largely defines the stability of the bulk powder, which in turn is critical to the long-term effective performance of the dry powder product. The section ''Fine Particles and the Solid State'' in this article is an introduction to understanding better the fundamental properties that underlie the behavior of bulk powders. Commentary on the various means of producing fine powders follows in the section ''Powder Production: Formulation and Processing.''

Drug containment in dry powder inhalers (DPIs) falls into two categories: the unit dose, in which the dose is premetered during manufacture, and the reservoir, in which the drug dose is metered during dose administration. Some devices store multiple unit doses for convenience. These are discussed in the section, ''Filling and Packaging.'' The next section, ''Devices for Forming the Dry Powder Aerosol,'' considers the various means of aerosolizing powder in the context of device design history and functionality. Advantages and disadvantages of different design types are considered.

''Performance and Regulatory Requirements'' describes various ways of characterizing the dry powder aerosols and provides information on product quality performance requirements and how these attributes must be reflected in registration applications.

''The Role of DPIs in Therapy'' briefly discusses factors affecting the therapeutic profiles of drugs delivered by DPI devices. The article concludes with ''The Burgeoning DPI Industry,'' which surveys the DPI products on the market in 1999 and a selection of those known to be under development. Thoughts on the potential of the DPI dosage form in future therapeutic applications conclude the article.

Methods of Drug Delivery

Delivery of drugs to the lung depends on administration by any one of three methods:

- Nebulizer,
- Metered dose inhaler (MDI), or
- Dry powder inhaler (DPI).

The nature of the drug substance and its therapeutic target may dictate which lung-delivery dosage form is most appropriate for the drug. Nebulization, for example, requires that the drug dissolve well in an aqueous medium at a concentration suitable for convenient dosing. Drugs developed as MDIs must dissolve or suspend well in a nonaqueous propellant medium at a concentration appropriate for doses metered in volumes generally less than 100 microliters (μL). For DPIs, the physical properties of the drug substance determine the ease with which processing yields a stable powder that can be effectively aerosolized in milligram (mg) quantities by the inhaler device to deliver the proper drug dosage.

Nebulizers

Nebulizers have a long history in pulmonary delivery. Although generally effective, traditional nebulizer systems require lengthy (10–20 min) administration periods during which drug solution is delivered with relative inefficiency using an external power source. More convenient hand-held systems are currently under development and offer the convenience of portability and metered-dose administration [1]. Depending on drug solubility and dose, these systems may require multiple actuations to deliver an effective dose.

Metered Dose Inhalers

Since the 1970s, MDIs have dominated inhalation delivery, especially in the United States. The MDIs are more convenient to use than the nebulizers, generally offering 100–300 metered doses per pocket-sized canister. Limitations to the reliability of their therapeutic effectiveness, however, typically arise from the need for the patient to coordinate MDI actuation with breath inhalation [2], and from the deposition of a sometimes significant amount of drug, driven by the propellant blast, to the back of the throat instead of the lung. Then in the late 1980s, the chlorofluorocarbon (CFC) propellants used in MDIs were identified as agents contributing to the depletion of the ozone layer. This led to the industry-wide reformulation efforts still underway to replace CFCs with environmentally more friendly propellants.

Dry Powder Inhalers

The technical challenges of MDI reformulation have contributed to a growing interest in the potential of DPI technology for the development of new products that satisfy similar therapeutic, market, and environmental needs.

The DPI device presents medication to the patient as a dry powder in a form that can be inhaled orally for delivery to the target lung tissues. The delivery system should assist in the generation of very fine particulates of medication in a way that enables them to avoid the impaction barriers that normally operate in the lung to prevent the ingress of potentially harmful particles. These barriers include the oropharynx and, for deep-lung delivery, the air-conducting bronchi and bronchioles.

Studies have shown that in order to clear the oropharyngeal impaction barrier (comprising the mouth, throat, and pharynx), particles with aerodynamic diameters smaller than 5 μm are required [3,4]. Only particles with aerodynamic diameters less than 3 μm reach the terminal bronchi and the alveoli in significant numbers [5]. Therefore, the particle diameter required to be produced by the delivery system depends to a great extent on the intended target lung tissue. Lung deposition is also affected substantially by the specific inhalation dynamics of the patient, which in turn are influenced by the delivery device. This article addresses various attributes of the dry powder inhalation product, from intrinsic material properties to final product performance.

More simple in concept than implementation, DPI technology is rapidly expanding to address a broadening therapeutic need, as well as market opportunity. Characteristics of the ideal DPI system will include most or all of the following attributes:

- Simple and comfortable to use,
- Compact and economical to produce,
- Highly reproducible fine-particle dosing,
- Reproducible emitted dose,
- A powder physically and chemically stable,
- Minimal extra-pulmonary loss of drug,
    Low oropharyngeal deposition
    Low device retention
    Low exhaled loss
- Multi-dose system,
- Powder protected from external environment,
    Usable in all climates
    Protected from moist exhaled air
- Overdose protection, and
- Indicating the number of doses delivered and/or remaining.

Fine Particles and the Solid State

Crystalline and Amorphous (Glassy) States

Pharmaceutical solids can generally be described as crystalline or amorphous (or glassy). In fact, the actual solid phase composition of a pharmaceutical formulation is usually characterized by an intermediate composition, both crystalline and amorphous in character. In a multicomponent system, such as a solid formulation comprising drug and excipient(s), certain components or even a single component may be

amorphous. Since the amorphous form of a material is always a less stable, higher energy form than its crystalline counterpart, the distinction between these forms relates to thermodynamic stability of the solid.

Crystalline materials are characterized by a three-dimensional long-range order that translates into a distinct and unique molecular pattern that can be characterized by x-ray diffraction (XRD) [6]. The molecular arrangement of the glassy state resembles that of the liquid state and lacks three-dimensional order. Thus, the classically glassy state has been designated as amorphous, that is, without structure. Pharmaceutical operations commonly used in the manufacture of DPI formulations, such as milling, spray drying, and lyophilization (freeze drying), produce materials possessing amorphous character [7].

Milling of crystalline materials introduces or increases amorphous character as the result of the significant mechanical activation that takes place during the process, including friction, deformation, attrition, and agglomeration [8]. The extent of disorder, or amorphous character, introduced by the milling process depends on the behavior of the material and its inherent resistance to the milling-imposed stresses, the amount of energy imposed by the process, and the time scale of energy release. Solid particles formed from the liquid phase, as in spray drying or freeze drying a product from solution, are predominantly amorphous materials.

Crystalline materials exhibit a characteristic melting point at which they convert into a liquid form, whereas amorphous materials show no such defined transition. Rather, amorphous materials change on heating from a brittle glassy state to a rubbery state over a narrow temperature range known as the glass-transition temperature, or T_g. Orders of magnitude of change in properties such as viscosity and molecular mobility take place near the T_g for amorphous materials; the temperature dependence of these properties near the T_g is typically non-Arrhenius [9]. Glassy-to-rubbery transition is also associated with a step-wise change in the heat capacity. Thus, the T_g of an amorphous material may be determined using a differential scanning calorimeter where a stepwise change in heat flow (corresponding to a change in heat capacity) is observed during sample heating [10]. For many hydrophilic drugs and excipients water acts as a plasticizer, increasing molecular mobility and reducing the glass-transition temperature. It is not uncommon for as little as 2–3% w/w water to depress the T_g by 30–40°C. The physical and chemical stability of a glassy material decreases as it approaches the T_g because of that mobility. Thus the presence of water promotes instability. The rate at which a compound crystallizes from an amorphous state likewise increases as the temperature of storage approaches the T_g and as moisture content is increased [11,12].

Effect of Physical State on the Stability of Dry Powder Formulations

The impact of even subtle changes in the physical properties of a DPI formulation can lead to substantial changes in aerosol behavior. Moisture uptake by the hydrophilic components of the formulation can result in surface dissolution and liquid bridging between particles. This in turn leads to crystal growth, particle fusion, and an increase in particle size which can result in strongly diminished aerosol performance [13]. Powder densification under vibration during unit dose or reservoir filling, as well as product shipping, can also affect the observed aerosol behavior of dry powders.

Because of their greater molecular mobility in the solid state, amorphous systems generally exhibit greater physical and chemical instability at any given temperature compared to their crystalline counterparts. Thus, DPI formulations are desirably prepared in a crystalline state. The low molecular weight of drugs in DPI products currently marketed supports their crystalline nature. Since the late 1980s there has been increased interest in delivering drugs of biological origin, such as proteins for systemic uptake. These molecules typically do not crystallize and tend to remain amorphous. In freeze-dried and spray-dried biologicals for pulmonary delivery, excipients that act as protectants, such as sugars, must also remain amorphous to interact with the protein and/or provide a rigid matrix around the protein molecules to restrict and stabilize their motion. As with any amorphous product, physical change can be minimized by storage at temperatures well below the T_g and protection from moisture during handling and storage.

The chemical stability of an amorphous formulation is usually also a function of its storage temperature relative to T_g. The enhanced molecular mobility achieved near the glass transition translates into an increase in translational diffusion-dependent degradation pathways, such as aggregation in proteins. It should be noted that the reaction kinetics near the T_g do not obey Arrhenius kinetics, and that extrapolation of the accelerated stability data generated near the T_g to stability at the storage temperature should be viewed with extreme caution. Amorphous materials must be stored well below the glass transition (at least 10°C, and typically 40 to 50°C below T_g) to maintain their physical and chemical stability.

When dealing with partially crystalline materials, such as those produced by milling, the effect of water uptake is intensified. The amorphous component likely absorbs greater quantities of water than its crystalline counterpart, leading to reduced T_g, increased molecular mobility, and both physical and chemical instability.

Bulk Powder Properties

The respirable powders of a DPI cannot be characterized adequately by single-particle studies alone; bulk properties must also be assessed since they contribute to ease of manufacture and affect system performance. Primary bulk properties include particle size, particle size distribution, bulk density, and surface area. These properties, along with particle electrostatics, shape, surface morphology, etc., affect secondary bulk-powder characteristics such as powder flow, handling, consolidation, and dispersibility.

The characterization and control of primary particle size and the particle size distribution of drug-containing particles are perhaps the most important factors in the design and manufacture of dry powders for inhalation. The size, density, and shape of a particle determine its aerodynamic behavior and therefore its likelihood of depositing in the desired region of the lung. The mean (average) or median (50th percentile) particle size may be based on the number of particles, the mass (or volume) of particles, or even the surface area of particles. The particle size distribution describes the range or frequency of particle sizes occurring in a sample, or the width of the particle size distribution in a sample. The variation in these size parameters due to sampling errors must be considered in characterizing blended powders, powders stored for prolonged periods, or powders that have been mechanically agitated.

The mass median diameter (MMD) is the most common descriptor of primary particle size and may be determined by sieving or centrifugal sedimentation. The volume median diameter, as determined by laser diffraction, may be used as an approximation of MMD, provided that the particle density is known and does not vary with size, and that the particle shape is near spherical. The MMD of a powder can be used as a predictor of aerodynamic diameter by Eq. (1),

$$\text{MMAD} = \text{MMD} \cdot \rho_{\text{true}}^{1/2} \tag{1}$$

where MMAD is the mass median aerodynamic diameter, and ρ_{true} is the true density of the particle, usually determined by helium pycnometry. Cohesion and adhesion between particles normally results in the MMAD being larger than predicted. Values of MMAD less than 5 µm are considered necessary to facilitate airborne particle transit past the larynx and deposition within the lung. Powders intended for delivery to the deep lung, such as treatments for asthma or for systemic delivery, require aerodynamic behavior reflected by MMAD values between 1 and 3 µm [14]. Particles of MMAD less than about 0.5 µm are likely to be exhaled.

Surface area is a bulk powder characteristic directly dependent upon particle size distribution, porosity, and morphology. It is commonly determined by nitrogen adsorption whereby the adsorption isotherm data are fit to a suitable mathematical model from which the surface area is derived. If the particle size distribution is sufficiently narrow and the particles are not hollow or porous, the surface area can be used as a measure of change in average particle rugosity or shape. Surface area may be a more sensitive means of monitoring process control during fine particle manufacturing (jet milling or spray-drying) than are particle-sizing techniques.

Bulk powder density, porosity, and consolidation rate are used as characteristics of powder structure and ease of flow. These properties are typically more difficult to determine for fine respirable powders than for coarse particles due to the formation of bridging structures caused by high interparticulate interaction. These forces must be overcome by introducing energy, such as ultrasonic vibration or mechanical agitation, to fluidize micron-sized powders in a controllable manner. Carrier-based powder formulations are designed in part to overcome the inherent cohesion of micron-sized particles. In these formulations, the microfine drug adheres to larger-sized carrier particles, improving powder flow and metering capability. For effective delivery, the drug particles must, of course, separate from their carrier upon aerosolization and/or inhalation.

Pelletization is often employed to improve the flow properties of micron-sized powders. Pelletization converts an ensemble of single particles into larger-sized agglomerates through the formation of weak solid bridges between particles. This process also results in increased bulk powder density (ρ_{bulk}). The solid bridges formed during pelletization may aid powder flow and metering but must be overcome during aerosolization [15].

Bulk powder density must be distinguished clearly from the true density of particles. Bulk powder density is simply the mass of a powder bed divided by its volume. The volume of the powder bed includes the spaces between agglomerates, between primary particles, and the volume of micropores within the particles. These voids

within the powder bed volume constitute collectively the powder porosity. The powder porosity, F, is calculated by Eq. (2).

$$F = (1 - \rho_{true}/\rho_{bulk}) \tag{2}$$

The average number of contact points between particles increases as bulk density increases, and the interparticulate forces at these contact points must be overcome to produce a dispersed aerosol cloud. Therefore, a powder of low bulk density may be more easily dispersed as an aerosol than an otherwise identical powder of high bulk density.

However, a powder with low bulk density may be more prone to consolidation than a powder with high bulk density. Powder consolidation can be envisioned as a process of densification or packing of the particles. Consolidation occurs most rapidly during the powder agitation that accompanies powder filling or product shipping; even the imperceptible vibrations a powder experiences during seemingly static storage cause consolidation with time. The rate and extent of consolidation are dependent upon particle size distribution and particle shape, and can be used to describe the dynamic behavior of powder structures. The extent of powder consolidation, or compressibility, can be evaluated by performing tap density measurements, and using the relationship given by Eq. (3),

$$100 \times (\rho_{tap} - \rho_{aerated})/\rho_{tap} = \% \text{ compressibility} \tag{3}$$

where, ρ_{tap} is the tapped bulk density and $\rho_{aerated}$ is the aerated bulk density.

An understanding of the behavior of a powder during the manufacturing process (e.g., during blending) may aid in the identification of an optimal filling and packaging process. Blended powders may also undergo segregation of active particles from carrier particles concurrently with consolidation. Although segregation can lead to poor drug content uniformity, it is in fact desirable at the point of aerosolizing a powder composed of drug blended with a larger-particle-size carrier.

Powder Production: Formulation and Processing

The primary factor influencing the manufacture of DPI powders is the need to produce material that can penetrate into the lung. The manufacturing of fine particles is challenging, especially with regard to reproducibility. This challenge has resulted in the development of various approaches to the controlled production of fine particles, largely depending on the nature of the drug. Of the processes described below, micronization and blending, and more recently spray-drying, are used most often.

Once manufactured, small particles present another challenge. At small particle size diameters, gravity ceases to be the major force exerted on the particles and interparticle forces become more prominent. The resultant increase in the cohesive and adhesive nature of the particles produces problems such as poor flowability, fillability, and dispersibility. These problems are typically minimized by blending with larger,

less cohesive excipient particles such as lactose, or by pelletization of the individual drug particles. The cohesive nature of particles can be reduced further by modifying the particle surface, the goal of several emerging technologies.

Secondary processing techniques are often employed in powder production to ensure that the stability of the manufactured drug product does not change. These major technologies are discussed below in more detail.

Formulation

Formulation of dry powders for inhalation must rely on a very short list of excipients to fulfill the customary roles of diluent, stabilizer, solubilizer, processing aid, and property modifier (e.g., flow enhancer). In the United States, only a few materials are approved for use in inhalation products, and of those (e.g., propellants, surfactants) many are of little help in dry powder formulation.

Where dose requirements and drug properties allow, drug may be processed in the absence of any excipient, as, for example, in Astra's Pulmicort Turbuhaler. Most DPIs marketed as well as under development, however, rely on lactose as filler and flow enhancer (see Blending below). Given the proprietary nature of product development, it is not known what additional excipient materials are likely to emerge in the future as safe for inhalation. It is expected, however, that the expansion of inhalation technology to systemic delivery will call for the addition of sugars, buffer salts, and other excipients common to parenteral dosage forms, to the list of acceptable inhalation excipients.

Controlled Crystallization or Precipitation

Crystallization is the process by which particles are produced by precipitation of the material in a suitable solvent. The level of control over this process determines the physical nature and size of the finished particles. Most pharmaceutical bulk material is produced through crystallization as the final stage of the manufacturing process. The formation of a pure, crystalline material is normally the target of this final step.

In the production of materials for use in DPI products, however, the particle size of the crystallized product is normally too large. Subsequent size reduction is necessary which can significantly alter the physical nature of the material [16].

Micronization

Micronization is a high-energy particle-size reduction technique that can convert coarse particles into particles of less than 5 μm in diameter. Different types of equipment are available for this purpose, for example, jet or fluid energy mills and ball mills. Although the different types have different operating parameters, the fundamental method of reducing the particle size is the same. All techniques involve applying a force on the particle, typically in the form of a collision, either particle–particle or particle–equipment. The force acts against imperfections in the crystal surface, initiating crack propagation through the particle. As the size of the particle is

reduced, the number of imperfections decreases, thereby making the task of reducing particle size more difficult.

Micronization has been used for the past 50 years to produce small particles for inhalation therapy. However, only in recent years have batch-to-batch reproducibility and stability problems been associated with the technique. Batch-to-batch variations can be caused by morphological differences in the starting material; thus, it is critical that a reproducible raw material supply be available. Stability issues typically derive from changes to the varying quantities of amorphous material that are produced by the micronizing process on the surface of the resulting particles [17]. This can be minimized through careful control of the micronization process, including processing conditions, batch size, and feed rate, or by the addition of a secondary processing procedure. Additionally, micronization can cause decomposition of some materials [18]. The issues associated with micronization are forcing many companies to investigate alternative methods of producing small particles.

Blending

The most common method for improving the flowability, fillability, and dispersibility of small cohesive particles is blending the drug with excipient particles, usually lactose, of considerably larger particle size, typically greater than 60 μm, whereas the drug particles are less than 5 μm. The objective of the mixing process is to produce an ordered powder in which the small particles attach themselves to the surface of larger ''carrier'' particles. The challenge is to ensure that the force of adhesion between the drug and the carrier is strong enough to withstand segregation during blending and product storage, and weak enough to allow separation of the drug particles from the carrier surface upon aerosolization [19,20]. During formulation, the components are blended with mortar and pestle and/or by geometric mixing in a tumbling blender. For high volume production, the process generally involves a high-shear mixer.

The final product performance of a powder blend in a DPI is ultimately dependent on the individual drug and carrier properties, as well as the process by which they are blended [21,22]. Small changes in carrier morphology can result in significant variations in the dose received by a patient [16]. Again, control of the raw material supply is critical to successful product development. Moreover, secondary processing may be required to ensure that carrier particles behave consistently from batch to batch. Steps that involve transport or storage of the finished blend should be closely monitored to avoid separation of the drug from the carrier or separation of carriers of different sizes. Segregation can be minimized by the careful selection of formulation and process equipment. For example, a hopper design can play a significant role in minimizing segregation.

Pelletization

Pelletization often does not require the use of excipients and may offer an alternative to blending for high dose therapeutics. The process involves deliberate agglomeration of the fine drug material into less cohesive, larger units [23]. Pelletization is usually achieved by vibratory sieving or any tumbling process. All processes require particu-

lar attention to time and energy parameters to ensure a consistent product. The resultant pellets must be used in a system capable of deaggregating to an appropriate particle size for aerosol drug delivery [15].

Secondary Processing

As discussed previously, materials used in dry powder inhalation are predominantly crystalline in nature, with varying degrees of ''amorphousness.'' This typically results from the high-energy milling process, which introduces regions of amorphous material within a crystalline material. Occasionally, however, the converse is true. Minimizing any change over time and ensuring that the material is physically stable before final packaging are major formulation challenges. These stability issues tend to be physical in nature, but occasionally chemical changes such as the formation of impurities occur.

The technique generally used to minimize the degree of change in crystallinity of the milled product is to eliminate the water or other solvents from the product, usually by packaging the material within a suitable barrier (for example, aluminum foil laminate). Other techniques include the production of a 100% crystalline material, which may eliminate the effects of moisture. This technique, however, may require a secondary production stage of annealing or a period to allow the product to equilibrate under controlled storage conditions.

The final measure of crystallization effects is assessed by appropriate rigorous stability data (for example, 6 months accelerated stability at 40°C and 75% RH). This may seem excessive, but measuring the degree of crystallinity is inherently difficult because of low analytical sensitivity (for example, of amorphous components below 5% w/w), and pure single-phase standards are difficult to prepare and subsequently measure.

Spray-Drying

Spray-drying, a process typically used in the production of coarser (up to 500 μm) food, pharmaceutical, and industrial powders, can also be used to prepare microparticulate powders for DPIs [13,25,26]. A typical first step involves creating a solution of the excipients and drug. This ensures a uniform distribution of all the excipients and the active drug in the finished powder in contrast to the heterogeneous nature of blended powders. The solution is atomized and mixed with a drying medium, usually air, or an inert gas if the feed consists of an organic solvent. The solvent is evaporated and removed from the drug solids.

Each spray-dried droplet forms a single particle whose size is determined by the droplet size, the dissolved solids of the feed solution, and the density of the resulting solid particle. For a given formulation and process, both the solid content and density of the powder remain constant within a batch and from batch to batch; therefore, the distribution of the primary particle size is determined by the droplet size distribution. A narrowly distributed particle size can be achieved with a well-designed atomizer and controlled process parameters.

The droplet has a relatively short residence time (on the order of seconds) in the spray-dryer, which minimizes the degradation of heat-sensitive components. Addi-

tionally, the drug is exposed to a temperature much lower than that of the drying gas due to the cooling effect of the solvent evaporation. Control of droplet residence time and the outlet temperature defines the amorphous vs. crystalline nature of the material.

A spray-dryer consists of a feed tank, a rotary or nozzle atomizer, an air heater, a drying chamber, and a cyclone to separate the powder from the air. A rotary atomizer uses centrifugal energy to form the droplet. Pressure-nozzle atomizers feed solution to a nozzle under pressure, which forms the droplet. Two-fluid nozzles feed solutions separately into a nozzle head, which produces high-speed atomizing air that breaks the solution into tiny droplets. Both the feed solution and the drying air are fed into the drying chamber in a standard cocurrent flow [27].

Lyophilization

Lyophilization, although a relatively expensive process, can offer advantages for relatively unstable compounds. In lyophilization, the solvent (usually water) is frozen and then removed by sublimation in a vacuum environment. The low temperature maintained during the entire process minimizes thermal degradation of the drug compound.

Typically, the drying process can be divided into a primary and a secondary phase. During the primary phase, the drug solution is filled into vials and placed within a temperature-controlled drying chamber. There the solution is frozen according to physicochemical principles as the shelf temperature is lowered to below freezing. The shelf temperature is subsequently increased but maintained below the freezing point. A vacuum is applied to the chamber to sublimate the solvent. This phase of the drying process extracts most of the solvent (50–80%).

During the secondary drying phase, the remainder of the solvent is removed at higher but still subfreezing temperature. During freezing, supercooling is necessary to encourage crystallization of the drug compound. The extent to which the compound is supercooled depends on the nature of the compound, the temperature program of the shelf, the heat-transfer properties of the container, and the presence of particulates in the solution. The degree of supercooling determines the size of the solvent crystal and, subsequently, the size of the channel formed during primary drying. Consequently, the degree of supercooling affects the rate of sublimation, the rate of secondary drying, and, eventually, the surface area of the finished powder. A goal of secondary drying is to minimize the moisture content of the product.

Therefore, it is most important to select a cooling temperature profile to achieve the desired objective(s). The objective could be simply to achieve a uniform degree of supercooling and freezing or to add an annealing process in order to allow the solute to crystallize or the ice crystal to grow. The possibility of allowing a long annealing process provides great flexibility to achieve the desired solid-state property for the powder.

The lyophilized cake must then be milled. The particle size of milled lyophilized powders generally has a broader distribution than spray-dried powder, which is formed one particle at a time in a continuous process. Despite the longer processing time necessary to create a dry powder through lyophilization (and the consequent

economic implications), this process can provide the formulator with better control of the powder in its solid state.

Supercritical Fluid Technology

Extraction by supercritical fluids, in particular carbon dioxide and propane, is currently being investigated as a means of controlling the size and shape of particles for inhalation. Supercritical fluids are liquids above their critical pressure and temperature [28]. Under these conditions the molecules exhibit the flow, polarity, and solvency properties common of liquids but have the diffusivities and reactivities characteristic of gases.

　　Precipitation of the particles occurs by two methods involving atomization of a feed:

- Rapid expansion of supercritical solutions containing dissolved drug, and
- Gas antisolvent recrystallization where the supercritical fluid acts as an antisolvent for dissolved drug contained in droplets of another miscible or partially miscible liquid, for example, ethanol, methanol, or acetone.

The second technique, sometimes described as SEDS (solution-enhanced dispersion by supercritical fluids), has been successfully scaled up to pilot plant manufacture for an inhalation application. As with spray-drying, this technique is a single-step process. The drug material must show solvency in the cosolvent but complete insolubility in the supercritical carbon dioxide. The resultant solvent-free particles are less cohesive than micronized material inasmuch as high crystallinity is achieved, reducing charging effects [29]. Particle size distributions for these powders are reported to be narrow with small median aerodynamic diameters (<2.5 μm). In addition, regular particle morphologies are obtained for these thermodynamically stable powders, making them amenable to further processing steps and handling [30].

Filling and Packaging

The primary consideration when developing systems for packaging dry powders for dose delivery is the goal of delivering the exact drug dosage to the patient. When dealing with drug powders intended for use in DPIs, some basic issues must be considered, specifically that the drug must usually be delivered in a small volume, which is often difficult to handle because of small particle size.

　　The greatest challenges faced in developing packaging systems for dry powders relate to the maintainance of dispersibility in packaging, which can be affected by compression and electrical charge. Compression of the drug powder may be a consequence of excessive handling, and can result in an unintended increase in drug concentration. The small drug particles are also vulnerable to alteration in electrical charge due to the motion of particles, both against themselves and the packaging equipment, and to the unintended absorption of water by the drug powder.

Package dose can be metered by weight or by volume. Dry powders developed for DPIs are formulated to deliver a specific dose of drug per a given unit of drug powder. Drug powders can be packaged in unit-dose or reservoir systems, each of which has certain advantages (Table 1).

Unit-Dose Systems

In unit-dose systems drug powders are divided into individual-use packages that contain a known quantity of drug. Patients may use one or several units to obtain a given dose. The greatest advantage of these systems is that a higher degree of control at the manufacturing level can be maintained. Individual drug doses can be metered by weight or volume. While metering by weight results in a high degree of accuracy, it is a slow process; thus, more commonly, unit dose packaging is metered by volume.

Metering by volume, while offering a reliable means for high-volume production, has disadvantages related to the dispersibility of the drug powder, which in turn can affect the accuracy and precision of the delivered dose. Dispersibility can be managed by devising a filling process that optimizes powder flow, which varies according to drug compound; by minimizing handling and thereby compression of the drug pow-

TABLE 1 Primary Packaging for Drug Formulations

Dosing System	Advantages	Disadvantages
Unit dose	Simpler, cheaper device, less prone to malfunction Protects powder up to the time delivered to the patient as an aerosol	Patient must handle and load individual unit-dose packages into the device before dosing Dose titration is limited to quanta available from drug supplier (similar to pills)
Multidose	More convenient to the patient	The device becomes more complex as means to load multiple doses are required Means for displaying number of doses left are required Device may be more prone to malfunction due to jamming or improper indexing
Reservoir	Multidose and dose titration easy Convenient, no separate unit-dose blisters to worry about	Powder not generally well protected after reservoir is opened. Physical and/or chemical characteristics may deteriorate with time Biological contamination may be an issue The dose is metered by the device. This increases the device complexity. Metering often is not adequately controlled, as the physical characteristics of the powder are often unknown at the time of dosing

der; and by minimizing the relative motion of drug particles against other drug particles and the filling equipment. Minimizing drug powder motion reduces electrostatic charging of particles and consequent equipment malfunctions and avoids packaging problems which include dose-extraction difficulties and particle dispersion at the time of drug administration.

Unit-dose systems typically rely on blister packaging or capsules to contain the drug until it is dispersed by the delivery device. Blister packages have several advantages over capsules. Those constructed of aluminum are usually impervious to moisture. Inner linings of polyvinyl chloride or polypropylene create means for sealing the package. Gelatin capsules generally contain approximately 12% water under ambient conditions, and thus are a potential source of moisture to powder not equilibrated to ambient relative humidities.

Reservoir Systems

Reservoir systems offer the advantage of variable dosing, generate less waste, are less expensive to manufacture, and are simpler to use than unit-dose systems. Relying on a metering system contained within the delivery device, they may be less precise in drug delivery. Because the drug reservoir must be accessed repeatedly, keeping the moisture level constant is difficult. Maintaining a highly flowable drug powder in this system may also create formulation challenges.

Devices for Forming the Dry Powder Aerosol

Design Objectives and Constraints

The ultimate goal of all pulmonary delivery devices is to reproducibly deposit the required quantity of drug in the target lung tissues. Many factors influence the selection of a particular DPI design, including the characteristics of the drug to be delivered, its powder formulation, and its associated therapeutic regime. Other factors that must be considered include drug cost, desired dose, market factors, and expected degree of patient compliance.

Drug cost plays an important role in determining the economic feasibility of a device by determining how much of a drug may be lost in administration and routine device use. Drug dose and side effects may determine the reproducibility limits of particle size and mass of drug delivered necessary for effective therapy. The drug target tissue may determine the importance of achieving a very small particle size, that is, local delivery of drugs to the upper airway may allow for a larger particle size than delivery of particles to the deep lung for systemic absorption. The degree of cohesiveness of the powder particles determines how much energy the device must transfer to produce a particle of a given size. The therapeutic regime and a range of market factors, such as degree of convenience and cost of other available drugs and/or therapies, may determine how portable or inexpensive the device must be. The anticipated degree of patient compliance may favor some technological solutions over others.

The DPIs cannot be considered only as devices, but must be considered as components of a larger delivery system, which includes the formulation of the drug powder, its manufacturing processes, and packaging.

Functional Description

Several DPI designs have been proposed, developed, and successfully marketed in the past three decades. Although these devices vary widely in characteristics and operation, they all perform certain basic functions:

- The dose is extracted from the bulk powder drug package,
- A fine cloud of drug particles is generated by deagglomerating the powder and diluting it with air, and
- The drug cloud is delivered to the patients' airways.

Bulk Powder Drug Package

The first task for a device delivering a dose is the extraction of the dose from the drug package. As discussed earlier, two main alternatives are available, that is, a number of doses may be stored in a powder reservoir, or each dose may be individually packaged as a unit dose. Reservoir systems are inherently multidose. With unit-dose systems, the device may require individual loading of a unit-dose package before inhalation or loading of several unit-dose packages into the device for multidosing.

Energy Sources

Energy input is required to extract the powder from its packaging, generate the fine particle cloud, and dilute it with air. Historically, this energy comes from the patient's inhalation effort (Table 2). In some cases, the energy for extracting and metering the powder comes from the mechanical manipulation of the device by the patient [31,32]. More recent designs use concepts adapted from the MDI industry or novel approaches involving other technologies.

Uncontrolled vs. On-demand Aerosol Generation

The DPI devices that rely on patient inhalation are inherently on-demand, that is, only when the patient inhales is the aerosol delivered. In contrast, MDIs are mostly uncontrolled, and the patient's breathing maneuver has to be carefully synchronized to the aerosol generation event for effective dosing.

The DPI devices that rely on sources of energy other than patient inhalation effort may face similar problems as MDIs. Devices may ''trigger'' the aerosol generation which is then uncontrollably inhaled. Alternatively, the delivery of energy to the powder may be modulated by the device and controlled based on monitoring the patient's inhalation maneuver. Some advantages and disadvantages of either approach are given in Table 3.

TABLE 2   Energy Sources for Drug Delivery

Mechanism	Features	Characteristics
Patient inhalation	Advantages	No need to coordinate aerosol generation with patient inhalation
		Device is generally very simple; in many cases, no moving parts are involved in powder deagglomeration
	Disadvantages	Delivery, dispersion performance, and hence dose, is affected by the patient's ability to inhale at a suitable high flow rate
Others, e.g., air pump, metered propellant, electrical, etc.	Advantages	Decouples aerosol generation from patient ability to perform a correct inhalation maneuver
		Allows the extraction and deagglomeration of more cohesive powders, as additional energy can be applied in the process
	Disadvantages	Adds complexity and cost to the device by increasing the number of subsystems

Homogeneous Powders and Blends

Dry powders must be able to flow readily in order to leave the capsule or powder reservoir, but must also generate a fine aerosol enabling the patient to inhale a proper dose. These two requirements are often difficult to achieve simultaneously. Fine powders tend to be cohesive and have poor flow properties. Blending with a carrier phase, pelletization, and other approaches have been used to overcome these limitations. The features of blends and homogeneous powders are compared in Table 4 from a DPI device perspective:

TABLE 3   Control of Aerosol Generation

Method	Feature	Characteristic
Uncontrolled	Advantages	Device is simpler, no feed-back systems are required to monitor the patient
		''Violent'' aerosol generation processes are allowable, permitting the delivery of large amounts of energy to the powder in a short period of time
	Disadvantages	More prone to patient misuse
		Typically requires the use of a holding chamber to store the aerosol between generation and patient inhalation, resulting in a larger device
On-demand	Advantages	Aerosol is delivered when patient can inhale it most effectively
		Better dose control
	Disadvantages	Device is more complex as feed-back systems (mechanical or electronic) are required
		Energy delivery to the powder has to be well controlled
		Device may be more prone to failure

TABLE 4 Influence of Powder Behavior on Device Design

Powders	Features	Characteristics
Blends (lactose carrier)	Advantages	Powder can be easily extracted from packaging
		Inclusion of the carrier phase usually facilitates dispersion
	Disadvantages	Upon delivery, coughing and other unpleasant sensations may be induced as the carrier particles deposit in the mouth and throat
		A larger amount of powder needs to be moved and dispersed; in terms of energy requirements, the gains in dispersion may be offset by the increase in payload
Homogeneous	Advantages	Little mouth and throat deposition; patient does not ''feel'' inhaling an aerosol
	Disadvantages	Formulation process becomes a key factor in the development of the product; the properties of the compound to be delivered dominate the performance of the resulting powder

Performance and Regulatory Requirements

Performance Characterization

The two critical attributes characterizing the performance of DPIs are the uniformity of the delivered dose and the aerodynamic assessment of particle size distribution. To determine the uniformity of the delivered dose, an apparatus capable of quantitatively retaining the dose leaving the device is used. For aerodynamic particle size assessment, a multistage liquid impinger or cascade impactor is used. All aerosol performance testing must be conducted under defined temperature and humidity conditions.

Performance Specifications

Currently the U.S., European, and British pharmacopeias specify different requirements for delivered dose uniformity. Table 5 describes these requirements as well as proposed FDA expectations [33]. The Japanese pharmacopeia does not specify a delivered dose uniformity requirement. Current compendia should be consulted as references.

Of the four pharmacopeias, the U.S. pharmacopeia (USP) has the strictest requirements for delivered dose uniformity. Although the British pharmacopeia (BP) allows the same performance range, the USP defines the range around the label claim and the BP defines the range around the average value. The FDA expectation for delivered dose uniformity is currently tighter than that stated in all the pharmacopeias.

The various pharmacopeias outline appropriate methods for aerodynamic assessment of particle size distribution. The USP defines the size distribution through mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD).

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TABLE 5 Product Quality Requirements for Delivereol Dose

Pharmacopeia	First-Stage Testing (N=10)	Second-Stage Testing (additional 20)
United States	NMT[a] 1 out of 10 outside the range of 75.0 to 125.0% of label claim None outside of the range of 65.0 to 135.0% of label claim. If 2–3 are outside of 75.0 to 125.0% and none are outside of 65.0 to 135.0%, proceed to second stage.	NMT[a] 3 out of 30 outside the range of 75.0 to 125.0% of label claim. None outside the range of 65.0 to 135.0% of label claim.
British	NMT[a] 1 out of 10 outside the range of 75 to 125% of average value. None outside the range of 65 to 135% of average value. If 2–3 are outside of 75 to 125% and none are outside of 65 to 135%, proceed to second stage.	NMT[a] 3 out of 30 outside the range of 75 to 125% of average value. None outside of the range of 65 to 135% of average value.
European	NMT[a] 1 out of 10 outside the range of 65 to 135% of average value. None outside the range of 50 to 150% of average value. If 2–3 are outside of 65 to 135% and none are outside of 50 to 150%, proceed to second stage.	NMT[a] 3 out of 30 outside the range of 65 to 135% of average value. None outside the range of 50 to 150% of average value.
FDA, proposed[b]	NMT[a] 1 out of 10 outside the range of 80 to 120% of label claim. None outside the range of 75 to 125% of label claim. If 2–3 are outside of 80 to 120% and none are outside of 75 to 125%, proceed to second stage.	NMT[a] 3 out of 30 outside the range of 80 to 120% of label claim. None outside of the range of 75 to 125% of label claim.
Japan	No delivered-dose uniformity specification	NA[c]

[a] NMT = not more than.
[b] Ref. 33.
[c] Not available.

None of the pharmacopeias state any requirements for particle size. However, the particle size specifications set should be appropriate for the intended use of the product. For example, if the particles are intended to reach the deep lung, the MMAD of particles exiting the device should be less than 5 μm. In general, the smaller the aerosol MMAD, the greater the deposition in the lung.

The two recent trends originating with the FDA may influence the assessment and reporting of aerodynamic particle size distribution. The first is determining the particle size distribution from a single or unit-emitted dose. This may pose an analytical challenge in some cases, since the amount of active ingredient on each stage may be present only in trace amounts. The second is setting a drug quantity specification for each stage of the impinger or cascade impactor.

Release and Stability Testing Parameters

Various dry powder attributes are assessed at release and on stability. These include physical characteristics such as appearance, content uniformity, delivered dose uniformity, and particle size distribution. Chemical attributes that may be assessed include drug content, purity, and identity, as well as the water content. Dry powders may also undergo microscopic evaluation for foreign particulate matter, unusual agglomeration, and particle size. Microbial limits should also be examined, including the total aerobic, yeast, and mold counts. The presence of specific pathogens should be ruled out. The dry powders may be dissolved to test for pH.

In addition, certain compendial requirements for content and delivered dose uniformity should be measured. The USP and EP propose that the total aerobic count not exceed 100 CFU/g (colony-forming units); that the total yeast and mold counts not exceed 10 CFU/g; and that no specific pathogens be detectable. Specifications for the other attributes should be based on the intended use and the historical performance of the product. As with other dosage forms, specifications must be met throughout the intended shelf life of the product.

The International Conference on Harmonization (ICH) has identified stability requirements for room temperature storage and testing intervals. It recommends that dry powders be stored at 25°C and 60% RH for real-time conditions; at 40°C and 75% RH for accelerated conditions; and 30°C and 60% RH if significant change is observed at accelerated conditions. The ICH recommends testing samples every three months for the first year, every six months for the second year, and yearly thereafter. In addition to these requirements, the FDA suggests a storage condition at 25°C and 75% RH if significant change is observed at the accelerated condition.

When an NDA is submitted, the FDA requires that 12 months of data be collected at real-time conditions and six months under accelerated conditions. If a significant change is observed at six months for the accelerated condition, six-month data at the 30°C and 60% RH condition must be submitted and the study must cover one year.

The Role of DPIs in Therapy

Some direct comparisons of DPI and MDI for the same drug have been made in the interest of developing alternative, but comparable, products for patients. The therapeutic performance of inhalation delivery systems is as dependent on the patient as it is on the product itself. Therefore, some demonstration of clinical comparability is generally required to support product substitution.

The deposition pattern of the inhaled dry powder aerosol can be strongly influenced by the patients, inhalation dynamics and lung anatomy. At high inhalation flow rates, a given particle has a greater tendency to impact the back of the throat or to deposit in the upper airways. For delivery systems requiring high flow in order to deaggregate the powder particles, deep-lung deposition is less accessible. The proliferation of device designs has been in part the result of attempts to minimize dosing variability, regardless of source [34].

The target for lung deposition varies depending on the therapy under consider-

ation. In the treatment of asthma by β-adrenergic agonists, the central airways are generally targeted. On the other hand, therapies for alveolar disease, chronic obstructive pulmonary disease, or systemic conditions must reach the peripheral regions of the deep lung.

Among the pharmacokinetic advantages offered by delivery to the lung are fast onset of action and lack of first-pass effect. Doses to the lung can prove 10 to 20 times more effective than oral dosing, and for local therapy can result in substantially reduced side effects.

In a study, three different fluticasone propionate products (an MDI and two DPI products, Diskhaler and Diskus) were directly compared in healthy volunteers and patients [35]. The systemic drug bioavailability was highest for the MDI, while the bioavailability was similar for the two DPIs. The pharmacokinetic results are consistent with the in vitro evaluation where the MDI gave the highest fine particle dose (FPD), while the two DPIs have similar FPD values. It was also reported that in a separate study in healthy volunteers, the pharmacokinetics of a non-chlorofluorocarbon formulation have been shown to be similar to that for the original chlorofluorocarbon formulation for the fluticasone propionate MDI product. Consistent with their similar pharmacokinetic results, the clinical performance of Diskhaler and Diskus is similar in children and in adults [36,37].

The Burgeoning DPI Industry

The first commercially available DPI system appeared on the market in 1949, developed and marketed by Abbott under the name Aerohaler. Like all early pulmonary drug-delivery devices, it delivered small-molecule compounds (bronchodilators or inhaled corticosteroids) to the airways (not necessarily the deep lung) for the treatment of asthma or chronic obstructive pulmonary disease. Table 6 lists some of the early DPI systems used for asthma and COPD; the energy sources in these devices were mechanical and patient inspiration.

TABLE 6   Early Dry Powder Inhalation Systems

Year Introduced	Trade Name	Manufacturer	Packaging, Metering	Blend
1949	Aerohaler	Abbott	Unit dose; ''sifter'' cartridge	No
1971	Spinhaler	Fisons (now Aventis)	Unit dose; hard gelatin capule	Yes
1977	Rotahaler	Allen and Hanburys (now Glaxo)	Unit dose; hard gelatin capsule	Yes
1988	Turbuhaler	Astra	Reservoir	No
	Diskhaler	Allen and Hanburys (now Glaxo)	Multidose blister	Yes
	Inhalator	Boehringer-Ingelheim	Unit dose; hard gelatin capsule	Yes

TABLE 7 More Recent Dry Powder Inhalation Systems[a]

Trade Name	Manufacturer	Packaging, Metering	Energy Source(s)
Pulvinal	Chiesi	Reservoir	Mechanical, patient inspiration
Easyhaler	Orion	Reservoir	Mechanical, patient inspiration
Clickhaler	ML Labs	Reservoir	Mechanical, patient inspiration
Discus	Glaxo	Multidose blister	Mechanical, patient inspiration
Monohaler	Astra	Unit dose	Mechanical, patient inspiration
AIR[b]	Alkermes	Unit dose	Mechanical, patient inspiration
Spiros[b]	Dura	Multidose blister	Mechanical; not driven by patient inspiration
TM[b] PDS	Inhale	Unit dose	Mechanical; not driven by patient inspiration

[a] This list is not exhaustive. Many other manufacturers, in both the United States and Europe, are developing dry powder inhalation drug-delivery systems.
[b] In clinical trials; not yet on the market.

Table 7 presents some of the newer DPI devices, most still focusing on local delivery of small-molecule drugs to the airway for asthma or COPD, but some in clinical trials for systemic delivery of macromolecules, such as insulin, via the deep lung.

New DPI technologies in development by Inhale Therapeutic Systems and Alkermes are enabling the delivery of macromolecules to the deep lung. Leading this field is Inhale's insulin product currently in the phase 3 trials conducted by Pfizer. It is expected that in the next years dry powder inhalation will become a broadly accepted and effective means of delivering a wide variety of therapeutics: antibiotics, analgesics, antibodies, hormones, proteins, and perhaps gene therapeutics. The potential of this technology continues to be explored.

Acknowledgements

Glossary

BP	British pharmacopeia
BDP	beclomethasone dipropionate
CFC	chlorofluorocarbon
COPD	chronic obstructive pulmonary disease
DPI	dry powder inhaler
EP	European pharmacopeia
F	powder porosity
FDA	United States Food and Drug Administration
FPD	Fine particle dose
GSD	geometric standard deviation

ICH	International Conference on Harmonization
MDI	metered dose inhaler
MMAD	mass median aerodynamic diameter
MMD	mass median diameter
NDA	New Drug Application (FDA)
NMT	not more than
$\rho_{aerated}$	aerated bulk density
ρ_{bulk}	increased bulk powder density
ρ_{tap}	tapped bulk density
ρ_{true}	true density of the particle (usually determined by helium pycnometry)
SEDS	solution enhanced dispersion by supercritical fluids
T_g	glass transition temperature
USP	U.S. pharmacopeia
XRD	X-ray diffraction

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Liquid Crystals in Drug Delivery

Definition and Formation of Liquid Crystals

Definition

The liquid crystalline state combines properties of both liquid and solid states. The liquid state is associated with the ability to flow, whereas solids have an ordered, crystalline structure [1]. Crystalline solids exhibit short as well as long-range order in context of both position and orientation of the molecules (Fig. 1a). Liquids are amorphous in general but may show short-range order in context of position and/or orientation (Fig. 1b). Liquid crystals show at least orientational long-range order and may show short-range order where the positional long-range order has disappeared (Fig. 1c) [2]. Accordingly, liquid crystalline phases represent intermediate states, also called mesophases.

A prerequisite for the formation of liquid crystalline phases is an anisometric molecular shape which is generally associated with a marked anisotropy of the polarizability. Molecules that can form mesophases are called mesogens. Depending on the molecular shape, rod-like mesogens form calamitic mesophases, whereas disk-like mesogens form discotic mesophases. Rod-shaped molecules are often drug excipients (e.g., surfactants). Even drug compounds, such as salts of organic acids or bases, with anisometric molecular shape fulfill the requirements for the formation of calamitic mesophases.

Formation

Starting with the crystalline state, the mesophase is reached by increasing the temperature or by adding a solvent. Accordingly, a differentiation can be made between thermotropic and lyotropic liquid crystals, respectively. As with thermotropic liquid crystals, a variation of the temperature can also cause a phase transformation between different mesophases with lyotropic liquid crystals.

Thermotropic Liquid Crystals

Calamitic mesophases were the first liquid crystals to be found more than 100 years ago. In 1888, the botanist Friedrich Reinitzer observed birefringence of cholesteryl esters after melting [3] and consulted the physicist Otto Lehmann, a specialist in crystallization microscopy, who interpreted this birefringence as a parallel orientation of the molecules within a liquid crystal, a new kind of state [4]. However, these cholesteric liquid crystals exhibit not only a parallel orientation of the anisometric molecules, but the direction of the orientation rotates layer by layer in a right- or left-handed helix (Fig. 2b). The layer distance where a 360° rotation has been performed is called pitch which is often in the range of visible light. The latter phenomenon as well as the variation of the pitch with temperature are responsible for the characteristic color play of the cholesteric liquid crystals. Cholesterics require chirality of the mesogen itself or on addition of a mesogen.
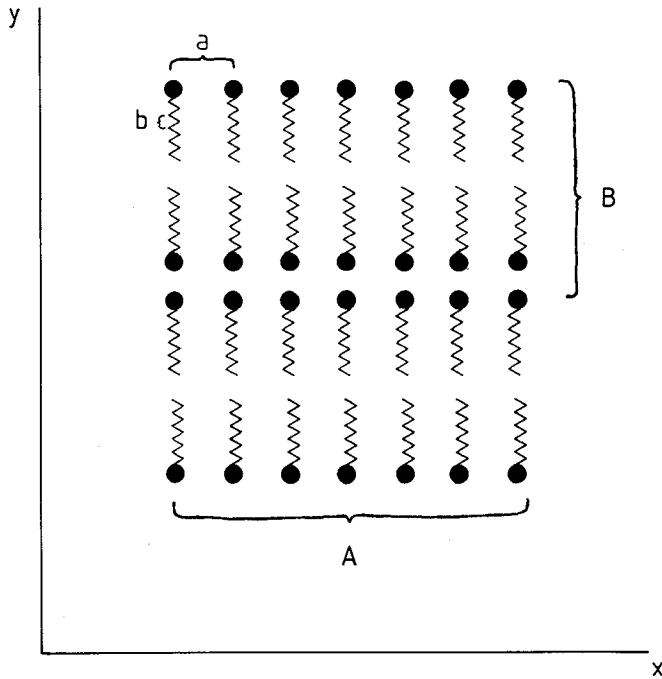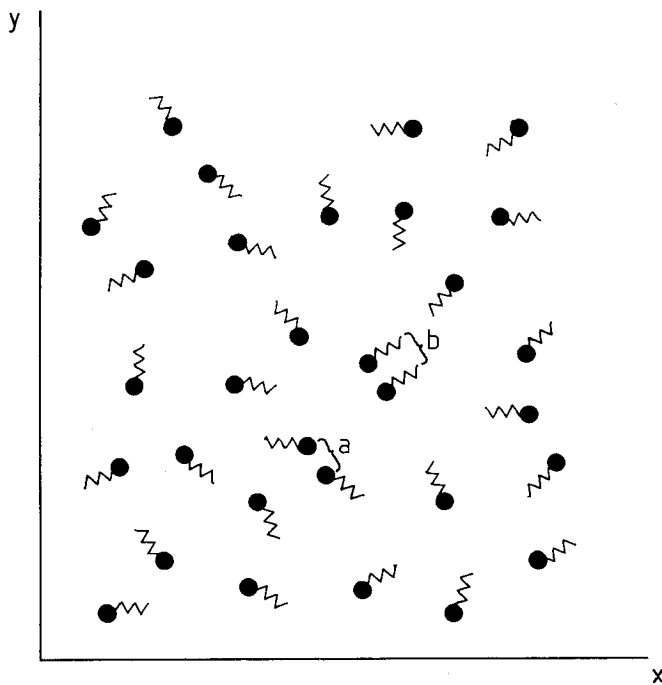
FIG. 1. Two-dimensional representation of short-range (a,b) and long-range order (A,B) in the crystalline (a), liquid (b), and liquid crystalline state (c). From Müller-Goymann, C.C., Flüssigkristalline Systeme in der Pharmazeutischen Technologie, *PZ Prisma*, 5:129–140 (1998).
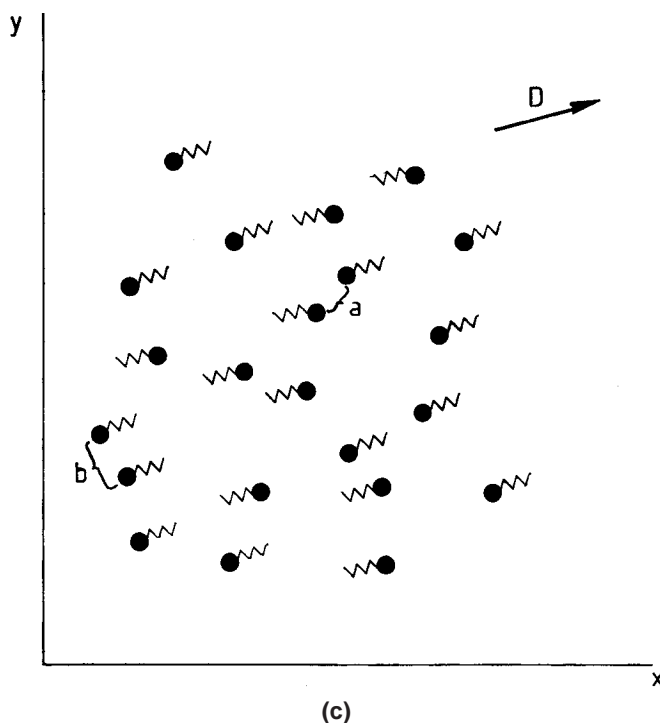
FIG. 1. (*continued*)

Similar to cholesteric liquid crystals, the nematics have an orientational long-range order with the deviation that the direction of the preferred orientation does not rotate (Fig. 2a). If, however, a chiral mesogen is dissolved in a nematic liquid crystal, the latter will be transformed into a cholesteric liquid crystal.

Calamitic mesophases with a parallel orientation of the molecules, which are additionally arranged in layers, are called smectic liquid crystals (Fig. 2c-e). The layer plane may be oriented perpendicular or tilted to the long axes of the molecules. Furthermore, the molecules may be arranged regularly within the layer (e.g., in a hexagonal arrangement), thus forming a three-dimensional lattice. In contrast to crystals, this smectic liquid crystalline state allows rotation of the molecules around their long axes. Due to the variety of arrangements, different smectics may be distinguished.

With increasing temperature, phase transitions occur, including crystalline to smectic C to smectic A to nematic to isotropic, or crystalline to nematic to isotropic. These examples demonstrate that not all possible transitions necessarily occur. Depending on the number of mesophases occurring, thermotropic mono-, di-, tri-, or tetramorphism may be distinguished.

Discotic liquid crystals arise from disk-shaped molecules as nematic or cholesteric mesophases. Their structural characteristics are similar to the respective calamitic mesophases, that is, the normals of the disks are oriented parallel. Instead of the smectic mesophases, discotic columnar liquid crystals arise from connecting the disks to each other. The columns of the discotic columnar mesophase form a two-dimensional lattice which is in a hexagonal or rectangular modification. In addition, the columns may be tilted (Fig. 2f,g).
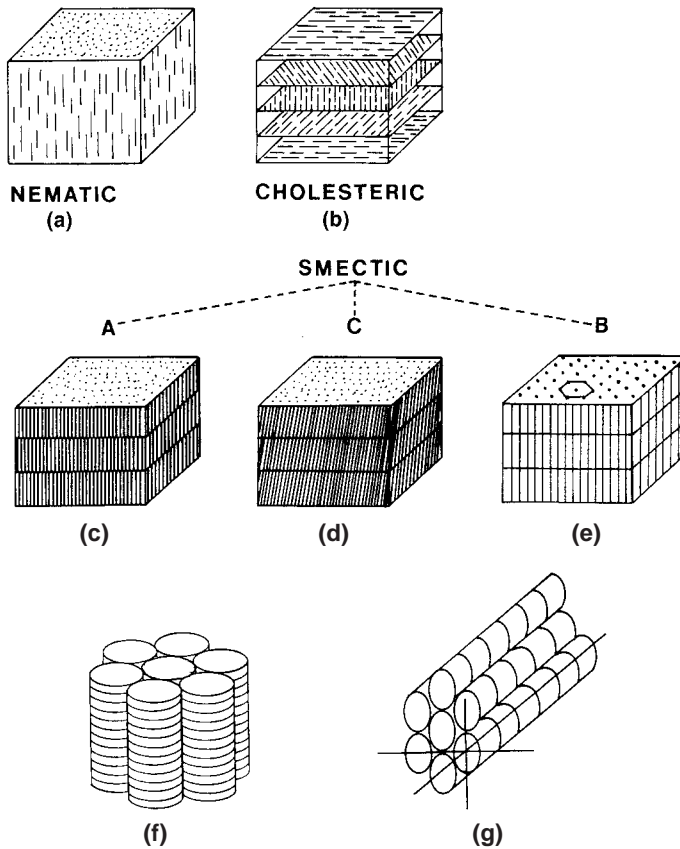
FIG. 2.   Schematic representation of different calamitic and discotic thermotropic liquid crystals: (a) nematic, (b) cholesteric, (c-e) smectic, (f) columnar hexagonal, (g) columnar hexagonal tilted; a-e adapted from Demus, D., and Richter, L., *Textures of Liquid Crystals*, Verlag Chemie, Weinheim, Germany, 1978; f,g adapted from Eidenschenk, R., Flüssige Kristalle, *Chem. Unserer Zeit*, 18, 168–176 (1978).

Lyotropic Liquid Crystals

Lyotropic liquid crystals differ from thermotropic liquid crystals. They are formed by mesogens which are not the molecules themselves but their hydrates or solvates as well as associates of hydrated or solvated molecules. In the presence of water or a mixture of water and an organic solvent as the most important solvents for drug molecules, the degree of hydration or solvation, respectively, depends on the amphiphilic properties of a drug molecule. Hydration of the mostly rod-shaped molecule—and the same holds for solvation—results in different geometries, cone or cylinder [5] (Fig. 3).

Cylinders arrange in layers, resulting in a lamellar phase with alternating polar and nonpolar layers (Fig. 4a). Water and aqueous solutions can be included in the polar layers, resulting in an increase of the layer thickness. Analogously, lipophilic molecules can be included in the nonpolar layers. In addition to the increased layer thickness of the lamellar phase, lateral inclusion between molecules is also possible with an increase in the solvent concentration, which changes the rod shape of the
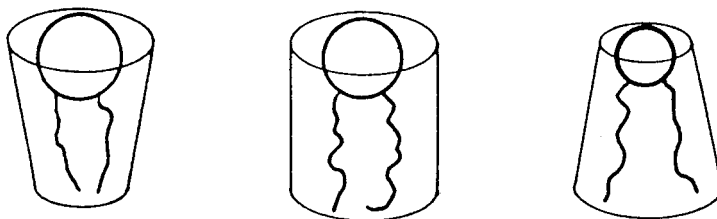
FIG. 3. Geometry of hydrated molecules : cylinders associate to a lamellar liquid crystal, cones to a hexagonal and an inverse hexagonal. Adapted from *The Physical Chemistry of Membranes* (Silver, B., ed.), Allen & Unwin, Inc. & Solomon Press, Winchester, MA, 1985.

solvated molecules to a cone shape (Fig. 3), leading to a phase change. Depending on the polar or nonpolar character of the solvating agent and the molecule itself, the transition results in a hexagonal or an inverse hexagonal phase (Fig. 4b,c).

The hexagonal phase is named after the hexagonally packed rod micelles of solvated molecules, with their polar functional groups pointing to the outside (Fig. 4b) or the inside of the structure (Fig. 4c, inverse hexagonal phase). In the hexagonal phase, the additional amount of water or nonpolar solvent which can be included is limited. As the molecular geometry changes further during solvation, another phase transformation takes place to a cubic form (type I) or inverse cubic form (type IV), consisting of spherical or ellipsoidal micelles and/or inverse micelles (Fig. 4d,e).

In addition to the cubic and/or inverse cubic forms described above, further transitional forms exist between the lamellar phase and the hexagonal mesophase (cubic, type II) or inverse hexagonal mesophase (cubic, type III) [6]. In contrast to the discontinuous phases of types I and IV, cubic mesophases of type II and III belong to the bicontinuous phases (Fig. 4f). A range of lyotropic mesophases are possible, depending on the mesogen concentration, the lipophilic or hydrophilic characteristics of the solvent, and the molecule itself [6].

micellar ←→ hexagonal ←→ lamellar ←→ inverse hexagonal ←→ inverse micellar

      ↘   ↙   ↘   ↙   ↘   ↙   ↘   ↙

    cubic I   cubic II   cubic III   cubic IV

————————————————————————————————————→

lipophilicity of the solvent and/or the amphiphilic compound (AC)
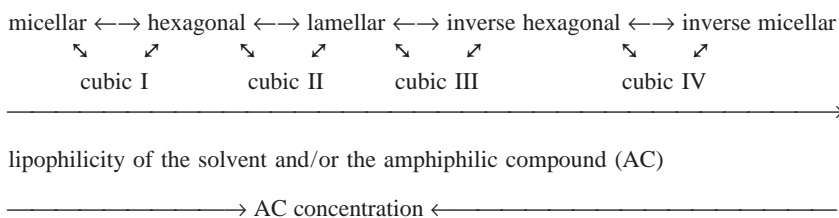
————————————→ AC concentration ←————————————————————

However, not all theoretically possible mesophases may occur in practice.

Liposomes

With some molecules, a high concentration results in a lamellar phase but no additional mesophases are formed if the concentration is reduced. The lamellar phase is dispersed in the form of concentric layered particles in an excess of solvent (water or aqueous solution). This results in a vesicular dispersion. If the mesogenic material consists of phospholipids, the vesicular dispersion is called a liposomal dispersion

(a)

(b)

(c)

(d)

(e)

(f)

Vesicle

multilamellar   oligolamellar

S U V   unilamellar   L U V

multivesicular
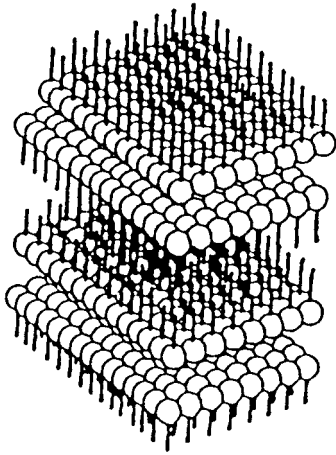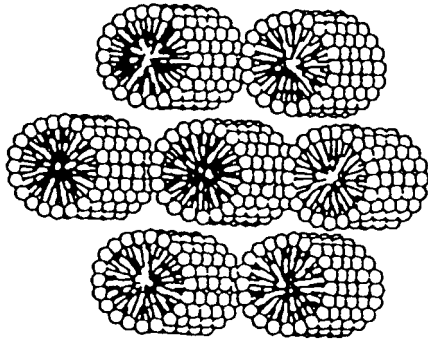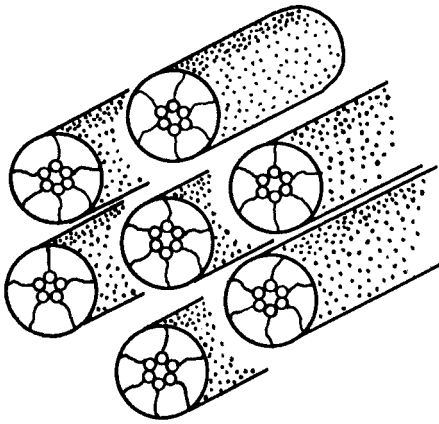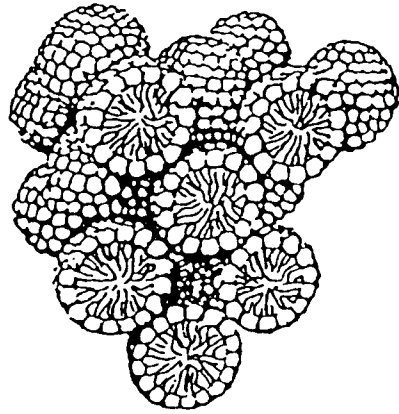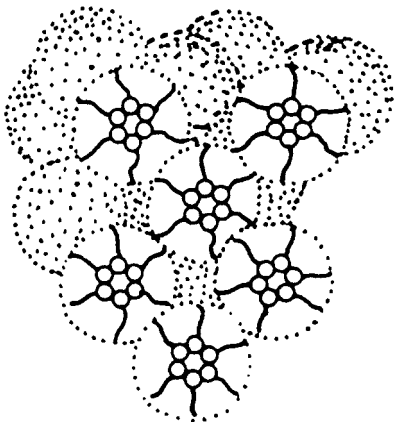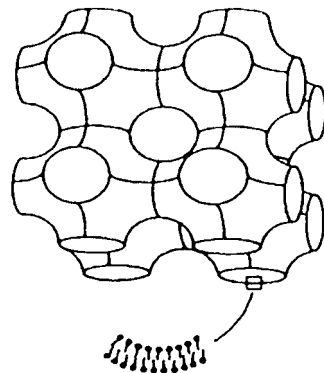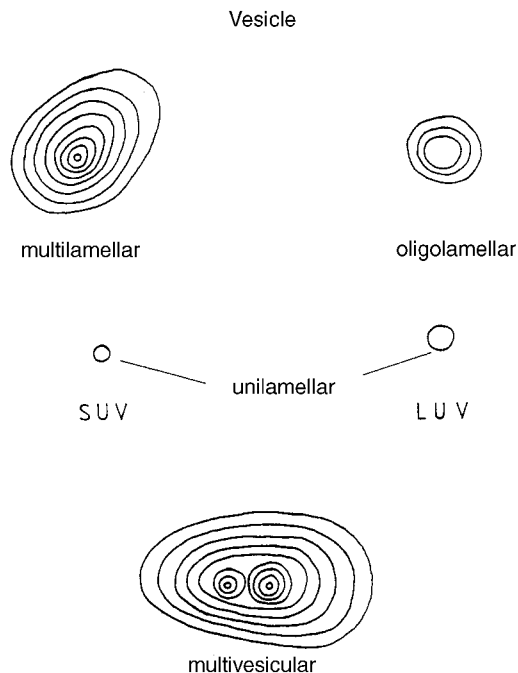
FIG. 5. Schematic cross-sections of vesicles with each line representing a bilayer of hydrated molecules. From Müller-Goymann, C.C., Flüssigkristalline Systeme in der Pharmazeutischen Technologie, *PZ Prisma*, 5, 129–140 (1998).

[7]. In principle, the liposomes may also be dispersed in oily continuous media, but these are of minor interest in drug formulation.

Liposomes consist of many phospholipid bilayers of only few, or just one bilayer (Fig. 5). Therefore multilamellar vesicles (MLV), oligolamellar vesicles (OLV), small unilamellar (SUV), and large unilamellar vesicles (LUV) have to be distinguished. Furthermore, multivesicular liposomes (MVL) may be formed.

The polar character of the liposomal core makes the encapsulation of polar drug molecules possible. Amphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer according to their affinity toward the phospholipids. Participation of nonionic surfactants instead of phospholipids in the bilayer formation results in Niosomes™. The term sphingosomes is suggested for vesicles from sphingolipids. However, the nomenclature is not consistent, and the term liposomes is used as a general term, although vesicles would be the better choice.

FIG. 4. Molecular structure of lyotropic liquid crystals: (a) lamellar, (b) hexagonal, (c) inverse hexagonal, (d) cubic type I, (e) inverse cubic type IV, (f) cubic type II; (a, b, d) adapted from Brown, G.H., and Wolker, J.J., *Liquid Crystals and Biological Structures*, Academic Press, New York, 1979; (c) adapted from Friberg, S.E., *Food Emulsions*, Marcel Dekker, Inc., New York, 1976; (e) from Müller-Goymann, C.C., Anwendung lyotroper Flüssigkristalle in Pharmazie und Medizin. In: *Lyotrope Flüssigkristalle: Grundlagen, Entwicklung, Anwendung* (H. Stegemeyer, ed.), Steinkopff Verlag, Darmstadt, Germany, 1999, p. 141; (f) adapted from Larsson, K., Structure of isotropic phases in lipid-water systems, *Chem. Phys. Lipids*, 9:181–195 (1972).

A standard procedure for manufacturing liposomes is the film-forming method where the phospholipids are dissolved in an organic solvent. By rotational evaporation of the solvent a thin, multilayered film of phospholipids arises at the inner wall of the vessel. Redispersion of this film in water or aqueous buffer results in the formation of vesicles. The size of these vesicles and the number of bilayers vary. Hence further manufacturing steps have to follow to obtain defined vesicular dispersions with a sufficiently long shelf life.

To reduce vesicle size and the number of bilayers, high pressure filtration via polycarbonate membranes as well as high pressure homogenization in a French press or in a microfluidizer are appropriate manufacturing procedures. Sonication may also be applied, although the resulting dispersion does not have the same particle sizes.

The injection method and reverse phase dialysis are additional procedures for the formation of SUV and LUV. Freeze–thaw procedures allow drug loading of the liposomes and offer an evaluation of the stability of the vesicular dispersion. For further reading, Refs. 7 and 8 are recommended.

Liquid Crystal Polymers (LCP)

Both thermotropic and lyotropic liquid crystal polymers exhibit characteristic microstructure features [9,10]. Anisometrical monomers such as rods or disks are connected to chains in an appropriate manner. These anisometrical monomers are considered the mesogens and may be part of main chain LCP, side chain LCP, or of both types together (Fig. 6). Flexible spacers of nonmesogenic character are located between the mesogens. A sufficient flexibility is a prerequisite for the liquid crystal formation with an increase in temperature or solvent concentration.
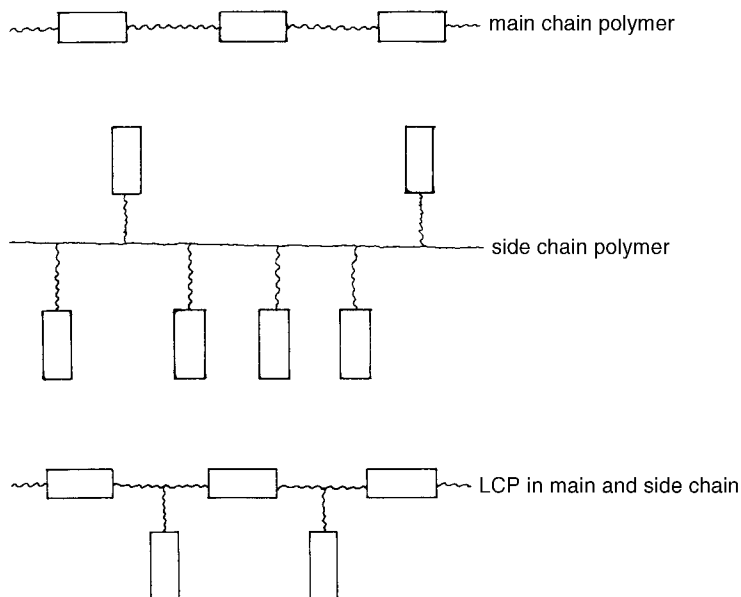
FIG. 6. Liquid crystal polymers with the mesogen within the main chain, the side chain, and both the main and side chain. From Müller-Goymann, C.C., Flüssigkristalline Systeme in der Pharmazeutischen Technologie, *PZ Prisma*, 5:129–140 (1998).

Methods for the Characterization of Liquid Crystals

Both macroscopic and microscopic methods appropriate for the investigation and characterization of lyotropic liquid crystals are frequently used by pharmaceutical laboratories in drug development.

Polarized Light Microscopy

Lyotropic liquid crystals, except for cubic mesophases, show birefringence like real crystals. Birefringence can be observed in the polarizing microscope, where two polarizers in cross-position are mounted below and above the birefringent object being examined. The cross-position of the polarizers provides plane-polarized waves perpendicular to each other. Therefore the light passing the polarizer below an isotropic object cannot pass the polarizer in cross-position above the object. In the case of an anisotropic material some parts of the light are able to pass the second polarizer because the plane-polarized beam has been rotated under an angle relative to the plane of the incoming beam.

Each liquid crystal shows typical black and white textures. The addition of a λ-plate with strong birefringent properties makes it possible to observe the color effects of the textures in yellow, turquoise, and pink. These effects arise due to the fact that the rotation of the plane-polarized light depends on the wavelength. The thickness of the λ-plate is constructed for the wavelength of 550 nm. After leaving the plate, this wavelength is swinging in the same plane as the incoming polarized white light. Therefore it will be totally absorbed by the second polarizer in cross-position. All the other wavelengths of the white light, except for 550 nm, are more or less rotated in context to the polarization plane. Hence they pass the polarizer with various intensities. White light minus the wavelength of 550 nm (green-yellow) gives a pink color impression. With an additional birefrigent liquid crystalline material in the microscope, small deviations of the wavelengths being absorbed occur, and turquoise and yellow textures can be observed.

Hexagonal mesophases can be recognized by their typical fan-shape texture (Fig. 7a). Lamellar mesophases typically show oily streaks with inserted maltese crosses (Fig. 7b). The latter are due to defects, called confocal domains, that arise from a concentric rearrangement of plane layers. In some lamellar mesophases these defects prevail. Hence no oily streaks occur but maltese crosses are the dominant texture (Fig. 7c).

The smectic mesophases of the thermotropic liquid crystals show a variety of textures but resemble mainly the fan-shape texture of the lyotropic hexagonal mesophase. For further reading more comprehensive literature is recommended [11].

Transmission Electron Microscopy (TEM)

Due to the high magnification power of the electron microscope, the microstructure of liquid crystals can be visualized. However, aqueous samples do not survive the high vacuum of an electron microscope without loss of water and thus change of the microstructure. Therefore, special techniques of sample preparation are necessary prior to the electron microscopy. Freeze fracture has proved to be successful (Fig. 8),
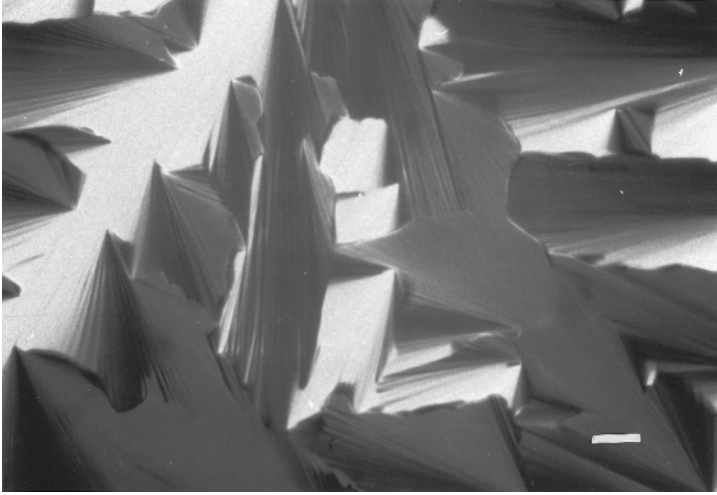
(a)

(b)
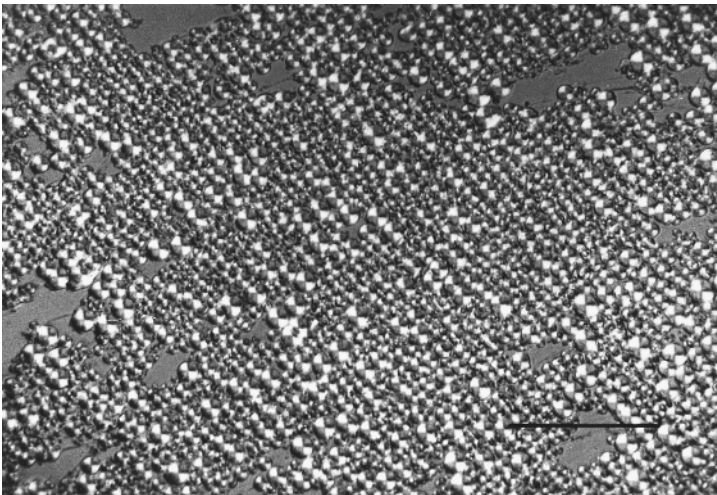
(c)
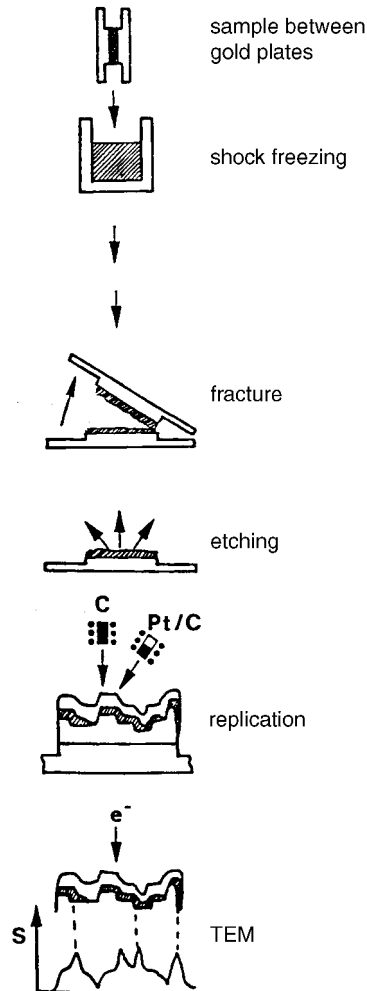
FIG. 8. Freeze fracture replication technique for transmission electron microscopy. From Heering, W., *Die Struktur des Gelgerüsts der Wasserhaltigen Hydrophilen Salbe DAB 8, Anwendung der Gefrierbruchätztechnik und TEM auf kolloide pharmazeutische Zubereitungen*, Ph.D. Thesis Technische Universität Braunschweig, Germany, 1985.

FIG. 7. Polarized light micrographs of (a) hexagonal and (b,c) lamellar liquid crystals, bar 50 μm. From Müller-Goymann, C.C., Flüssigkristalline Arzneimittel. In: *Pharmazeutische Technologie: Moderne Arzneiformen*. (R.H. Müller, and G.E. Hildebrand, eds.), 2nd ed., Wissenschaftliche Verlagsges. mbH Stuttgart, Germany, 1998, pp. 219–242.

where a replicum of the sample is produced and viewed in the electron microscope. To preserve the original microstructure of the sample during the replication, the first step is the shock freezing. To reach freezing rates of 10^5–10^6 K/s, a thin layer of the sample is sandwiched between two gold plates and shock frozen with nitrogen-cooled liquid propane at $-196°C$ or with slush nitrogen at $-210°C$. If the temperature of the cooling medium is far below its boiling temperature, an efficient freezing rate can be obtained.

The frozen sample is transferred into a freeze-fracture apparatus and processed at $-100°C$ at a vacuum between 10^{-6} and 5×10^{-7} bar. Within a homogeneous material the fracture happens randomly because all structural elements have equal probabilities for fracture. However, even a homogeneous material often consists of more or less polar areas. Within the polar areas, stronger interactions via hydrogen bonds prevent the fracture, which is thus less probable than the fracture within apolar areas. Therefore, the sample profile obtained after fracturing represents the microstructure of the sample only qualitatively but not quantitatively.

Immediate etching after freeze fracture provides sublimation of temporary constituents, commonly ice, with the effect, that unevenness in the sample surface will become more pronounced. The sample surface is then coated with platinum of 2 nm thickness under an angle of 45°. Additional vertical coating with a carbon layer of 20 nm platinum provides a high mechanical stability of the replicum which means easier handling, removing, cleaning, drying, and finally observation in the transmission electron microscope.

The coating with platinum under an angle of 45° illuminates the differences in contrast because platinum precipitation takes place preferably at sample positions facing the platinum source in luff, whereas sample positions in lee are less coated or not at all. In the transmission electron microscope (TEM), these different thicknesses of platinum absorb the electron beam differently, thus providing the formation of shadows. This phenomenon produces the plastic impression of the transmission electron micrographs.

Figures 9a–c represent transmission electron micrographs of different lyotropic liquid crystals after freeze fracture without etching. The layer structure of the lamellar mesophase including confocal domains, hexagonal arrangement of rodlike micelles within the hexagonal mesophase, as well as close-packed spherical micelles within the cubic liquid crystal can be clearly seen.

Figures 9d,e show aqueous dispersions of vesicles. The smaller the vesicles, the less probable is an upcoming cross-fracture. Thus the question whether the vesicle is uni- or multilamellar can hardly be answered. At least for fluid vesicle dispersions it is possible to solve the problem with the help of cryotransmission electron microscopy.

For this purpose it is necessary to give sufficient contrast to a thin film of the frozen sample, for example, by use of osmium tetroxide. Then the sample can be viewed directly in the TEM (at $-196°C$). The adjustment of the temperature to $-196°C$ produces a very low vapor pressure, especially of water, so that the examination of the probe is possible by preservation of the microstructure despite the high vacuum. A disadvantage of cryo-TEM is the classification of vesicles according to their size. Due to the fluid property of the vesicle dispersion prior to freezing, the thickness of the sample film varies from the center to the outside. Hence the smaller vesicles stay in the center, where the film is thin, while the larger ones remain at the outside margin in the thicker part of the film. In this outer part, the vesicles evade

the examination and cannot be detected. Hence the resulting distribution does not represent the true size distribution.

X-Ray Scattering

In x-ray scattering experiments characteristic interferences are generated by an ordered microstructure [12]. A typical interference pattern arises due to specific repeat distances of the associated interlayer spacings *d*. According to Bragg's equation *d* can be calculated by:

$$d = n \times \lambda/2 \times \sin\delta$$

where λ is the wavelength of the x-ray being used (e.g., 0.145 nm by using a copper anode or 0.229 nm by using a chromium anode), *n* is an integer indicating the order of the interference, δ is the angle under which the interference occurs and reflection conditions are fulfilled (Fig. 10).

Bragg's equation points at the inverse proportionality between the interlayer spacing *d* and the angle of reflection δ. Large terms for *d* in the region of long-range order are registered by small-angle x-ray diffraction technique (SAXD), whereas small terms for *d* in the region of short-range order are registered by wide-angle x-ray diffraction technique (WAXD). For the exact determination of the distances of interlayer spacings of liquid crystalline systems SAXD is especially important. With WAXD the loss of the short-range order of liquid crystalline systems can be recognized in terms of the absence of interferences, which are characteristic for the crystalline state.

There are two possibilities for the detection of the interferences: the film detection and the registration of x-ray counts with scintillation counters or position-sensitive detectors. However, the SAXD method does not detect interferences from which the interlayer spacings can be calculated. It rather makes it possible from the sequence of the interferences to decide the type of liquid crystal [13,14].

The sequence of the interferences for the different liquid crystals is as follows:

Lamellar $1 : 1/2 : 1/3 : 1/4$
Hexagonal $1 : 1/\sqrt{3} : 1/\sqrt{4} : 1/\sqrt{7}$
Cubic $1 : 1/\sqrt{2} : 1\sqrt{3} : 1/\sqrt{4}$
Cubic $1 : 1/\sqrt{4} : 1/\sqrt{5} : 1/\sqrt{6}$

Differential Scanning Calorimetry (DSC)

Phase transitions go along with changes in energy content of the respective system. This phenomenon is caused by changing the enthalpy ΔH or the entropy ΔS. Enthalpy changes cause endothermic or exothermic signals, depending on whether the transition is due to consumption of energy (e.g., melting of a solid) or a release of energy (e.g., recrystallization of an isotropic melt).

The transition from crystalline to amorphous state requires much energy whereas the transition from crystalline to liquid crystalline or liquid crystalline to amorphous
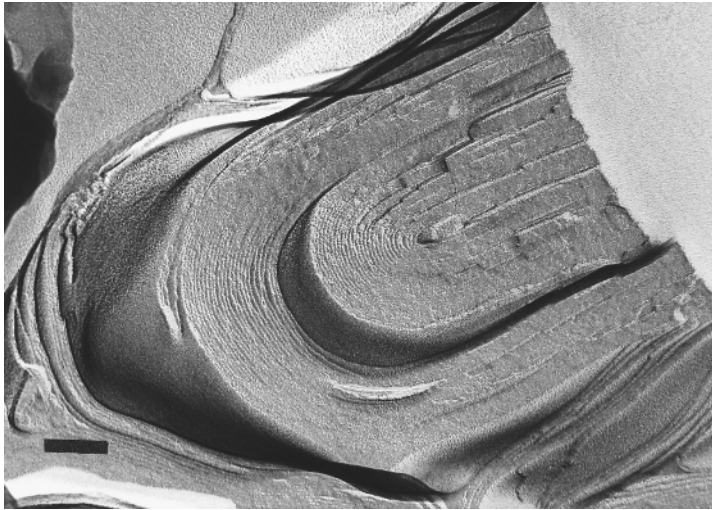
(a)
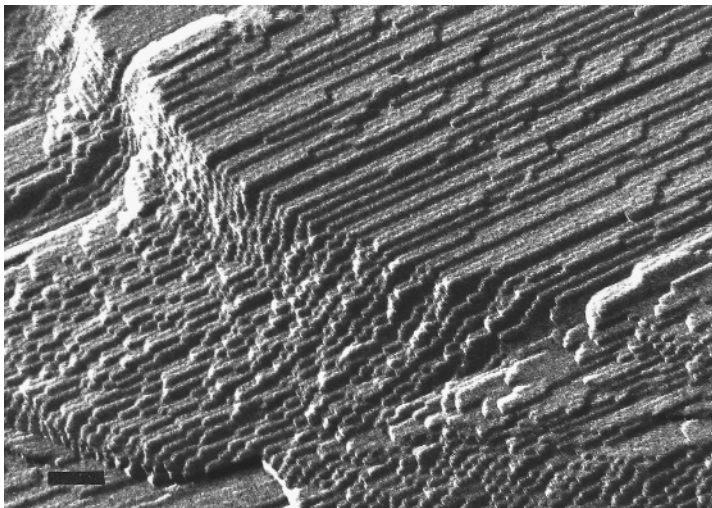
(b)

FIG. 9.   Transmission electron micrographs of freeze-fractured liquid crystals: (a) lamellar with confocal defects, bar 100 nm, (b) hexagonal, bar 100 nm, (c) cubic of type I, bar 100 nm, (d) multilamellar vesicle consisting of dodecyl-PEG-23-ether, cholesterol and water, bar 200 nm, (e) multivesicular vesicle, bar 1 μm; (a,b) from Mueller-Goymann, C., Liquid crystals in emulsions, creams and gels, containing ethoxylated sterols as surfactant, *Pharm. Res.*, 1:154–158 (1984); (c) from Schuetze, W., and Mueller-Goymann, C.C., Mutual interactions between nonionic surfactants and gelatin-investigations in cubic liquid crystalline systems and micellar systems, *Colloid Polym. Sci.*, 269:85–90 (1992); (d) from Usselmann, B., *Beitrag zur Strukturaufklärung topischer Zubereitungen mit Fettalkoholpolyethylenglykolethern und Cholesterol als Tensiden*, Ph.D. Thesis Technische Universität Braunschweig, Germany, 1987; (e) from Schütze, W., *Diffuse Röntgenkleinwinkelstreuung an kolloidalen Drug Delivery Systemen*, Ph.D. Thesis Technische Universität Braunschweig, Germany, 1998.

(c)

(d)

(e)

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FIG. 10. Schematic representation of the reflection conditions according to Bragg' equation.

as well as particularly the transition between different liquid crystals consumes low amounts of energy. Therefore care has to be taken with regard to the sensitivity of the measuring device and a sufficiently low detection limit [15].

Entropically caused phase transitions may be recognized by a change in the baseline slope indicating a change in the specific heat capacity. In particular, the phase transitions of liquid crystalline polymers are due to entropy and are thus being considered second-order transitions, usually called glass transitions. They can be outweighed by an enthalpic effect impeding their detection.

Rheology

Different types of liquid crystals exhibit different rheological properties [16,17]. With an increase in organization of the microstructure of the liquid crystal its consistency increases and the flow behavior becomes more viscous. The coefficient of dynamic viscosity η, although a criterion for the viscosity of ideal viscous flow behavior (Newtonian systems), is high for cubic and hexagonal liquid crystals but fairly low for lamellar ones. However, the flow characteristics are not Newtonian but plastic or pseudoplastic, respectively.

The viscosity of thermotropic liquid crystals increases following the sequence nematic<smectic A<smectic C.

The poor flowability of lyotropic liquid crystals, such as cubic and hexagonal mesophases, is due to their tridimensional and two-dimensional order, respectively. Lamellar mesophases with unidimensional long-range order have a fairly good flowability. Due to their gel character, cubic and hexagonal mesophases exhibit a yield stress until flow occurs. Unlike the corresponding inverse liquid crystals the gel character is more pronounced as a result from the interactions between polar functional groups being located at the surface of the associates. The associates may form strong networks with each other via polar interactions (e.g. hydrogen bonds). In contrast, the surface of the associates of inverse mesophases consists of apolar groups of the associated molecules. Thus the resulting interactions are less strong and the deformation of the gel is obtained easier.

The elasticity of liquid crystalline gels is determined by a mechanical oscillation measurement. Without applying a superposition of shear strain, the viscoelastic properties of liquid crystals may be studied without a change in network microstructure which usually happens with rheological investigations in terms of mechanical deformation. With the oscillation experiments the viscoelastic character of cubic and hexagonal mesophases can be quantified as well as that of lamellar mesophases and highly concentrated dispersions of vesicles which show viscoelastic behavior. A vesicle dispersion of low content of the inner phase, however, exhibits ideal viscous flow property. According to the Einstein equation, the dynamic viscosity coefficient η is larger than η_0 of the continuous phase, usually pure water or solvent, by a factor of $2.5 \times$ volume ratio of the dispersed phase φ.

$$\eta = \eta_0(1 + 2.5\ \varphi),$$

where η_0 = viscosity of pure solvent as the continuous phase
φ = volume ratio of the inner phase

Determination of Vesicle Size by Laser Light Scattering

The vesicle size is an important parameter not only for in-process control but particularly in quality assurance, because the physical stability of the vesicle dispersion depends on particle size and particle size distribution. An appropriate and particularly quick method is laser light scattering or diffraction. Laser light diffraction can be applied to particles > 1 μm and refers to the proportionality between the intensity of diffraction and the square of the particle diameter according to the diffraction theory of Fraunhofer.

For particles below 200 nm, Rayleigh's theory holds, which considers the scattering intensity to be proportional to the 6th potency of the particle diameter. Both Fraunhofer's and Rayleigh's theories are only approximations of Mie's theory which claims that the scattering intensity depends on the scattering angle, the absorption, the size of the particles as well as on the refractive indices of both the particles and the dispersion medium.

Unfortunately, these indices are difficult to determine. Furthermore, most vesicle dispersions contain a dispersed mesophase with particle sizes below 200 nm up to 1 μm. Therefore photon correlation spectroscopy (PCS), on the basis of laser light scattering, provides an appropriate method of investigation [18].

Dynamically raised movements in the dispersion (e.g., Brownian molecular motion) cause variations in the intensities of the scattered light with time, which is being measured by PCS. Particularly the Brownian motion causes higher fluctuations the smaller the particles are. Thus a correlation between the different intensities measured is only possible for short time intervals. This means in the case of a monodisperse system following 1st-order kinetics, that the autocorrelation function decreases fast. In a half-logarithmic plot of the autocorrelation function the slope of the graph can be used for the calculation of the hydrodynamic radius according to the Stokes-Einstein equation. With commercial PCS devices, the z-average is determined, which corresponds to the hydrodynamic radius.

In the case of a polydisperse system the calculation of the particle size distribution is possible by using special transformation algorithms. For this purpose certain requirements need to be fulfilled, such as a spherical particle shape, sufficient dilution, and a large difference between the refractive indices of the inner and the outer phases. Since usually not all requirements can be fulfilled, the z-average is preferred as a directly accessible parameter rather than the distribution function depending on models.

Applications of Liquid Crystals in Drug Delivery

Liquid Crystalline Drug Substances

Some drug substances can form mesophases with or without a solvent [19–26]. In the absence of a solvent, an increase in temperature causes the transition from the solid state to the liquid crystalline state, called thermotropic mesomorphism. Lyotropic mesomorphism occurs in the presence of a solvent, usually water. A further change in temperature may cause additional transitions. Thermotropic and/or lyotropic liquid crystalline mesophases of drug substances may interact with mesomorphous vehicles as well as with liquid crystalline structures in the human organism. Table 1 presents drug substances for which thermotropic or lyotropic mesomorphism has been proved.
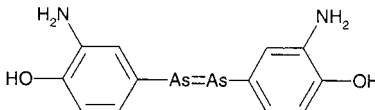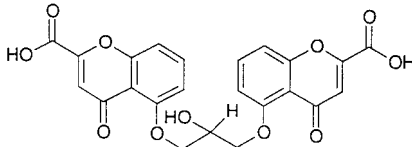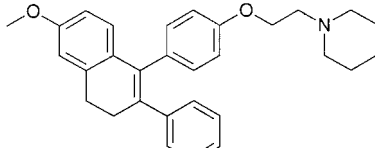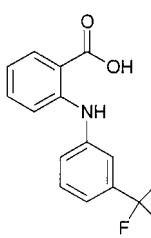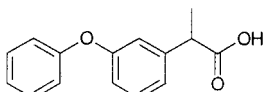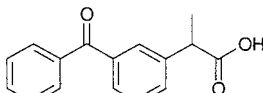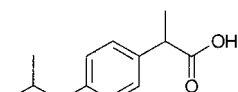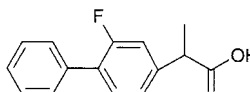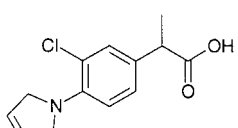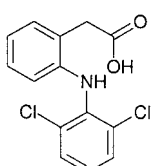
Arsphenamin was the first drug substance with thermotropic mesomorphism [19] to be used therapeutically as Salvarsan during the first half of the 19th century. This drug is effective against microorganisms and offered for the first time a therapy for venereal diseases. Today it has been replaced by antibiotics with less serious side effects.

The molecular structure of arsphenamin is a typical representative of a thermotropic mesogen. With its symmetrical arrangement of the atoms the same holds for disodium cromoglycate, DNCG [20], which forms both thermotropic liquid crystals and lyotropic mesophases in the presence of water. Micronized DNCG powder applied to the mucosa of the nose or the bronchi absorbs water from the high relative humidity of the respiration tract and is first transformed into a lyotropic mesophase and then into a solution depending on the amount of water available.

Disodium cromoglycate serves as a mast-cell stabilizer. Mast cells are located on the mucosa of the respiratory tract and act by releasing the mediator histamine on contact with an allergen, provided that the patient had been sensitized before. Due to its mast-cell stabilizing effect, DNCG acts as prophylactic against allergic reactions associated with asthma and hay fever. In addition, DNCG exhibits a second mode of action in asthma therapy which has not yet been fully clarified. According to new findings, DNCG relieves the inflammation of the mucosa of the bronchi.

For therapeutical purposes, a likewise frequently used group of drug compounds are the nonsteroidal anti-inflammatory drugs (NSAID). Among the best known representatives of the aryl acetic acid derivatives is diclofenac as well as ibuprofen, an aryl propionic acid derivative. As both have acidic properties, they dissociate while being dissolved and may form salts with amphiphilic properties. Together with appropriate counterions these amphiphilic organic acids may form lyotropic mesophases with water even at room or body temperature, for example, diclofenac diethylamine

TABLE 1 Liquid Crystalline Drug Substances

Drug	Crystal Type	Formula	Reference
Arsphenamine	Nematic		19
Disodium cromoglycinate	Nematic, hexagonal		20
Nafoxidin HCl[a]	Hexagonal, cubic, lamellar		21
Diethylammonium flufenamate	Lamellar		22
NSAID salts			
Fenoprofen	Lamellar		23
Ketoprofen	Lamellar		23
Ibuprofen	Lamellar		23
Flurbiprofen	Lamellar		23
Pirprofen	Lamellar		23
Diclofenac	Lamellar		25
Peptide hormone LH-RH analogue			24

[a] So far the only example that has not been used as a therapeutic.

or ibuprofen lysinate [23,25]. Furthermore, some NSAID anhydrates exhibit thermotropic mesomorphism after thermal dehydration of the crystalline salt, such as fenoprofen calcium [26].

Liquid Crystalline Formulations for Dermal Application

Drug molecules with amphiphilic character may form lyotropic mesophases, and amphiphilic excipients in drug formulations also form lyotropic liquid crystals. Especially surfactants, which are commonly used as emulsifiers in dermal formulations, associate to micelles after dissolution in a solvent. With increasing concentration of these micelles the probability of interaction between these micelles increases and thus the formation of liquid crystals.

Surfactant Gels

The use of monophasic systems of lyotropic liquid crystals is relatively rare and is limited to gels. A variety of polar surfactants (e.g., ethoxylated fatty alcohols) are hydrated in the presence of water and form spherical or ellipsoidal micelles. At high surfactant concentrations these associates are densely packed and thus are identified as cubic liquid crystals [27]. Figure 9c shows a transmission electron micrograph of a liquid crystalline surfactant gel of this type. Such gels are optically transparent. If agitated mechanically, their elastic properties become evident. Due to resonance effects in the audible range, they are also called ringing gels. The lipophilic components are solubilized together with the active ingredients in hydrated associates of the surfactants. However, the solubilization capacity for lipophilic components is generally limited. By exceeding this capacity, the excess of the lipophilic component is dispersed dropwise in the liquid crystalline phase (Fig. 11b). Such systems appear white according to the change in refractive index at the interface between the continuous liquid crystalline and dispersed oil phases. The dispersed drops are mechanically stabilized because the liquid crystalline phase of hexagonal (Fig. 11a) or cubic character (Fig. 11b) has a high yield stress.

Ringing gels with cubic liquid crystalline microstructure are used in commercial drug formulations, especially for topical NSAID formulations. Examples on the German market include Contrheuma Gel Forte N™, Trauma-Dolgit™ Gel, and Dolgit Mikrogel™. The last was introduced in 1996 and contains ibuprofen as active ingredient. The high surfactant concentration of such gels is necessary to verify the liquid crystalline microstructure and also influences the microstructure of the stratum corneum lipids in context of an increase in permeability. The latter effect is also achieved by alcohol, which is part of the formulation. Figure 12 shows the result of permeation tests with excised human stratum corneum. The amount of ibuprofen permeating per time and surface area is much higher for Dolgit™ Mikrogel than for an aqueous mixed micellar solution of the drug. Although relatively high permeation rates are possible for the liquid preparation, the commercial formulation is significantly more effective since the high surfactant content and the alcohol favor a high permeability.

A ringing surfactant gel of liquid crystalline microstructure containing the antimykotic bifonazole was introduced in 1995 into the German market (Bifomyk™ Gel).
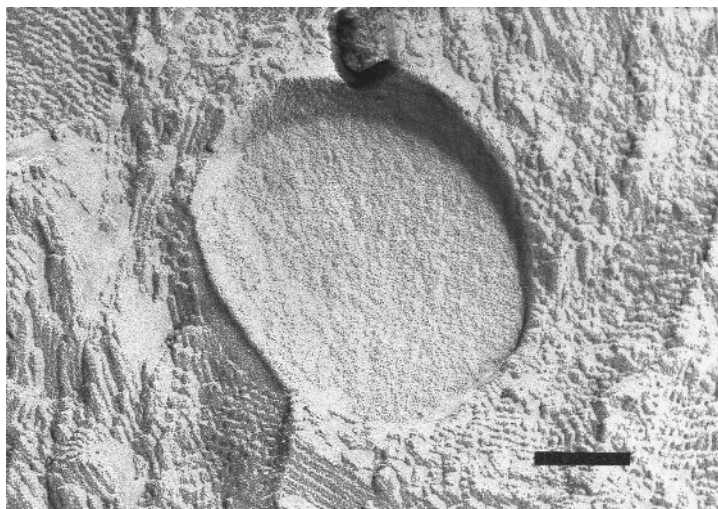
(a)
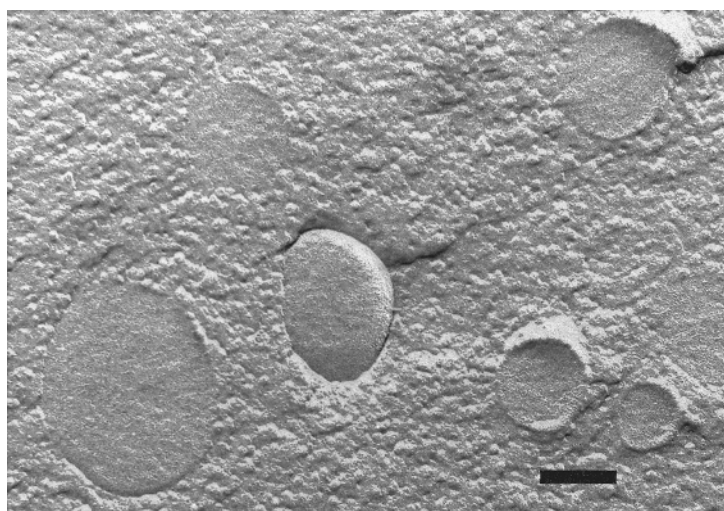
(b)

FIG. 11. Transmission electron micrographs of freeze fractured oily droplets dispersed (a) in a hexagonal and (b) in a cubic liquid crystalline phase, bar 100 nm. From Mueller-Goymann, C., Liquid crystals in emulsions, creams and gels, containing ethoxylated sterols as surfactant, *Pharm. Res.* 1:154–158 (1984).

Similar to surfactant gels containing NSAID, an improved penetration of the active ingredient is also desired in antifungal dermal therapy. However, since the liquid crystal structure only forms with a relatively high surfactant concentration, the positive effect of improved penetration must be considered together with the potential of irritation. The objective is as little irritation as possible and as much improvement of penetration as possible via a change in skin structure. Since hyphae of fungi (mycelium) can penetrate deep into the epidermal layers by sliding past the corneocytes
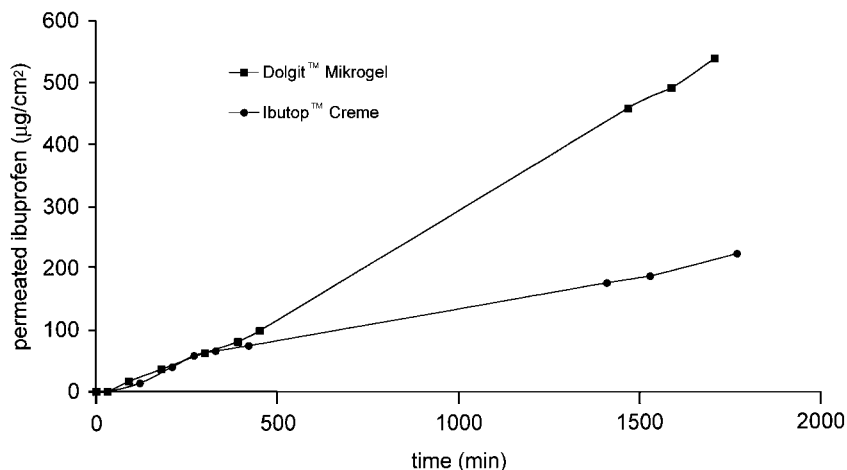
FIG. 12. Permeability of ibuprofen from different formulations via excised human stratum corneum. Redrawn from Stoye, I., *Permeabilitätsveränderung von humanem Stratum corneum nach Applikation nicht-steroidaler Antirheumatika in verschiedenen kolloidalen Trägersystemen*, Ph.D. Thesis TU Braunschweig, 1997.

of the horny layer, improvement of the antimycotic therapy is of particular importance. The same holds for the penetration of NSAID through several epidermal layers, because they have to arrive at the deeper muscle and joint tissue.

Ointments and Creams

Commonly the surfactant concentration in ointments and creams is significantly lower than in surfactant gels. Ointments are nonaqueous preparations, whereas creams derive from ointments by adding water. The microstructure of both ointments and creams may consist of liquid crystals, as far as a liquid crystalline network or matrix is formed by amphiphilic molecules. In the case of a liquid crystalline matrix the system is easier to be deformed by shear. Such formulations show plastic and thixotropic flow behavior on shear. A liquid crystalline matrix exhibits a short regeneration time of the sheared matrix, whereas the crystalline matrix is usually destroyed irreversibly by shear. To obtain a liquid crystalline matrix, amphiphilic surfactants have to be selected which form lyotropic liquid crystals at room temperature. Preferably lamellar liquid crystals should be formed that are able to solubilize high amounts of ingredients and to spread through the whole formulation as a network-forming cross-linked matrix. In contrast, ointments which contain long-chain fatty alcohols such as cetyl and/or stearyl alcohol, have a crystalline structure at room temperature [28].

The so called α phase of the fatty alcohols is a thermotropic type smectic B liquid crystal with a hexagonal arrangement of the molecules within the double layers. It is initially formed from the melt during the manufacturing process and normally transformed into a crystalline modification on cooling. However, the crystallization of the gel matrix can be avoided if the α phase can be kept stable as it cools to room temperature. This can be achieved by combining appropriate surfactants, such as myristyl or lauryl alcohol and cholesterol, a mixture of which forms a lamellar liquid

crystal at room temperature [29]. Due to a depression of the melting point, the phase transition temperature of crystalline to liquid crystalline as well as liquid crystalline to isotropic decreases. Therefore a liquid crystalline microstructure is obtained at room temperature.

The polar character of a surfactant molecule allows the addition of water to form creams. Depending on whether the surfactant or the surfactant mixture has a strong or weak polar character, creams of type o/w or w/o, respectively, are obtained. Creams of the w/o type are produced from systems which are solely stabilized with weakly polar surfactants such as fatty alcohols, cholesterol, glycerol monostearate, or sorbitan fatty acid esters. The surfactants or surfactant mixtures are adsorbed at the interface between the dispersed aqueous and the continuous lipophilic phase. Even multiple layers of the surfactants are adsorbed, if the concentration of mesogenic molecules is high enough to form their own liquid crystalline phase (Fig. 13). Apart from the reduction of the surface tension and/or surface energy, the liquid crystalline interface also has a mechanically stabilizing effect on the drops of emulsion.

Surfactants such as sulfated fatty alcohols may be hydrated to a higher extent than the fatty alcohols alone and thus stabilize o/w emulsions. The combination of an anionic and a nonionic surfactant has proved to be particularly effective, since the electrostatic repulsion forces between the ionic surfactant molecules at the interface are reduced by the incorporation of nonionic molecules, thus improving the emulsion stability. The combination of cetyl/stearyl sulfate (Lanette E) and cetyl/stearyl alcohol (Lanette O) to yield an emulsifying cetyl/stearyl alcohol (Lanette N) is an example of this approach. The polar properties of this surfactant mixture are dominant, and o/w creams are formed. In contrast to w/o systems, the stabilizing effect of the surfactant mixture is not mainly due to adsorption at the interface. Instead, the mixed surfactants are highly hydrated and form a lamellar network, which is

FIG. 13. Transmission electron micrograph of a freeze fractured w/o cream. The aqueous phase is dispersed as droplets within the continuous lipophilic phase; the interface consists of multiple bilayers of hydrated surfactant molecules, bar 500 nm. From Müller-Goymann, C., Halbfeste emulsionsähnliche Zustände, *Seifen, Öle, Fette, Wachse*, 110:395–400 (1984).

dispersed throughout the continuous aqueous phase whereas the dispersed lipophilic components are immobilized within the gel network. However, this hydrated gel matrix is not crystalline at room temperature as the corresponding w/o creams with cetyl/stearyl alcohol, but is in its α-phase, which belongs to the thermotropic smectic liquid crystals and shows a strong similarity to lyotropic lamellar liquid crystals.

Analogous gel matrices of liquid crystalline lamellar phases can also be formed with nonionic mesogens, such as the combination of cetyl/stearyl alcohol and ethoxylated fatty alcohol, provided that the hydrophilic and lipophilic properties of the surfactant molecules are more or less balanced to favor the formation of lamellar structures.

Liposome Dispersions

Although liposomes have been studied intensely since 1970, only a few commercial drug formulations contain liposomes as drug carriers [30,31]. The first commercial drug formulation for topical administration containing liposomes was registered in Italy. The antimykotic econazol was encapsulated in liposomes being dispersed in a hydrogel (Ecosom™ Liposomengel, formerly Pevaryl™ Lipogel). Due to the formation of a highly hydrated gel network of hydrophilic polymers, liposomes are immobilized within the gel network and thus mechanically stabilized. Stabilization via gelation of the continuous aqueous phase can also be applied to other disperse systems, such as suspensions or emulsions. Hepaplus™ Liposom™ is an example for such an emulsion/hydrogel combination which contains heparin sodium as active ingredient and liposomes as additional dispersed phase (actually the latter just since 1995). A formulation with an analogous emulsion/hydrogel combination but without liposomes is Voltaren™ Emulgel. A transmission electron micrograph (Fig. 14) reveals an adsorption of lamellar liquid crystals at the interface between the dispersed oil drops and the aqueous continuous phase. The aqueous continuous phase is again a hydrogel based on polyacrylate in which the lipophilic phase is immobilized. The interface is composed of multilamellar layers consisting of both surfactant and drug molecules. Thus the hydrogel is not only stabilized by the hydrogel network itself but by the liquid crystalline interface which provides an additional stabilization. The active ingredient is diclofenac diethylamine which diffuses slowly from the dispersed phase via the multilamellar interface into the continuous phase from where it penetrates into the epidermis.

Similar to Voltaren™ Emulgel, oily droplets of an eutectic mixture of lidocaine and prilocaine are dispersed in a hydrogel to provide local anesthesia to the skin for injections and surgical treatment (Emla™ cream). A further possibility is the dermal administration of a liposome dispersion as a spray (Heparin PUR ratiopharm Sprühgel™). After administration, water and isopropylic alcohol evaporate partially resulting in an increase of concentration and in a transition from the initial liposome dispersion into a lamellar liquid crystal [32]. The therapeutic effect appears to be influenced favorably by the presence of lecithins rather than by the degree of liposome dispersion.

Liposome Dispersions for Parenteral Administration

Depending on their size and surface charge, parenterally administered liposomes interact with the reticuloendothelial system (RES) and provoke an immune response.
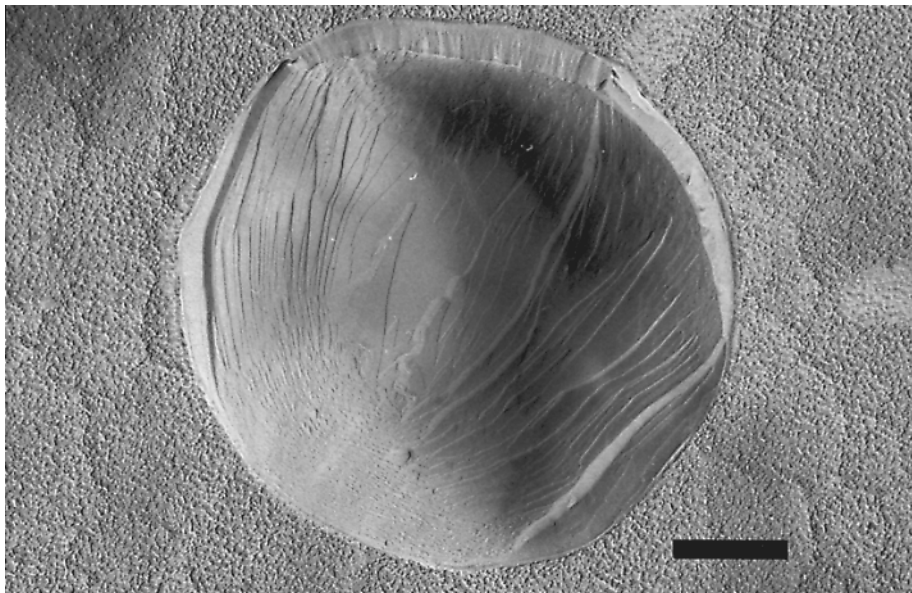
FIG. 14. Transmission electron micrograph of Voltaren™ Emulgel; the interface between the continuous hydrogel and the dispersed emulsion droplets consists of multiple bilayers of hydrated surfactant molecules, bar 500 nm. From Müller-Goymann, C., and Schütze, W., Mehrschichtige Phasengrenzen in Emulsionen, *Dtsch. Apoth. Ztg*., 130:561–562 (1990).

After being marked by the adsorption of certain serum proteins, so called opsonines, they are identified as invaders and destroyed by specific immune cells, mainly in the liver, spleen, and bone marrow.

This passive drug targeting provides an efficient therapy of diseases of these organs or affected cells of them. Clinical tests in the therapy of parasitic diseases, concerning especially the liver and spleen, have been most efficient by having encapsulated the drug substance in liposomes. Apart from passive drug targeting, drug encapsulation within liposomes offers a modification of the therapeutical effect in terms of intensity and duration combined with a minimum of undesired side effects. For this purpose, the liposomes have to circulate as long as possible in the vascular system remaining unrecognized by phagocytic cells.

The antimykotic amphotericine is encapsulated in liposomes and marketed as AmBisome™ against severe systemic mycosis. The liposomal encapsulation reduces the toxicity of amphotericine while increasing the half-life of the drug and plasma level peaks [31]. For stability reasons, the parenteral formulation is a lyophilized powder which has to be reconstituted by adding the solvent just before administration.

The cytostatic daunorubicine, which is administered in the later state of Kaposi sarcoma of AIDS patients, is encapsulated in liposomes of about 45 nm size [31]. The liposome dispersion is marketed as a sterile, pyrogen-free concentrate (DaunoXome™) and has to be diluted with a 5% glucose solution just before being administered as an infusion. Although daunorubicine itself is cardiotoxic, the liposomal formulation attacks cardiac tissue only insignificantly but strongly affects the tumor cells by being taken up preferably. It is postulated that small unilamellar vesicles (SUV) may pass endothelial gaps in newly formed capillaries of the tumor entering

the tissue. The drug is released at this site from the liposomal carrier and inhibits the proliferation of the tumor cells.

Liposome Dispersions for Instillation into the Lung

A liposomal formulation containing a surfactant, which usually coats the mucosa of the bronchi and prevents a collapse of the alveolar vesicles of the lung, has been developed for patients who suffer from infant respiratory distress syndrome (IRDS) or adult-acquired respiratory distress syndrome (ARDS). Premature babies often suffer IRDS before the development of a functional lung surfactant and pulmonary gas exchange. ARDS is also a life-threatening failure and loss of the lung function and is usually acquired by illness or accident. Clinical trials with liposomal surfactant have proved to be effective in prophylactic treatment of IRDS and ARDS.

The surfactant is obtained by extraction from the lungs of cattle by washing and centrifugation several times. The raw extract is treated with the appropriate organic solvents, sterilized by filtration, dried by solvent evaporation under aseptic conditions, resuspended in water, and finally homogenized in a French press under cooling. Care has to be taken to maintain sterility of the extract during all procedures. Special attention has to be paid to TSE (transmissible spongiform encephalopaties). The complete manufacturing process has been validated in terms of a decrease of infectious material by a factor of 10^{21} although a factor of 10^8 would have been sufficient. The result is a formulation (Alveofact), which is considered safe with regard to TSE and viruses and which contains all relevant components of the lung surfactant in terms of the pulmonary exchange of gas [31].

Transdermal Patches

A systemic effect via percutaneous penetration of a drug compound requires a high permeability through the stratum corneum and the living tissue beneath as well as a high potency of the drug for the low dose to be administered. In the cases of a short biological half-life, controlled release transdermal systems are a good choice.

Transdermal patches are high-tech devices which contain the drug substance in a reservoir from which it is released in a controlled manner, that is, zero-order kinetics. The control element is a membrane or a matrix. Membrane-controlled patches were the first to be marketed. However, these patches have the disadvantage of so-called dose-dumping if the membrane is damaged during handling. To ensure the desired drug control, even liquid crystalline polymers have been investigated with regard to their usefulness in membrane-controlled transdermal patches [33]. The matrix-controlled transdermal patch consists of only one functional element, the porous polymer matrix, which not only controls drug release but acts as drug reservoir and adhesive element.

Transdermal patches are marketed worldwide, containing drug substances such as glycerole trinitrate, estradiol, testosterone, clonidine, scopolamine, fentanyl, and nicotine. The patch has to remain for up to one week at the appropriate body site. In this case the drug amount in the reservoir is rather high. Since liquid crystalline vehicles with lamellar microstructure have high solubilization capacities, they are recommended as reservoirs for transdermal patches [34], although the high surfactant

concentration of the lamellar liquid crystal might irritate the skin. In the membrane-controlled patch, the liquid crystalline vehicle is not in direct contact with the skin and an irritating effect on the skin is avoided.

Sustained Drug Release from Solid, Semisolid, and Liquid Formulations

The therapy of a chronic disease requires repeated drug dosing. In the case of a short biological half-life, the drug has to be administered up to several times daily within short intervals. To reduce the application frequency, sustained formulations have been developed. For this purpose liquid crystalline excipients are appropriate candidates, because in a liquid crystalline vehicle the drug diffusion is reduced by a factor of 10 to 1000 in comparison with a liquid vehicle such as a solution [35–37]. The factor depends on liquid crystal.

Solid Formulations

Solid formulations for sustained drug release may contain mesogenic polymers as excipients forming a matrix which is usually compressed into tablets. Some of the most frequently used excipients for sustained release matrices include cellulose derivatives belonging to the group of lyotropic liquid crystals which gradually dissolve in aqueous media. Cellulose derivatives such as hydroxypropyl cellulose or hydroxypropyl methylcellulose form gel-like lyotropic mesophases in contact with water [38] through which diffusion takes place relatively slowly. Increasing the dilution of the mesophase with water transforms it to a highly viscous slime and finally to a colloidal polymer solution.

Semisolid Formulations

The solubilization of a drug substance in monophasic liquid crystalline vehicles gives semisolid formulations which are preferably used for topical application (see Surfactant Gels and Transdermal Patches above).

Liquid Formulations

Sustained release from disperse systems such as emulsions and suspensions can be achieved by the adsorption of appropriate mesogenic molecules at the interface. The drug substance, which forms the inner phase or is included in the dispersed phase, cannot pass the liquid crystals at the interface easily and thus diffuses slowly into the continuous phase and from there into the organism via the site of application. This sustained drug release is especially pronounced in the case of multilamellar liquid crystals at the interface.

A further possibility is the formation of liquid crystals on contact with body fluids at the site of application. The initially applied drug solution interacts with body fluids such as plasma, tears, or skin lipids and undergoes a phase transition into a mono- or multiphasic system of liquid crystals (Fig. 15). For example, oily solutions of reverse micellar solutions of phospholipids, which solubilize additional drug, trans-
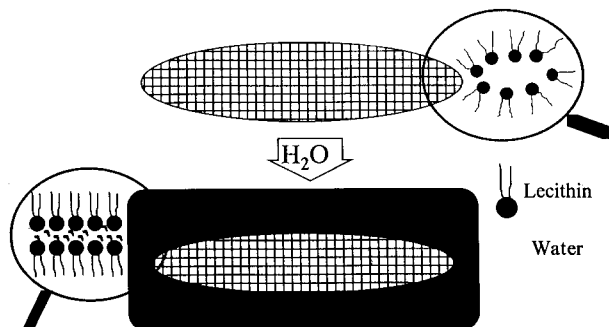
FIG. 15. Application induced transformation of a reverse micellar solution into a liquid crystal on contact with aqueous media. From Müller-Goymann, C.C., Anwendung lyotroper Flüssigkristalle in Pharmazie und Medizin. In: *Lyotrope Flüssigkristalle: Grundlagen, Entwicklung, Anwendung* (H. Stegemeyer, ed.), Steinkopff Verlag, Darmstadt, Germany, 1999, p. 141.

form into liquid crystalline lamellar phases by the absorption of water when applied to the mucosa. Drug release is controlled by the liquid crystals because the diffusion within the liquid crystalline phase is slowest and thus rate-controlling [37]. This principle can be used for ophthalmological administration as well as for nasal, buccal, rectal, vaginal, or even parenteral subcutaneous application [39]. However, the peroral administration of such reverse micellar solutions directly or encapsulated within soft gelatin capsules is not recommended [40], because the sustained release effect is limited by interindividual variations in digestion, that is, the amount and composition of the gastric fluid as well as its ability for emulsification and solubilization in terms of enteral absorption.

For the treatment of paradontitis of infected gum pockets, the chemotherapeutic metronidazol has been proved to be effective. The crystalline prodrug metronidazolbenzoate, which has to provide the active metronidazol as a result of dissolution and hydrolysis, is suspended in an oleogel (Elyzol™ Dentalgel). The oleogel consists of glycerol monooleate and sesame oil, which is immobilized within the matrix structure of the surfactant. The base melts at body temperature and spreads evenly over the inner surface of the gum pockets. The molten system absorbs water and is transformed into a reverse hexagonal phase. This liquid crystalline structure has a high viscosity. The resulting system adheres well to the surface of the mucosa and releases the active ingredient slowly [41].

Liquid Crystals in Cosmetics

Liquid crystals are mainly used for decorative purposes in cosmetics. Cholesteric liquid crystals are particularly suitable because of their iridescent color effects, and find applications in nail varnish, eye shadow, and lipsticks. The structure of these thermotropic liquid crystals changes as a result of body temperature, resulting in the desired color effect. In recent times, such thermotropic cholesteric liquid crystals have been included in body care cosmetics, where they are dispersed in a hydrogel. Depending whether this dispersion requires stirring or a special spraying process, the iridescent liquid crystalline particles are distributed statistically in the gel (Estée Lau-

der Time Zone Moisture Recharging Complex) or concentrated locally (Vichy Restructure Contour des Yeux) to give the formulation the required appearance. Tests of the cosmetic efficiency of the liquid crystalline constituents have not been published up to now.

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CHRISTEL C. MUELLER-GOYMANN

Medication Errors: A New Challenge for the Pharmaceutical Industry

Introduction

A report issued by the Institute of Medicine [1] in November 1999 has drawn unprecedented national attention to the prevalence of medical error (including medication error). The issues and complexities surrounding this problem are compounded by the array of players and solutions that must address this concern for the public health and safety of the U.S. population. Medical products and their manufacturers are reported not only as part of the problem but also as part of the solution. Ongoing national efforts in medication error reporting and prevention place the pharmaceutical industry far ahead of other medical product manufacturers. These efforts headed by the U.S. Pharmacopeia have involved the industry since 1991 by sharing reports received from health care practitioners and documenting industry actions directed to the reported problems. The Institute of Medicine fosters a systems approach to error analysis that focuses on identifying the root cause of error within the system and not focusing on blame of the individual. The report postulates that individuals who commit errors are often well-trained, experienced, well-intentioned individuals whose misfortune is a product of the unsafe systems in which they operate. With the advent of new technologies and the health care delivery processes surrounding them, new problems are likely to surface, particularly as health systems are redesigned in response to this national call to action. The challenge to the pharmaceutical industry is to learn from its own experiences and the USP national database of errors. The industry will be expected to be knowledgeable of the medication use process for the health setting in which their products are used and to anticipate misuse by designing error-free products.

Scope of the Problem

Incidence of Medication Errors and Related Morbidity and Mortality

That medication errors occur frequently in U.S. hospitals has been well-documented [2–4]. In observation studies done between 1962 and 1995 on the rate of administration errors in a variety of in-patient settings, rates ranged from 0 to 59% [5]. Estimates that medication errors occur in almost 7% of hospitalized patients have been reported [6]. One study found that the frequency of medication errors was 1.4 per admission [4]. When approximately 290,000 medication orders were analyzed, Lesar et al. estimated that there were almost two serious errors for every 1,000 orders written. Based on a review of death certificates, it was estimated that almost 8,000 people died from medication errors in 1993, as opposed to almost 3,000 people in 1983 [3]. Researchers found an error rate at two children's hospitals of 4.7 per 1,000 orders [7]. Several

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excellent and comprehensive reviews of the literature on medication errors have recently been published [1,5].

A variety of error rates for different aspects of the medication use process have been reported. Researchers use different methodologies and definitions of ''medication error,'' and study different aspects of the medication use process (i.e., prescribing, dispensing, administering). Because there is no national standardization for the denominator used to report medication error rates, the denominator can vary among several, including doses dispensed, doses administered, doses ordered, and patient days. Therefore, the rates reported in the literature are limited in their use for comparative purposes [5].

Research supports a systems approach to error prevention as well as investigation of errors [8–11]. This means that all aspects of the medication use process, including characteristics of the products themselves should be explored for ways to improve safety in use.

Cost of Medication Errors

Medication errors are costly to both the patient (direct costs such as additional treatment and increased hospital stay) and to society (indirect costs such as decreased employment, costs of litigation) [1,5]. The cost of medication errors in a 700-bed teaching hospital based on a study in eleven medical and surgical units in two hospitals over a six-month period, was estimated to be $2.8 million dollars annually [2]. The increased length of stay associated with a medication error was estimated to be 4.6 days [2]. In a four-year study of the costs of adverse drug events (ADEs) in a tertiary care center, 1% of these events were classified as medication errors. The excess hospital costs for ADEs over the study period were almost $4,500,000 with almost 4,000 days of increased hospital stay [12].

Harm caused by drugs is a major cause of malpractice claims associated with medical procedures [8]. The average compensation for medication errors between 1985 and 1992 was almost $100,000. Most compensation for medication errors is for larger amounts that are agreed upon in out-of-court settlements [5]. None of the costs cited above include the cost of patient harm or subsequent hospital admissions [3,1].

USP's Efforts to Standardize Medication Errors

History of USP and its Involvement in Medication Errors

The United States Pharmacopeia (USP) is a private, not-for-profit organization whose mission is to promote public health through the creation of standards and authoritative information for the use of medicines and related technologies. USP's authority to set standards was created in the Pure Food and Drug Act and in the Federal Food Drug and Cosmetic Act. These standards include those for quality, strength, purity, packaging, labeling, and storage of drug products. USP creates the official name for drug products and is a member of the United States Adopted Names (USAN) Council that sets the nonproprietary name for drugs in the United States. The USP has been in-

volved in reporting programs for health professionals for nearly 30 years through its USP Practitioners' Reporting Network (USP PRN). These programs support the standards-setting activity by providing practitioner-based experiences about the quality and safe use of medicines in the market place.

Nearly a decade ago, USP agreed to coordinate the medication errors reporting program for the Institute for Safe Medication Practices (ISMP). The Institute was seeking a home for its grass-roots program and believed that the program could have greater impact on the national level. Through the program, USP hoped to learn of those circumstances where the product labeling, packaging, or name of the product caused or contributed to an error. Then USP envisioned setting standards to address the issues and thereby prevent future errors. In 1994, USP signed an agreement to purchase the program from the Institute, established the USP Medication Errors Reporting (MER) Program, and began its long-term commitment to the program as an important part of its standards-setting process. As a condition of the agreement, the ISMP continues to receive copies of reports submitted to this program for its education and advocacy work.

Health care professionals report errors in which they are involved as well as errors that they observe or are party to. Reported information forms a database used by USP to identify problematic situations, heighten practitioners' awareness of these situations, and make appropriate interventions regarding issues with drug products.

USP Medication Errors Reporting (MER) Program

Prevention of medication errors is the primary objective of the USP Medication Errors Reporting Program. It collects and analyzes potential and actual medication errors submitted by health care practitioners. The program affords health care professionals the opportunity to report medication errors and thereby contribute to improving patient safety by sharing their experiences.

To report an error, practitioners may phone USP toll-free at 1-800-23ERROR. A voice-mail system allows a report to be left 24 hr a day, seven days a week. Callers may submit reports anonymously, or speak directly to one of USP health professional staff. Alternatively, a report may be submitted to USP in writing. Report forms (Fig. 1A and B) may be obtained by calling USP directly or via an on-demand faxback system. Practitioners may also employ the form online on the USP website.

Medication error information submitted to USP is entered into a nationally recognized repository for medication error reporting. This database serves to track, monitor, and analyze medication errors from a systems-based perspective. The USP develops educational resources and materials to disseminate best practice solutions and error-avoidance strategies to students and practitioners.

The MER Program is presented in cooperation with the Institute for Safe Medication Practices and is a partner in MEDWATCH, the FDA's medical products reporting program. Although the FDA does not usually assert jurisdiction over practice issues, which are often involved in medication errors, it is concerned with issues relevant to product quality, such as labeling, packaging, and product names, both brand and generic. When medication errors concerning product labeling and packaging are reported through the MER Program, pharmaceutical manufacturers are noti-

USP MEDICATION ERRORS REPORTING PROGRAM
Presented in cooperation with the Institute for Safe Medication Practices
The USP Practitioners' Reporting Network℠ is an FDA MEDWATCH partner

MEDI-CATION ERRORS REPORTING PROGRAM

☐ ACTUAL ERROR   ☐ POTENTIAL ERROR

Please describe the error. Include sequence of events, personnel involved, and work environment (e.g., code situation, change of shift, short staffing, no 24-hr. pharmacy, floor stock). If more space is needed, please attach separate page.

Was the medication administered to or used by the patient? ☐ No ☐ Yes   Date and time of event:

What type of staff or health care practitioner made the initial error?

Describe outcome (e.g., death, type of injury, adverse reaction).

If the medication did not reach the patient, describe the intervention.

Who discovered the error?

When and how was error discovered?

Where did the error occur (e.g., hospital, outpatient or retail pharmacy, nursing home, patient's home)?

Was another practitioner involved in the error? ☐ No ☐ Yes If yes, what type of practitioner?

Was patient counseling provided? ☐ No ☐ Yes If yes, before or after error was discovered?

If a product was involved, please complete the following:

	Product #1	Product #2
Brand name of product involved		
Generic name		
Manufacturer		
Labeler (if different from mfr.)		
Dosage form		
Strength/concentration		
Type and size of container		
NDC number		

If available, please provide relevant patient information (age, gender, diagnosis, etc.) Patient identification not required.

Reports are most useful when relevant materials such as product label, copy of prescription/order, etc. can be reviewed. Can these materials be provided? ☐ No ☐ Yes If yes, please specify.

Suggest any recommendations you have to prevent recurrence of this error or describe policies or procedures you have instituted to prevent future similar errors.

A copy of this report is routinely sent to the Institute for Safe Medication Practices (ISMP), to the manufacturer/labeler, and to the Food and Drug Administration (FDA). **USP may release my identity to: (check boxes that apply)**

☐ ISMP ☐ The manufacturer and/or labeler as listed above ☐ FDA ☐ Other persons requesting a copy of this report ☐ Anonymous to all

Your name and title

Your facility name, address, and ZIP

Telephone number (include area code)

Signature

Date

Return to the attention of:
Diane D. Cousins, R.Ph.
USP PRN
12601 Twinbrook Parkway
Rockville, MD 20852-1790

Call Toll Free: 800-23-ERROR (800-233-7767)
or FAX 301-816-8532
USP home page: http://www.usp.org
Electronic reporting forms are available. Please call for additional information and/or your free diskette.

Date Received by USP: File Access Number:

Additional forms can be found in the USP DI Vol. I and Vol. III.

FIG. 1A.   USP Medication Error Report Forms.

MEDICATION ERRORS REPORTING PROGRAM

Medication Errors Do Occur

Medication errors can occur anywhere, any time along the drug therapy course, from prescribing through transcribing, dispensing, administering, and monitoring. An error can cause confusion, alarm, and frustration for the health care provider and for the patient. And YES, an error can even cause a death or injury to your patient. The causes of errors are many; for example, lack of product knowledge or training; poor communication; ambiguities in product names, directions for use, medical abbreviations, handwriting, or labeling; job stress; poor procedures or techniques; or patient misuse. Along this continuum, any health care professional may be the cause of or contribute to an actual or potential error.

A Safer Environment for Your Patients

It is important to recognize that health care providers learn from medication errors. By sharing your experience through the nationwide USP Medication Errors Reporting (MER) Program you help your colleagues to gain an understanding of why errors occur and how to prevent them. You can also have a positive impact on the quality of patient care and influence drug standards and information. When others are informed about an error, the chance of recurrence may be lessened. Education regarding medication errors assists health care professionals to avoid errors by recognizing the circumstances and causes of actual and potential errors.

Easy Access

Just call 800-233-7767 to reach a USP health care professional, who will take your report and respond to your concerns. Reports may also be submitted in writing or faxed. All reported information is reviewed by USP for possible impact on USP standards and information development. Reports are forwarded to the Food and Drug Administration, the ISMP, and when appropriate, the product manufacturer/labeler. If you wish to remain anonymous to any of these sources, the USP will act as your intermediary in all correspondence. While including your identity is optional, it does allow for appropriate follow-up with you to discuss your observations or provide feedback.

USP: A Partner in MEDWATCH

The USP Practitioners' Reporting Network is a partner in MEDWATCH, the FDA's medical products reporting program. As a partner, USP PRN contributes to the FDA's efforts to protect the public health by helping to identify serious adverse events for the agency. This means that your reported information is shared with the FDA on a daily basis, or immediately if necessary.

USP PRN

The USP PRN™ is designed to collect experiences and observations from health care providers through three separate reporting programs:

- The USP Drug Product Problem Reporting Program
- The USP Medication Errors Reporting Program
- The USP Veterinary Practitioners' Reporting Program

The Institute for Safe Medication Practices, and the American Veterinary Medical Association cooperate in presenting the USP PRN.

Your Input Could Make the Difference!
USP PRN...CALL US WHEN YOU NEED US.

U.S. Pharmacopeia
12601 Twinbrook Parkway
Rockville, MD 20852-1790

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THE USP PRACTITIONERS' REPORTING NETWORK
12601 TWINBROOK PARKWAY
ROCKVILLE MD 20897-5211

FIG. 1B.

fied. They respond frequently and voluntarily make changes in labeling and packaging. Depending upon the nature of the medication error, the MER Program reports provide material for ongoing discussions between FDA and manufacturers, and if warranted, regulatory action. Furthermore, reported information identifies broader issues that may become the basis for instituting industry-wide changes. The reported concerns of practitioners have prompted the USP, FDA, and various drug manufacturers to institute numerous changes and improvements to drug products and have contributed to safer medication prescribing and use.

Facility-Based Reporting May Help Define the Denominator of Errors

Because of its leadership and experience in the area of medication errors, USP began to receive inquiries from hospitals looking for a nationally standardized database that would help the hospital to meet its accreditation requirements and also to compare rates of medication errors among hospitals. Hospitals were willing to share their adverse experiences with other participating hospitals but only if the report could be shared on an anonymous basis. In 1998, USP developed MedMARx^{SM}, an internet-accessible database of medication errors for hospitals. Reports submitted to the system are anonymous so that the participating hospitals will share information openly. The database is structured to become part of the hospital's internal quality improvement program and captures not only errors but the prevention strategies taken by each hospital in response to its errors. This valuable aspect of the national database enables hospitals to practice risk prevention, not just risk management, by learning from the unfortunate experiences of others before it happens to them. It is expected that this database will become a rich repository of information not only for hospitals but for the pharmaceutical industry as well.

The First USP Advisory Panel on Medication Errors

In 1996, USP created an ad hoc Advisory Panel on Medication Errors. Its mission is to provide a practitioner review of reports received through the USP Medication Errors Reporting Program, and to make recommendations to USP standards-setting, information, and reporting programs. The panel chairperson also has the unique opportunity to make broad recommendations through its seat on the National Coordinating Council for Medication Error Reporting and Prevention (NCC MERP). The chairperson of the Advisory Panel on Medication Errors is an exofficio nonvoting member of the NCC MERP.

The USP Advisory Panel on Medication Errors offers a unique and unprecedented opportunity among health care professionals to provide peer review of medication errors occurring nationally and to recommend far-reaching strategies for medication error prevention.

The Panel consists of 12 actively practicing volunteers representing medicine, nursing, and pharmacy. In the year 2000, a Safe Medication Use Committee will be elected to replace the ad hoc panel. For the first time, with the formation of this committee, a formal mechanism will be in place in the standards development process for the purpose of providing direct practitioner input to standards development for the safer use of pharmaceuticals.

A National Coordinating Council is Initiated

After a few short years operating the MER Program, USP realized that the solutions addressing the myriad issues identified through the program were beyond the mission of USP in its standards-setting capacity. Indeed, errors proved to be multidisciplinary in origin and multifactorial in cause. These other practice- and process-related aspects surrounding medication errors needed to be addressed. In 1995, USP spearheaded the formation of the National Coordinating Council for Medication Error Reporting and Prevention (NCC MERP). It promotes the reporting, understanding, and prevention of medication errors relative to professional practice, health care products, procedures, and systems. The goals of the NCC MERP are far-reaching and encompass the full spectrum of health care including:

- Examination and evaluation of the causes of medication errors
- Increased awareness of medication errors and methods of prevention throughout the health care system
- Recommendation of strategies relative to system modifications, practice standards, and guidelines
- Stimulation of development and use of medication error reporting and evaluation systems and stimulation of reporting to a national system for review, analysis, and development of recommendations to reduce and prevent medication errors

The Council is composed of 17 national organizations and agencies, representative of health professions, licensing boards, health care facilities, pharmaceutical manufacturers, regulators, standards setters, and others (Table 1). The membership of the NCC MERP is interdisciplinary and represents crossfunctional groups in the delivery of health care products and services. USP is a founding member and Secretariat to the Council.

TABLE 1 National Coordinating Council for Medication Error Reporting and Prevention, Organizations Represented

American Association of Retired Persons
American Health Care Association
American Hospital Association
American Medical Association
American Nurses Association
American Pharmaceutical Association
American Society of Health-System Pharmacists
American Society for Healthcare Risk Management
Department of Veterans Affairs
Food and Drug Administration
Generic Pharmaceutical Industry Association
Institute for Safe Medication Practices
Joint Commission on Accreditation of Healthcare Organizations
National Association of Boards of Pharmacy
National Council of State Boards of Nursing, Inc.
Pharmaceutical Research and Manufacturers of America
United States Pharmacopeia

Since its formation, the Council has prepared several important work products. Among them are a standardized definition of a ''medication error,'' a series of recommendations designed to reduce errors in the medication use process, and the adoption of a severity index for categorizing the outcome of medication errors. The ''Recommendations to Correct Error-Prone Aspects of Prescription Writing,'' the first set of suggestions issued by the Council, included a list of ''Dangerous Abbreviations,'' that is, abbreviations that are frequently misunderstood or have often been implicated in medication errors, and should never be used (Table 2). In addition to being used in prescription writing, these abbreviations can be found in proprietary product names, on product labels, and in advertising of pharmaceutical manufacturers. The pharmaceutical industry can support this effort by avoiding the use of these abbreviations. The Council also produced an extensive set of recommendations to reduce errors due to labeling and packaging. These recommendations are targeted to regulators and standards setters, health care organizations and professionals, and the industry (see Appendix, Tables 3–6). The practical importance of the Council's recommen-

TABLE 2   These dangerous abbreviations should never be used because they have been misunderstood or misinterpreted.

Abbreviation	Intended Meaning	Common Error
U	Units	Mistaken as a zero or a four (4) resulting in overdose. Also mistaken as ''cc'' (cubic centimeters) when poorly written.
μg	Micrograms	Mistaken for ''mg'' (milligrams) resulting in overdose.
Q.D.	Latin abbreviation for every day	The period after the ''Q'' has sometimes been mistaken for an ''I,'' and the drug has been given ''QID'' (four times daily) rather than daily.
Q.O.D.	Latin abbreviation for every other day	Misinterpreted as ''QD'' (daily) or ''QID'' (four times daily). If the ''O'' is poorly written, it looks like a period or ''I''.
SC or SQ	Subcutaneous	Mistaken as ''SL'' (sublingual) when poorly written.
TIW	Three times a week	Misinterpreted as ''three times a day'' or ''twice a week.''
D/C	Discharge; also discontinue	Patient's medications have been prematurely discontinued when D/C, (intended to mean ''discharge'') was misinterpreted as ''discontinue,'' because it was followed by a list of drugs.
HS	Half strength	Misinterpreted as the Latin abbreviation ''HS'' (hour of sleep).
Cc	Cubic centimeters	Mistaken as ''U'' (units) when poorly written.
AU, AS, AD	Latin abbreviation for both ears; left ear; right ear	Misinterpreted as the Latin abbreviation ''OU'' (both eyes); ''OS'' (left eye); ''OD'' (right eye)

dations lies in their joint endorsement by a diverse group of organizations, ranging from experts in safety issues to manufacturers of drug products to regulators. The importance of achieving consensus through a collaborative effort by these national leading health care and consumer organizations furthers the adoption of nonpunitive, systems-based approaches to reducing medication errors.

Error Avoidance Strategies for the Industry, Designing Error out of Products

The USP medication error reporting programs have uncovered a number of reported error-prone situations that could help industry to consider the problems that should be addressed directly, starting with the selection of a drug name and including the development of labeling, packaging, and dosing devices. Some of those cases are presented below. In many cases the manufacturer corrected design flaws immediately and successfully. These should be considered examples of industry responsiveness, and should also serve to demonstrate that certain designs in labels or packaging should be avoided. Finally, these cases also show how products can be misused because of the systems with which they interface.

Characteristics of Product Errors

It must be kept in mind that the medication use process is a complex continuum that requires the successful interaction of numerous allied health professionals, technology, and the patient. It can be described as a succession of joined, but distinct processes, known as nodes, including:

- Prescribing
- Documenting
- Dispensing
- Administering
- Monitoring

Each node in the medication use process is, in actuality, a discrete system and presents an opportunity for the occurrence and prevention of medication errors.

Medication errors have been defined in many ways, depending on research methodologies, incident reporting systems, risk management, or total quality improvement systems. USP uses the broad definition of medication error from the NCC MERP:

> A medication error is any preventable event that may cause or lead to inappropriate medication use or patient harm, while the medication is in the control of the health care professional, patient, or consumer. Such events may be related to professional practice, health care products, procedures, and systems including: prescribing; order communication; product labeling, packaging, and nomenclature; compounding; dispensing; distribution; administration; education; monitoring; and use.

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Thorough documentation of medication errors provides information about the severity of the error as it relates to the effect on the patient, the product(s) involved, the competence of staff handling the product or processing the order, any contributing factors that may predispose a product to misuse, and the suspected root cause of the error. USP adds certain codes to MER Program data in order to characterize the error as it was reported. These codes include the type of error and the possible cause(s) of error. The following list shows some of the product characteristics that have been recorded over 9 years to have caused or contributed to a medication error.

Product Characteristics from Medication Errors as Reported to USP

- Abbreviations—Includes symbols and acronyms used in drug names as well as directions for use.
- Dosage form confusion—Confusion due to similarity in color, markings, shape, and/or size to another product, or to a different strength of the same product.
- Equipment design confusing and/or inadequate for proper use—Example: Administration pump makes it difficult to set precise fluid rate or is confusing to use.
- Label (manufacturer's) design—Physical label design, e.g., contrast of label information and background, letter font, symbol(s), or logo, causes information to be overlooked or difficult to read.
- Measuring device inaccurate and/or inappropriate—Scale of graduation markings on medical device (e.g., syringe, dropper) is inaccurate or inappropriate for administering the correct dose.
- Names, a brand name/generic name of different products look alike—self-explanatory.
- Names, a brand name/generic name of different products sound alike—self-explanatory.
- Names, brand names look alike—Brand names of different products look alike.
- Names, brand names sound alike—Brand names of different products sound alike.
- Names, generic names look alike—Generic names of different products look alike.
- Names, generic names sound alike—Generic names of different products sound alike.
- Non-metric units of measurement (apothecary)—Use of apothecary units of measurement results in misinterpretation (e.g.: ''cc'' (cubic centimeter) written and misinterpreted as ''u'' (units)).
- Packaging/container design—The design of the package, bag, syringe, etc., caused or contributed to the error.
- Similar packaging/labeling—Packaging/labeling of two or more different products look similar causing one product to be mistaken for the other.

The pharmaceutical industry should pay close attention to these factors in the earliest stages of product development including clinical stages. Several years ago the drug, zidovudine (antiviral), was referred to as AZT in clinical trials. The abbreviation was also used in the marketing of the product. However, AZT has been a common abbreviation for azathioprine (immunosuppressant) and several errors were made. The Institute of Medicine Report [1] suggests that the FDA develop and enforce standards for the design of drug packaging and labeling that will maximize safe product use. The above list could serve as a starting point of areas to examine.

Case 051133: Poor Label Design; Confusing or Incomplete Label Information; Packaging

A pediatric patient was presented to the emergency room (ER) experiencing seizures for which 150-mg Cerebyx® (fosphenytoin anticonvulsant) IV was ordered. The pharmacy technician took the call for Cerebyx and delivered three 10-mL vials of Cerebyx 50-mg PE (phenytoin sodium equivalents) per mL to the ER as a floor stock transaction. A nurse misread the 50-mg PE/mL on the 10-mL container label, making the assumption that the entire vial contained 50 mg PE. The contents of all three vials were prepared for administration. Instead of 150 mg PE, the patient was administered ten times the intended dose, or 1,500 mg PE. The patient later died. The ER staff only discovered the error after the patient's blood phenytoin levels were returned from the laboratory.

Discussion

Serious medication errors, including some leading to death, have resulted from the interpretation of the Cerebyx® product labeling. The terminology on the label, which previously indicated the concentration as being 50 mg of phenytoin equivalents (PE) per milliliter, was misinterpreted as the total number of PEs per vial. Furthermore, health professionals were reportedly confused by the expression ''phenytoin equivalents,'' a prodrug concept introduced for this product. As a result, massive fosphenytoin overdoses were mistakenly administered.

Fosphenytoin is a prodrug, a compound that undergoes chemical conversion in the body to become the therapeutically active compound phenytoin. The Cerebyx dosage will continue to be expressed in phenytoin equivalents. This terminology was adopted in an effort to simplify therapeutic conversions between phenytoin sodium and fosphenytoin sodium (i.e., 500 mg of phenytoin sodium injection is equal to 500 mg PE of fosphenytoin sodium injection). The manufacturer pointed out that by using phenytoin sodium equivalents (PE), prescribers will not have to make dosing adjustments when converting from phenytoin sodium to Cerebyx or vice versa. To reduce the risk of incorrect dosing, all health care providers should prescribe and dispense Cerebyx in PEs.

Parke-Davis has taken action to prevent future errors. The labeling for Cerebyx vials and packaging has been changed to further clarify the total amount of drug for both the 2-mL and the 10-mL vials of the product. Although the new labeling clarifies the total quantity of drug contained in each vial, the concentration of Cerebyx remains 50mg PE/mL.

Case 51832, 51845: Line Extension Creates Confusion

Muro Pharmaceutical, Inc. introduced a new line extension, Prelone® Syrup 5 mg/ 5 mL (Prednisolone steroid), to the existing product, Prelone® 15 mg/5 mL. Because only one strength of Prelone had been available for many years, it was a general practice to prescribe Prelone Syrup without specifying the strength.

Discussion

Manufacturers have to consider the transition time needed by practitioners to become familiar with the introduction of a new strength. This type of confusion is also seen when ''long-acting'' versions of a product are added to a line, thereby changing the dosing regime to less frequent intervals. The product name is prescribed without the ''long-acting'' designation and a medication error results. In similar cases, suffixes cause errors when the product line extension adds a second strength and places a suffix such as XL after the product name to indicate long-acting release. Prescribers omit the suffix out of habit (for the initial formulation) and the patient receives the shorter acting medication at the long-acting interval.

Muro Pharmaceutical, Inc. anticipated that a new concentration of an established product could indeed cause confusion and developed new packaging for both concentrations. Muro also sent mailers that announced the availability of two concentrations of Prelone to 32,000 pediatricians and 65,000 pharmacies, wholesalers, HMOs, and Preferred Provider Organization (PPOs).

Cases 50446, 50499, 50519, 50534, 50736, 50820, 50918: Poor Contrast Compromises Readability

The unit-dose packaging of the quinolone, Levaquin (Levofloxacin antibacterial), is silver foil with black letters. The dose is reverse shaded. A reporter noted that the packages have to be held at just the right angle to be able to read the label. It was reported to be especially difficult to differentiate between the 250 and 500 mg strengths because the numbers were so difficult to see.

Discussion

Ortho-McNeil is redesigning the packaging for Levaquin to improve readability. Manufacturers should be aware that practitioners are often operating in poorly lit areas. This makes double-checking the label to prevent errors even more difficult. In some situations, there may not be adequate time to read the label carefully the first time without having to look again because of poor contrast. The use of embossed printing on plastic containers has also been reported to be difficult to read because there is no contrast and no paper label to distinguish the products or identify them properly.

Total Volume is the Key

USP received eight reports from pharmacists expressed concern about the labeling on the Bentyl® (dicyclomine hydrochloride antispasmodic) 2-mL ampule. Practitioners reported that the label indicates only the drug concentration, 10 mg/mL, not the total volume, and some regarded the label information as incomplete. In one report, a 20-mg dose was ordered, but two ampules were administered (a total of 4 mL instead of 2 mL), leading to an overdose. This happened because the practitioner mistook 10 mg as the total contents of one vial.

Discussion

Reports received by Hoechst Marion Roussel have prompted the company to return to the old-style labeling that includes the total volume data. According to the firm, this change will be implemented as quickly as possible.

Reports received at USP have identified the need for the following information to appear on the vial or ampule:

- The total volume,
- The strength per milligram,
- The total strength per total volume.

Although some manufacturers regard it as unreasonable to include all this information on the label (especially on containers of 1- and 2-mL size), it would greatly minimize the chance of misinterpretation of the contents or strength.

Cases 040925, 050419, 041485: Wholesaler Errors due to Label Similarity

A pharmacist reported that 1- and 10-g vials of Marsam cefazolin sodium appear to be identical in shape and have the same color flip-top closures. The pharmacy ordered the 1-g product from the wholesaler. However, the wholesaler sent 10-g bulk vials of cefazolin sodium along with stickers for the 1-g vial. The pharmacy, which does not normally stock the 10-g vials, interspersed the 10-g vials with the 1-g vials in their stock. Several vials were reconstituted in error. Fortunately no patient received the wrong dose.

In another reported incident, a pharmacist ordered the 10-g vials of cefazolin sodium but received the 1-g vials in error. Intending to reconstitute and divide the 10-g vials into 1-g doses, a technician inadvertently reconstituted the 1-g vials and proceeded to divide the total solution of each vial into ten 100-mg doses. Some of the prepared 100-mg doses of cefazolin sodium were administered to patients instead of their scheduled 1-g doses, but no adverse effects were reported. The pharmacist thought that the error occurred partly because the vials are identical in size and have similar labels.

Discussion

The pharmacist suggested that the color of the flip-top of the 10-g vial be changed. The company replied that although it is common practice to use color-coded labels and flip-tops to differentiate product lines or strengths, it tries to stress the individual products in other ways, for example, by varying the style and format of the label. Marsam revised the labeling of the cefazolin sodium 10-g bulk vial in order to distinguish it from the 1-g single-dose vial. The newly revised labeling includes:

- The word ''BULK'' was added to two places on the side panel.
- Screened color was added to the box surrounding the product name.
- ''10 grams'' is now printed in color.
- The product name and strength on the back of the label is printed in color.

The use of color-differentiation is favored whereas the use of color coding is controversial because of the limited number of colors, the prevalence of color blindness in the general population, and inappropriate reliance on color in lieu of reading the label.

Case 042031: Packaged Measuring Devices

An order was written for 30 mg Cyclosporine (immunosuppressant) oral solution to be administered to a pediatric patient. However, for several days, the nurse administered 300 mg believing that the syringe was calibrated in mg not mL. The oral solution is available as 100 mg/mL. As the pharmacist reviewed the error, he noted that the syringes accompanying the medication were never designed for pediatrics. It is not possible to calculate any dose less than 50 mg. It is understandable how the nurse assumed that the ''3'' mark was for 30 mg since it is positioned between ''2,5'' and ''3,5'' (which are European style for the decimals 2.5 and 3.5). To harmonize products in the global market, the manufacturer chose to follow European convention for expressing numbers which uses commas and decimals in the reverse manner as in the United States.

Discussion

This error is unusual because it involves a global trade issue. Manufacturers would prefer to harmonize products used in the United States with those available in other markets. If the dose preparation had been centralized in the pharmacy, this error might have been avoided.

Other medication errors involving medication-dispensing devices reported to USP have included the interchange of devices supplied with specific products. Each device packaged with a medication is calibrated for that medication based on the viscosity and concentration of the specific liquid it delivers. These devices are not calibrated in any standardized way. (Some measure in milligrams (mg), others in milliliters (mL), and others in cubic centimeters (cc). Still others have calibrations for the strength per drop or per teaspoonful. Policies should be in place restricting the dispensing or use of droppers or calibrated cups provided with specific medications to those medications. Manufacturers who supply droppers with a stock bottle, should supply enough droppers to enable breakdown of the liquid to usable volumes. For example, one company supplied only one dropper with its morphine sulfate 8-oz. bottle even though the more common quantities dispensed are 2 and 4 oz. Alternatively, manufacturers should package medication in the volume expected to be dispensed per medication order.

Case 52348: Abbreviations

USP received a medication error report involving the products Neumega® (oprelvekin) and Proleukin® (aldesleukin). Oprelvekin, a recombinant human interleukin-11 product used to stimulate platelet production in selected patients undergoing chemotherapy, is sometimes abbreviated as IL-11. Aldesleukin, a recombinant human interleukin-2 derivative indicated in designated patient populations for the treatment of metastatic renal-cell carcinoma, is sometimes abbreviated as IL-2.

In the reported error, a physician used the abbreviation ''IL-11'' when ordering oprelvekin for a patient. Unfortunately, the order was misinterpreted to be interleukin-2 (i.e., the number eleven was perceived to be the Roman numeral two). Five or more health care professionals, including pharmacists and nurses, mistook the order to be aldesleukin. The error was not detected for four days, until it was noted that the inventory of aldesleukin was nearly depleted.

Discussion

Practitioners should be especially vigilant when orders for these interleukin products are received. If abbreviations have been used in an order, the order should be clarified to ensure that patients receive the intended medication. This medication error exemplifies the value of implementing prescribing guidelines, such as the recommendations adopted by the National Coordinating Council for Medication Error Reporting and Prevention. Specifically, when writing an order, prescribers should avoid the use of abbreviations, especially those for drug names. Abbreviations for drugs names should not be accepted. Reference materials sometimes refer to these abbreviations as synomyns for the approved drug names. Manufacturers should discourage use of abbreviations because of the potential to cause medication errors.

The following cases demonstrate how products can be misused because of the systems with which they interface.

Case 052718: Electronic Drug Reference Products

A pharmacist asked a clinical pharmacist for information about Cartia®. Because an electronic drug reference listed the active ingredient as aspirin, the pharmacist was prepared to substitute an aspirin product for Cartia®. The clinical pharmacist recognized the new product as Cartia XT® (Diltiazem, a calcium channel blocker) and prevented the error.

Discussion

The manufacturer of Cartia XT®, shared the concern of the reporting pharmacist and contacted the electronic reference source to investigate this matter. The publisher of the electronic reference stated that a salicylate product called Cartia® is manufactured by Lusofarmaco in Portugal and Smithkline Beecham in Australia. Both Cartia® products were verified as active current products by the publisher who stated that there was no way of excluding the Cartia® marketed abroad because it is an active product imported from a master database that contains many foreign drug products. The electronic reference is published in quarterly volumes. Cartia XT® was entered into the database which is currently being shipped to customers, who will now be able to choose between Cartia® and Cartia XT®. This should lessen the confusion between the products.

As with many hard copy drug reference books, electronic drug references have a lag time between production and the customer's receipt of the databases. Unfortunately, this may result in inaccurate and/or outdated information and omission of current drug information. This may cause confusion and lead to misinterpretation of

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drug information by the users. The health care providers should realize that reference sources, including electronic reference databases, are not infallible and that they are only as good as their contents of updated information. As a safeguard, the health care providers should make it practice to check at least two different drug information sources for confirmation.

Case 52125: Computers and Processing Software

A pharmacist entered an order for Diflucan (antifungal) for a patient who had been receiving Propulsid (gastrointestinal emptying adjunct), which would result in a documented drug interaction. The pharmacy computer system had multiple-drug interaction screens. The pharmacist passed these screens by pressing ''next screen'' without any resistance by the system for this dangerous interaction. The patient received two doses of Diflucan. On the second day the patient ''coded'' and later died.

Case 51088

The patient died after 12 mg IV Colchicine (antigout) was given instead of 2 mg IV, ''until diarrhea,'' as ordered. The physician was contacted by the pharmacist but insisted on the dose. There was no computer warning about the dangerous dose and the nurses had no idea that they were giving an overdose.

Case 50908

Amoxicillin was prescribed and dispensed to a patient with a Penicillin allergy. The front of the chart was not marked for an allergy and the problem list showing the allergy was covered with a misfiled document. The pharmacy software program does not screen for allergies and the pharmacy profile was not marked with any allergies.

Digoxin Pediatric Elixir

Because the computer in one facility was limited to entering doses in milligrams, a neonate's 20 microgram dose of digoxin first had to be converted to the equivalent milligram dose before it could be entered into the computer. A pharmacist incorrectly converted the 20 mcg dose and then entered it into the computer as 0.2 mg (instead of 0.02 mg). Consequently, the neonate received four 200 microgram doses of digoxin instead of the 20 microgram dose as ordered. The neonate experienced digoxin toxicity before the error was discovered.

Teaspoonful vs. mL

By default, a certain software program printed ''teaspoonful'' for any syrup preparation when a numerical figure was not followed by a specific measure, such as mL, for the dose. A prescription for ½ mL albuterol syrup every 6 hr for a 9-week-old infant was presented to the pharmacy, and the pharmacist entered ''1/2'' into the

computer but did not enter mL. Therefore, by default, the label printed ½ teaspoonful every 6 hr if needed for wheezing. The child was administered the overdose and consequently admitted to the hospital emergency room for observation. Fortunately, the child was released with no permanent damage.

One vs. One-half

New computer software was used to enter the directions for a cough medicine with a dose as ''1–2 teaspoonsful . . .''. Instead, the new software printed a label as ½ teaspoonful. The pharmacist did not check the label against the prescription and dispensed the product with the incorrect directions on the label.

Discussion

Computerized systems have become important tools in today's pharmacies. Computers have made prescription processing faster, easier, and more efficient. Computers have also provided that patients' information be readily available. However, as reliance on computers systems grow, care should be taken not to become totally dependent on these systems as the sole check in preventing medication errors.

Similar Drug Names

Confusion caused by similarity of drug names when written or spoken, accounts for approximately one-quarter of all reports to the USP MER Program. Such confusion is compounded by illegible handwriting, incomplete knowledge of drug names, newly available products, similar packaging or labeling, and incorrect selection of a similar name from a computerized product list. USP has produced a list of more than 1,000 drug name pairs that have been reported as confusing. Manufacturers should refer to this list when selecting drug names. Recently USP voted to change Amrinone to Inamrinone because it was being confused with Amiodarone and caused fatal errors. This type of change is expensive for the industry and can be avoided by considering the potential for similarity first. Technologies and testing protocols, including voice and handwriting recognition are available to help determine if a drug's name looks or sounds like another.

Summary

The ability to predict error and thus avoid it is the focus of the science of human factors engineering. The adaptation of this science to the medication use process can help to predict the chances that a medication error will occur. Pharmaceutical manufacturers should design products including their names, labeling, and packaging so that errors can be avoided and systems and health care delivery will be safer.

Appendix

TABLE 3   Recommendations on Labeling and Packaging to Industry Manufacturers of Pharmaceuticals and Devices (Adopted May 12, 1997)

The Council recommends that industry not use any printing on the cap and ferrule of injectables except to convey warnings.

The Council encourages industry to employ failure mode and effects analysis in its design of devices, and the packaging and labeling of medications and related devices.

The Council encourages industry to employ machine-readable coding (e.g. bar coding) in its labeling of drug products. The Council recognizes the importance of standardization of these codes for this use.

The Council encourages printing the drug name (brand and generic) and the strength on both sides of injectables, and IV bags, containers, and overwraps. For large volume parenterals and IV piggybacks (minibags), the name of the drug should be readable in both the upright and inverted positions.

The Council encourages industry to support the development of continuing education programs focusing on proper preparation and administration of its products.

The Council encourages industry to use innovative labeling to aid practitioners in distinguishing between products with very similar names, for example, the use of tall letters such as VinBLAStine and VinCRIStine.

The Council encourages industry to avoid printing company logos and company names that are larger than the type size of the drug name.

The Council encourages collaboration among industry, regulators, standards-setters, health care professionals, and patients to facilitate design of packaging and labeling to help minimize errors.

TABLE 4 Recommendations on Labeling and Packaging to Regulators and Standards Setters (Adopted May 12, 1997)

The Council recommends that FDA restrict the use of any printing on the cap and ferrule of injectables except to convey warnings.

The Council recommends the use of innovative labeling to aid practitioners in distinguishing between products with very similar names, for example, the use of tall letters such as VinBLAStine and VinCRIStine.

The Council recommends that FDA discourage industry from printing company logos and company names that are larger than the type size of the drug name.

The Council supports the recommendations of the USP-FDA Advisory Panel on Simplification of Injection Labeling. Furthermore, the Council encourages USP/FDA to consider expansion of the concepts of simplification to apply to:

   package inserts; and

   labeling of other pharmaceutical dosage forms.

The Council encourages further development of FDA's error prevention analysis efforts to provide consistent regulatory review of product labeling and packaging relative to the error-prone aspects of their design.

The Council encourages collaboration among regulators, standards-setters, industry, health care professionals, and patients to facilitate design of packaging and labeling to help minimize errors.

The Council encourages USP/FDA to examine feasibility and advisability of use of tactile cues in container design and on critical drugs. Such cues may be in the design of the container or embedded in the label.

The Council encourages the printing of the drug name (brand and generic) and the strength on both sides of injectables and IV bags, containers, and overwraps. For large volume parenterals and IV piggybacks (minibags), the name of the drug should be readable in both the upright and inverted positions.

TABLE 5   Recommendations to Health Care Professionals to Reduce Errors Due to Labeling and Packaging of Drug Products and Related Devices (Adopted March 30, 1998)

The Council encourages health care professionals to routinely educate patients and caregivers to enhance understanding and proper use of their medications and related devices. Furthermore, the Council encourages health care professionals to regularly participate in error prevention training programs and, when medication errors do occur, to actively participate in the investigation.

In addition, the Council makes the following recommendations to health care professionals to reduce errors due to labeling and packaging of drug products and related devices:

1. The Council encourages health care professionals to use only properly labeled and stored drug products and to read labels carefully (at least three times—before, during, and after use).

2. The Council encourages collaboration among health care professionals, health care organizations, patients, industry, standard-setters, and regulators to facilitate design of packaging and labeling to help minimize errors.

3. The Council encourages health care professionals to take an active role in reviewing and commenting on proposed regulations and standards that relate to labeling and packaging (i.e. Federal Register, and Pharmacopeial Forum).

4. The Council encourages health care professionals to report actual and potential medication errors to national (e.g., FDA MedWatch Program and/or the USP Practitioners' Reporting Network), internal, and local reporting programs.

5. The Council encourages health care professionals to share error-related experiences, case studies, etc., with their colleagues through newsletters, journals, bulletin boards, and the Internet.

TABLE 6   Recommendations to Health Care Organizations to Reduce Errors Due to Labeling and Packaging of Drug Products and Related Devices (Adopted March 30, 1998)

The Council recommends the establishment of a systems approach to reporting, understanding, and prevention of medication errors in health care organizations. The organization's leaders should foster a culture and systems that include the following key elements:

a. an environment that is conducive to medication error reporting through the FDA MedWatch Program and/or the USP Practitioners' Reporting Network;

b. an environment which focuses on improvement of the medication use process;

c. mechanisms for internal reporting of actual and potential errors including strategies that encourage reporting;

d. systematic approaches within the health care organization to identify and evaluate actual and potential causes of errors including Failure Mode and Effects Analysis (FMEA) and root cause analysis;

e. processes for taking appropriate action to prevent future errors through improving both systems and individual performance.

In addition, the Council makes the following recommendations to health care organizations to reduce errors due to labeling and packaging of drug products and related devices:

1. The Council recommends that health care organizations employ machine readable coding (e.g., bar coding) in the management of the medication use process.

2. The Council recommends reevaluation of existing storage systems for pharmaceuticals by health care organizations and establishment of mechanisms to insure appropriate storage and location throughout the organization from bulk delivery to point of use. The following issues should be considered when applicable: storage and location that will help distinguish similar products from one another; storage and location of certain drugs, (e.g., concentrates, paralyzing agents) that have a high risk potential; scope, access, and accountability for floor stock medications; safety and accountability of access to pharmaceuticals in the absence of a pharmacist (e.g., floor stock, eliminate access to pharmacy after hours); labeling and packaging of patient-supplied medications.

3. The Council recommends the development of policies and procedures for repackaging of medications that will clarify labeling to help avoid errors.

4. The Council encourages collaboration among health care organizations, health care professionals, patients, industry, standard-setters, and regulators to facilitate design of packaging and labeling to help minimize errors.

5. The Council recommends that health care organizations develop and implement (or provide access to) education and training programs for health care professionals, technical support personnel, patients, and caregivers that address methods for reducing and preventing medication errors.

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DIANE D. COUSINS

Mucoadhesive Hydrogels in Drug Delivery

Introduction

This article focuses on defining the principles of bioadhesive delivery systems based on hydrogels to biological surfaces that are covered by mucus. An overview of the last decade's discoveries on mucoadhesion and applications of mucoadhesive hydrogels as drug carriers is given. Techniques that are frequently used to study the adhesion forces and physicochemical interactions between hydrogel, mucus, and the underlying mucosa are reviewed. Typical examples of applications of mucoadhesive hydrogels to mucosal routes of delivery are given. Finally, the perspectives of application of these polymers in drug delivery are discussed.

Noninvasive drug delivery may require the administration of the drug delivery system (DDS) at an epithelium as a suitable site of absorption of the active compound. Such regions are usually called mucosae. In the human body several mucosal sites can be identified, the one mostly used for administration and absorption of therapeutics being the gastrointestinal route. In order to increase the residence time at these absorption sites, a so-called mucoadhesive delivery system has to be used. Generally, these systems consist of one or more types of hydrogels.

By definition, mucoadhesive hydrogels are a class of polymeric biomaterials that exhibit the basic characteristic of an hydrogel to swell by absorbing water and interacting by means of adhesion with the mucus that covers epithelia.

Bioadhesion has been defined as the attachment of synthetic or biological macromolecules to a biological tissue [1]. The term mucoadhesion refers to the special case of bioadhesion where the biological tissue is an epithelium covered by mucus. Mucus is a thin blanket covering all epithelia that are in contact with the external environment in the gastrointestinal, respiratory, and urogenital tracts. The function of mucus is mainly the protection and lubrication of the underlying epithelium, but it may have additional functions dependent on the type of the covered epithelia. In each case of these mucosal routes, mucus characteristics (i.e., thickness) and functions are different. By this definition, the mucosal routes for drug delivery are:

- The buccal/oral route,
- The nasal route,
- The ocular route,
- The vaginal route, and
- The gastrointestinal route.

The concept of mucoadhesion in drug delivery was introduced in the field of controlled-release drug delivery systems in the early 1980s [2,3]. Thereafter, several researchers have focused on the investigations of the interfacial phenomena of mucoadhesive hydrogels (and of other type mucoadhesive compounds) with the mucus. Several techniques of studying these interactions were evaluated both in vitro and

in vivo. These techniques have been recently reviewed by Harding et al. [4] and are given in Table 1.

A mucoadhesive hydrogel used as a drug delivery system should

1. Be loaded substantially by the active compound;
2. Not interact physicochemically with the active compound or create a hostile artificial environment that would lead to inactivation and degradation of the active compound;
3. Swell in the aqueous biological environment of the delivery–absorption site;
4. Interact with mucus or its components for adequate adhesion;
5. Allow, when swelled, controlled release of the active compound;
6. Be biocompatible (biomaterial) with the underlying epithelia by means of complete absence of cytotoxicity, ciliotoxicity, or other type of irreversible alterations of the cell membrane components;
7. Have the appropriate molecular size and conformation in order to escape systemic absorption from the administration site; and
8. Be excreted unaltered or biologically degraded to inactive, non-toxic oligomers/monomers that will be further subject of physical clearance.

Additionally, a mucoadhesive delivery system designed for controlled release of active compounds should be localized at specific sites of administration and absorption,

TABLE 1　Methods of Studying Mucoadhesion[a]

Method	Comment
Direct assays	
Tensiometry	Force required to dislodge two surfaces, one coated with mucus, the other solid dosage form consisting of mucoadhesive hydrogel
Flow through	Flow rate dV/dt required to dislodge two surfaces; useful for microparticulate dosage forms
Colloidal gold staining	Measures the ''adhesion number''
In vivo techniques	Endoscopy, gamma scintigraphy
Molecular mucin-based assays	
Viscometry and rheology	Intrinsic viscosity [η] can be related to complex size via MHKS,[b] α coefficient
Dynamic light scattering	Diffusion coefficient, D, can be related to complex size via MHKS ε coefficient
Turbidity, light scattering	SEC MALLS[c] particularly useful for determining MW of mucin, turbidity, semi-quantitative indicator
Analytical ultracentrifugation	Change in MW (sedimentation equilibrium), sedimentation coefficient ratio of complex to mucin
Surface plasmon resonance imaging methods	Needs mobile and immobile phase, atomic force microscopy (conventional and gold labelled), scanning tunneling microscopy

[a] Adapted from Ref. 4.
[b] Mark-Houwink-Kuhn-Sakurada.
[c] Size exclusion chromatography multiangle laser light scattering.

and prolong the residence time of the active compound at the site of administration to permit, if possible for once daily dosing.

The mucoadhesive delivery system, designed for the administration of macromolecular therapeutics like peptide or protein drugs, should have permeation-enhancing properties by means of alteration of the permeability properties of the underlying epithelium, and protect the peptide drug from degradation by inhibiting the proteolytic enzymes usually present at the site of administration or by stabilizing the intrinsic environment of the delivery system by sustaining the suitable pH.

Solid dosage forms based on mucoadhesive polymers are used mainly for buccal delivery of drugs, whereas micro- or nanoparticulate formulations are preferred for the delivery of therapeutics in the nasal and intestinal tract [5].

During the last decade, research was particularly focused on the delivery of mucoadhesive dosage forms in the gastrointestinal tract, which is the most favorable route of delivery with regard to patient compliance and ease of application. For this reason microparticulate formulations, consisting of mucoadhesive hydrogels, were designed and evaluated by different techniques [6,7]. However, solid dosage forms are more suitable for smaller cavities in the human body, like the oral cavity, either for systemic absorption of compounds or for local treatment of inflammatory diseases. In this case, monolithic devices (tablets) made from mucoadhesive hydrogels were evaluated [8]. Ocular and vaginal applications (for local or systemic absorption) of mucoadhesive hydrogels were also investigated.

The mucoadhesive properties of several classes of hydrogels have been identified, and two types of polymers have attracted special attention. Polyacrylates and their cross-linked modifications represent the anionic type, chitosan and its derivatives the cationic group. In addition, both types of polymers show a number of interesting characteristics beneficial for the administration of a wide range of therapeutics.

Specific type of mucoadhesive compounds, like lectins, have been evaluated to solve the difficulties presented by conventional mucoadhesive hydrogel systems, for instance in the gastrointestinal route. Since these compounds do not belong to the class of hydrogels, they are not extensively discussed here.

Mucus

In higher organisms epithelia are covered by a protective gel layer defined as mucus. By weight, mucus consists mainly of water (95–99.5%) in which the mucous glycoprotein mucin (0.5–5%) is dispersed. Mucins are the major components responsible for the gel-like structure of the mucus. They possess a linear protein heavily glycosylated by oligosaccharide side chains. This protein core consists of a single polypeptide chain. One in every three residues is L-serine or L-threonine, in which the O-3 atoms provide the sites for glycosidic linkage. A mucus glycoprotein is composed typically of about 80% of carbohydrates, which for humans are restricted to five monosaccharides:

- L-fucose,
- *N*-acetylglucosamine,
- D-galactose,

- *N*-acetylgalactosamine, and
- Sialic acid.

The sialic acid residues are usually in a terminal position on the carbohydrate chain, whereas the ester sulfate residues are in a more internal position; both contribute to give the molecule a net negative charge. The molecular weights of mucus glycoproteins range from 0.5 to 16×10^6 Da [4].

Mucins can be divided into two classes, membrane bound and secretory forms. Membrane-bound mucins are attached to cell surfaces and may affect immune responses or inflammation. It has been suggested that the high expression of cell-surface mucins, such as sialomucin MUC1, may result in cell–cell and cell–matrix interactions [10]. Secretory mucins eminate from mucosal absorptive cells and specialized goblet cells. They constitute the major component of mucous gels in the gastrointestinal, ocular, respiratory, and urogenital epithelia. This type of mucus gel layer is functioning mainly as a physical barrier and lubricant. Important constituents in the mucus include growth factors and trefoil peptides, both secreted by the specialized cells located next to ulcerated mucosal tissue. This appears to be an adaptive phenomenon, important in maintaining the barrier function of the mucosal tissue, enhancing cell migration, and healing after injury. Other substance present in mucus include secretory immunoglobulin A (IgA), lysozyme, lactoferin, α_1-antitrypsin, salts, and *N*-glycosylated glycoproteins [10].

At present, nine different human epithelial mucin genes have been identified, each of which contains distinct sequences repeated in tandem that encode (apo)mucin core polypeptides (MUC) [10].

Leung and Robinson [11] defined four characteristics of the mucus layer related to mucoadhesion:

1. Mucus is a network of linear, flexible, and random-coil macromolecules.
2. Mucin is negatively charged due to sialic acid and sulfate residues.
3. Mucus is a cross-linked network connected by disulfide bonds between mucin molecules.
4. Mucin is heavily hydrated.

Several techniques have been used to estimate the rate of mucus secretion, but their accuracy seems to be doubtful. Nevertheless, it has been concluded that a slow baseline secretion of mucus is maintained by exocytosis from goblet cells in the gastrointestinal tract which appears to be under cholinergic control [12]. Rubinstein and Tirosh used carbachol (cholinergic agonist) at different doses to increase the mucus thickness in different parts of the gastrointestinal tract [13].

Attempts were also made to estimate the rate of turnover of the mucus gel. Lehr et al. [14] measured the amount of mucus produced per time unit, using an in situ perfused intestinal loop model in the rat. They found that this turnover time is varying between 0.8 and 4.5 hr.

Mechanism and Theories of Mucoadhesion

The mechanistic processes involved in mucoadhesion between hydrogels and mucosa can be described in three steps:

1. Wetting and swelling of the polymer to allow for intimate contact with the biological tissue,
2. Interpenetration of the bioadhesive polymer chains and entanglement of polymer and mucin chains, and
3. Formation of weak chemical bonds between entangled chains (Fig. 1).

Several theories have been proposed to explain the biomucoadhesive phenomena [1,15–17].

The electronic theory is based on the assumption that the mucoadhesive hydrogel and the target biological tissue have different electronic structures. When two materials come into contact with each other, electron transfer occurs, causing the formation of a double layer of electrical charge at the bioadhesive-biological interface. The bioadhesive force is believed to be due to attractive forces across this electrical double layer.

The adsorption theory states that the bioadhesive bond formed between an adhesive substrate and tissue or mucosae is due to van der Waals interactions, hydrogen bonds, and related forces. Alternatively, when mucus or saliva are interacting with a solid dosage form, the molecules of the liquid are adsorbed on the solid surface. This is an exothermic process. The free energy of adsorption is given by Eq. (1).

$$\Delta G_{AD} = \Delta H_{AD} - T \, \Delta S_{AD} \tag{1}$$

where ΔH_{AD} and ΔS_{AD} are the enthalpy and entropy changes, respectively. When adsorption takes place spontaneously, ΔG_{AD} is negative.

The contact angle θ of a liquid on a solid is the reflection of its wetting power. If $\theta = 0$, the liquid spreads freely on the solid surface and wets it. The relationship between the contact angle of a liquid on a solid and the surface tensions in the presence of saturated vapor of the liquid, is given by the Young's equation, Eq. (2).

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} \cos\theta \tag{2}$$

<div style="text-align:center">Contact   Interpenetration   Interaction</div>
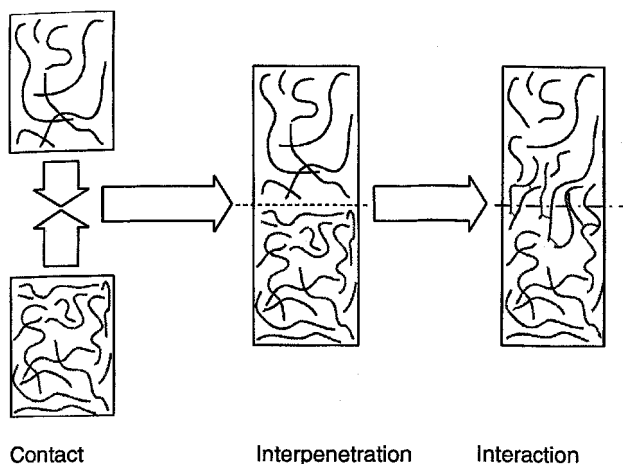
FIG. 1. Three stages in the interaction between a mucoadhesive polymer and mucin glycoprotein according to the interpenetration theory.

where γ_{sv} = solid/vapor surface tension, γ_{sl} = solid/liquid surface tension, and γ_{lv} = liquid/vapor surface tension.

Another way to relate the interfacial tension γ_{sl} to the individual surface tensions of a liquid and solid is given by the Good equation, Eq. (3).

$$\gamma_{sl} = \gamma_{sv} + \gamma_{lv} - 2\phi(\gamma_{sv}\gamma_{lv})^{1/2} \tag{3}$$

Equation (3) represents the reduction in interfacial tension resulting from molecular attraction between liquid and solid. The term ϕ is defined by Eq. (4),

$$\phi = W_a/(W_{cl}W_{cs})^{1/2} \tag{4}$$

where W_{cl} and W_{cs} are the work of cohesion of the liquid and the solid, respectively, and W_a is the work of adhesion.

The diffusion theory states that interpenetration and entanglement of polymer chains are additionally responsible for bioadhesion. The intimate contact of the two substrates is essential for diffusion to occur, that is, the driving force for the interdiffusion is the concentration gradient across the interface. The penetration of polymer chains into the mucus network, and vice versa, is dependent on concentration gradients and diffusion coefficients. It is believed that for an effective adhesion bond the interpenetration of the polymer chain should be in the range of 0.2–0.5 μm. It is possible to estimate the penetration depth (l) by Eq. (5),

$$l = (tD_b)^{1/2} \tag{5}$$

where t is the time of contact and D_b is the diffusion coefficient of the bioadhesive material in the mucus.

The fracture theory is the most widely applied theory in studying mucoadhesion mechanisms. It accounts for the forces required to separate two surfaces after adhesion. The maximum tensile stress (σ) produced during detachment can be determined by Eq. (6) by dividing the maximum force of detachment F_m by the total surface area (A_0) involved in the adhesive interaction:

$$\sigma = F_m/A_0 \tag{6}$$

According to Duchêne et al. [17], when tensiometry is used to measure the maximum detachment force as a function of the displacement of the upper support (function of the joint elongation), the work of bioadhesion can be defined as in Eq. (7).

$$W_b = F \times l \tag{7}$$

Additionally, the fracture energy for a zero extension rate can be defined as the bioadhesion work to the initial surface between the bioadhesive material (in a form of a tablet or disk) and the biological support of a surface A_0, which allows for calculation of the fracture energy (ε) using Eq. (8).

$$\varepsilon = W_b/A_0 \tag{8}$$

Thermodynamically, the fracture energy is the sum of the reversible work (W_r, representing the reversible molecular interactions at the interface) and irreversible work (W_i, representing the irreversible deformation of the interfacial joint); both W_r, and W_i are expressed per unit area of the fracture energy by Eq. (9).

$$\varepsilon = W_r + W_i \qquad (9)$$

When a low extension rate is used for the measurements (for instance 1 mm/min), W_i can be considered as negligible and the fracture energy for zero extension rate (ε_0) is then equal to the reversible work of bioadhesion W_r, as shown by Eq. (10).

$$\varepsilon_0 = W_r = W_b/A_0 \qquad (10)$$

The fracture theory does not take into account biological phenomena such as stress caused, for example, by movement of the tissue.

The Study of Mucoadhesion of Hydrogels

During the last decade several methods to study mucoadhesion phenomena or mucoadhesive properties of hydrogels were used (Table 1). Tensiometry has already been reported as a suitable method during late 1980s, and is still the most frequently used technique to study mucoadhesiveness of hydrogels. Peppas and co-workers developed a tensile technique for measurements of the bioadhesive strength of tablets containing polyacrylic acid to bovine mucosae [16,18]. A tensiometry set-up for the investigation of solid devices is shown in Fig. 2. The system is better mimicking the in vivo conditions when the experiment is performed in an aqueous environment and the mucosa of interest is originating from freshly prepared biological tissue. It has been suggested that this system is most suitable for buccal or vaginal application, where biological liquid is controllable. Tensiometry has been shown to be very useful in comparing the mucoadhesive properties of different hydrogels [19].

Mikos and Peppas [20] described the flow channel to measure the bioadhesion of polymer microparticles on mucin gels. Later Lehr et al. [21] used an in situ loop model in the rat for the investigation of mucoadhesive microspheres (Fig. 3). They concluded that this approach allowed the study of the transit of particles. Another technique to study the mucoadhesive properties of microspheres is the electrobalance method, as described by Chickering et al. [22,23]. Environmental conditions like temperature and pH can be easily controlled and several parameters can be obtained from one single experiment. The authors suggested that mucoadhesion in the bioerodible materials used to conduct the studies, is not attributable to chain entanglement, but to hydrogen bonding between hydrophilic functional groups (—COOH) and mucus glycoproteins.

Colloidal gold staining has been introduced by Park [24] for studying the ''adhesion number.'' The polymer in a form of strips is incubated with colloidal gold–
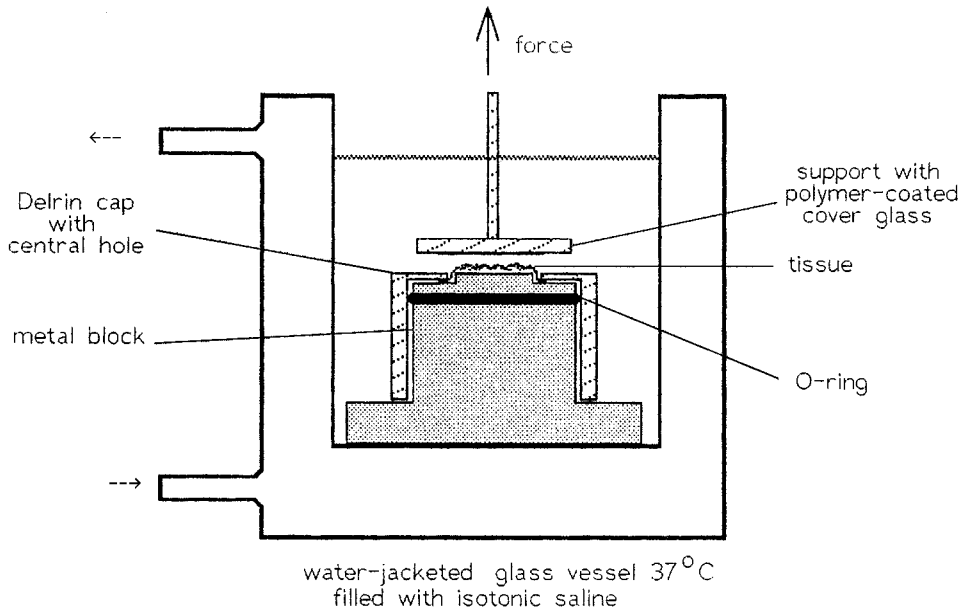
FIG. 2. Experimental setup to measure the force of detachment of mucoadhesive polymer films from mucosal tissue.
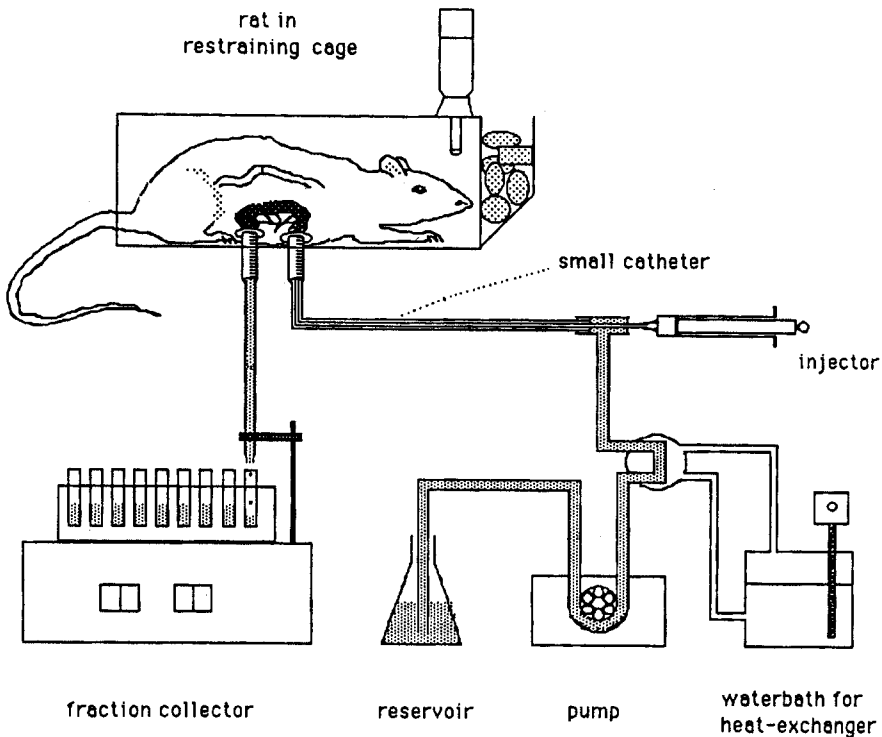
FIG. 3. Experimental setup to study the intestinal transit of mucoadhesive microspheres in a chronically isolated loop. (Adapted from Ref. 21.)

mucin conjugates, and after a rinsing procedure the absorbance of strips is measured. Colloidal gold staining has also been used to investigate mucin–chitosan interactions [25].

Rheology measurements were used by Mortazavi et al. [26,27] to investigate mucus–Carbopol 934P interactions at different pH values and the role of water movement in mucoadhesion. Similar rheological techniques were applied for studying four different types of polymers, and it was concluded that molecular interpenetration is an important factor in mucoadhesion by strengthening the mucus in the mucoadhesive–mucosal interface [28]. Rheological methods were used to investigate the interpenetration between ion-sensitive polymers (Carbopol and deacetylated gellan gum, Gerlite) with two commercially available mucins, submaxillary gland mucin and porcine gastric mucin [29]. It was suggested that the increase in the elastic modulus of a polymer–mucin mixture (compared to the elastic modulus of polymer alone) indicates a positive interaction caused by mucoadhesion. However, the concentration of the polymer, the type of the mucin used, and the quantity of ions present appeared to have a strong influence on the interactions between mucins and ion-sensitive polymers, indicating that the explanation of mucoadhesion by means of interpenetration should be applied only to interpret special cases of mucoadhesion between polymers and mucins.

Rossi et al. [30] evaluated rheologically mucins of different origin with polyacrylic acid and sodium carboxymethyl cellulose. The same group also reported a novel rheological approach based on a stationary viscoelastic test (creep test) to describe the interaction between mucoadhesive polymers and mucins [31,32]. Jabbari et al. [33] used attenuated total-reflection infrared spectroscopy to investigate the chain interpenetration of polyacrylic acid in the mucin interface.

Other techniques used for studying molecular interactions between polymers and mucus include ultracentrifugation, surface plasmon resonance, and electromagnetic transduction [4,34]. Illum and co-workers [35] investigated the interaction of chitosan microspheres using turbidimetric measurements and adsorption studies of mucin to the microspheres.

Microscopic visualization techniques have also been used to investigate mucus–polymer interactions [36–39]. Transmission electron microscopy was used by Fiebrig et al. [36], whereas different microscopical techniques were used by Lehr et al. [37] for the visualization of mucoadhesive interfaces. Transmission electron microscopy in combination with near-field Fourier transform infrared microscopy (FT-IR) has been shown to be suitable for investigating the adhesion-promoting effect of polyethyleneglycol added in a hydrogel [38]. Moreover, scanning force microscopy may be a valuable approach to obtain information on mucoadhesion and specific adhesion phenomena [39].

The Mucosal Routes of Delivery

Buccal Route

The buccal route of drug administration is the most widely used method for application of mucoadhesive delivery systems. Both for local treatment of inflammation (i.e., apthae) and for rapid absorption of compounds (nitroglycerin), formulation technolo-

gies have employed the buccal route for over two decades, and sublingual or gingival dosage forms are already established at the market [8] (Table 2).

Oral mucosae are composed of multiple layers of cells, which show various patterns of differentiation dependent on the functions of different regions in the oral cavity [40]. The oral mucosa is covered by a stratified, squamous epithelium and three different types of mucosa can be distinguished: the masticatory, the lining, and the specialized mucosa. Blood supply to the oral cavity tissues is delivered via the external carotid artery, which branches to the maxiliary lingual and facial artery. There are no mucus-secreting goblet cells in the oral mucosa, but mucins are found in human saliva. These mucins are water-soluble and form a gel of 10–200μm thickness. Saliva, mainly composed of water (99%), is continuously secreted in the oral cavity and exists as a film with a thickness of 0.07–0.1 mm [40].

In the 1980s, Machid and Nagai [41] evaluated spray dosage forms based on hydroxypropyl cellulose (HPC) for the delivery of beclomethasone to treat recurrent and multiple apthae. Previously a double-layered tablet of HPC and Carbopol 934P was introduced in the market for the treatment of apthous stomatitis [41].

Bouckaert et al. [42] tested buccal tablets of miconazole based on modified starch–polyacrylic acid mixtures. Although these tablets showed different mucoadhesion properties in vitro, no significant differences in the salivary content of miconazole could be observed in human volunteers.

Lee and Chien [43] evaluated mucoadhesive devices of a bilayer type, consisting of a fast-release layer containing polyvinylpyrrolidone (PVP) and a sustained-release layer of Carbopol 934P and PVP for prolonged delivery of luteinizing hormone-releasing hormone (LHRH) onto the porcine gingival and alveolar mucosa for 24 hr. This device contained also an absorption enhancer (sodium cholate) and cetylpyridinium chloride to protect LHRH from degradation by microflora. The LHRH permeation appeared to increase by raising the loading of LHRH or enhancer in the fast-release layer. The formulation of the devices could be varied to achieve specific rates of transmucosal peptide drug permeation.

Nair and Chien [44] compared patches and tablets of different polymers (sodium carboxymethylcellulose, carbopol, polyethylene oxide, polymethyl vinyl ether–maleic anhydride, tragacanth) regarding their release characteristics of four drugs (chlorheximide, clotrimazole, benzocaine, and hydrocortisone). They observed sus-

TABLE 2 Drug Products Available for Buccal and/or Sublingual Application Using Mucoadhesive Polymers[a]

Drug	Therapeutic area	Product names
Nitroglycerin	Angina pectoris	Suscard, Cardilate, Nitrobid, Nitromex, Nitrong
Isorbide mononitrate	Angina pectoris	Imdur, Isordil, ISMO
Buprenorphine	Analgesia	Temgesic, Buprenex
Nicotine	Smoking cessation	Nicotinelle, Nicorette
Ergotamine	Migraine	Ergostat, Ergomar
Methyl testosterone	Hypogonadism, delayed puberty	Oreton Methyl, Testred, Virilon
Lorazepam	Anxiety, insomnia	Ativan

[a] Adapted from Ref. 8.

tained release of all four compounds from the mucoadhesive tablets, but only two of the active compounds, chlorheximide and clotrimazole, could be released in a controlled manner from the mucoadhesive patches.

Buccal bilayer devices (films and tablets) are comprised of a drug-containing mucoadhesive layer and a drug-free backing layer [45]. The former consists of chitosan, free or cross-linked by an anionic polymer (polycarbophil, sodium alginate, gellan gum), and the latter of ethylcellulose. The in situ cross-linking of chitosan by polycarbophil gives tablets that exhibit controlled swelling, drug release, and adequate mucoadhesion to bovine sublingual mucosa.

The periodontal pocket is another site for drug delivery in the oral cavity. Needleman et al. [46] investigated three mucoadhesive polymers (cationic chitosan, anionic xanthan gum, neutral polyethylene oxide) in vitro, using organ cultures, and in vivo in patients on their periodontal and oral mucosa. Of the polymers studied, chitosan displayed the longest adhesion in vitro and on the periodontal pockets, and the shortest adhesion on oral mucosa.

Nasal Route

The nasal route of drug administration is the most suitable alternative of delivery for poorly absorbable compounds such as peptide or protein drugs. The nasal epithelium exhibits relatively high permeability, and only two cell layers separate the nasal lumen from the dense blood-vessel network in the lamina propria. The respiratory epithelium is the major lining of the human nasal cavity and is essential in the clearance of mucus by the mucociliary system. This epithelium is composed of ciliated and nonciliated columnar cells, goblet cells, and basal cells. The respiratory epithelium is covered by a mucus layer, which can be divided into two distinctive layers (the periciliary layer and a more gel-like upper layer). The periciliary layer consists of a liquid of lower viscosity. Mucus is secreted from goblet cells as highly condensed granules by exocytosis. The mucus layer is propelled by the cilia toward the nasopharynx, and the function of the mucociliary clearance is to remove foreign substances and particles from the nasal cavity, preventing them to reach the upper airways [47].

Various structurally different mucoadhesive polymers were tested for their ability to retard the nasal mucociliary clearance in rats [48]. Methylcellulose, sodium carboxymethyl cellulose, hydroxypropyl methylcellulose, chitosan glutamate, Carbopol 934P, polyethylene oxide 600K, and Pluronic F127 were applied in gel form and their clearance was measured using microspheres labeled with a fluorescent marker incorporated into the formulation. The clearance rate of each polymer gel was found to be lower than that of a control microsphere suspension, resulting in an increased residence time of the gel formulations in the nasal cavity. Methylcellulose (3%) gel gave the longest nasal residence time, whereas a Carbopol 934P (0.2%) aqueous gel was the least effective.

Illum et al. [49] evaluated chitosan solutions as delivery platforms for nasal administration of insulin to rats and sheep. They reported a concentration-dependent absorption-enhancing effect with minimal histological changes of the nasal mucosa in all concentrations applied.

Oechslein et al. [50] studied various powder formulations of mucoadhesive polymers for their efficacy to increase the nasal absorption of octreotide in rats. Although

chitosan showed the highest water uptake (chitosan > microcrystalline cellulose > semicrystalline cellulose ≫ pectin = hydroxyethyl starch = alginic acid = Sephadex G25), the highest peptide drug bioavailability was found after coadministration of alginic acid and Sephadex G25 powders (4,1 and 5.56%, respectively). The authors concluded that the calcium-binding properties of the polymers used correlated better with the increased octreotide bioavailability.

Nakamura et al. [51] studied the adhesion of water-soluble and neutral polymers [hydroxypropyl cellulose (HPC), xanthan gum (XG), tamarind gum (TG), and polyvinyl alcohol (PVA)] to nasal mucosa in vitro and in vivo. The polymers, mixed with a dye, were applied as powders to the nasal cavity of rabbits, and the remaining dye residue was determined at 2, 4, and 6 hr after nasal instillation with a thin fiberscope. The polymer XG showed the longest residence time of the dye in the cavity, followed by TG, HBC, and PVA in decreasing order. For the mixture XG and XG-PVA (2: 8), some residue of dye could still be observed 6 hr after administration. The order of adhesion of these polymers to agar plates in vitro agreed with that of their mucoadhesion in vivo. Illum et al. [52] introduced bioadhesive microspheres for nasal delivery of poorly absorbable drugs. Radiolabelled microspheres made from diethylaminoethyl (DEAE)-dextran, starch microspheres, and albumin microspheres were administered to human volunteers and appeared to be cleared significantly slower than solutions or nonmucoadhesive powder formulations. However, starch or hyaluronic acid microspheres significantly increased the absorption of peptide drugs from nasal mucosa [53].

Nakamura et al. [54] describe a microparticulate dosage form of budesonide, consisting of novel bioadhesive and pH-dependent graft copolymers of polymethacrylic acid and polyethylene glycol, resulting in elevated and constant plasma levels of budesonide for 8 hr after nasal administration in rabbits.

Recently, starch and chitosan microspheres as well as chitosan solutions were tested for their clearance characteristics in human volunteers using gamma scintigraphy [55]. The results revealed a 4-, 3-, and 2-times longer clearance half-life (compared to controls) for chitosan microspheres, starch microspheres, and chitosan solutions (Fig. 4). These observations support the hypothesis that chitosan delivery systems can reduce the rate of clearance from the nasal cavity, thereby increasing the contact time of the delivery system with the nasal mucosa and providing the potential for raising the bioavailability of drugs incorporated into these systems.

Ocular Route

The ocular route is used mainly for the local treatment of eye pathologies. Absorption of drugs administered by conventional eyedrops can result in poor ocular bioavailabilities (2–10%). This is due to the limited area of absorption, the lipophilic character of the corneal epithelium, and a series of elimination factors that reduce the contact time of the medication with the corneal surface, such as drainage of instilled solutions, lacrimation, and tear turnover and tear evaporation [56].

The first structure encountered by an ocular dosage form is the precorneal tear film, consisting of three layers:

- The outer layer, oily and lipid, mainly prevents tear evaporation,
- The middle layer which is an aqueous salt solution layer, and
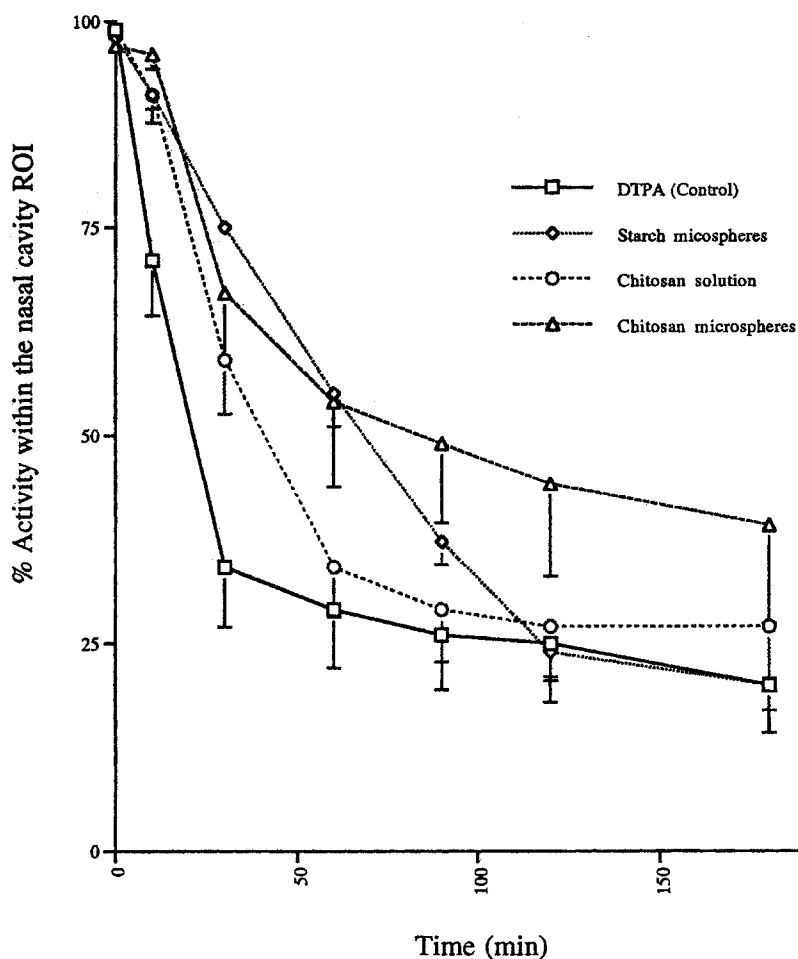
FIG. 4. The nasal clearance of bioadhesive formulations and a control in human volunteers. (From Ref. 55.) DTPA = diethylenetriaminepentaacetic acid.

• The inner layer, a mucus layer secreted by the conjuctiva goblet cells and the lacrymal gland. This layer is important for wetting the corneal and conjuctival epithelia.

The ocular membranes comprise the cornea (not vascularized) and the conjuctiva (vascularized). The corneal epithelium consists of five or six layers of nonkeratinized squamous cells, and is considered to be the major pathway for ocular drug penetration [57].

The following types of mucoadhesive preparations have been evaluated for ocular drug delivery: hydrogels, viscous liquids, solids (inserts), and particulate formulations [57]. Hui and Robinson [58] introduced hydrogels consisting of cross-linked polyacrylic acid for ocular delivery of progesterone in rabbits. These preparations increased progesterone concentrations in the aqueous humor four times over aqueous suspensions.

Davies et al. [59] compared the precorneal clearance of Carbopol 934P to that of an equiviscous nonmucoadhesive polyvinyl alcohol solution (PVA) and phosphate buffer saline (PBS) using lacrimal dacryoscintigraphy in the rabbit. The precorneal retention of the Carbopol 934P was shown to be significantly longer than that of PVA, which, in turn, was significantly longer than that of PBS. In the same study, Carbopol 934P solution produced a significant increase in bioavailability of pilocarpine as compared to PVA and PBS. The same authors [60] describe phospholipid vesicles coated with Carbopol 934P or Carbopol 1342 (a hydrophobic modified Carbopol resin). The mucoadhesive polymer-coated vesicles demonstrated substantially enhanced precorneal retention compared to noncoated vesicles at pH 5. However, the polymer-coated vesicles did not increase the ocular bioavailability of entrapped tropicamide compared to noncoated vesicles and aqueous solutions.

Lehr et al. [61] investigated two gentamicin formulations of polycarbophil (neutralized vs. nonneutralized) to pigmented rabbit eye. Both polymeric formulations doubled the uptake of gentamicin by the bulbar conjunctiva.

Saettone et al. [62] evaluated low viscosity polymers (polygalacturonic acid, hyaluronic acid, carboxymethylamylose, carboxymethylchitin, chondroitin sulfate, heparan sulfate, and mesoglycan) as potential mucoadhesive carriers for cyclopentolate and pilocarpine in a study of their influence on miotic activity in rabbits. Small but significant increases in bioavailability were observed and a correlation was found between the bioavailability of the two drugs and the mucoadhesive bond strength of the polymers investigated.

Calvo et al. [63] studied chitosan (CS)- and poly-L-lysine (PLL)-coated poly-ε-caprolactone (PECL) nanocapsules for ocular application. In comparison with commercial eye drops, the systems investigated (uncoated, PLL-coated, and CS-coated nanocapsules) significantly increased the concentrations of indomethacin in the cornea and aqueous humor of rabbit eyes. The chitosan-coated formulation doubled the ocular bioavailability of indomethacin over the uncoated particles, whereas the PLL coating was ineffective. The authors concluded that the specific nature of CS was responsible for the enhanced indomethacin uptake and not the positive surface charge. Both the PLL- and CS-coated nanocapsules displayed good ocular tolerance [63].

A recent approach to ocular inserts was presented by Chetoni et al. [64] in a study of cylindrical devices for oxytetracycline, made from mixtures of silicone elastomer and grafted on the surface of the inserts with an interpenetrating mucoadhesive polymeric network of polyacrylic acid or polymethacrylic acid. The inserts were tested for drug release and retention at rabbit eyes. It was shown that some of the inserts are able to maintain prolonged oxytetracycline concentrations in the lacrimal fluid for 36 hr.

Vaginal Route

The vaginal route is considered to be suitable for the local application and absorption of therapeutics like estrogens for hormone replacement therapy or contraception. Systemic absorption of peptide drugs such as LHRH agonists and calcitonin can also be achieved [65].

The vagina offers a substantial area for drug absorption, because numerous folds in the epithelium increase the total surface area. A rich vascular network surrounds

the vagina, whereas the vaginal epithelium is covered by a film of moisture consisting mainly of cervical mucus and fluid secreted from the vaginal wall.

Conventional vaginal delivery systems include tablets, foams gels, suspensions, and pessaries. Mucoadhesive gel formulations based on polycarbophil have been reported to remain 3 to 4 days at the vaginal tissue, providing an excellent vehicle for the delivery of progesterone and nonoxynol-9 [66].

The benzyl ester of hyaluronic acid (HYAFF 11) is a highly mucoadhesive polymer which can be processed into microspheres. Such microspheres containing salmon calcitonin were intravaginally administered to rats as a dosage form for the prevention of ovariectomy osteopenia [65]. In recent studies, HYAFF 11–salmon calcitonin microspheres were formulated as single-dose pessaries, resulting in sustained plasma concentrations of calcitonin [67].

Gastrointestinal Route

The peroral route represents the most convenient route of drug administration, being characterized by high patient compliance. The mucosal epithelium along the gastrointestinal tract varies. In the stomach the surface epithelium consists of a single layer of columnar cells whose apical membrane is covered by a conspicuous glycocalyx. A thick layer of mucus is covering the surface to protect against aggressive luminal content. This site of the tract is of minor interest for drug delivery, since the low pH and the presence of proteolytic enzymes make the stomach a rather hostile environment. However, there are examples of dosage forms specially designed to be retained in the stomach such as some gastroretentive systems consisting of mucoadhesive hydrogels [68]. Akiyama et al. [69] evaluated microspheres for prolonged residence time in the gastrointestinal tract of rats. They prepared two types of polyglycerol fatty acid esters (PGEF)-based microspheres, Carbopol 934P-coated microspheres, and Carbopol 934P-dispersion microspheres. Significantly longer residence times were observed after administration of the dispersion-microspheres than with the coated ones. Additionally, it was shown that the microspheres were retained in the stomach of the animals.

The small intestine is characterized by an enormous surface area available for the absorption of nutrients and drugs. This large area is formed by crypts and villi. The intestinal epithelium consists of a single layer of three types of columnar cells, enterocytes, goblet cells, and enteroendocrine cells. The enterocytes are linked to each other by tight junctions and desmosomes. The goblet cells are mucin-producing unicellular glands intercalated between the enterocytes. The enteroendocrine cells are scattered between the enterocytes and goblet cells and release hormones that can modify the local environment or influence the intestinal motility. At the terminal ileum, the Peyer's patches, a particular specialization of the gut-immune system, are located. This domain contains the M cells, which are specialized in endocytosis and processing luminal antigens. The large intestine (colon) has the same cell populations as the small intestine, and its main function is the absorption of water and electrolytes. The role of mucus in the intestine is to facilitate the passage of food along the intestinal tract and to protect the gut from bacterial infections [70].

In the past decade several difficulties have been encountered in the design of successful mucoadhesive delivery systems for peroral applications. The reasons may

be due to shortcomings of the mucoadhesive properties of the polymers or to the peculiar physiological limits of the digestive tract, soluble mucins, and shed-off mucus, food, or other contents of the intestinal lumen which would inactivate the mucoadhesive properties of the delivery system before having reached the absorbing membrane. Furthermore, the adhesion of the delivery system can last as long as the gel-state mucus itself remains attached to the intestinal mucosal tissue. Mucus turnover is continuously removing the mucus gel layer attached to the epithelium by a steady-state process [71].

The failure in increasing residence time of mucoadhesive systems in the human intestinal tract has led scientists to the evaluation of multifunctional mucoadhesive polymers. Research in the area of mucoadhesive drug delivery systems has shed light on other properties of some of the mucoadhesive polymers. One important class of mucoadhesive polymers, poly(acrylic acid) derivatives, has been identified as potent inhibitors of proteolytic enzymes [72–74]. The interaction between various types of mucoadhesive polymers and epithelial cells has a direct influence on the permeability of mucosal epithelia by means of changing the gating properties of the tight junctions. More than being only adhesives, some mucoadhesive polymers can therefore be considered as a novel class of ''multifunctional macromolecules'' with a number of desirable properties for their use as delivery adjuvants [72,75].

Lueßen et al. [73,74] evaluated the mucoadhesives polyacrylates polycarbophil and Carbopol 934P for their potency to inhibit intestinal proteases. These polymers are able to inhibit the activities of trypsin, α-chymotrypsin, and carboxypeptidase A and B as well as of cytosolic leucine aminopeptidase. Carbopol 934P was found to be more efficient to reduce proteolytic activity than polycarbophil [74]. The pronounced binding properties of polycarbophil and Carbopol 934P for bivalent cations, such as zinc and calcium, were demonstrated to be a major reason for the observed inhibitory effect. These polymers have been shown to remove Ca^{2+} and Zn^{2+}, respectively, from the enzyme structures, thereby inhibiting their activities. Carboxypeptidase A and α-chymotrypsin activities were observed to be reversible upon the addition of Zn^{2+} and Ca^{2+} ions, respectively. Therefore, it was concluded that polyacrylates are promising excipients to protect peptide drugs from intestinal degradation. In vitro studies, using the Caco-2 cell intestinal epithelium model, showed that Carbopol 934P was able to substantially increase the transport of a macromolecular paracellular fluorescent marker (dextran) and the peptide drug 9-desglycinamide, 8-L-arginine vasopressin [76].

Carbopol 934P and chitosan gels were also tested in vivo for their ability to increase the absorption of the peptide analogue buserelin when administered intraduodenally in rats [77]. Both polymers increased the absorption of the peptide significantly, probably due to both permeation-enhancing and enzyme-inhibition properties; mucoadhesion played a secondary role. Chitosan was found to remarkably increase the peroral bioavailability of the peptide in comparison to Carbopol 934P [77], indicative of a more specific effect of chitosan with the tight junctions, than previously suggested by Artursson et al. [78].

Chitosan and chitosan salts, however, lack the advantage of good solubility at neutral pH values. They aggregate in solutions at pH values above 6.5, and recent studies have shown that only protonated chitosan (i.e., in its uncoiled configuration) can trigger the opening of the tight junctions, thereby facilitating the paracellular transport of hydrophilic compounds [79]. This property implies that chitosan can be

effective as an absorption enhancer only in a limited area of the intestinal lumen where the pH values are close to its pK_a. For this reason, chitosan and its salts may not be suitable carriers for targeted peptide drug delivery to specific sites of the intestine, for instance, the jejunum or ileum. To overcome this problem the chitosan derivative N,N,N-trimethylchitosan chloride (TMC) has been synthesized and characterized [80]. This quaternized chitosan shows higher aqueous solubility than chitosan in a much broader pH range. Chitosan HCl and TMCs of different degrees of trimethylation were tested for enhancing the permeability of the radiolabelled marker ^{14}C-mannitol in Caco-2 intestinal epithelia at neutral pH values (for instance, pH 7.2). Chitosan HCl failed to increase the permeation of these monolayers and so did TMC with a degree of trimethylation of 12.8%. However, TMC with a degree of trimethylation of 60% significantly increased the permeability of the Caco-2 intestinal monolayers, indicating that a threshold value at the charge density of the polymer is necessary to trigger the opening of the tight junctions [81]. Because of the absence of significant cyto- and ciliotoxicity, TMC polymers (particularly with a high degree of trimethylation) are expected to be safe absorption enhancers for improved transmucosal delivery of peptide drugs [82].

In recent studies both in vitro (Caco-2 cells) and in vivo in rats, TMC with a degree of trimethylation of 60% was proven to be an excellent intestinal absorption enhancer of the peptide drugs buserelin and octreotide. The observed absolute bioavailability values were 13 and 16% for buserelin and octreotide, respectively [83] (unpublished data; Fig. 5). Permeation enhancing effects were more responsible for these increased bioavailabilities, rather than the mucoadhesive properties of the TMC polymers. Nevertheless, mucoadhesion is a prerequisite for these polymers in order to further act as absorption enhancers.

Mucus also appears to be a barrier to the permeation enhancing effect of polymeric or monomeric absorption enhancers. In the above mentioned TMC studies, the enhancement effect (enhancement ratio = permeation rate of the drug in the presence
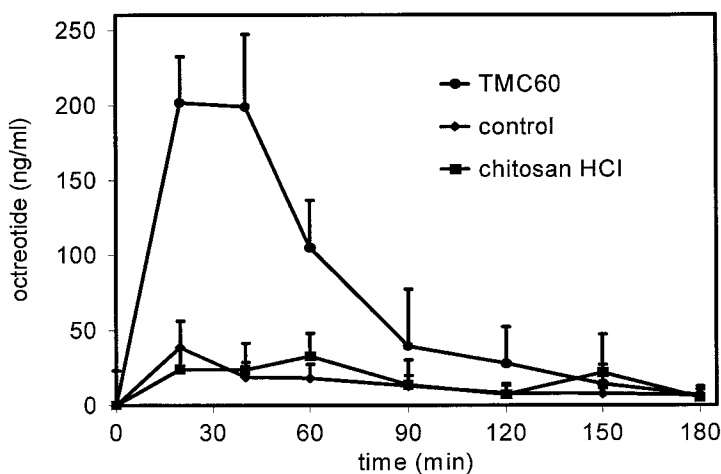
FIG. 5. Intestinal absorption of octreotide acetate in rats using mucoadhesive polymers. (From Thanou et al. unpublished data).

of polymer vs. permeation rate of the drug alone) was higher in vitro (Caco-2 cells; no mucus secretion) than the absorption enhancement in vivo. Meaney and O'Driscoll [84] studied the effect of mucus on the permeation properties of a micellar system consisting of sodium taurocholate in a coculture of Caco-2 and Ht29GlucH (mucin-secreting) cells. They found that the effect of bile salts on the permeation of hydrophilic paracellular markers was increased in the cocultures that were pretreated with the mucolytic compound N-acetylcysteine.

Bernkop-Schnürch [85] prepared a series of conjugates of protease inhibitors (pepstatin, Bowman-Birk, chymostatin, elastatinal, antipain bacitracin) and/or EDTA to three different types of polymers (carboxymethyl cellulose, polyacrylic acid, and chitosan). In addition to their mucoadhesive properties, most of these conjugates exhibited enzyme-inhibitory properties. Furthermore, the toxicity of these protease inhibitors was reduced by being covalently bound to the polymers. Chitosan-EDTA conjugates have proven to be potent inhibitors of the zinc-containing proteases as well as to be strong mucoadhesives [86].

In order to design highly mucoadhesive platforms for peroral drug delivery, Bernkop-Schnürch et al. [87] proposed thiol groups-bearing polycarbophil modifications, based on the mucolytic activity of thiols caused by disulfide exchange reactions between mucin glycoproteins and the mucolytic agent. Polycarbophil–cysteine conjugates appeared to exhibit superior mucoadhesiveness compared to polycarbophil itself, due to improved cohesion and rapid hydration of the gels. However, again mucus turnover is still the limiting factor of adhesion to the cell surfaces.

All the above mentioned polymers have been evaluated mainly for application in the intestine. Finally, the last part of the gastrointestinal tract, the rectum should also be mentioned as a suitable site for delivery and fast absorption of therapeutics. Kim et al. [88] developed an in situ gelling and mucoadhesive acetaminophen liquid suppository prepared with poloxamers and sodium alginate. It was found that this particular formulation of acetaminophen in humans resulted in shorter T_{max} and higher maximum plasma concentrations of drug (C_{max}) than the conventional acetaminophen suppositories.

Suppositories are the preferable dosage form for patients that experience nausea. Yahagi et al. [89] evaluated a mucoadhesive suppository consisting of Witepsol H-15 and 2% Carbopol 934P for rectal delivery and absorption of the anti-emetic drug ramosetron hydrochloride (serotonin antagonist) in rabbits. These suppositories increased the $AUC_{(0-24h)}$ 2.5 times and prolonged the residence time compared to suppositories without mucoadhesive polymer. The anti-emetic effect of the formulation was tested in ferrets and it was found that the Carbopol 934P-containing suppositories had the same effect as intravenous administration. This formulation was suggested as a once-a-day dosage form for the treatment of chemotherapy-induced nausea.

Trends and Perspectives

In this article a large number of polymer modifications have been described as novel drug delivery platforms, being second-generation mucoadhesive hydrogels. These polymers, both as safe absorption enhancers [75] or as improved mucoadhesive hy-

drogels, are the most recent developments in mucoadhesive delivery platforms for intestinal absorption of drugs.

Another trend observed during the past decade was the coating of liposomes with mucoadhesive polymers. Liposomes are coated with chitosan, long-chain polyvinyl alcohol, and polyacrylates bearing a cholesteryl group [90]. Chitosan-coated liposomes showed superior adhesion properties to rat intestine in vitro than the other polymer-coated liposomes. In vivo, chitosan-coated liposomes containing insulin substantially reduced blood glucose levels after oral administration in rats, which were sustained up to 12 hr after administration [90].

Another type of novel mucoadhesive formulations was suggested to be submicron emulsions (o/w), bearing droplets coated with Carbopol 940. These formulations have been shown to generate a 12-fold enhancement in rats in the oral bioavailability of the antidiuretic peptide drug desmopressin [91].

Specific adhesion approaches also show promise. The more specific bindings of plant lectins, mussel glue protein, and K99-fimbriae have been suggested as an alternative to the classical nonspecific mucoadhesive hydrogels. Tomato lectins were found to bind specifically onto both isolated porcine enterocytes and Caco-2 cells with the same affinity [92]. However, lectin binding was inhibited in the presence of crude porcine gastric mucin, indicative of a marked cross-reactivity. Irache et al. [93] investigated three different plant lectins conjugated to latex, tomato lectin, *asparagus pea* lectin, and *mycoplasma gallisepticum* lectin. The extent of interactions of these three lectin–latex conjugates decreased from the duodenum to the ileum, when tested on rat intestinal mucosa without Peyer's patches. However, when mucosa containing Peyer's patches was used, a substantial increase in the interaction of the conjugates with the mucosa was found, which was more pronounced for the mycoplasma and asparagus lectins than for the tomato lectin [93].

A natural example of mucodhesion can be the colonization of the small intestine by *Escherichia coli* strains mediated by cell-surface antigens, called fimbriae [94]. Fimbriae are long, thread-like protein polymers found on the surface of many bacterial strains. They enable bacteria to adhere to the brush border of epithelial cells. A special fimbriae antigen, K99-fimbriae, has been isolated from *E. coli* and bound to polyacrylic acid. The conjugate was tested by a hemagglutination assay for its ability to bind to equine erythrocytes, which have the same K-99-receptor structures as gastrointestinal epithelial cells. A 10-times stronger retention of erythrocyes was observed for the matrix-bound K-99 antigen than for the matrix-bound ovalbumin [94].

Mussel adhesive protein (MAP) is a 130-kDa protein produced by the blue mussel (*Mytilus edulis*), which provides strong adhesion to submerged surfaces. MAP films were prepared by drying and stored under nitrogen atmosphere. These films showed twice the adhesion strength of polycarbophil when tested on porcine duodenum in vitro [95].

All these examples of the applications of mucoadhesive polymers, demonstrate that the use of mucoadhesive hydrogels is a powerfull strategy to improve the absorption of therapeutics across mucosal epithelia. With respect to buccal, nasal, or ocular delivery of drugs, the use of such carriers has already been successfully established. However, the application of the mucoadhesive polymers in the gastrointestinal tract is still waiting for a break-through. Specific-binding principles may be applied in the near future to design a third generation of mucoadhesive polymers for application in the gastrointestinal tract.

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Peptides and Proteins: Buccal Absorption

Introduction

Background and Rationale

In recent years, proteins and peptides are emerging as a major class of therapeutic agents. Pharmaceutical scientists are faced with the challenges of selection of a suitable route of drug delivery and the formulation of these bioengineered drugs. The most common route of protein and peptide drug delivery has been parenteral. The gastrointestinal (GI) route has some disadvantages because it presents a hostile environment. Drugs administered by this route are subjected to acid hydrolysis and extensive gut and/or hepatic ''first-pass'' metabolism. Thus, protein drugs may exhibit poor oral bioavailability.

The parenteral route has direct access to the systemic circulation and therefore produces effective plasma levels. However, this route is associated with pain on administration, resulting in poor patient compliance; in addition, the formulation needs to be sterile. The parenteral route may be particularly unsuitable for long-term treatment as it causes occasional allergies, hypertrophies, or atrophy of the subcutaneous fat at the site of injection, and psychological distress [1]. Noninvasive mucosal and transdermal routes offer effective alternative routes for systemic drug delivery. The transdermal delivery route is limited to potent, lipophilic compounds, and does not rapidly attain the required blood levels. Furthermore, the dermal epithelium is less permeable than oral mucosa [2,3]. Various absorptive mucosae have been identified and investigated for systemic drug delivery. These include nasal, ocular, pulmonary, rectal, vaginal,buccal, and sublingual. The nasal mucosa consists of microvilli and has a rich blood supply [4]. Large individual variations in mucus secretion and turnover [5], negative effects of drugs and/or excipients on the ciliary cells [6], and pathological changes of the nasal mucosa with long-term treatment [5] limit extensive utilization of this mucosa. The rectal route has met with variable patient acceptance, and depending on the site of administration, the drug may undergo a first-pass effect [7]. The vaginal route is gender specific. The buccal and sublingual routes do not have many of these limitations, and therefore both routes seem to provide attractive alternatives for systemic drug delivery. In this article, only buccal drug delivery is discussed.

Advantages

Because of its rich vascularity, drugs delivered by the buccal route gain direct access to the systemic circulation and are not subject to first-pass metabolism. Furthermore, therapeutic agents do not come in contact with the acidic digestive fluids secreted by the GI tract and thus are protected from its harsh environment.

Compared to the nasal and rectal routes, the buccal mucosa has low enzymatic activity, and drug inactivation due to biochemical degradation is not as rapid and extensive [8].

Excellent accessibility to the buccal mucosa makes application of the dosage form painless. It can be precisely located, and is easily removed without discomfort at the end. The oral cavity consists of a pair of buccal mucosae. Hence, a drug delivery system can be applied at various sites, on the same mucosa or on the left or right buccal mucosa on different applications. This is particularly advantageous if the delivery system contains a drug or excipient which mildly and reversibly damages or irritates the mucosa.

A buccal drug delivery system is applied to a specific area on the buccal membrane. Moreover, the delivery system can be designed to be unidirectional in drug release so that it can be protected from the local environment of the oral cavity. It also permits the inclusion of a permeation enhancer/protease inhibitor or pH modifier in the formulation to modulate the membrane or the tablet-mucosal environment at that particular application site. While the irritation is limited to the well-defined area, the systemic toxicity of these enhancers/inhibitors and modifiers can be reduced. The buccal mucosa is well suited for this type of modification as it is less prone to irreversible damage [9]. In the event of drug toxicity, delivery can be terminated promptly by removal of the dosage form.

In addition, the buccal route may be useful for unconscious patients and in patients who have recently undergone surgery or have experienced upper GI tract disease which would affect oral drug absorption.

Disadvantages

The surface area available for absorption in the buccal mucosa is much smaller than that in the GI, nasal, rectal, or vaginal mucosae. The buccal mucosa is continuously bathed by saliva which reduces the drug concentration at the absorbing membrane. These two factors, along with the permeability coefficient of the drug, affect the overall absorption rate of a drug by this route. In addition, the buccal mucosa is less permeable than any of the above mentioned mucosae.

Involuntary swallowing of saliva containing dissolved drug or swallowing the delivery system itself would lead to a major loss of drug from the site of absorption. Talking, eating, and drinking affect the retention of the delivery system and therefore may constitute limitations associated with this route of administration [3]. In addition, there is a risk of choking on the dislodged drug delivery device.

Taste, irritancy, and allergenicity may also limit the number of drugs that can be delivered by the buccal route.

Anatomy and Physiology of the Oral Mucosa

Structure

The oral mucosa is anatomically divided into three tissue layers (Fig. 1) [10]: the epithelium, the basement membrane, and the connective tissues.
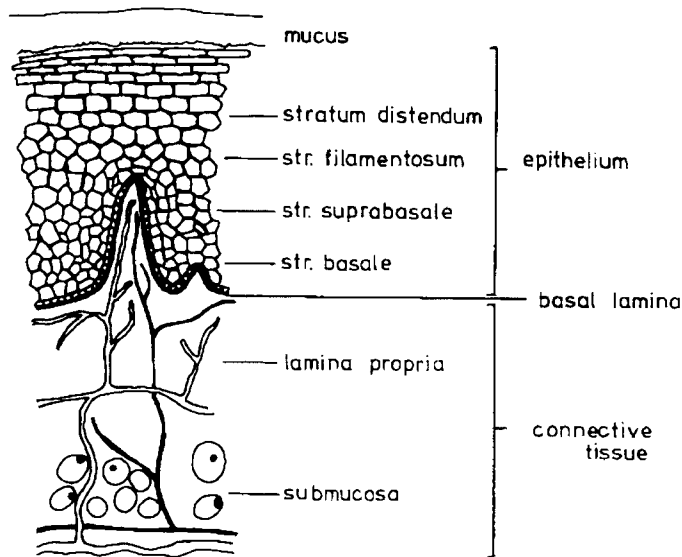
FIG. 1. Schematic diagram showing the principal components of oral mucosa (from Ref. 8).

Epithelium

The epithelium consists of approximately 40–50 layers of stratified squamous epithelial cells. These cells originate from a layer of basal cells, which are cuboidal in shape, and undergo continuous mitosis and move to the surface. As the cells migrate to the surface through the intermediate layers, they diversify and become larger, flattened, and surrounded by an external lipid matrix (membrane-coating granules). This matrix determines the permeability of the tissue to the drug. In comparison, the epithelium of gastrointestinal mucosa consists of columnar epithelial cells specialized for absorption and also of goblet cells for mucus secretion [11]. Gingiva (gum) and the hard palate are keratinized, whereas areas such as buccal, sublingual, and the soft palate are not. The nonkeratinized cells retain nuclei and other organelles and undergo greater morphological changes during their migration to the surface. These cells are devoid of acylceramides and contain only small amounts of neutral, but polar lipids like cholesterol sulfate and glucosyl ceramides. In contrast, the keratinized cells contain large amounts of acylceramides and ceramides [12,13]. The thickness of buccal epithelium varies with location and typically ranges from 500 to 800 μm in humans, dogs, and rabbits. The estimated cell turnover time is five to six days [14]. In addition, the buccal epithelium is characterized by the presence of intercellular gap junctions.

Basement Membrane

The basement membrane (BM) is a continuous layer of extracellular materials, forming a boundary between the basal layer of the epithelium and the connective tissues of the lamina propria and the submucosa. It can be subdivided into the lamina lucida,

the lamina densa, and a sublayer of fibrous material. The BM functions include providing adherence between the epithelium and underlying connective tissues, mechanical support for the epithelium, and a barrier to the passage of cells and some large molecules.

Connective Tissues

The connective tissues consist of lamina propria and submucosa, if present. The lamina propria is a continuous sheet of connective tissue composed of blood capillaries and nerve fibers serving the oral mucosa.

Vascular drainage from the oral mucosa is principally by the lingual, facial, and retromandibular veins. These veins open into the internal jugular vein and thus avoid first-pass metabolism.

The buccal mucosae from monkeys, apes, dogs, pigs, and rabbits exhibit physiology similar to human buccal mucosa [14].

Permeability

The permeability of buccal mucosa is intermediate between that of the skin epidermis and intestinal mucosa.

Permeability Barriers

Epithelium. The predominant barrier to drug diffusion resides approximately within the outermost one-third of the epithelium. This is true of both keratinized and nonkeratinized epithelia. Therefore, keratinization is unlikely to offer major resistance to buccal permeation.

Membrane Coating Granules (MCG). The MCGs are spherical or oval organelles (100–300 nm in diameter) found in the intermediate cell layer of many stratified epithelia. The granules are found both in keratinized as well as nonkeratinized epithelia, but are different with regard to composition. The MCGs discharge their contents into the intercellular space forming the permeability barrier.

Permeant Factors

The permeation of a drug molecule across the buccal mucosa is dependent on the

- Molecular size. For hydrophilic substances, as molecular weight and molecular size and radius increase, permeability typically diminishes. Small molecular weight permeants (MW < 100 Da) are rapidly transported through the buccal mucosa.
- Lipid solubility. For nonionizable compounds, as the lipophilicity increases, the drug permeability typically increases. To maximize the absorption rate, a drug should be available in the salivary film at its solubility limit.

- Ionization. For ionizable drugs, maximal permeation occurs at the pH where ionization is minimal, where the drug is predominantly in the unionized form [15,16].

The rate of drug absorption for the transcellular route is pH dependent. Such dependency results from the fact that the membrane–aqueous partition coefficient for an ionizable drug is pH dependent.

Basement Membrane

The BM has an enormous surface area compared to the epithelium due to connective tissue papillae on which the effective diffusional path length may depend.

Mechanism of Drug Transport

The major pathway of drug transport across buccal mucosa seems to follow simple Fickian diffusion [17]. Passive diffusion occurs in accordance with the pH-partition theory. Considerable evidence also exists in the literature regarding the presence of carrier-mediated transport in the buccal mucosa [18,19]. Examination of Eq. (1) for drug flux,

$$J = \frac{DK_\mathrm{p}}{h} \Delta C_\mathrm{e} \tag{1}$$

where J = drug flux, D = diffusivity, K_p = partition coefficient, ΔC_e = concentration gradient, and h = diffusional path length, shows that the flux may be increased by decreasing the diffusional resistance of the membrane by making it more fluid, by increasing the solubility of the drug in the saliva immediately adjacent to the epithelium, or by enhancing lipophilicity through prodrug modification. Because of the barrier properties of the tight buccal mucosa, the rate-limiting step is the movement of drug molecules across the epithelium.

The two pathways of permeation across buccal mucosa are:

- Transcellular in which the passage of drug occurs through the individual cells of the mucosa and which appears to be the preferred pathway for drugs with a large partition coefficient, and
- Paracellular, in which the passage of drug occurs through intercellular junctions of the mucosa. The paracellular flux of drug molecules usually does not depend on the partition coefficient.

Permeability coefficients typically range from 1×10^{-5} to 2×10^{-9} cm/s for oral mucosae [20]. The pathway of drug transport across oral mucosa may be studied by

microscopic techniques using fluorescent dyes [20], autoradiography [14], or confocal laser scanning microscopic procedures [14].

Factors Affecting Systemic Oral Mucosal Delivery

Membrane Factors

Regional differences in both permeability and thickness affect both the rate and extent of drug reaching the systemic circulation [21]. Keratinization and composition, although not major factors, of the various oral mucosae affect systemic mucosal drug delivery. Approximately 50% of the total surface area of the mouth consists of keratinized tissue containing neutral lipids such as ceramides [22], whereas only 30% of the total surface area is composed of nonkeratinized tissue containing predominantly polar lipids such as cholesterol sulfate and glucosylceramides [22]. The basement membrane has an enormous surface area compared to the epithelium due to the presence of connective tissue papillae which may affect the effective diffusional path length [2]. Additional factors like absorptive membrane thickness, blood supply, blood/lymph drainage, cell-renewal rate, and enzyme content govern the rate and extent of drug absorption into the systemic circulation.

Environmental Factors

Saliva

Saliva contains 99% water and has a pH of 6.5–7.5, depending on the flow rate and location [23]. Salivary secretion may be stimulated by chewing paraffin wax, applying citric acid to the tongue, or administering systemic pilocarpine or other cholinergic drugs [24]. Production is also influenced by hormones, salivary gland disease, and changes in the plasma concentration of electrolytes [24]. These factors increase the salivary flow rate, leading to the secretion of watery saliva. Such stimulated salivary secretion, in turn, affects the film thickness and aids in easy migration of test compounds from one region of the mouth to another. Salivary pH is also important since passive diffusion of unionized drug is the major mechanism of oral absorption [25,26].

Salivary Glands

Stimulation of saliva production is under sympathetic and parasympathetic control. Parasympathetic stimulation produces a serous watery secretion, whereas sympathetic stimulation produces much thicker saliva. Drug delivery systems, therefore, should not be placed over a duct or adjacent to a salivary duct, as this may dislodge the retentive system or may result in excessive wash-out of the drug or rapid dissolution/erosion of the delivery system making it difficult to achieve high local drug concentrations. If a retentive system is placed over salivary ducts, the reduced salivary flow rate may produce less or no mucus which is required for the proper attachment of a mucoadhesive delivery device.

Movement of the Oral Tissues

Talking, eating, and swallowing may cause some mouth movement leading to dislodgment of the delivery device [3]. The movement of the tongue may influence the drug delivery from a mucoadhesive, retentive system due to the tongue swiping across the dosage form and adjacent tissues as well as the induction of suction pressures from the tongue pressing against the hard palate.

Dosage Form Design

A mucoadhesive buccal drug delivery system should

1. Be convenient to apply and unobtrusive when in place,
2. Be free of bitter-tasting constituents,
3. Have a smooth surface rather than a textured surface,
4. Preferably achieve unidirectional release of the drug, and
5. Utilize nontoxic excipients (both diluents and the mucoadhesive polymers) which do not irritate or damage the mucosa and do not stimulate salivary secretion.

The size of the delivery system varies with the type of formulation; a buccal tablet may be approximately 5–8 mm in diameter, whereas a flexible buccal patch may be as large as 10–15 cm^2 in area. Mucoadhesive buccal patches with a surface area of 1–3 cm^2 are most acceptable [27]. It has been estimated that the total amount of drug that can be delivered in one day across the buccal mucosa from a 2-cm^2 system is approximately 10–20 mg [20]. The shape of the delivery system may also vary, although for buccal drug administration, an ellipsoid shape appears to be preferred [27]. The thickness of the delivery device is usually restricted to only a few millimeters, and its location also needs to be considered. A mucoadhesive retentive system is preferred over a conventional dosage form. In comparison to sublingual, which is constantly bathed by saliva, the buccal mucosa seems to be an attractive alternative as it can be easily accessed. A bioadhesive buccal patch would appear to be the most appropriate delivery system because of its flexibility and the area of the buccal mucosa available for its application. The maximal duration of buccal drug retention and absorption is approximately 4–6 h as meal intake and/or drinking may require the removal of the delivery device.

Bioadhesion

The term bioadhesion can be defined as the ability of a material (synthetic or natural) to ''stick'' (adhere) to a biological tissue for extended periods of time [27]. The phenomenon of bioadhesion can be visualized as a two-step process. The first step involves the initial contact between polymer and the biological tissue. The second step is the formation of secondary bonds due to noncovalent interactions. The strength of bioadhesion (expressed as the force of detachment) for a novel oligosaccharide gum (*Hakea Gibbosa*) contained in a buccal tablet developed by Alur et al. [28,29]

appeared to be saturable both as a function of time and the amount of the bioadhesive polymer in the formulation (Figs. 2 and 3). However, the force of detachment for this mucoadhesive buccal tablet containing Hakea seemed to increase linearly as a function of the applied force of attachment of the dosage form to the tissue (Fig. 4) [28,29]. This may be due to the finite number of contact points of the retentive system with the mucosa and through which it may interact with the mucus. Therefore, the maximum strength of bioadhesion is also dependent on the area of contact between the mucoadhesive system and the biological membrane.

Biomembrane Characteristics

Oral mucosae are covered with mucus which serves as a link between the adhesive and the membrane. Mucus is a glycoprotein consisting of a large peptide backbone with pendant oligosaccharide side chains. The side chains terminate in sialic or sulfonic acids, L-fucose, sulfated galactose, or N-acetylglucosamine. The glycoprotein component imparts the viscous or gel-like (non-Newtonian) nature due to its capacity
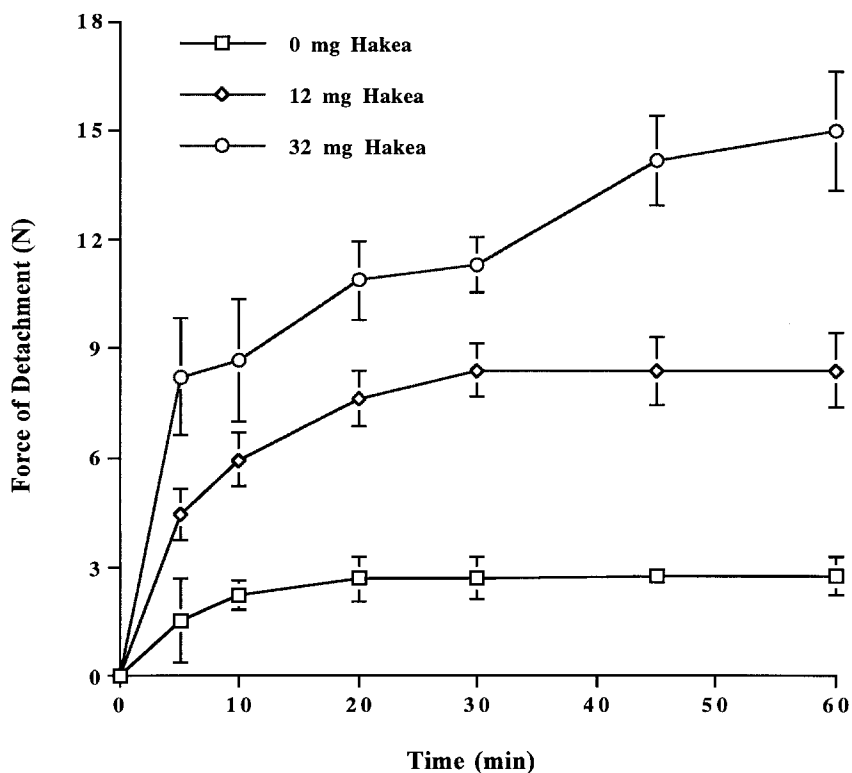
FIG. 2. The force of detachment from excised rabbit intestinal mucosa for directly compressed buccal tablets which contained 40 μg sCT (salmon calcitonin) and zero Hakea (—□—), 12 mg Hakea (—◊—), or 32 mg Hakea (—○—). All data points represent the mean value ± standard deviation of five experiments. Lines through mean values are included to illustrate the trend and do not represent a mathematical fit of the data (from Ref. 28).

$y = 1.811x^{0.234} \quad r^2 = 0.974$ □   **5 N**

$y = 2.679x^{0.204} \quad r^2 = 0.974$ ◇   **10 N**

$y = 4.792x^{0.111} \quad r^2 = 0.954$ ○   **15 N**

$y = 5.278x^{0.115} \quad r^2 = 0.850$ △   **20 N**
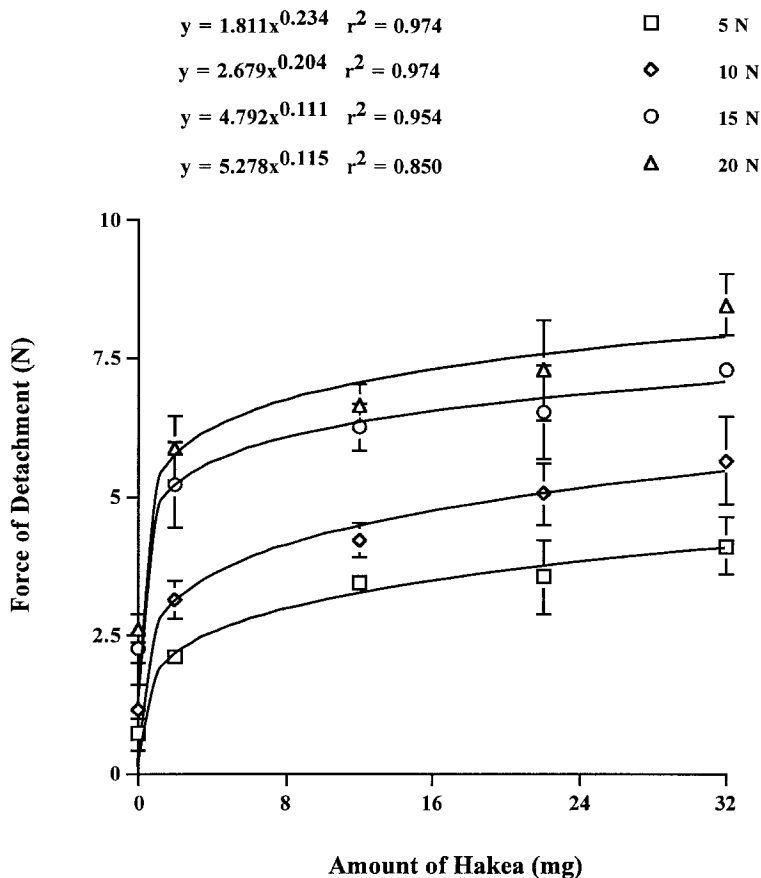
FIG. 3. The force of detachment from excised rabbit intestinal mucosa for directly compressed buccal tablets which contained 40 mg CPM (chlorpheniramine maleate) and 0, 2, 12, 22, and 32 mg Hakea applied to the mucosa with a force of 5 N (—□—), 10 N (—◇—), 15 N (—○—), and 20 N (—△—). All data points represent the mean value ± standard deviation of ten experiments. Lines through mean values are included to illustrate the trend and represent a mathematical fit of the data as well (from Ref. 29).

to retain 40 times its weight in water. Oligosaccharide chains are covalently linked to the hydroxyamino acids, serine and threonine, along the peptide backbone. Mucin is a polyelectrolyte under neutral or slightly acidic conditions because of the terminal sialic acid residues having a pK_a of 2.6 [30]. At physiological pH (7.4), the mucin molecule is polyanionic which contributes to bioadhesion.

Adhesive Characteristics

A variety of polymers including water-soluble and insoluble, ionic and nonionic hydrocolloids, and water-insoluble hydrogels can be used in bioadhesive systems [31]. The bioadhesive properties of the polymer are affected by the:

$$y = 0.137x - 0.035 \quad r^2 = 0.960$$
$$y = 0.268x + 0.740 \quad r^2 = 0.964$$
$$y = 0.232x + 2.225 \quad r^2 = 0.935$$
$$y = 0.242x + 2.625 \quad r^2 = 0.983$$
$$y = 0.294x + 2.695 \quad r^2 = 0.995$$

□ 0 mg
◇ 2 mg
○ 12 mg
△ 22 mg
■ 32 mg
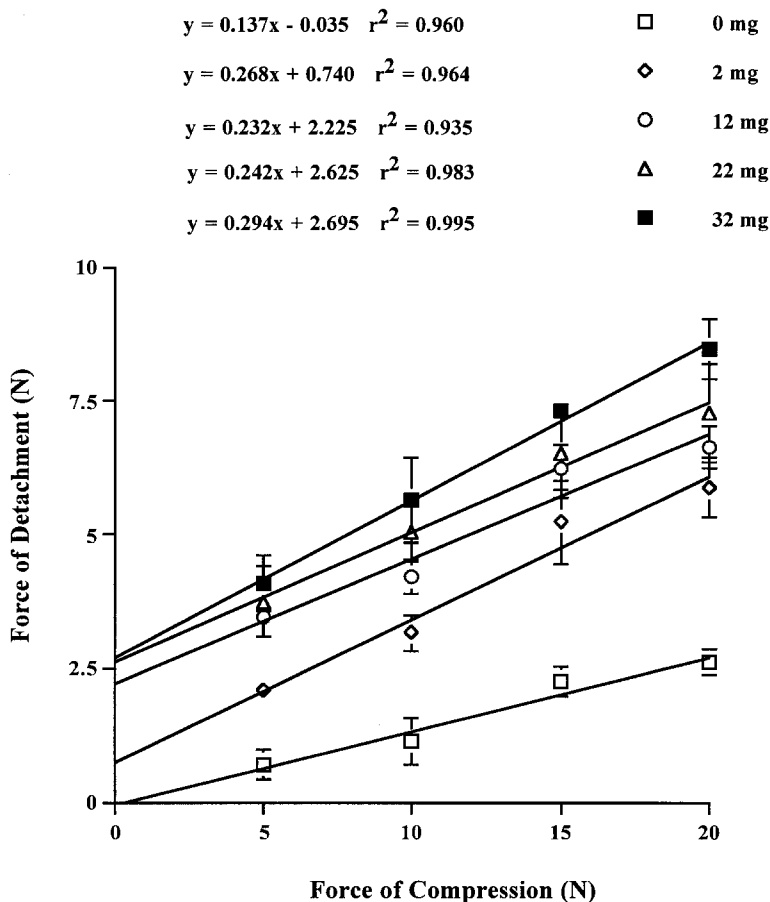
FIG. 4. The force of detachment from excised rabbit intestinal mucosa for directly compressed buccal tablets which contained 40 mg CPM (chlorpheniramine maleate) and zero Hakea (—□—), 2 mg Hakea (—◇—), 12 mg Hakea (—○—), 22 mg Hakea (—△—), and 32 mg Hakea (—■—) applied to the mucosa with a force of 5, 10, 15, and 20 N. All data points represent the mean value ± standard deviation of ten experiments. Lines through mean values are included to illustrate the trend and represent a mathematical fit of the data as well (from Ref. 29).

- Molecular weight and polymer conformation. In general, adhesive strength of a polymer increases with molecular weight above 100,000 [32]. The molecule must have adequate length to allow chain interpenetration into the mucus layer. However, the size and conformation of the polymer molecule plays an important role as well [33].
- Cross-linking density of the polymer. The strength of mucoadhesion decreases with an increase in cross-linking as this leads to a decrease in the polymer's diffusion coefficient [34] and in chain segment flexibility and mobility (which in turn reduces interpenetration).
- Charge and ionization of the polymer. Anionic polymers provide better efficiency than cationic or uncharged polymers with respect to both adhesiveness and toxicity [35]. Furthermore, polymeric adhesives with carboxyl groups are preferred over those with sulfate groups [36].

- Concentration of the polymeric adhesive. In general, the more concentrated the polymeric adhesive, the lower its bioadhesive strength. The coiled molecules become solvent poor in a concentrated solution which, in turn, reduces the available chain length for interpenetration into the mucus layer. Therefore, a critical concentration of the polymeric adhesive is required for optimum bioadhesion [37].
- Medium pH. The pH influences the charge on the surface of the mucus and the polymer [38]. Charge density on the surface of mucus varies with pH due to the differences in dissociation of the functional groups on the carbohydrate and amino acid moieties.
- Hydration of the polymer. Swelling affects bioadhesion [39], although an increase in swelling does not necessarily always result in an increase in the bioadhesive strength. However, a sufficiently high water activity is required to hydrate the mucoadhesive component to expose the bioadhesive site(s) for secondary bond formation, to expand the gel to create pores of sufficient size, and to mobilize all flexible polymer chains for interpenetration. A critical degree of hydration of the mucoadhesive polymer is needed for optimum bioadhesion [31]. A high degree of hydration lowers the adhesive strength due to the formation of a slippery mucilage [2].

Theories of Bioadhesion

In general, both physical and weak chemical bonds are responsible for mucoadhesion. Physical/mechanical bond formation can be explained as the entanglement of the adhesive polymer and the extended mucin chains. When this diffusion is mutual, it leads to maximum bioadhesive strength.

Chemical bonds may be primarily due to covalent bonding, or secondarily due to electrostatic, hydrogen, or hydrophobic bonding. Electrostatic or hydrogen bonding results primarily because of hydroxyl ($-OH$), carboxyl ($-COOH$), sulfuric acid ($-SO_3H$), and amino ($-NH_2$) groups. Hydrogen bonding energy is approximately 25 kJ/mol (6 kcal/mol). In bioadhesion, hydrophobic bonding is considered more important than hydrogen bonding. Nonpolar groups associate with each other as they are excluded from water. Van der Waals binding energies range from 4.18 to 41.8 kJ/mol (1–10 kcal/mol). Several theories of bioadhesion have been proposed, such as wetting, diffusion, electronic, fracture, and adsorption. The mechanism of bioadhesion appears to be best explained by a combination of the wetting, diffusion, and electronic theories.

Measurement of Bioadhesion

Measurement of bioadhesion not only helps in screening the candidate polymer, but to study the mechanism of bioadhesion as well. However, performance of the final dosage form containing the polymer and the drug is the best test.

In Vitro Measurements

Measurement of tensile or shear stress is the most commonly used in vitro method to determine bioadhesion. All in vitro measurements provide a rank order of bioadhesive strength for a series of candidate polymers. Measurement of tensile strength involves quantitating the force required to break the adhesive bond between the test polymer

and a model membrane. This method typically employs a modified balance or tensile tester. A section of freshly excised rabbit stomach tissue with the mucosal side exposed is secured on a weighed glass vial and placed in a beaker containing USP-simulated gastric fluid. Another section of the same tissue is secured onto a rubber stopper by a vial cap with the mucus side exposed (Fig. 5). A small quantity of the test polymer is placed between the two mucosal tissues and the force required to detach the polymer from the tissue is recorded [40]. Measurement of shear strength involves quantitating the force that causes the polymer to slide in a direction parallel to the plane of contact between the polymer and the mucus. A glass plate is suspended from a microbalance on which the test polymer is coated (Fig. 6) and dipped in a temperature-controlled mucus sample. The force required to pull the plate out of the mucus sample is determined under constant experimental conditions [33].

Other in vitro methods include the determination of the weight needed to break the adhesion [41], the fluorescent probe [35], the flow channel [42], mechanical spectroscopy [43], the falling film [44], colloidal gold staining [45], viscometry [46], the thumb test [47], the adhesion number [47], and electrical conductance [47].

In Vivo Measurements

In vivo methods, which are few, measure the residence time of bioadhesives at the application site [47]. Techniques like γ-scintigraphy, the perfused intestinal loop and radiolabeled transit studies using ^{55}Cr-labeled bioadhesive polymer [48] and ^{99m}Tc-labeled polycarbophil [49] have been employed for this purpose.

Bioadhesive Polymers in Buccal Drug Delivery

A variety of water-soluble and water-insoluble polymers of both synthetic and natural origin [31] have been studied as bioadhesives. These polymers are used mainly to
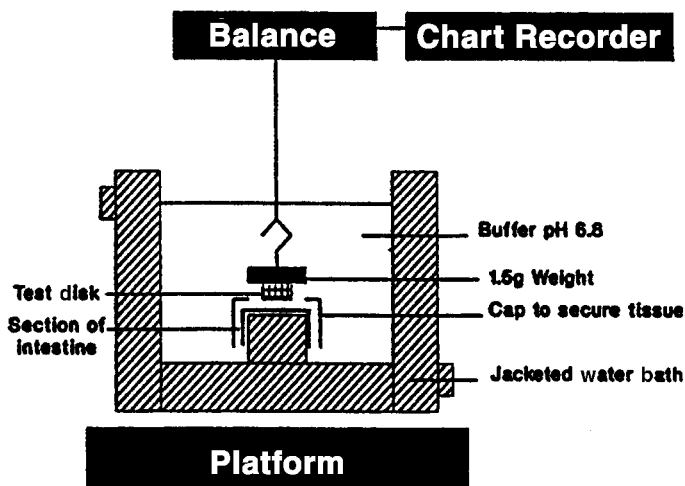
FIG. 5.   Schematic diagram showing the apparatus and setup for measuring tensile strength (from Ref. 104).
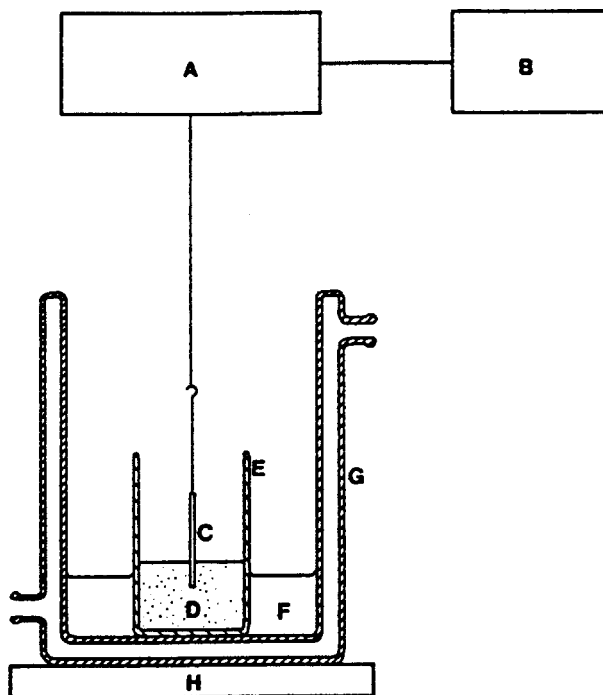
FIG. 6. Schematic diagram showing the apparatus and the setup for measuring shear strength (from Ref. 33). Key: A, microforce balance; B, chart recorder; C, glass plate (sideways); D, 1 mL homogenized mucus; E, glass vial; F, water; G, water jacket at 20°C; H, platform moving in vertical direction.

overcome the short residence time of the drug and the dosage form, improve localization of the drug, and achieve controlled or sustained release of the drug. Bioadhesive polymers can be divided into three categories:

- ''Wet'' adhesives, that is, polymers that become sticky upon hydration,
- Polymers that are electrostatic in nature and adhere primarily due to nonspecific and noncovalent interactions, and
- polymers that can bind to a specific site on the cell surface [50].

An ideal bioadhesive should be nontoxic, nonabsorbable, and nonirritating to the mucus membrane, form a strong noncovalent bond with the mucin–epithelial cell surfaces, allow easy incorporation of drug and should not offer hindrance to drug release, and should not decompose on storage or during the shelf-life of the dosage form. Some of the other desirable characteristics of the polymer have been discussed under bioadhesion.

Drug release from soluble polymers is accompanied by the gradual erosion-type dissolution of the polymer. Therefore, polymer dissolution and drug diffusion may be the overall hybrid mechanism of release. Drug release from nonsoluble hydrogels generally follows Fickian or non-Fickian diffusion kinetics [51]. The mechanism of

release may be determined by modeling the drug release (first 60%) to the empirical Eq. (2),

$$\frac{M_t}{M_\infty} = kt^n \tag{2}$$

where M_t/M_∞ = fraction of drug released, k = kinetic constant, t = time, and n = diffusional exponent. The mechanism of drug release may be Fickian diffusion when the value of $n = 0.5$, anomalous (non-Fickian) transport when $n = 0.5 < n < 1.0$, and case-II transport when $n = 1.0$. A value of n above 1 signifies super case-II transport as the mechanism of drug release [52].

Dosage Forms

Several biomucoadhesive dosage forms have been developed and the mechanism(s) of bioadhesion have been elucidated.

Buccal Dosage Forms

Buccal mucosa presents a relatively smooth and immobile surface for the placement of a bioadhesive dosage form. The amount of drug that can be incorporated is limited by the size limitation of the buccal dosage form. In general, a drug with a daily requirement of 25 mg or less is suitable for buccal delivery. Drugs with short half-lives, requiring sustained and controlled delivery, with poor aqueous solubility, which are sensitive to enzymatic degradation may be successfully delivered across the buccal mucosa. The dosage forms developed for this purpose include tablets, adhesive patches, adhesive gels, and adhesive ointment. Adhesive tablets and patches can be formulated to release the drug unidirectionally or multidirectionally by varying the extent and permeability of the backing (Fig. 7).

Adhesive tablets are more ''user-friendly'' than conventional tablets and allow drinking and talking without much discomfort. Some of the disadvantages include inflexibility, a rather small surface area for contact with the buccal mucosa, and on demand, drug delivery cannot be accelerated [53].

Adhesive patches are preferred over adhesive tablets because they are flexible and may be formulated in any shape. The size of the patches may vary from 1 to 15 cm^2. Small patches are more convenient and comfortable to wear [27].

Adhesive gels may provide greater bioavailability by allowing a longer residence time than solutions. Adhesive gels may be preferred for local therapy [54,55].

Adhesive ointments have not been investigated as extensively as have adhesive tablets or adhesive patches. Nevertheless, attempts have been made to utilize this dosage form primarily for local therapy [56].

Sublingual Dosage Forms

The sublingual route is more suitable for the delivery of drugs which require a rapid onset of action. The sublingual region, although more permeable than the buccal
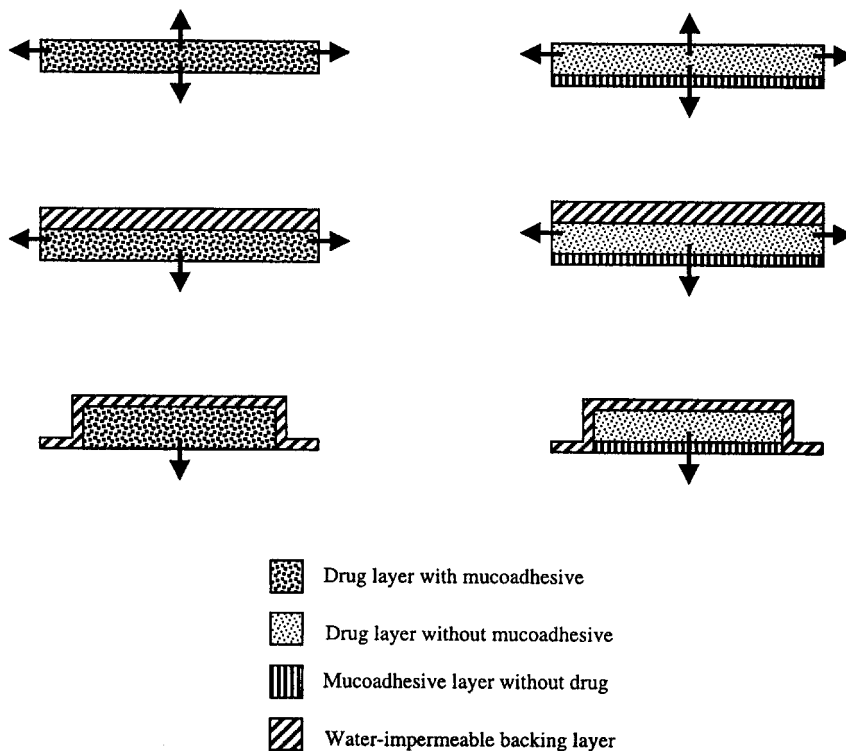
FIG. 7. Schematic diagram showing the geometric designs of buccal delivery devices (from Ref. 97).

region, is not suitable for retentive systems because of the physical structure and mobility of this area, and the dosage form being continuously bathed by saliva. Many attempts have been made to deliver drugs sublingually [37].

Dental-Gingival Dosage Forms

Denture adhesives have been used to deliver drugs in a sustained fashion. Because of the disadvantages such as nausea, damage to the prosthesis, and short and variable duration of action, their use has been limited [57]. Gingival plasters have been tried for gingival drug delivery or local therapy [58]. These formulations seem to be well tolerated and do not give any signs of irritation.

Formulation Development of Buccal Dosage Forms

Buccal dosage forms can be of the tablet, patch, gel, or ointment type and can be employed for local or systemic delivery. For local delivery, conventional dosage forms such as solutions and various types of tablets (immediate release, effervescent, etc.) are more suitable. These forms generally have uncontrolled drug release with subsequent variable absorption and short residence times, and may not provide sufficient bioavailability. Novel dosage forms such as adhesive tablets, patches, gels, and

ointments have been developed primarily for systemic delivery of therapeutic agents. These dosage forms are also capable of providing sustained drug delivery.

Buccal dosage forms can be of the reservoir or the matrix type. Formulations of the reservoir type are surrounded by a polymeric membrane, which controls the release rate. Reservoir systems present a constant release profile provided (1) that the polymeric membrane is rate limiting, and (2) that an excess amount of drug is present in the reservoir. Condition (1) may be achieved with a thicker membrane (i.e., rate controlling) and lower diffusivity in which case the rate of drug release is directly proportional to the polymer solubility and membrane diffusivity, and inversely proportional to membrane thickness. Condition (2) may be achieved, if the intrinsic thermodynamic activity of the drug is very low and the device has a thick hydrodynamic diffusion layer. In this case the release rate of the drug is directly proportional to solution solubility and solution diffusivity, and inversely proportional to the thickness of the hydrodynamic diffusion layer.

In a matrix type system, the drug is uniformly dispersed in the polymer and drug release is controlled by the matrix. Drug molecules dispersed in the polymer have to dissolve in the medium and then diffuse through the polymer network. Therefore, a drug dispersion and drug depletion zone always exists in the matrix. A thin hydrodynamic diffusion layer also exists at the interface of the drug and the matrix. A matrix system may result in a constant release profile only at early times when the drug depletion zone is rather insignificant.

The parameters that determine the release rate of a drug from a delivery device include polymer solubility, polymer diffusivity, thickness of the polymer diffusional path, and the aqueous solubility, partition coefficient, and aqueous diffusivity of the drug. Finally, the thickness of the hydrodynamic diffusion layer, the amount of drug loaded into the matrix, and the surface area of the device all affect the release rate.

Adhesive Tablets

Adhesive tablets may be monolithic or multilayered devices. Monolithic tablets can be prepared by the conventional techniques of direct compression or wet granulation. These tablets are capable of holding large amounts of drug. Unidirectional drug release may be achieved by a partial compression or spray coating of every face, except one that is in contact with the mucosa, with a water-impermeable material such as cellophane, hydrogenated castor oil, teflon, or ethylcellulose. Multilayered tablets may be prepared by adding each formulation ingredient layer by layer into a die and compressing it on a tablet press (Fig. 7). These tablets can be designed to deliver drugs systemically or locally. For multilayered tablets, incorporation of the drug into the adhesive layer which is immediately adjacent to the mucosal surface may aid in optimizing bioadhesion.

Adhesive Patches

Adhesive patches may also be monolithic or multilayered devices of the reservoir or matrix type for either systemic or local drug delivery (Fig. 8). The two main manufacturing processes to prepare adhesive patches are solvent casting and direct milling (with or without a solvent). The intermediate product is a sheet from which
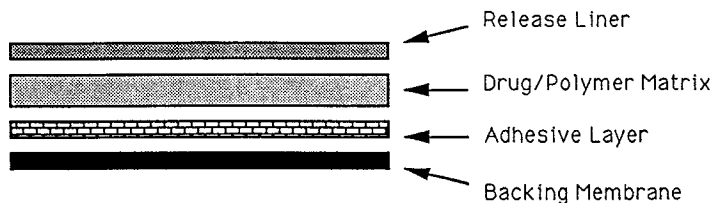
FIG. 8. Schematic diagram showing the design of mucoadhesive buccal patch (from Ref. 105).

patches are punched. A backing is applied to control the direction of drug release and minimize deformation and disintegration of the device during its residence in the mouth. Preparation of adhesive patches by the solvent casting method involves casting volumes of appropriately prepared aqueous solutions of polymer (for drug-free patches) or of a drug/polymer mixture onto a backing layer sheet mounted on top of a stainless steel plate by means of a frame. The assembly may be dried by perfusing with a thermostated stream of water or by air-drying. The temperature is typically selected according to the formulation exicipients. Upon complete drying, the laminate may be cut into the desired shape and size using a suitable punch and a die set.

In the preparation of adhesive patches by direct milling on a two-roll mill, the drug and the bioadhesive are homogeneously mixed with or without the aid of a solvent. The polymer/drug mixture may then be compressed to its desired thickness and patches of appropriate size may be cut or punched out. The polymer/drug mixture prepared with a solvent may require an additional drying step by air or in an oven.

Design of Experiments

A formulation may be evaluated for both in vitro and in vivo release and for permeation by the appropriate experiments.

In Vitro Release and Permeation Studies

In vitro release studies are designed according to the shape and application of the dosage form because no standard method is available. A survey of the literature indicates that the apparatus used for release studies varied from a typical USP-type I/II apparatus to a rotating basket immersed in a beaker with agitation conditions being rotational (50 to 250 rpm), to magnetic stirring, or mechanical shaking [50]. A variety of testing media have been used such as distilled water, chloroform, phosphate buffer, saline, a mixture of methanol and water, and others [50]. In vitro release studies provide valuable information regarding the behavior of the delivery device and the mechanism of drug release.

In vitro permeation studies employ a glass diffusion cell with the buccal tissue mounted between the two halves of the cell which may be filled with constantly stirred buffer solutions. The buccal mucosa is excised from the canine, porcine, or rabbit cheek immediately after sacrifice. Permeation studies may provide meaningful results on the simultaneous processes of drug transport and metabolism in the tissue

[59]. Attention must be paid to the viability of the excised tissue under in vitro conditions. Electrophysiologic characterization appears to be a valuable tool to indicate the viability of the tissue following excision. The viability of the tissue can be retained by continuous bubbling with O_2/CO_2 mixtures and addition of glucose to the buffer medium [11].

In Vivo Absorption Studies

Only a small number of absorption studies have been conducted on animals. This may be due to the fact that buccal administration of drugs to animals is difficult and often produces misleading results [60]. Animals selected as models should be representative of human absorption, tissue, enzymes, degree of keratinization, etc. Conscious or anesthetized animals may be used for absorption studies. Conscious animals should be preconditioned by receiving placebos for several consecutive days. The experimental dosage form may then be administered by application to the buccal mucosa. Absorption is followed by monitoring plasma and/or urinary drug concentrations as well as the amount of drug remaining in the dosage form following the experiment.

Human studies are conducted after the dosage form has been optimized with respect to release of the drug, shape of the dosage form, and amount of the drug to be incorporated, and after preclinical toxicity studies in animals have been completed. Human volunteers are asked to place the dosage form on the buccal mucosa and the absorption process may be followed by monitoring the amount of drug remaining in the dosage form after a certain time interval and/or by sampling biological matrices (blood, urine, etc.) with appropriate pharmacokinetic/pharmacodynamic analysis. The amount of drug remaining in the dosage form may reflect absorption only if the absorption step is not rate-limiting and an attempt to prevent swallowing a drug/saliva solution has been made [61].

Evaluation of Toxicity and Irritation

Irritation is very subjective and may differ widely from treatment to control subjects. Most irritation occurs as a result of penetration enhancers. Evaluation of toxicity and irritation should be concerned with mucosal tissue irritation, the extent of damage to the mucosal cells, and the rate of recovery.

Mucosal Tissue Irritation.   Irritation is a complex phenomenon involving interaction among the solution properties of the vehicle, mucosal transport, biological transport, and local drug disposition. No definite relationship has so far been established between the structure of a penetration enhancer and the degree of irritation it may cause following buccal application. However, a relationship between the pK_a of an ionizable compound (benzoic acid derivatives) and irritation as measured by the degree of erythema has been reported [63]. Azacycloalkanone enhancers demonstrated more irritancy with alkyl than those with alkenyl chains [64]. In general, it would appear that the most effective penetration enhancers induce the highest degree of irritation to mucosal tissues.

Extent of Damage to Mucosal Cells.   Permeation enhancement may result in a change in the protective permeability barrier by either an increase in the fludity of intercellular lipids (relatively nontoxic) and/or the extraction of intercellular lipids or denaturation of cellular proteins (much more damaging and toxic). Therefore, it is imperative that the permeation enhancer exert a reversible effect, is not systemically absorbed, and does not cause cumulative toxicity or permanent changes in the barrier properties. Application of up to 1% sodium lauryl sulfate or cetylpyridinium chloride to the ventral surface of the tongue of dogs resulted in desquamation, widening, and separation of keratin [65]. The buccal mucosa of rabbits treated with 0.5% sodium deoxycholate or 0.1% sodium lauryl sulfate demonstrated a loss of surface epithelial cells [66]. Sodium taurocholate and lysophosphatidyl chloride increased buccal insulin absorption in dogs with no mucosal irritation [67].

Assessment of Membrane Damage.   Several methods are commonly used to estimate the damage to biological membranes caused by various permeation enhancers. The following methods comprise a partial listing:

1. Morphological examination by scanning or transmission electron microscopy [68],
2. Morphological examination using light microscopy and appropriate staining (e.g., hematoxylin and eosin, H&E) [69],
3. Determination of the extent of hemolysis caused by a permeation enhancer [70],
4. Determination of the release of cellular constituents (e.g., lactate dehydrogenase, LDH) [71],
5. Measurements of the changes in the electrical resistance of the membrane [72],
6. Measurements of the changes in the permeability to various markers (e.g., inulin, mannitol, and FITC-dextran) [73], and
7. Measurements of changes in cilia movement (e.g. with nasal mucosa) [74].

Recovery of Mucosal Membranes.   There is no information available on the chronic effects of penetration enhancers on barrier function. The rate of recovery is generally inversely related to the extent of membrane damage. A greater and more rapid recovery is observed for permeation enhancers which induce minimal damage such as acylcarnitines [75] and sodium glycocholate [76] compared to enhancers like sodium deoxycholate [77] and polyoxyethylene-9-lauryl ether [76]. Permeation enhancers must be used with caution, especially if they affect intra- or extracellular calcium concentrations. The permeability of the tight junction is sensitive to the extracellular calcium concentration. Resealing of tight junctions has been shown to be accelerated by high extracellular calcium concentration rather than by elevated cytoplasmic calcium concentration [78,79].

Other Toxicity Concerns.   Additional toxicity concerns include interference with normal metabolism and function of mucosal cells, for example, water absorption by these cells [80]. The unconjugated bile acids are known to block amino acid metabolism [81] and glucose transport [82]. There is a possibility of biotransformation of these enhancers to toxic or carcinogenic substances by hepatic monooxygenases [83]. Absorption of permeation enhancers into the systemic circulation can also cause toxicity, for example, azone [84] and hexamethylene lauramide [85] which are absorbed

across the skin. Moreover, changes in membrane fluidity may alter the activity of membrane-bound transport proteins and enzymes [86].

Buccal Drug Delivery of Peptides and Proteins

Buccal drug delivery avoids acid- and enzyme-mediated degradation and hepatic first-pass metabolism. However, the bioavailability of therapeutic polypeptides and proteins is generally very low (<5%) due to low lipid solubility and their inherently larger molecular weight compared to conventional small molecules. Degradation of proteins and peptides by enzymes, such as aminopeptidases, carboxypeptidases, several endopeptidases, and several esterases, is also a reason for their low bioavailability [87]. A chronological survey of in vivo experiments performed on buccal absorption of proteins and peptides is given in Ref. [60]. The premise of passive transport as the mechanism of peptide and protein absorption across the buccal mucosa is widely accepted. Endocytotic processes are not apparent in buccal epithelium. No active or carrier-mediated peptide transport systems are present in the buccal epithelium except those that are responsible for the absorption of a few amino acids such as glutamic acid (anionic) and lysine (cationic) [88].

Biological Activity

Biological activity is the most important concern with the delivery of therapeutic peptides and proteins. Susceptibility of these molecules to denaturation by various manufacturing processes may seriously limit the methods that can be employed in the fabrication of delivery systems. Important process variables such as temperature, pressure, exposure to organic solvents, etc., during manufacturing need to be considered.

Temperature

High temperatures can break native S–S bonds and form new S–S bonds which can ''lock'' the protein into a denatured configuration [89]. Low pH, sodium dodecyl sulfate, Tween 80, chaotropic salts, and exogenous proteins have been used to protect proteins from thermal inactivation [90]. Ethylene glycol at 30–50% was used to protect the antiviral activity of β-interferon preparations [91]. Human serum albumin was used in recombinant human interferon-$β_{ser-17}$ which resulted in increased thermal stability [62]. Water-soluble polysaccharides such as dextrans and amylose [92], as well as point-specific (site-directed) mutagenesis [93] have also been used to increase thermal stability of therapeutic proteins and peptides.

Pressure

Proteins are not very sensitive to pressure changes, and only at high pressure do they undergo conformational changes when denatured by heat or changes in solution pH

[94]. A model enzyme (protein), urease, did not lose much of its activity until the compaction pressure exceeded 474 mPa (63 mm Hg) above which 50% of the relative activity was lost [95].

Pharmacokinetics and Pharmacodynamic Response

Pharmacokinetics and pharmacodynamic response have to be evaluated separately. Initial consideration for a drug candidate for buccal drug delivery is the low biological half-life ($t_{1/2}$) with factors like high first-pass metabolism and gastrointestinal degradation. It should be noted that both the t_{max} and C_{max} increase with an increase in the $t_{1/2}$ [96]. The fraction of the therapeutic peptide absorbed via the oral mucosa should not be calculated from pharmacodynamic response data alone since the efficiency of peptide absorption with respect to its pharmacodynamic response depends not only on the total dose absorbed, but also on the rate at which the peptide is taken up by the target organ. Furthermore, the relationship between pharmacodynamic response and the dose is sigmoidal in nature, that is, at a certain dose, the pharmacodynamic response reaches a plateau without further increase. Therefore, it may lead to an overestimation of the fraction of the peptide absorbed [28] unless a linear relationship of response and peptide concentration is generated.

Enzymatic Degradation

The proteolytic activity of the buccal mucosa presents a barrier against the delivery of proteins and peptides. Proteolytic enzymes include aminopeptidases, carboxypeptidases, and endopeptidases. Compared to the nasal and rectal routes, the buccal mucosa has low enzyme activity, and inactivation due to enzyme degradation is not as rapid with this route of drug administration [8]. Buccal homogenate studies may provide initial data concerning the rate and extent of biochemical degradation of peptides delivered by the buccal route [86]. The disadvantage of homogenate studies includes the inability to distinguish between cytosolic, membrane-bound, and intercellular proteolytic activity. Since protein and peptide transport can be either transor paracellular in nature, the exact location of these proteolytic enzymes is important.

A novel concept of using bioadhesive polymers as enzyme inhibitors has been developed [97]. Included are derivatives of polyacrylic acid, polycarbophil, and carbomer to protect therapeutically important proteins and peptides from proteolytic activity of enzymes, endopeptidases (trypsin and α-chymotrypsin), exopeptidases (carboxypeptidases A and B), and microsomal and cytosolic leucine aminopeptidase. However, cysteine protease (pyroglutamyl aminopeptidase) is not inhibited by polycarbophil and carbomer [97].

Enzyme Inhibitors

Protease inhibitors are generally employed as enzyme inhibitors [98]. These include aprotinin [99], bestatin [100], chondroitinase [101], and hyaluronidase [101]. According to several published reports, these inhibitors appear to lack adequate effectiveness when administered simultaneously with various peptides in vivo [99,101].

Permeation Enhancers

Enhancers proposed for potential use for the buccal route include those studied for transdermal delivery [102], such as dimethyl sulfoxide (DMSO), dimethylformamide, ethanol, propylene glycol, 2-pyrxolidones, decylmethyl sulfoxide, azone, sodium lauryl sulfate, oleic acid, bile salts, and nonionic surfactants (polysorbates). These enhancers fall into six categories [103]:

1. Chelators, e.g., EDTA, EGTA (ethyleneglycol tetraacetic acid)
2. Surfactants
   Nonionic, e.g., laureth-9, polysorbate 80, sucrose esters, dodecylmaltoside
   Cationic, e.g., cetylmethylammonium bromide
   Anionic, e.g., sodium dodecyl glycocholate, sodium lauryl sulfate
3. Bile salts and other steroidal detergents, e.g., sodium glycocholate, sodium taurocholate, saponins, sodium taurodihydrofusidate, sodium glycodihydrofusidate
4. Fatty acids, e.g., caprylic acid
5. Nonsurfactants, e.g., 1-dodecylazacycloheptane-2-one (azone), salicylates, sulfoxides
6. Enzymes, e.g., phopholipases, hyaluronidases, neuraminidase, chondroitinase ABC

Different mechanisms of absorption enhancement for these permeation enhancers have been proposed. Most permeation enhancers are thought to disrupt the lipid bilayer which increases membrane fluidity. Enhancers may interact with the polar head groups of the proteins in the lipid bilayer. It is suggested that this interaction causes the proteins to absorb additional water and separate, thus increasing paracellular transport. Some enhancers (nonionic, and presumably ionic surfactants) may solubilize and extract lipids [100]. However, extraction of membrane lipids is much more disruptive to membrane integrity than increasing membrane lipid fluidity. Hence, membrane lipids and membrane integrity are more slowly restored with permeation enhancers of this type [100]. Bile salts enhance absorption, stabilize enzyme-labile drugs, and potentially inhibit proteolytic degradation and aggregation of therapeutic proteins by formation of micelles [100]. Permeation enhancers that open tight junctions are of little benefit for oral mucosal drug delivery since tight junctions are rarely present in these tissues. Structure/absorption enhancement activity relationships have not been completely characterized for permeation enhancers. However, for surfactants, the structure of the polar head groups strongly influence the permeability. For example, it has been reported that, for surfactants, absorption enhancement was highest for ether-based surfactants rather than for esters with similar structure [100].

Summary

Recent years have witnessed an explosive growth in the understanding of the mechanisms associated with the absorption of drugs, especially therapeutic peptides and proteins. Scientists from a variety of disciplines continue to elucidate the variables associated with the optimal formulation and delivery of drugs via the oral mucosa. A greater understanding of the para- and transcellular route of drug absorption, pro-

teolytic enzyme activity that may potentially degrade therapeutic peptides, and metabolism of compounds during the mucosal transport process are essential to the development of buccal delivery systems. Moreover, methods to increase drug flux (e.g., permeation enhancers) without associated toxicity, strategies to inactivate proteolytic enzymes, and innovative approaches with regard to controlled drug delivery and mucoadhesive dosage forms will all improve the delivery of drug substances via the oral cavity.

Acknowledgement

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Pharmaceutical Quality Assurance Microbiology Laboratories

The Role of the Pharmaceutical Microbiologist in Establishing the Quality, Purity, Efficacy, and Safety of Pharmaceutical and Over-the-Counter Drug Products

The pharmaceutical microbiologist has an important role in product development, manufacturing process development and assuring control of microorganisms in the manufacturing environment, and routine raw material, in-process material, and product testing. The involvement of an experienced microbiologist in each stage of the product life cycle is important to maintain product quality. Typically, microbiologists are involved in formulation, manufacturing process development, and specification-setting decisions that can prevent microbial contamination of pharmaceutical products. In this article the author discusses what he believes is the appropriate level of involvement of microbiologists in establishing the quality, purity, efficacy, and safety of pharmaceutical and over-the-counter drug products and he will explore areas of future challenge to the pharmaceutical microbiologist.

A review of the regulations governing the pharmaceutical industry outlines some of the formal responsibilities of the microbiologist working in this industry. The U.S. Federal Regulations that govern the pharmaceutical industry are termed current Good Manufacturing Practices (cGMPs). The U.S. Federal Food and Drug Administration (FDA) was mandated by the 1962 New Drug Amendments to the United States Federal Food, Drug, and Cosmetics Act to promulgate regulations that have the force of law to assure that drug manufacturers maintain the safety, identity, strength, quality, and purity of their products. These official regulations were published the Federal Registrar, September 29, 1978 as 21 CFR Parts 211 through 226 [1] as they pertain to drugs. The regulations are considered minimum requirements, and failure to comply with any part of the regulations during the manufacture, processing, packaging, or holding of a drug causes that product to be adulterated under section 501(a) (2) (B) of the Federal Food, Drug, and Cosmetics Act. The drug product as well as the persons who are responsible for the failure to comply with the regulations, may be subject to regulatory action.

The sections of the cGMPs most pertinent to the pharmaceutical microbiologist include:

- Subpart B, Organization and Personnel
    - 211.22 Responsibilities of the quality control unit
    - 211.25 Personnel qualifications
    - 211.113 Control of microbiological contamination
- Subpart F, Production and Process Controls
- Subpart I, Laboratory Controls
    - 211.167 Special testing

The cGMP regulations require that a pharmaceutical manufacturer shall have an independent quality unit responsible for approval or rejection of all components, drug product containers, closures, in-process materials, packaging materials, labels and drug products, the review of production records for possible error, and the investigation of manufacturing deviations. The quality unit also reviews and approves all specifications and procedures impacting on the products and leads the investigation of manufacturing deviations and product failures.

The quality unit must have access to an adequate testing laboratory to aid in the approval of the materials under its control. One of these laboratory facilities would be suitably equipped and staffed to conduct microbiological testing. The quality unit need not manage the microbiology laboratory. The laboratory could be administered by quality control, research and development, and even manufacturing, or it could be a contract testing laboratory, provided its meets cGMP requirements and is responsive to the needs of the quality unit. Although not a regulatory requirement, it is industry practice to use the audit process as a tool to ensure that the microbiology laboratory meets all regulatory requirements and company internal policies.

The 211.25 Personnel qualifications requirement stating that each person engaged in all phases of pharmaceutical manufacture has the education, training, and experience to enable them to do their job and have a working knowledge of the cGMP regulations applies equally to laboratory personnel. For example, bench microbiologists should have a bachelor degree in microbiology or allied life sciences and be adequately trained in laboratory procedures and testing documentation conducted in the microbiology laboratory. They need to be assigned responsibilities in keeping with their level of skill and experience. Microbiologists with supervisory or managerial responsibilities need training in supervisory skills, scheduling, budgeting, laboratory investigations, technical report writing, pharmaceutical products, and the manufacturing processes. They need to understand the requirements of the quality unit and ensure that the unit is supplied with quality test results in a timely and cost-effective manner. Since the pressures to manufacture, test, and release products in a timely fashion can be considerable, they need to work well under pressure and enjoy team work.

The educational background of bench microbiologists, supervisors, and managers is now even more important, given the current transition from classical to nucleic acid-based testing methods.

The demands of microbiological testing require that the core educational background of the staff, supervisors, and managers be in microbiology. Training and experience in aseptic techniques is necessary. The author believes that the skill sets of chemists, pharmacists, and even biologists do not allow them to readily act effectively as microbiologists without extensive training. The course work invaluable to the pharmaceutical microbiologist includes:

- Isolation, enumeration, and identification of bacteria and fungi,
- Pathogenic microbiology,
- Microbial physiology and biochemistry,
- Introductory chemistry including organic, inorganic and physical chemistry and quantitative analysis,
- Introductory physics,
- Introductory mathematics,

- Introductory pharmaceutical manufacturing,
- Introductory statistics and probability, and
- Written and oral expression with emphasis on technical report writing.

According to 211.113 Control of microbiological contamination, pharmaceutical manufacturers need written procedures describing the systems designed to prevent objectionable microorganisms in both nonsterile and sterile drug products. All sterilization processes used to manufacture parenteral drugs need to be validated.

Prior to release, each batch of drug product needs a laboratory test to determine that the product conforms to specification, including the identity and strength of each active ingredient. Where sterility and/or pyrogen testing are conducted on specific batches of short-lived radiopharmaceuticals, batches may be released prior to completion of testing, provided testing is completed as soon as possible. The 211.165 ''Testing and release for distribution'' regulation states that there shall be appropriate laboratory testing, as necessary, of each batch of drug product required to be free of objectionable microorganisms. This implies that each and every batch of product need not be subject to microbial evaluation.

In the product development organization, the pharmaceutical microbiologist plays a major role in bringing safe products to the market. Typically, Research & Development (R&D) microbiologists are found in the analytical development group. Their role is to develop and validate the microbial tests that may be applied to the new pharmaceutical products to confirm that they are not contaminated by excessive numbers of microorganisms or objectionable microorganisms that may infect patients or degrade the quality of the product during its shelf life.

These tests would include compendial tests for Microbial Limits, Sterility, Bacterial Endotoxins, and Antimicrobial Effectiveness.

These compendial tests need to be developed and qualified for each new product prior to the Phase II clinical trials that involve 100 to 200 subjects. Considering the large investment to bring a new pharmaceutical drug product to market, estimated at 250 to 500 million U.S. dollars, it is important not to jeopardize the future of a new product or the subjects of the clinical trial by administering a drug product that may be contaminated with objectionable microorganisms. However, for the results of microbial testing to have any meaning, the microbial test needs to be qualified as suitable for use with each product.

Many dosage forms have active pharmaceutical ingredients or contain preservative systems that may inhibit the recovery of any bioburden associated with the product. This inhibition may be overcome by neutralizing the active ingredient or preservative system with the appropriate agents, by dilution to overcome the inhibition, or by a combination of both strategies. The USP Chapters ⟨51⟩ Antimicrobial Effectiveness Test, ⟨61⟩ Microbial Limit Tests, and ⟨71⟩ Sterility Testing [2] contain specific instructions on how to qualify the test for specific pharmaceutical drug products and the microorganisms to use during this process.

A major concern for pharmaceutical operations, that is, materials management, manufacturing, and quality assurance (QA), is whether the R&D microbiologists develop microbial tests that meet the QA requirements of robustness, simplicity, and standard for ease of testing for the routine release testing of pharmaceutical products. Good communications between the two microbiology groups ensures the smooth technology transfer of the most appropriate tests from R&D to QA.

During the development of the manufacturing process, an experienced microbiologist should be consulted as to the potential for microbial contamination. Issues may include the selection of appropriate pharmaceutical ingredients, the ability of the manufacturing steps to control microbial contamination, the validation of sterilization processes, the cleaning and sanitization of process equipment, the adequacy of the water system, the holding times for intermediates, training of personnel, and the design of the packaging.

Current Microbiological Testing Practices

The compendial microbial methods currently used for the routine testing of pharmaceutical products are generally conservative and may be used to resolve disputes concerning the microbial contamination of pharmaceutical products. The *US. Pharmacopoeia* (USP) is recognized as an official compendium by the United States Federal Food, Drug and Cosmetic (FDC) Act. The USP standards are used to determine the identity, strength, quality, and purity of pharmaceutical articles. The General Chapters section of the USP, numbered from ⟨1⟩ to ⟨999⟩, includes requirements for tests and assays; general information is found in Chapter ⟨1000⟩ and above. Of particular interest to the pharmaceutical microbiologist are the following USP 24 Informational Chapters: ⟨1116⟩ Microbiological Evaluation of Clean Rooms and other Controlled Environments; ⟨1111⟩ Microbiological Attributes of Pharmaceutical Articles; ⟨1225⟩ Validation of Compendial Methods; and ⟨1231⟩ Water for Pharmaceutical Purposes [3]. Testing chapters pertinent to pharmaceutical microbiology and their Japanese and European (JP and Ph. Eur.) counterparts are as follows:

1. USP Chapter ⟨51⟩ Antimicrobial Effectiveness Test

Antimicrobial preservatives are added to multiuse nonsterile liquids, ointments, and creams, and sterile injectable products to protect them from microbial contamination that may be introduced inadvertently during use of the product (postmanufacturing).

The test for antimicrobial effectiveness is used to demonstrate the effectiveness of any added antimicrobial preservative(s). Compendial references include: USP 24 Chapter ⟨51⟩; Antimicrobial Effectiveness Test; JP XIII; General Information 3, Preservatives-Effectiveness Tests; and the Ph. Eur. 3rd ed., Biological Tests, 5.1.3. Efficacy of Antimicrobial Preservation.

2. USP Chapter ⟨61⟩ Microbial Limits Tests

The tests for microbial limits and recommendations for microbial quality criteria of raw materials, excipients, drug substances, and pharmaceutical products have been established in pharmacopoeial compendia for over 30 years. These tests are listed in the USP 24 Chapter ⟨61⟩ Microbial Limits Tests; and in the Ph. Eur. 3rd ed., Biological Tests 2.6.12 and 2.6.13, Microbial Contamination of Products Not Required to Comply with the Test for Sterility (total viable count, tests for specified microorganisms); and the JP XIII 30 Microbial Limit Test.

3. USP Chapter ⟨71⟩ Sterility Test

The sterility test is applicable for determining whether drug substances, preparations, or other pharmacopeial articles are sterile as defined by the compendial method. A satisfactory result only indicates that no contaminating microorganisms have been found in the sample examined under the conditions of the test. Therefore, the result is a function of the efficiency of the adopted sampling plan. Compendial references to sterility testing include: USP 24 Chapter ⟨71⟩ Sterility Tests; the Ph. Eur. 3rd ed., Biological Tests 2.6.1, Sterility; and the JP XIII 45, Sterility Test.

4. General Informational Chapter ⟨1116⟩ Microbiological Evaluation of Clean Rooms and Other Controlled Environments

The microbiological monitoring of air, surfaces, and personnel in facilities used for sterile pharmaceutical manufacturing is discussed in the USP 24 Informational Chapter ⟨1116⟩ Microbiological Evaluation of Clean Rooms and Other Controlled Environments. This chapter also covers the design and implementation of a microbiological monitoring program, and suggests monitoring frequencies and microbiological acceptance criteria.

5. USP Informational Chapter ⟨1231⟩ Water for Pharmaceutical Purposes

Types of water, methods, and specifications for testing are listed in the USP Informational Chapter ⟨1231⟩ Water for Pharmaceutical Purposes. The USP also references *Standard Methods for the Examination of Water and Waste Water*, American Public Health Association (APHA), 19th ed., for specific test methods [4].

Other Testing Methods

New microbial testing methods are coming to the market place based on advanced technologies. These new tests represent improvements in the timeliness and quality of testing.

The USP 24 General Notices state that alternative methods may be used to determine that products comply with the pharmacopoeial standards for the advantages in accuracy, sensitivity, precision, selectivity, adaptability to automation or computerized data reduction, or any other special circumstances. Such alternative or automated methods shall be validated: However, when disputed, the compendial method is conclusive as it is the official or referee test. In addition, USP Chapter ⟨61⟩ Microbial Limit Tests states that automated methods may be substituted provided they are validated and give equivalent or better results, whereas USP Chapter ⟨71⟩ Sterility Tests states that alternative procedures may be employed to demonstrate that an article is sterile, provided the results obtained are at least of equivalent reliability.

It is not required to have prior FDA approval to use an alternative method to a compendial test. According to 21 CFR 314.70 ''Supplements and Other Changes to an Approved Application,'' the addition or deletion of an alternative analytical method does not require prior approval and may be filed in the *Annual Product Report*. However, the equivalency of the alternative method needs to be documented

and the validation report must be available for an FDA investigator to inspect at the manufacturing site. When the test method is particularly novel, it may be advisable to include the test in an NDA supplement for FDA review. The company can then get prior FDA approval before the new test method is implemented.

Organization of the Pharmaceutical Quality Assurance Microbiological Testing Laboratory

The microbiology manager has three major functions in the QA organization:

- Establishing, staffing, and running the microbiological testing laboratory,
- Monitoring pharmaceutical ingredients, water for pharmaceutical purposes, the manufacturing environment, and finished products submitted to the laboratory to demonstrate control of microbial contamination of the pharmaceutical products manufactured at the site, and
- Providing microbiological expertise to the QA organization to prevent microbial contamination.

The microbiology manager is responsible for the establishment of a suitably constructed and equipped laboratory, recruiting and retaining an appropriately educated, skilled, and experienced staff, and running the laboratory in compliance with company policies and cGMP regulations. The needs of business require that microbial testing be conducted in a timely fashion in order to release products to the market.

The microbiological monitoring program established at a pharmaceutical manufacturing site depends on the range of products manufactured at that site. Typical microbiological monitoring programs for release testing and environmental monitoring for nonsterile and sterile product manufacturing sites are given in Table 1.

The general procedures to be followed when selecting a microbial testing strategy within a marketed product stability program for pharmaceutical drug products based on cGMP and compendial requirements and commitments made in regulatory filings are given in Table 2.

These approaches are in general accord with CFR 21 Parts 211.113 Control of Microbiological Contamination, Section (b); 211.137 Expiration dating; 211.167 Special Testing Requirements, Section (a); USP 24 ⟨51⟩ Antimicrobial Effectiveness Testing, ⟨61⟩ Microbial Limit Tests, ⟨71⟩ Sterility Tests, ⟨85⟩ Bacterial Endotoxin Tests, General Informational Chapter ⟨1151⟩ Pharmaceutical Dosage Forms, and the June, 1998 FDA *Draft Guidance for Industry—Stability Testing of Drug Substances and Drug Products* [5]. The tests that may be included in the program include: antimicrobial effectiveness testing, microbial limit testing, sterility testing, bacterial endotoxin testing, and container-closure integrity testing (Table 2).

Antimicrobial Effectiveness Testing

The following principles apply to preservative effectiveness testing within a pre- and postmarketed product stability program.

TABLE 1 Microbiological Monitoring Programs

Dosage form	Monitoring	Frequency
Tablets and capsules	Pharmaceutical ingredients	Periodic after history is established; accepted on supplier certificate of analysis
	Purified water	Loop daily and taps weekly
	Manufacturing environment	Quarterly
	Products	Periodic after history is established due to low water activities of tablets and capsules
Topicals, otics, vaginal and rectal products	Pharmaceutical ingredients	As above
	Purified water	Loop daily and taps weekly
	Manufacturing environment	Weekly or monthly
	Products	Routine for products with high water activity. Periodic after history is established for product with a low water activity
Nasal sprays, inhalants	Pharmaceutical ingredients	As above
	Purified water	Loop daily and taps weekly
	Manufacturing environment	Daily or weekly
	Products	Routine for products with high water activity. Periodic after history is established for product with a low water activity
Injectable products, ophthalmic products, inhalation solutions	Pharmaceutical ingredients	As above
	Purified water	Loop and taps daily
	Manufacturing environment	Every shift in critical aseptic processing areas
	Products	Every batch with the exception of terminally sterilized products approved for parametric release

1. The selection of the preservative system for multiuse new products is the responsibility of the R & D formulation group. Typical shelf specifications are 80 to 120% of label specifications. The appropriate preservative system for the particular formulation should be demonstrated to be effective by microbial challenge to at least 75% and preferably 50% of the target concentration. It is recommended that during development the product be formulated with preservative concentrations of 100, 75, and 50% of the labeled amount and be subjected to antimicrobial effectiveness testing to determine the lowest effective preservative concentration.

2. The release and shelf-life specifications are established based on both the premarketed stability data for the preservative system concentration and the antimicrobial effectiveness test results.

TABLE 2 Microbial Testing for Marketing Stability

Dosage Form	Microbial Test[a]	Testing Plan	Test Intervals[b]
Tablets, powder- and liquid-filled capsules	Microbial limit test, TAMC, TCYMC only	Test development, scale-up and validation batches only	0, 6, 12, 24, 36 months
Topical liquids, ointment, creams	a. Microbial limit test, TAMC, TCYMC only b. USP AET for various use	$A_w < 0.75$ test development, scale-up and validation batches only; $A_w > 0.75$ all batches on stability for microbial limit; first three batches for AET	a. 0, 6, 12, 24, 36 months b. 0, middle of stability period and expiry
Vaginal creams, suppositories	Microbial Limit Test, TAMC, TCYMC only and AET	As above	0, 6, 12, 24, 36 months
Rectal creams, suppositories	As above	As above	0, 6, 12, 24, 36 months
Nasal sprays	As above	As above	0, 6, 12, 24, 36 motnhs
Inhalation sprays and aerosols	As above	As above	0, 6, 12, 24, 36 months
Ophthalmic ointments and solutions	Sterility test, CCI, AET	Test all batches on stability with the exception of the first three batches for AE[c]	0, 12, 24, 36 months
Injectables, Inhalation solutions	a. Sterility test, b. CCI, AET. c. Bacterial endotoxin test	Test all batches on stability with the exception of the first three batches for AE[c]	a. 0 only b. 0, 12, 24, 36 months c. 0 and expiry only

[a] Abbreviations: TAMC = total aerobic count; TCYMC = total combined yeasts and molds; AET = antimicrobial effectiveness test; CCI = container-closure integrity; A_w = water activity.
[b] Time intervals suggested in the 1998 FDA stability guide [5]. More annual test intervals can be added if the expiration dating exceeds 36 months.
[c] A stability-indicating presrvative assay can be justified only at all time intervals as a substitute for the USP Antimicrobial Effectiveness Test by confirming preservative efficacy at 50, 75, and 100% of the lower shelf-life specification.

3. If the study outlined above in paragraph 1 was not conducted during product development, it is recommended that QA and/or the technical services groups undertake a study to justify the current specifications and the elimination of routine antimicrobial effectiveness testing within the stability program.

4. All preservative systems for both parenteral and nonsterile dosage forms should meet the 3 log reduction at 14 days for bacteria, that is, USP category I requirements. Both EP and BP Antimicrobial Effectiveness Testing would be run only if specially requested by the marketing group.

5. Preservative effectiveness testing should be included at the 3, 12, 24, and 36 months intervals for pilot validation batches or for the first three commercial batches of a new product.

6. With subsequent batches, chemical assays would be only used to confirm the preservative level, since the effectiveness during shelf life is being demonstrated. If the formulation is changed, the preservative effectiveness must be verified with at least one batch throughout the shelf life.

7. The choice of additional challenge organisms used in the formulation development is determined by

- The range of activity of the preservative system, that is, if a preservative system has reduced activity against *Pseudomonas* spp. additional organisms from this genera or related genera could be added to the challenge organisms.
- The organisms considered objectionable for that product and dosage form, and
- The frequency of isolation of organisms from the manufacturing environmental and product monitoring.

8. Repeat challenges should be limited to the evaluation of preservative systems that cannot be improved because of formulation difficulties and limitations due to the intended site of use and products that may be misused during multiple consumer use.

Microbial Limit Test

The following principles apply to microbial limit testing within a pre- and postmarketed product stability program.

1. The inclusion of the routine microbial limit test in a marketed product stability protocol depends on the pharmaceutical dosage form. Typically, the test would be used only for nonsterile products, especially oral liquids, nasal sprays, and topical liquids, lotions, and creams that have sufficient water activity to support the growth of microorganisms. In contrast, tablets, powder- and liquid-filled capsules, topical ointments, vaginal and rectal suppositories, nonaqueous liquids and inhalation aerosols with a water activity too low to allow for the product to support the growth of microorganisms would not be routinely tested.

2. To establish a microbial-limit testing history, all development, clinical, scale-up and process validation batches of new nonsterile dosage forms would be tested to verify that the pharmaceutical ingredients, manufacturing process, and packaging does not contribute to the bioburden of the product. After the testing history has been

established, the stability protocol of products with a water activity below 0.75 should not include microbial limit testing.

Sterility Testing

The following principles apply to sterility testing within a pre- and postmarketed product stability program.

1. All injectable and ophthalmic products, with the exception of a terminally sterilized product subject to parametric release, shall be subject to sterility testing at release.

2. Since the sterility assurance of an injectable or ophthalmic products is established through media fill or sterilization validation for aseptically filled and terminally sterilized products, respectively, sterility testing has been included in past stability protocols as a measure of container-closure integrity of the product throughout its shelf life. If there is a continued need due to prior regulatory commitments to include sterility testing in a protocol, testing at release and expiry is recommended.

3. Whenever possible, container-closure integrity testing shall be substituted for sterility testing as recommended in the draft FDA stability guide.

Bacterial Endotoxin Test

The following principles apply to endotoxin testing within a pre- and postmarketed product stability program.

1. All injectable products should be tested for endotoxin at release.

2. Since, in the absence of bacterial growth in the product, the endotoxin level does not increase on storage during the shelf life, bacterial endotoxin testing is not indicated in stability protocol. If there is a continued need to include endotoxin testing in a protocol, testing at release and at expiry is recommended.

Container-Closure Integrity Test

The following principles apply to integrity testing within a pre- and postmarketed product stability program.

1. The integrity of the container-closure system as a microbial barrier should be assessed using a sensitive and adequately validated test.

2. One of a number of physical container-closure integrity tests may be selected and validated against the bacterial liquid immersion test. The physical leak test shall be correlated to bacterial ingress.

3. The physical container-closure integrity test method shall be chosen after consideration of the container-closure type, the performance criteria, and the available validated test methods.

4. Test methods described in the literature include the bubble method, helium mass spectrometry, liquid tracer (dye), head space analysis, vacuum and pressure decay, weight loss and gain, and high voltage leak detection [6].

5. The number of samples tested should reflect the sampling requirements provided in the USP ⟨71⟩ Sterility Tests.

6. The testing intervals shall be annually and at expiry.

Consulting and Testing Considerations

Supplying microbiological expertise to the manufacturing and quality assurance section is important, as a depth of experience in microbiology may sometimes be lacking within these departments in some companies.

Typically, the management of these organizations is more often trained in chemistry, engineering, business, or pharmacy. Microbiologist should assist management to exercise the best judgment on microbiological issues. Given that the ultimate objective is to prevent microbial contamination of pharmaceutical and OTC drug products, it is important that a pharmaceutical microbiologist is knowledgeable in the areas of microbiological testing, infectious diseases, compendial changes, regulatory issues, product formulation, and manufacturing processes in order to give credible advice.

A common practice in microbiological testing is that pharmaceutical ingredients are tested without full consideration as to their criticality. Sometimes all raw materials purchased and products manufactured are submitted to the microbiology laboratory and tested or if materials for testing are selected, insufficient judgment is made with respect to which materials are tested. Sometimes materials with a low risk of microbial contamination are tested while materials with a high risk are not tested. Testing should always reflect the risk of microbial contamination. An important managerial tool to rationalize the microbial testing is the reduced testing program. An important aspect of a reduced pharmaceutical ingredient monitoring program, after supplier audit and an evaluation of the equivalency of results from the supplier's certificate of analysis and the manufacturing site microbiological testing laboratory, is an understanding of the potential risk of the microbial contamination of a pharmaceutical ingredient, manufacturing environment, or pharmaceutical product. A microbiologist needs to make a judgment based on the source of the ingredient, how it is processed, its water activity and testing history, and how it is used in formulations, as to whether periodic microbial monitoring to ensure that the testing laboratory confirms the microbial results reported on the supplier certificate of analysis is justified.

New Microbiological Testing Methods

Opportunities exist to implement new microbiological testing methods as alternatives to the compendial methods to improve the quality of the test results and reduce the product release cycle time. The selection of candidate test methods, proof of concept studies, assay development and validation, regulatory approval and implementation of the new microbiological testing methods are major issues that need to be addressed to take advantage of the new technologies. These new methodologies offer significant improvements in terms of speed, accuracy, precision, specificity, etc., with which testing can be performed.

Most of the tests performed today rely on century-old methods based on the recovery and growth of microorganisms using solid or liquid microbiological growth media. This is true in part because these methods can be highly effective and have a long history of application. However, they are often limited by slow microbial growth rates, unintended selectivity for microorganisms that grow in nutrient-rich culture media, and the inherent variability of microorganisms in their response to culture methods. In spite of the limitations of current methods, acceptance of new and poten-

tially superior methods is often slow due to the understandable conservatism of micro-
biologists. This may be due in part to a lack of clear guidance regarding the demon-
stration of their equivalence to existing methods acceptable to regulatory agencies
and validation of the equipment associated with the new methods.

Guidance can be found regarding the validation of chemical methods applicable
to microbial testing. Examples include the USP Chapter ⟨1225⟩ Validation of Com-
pendial Methods, and a recent publication by the International Conference on Harmo-
nization (ICH) *Validation of Analytical Methods*. These publications provide specific
instruction regarding the demonstration of new chemical analytical methods and their
equivalence to existing methods.

When instrumentation is developed for existing microbiological methods to auto-
mate sample handling, result reading, or data management, it is not difficult to demon-
strate the equivalency of the alternative method using guidelines developed for chem-
ical assays because the tests remain essentially the same. In a similar fashion, when
a new technology continues to rely on the measurement of microbial growth (e.g.,
impedance, ATP bioluminescence, or other metabolic changes in a microbial culture),
equivalence can be readily demonstrated. However, when a new method is based
on novel technology without a connection to the existing method (e.g., microbial
identification by rRNA amplification vs. patterns of biochemical reactions, or count-
ing fluorescent-labeled bacterial cells instead of colony-forming units on an agar
plate), demonstration of equivalency may require a new application of the validation
principles although the method provides higher quality results. The principles that can
be applied to the validation of new microbiological testing methods are found in the

TABLE 3   New Microbiological Testing Methods

Methods	Technologies
Growth-based technologies	ATP bioluminescence impedance and conductivity
	Hydrophobic grid membrane filter methods
Viability-based technologies	Direct epifluorescent filter microscopy
	Membrane laser scanning
	Fluorescence cytometry
	Fluorescence flow cytometry
Cellular component or artifact-based technologies	Gas-liquid chromatographic fatty acid profiles
	MALDI-TOF[a] mass spectrometry
	Fluorescence antibody techniques
	Enzyme-linked imunosorbent assay
	Limulus amebocyte lysate-endotoxin assay
Nucleic acid-based technologies	Nucleic acid probe
	Polymerase chain reaction, DNA ampli-cation
	16S rRNA sequencing techniques auto-mated riboprinting

[a] Matrix-Assisted Desorption Laser Ionization-time-of-flight.

recently published PDA Technical Report [7]. For convenience, the technologies are divided into growth-based technologies, viability-based technologies, cellular component or artifact-based technologies, and nucleic acid-based technologies (Table 3).

Risk-based Microbiological Testing Program

To be both cost-effective and control microbial contamination, a testing program must reflect the potential risks of microbial contamination of pharmaceutical drug products. A knowledge of product formulation, manufacturing processes, packaging, and ability of product to support microbial growth can be applied to develop rational specifications and a monitoring program that reflects the potential risk to the consumer of each dosage form. This emphasis on potential risk involves the pharmaceutical microbiologist most closely with products with a higher potential for microbial contamination and serves the needs of pharmaceutical companies and the consumer. A recall is a removal or correction of a marketed product by the pharmaceutical manufacturer when that product violates the laws enforced by FDA. Unlike other FDA tools for achieving compliance, such as court-ordered seizures and injunctions, recalls are almost always voluntary. The FDA cannot order a company to recall a product, except in cases involving infant formulas, biological products, and devices that present a serious hazard to health. In a Class-I recall, there is a reasonable probability that the use of or exposure to a certain product causes serious adverse health consequences or death. In a Class-II recall, use of or exposure to a certain product may cause temporary or medically reversible adverse health consequences and where the probability of serious adverse health consequences is remote. In a Class-III recall, use of or exposure to a certain product is not likely to cause adverse health consequences. When the FDA headquarters receives a recall report from the FDA district office, it evaluates the health hazard presented by the product and categorizes it as a Class I, II, or III. The classification is determined by an ad hoc Health Hazard Evaluation Committee made up of FDA scientists chosen for their expertise. Classification is done on a case-by-case basis, considering the potential consequences of a violation.

The average number of recalls per annum for microbial contamination of nonsterile pharmaceutical and OTC drug products is six recalls (Table 4). The emphasis on waterborne gram-negative bacteria of the species *Bulkholderia (Pseudomonas) cepacia* (nine recalls), *Pseudomonas putida* (three recalls), *Pseudomonas aeruginosa* (three recalls), *Pseudomonas* spp. (two recalls), *Ralstonia* (*Pseudomonas*) *pickettii* (one recall) is notable and reflects the concern for bacteria capable for growth in liquid oral dosage forms overwhelming the preservative system.

Analysis of the underlying probable causes of the microbial contamination of nonsterile products suggests that they are based on

• Microbial contamination of water used for pharmaceutical purposes,
• Pharmaceutical ingredients with high microbial counts,
• Failure of preservative systems to protect liquid products,
• Microbial contamination during the manufacturing process, and
• Improper use and/or storage of the products during their shelf life.

TABLE 4 Nonsterile Pharmaceutical and OTC Products Recalled by the FDA due to Microbial Contamination Problems, 1991–1998 (n = 46)[a]

Year	Recalls	Class I	Class II	Class III	% Pseudomonads
1998	7	2	3	2	57
1997	5	1	2	2	60
1996	4	1	2	1	75
1995	4	0	3	1	0
1994	8	1	4	3	38
1993	9	0	6	3	44
1992	6	1	5	0	33
1991	3	0	0	3	0
1991–98	46	6	25	15	38

[a] After *The Gold Sheet*, January 1991–1998 [8].

Suitable water systems, correct management of the equipment, and appropriate monitoring programs were emphasized during past regulatory inspections, so that manufacturers have no excuse for using unacceptable water during the manufacture of their products. In most cases, pharmaceutical ingredients of high microbial quality can be selected for pharmaceutical manufacturing. More emphasis must be placed on preservative systems during formulation development, and to optimize the preservative system by manipulating the pH, surfactant properties, and water activity to exclude microbial growth. A comprehensive discussion of the application of water activity determination to product formulation and the development of microbial monitoring programs has recently appeared in the literature [9].

A greater appreciation of the ability of different manufacturing steps to affect the microbial content of a formulation by formulators, microbiologists, and manufacturing personnel would be helpful. Exposure of pharmaceutical products to high humidity during their shelf life may increase the water activity of a liquid, ointment, cream, or tablet allowing for the growth of microorganisms on the surface of the product. Therefore suitable packaging and appropriate patient handling of the drug product is important.

Future Trends

The four major trends in the pharmaceutical microbiological testing laboratory are:

- The drive to introduce new microbiological testing technologies,
- The organization of the laboratory based on work stations,
- The use of computerized information management systems, and
- The change of emphasis from testing to prevention of microbial contamination.

If the pioneering German bacteriologist Robert Koch visited today a routine microbiological testing laboratory in the pharmaceutical, biotechnology, or medical device industry, he would see that most current techniques were first developed or used in his laboratory during the last three decades of the 19th century.

These methods include the fixing and staining of bacterial cells on glass slides for microscopic examination and photomicroscopy, growth of colonies on solid media, streaking for isolation of pure cultures on solid media, the use of agar–agar as a support for microbiological media in Petri dishes, serial dilution and plating on solid media to enumerate the microbial population in water, monitoring bacteria within the air, the classification of bacteria by their cellular morphology and differential staining, sterilization of microbiological media by filtration or steam sterilization, disinfectant testing, and aerobic and anaerobic incubation. A major trend is under way in the pharmaceutical QA microbiological testing laboratories where the classical microbiological cultural methods developed in the late 19th century for routine testing are replaced by biochemical, fluorescent cytometric, and nucleic acid-based techniques. Although many companies have developed instruments to automate the running or miniaturizing of existing test methods, technological improvements are progressing rapidly with new methods based on fluorescent laser and nucleic acid-based detection. The future of microbiological testing will lie with the commercialization of automated specific detection methods that will reduce reliance on cultural methods. This should result in routine testing with significantly shorter test cycle time and improved quality of results.

Organization of the microbiology laboratory is more and more frequently based on self-directed work teams with the widespread use of workstations based on the newer testing technologies. The workstations will reflects the product mix that is manufactured by the pharmaceutical company. This will rationalize the deployment of lab personnel and the flow of materials and information through the laboratory. Common workstations include sample receipt and distribution, water for pharmaceutical purposes monitoring, microbial limits of pharmaceutical ingredients and nonsterile products, antimicrobial effectiveness testing, microbial identification, environmental monitoring, growth promotion, sterility testing, microbial assay of antibiotics, and vitamins, and information management.

Each testing workstation is managed by trained laboratory personnel who rotate through the laboratory, which contains dedicated testing equipment interfaced to the Laboratory Information Management Systems (LIMS) media, reagents, and supplies, with accompanying SOPs, training documents, and calibration and preventative maintenance logs. Test specimens would be received, inspected, entered into the information management system via a keyboard or bar code scanning, and distributed to the appropriate workstation. The results would be generated at the workstations, and test results would be reviewed and transferred the into LIMS.

In conclusion, the important role of the microbiologists in the pharmaceutical industry should be reinforced. The need to involve experienced microbiologists in each stage of the product life cycle to maintain high quality, safety, and efficacy is highlighted in this article.

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ANTHONY M. CUNDELL

Process Chemistry in the Pharmaceutical Industry

Introduction

In the pharmaceutical industry, identification of a development drug candidate (pre-clinical lead profile, PLP, or early candidate notification, ECN), filing of investigational new drug application (IND), and new drug application (NDA) are important milestones before the launch of a new drug. The IND and the NDA are the events where the industry interacts with the Food and Drug Administration (FDA), prior to launching. Various governmental departments play specific roles in furthering drug development programs. The medicinal or discovery chemists identify the new drug candidates to treat or prevent a particular medical indication, whereas the process chemists are responsible for devising a synthesis and supplying the active pharmaceutical ingredient (API) or bulk drug substance (BDS) in multigram quantities for various studies needed to file the IND application and support other drug development programs. On approval of the IND, the compound can be administered to humans for the first time as part of the phase-I clinical studies, also known as first-in-man trials (FIM). Studies which are reported in an IND include:

- Synthesis,
- Animal toxicology,
- Pharmaceutics and formulation,
- Drug substance and drug product stability and safety, and
- Metabolic and pharmacokinetics.

The clinical development is the most expensive and resource-intensive segment of the process. Process research and development play a key role in shortening the overall timeline from candidate identification to drug launch. On an average, it takes about 10–15 years from discovery to launch a drug into the market at a cost in excess of $400 millions. The paradigm shift, in the 1990s, of increasing the number of compounds entering development has made a tremendous impact on chemists responsible for preparing supplies of these new drug candidates. Early and effective interaction of process research personnel with medicinal chemists and early innovations in process development is believed to shorten the IND and NDA timelines, respectively. These overall reduced timelines would allow for an early launch of the drug into the market [1].

Pharmaceutical Drug Development Events, the Chemist's Viewpoint

A simplified view of the pharmaceutical drug development events (not to the time scale) is given in Fig. 1.
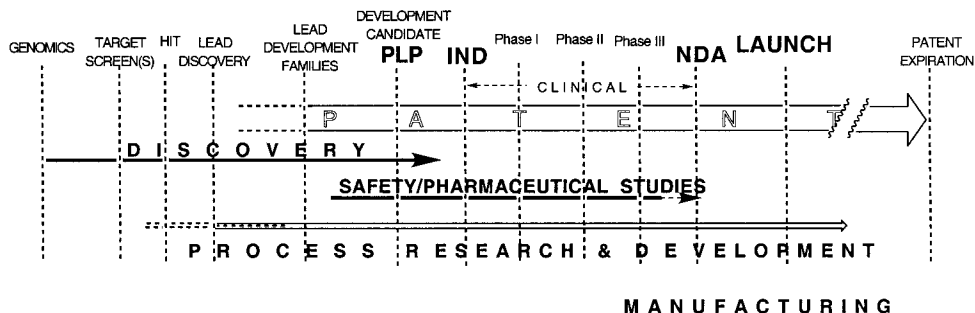
FIG. 1.   Pharmaceutical drug development events, a simplified view. *Preclinical*: Medicinal chemistry, combinatorial chemistry, process research. *Clinical*: Process R&D & Manufacturing (operations).

Genomics to Lead Development Candidate (Preclinical Lead Profile, PLP)

During the past decade, the pharmaceutical industry has seen a paradigm shift in the drug discovery process. The driving force for such change, arguably, was propelled by a variety of factors including:

- Generation of new targets (for human diseases) from advances in genomics and functional genomics,
- Advances in combinatorial chemistry methods to increase the number of compounds for high throughput screening (HTS), and
- Advances in automation and high throughput screening techniques for rapid identification of lead compounds.

Although these approaches promise to provide an increased number of novel drug candidates for evaluation in the treatment of a greater number of diverse diseases, successful realization of the potential benefit of these compounds is very much dependent on the ability of the pharmaceutical industry to develop suitable manufacturing processes.

PLP Development Candidate to IND and the Kilo Lab

The IND studies require relatively large amounts of the drug substance, substantially more than what was prepared during the course of the medicinal chemistry programs. Generally, it becomes the responsibility of the process research group to produce this material within the shortest feasible time frame [2]. Most of the major pharmaceutical companies have kilo-lab facilities as an interface between the process research labs and the pilot plants. Pharmaceutical process groups are organized in different ways. In some companies, process research is responsible for providing the supplies of bulk drug substance required for an IND filing, including the initial clinical batch (or batches). Typically, the initial supplies are prepared by the combination of modified medicinal chemistry synthesis and new, alternative synthetic processes more suitable for scale-up. In some companies, the chemists in process research labs and kilo labs share this responsibility. Kilo-lab scale-up work typically is performed in 22-L glass-

ware and small reactors (50 to 100-L). The process development group, on the other hand, is responsible for further optimization and scale-up in the pilot plant and interfaces with the manufacturing.

Clinical Phase, NDA, and Launching of the Drug

The clinical phase (phase I) of the drug development begins after the IND where humans are subjected to the clinical trials. After a successful phase-I trial, efficacy of the drug is tested on a large number of patients as part of the phase-II/III clinical trials. Once the phase-II/III clinical trials are completed, the NDA is filed with the FDA. Clinical trials and NDA are prerequisites for launching the drug.

Process Research and Development

The goal of the medicinal or drug discovery chemist is to identify a new lead to treat or prevent some particular medical indication and define a synthetic strategy to allow for the preparation of as many analogs as possible. The focus of process chemistry differs from routine organic chemistry. It emphasizes optimization and defines the controls to make the sequence of chemical reactions amenable to scale-up. A viable process should reliably yield a high purity product made by a process unencumbered by a patent. In short, the overall thrust of the scientists engaged in process chemistry is to develop the shortest, least expensive, safest, and most environmentally friendly processes to produce the API in multi-kilogram quantities.

The term ''process'' is, in general, misinterpreted as scale-up work by the overwhelming majority of the scientists and technologists involved with drug discovery and development programs in the pharmaceutical industry. Scientists and engineers engaged in the various aspects of pharmaceutical process research and development have a highly refined appreciation for the challenges of large-scale synthesis and the purification of the API. An algorithm for process research and development is shown in the following diagram.
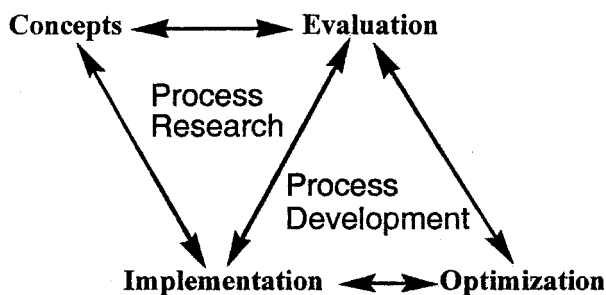
The mission of process chemistry in the pharmaceutical industry is to provide documented, controlled synthetic processes for the manufacture of the supplies to support the development programs and future commercial requirements of the API. The science and technology associated in accomplishing this mission provides a

tremendous challenge to the individuals or the group of individuals for the drug supply progress from milligram to metric ton quantities.

Route Scouting

As the development drug candidate moves from discovery to process, a workable synthesis is available, which, however, may not be scaleable. Route scouting plays a key role in identifying synthetic transformations which are safe, practical, scaleable, cost effective, and environmentally friendly, thus setting the stage for eventually delivering a manufacturing synthetic process. An integral part of route scouting is to identify key intermediates that could be easily outsourced.

An Example of Route Scouting and Process Improvements

Scheme 1 depicts the medicinal chemistry approach to the synthesis of BMS-180291 (Ifetroban).

A condensed schematic of the overall alternative synthesis of BMS-180291 (Ifetroban) is provided in Scheme 2. The synthesis of the key acid ester intermediate A has been reduced dramatically from 16 to four steps, and the yield has been increased ten-fold from 5 to 52%. The overall synthesis of Ifetroban has been cut virtually in half (from 23 to 12 steps) and the yield (via the longest linear sequence) improved from 3 to 28%.
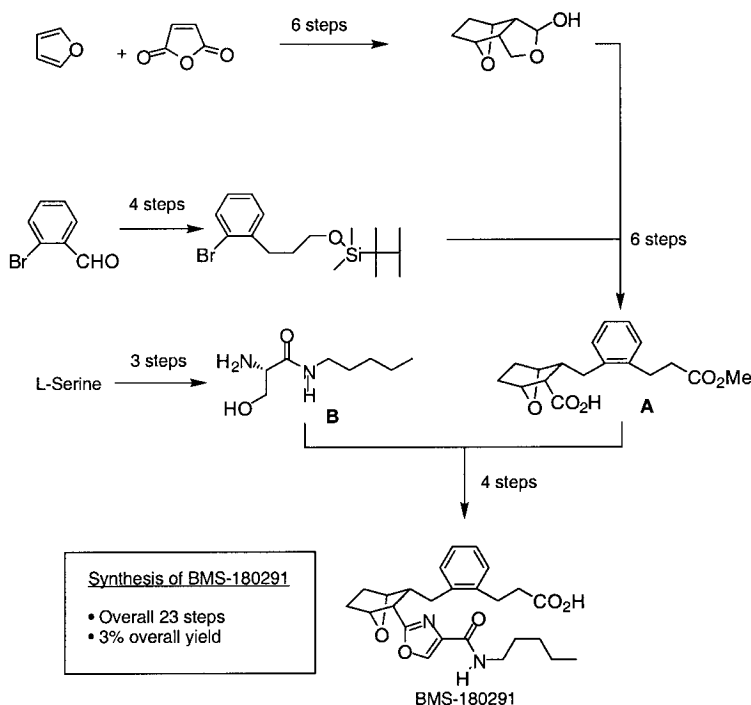
Scheme 1. Original Synthesis of BMS-180291.
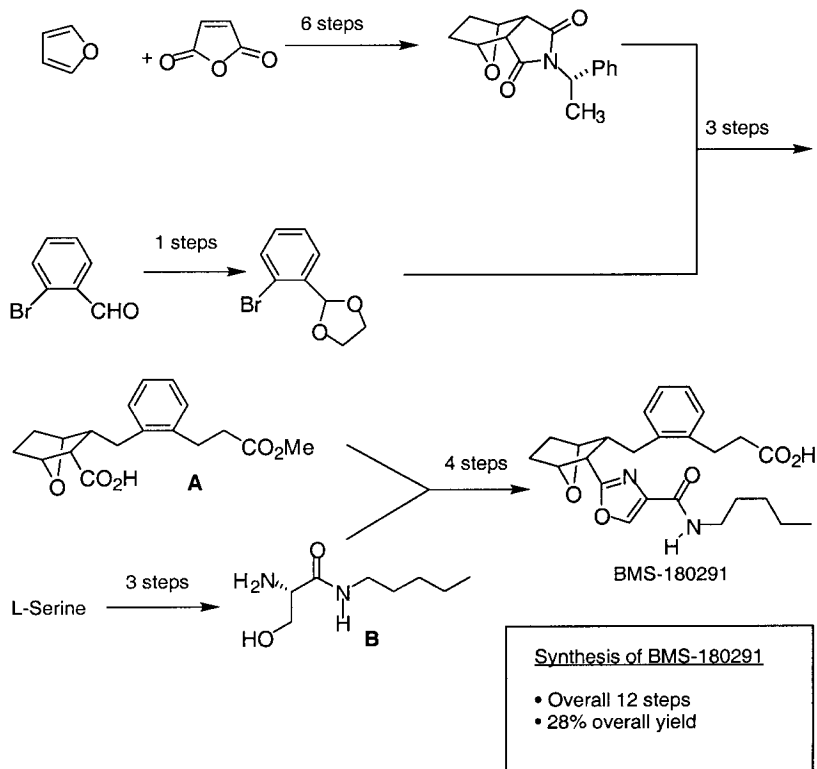
Scheme 2. Alternative Synthesis of BMS-180291.

Process Development and Optimization

Human safety is the first priority when developing a process, followed by quality (purity, crystallinity of the product, etc.) and product yield. In addition, the process chemist takes into account the following factors:

- Hazards associated with the chemical step (human and equipment safety),
- Waste generated (disposal cost, environmental concerns),
- Cost and availability of raw materials, reagents, and solvents (cost of goods, sourcing issues),
- Ease of performing the reaction on-scale (savings in capital and labor),
- Ease of product isolation (crystallization), and
- Opportunity to combine two or more chemical steps with no intermediate isolation (telescoping to increase throughput).

Once a route has been selected, the next step is to understand the effect of interactions of variables with each other on the reaction yield and quality for each and every step of the synthetic scheme and to define the limits for these process variables. This requires designing appropriate experiments including controls. This is important because as the scale of the reaction increases, every unit operation, such as addition

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CHO

NH$_2$

OMe

+

benzene

azeotrope

N

OMe

+ AcO

O

Cl

Triethylamine,
Dichloromethane

[2+2] Cycloaddition

AcO

O

N

OMe

BMS-182205

N

OMe
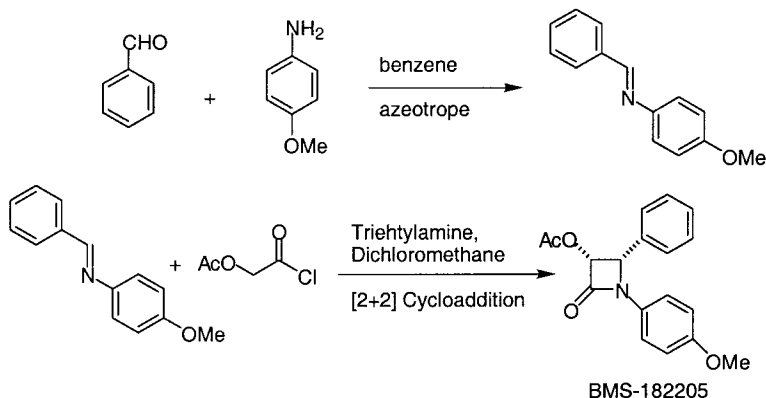
Scheme 3. Synthesis of BMS-182205.

of reagents, solvents, distillation, phase splits, etc., takes a considerably longer time. Mixing begins to play a major role as the size of the vessel increases. Due to uneven mixing, localized differences in variables such as temperature and concentration are expected, which may have an adverse effect on the reaction.

Example 1: Process Development of BMS-182205, Scheme 3.

Issues: environmental concerns, human safety, waste management, process inefficiency.

The first step in the synthesis of the Paclitaxel® side chain has been reported in the literature by Holton and co-workers [4]. The drawbacks of this procedure from a process development standpoint are:

1. The use of benzene and dichloromethane (human and environmental concerns),
2. No opportunity for telescoping (since the first reaction is conducted in benzene and the second in dichloromethane),
3. Low yield (68% after crystallization from ethylacetate and hexane), and
4. Brown-black product (color).

Process Improvements.   Phase I: Benzene was replaced with a safer solvent, toluene, which was also used for the [2 + 2] cycloaddition reaction, thus facilitating the telescoping of the two reactions. However, dichloromethane was added during the work-up to prevent product precipitation. The product was crystallized from 2-propanol in 80% yield. The phase I of development eliminated the use of benzene and allowed for telescoping of the two chemical steps. However, a solvent exchange from toluene–dichloromethane to 2-propanol was still needed to crystallize the product. The color of the product after this modification was dark-brown.

Phase II: Additional crystallization studies revealed that the product could be crystallized from toluene–heptane and acetone–water. The product also exhibited limited solubility in toluene. With this information at hand, both reactions were conducted in toluene as before, however, during the work-up, dichloromethane was not added. Instead, aqueous hydrochloric acid was added to neutralize excess triethylamine. Heptane was then added to precipitate the product directly from the reaction

mixture. The crude wet product was crystallized from acetone–water with an overall yield of 80%. The color of the product was brown. The advantages of this modification are the elimination of several extraction and back-extraction steps, and a reduction in processing time and in solvent consumption.

In an attempt to further improve the yield and address the product color issue, the following process variables were studied: the rate of addition of acetoxyacetyl chloride, the reaction temperature, and the effect of other amine bases.

The rate of addition of acetoxyacetyl chloride was optimized to be 2 to 3 hr, at 3, 10, and 17°C. Reactions conducted at 3°C afforded the product with the highest quality. Two amines, N-methylmorpholine (NMM) and diisopropylethylamine (DIPEA) were evaluated in addition to triethylamine. In the presence of NMM, the reaction stopped after 65% conversion. In triethylamine the reaction took an additional 3 hr after the addition of acetoxyacetyl chloride to completion. However, in DIPEA, the reaction was instantaneous, the yield was further improved to 87%, and the product was free of brown colored impurities.

Example 2: Process Development of Ifetroban, Scheme 4.

Issues: environmental concerns, human safety, cost of goods [5].

In the improved synthesis of Ifetroban described above, environmental concerns due to special handling of copper bromide waste and hazards associated with hexamethylene tetramine (HMT) on manufacturing scale led to further perfection of the synthesis. Mechanistic considerations suggested that an oxidized form of aminoamide B (Scheme 4) would eliminate the necessity for a late-stage copper-mediated oxidation. This was indeed accomplished. The cyclization–elimination sequence was initiated by a Lewis acid and completed by base-mediated elimination to afford the Ifetroban penultimate. In addition to eliminating the need for copper bromide and HMT, this modification helped to reduce the cost of the product by an additional 15%.
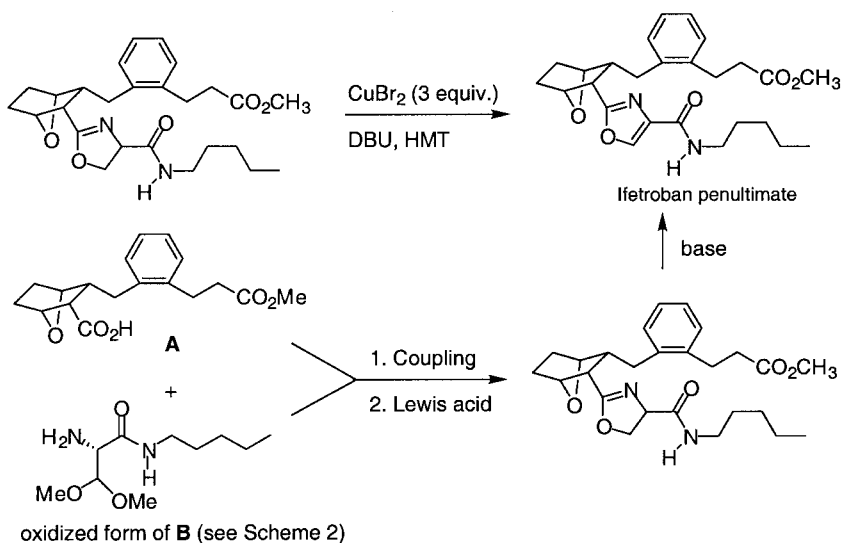
Scheme 4. Process Development of Ifetroban.
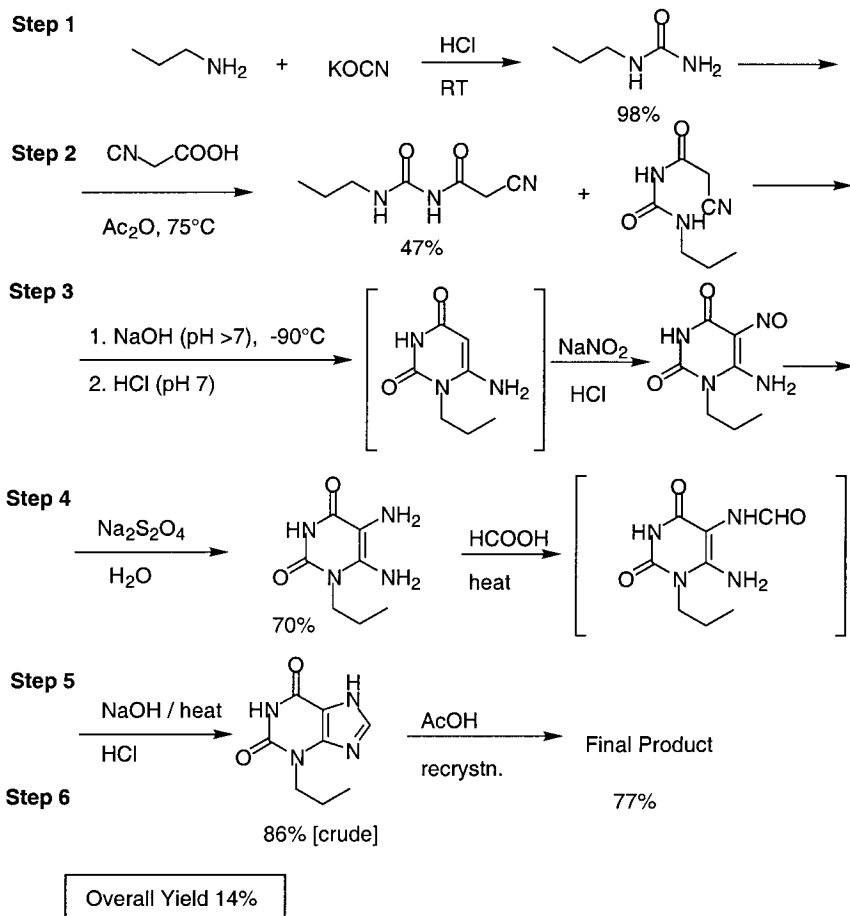
Scheme 5.   Original Synthesis of Enprophylline by the Medicinal Chemistry Department at Astra.

Manufacturing Process, Route from Medicinal Chemistry to Multi-Kilos for Clinical Study Supplies

A highly optimized and concise large-scale synthesis of a purine bronchodilator was developed by the Astra Production Chemical company from Sweden [6]. Supplies for the initial biological studies were generated by the medicinal chemistry route shown in Scheme 5. The overall yield was about 14%, which was improved in the environmentally friendly manufacturing process to about 51% (Scheme 6 and Scheme 7).

Process Hazards and Safety

Process safety has become an integral part of the development of new synthetic routes and more and more relevant information is gathered in typical process development labs [7]. Many development facilities have now established special process safety
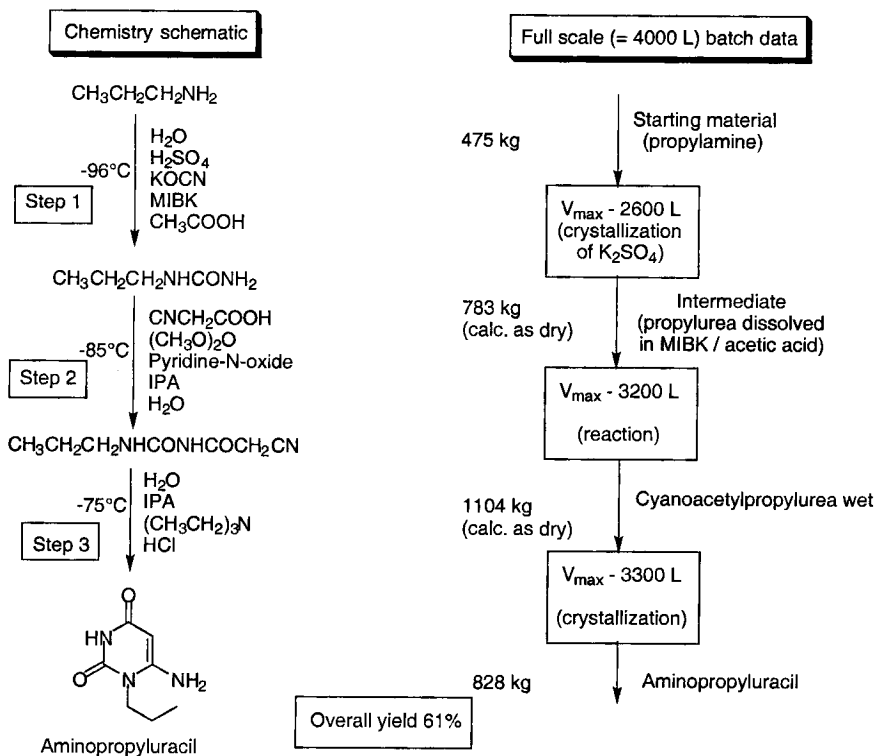
Scheme 6. Astra commercial process of 6-amino-1-propyluracil.

departments with state of the art equipment and well trained personnel. It is important for process chemists considering alternative process routes to know the potential hazards from the main reaction and from the unwanted side reactions in each case so that the hazards of reactivity are included in the factors reviewed in developing and selecting the final process route; three main parameters determine the design of safe chemical processes:

1. The potential energy of the chemicals involved and understanding of the inherent energy (exothermic release or endothermic absorption) during a chemical reaction.
2. The rates of reaction (energy release in the form of heat or pressure) which depend on the temperature, pressure, and concentrations. In any hazard evaluation process, the rates of reaction during normal and abnormal operations (including the worst credible case) must be considered in order to design an inherently safe process.
3. An equipment train must adequately remove any heat or pressure generated in a reaction. The effects and requirements of scale-up must be considered.

In most cases, a team of development chemists, engineers, and safety personnel evaluate and assess hazards associated with each and every step of a process, from performing a reaction to storage of waste streams in drums. The team recommends a set of safety experiments to be conducted, and data are collected for reaction exo-
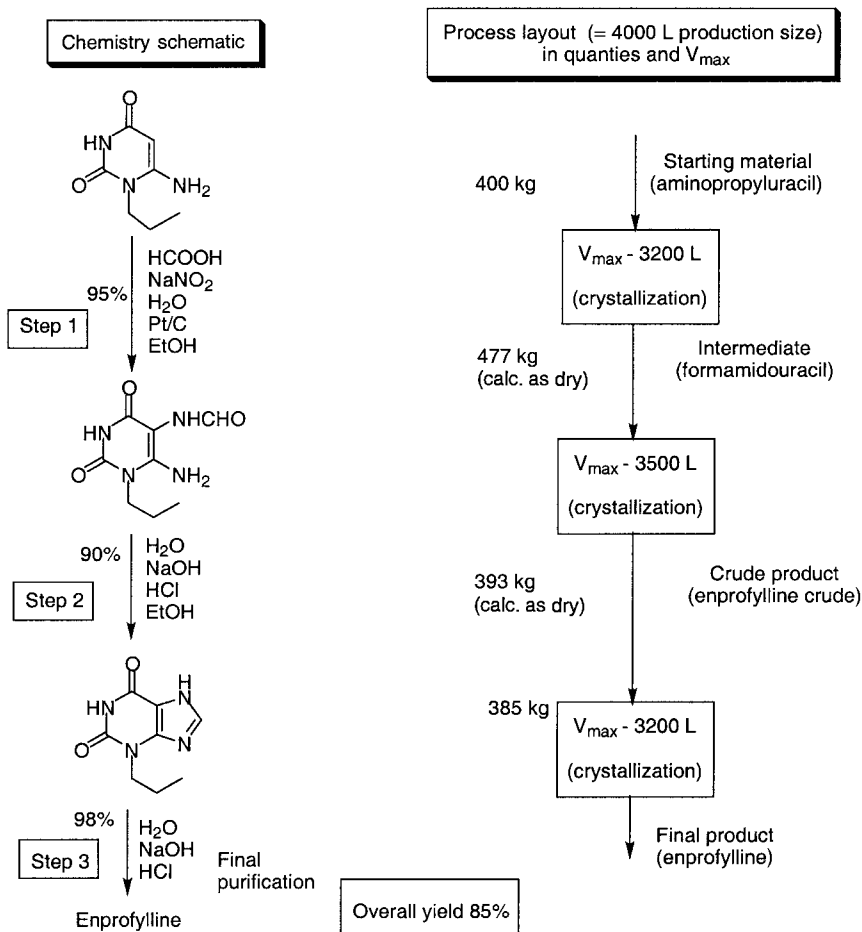
Scheme 7.   Astra Full-Scale Synthesis of Enprofylline from 6-Amino-1-propyluracil.

therms, powder explosivity, gas evolution, and compatibility of reaction mixtures or reaction waste with pilot-plant equipment and storage drums. Experiments are designed and conducted to determine the potential for initiation of a runaway reaction and the effect of decompositions that may occur on runaway. Based on the safety assessment of a process, appropriate measures are taken in the pilot plant to eliminate or minimize any hazard. During the early stages of process research (route scouting), only small amounts of materials are available. In many cases, only theoretical information from the literature or from calculations is readily available. Screening tests can be run to identify the reaction hazards. As the route-scouting efforts enter the process development phase, additional material becomes available, so that the reaction hazards can be studied more extensively to test "what-if" scenarios. During full-scale production, the chemical hazards may be reevaluated to address changed production requirements, or other process changes such as the use of a different source of raw materials.
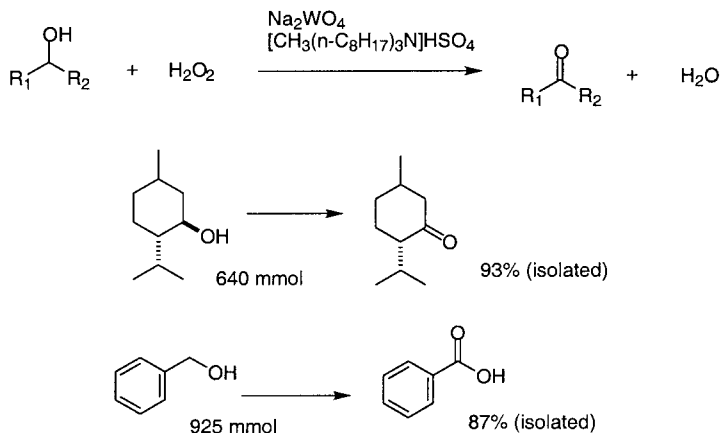
Scheme 8. Noyori's Oxidation of Alcohols.

Numerous test methods are available using a variety of sample sizes and conditions. The tests provide qualitative or quantitative data on onset of temperature, reaction enthalpy, instantaneous heat production as a function of temperature, maximum temperature, and/or pressure excursions as a consequence of a runaway, and additional data useful for process design and operation.

"Green Chemistry," Environmental Concerns

The research that involves the end-of-process treatments to eliminate pollutants is termed "green chemistry." As Ronald Breslow (Columbia University) pointed out [8], concern for the environment is as old as the biblical injunction, "hurt not the earth, neither the sea, nor the trees." The following example indicates approaches to the environmentally benign chemistry. The process described is high yielding with water as the by-product. Sato and co-workers [9] have developed an efficient, environmentally friendly method for oxidizing primary and secondary alcohols (Scheme 8). The Japanese scientists use hydrogen peroxide as an oxidant, a quaternary ammonium hydrogen sulfate as a phase-transfer agent, and tungsten as catalyst.

Enzymatic Intervention

The recent trend in pharmaceutical industries is to incorporate a microbial technology (MT) or enzymatic technology division in the process R&D and manufacturing (need basis) departments. The role of the MT division is usually to provide pharmaceutical drugs (e.g., pravastatin, β-lactam drugs, etc.), and chiral (optically pure) building blocks or synthons for the ongoing synthetic programs.

A number of cholesterol-lowering drugs (Pravachol, zocor, and mevacor) are prepared by enzymatic processes. Penicillin antibiotics that have been on the market for decades are produced in large quantities by the enzymatic process.

Enzymatic Catalysis

The extensive use of enzymatic catalysts in organic synthesis has been documented [10]. Enzymes represent a broad range of efficient chemical catalysts. They are classified mainly into six categories:

1. Hydrolases (hydrolysis of amides, esters, glycosides, and lactones)
2. Isomerases (C—C bond migration, E/Z isomerization, and racemization)
3. Lyases (addition to π-bonds)
4. Oxidoreductases (reversible oxidations and reductions)
5. Synthetases formation and breaking of C—C, C—N, C—O, C—S, and phosphate ester bonds
6. Transferases (transfer of acyl, glycosyl, or phosphoryl groups from one molecule to another)

Scheme 9 gives three examples.

9.1. Enantioselective enzymatic oxidation and lactonization
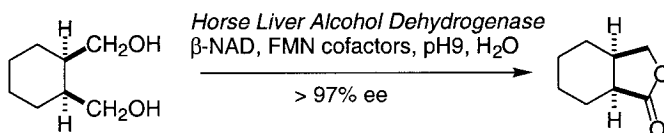
9.2. Enzyme reduction with Baker's yeast and enantioselective rule
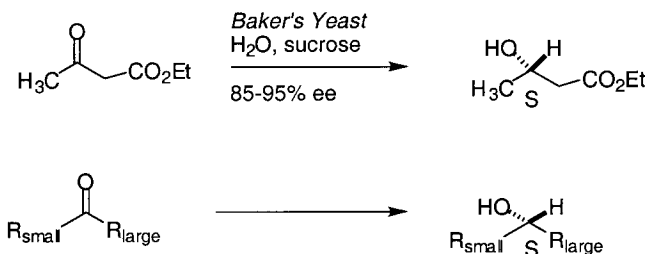
9.3. Enzymatic hydrolytic desymmetrization
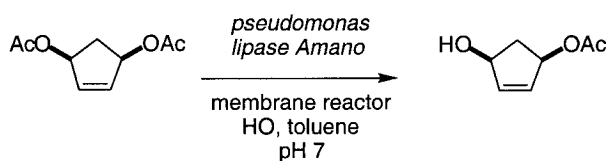
Scheme 9.   Enzyme-mediated chemical transformations.

Design of Experiments (DOE)

An understanding of how various process variables affect the chemistry is necessary for the design of a chemical process [11] that can reliably provide the product in high yield and quality. This understanding can be obtained from experimentally determined rate laws for the main and side reactions that relate temperature, concentration, pressure, solvent effects, and equivalents of each reagent to yield and impurity levels. The process chemist may take the approach of evaluating the importance of process variables by changing one variable at a time. This method can help generate chemical knowledge but is not efficient and does not easily provide the quantitative information needed to rank the importance of process variables. Furthermore, the process chemist is often faced with a task of quickly scaling up the synthesis without the benefit of completely understanding the mechanism of the chemistry involved. With the aid of statistical design approach, a great deal of useful information can be obtained with relatively few experiments. Statistical approaches involving factorial designs are ideal for studying processes where the underlying principles are not well developed or are extremely complicated.

Factorial experiments consist of a systematic variation of two or more process variables at a time. For a two-level experiment, each variable is set to a high or a low value according to a standard pattern. An experimental run is conducted for each possible combination of variable settings. Selection of the low and high levels for each variable is important for obtaining meaningful results. If the levels selected are too close together, the calculated effect could be no larger than the experimental noise. Selecting widely separated levels could result in running the reaction under unrealistic conditions, for example, above the solvent boiling point, or above the decomposition temperature for reagents and reactive intermediates.

Data analysis of factorial designs involves a comparison of the experimental responses at the high and low settings of each variable. The results can be plotted in several different ways to develop an understanding of the effect of changing two or more process variables at a time with regard to reaction yield and quality of the product.

Drug Substance Crystal Form (Final Form)

The crystal form of the drug substance [12] is important for pharmaceutical industries, where products are specified not by chemical composition but by their performance. Good crystallinity, good bioavailability, satisfactory aqueous solubility and dissolution rate, and satisfactory physicochemical properties, such as stability, hygroscopicity, and flowability are required. Choosing the appropriate crystal form (final form) often involves crystallizing the drug substance from various solvent systems to search for polymorphism and screening of various salts of the drug substance (if chemically possible).

The chemical, biological, and physical characteristics of the drug substance can be manipulated and hence optimized by conversion to a salt form. Every compound that exhibits acid or base characteristics can participate in salt formation. Various salts of the same compound often behave quite differently because of the physical, chemical, and thermodynamic properties they impart to the parent compound. Table 1 lists the top ten FDA approved commercially marketed final drug forms and Table 2

TABLE 1   Salts Approved by the FDA

Anion	Percent	Cation	Percent
Hydrochloride	43	Sodium	62
Sulfate	7.5	Potassium	11
Bromide	5	Calcium	10.5
Chloride	4	Zinc	3
Tartarate	3.5	Meglumine	2.3

the top ten salts that are not approved by the FDA but that are in use in other countries. Only salts of organic compounds have been considered here because most drugs are organic substances. The relative frequency with which each salt type has been used is calculated as a percentage, based on the total number of anionic or cationic salts in use.

The salt form is known to influence a number of physicochemical properties of the parent compound, such as dissolution rate, solubility, stability, and hygroscopicity. These properties, in turn, influence the absorption, distribution, metabolism, and excretion of the drug. This knowledge is essential for a complete understanding of the onset and duration of action, the relative toxicity, and the possible routes of administration. For example, certain salts of the strong base choline have proved to be considerably less toxic than their parent compound. This observation led to the preparation of choline salicylate as an attempt to reduce the gastrointestinal (GI) disturbances associated with salicylate administration. Clinical studies indicated that choline salicylate elicited a lower incidence of GI distress, was tolerated in higher doses, and was of greater benefit to the patient than was acetylsalicylic acid (aspirin).

The chemical and physical stability of a drug can influence the choice of dosage form, the manufacturing and packaging, and the therapeutic efficacy of the final preparation. Systematic determination of the thermal stability, solution stability (at various pHs), and light sensitivity of a drug and its derivatives both alone and in the presence of additives (excepients), provides essential information toward selecting the most suitable salt and dosage forms.

Depending on the mechanism of degradation, different salt forms impart different stability characteristics to the parent drug. Sparingly soluble salts used in the formulation of suspensions, reduce the amount of drug in solution and hence its degradation. Differences in hygroscopicity of several salts influence the stability of the drug in the dry state. For example, the stability of penicillin G and its salts has been widely studied because of the drug's therapeutic importance and its characteristic instability.

TABLE 2   Salts Not Approved by the FDA

Anion	Percent	Cation	Percent
Glycerophosphate	0.88	Piperazine	0.98
Aminosalicylate	0.25	Bismuth	0.98
Aspartate	0.25	Diethylamine	0.33
Bisulfate	0.25	Tromethamine	0.33
Hydroiodide	0.25	Barium	0.33

A solution of penicillin is not stable beyond two weeks even at refrigerator temperatures. However, the use of suspensions of sparingly soluble amine salts (procaine and hydrabamine salts) in aqueous vehicles allowed marketing of a ''ready-made'' penicillin product.

Crystallization of the Drug Substance and its Salt Forms

In academia, a synthetic organic chemist rarely thinks about isolating a compound by crystallization, unless a single crystal structure is required. Most of the time, the chemist depends upon column chromatography to purify the compound. In contrast, in the pharmaceutical industry much depends upon crystallization. For example, crystal morphology and particle size have a direct impact on the filtration of a crystal slurry, cake compressibility, bulk stability and dissolution, bulk density, and flow characteristics. Crystallization is the preferred way of isolating the product from a reaction mixture. Knowledge of various crystallization systems from which the drug substance could be crystallized can provide information on polymorphism, thus expediting the selection of final crystal form. In polymorphism, two crystal forms with the same molecular structure are distinguished by the way in which the molecules are packed within the crystal lattice; each form has distinct physical and thermodynamic properties. In 1998, Abbott Laboratories withdrew its HIV drug, Ritonavir, because of the unexpected appearance of a new crystal form that had different dissolution and absorption characteristics than the standard product [13].

Crystallization is generally preceded by two types of nucleation. The primary nucleation occurs with the formation of clusters of molecules at the submicron level. When the concentration exceeds saturation to afford supersaturation, the clusters become nuclei. The secondary nucleation is caused by particles due to primary nucleation or seeds. There are many strategies to achieve supersaturation to initiate crystallization such as cooling, evaporation, and antisolvent addition.

Early on in the drug development program, only small amounts of material are available for crystallization studies. Parallel crystallization technique in test tubes allows for the identification of many solvent systems using small amounts of material. On a small scale, it is not easy to control the rate of cooling or the rate of evaporation to achieve supersaturation. However, the antisolvent addition strategy to achieve supersaturation in combination with seeding, allows rapid identification of several crystallization systems using a minimum amount of compound.

Salts of a substrate having an acid or an amine functionality are prepared by dissolving the substrate and the counter-base (or acid) separately in appropriate solvents, mixing the two solutions in equimolar ratios, and removing the solvents under vacuum to afford a solid. This method has two distinct advantages: First, the composition of the substrate remains the same during various crystallization attempts, and second, this portion of the work can be automated with the use of liquid handling systems.

The next step is to determine the solubility of the substrate (or its salts) in different solvents. This can also be performed by an automated liquid handling system. Depending upon the solubility of the substrate in water-miscible solvent (alcohols, acetone, tetrahydrofuran, etc.) and water-immiscible solvents (ethyl acetate, methyl-*tert*-butyl ether, heptane, etc.) the process chemist can identify one or many solvent systems from which the substrate (or its salts) could be crystallized using the antisolvent addition strategy.
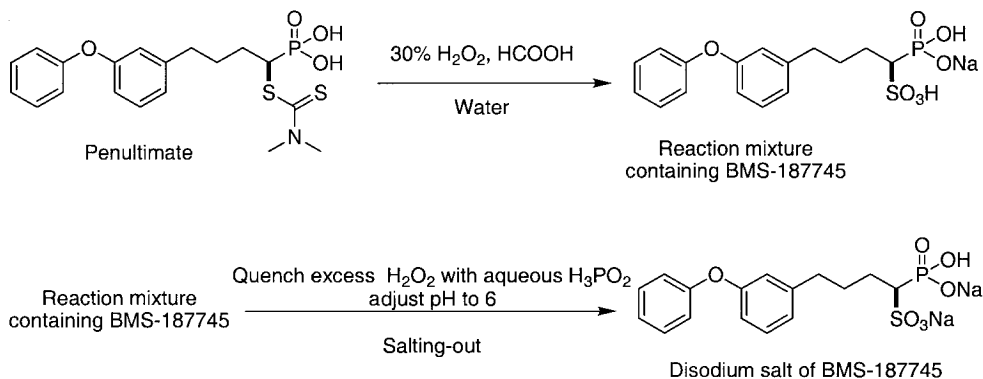
Scheme 10.   BMS-187745 Crystallization by Salting-out.

Another crystallization technique is used when the isolation of a highly water-soluble compound in its salt form is required from aqueous reaction mixtures. This technique takes advantage of the common-ion effect and is based on the le Chatelier's principle, which states that, ''if, to a system in equilibrium, a stress is applied, the system will react so as to relieve the stress.'' Thus, in aqueous solutions, the solubility of the compound in salt form can be reduced by adding large amounts of a common ion which is more soluble than the salt of the compound.

Example: BMS-187745 is a potent inhibitor of squalene synthase and an efficient cholesterol-lowering agent in orally dosed animals. The final step of the synthesis involved oxidation of the penultimate in water with hydrogen peroxide and formic acid. The disodium salt was chosen as the final crystal form. Because of extremely high solubility of the disodium salt in water, it cannot be extracted from water by common organic solvents. However, by employing the concept of the common-ion effect, the disodium salt can be easily crystallized from water. To accomplish this, the pH of the reaction mixture, after quench with hypophosphorous acid, is adjusted to the desired range of 6.05 to 6.25 (pKa of disodium salt of BMS-187745 is 6). In this pH range, sodium salts of formic, hypophosphorous, and phosphoric acids are present in high enough concentration to help lower the solubility of the disodium salt of BMS-187745 and effect crystallization (Scheme 10).

Automation in Process Chemistry Laboratories

Since the 1960s, automation has been the major tool for dramatically improving productivity [14]. Automation techniques were introduced on the plant floor to improve quality, increase safety, and streamline the work. Significant savings were realized initially from shorter delivery times, lower unit costs, smaller inventories, and fewer product failures. Due to an escalating cost of bringing a drug to market, companies are under immense pressure to shorten development time. The pharmaceutical industry has embraced new technologies in its discovery programs to increase throughput, generate precise data, and ensure accuracy.

Drug discovery scientists have already adopted tools such as combinatorial chemistry synthesizers; robotic systems for high-throughput screening (HTS); and software

packages for computational chemistry, molecular modeling, and design of experiments to identify lead compounds (''hits''). As the number of ''hits'' grows, there is a potential for process R&D to become the ''bottleneck.'' The number of compounds that can enter process development is limited by the number of process chemists available to work on these compounds. Without some type of automated process-development machines to increase throughput, it will not be feasible to evaluate all the potential ''hits.'' Created by the results from the HTS, demand for high-throughput development (HTD) tools is growing. Various companies, who in the past may have only addressed the needs of drug discovery scientists, are in the process of inventing and developing instruments that can be used by process chemists to perform many experiments in parallel. Examples of commercially available HTD tools are described below.

Parallel Reactions

Charybdis Technologies.   In the Calypso Reaction Block™, multi-well reaction arrays are designed for both solution and solid-phase synthesis applications. Well volumes range from 2 to 10 mL, pressures up to 207 kPa (30 psi), and temperatures from -80 to $+180°$C.

Quest Synthesis Technology.   Quest 210 is designed for up to 20 reactions in 5- or 10-mL reaction vessels. Each of the 20 vessels can be efficiently stirred, heated, or cooled, and maintained under an inert atmosphere. Quest 205 is designed for synthesis on a large scale, with two banks of five 100-mL reaction vessels.

Argonaut Technologies.   Endeavor™ allows parallel reactions under pressures up to 3.3 MPa (33 atm) and temperatures up to 200°C. Each of the eight vessels (working volume 15 mL) can be independently controlled for temperature, pressure, and gas delivery.
    Surveyor™ is suitable for parallel process development and optimization with on-line sampling and integrated HPLC analysis. It employs ten reaction vessels (working volume 15–45 mL), with individually controlled reaction temperatures from $-40°$ to $+150°$C, with the ability to reflux. Reagent addition, reaction parameter control, sampling, and HPLC injection are controlled by built-in software.

Bohdan Automation Inc.   The process development workstation can run up to 12 independent reactions with working volumes of 25 mL. The following operations are automatically carried out: reagent preparation and addition, individual heating, cooling, and mixing, reaction sampling and quenching, transfer to optional HPLC or FTIR modules.

Parallel Purification

Isco, Inc.   The CombiFlash™ Sg 100c System is suitable for purifying 10 to 35 g of material. It provides time- or peak-based fraction collection with on-line UV detection, and a linear and/or step gradient with two solvents at a flow rate of 10 to 100 mL/min.

The CombiFlash™ Si 10x System simultaneously purifies up to 10 samples, with a linear and/or step gradient with two solvents with a total flow rate of 10 to 100 mL.

Automated Sample Handling and Analysis

Bohdan Automation Inc.   The Balance Automator™ offers a cost-effective alternative to manual weighing, reduces errors and operator tedium, and processes up to 120 samples per hour. This system can accommodate various container sizes and balances with the help of interchangeable parts.

Gilson.   The Gilson 215 Liquid Handler is a versatile, large-capacity, septum-piercing liquid handler for safe and efficient transfer. It helps transferring samples to other analytical systems or inject samples directly to an on-line HPLC system.

Outsourcing

Outsourcing covers a broad range of services [15]. It can eliminate the need for additional staff, facilities, and/or equipment. Outsourcing in drug discovery and development is expected to continue its remarkable growth over the next decade as drug synthesis becomes more complex. This dynamic growth is due to a number of factors, including the ongoing consolidation in the pharmaceutical industry and a tight labor market. As larger companies consolidate, they seek synergies resulting in release of R&D personnel. Retrospectively, companies need to get more done with fewer internal resources and this promotes outsourcing. The tight labor market has made it increasingly difficult to recruit and retain staff, and many companies are turning to outsourcing to gain access to highly trained scientists who are available. Pharmaceutical companies are also looking into long-term relationships with outsourcing partners.

FDA Guidelines and Regulatory Issues

The guidelines on drug preparation (GLP and cGMP compliance), regulatory issues are described in details in the Center for Drug Evaluation and Research (CDER) by the FDA [16]. A complete section is dedicated to active pharmaceutical ingredient (API) and the GMP issues. The readers are encouraged to seek the reference for further details.

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Radiolabelling of Pharmaceutical Aerosols and Gamma Scintigraphic Imaging for Lung Deposition

Introduction

Inhalation aerosols have been successfully used to deliver drugs to the lung for local and systemic therapeutic effects. In vivo evaluation of pharmaceutical inhalation products is achieved by gamma scintigraphic imaging of the aerosol deposited in the lung. Imaging provides direct information on the amount and location of the drug deposited in the lung after inhalation [1]. This local bioavailability, rather than the systemic bioavailability after absorption, is pertinent to drugs that act directly on the lung. For drugs that act systemically, the deposition site affects the rate and extent of absorption. As a result, lung deposition using gamma scintigraphy has been proposed for bioequivalence studies of aerosol products [2]. The deposition data can further be linked to the clinical response and in vitro particle size distribution, adding a new dimension to the interrelationship between them.

To measure lung deposition by imaging, the aerosol must be first labelled or tagged with a suitable radionuclide. Radiolabelling techniques have been developed for current inhalation products including nebulizers, propellant-driven metered dose inhalers, and dry powder inhalers.

Lung imaging is achieved using a gamma camera, which creates an image of the gamma rays emitted by the radionuclide in the lung [3]. In the past, numerous lung deposition studies on radiolabelled aerosol products have been carried out using planar imaging by which only the anterior or posterior two-dimensional view of the lung is collected. However, since the lung is a three-dimensional object, spatial distribution of the aerosol in the lung can be best obtained using tomographic (rather than planar) imaging such as SPECT (single photon emission computed tomography), a technique for producing cross-sectional images of the radionuclide distribution in the body. This is achieved by imaging the lung at different angles (e.g., 64 or 128 images at 180 or 360°, respectively) around the thorax using a rotating gamma camera, followed by computational image reconstruction.

Radiolabelling Pharmaceutical Aerosols

It is a prerequisite that the pharmaceutical aerosols be suitably radiolabelled before any lung scintigraphic imaging can commence. Ideally, the drug can be directly radiolabelled, that is, chemically by substitution of an atom in the drug molecule with a radioactive isotope. This can be achieved using positron emitters, such as C-11, N-13, and O-15 atoms. Positrons are positively charged electrons which, when combined with an electron, produce two gamma rays with equal energy (511 keV) emitted

at 180° to each other. Theoretically, since C, N, and O atoms are present in all organic molecules, they can be used to label virtually any drug. In reality, the use of these atoms is limited by their short half-lives (20, 10, and 2 min for C-11, N-13, and O-15 atoms, respectively) relative to the time taken for manipulating the drug (including organic synthesis for radiolabelling and successive processing for product characteristics assurance such as particle size distribution of the aerosol particles). Furthermore, to produce these positron emitters, it is necessary to have a nearby cyclotron facility, which means extra cost. So far, only three anti-asthmatic compounds have been successfully radiolabelled by positron emitters: ipratropium bromide (Br-77) [4], triamcinolone acetonide (C-11), and fluticasone proprionate (F-18) [5]. This is indicative of the difficulties of direct radiolabelling.

Because of the forementioned limitation, gamma emitters have been employed to radiolabel the drug indirectly for gamma scintigraphy. In this case, the radiolabel associates with the drug by physical means instead of chemically incorporating into the drug molecule via covalent bonds. Hence, instead of being a direct chemical approach, it is indirect radiolabelling. Technetium-99m is the most commonly used pure gamma emitter for indirect radiolabelling of pharmaceutical aerosols. The gamma ray of ^{99m}Tc has sufficient energy (140 keV) to penetrate body tissues without significant absorption or scattering, but when it reaches the detector of the gamma camera, it is absorbed and converted into light photons, thus optimal for gamma-camera imaging. The half-life of ^{99m}Tc is 6 hr which is long enough for handling and imaging but not too long to increase the radiation dose to the subject unnecessarily. As a result, ^{99m}Tc is used for the majority of nuclear medicine imaging studies. Once inhaled into the lung, ^{99m}Tc can have a much shorter biological half-life, depending on the physical form, as diethylenetriamine pentaacetic acid or DTPA complex. It will be absorbed rapidly from the lung into systemic circulation, followed by glomerular filtration in the kidney to the bladder where it is excreted in the urine, and the whole process can take less than 2 hr, if the subject drinks plenty of water.

Regardless of the type of aerosol products to be radiolabelled, a fundamental requirement in radiolabelling aerosol products is that the radiolabel must associate with the drug in such a way that not only the radiolabel distribution matches the drug distribution but also that the radiolabel distribution matches that of the unlabelled commercial product.

Nebulizer Solutions

Nebulizer solutions are by far the simplest among the aerosol products, and radiolabelling is achieved by simply mixing the radionuclide with the drug solution. Since the radionuclide and the drug are uniformly distributed in the solution and provided that no precipitation of the ingredients occurred, each nebulized aerosol droplet would contain both radioactivity and drug in proportion to the droplet size. Technitium-99m, complexing with DTPA or human serum albumin, is widely used as the radionuclide. Sodium pertechnetate is not suitable since the free anion, like iodide, has a high affinity for the thyroid. Lung deposition of nebulized salines and drug solutions, including nedocromil sodium [6], salbutamol, fenoterol, ipratropium bromide, carbenicillin, pentamidine isethionate [7], flunisolide [8], and liposomes containing beclo-

methasone dipropionate [9], have been studied using this radiolabelling technique of mixing the radionuclide with the drug solution.

Propellant-Driven Metered Dose Inhalers (MDIs)

Historically, there are three major methods of radiolabelling suspension MDIs, developed by Few et al. [10], Newman et al. [11], and Kohler et al. [12]. The first two were initially developed for polymeric particles but were later modified for drugs.

Early in 1970, Few et al. [10] radiolabelled polystyrene particles for a mucociliary clearance study. The radiolabelled aerosols were produced by a spinning-disk generator. The technique involves the key steps of extracting sodium pertechnetate ($Na^{99m}TcO_4$) into chloroform as tetraphenylarsonium pertechnetate, followed by evaporation of the chloroform. A solution of polystyrene is added to the radioactive residue and dispersed (*Scheme* 1). This technique has subsequently been adopted by

TABLE 1 Radiolabelling Pharmaceutical MDIs

Drug MDI	Study Objective	References
Salbutamol sulfate	Development of radiolabelling method[a]	19
Salbutamol	Radiolabelling method development, simultaneous measurement of lung deposition and bronchodilator response[b]	16
Salbutamol	Comparison of lung deposition and bronchodilator response between MDI, DPI, and nebulizer[b]	17
Sodium cromoglycate	Development of radiolabelling method[c]	32
Sodium cromoglycate	Comparison of MDI with DPI	33
Sodium cromoglycate	Effect of spacer	13
Sodium cromoglycate	Effect of higher dose (5 mg/puff) Effect of spacer	14
Nedocromil sodium	Development of radiolabelling method	18
Nedocromil sodium	Ventilator suitability	21
Terbutaline sulfate, salbutamol	Development of radiolabelling method	22
Nacystelyn	Lung deposition	23
Salbutamol and fenoterol	Development of radiolabelling method and lung deposition	24
Flunisolide and fenoterol	Lung deposition and effect of a spacer	25
Salbutamol	Development of radiolabelling method and comparison of MDI with DPI	26
Beclomethasone diproprionate in hydrofluorocarbons	Correlation of in vitro particle sizing with in vivo deposition	27
Fenoterol	Comparison of nebulizer with MDI plus a holding chamber	28
Salbutamol	Aerosol delivery by microprocessor control (SmartMist™)	29

[a] Drug particles were suspended in the chloroform phase and spray-dried.
[b] Radiolabelled Teflon was used (the drug itself was not radiolabelled).
[c] Spray-drying of the drug was followed by reconstitution in an MDI.

other workers for radiolabelling pharmaceutical MDIs of sodium cromoglycate [13,14]. It is important to note that in this technique the complexing agent tetraphenylarsonium chloride is classified as poisonous [15]. Although the actual amount of the compound inhaled is in the nanogram range, safety to the researchers and the subjects inhaling the aerosols has to be carefully ensured.

MDI radiolabelling—Scheme 1 [10]

Elution of ^{99m}Tc as sodium pertechnetate from a ^{99}Mo–^{99m}Tc generator
Adjustment of pH to 7–9 with concentrated NH_4OH
Addition of a drop of 5% tetraphenylarsonium (TPA) chloride aqueous solution
Extraction of ^{99m}Tc-TPA into chloroform (1)
Separation by passing through phase-separation paper (2)
Collection of the filtrate (the $CHCl_3$ phase containing the ^{99m}Tc-TPA)
Evaporation of the $CHCl_3$ to dryness
Reconstitution in the appropriate medium containing the compound for radiolabelling
Note: (1) e.g., by mechanical shaking or sonication the solution with $CHCl_3$.
　　　(2) e.g., Whatman 1_s^p silicone-treated phase-separation filter paper.

Another way to radiolabel the pharmaceutical MDI is to use Teflon particles with a size distribution similar to that of the drug of interest (*Scheme* 2). This was carried out in 1981 by Newman et al. [11] who employed Teflon particles of mean size 2 ± 0.4 μm to mimic the MDI aerosols of bronchodilators. Hence, the Teflon particles were used as a surrogate for the drug. The Teflon particles were almost monodisperse and were produced by a spinning-disk aerosol generator. However, this approach is limited by the physicochemical characteristics of the Teflon particles being different from those of the drug particles. Furthermore, the aerosol particle size distribution of the Teflon may not match that of the drug. By coadministering a physical mixture of the radiolabelled Teflon particles with the drug salbutamol, the lung deposition and clinical response had been monitored simultaneously [16,17].

MDI radiolabelling—Scheme 2 [11]

Elution of ^{99m}Tc as sodium pertechnetate from a ^{99}Mo–^{99m}Tc generator
Removal of sodium by passing the eluate through a cation exchange column
Collection of the filtrate
Evaporation of the filtrate to dryness
Addition of Teflon particles suspended in 40% alcohol
Generation of radiolabelled Teflon particles by spinning-disk technique (1)
Collection of radiolabelled Teflon particles
Transfer of the radiolabelled particles to an empty canister
Addition of the content from a commerical MDI (2) into the canister (3) containing the radiolabelled particles, followed by recrimping of the MDI
Mixing by sonication
Note: (1) e.g., a spinning speed of 62,000 rpm has been used
　　　(2), (3) both canister, are chilled at −60°C before the transfer is carried out.

The first attempt to radiolabel drug particles (instead of polymers like polystyrene or Teflon particles) for pharmaceutical aerosols was carried out on fenoterol and salbutamol by Kohler et al. [12] (*Scheme 3*). However, it was later found that their method would change the particle size distribution of the labelled aerosol, resulting in a coarser aerosol than the unlabelled product. After subsequent improvement by Summers et al. [18] (*Scheme 4*), this method has become widely used for radiolabelling MDIs. It is preferred over other methods as it does not involve extraction with tetraphenylarsonium chloride and chloroform.

MDI radiolabelling—Scheme 3 [12]

Elution of ^{99m}Tc as sodium pertechnetate from a ^{99}Mo–^{99m}Tc generator
Extraction of ^{99m}Tc-TPA into methyl ethyl ketone (MEK) (1). Repeat the extraction if necessary
Separation of the aqueous and MEK phases (2)
Collection of the MEK phase (containing the pertechnetate)
Evaporation of the MEK to dryness in a glass beaker
Addition of propellant (3) and surfactant (4) to the dry pertechnetate
Concentration by evaporating the propellant to 0.2 mL
Transfer of the propellant containing the pertechnetate and surfactant into an MDI canister to be radiolabelled, followed by recrimping of the MDI
Mixing by shaking
Note: (1) e.g., by shaking the pertechnetate solution with approximately equal volume of MEK
     (2) e.g., in a separating funnel
     (3) e.g., Freon 11, 5–10 mL has been used
     (4) e.g., 1% sorbitan trioleate has been used

MDI radiolabelling—Scheme 4 [18]

The first four steps are the same as in Scheme 3
Evaporation of the MEK (containing the pertechnetate) to dryness in an empty canister
Transfer of the content from a commercial MDI into the canister containing the pertechnetate for radiolabelling, followed by recrimping of the MDI
Mixing by sonication
Note: This method is simpler than Scheme 3 as it does not involve the propellant concentration step and the subsequent transfer of the concentrate.

It is worth noting that each of the above mentioned radiolabelling methods can be further modified for the study need. For example, the drug particles can be suspended in the organic phase containing the radiolabel and spray-dried, followed by reconstitution in the propellants, as has been carried out on salbutamol sulfate [19].

Although the radiolabelling methods have been widely used, the mechanism of association between the radiolabel and the drug particles has been studied only recently. The study by Farr [20] on MDI systems indicated that in the chlorinated fluorocarbons (CFC) formulation the radiolabel ^{99m}TcO$_4^-$, being hydrophilic, would associate with hydrophilic domains including the surface of hydrophilic drug particles and the interior of surfactant reverse micelles. There is a need to extend the study to other systems, such as hydrophobic drugs and non-CFC propellants.

Dry Powder Inhalers (DPI)

A method of wide application to radiolabelling dry powders is by adsorbing the radiolabel on the particles in a suitable liquid (*Scheme 5*). The drug particles are wetted with a nonsolvent containing the radiolabel, followed by evaporation of the solvent, leaving the radiolabel on the surface of the drug particles. This method has been applied to radiolabel terbutaline sulfate and budesonide [30,31]. Factors influencing the radiolabelling of dry powder formulations include the physicochemical nature of the drug, choice of non-solvent for the drug, solubility of radiolabel in the non-solvent, moisture level, electrostatic charge, and the number of processing steps [32]. Unfortunately, the details still remain largely as proprietary information and are not available in the literature. For example, exactly how the spherical agglomerates of budesonide powder are to be wetted with the ^{99m}Tc solution in order to ensure reproducible radiolabelling has not been reported.

DPI radiolabelling—Scheme 5

The first four steps are the same as in Scheme 3
Evaporation of the MEK (containing the pertechnetate) to dryness
Redissolution of the pertechnetate in a suitable liquid (1)
Addition of the pertechnetate solution to (1) the drug powder to be radiolabelled (2)
Evaporation of the liquid (3)
Filling of the radiolabelled powder to the DOI (4)
Note: (1) the liquid must be a non-solvent for the drug powder to be radiolabelled; some suitable ones include water for budesonide, chlorofluorocarbon 11 for salbutamol
      (2) mixing, e.g., by sonication for 20 min, if required
      (3) freeze-drying has been used for water removal
      (4) blend with lactose carrier, if required

Alternatively, a method resembling Scheme 1 can be used, provided that the drug (e.g., terbutaline sulfate) to be radiolabelled does not dissolve in $CHCl_3$. This method involves the same first six steps as Scheme 1. After the filtrate (the $CHCl_3$ phase

TABLE 2 Radiolabelling Pharmaceutical DPIs

Drug DPI	Study Objective	References
Terbutaline sulfate	Development of radiolabelling method for Turbuhaler in lung deposition study	31
Nedocromil sodium	Lung deposition study and comparison with an MDI	34
Budesonide	Nasal distribution study	30
Budesonide	Lung deposition in children with cystic firbrosis	38
Salbutamol	Lung deposition study using a new DPI	32
Disodium cromoglycate	Lung deposition comparison between a conventional MDI and a new DPI	34
Salbutamol sulfate	Development of radiolabelling method and comparison with an MDI	26
Cromoglycic acid and nedocromil	Development of radiolabelling method	37

containing the ^{99m}Tc-TPA) is collected and added to the drug powder. The CHCl$_3$ is then evaporated (e.g., at 70°C), leaving the radiolabel with the powder. The powder is ready for filling into the DPI. Bronchodilators (salbutamol, terbultaline sulfate) and prophylactics (nedocromil sodium) for asthma have been successfully labelled by this technique for lung deposition studies [33,34].

An earlier method of radiolabelling dry powders (mainly sodium cromoglycate) involved spray-drying [35,36]. The basic principle can be considered the same as the one in Scheme 1 for MDI in that radiolabelled particles were produced by evaporation of radiolabel-containing atomized droplets. The method is straight forward (*Scheme 6*) but suffers the limitation that the spray-dried particles may not be physicochemically the same as those in the commercial products because milling rather than spray-drying is normally used for micronization of the drug particles. Spray-drying, following ^{99m}Tc adsorption to the surface of the particles, has also been used to prepare radiolabelled cromoglycic acid and nedrocromil powders [37].

DPI radiolabelling—Scheme 6

Preparation of solution of the drug to be radiolabelled (1)
Addition of sodium pertechnetate to the drug solution (2)
Spray-drying of the solution (3)
Collection of dry powder
Filling of the radiolabelled powder into the DPI (4)
Note: (1) e.g., 6% w/w for sodium cromoglycate
     (2) e.g., 1 mL of pertechnetate in normal saline
     (3) e.g., Buchi Minispray dryer, model 190, liquid feedrate 60 mL/min, inlet air temp
     180°C, air throughput 2.4 m^3/min and nozzel air pressure 800 kPa (116 psi)
     (4) blend with lactose carrier, if required

The exact mechanism of association between the radiolabel and the drug particles is unknown and is generally regarded as a surface-coating phenomenon. However, the surface is proportional to the square of the particle size, whereas the volume (drug mass) is proportional to the cube of the particle size. It follows that to have a match between the radiolabel and drug mass in the aerosol, the radiolabelled particles must exist as agglomerates rather than as single particles.

Gamma Scintigraphic Imaging for Lung Deposition

Aerosol deposition in the lung is measured by gamma scintigraphy routinely used in nuclear medicine. A gamma camera consists of a collimator located in front of a detector behind which is an array of photomultiplier tubes [3]. The collimator allows only gamma rays with a defined angle to reach the detector. The detector, which is a scintillation crystal made of sodium iodide, converts the gamma rays to light photons. The photomultiplier tubes transform the light photons into electrical signals that can be displayed in the X and Y position on a monitor such as a cathode ray tube or a computer. Planar imaging, which provides two-dimensional anterior and/or posterior views of the lung, has been commonly employed in the past to study deposition.

This involves inhalation of the radiolabelled aerosols with a collimated gamma camera placed in front or behind the chest of the subject. The image is formed as the gamma ray photons emitted from the radiolabelled aerosol in the lung fall on the camera detector. The main advantage of planar imaging is that the image acquisition and data processing demand less effort. Furthermore, compared to tomographic imaging (see SPECT below), it can utilize a lower radioactivity dose. However, a severe limitation of planar imaging is that it compresses the three-dimensional lung into a two-dimensional view, and hence it cannot provide 3D spatial information about the aerosol distribution in the lung. This may become more critical if the lung deposition data are to be used for bioequivalence comparison [1,2].

The 3D image data can be produced by single-photon emission computed tomography (SPECT). This technique has been used to measure lung deposition of aerosols in a number of studies [6,39–42]. It involves imaging the lung at different angles by a SPECT camera rotating around the chest of the subject after radioaerosol inhalation. The acquired raw data are then processed by high-speed computers to reconstruct the lung images in the coronal, saggital, or transverse section (e.g., Fig. 1 shows the coronal-section images). A question remains as to whether SPECT offers any advantage over planar gamma scintigraphy for the quantitation of total (as opposed to regional) lung deposition. This can be answered by comparing the measurement
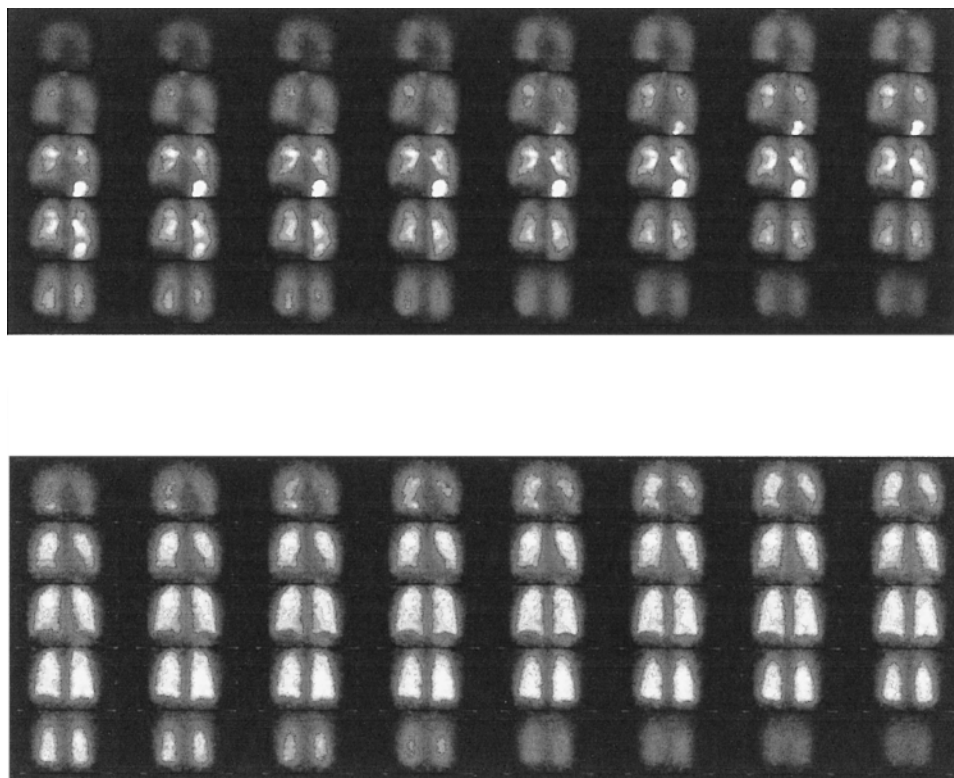
FIG. 1. Fast 1-min SPECT coronal-section lung images of a healthy subject after inhalation of normal saline aerosols containing ^{99m}TC-DTPA in large and small droplet sizes (7 µm, top panel; 3 µm, bottom panel) generated by air jet nebulizers. The difference in deposition between the two aerosols is clearly shown in these images.

of a known amount of radioactivity in a lung phantom (e.g., a perspex container with size and shape similar to the human lung) using both techniques. The results of this comparison have not yet published.

Misconceptions of SPECT

High radiation dose has often been quoted as a disadvantage of SPECT [1]. Obviously, the radioactive dose should never be administered higher than required, regardless of SPECT or planar imaging. It is interesting to note that a number of planar imaging studies were carried out in the past using radiation doses comparable to those for the SPECT [43]. In practice, the dose consideration must be balanced by the benefits of the deposition details gained using SPECT. If planar imaging cannot provide the required deposition details, then SPECT should be considered. Otherwise, carrying out a suboptimal study using planar imaging without obtaining the deposition data required is not justified as it exposes the subjects to unwanted radiation exposure. The amount of radioactivity delivered to the lung for SPECT is about 60 MBq which is equivalent to an effective whole body dose of 0.4 mSv. In comparison, the average radiation dose from background radiation is about 2–3 mSv in a year, depending on the geographical location.

Another common misconception about SPECT is the long image acquisition time and the associated problem of relocation of deposited aerosol particles in the lung [1]. Historically this was true when a single-head SPECT gamma camera was used in the 1980s for lung deposition as the imaging process took about 15–20 min [43]. During this length of time, relocation of the radiolabel occurred as confirmed by comparing lung images immediately before and after the SPECT acquisition. Radiolabel movement would create problems of inconsistent position during image reconstruction, leading to artefacts in the reconstructed images. Despite these limitations, SPECT has been shown to be superior to planar imaging in differentiating lung deposition between aerosols [39]. In a more recent study on a novel inhaler using different breathing maneuvres [42], SPECT was able to detect a subtle difference in the aerosol deposition that was not observed using planar imaging (Fig. 2). The problem of long acquisition time has recently been solved by fast dynamic SPECT [44]. Using a triple-head gamma camera, the SPECT acquisition time has been reduced to only one minute (Fig. 1). Hence, 20–30 complete individual 1-min SPECT image acquisitions can be collected in 20–30 min, making dynamic study feasible. The technique can also be applied to study clearance of an aerosol from the lung after deposition (Fig. 3).

Attenuation and Scatter Correction

Attenuation occurs when the gamma ray photons emitted from the radionuclide in the lung are absorbed by the body tissues before reaching the camera detector. Attenuation causes a reduction in measured radioactivity counts. Because of the heterogeneous nature of the thorax tissues (lung, muscles, bones), the attenuation cannot be assumed to be uniform and has to be measured. The attenuation information can be obtained experimentally from a transmission scan that uses an external gamma radiation source for imaging the thorax. To avoid uncertainty in image realignment between studies, transmission imaging is best carried out simultaneously with the aero-
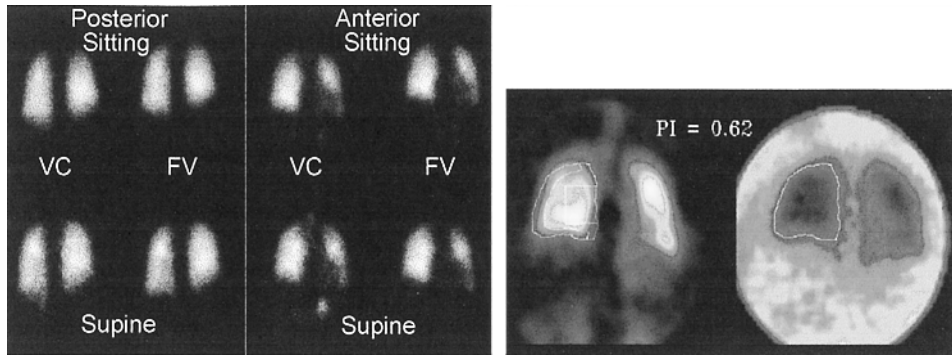
FIG. 2. Lung images of a healthy subject after inhalation of an aqueous aerosol containing [99m]TC-DTPA generated by a novel aerosol inhaler, the AERx system of Aradigm Inc. (for details, see Ref. 42). Left panel: planar images from aerosols inhaled using two different breathing maneuvres (vital capacity (VC) or fixed volume (FV) of 1L above the functional residual capacity) and two different postures as stated. Right panel: a mid-coronal image acquired by single photon emission computed tomography (SPECT). Superposition of the lung outline from the transmission SPECT image (on the right) on the emission SPECT image confirmed the excellent peripheral deposition of the aerosol.

sol (emission) study [45]. This can be achieved by attaching the radiation source to the gamma camera during the aerosol lung image acquisition. The use of the same radionuclide for the transmission as the emission imaging would eliminate uncertainties due to differences in the photon energy. When simultaneous emission-transmission imaging is not feasible, the attenuation information can also be obtained from a separate transmission study prior to the aerosol inhalation. Alternatively, an x-ray computerized tomography or CT scanning can be carried out but involves a higher radiation dose to the subject. In addition, the separate transmission scan would require extra time, and image realignment may be more difficult.

Scatter occurs when the gamma ray photons emitted from the radionuclide in the lung encounter the thorax tissues and change the travelling direction from their origi-
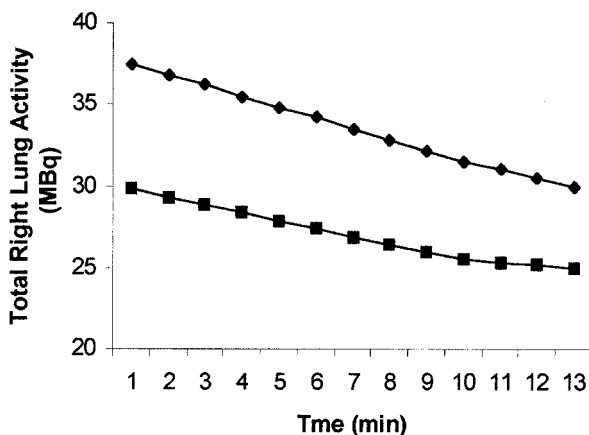
FIG. 3. The time course of activity clearance from the lung measured by fast 1-min SPECT on a healthy subject after inhalation of normal saline aerosols containing [99m]TC-DTPA in large and small droplet sizes (7 μm, ◆; 3 μm, ■). In the plot, 2-min frames are used for clearer illustration.

nal path. Scatter therefore causes mispositioning of the detected counts, leading to a loss of image contrast and quantitative accuracy. Quantitative assessment of aerosol deposition would require both attenuation and scatter correction. The latter is also important for proper image reconstruction in SPECT. For further reading, texts of SPECT imaging are recommended [46,47].

Data Analysis

Image Processing

Planar images can be analyzed on the anterior or posterior view. Because the lung has a thickness, the radiolabel may occur anywhere along the thickness. The effect due to deposition at varying thickness can be minimized by taking the geometric mean of the anterior and the posterior images.

Compared with planar images, SPECT data require more rigorous treatment involving mathematical image-reconstruction algorithms. Because of simplicity and speed, the filtered back-projection algorithm has been used in clinical SPECT studies including aerosol deposition in the lung. Filtered back-projection employs summation techniques using projections (count-rate profiles) of the acquired image data. Artefacts in the back-projected image are corrected by filtering the data (using mathematical operations) before or after reconstruction. Due to the nonuniform attenuation regions of the thorax, other iterative reconstruction techniques involving attenuation and scatter correction have also been used [46,47].

Drawing of the Lung Region

The lung outline is obtained from the transmission image, or from ventilation image using radioactive gases such as [81m]Kr, when available. After superimposing the lung outline on the aerosol emission image, the lung is divided into different regions of interest and the amount of activity in each region is measured. Regions such as apex and basal or central, middle, and peripheral are most commonly used. A conventional way to analyze aerosol distribution (or deposition pattern) is to express it as the ratio of the activity in one region to another (e.g., peripheral to central) which is conventionally defined as the penetration index. However, there is no consensus on how the size and shape of each region should be drawn. For example, both rectangular regions and lung shape regions have been used for the planar images in the past but the regions can vary from laboratory to laboratory. Hence there is a need to standardize the way for drawing lung regions for comparing studies between different laboratories. The rationale of drawing the lung regions is that the peripheral region should contain more small airways than the central region. In a planar image there is inevitably extensive overlapping of structures in the lung. In relative measurement or comparative studies using the same subject as his or her own control, the region size and shape may not be as crucial as in the absolute measurement when the aim is to assign the deposition to certain anatomical structures. The latter is a formidable task since the whole lung contains about 1.6 million airway branches. It has recently been attempted on SPECT images with the aid of computer modelling [48]. The success will provide valuable information for interpreting the gamma-camera images in relation to the aerosol distribution in the airways.

Summary

Radiolabelling of nebulizer solutions, propellant-driven metered-dose inhalers, and dry-powder inhalers is generally well documented. For some dry-powder inhalers the procedure may still be lacking sufficient details to ensure reproducible radiolabelling. Deposition of radiolabelled aerosols in the lung has been mainly measured by planar imaging. Tomographic imaging using SPECT can provide 3D information about the spatial distribution of the aerosol in the lung. The recent development of the fast dynamic SPECT has made it the method of choice for aerosol imaging in the new millennium.

Acknowledgements

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Super Disintegrants: Characterization and Function

Introduction

Disintegrating agents are substances routinely included in tablet formulations and in some hard-shell capsule formulations to promote moisture penetration and dispersion of the matrix of the dosage form in dissolution fluids. An oral solid dosage form should ideally disperse into the primary particles from which it was prepared. Although various compounds have been proposed and evaluated as disintegrants, relatively few are in common usage today. Traditionally, starch has been the disintegrant of choice in tablet formulations where it is still widely used. However, starch is far from ideal. For instance, starch generally has to be present at concentrations above 5% which could adversely affect compactibility, especially in direct compression. Moreover, intragranular starch in wet granulations is not as effective as dry starch. In recent years, several new disintegrants have been developed. Often called super disintegrants, they can be used at lower levels than starch. Because they can be present in lower concentrations in the overall formulation than starch, any possible adverse effect on fluidity or compactibility would be minimized. These newer disintegrants may be organized into three classes based on their chemical structure (Table 1).

General Chemistry and Surface Morphology

Sodium Starch Glycolate

Sodium starch glycolate is a super disintegrant made by cross-linking sodium carboxymethylstarch [1]. Cross-linking involves a chemical reaction with phosphorus oxytrichloride or sodium trimetaphosphate, or a physical manipulation. Carboxymethylation is performed by treating starch with sodium chloroacetate in an alkaline medium and neutralizing with citric or acetic acid, a process known as a Williamson ether synthesis. It yields carboxymethylation of about 25% of the glucose units. The by-products, which include sodium chloride, sodium glycolate, and sodium citrate or acetate, are partially washed out. The particle size of the disintegrant is increased by the substitution and cross-linking processes [3].

Sodium starch glycolates are generally spherical, a characteristic which accounts for their good flowability [4]. Figure 1 shows the scanning electron photomicrographs (SEMs) of some the commercial sodium starch glycolates.

Croscarmellose Sodium

Croscarmellose sodium is derived by internally cross-linking the ether, sodium carboxymethylcellulose. This water-soluble polymer is composed of repeating units of

TABLE 1   Classification of Super Disintegrants (Partial Listing)

Structural Type (NF Name)	Description	Trade Name (Manufacturer)
1. Modified starches (Sodium starch glycolate, NF)	Sodium carboxymethyl starch; the carboxymethyl groups induce hydrophilicity, cross-linking reduces solubility	Explotab (Edward Mendell Co.) Primojel (Generichem Corp.) Tablo (Blanver, Brazil)
2. Modified cellulose (Croscarmellose, NF)	Sodium carboxymethyl cellulose which has been cross-linked to render the material insoluble	AcDiSol (FMC Corp.) Nymcel ZSX (Nyma, Netherlands) Primellose (Avebe, Netherlands) Solutab (Blanver, Brazil)
3. Cross-linked polyvinylpyrrolidone (Crospovidone, NF)	Cross-linked polyvinylpyrrolidone; the high molecular weight and cross-linking render the material insoluble in water	Crospovidone M (BASF Corp.) Kollidon CL (BASE Corp.) Polyplasdone XL (ISP Corp.)

cellobiose units, with each unit consisting of two anhydroglucose units linked by 1,4-β-glucoside. Each unit carries three hydroxyl groups. The degree of substitution refers to the average number of hydroxyl groups substituted by carboxymethyl groups.

To prepare croscarmellose sodium, crude cellulose is steeped in sodium hydroxide solution [1] and treated with sodium monochloroacetate to form carboxymethylcellulose sodium. After completion of the reaction, the excess sodium monochloroacetate slowly hydrolyzes to glycolic acid. The glycolic acid converts a few of the sodium carboxymethyl groups to the free acid and catalyzes the cross-linkage to form croscarmellose sodium. The by-products sodium chloride and sodium glycolate can be removed by extraction with alcohol to achieve 99.5% purity. Croscarmellose sodium may be milled to break the polymer fibers into shorter lengths and hence improve flow properties.

Unlike sodium starch glycolate, crude croscarmellose sodium particles do not flow well because of their twisted fibrous morphology and varying lengths. Therefore, they are cryogenically milled to improve flowability. The scanning electron photomicrographs show that the croscarmellose sodium particles are fibers with fairly sharp ends, probably because of the milling process (Fig. 2).

Crospovidone

Crospovidone is a cross-linked homopolymer of *N*-vinyl-2-pyrrolidone. Acetylene and formaldehyde react to form butynediol. Hydrogenation and subsequent cyclodehydrogenation gives butyrolactone. The reaction of butyrolactone with ammonia produces pyrrolidone, which is vinylated with acetylene under pressure. The linear polymerization of the vinylpyrrolidone yields polyvinylpyrrolidone, a soluble binder, whereas the popcorn (branched) polymerization yields crospovidone, an insoluble
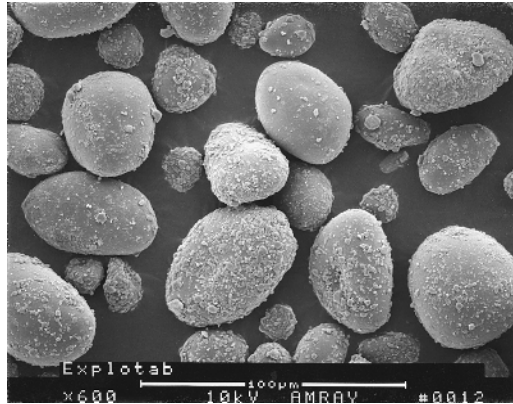
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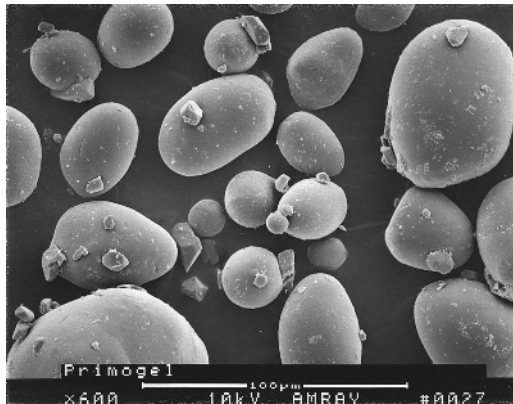
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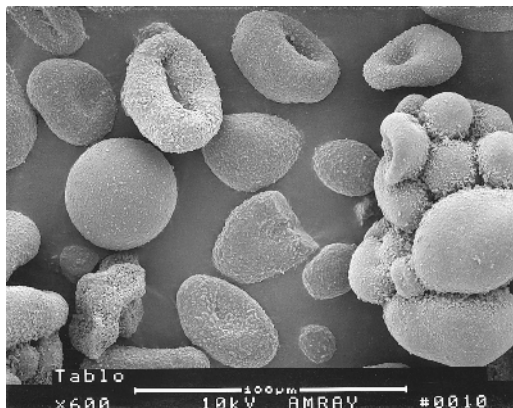
(C)

FIG. 1. Scanning electron photomicrographs of sodium starch glycolates: (A) Explotab, (B) Primojel, and (C) Tablo; 600× Magnification.
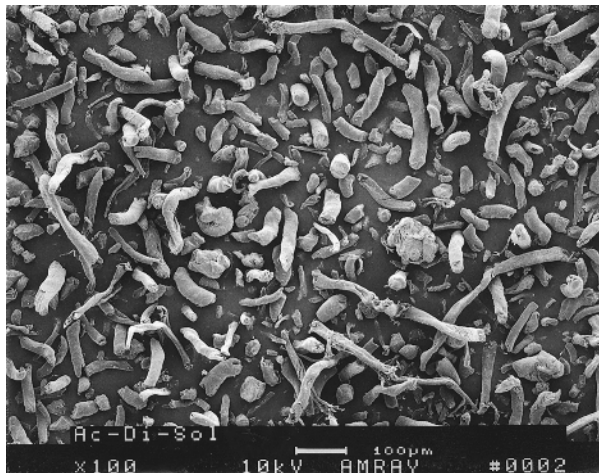
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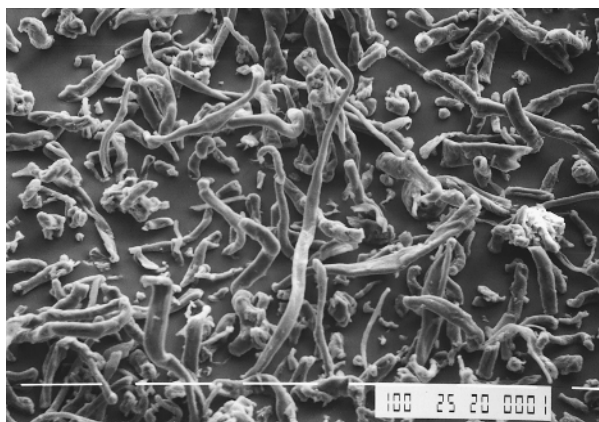
(B)

FIG. 2. Scanning electron photomicrographs of croscarmelloses: (A) AcDiSol, (B) Nymcel ZSX, (C) Primellose, and (D) Solutab; 100× Magnification.

super disintegrant. The by-products of popcorn polymerization include vinylpyrrolidone and polyvinylpyrrolidone. Crospovidone contains less than 1.5% of the soluble material, which has been determined to be polyvinylpyrrolidone by infrared spectroscopy [4].

Crospovidone particles have a different appearance from those of the other two classes of super disintegrants. Crospovidone particles seem to consist of aggregates of smaller particles that are fused together. This aggregation gives crospovidone a spongy, highly porous appearance (Fig. 3). Scanning electron photomicrographs show that the reduction of particle size of crospovidone increases the surface area per unit weight, but decreases the intraparticulate porosity and the spongy appearance [4].
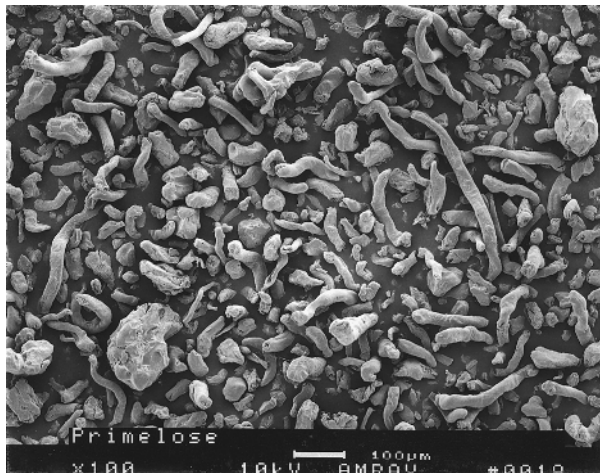
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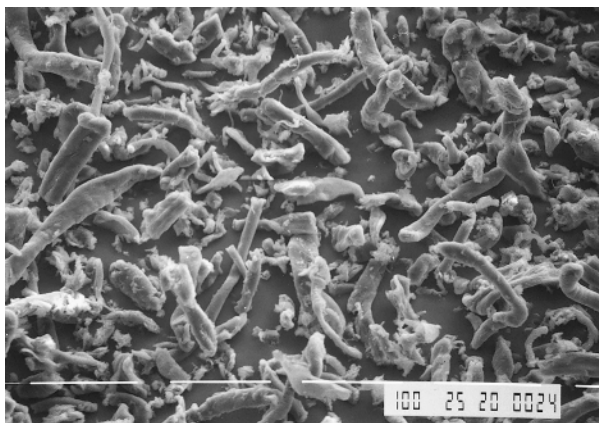
(D)

FIG. 2. (*continued*)

Disintegrant Action

Although disintegrants are important components in solid dosage forms, their mechanism of action has not been clearly elucidated. The mechanisms proposed in the past include water wicking, swelling, deformation recovery, repulsion, and heat of wetting. It seems likely that no single mechanism can explain the complex behavior of the disintegrants. However, each of these proposed mechanism provides some understanding of different aspects of disintegrant action.

Water Wicking

The ability of a disintegrant to draw water into the porous network of a tablet is essential for effective disintegration. For crospovidone, water wicking has been thought to be the main mechanism of disintegration. Kornblum and Stoopak [5] ob-

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Crospovidone M
100µm
×150
10kV
AMRAY
#0006
Kollidon CL
100µm
×150
10kV
AMRAY
#0026
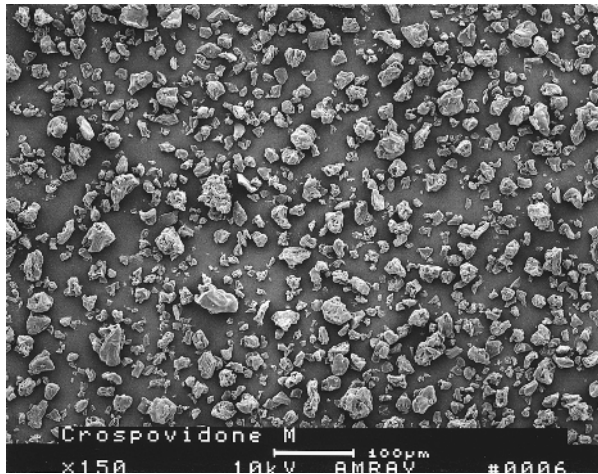
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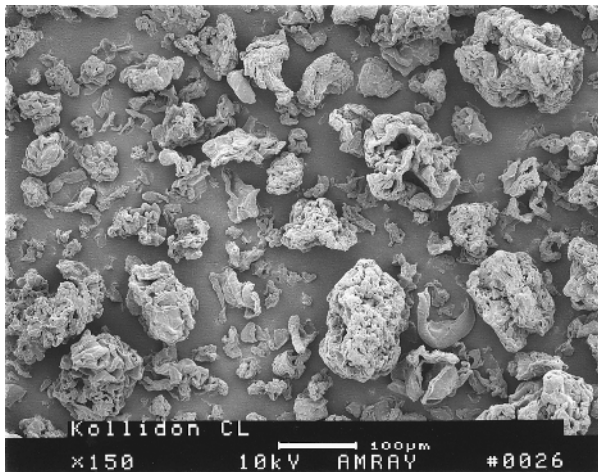
(B)

FIG. 3. Scanning electron photomicrographs of crospovidones: (A) Crospovidone M, (B) Kollidon CL, (C) Polyplasdone XL-10, and (D) Polyplasdone XL. 150× Magnification.

served that crospovidone swells very little, yet rapidly absorbs water into its network. Even the extensively swelling sodium starch glycolate shows improved disintegration when the molecular structure was altered to improve water uptake, as observed by Rudnic et al. [6]. Unlike swelling, which is mainly a measure of volume expansion with accompanying force generation, water wicking is not necessarily accompanied by a volume increase.

The ability of a system to draw water can be summarized by the Washburn equation (1).

$$L^2 = \left(\frac{\gamma \cos\theta}{2\eta}\right) rt \tag{1}$$
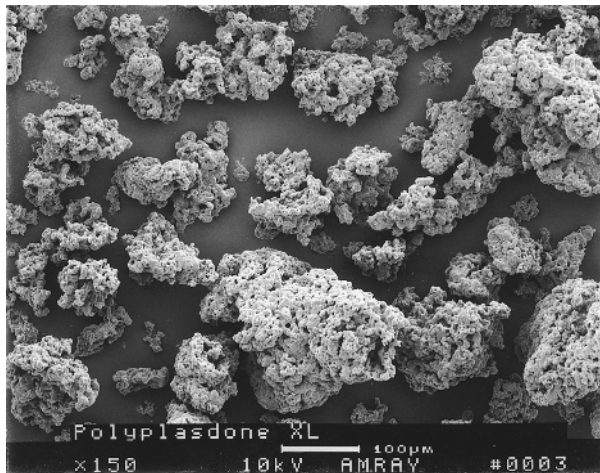
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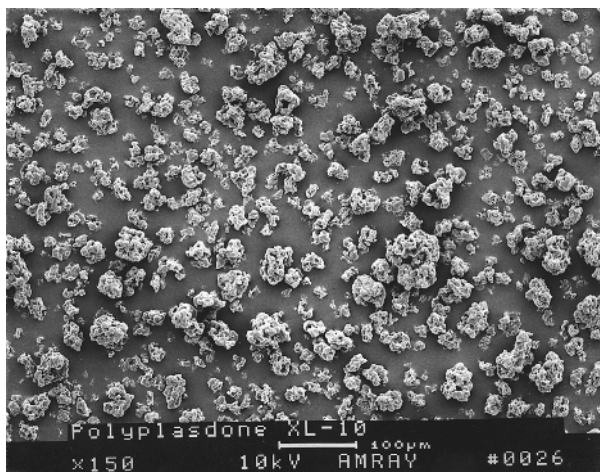
(D)

FIG. 3. (*continued*)

This equation [7] is too simplistic to apply to a dynamic tablet-disintegration process, but it does show that any change in the surface tension (γ), pore size (r), solid–liquid contact angle (θ), or liquid viscosity (η) could change the water wicking efficiency (L = length of water penetration in the capillary, t = time). For example, when Rudnic et al. [8] evaluated the disintegration efficiency of crospovidone of different particle sizes, the samples with the largest particle size range (50–300 μm) gave the shortest disintegration time. Large particle sizes probably yielded greater pore size and altered the shape of the pore. Indeed, fiber length increased by greater particle size could improve the efficiency of capillary uptake of water into the dosage form matrix.

Super disintegrants draw water into the matrix system at a faster rate and to a greater extent when compared to traditional starch [9]. Van Kamp et al. [10], utilizing

a water-uptake measurement device, showed that tablets that demonstrate greater uptake volume and rate, such as those containing sodium starch glycolate, disintegrated more rapidly. Although the hydrophobic lubricant, magnesium stearate, seemed to negatively affect the wicking process, those containing sodium starch glycolate were less affected by the detrimental effect of mixing with the hydrophobic lubricant. Lerk et al. [11] also observed a lower rate of wetting when disintegrants were mixed with magnesium stearate for various mixing times. The decrease in the rate of wetting was proportional to the time of mixing. Most likely, this observation reflects a greater delamination of magnesium stearate at longer mixing times.

Swelling

Although water penetration is a necessary first step for disintegration, swelling is probably the most widely accepted mechanism of action for tablet disintegrants. Indeed, most disintegrants do swell to some extent, but the variability of this property between disintegrants reduces its plausibility as a sole mechanism.

The earliest attempt to measure swelling was to measure the sedimentation volume of slurries. Nogami et al. [12] developed a reliable test to measure both swelling and water uptake. Gissinger and Stamm [13] modified this apparatus and found a positive correlation between the rate of swelling and the disintegrant action for some disintegrants. List and Muazzam [14] later adapted this apparatus to measure both the rate of swelling and the swelling force by the application of force and displacement transducers. They found that disintegrants which generate large swelling forces are generally more effective.

For swelling to be effective as a mechanism of disintegration, there must be a superstructure against which the disintegrant swells. Swelling of the disintegrant against the matrix leads to the development of a swelling force. A large internal porosity in the dosage form in which much of the swelling can be accommodated reduces the effectiveness of the disintegrant. At the same time, a matrix which yields readily through plastic deformation may partly accommodate any disintegrant swelling if swelling does not occur at sufficient rapidity.

The swelling of some disintegrants is dependent on the pH of the media. Shangraw et al. [3] reported that sedimentation volumes of anionic cross-linked starches and celluloses are altered in acidic media. Polyplasdone XL and Starch 1500 were unchanged. In a separate study, Chen et al. [15] showed that acetaminophen tablets containing Primojel and AcDiSol have longer disintegration and dissolution times in acidic than in neutral medium. Those containing Polyplasdone XL showed no such differences. Mitrevej and Hollenbeck [16] verified the remarkable swelling capacity of some super disintegrants by exposing individual particles deposited on slides to high humidities and observing their degree of swelling through a microscope.

On the other hand, when Caramella et al. [17–19] evaluated different disintegrants for their ability to swell, no correlation could be observed between the maximum disintegrating force and the degree of particle swelling. However, they did observe a correlation between the rate of disintegrating force development and the disintegration time. Therefore, these authors suggested that the rate of development of a disintegrating force is all-important. Swelling capable of rapid force development may be

preferred since a slowly developing force could hypothetically allow tablets to relieve the stress generated without bond disruption.

Deformation Recovery

The deformation recovery theory implies that the shapes of the disintegrant particles are distorted during compression, and that the particles return to their precompression shape upon wetting, thereby causing the tablet to break apart. Hess [20], with the aid of photomicrographs, showed that deformed starch particles returned to their original shape when exposed to moisture.

Fassihi [21] concluded that at higher compression forces, disintegration may become dependent on mechanical activation of the tablet, resulting from the stored energy imparted by the compression process. He examined the disintegration times of tablets made of Emdex powder, magnesium stearate, and 5% disintegrant. Regardless of the disintegrant used (sodium starch glycolate, microcrystalline cellulose, corscarmellose sodium, or starch), the disintegration time increased with increasing compression force, then decreased again when the compression force was above 120 MNm^{-2}.

Research on deformation and its recovery in situ as a disintegration mechanism is incomplete. However, such a mechanism may be an important aspect of the mechanism of action of disintegrants such as crospovidone and starch which appear to exhibit little or no swelling. The efficacy of such disintegrants is likely to be dependent on the relative yield strength of the disintegrant and of the matrix in which it is compressed, since disintegration efficiency would surely depend on how much deformation is sustained by the disintegrant particles. Time-dependent stress relaxation could also be a factor in the aging of such tablets, in that any deformation induced into the disintegrant which cannot be sustained by intraparticulate bonding may gradually recover as the matrix relaxes.

Repulsion Theory

Guyot-Hermann and Ringard [22] have proposed a particle–particle repulsion theory to explain the observation that particles which do not swell extensively, such as starch, could still promote disintegration. According to this theory, water penetrates into the tablet through hydrophilic pores and a continuous starch network which conveys water from one particle to the next, imparting a significant hydrostatic pressure. The water then penetrates between starch grains because of its affinity for starch surfaces, thereby breaking hydrogen bonds and other forces holding the tablet together. At present, this theory is not supported by adequate data.

Heat of Wetting

Matsumara [23] noticed that starch particles exhibit slight exothermic properties during wetting, which was thought to cause localized stress resulting from the expansion of air retained in the tablet matrix. Unfortunately, this explanation, if valid, would be limited to a few substances such as aluminium silicate and kaolinite. List and
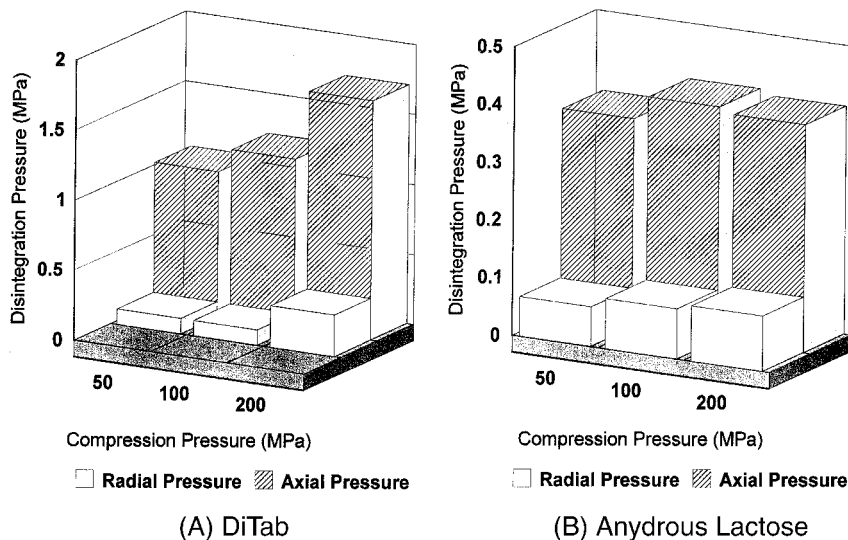
FIG. 4. The effect of compression pressure on the axial and radial disintegrating pressures of compacts made with AcDiSol (5%) and (A) DiTab or (B) lactose. (Data from Ref. 27.)

Muazzam [24] found that exothermic wetting reactions were not exhibited by all disintegrants and that even when a significant heat of wetting was generated, disintegration time did not always decrease. Caramella et al. [25] observed that an increase in temperature, which should cause air expansion, did not enhance maximum force generation in several formulations. Therefore, they concluded that expansion of air in pores due to heat of wetting could not be supported by the data. More recently, Luangtana-anan et al. examined the heat of wetting of powders and tablets of magnesium carbonate and Emcompress [26]. Magnesium carbonate tablets with significantly higher heat of wetting disintegrated more readily than the Encompress tablets. Indeed, a thermodynamic approach would be an interesting way to develop a model for the mechanism of tablet disintegration. However, heat of wetting alone is probably inadequate to explain disintegration.

Generation of a Disintegrating Force or Pressure as a Unifying Principle

The rate of generation of a disintegrating force or pressure may be a unifying factor in the mechanisms of disintegration [19]. Many proposed mechanisms may be imagined as giving rise to a force. Brzeczko [27] developed techniques to simultaneously measure the rate of liquid uptake into a tablet and the rate of generation of both axial and radial swelling forces. As indicated in Figs. 4–6 in a study of the three main super disintegrant classes in model tablet formulations, tablet compaction contributes more to the axial pressure than to the radial pressure. In all three cases, the maximum axial pressure in an anhydrous lactose matrix was well below that observed with a dicalcium phosphate dihydrate matrix when the disintegrants are compared at the same concentration. The differences in disintegrant performance in soluble and insoluble matrices could be rationalized in terms of pressure development and liquid
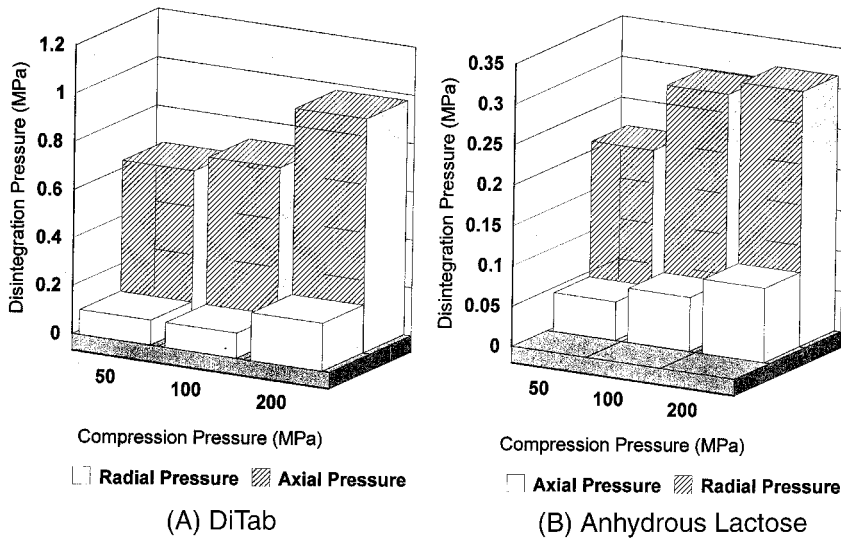
FIG. 5. The effect of compression pressure on the axial and radial disintegrating pressures of compacts made with Primojel (5%) and (A) DiTab or (B) lactose. (Data from Ref. 27.)
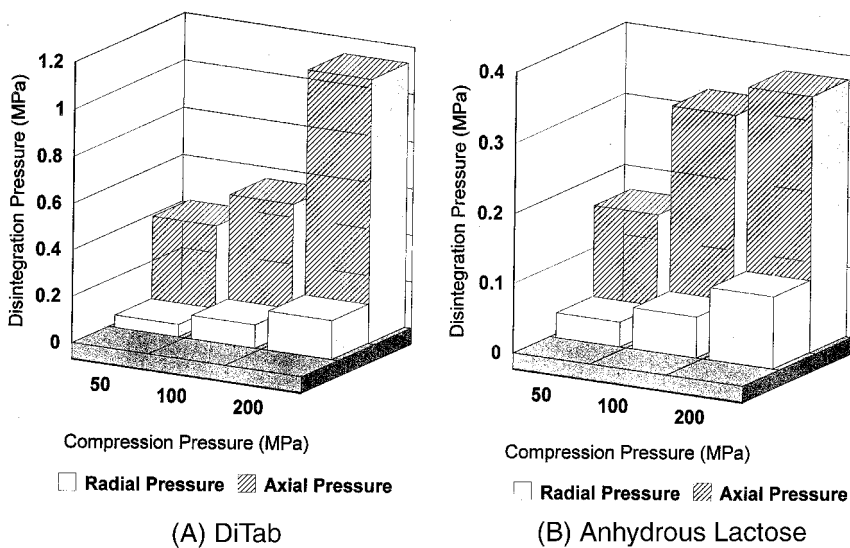
FIG. 6. The effect of compression pressure on the axial and radial disintegrating pressures of compacts made with Polyplasdone XL (5%) and (A) DiTab or (B) lactose. (Data from Ref. 27.)
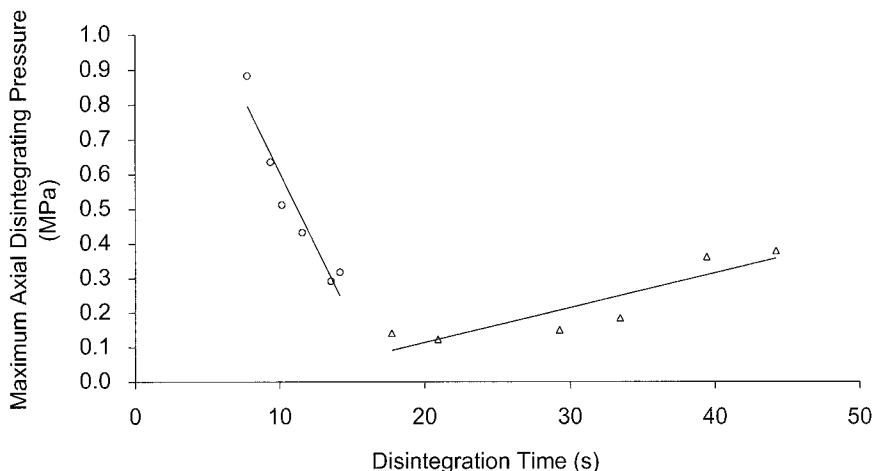
FIG. 7. Maximum axial disintegrating pressure vs. disintegration time of dicalcium phosphate and lactose tablets containing 2% super disintegrants. ($\bigcirc$) Dicalcium phosphate: $r^2 = 0.92$, $p < 0.05$, significant correlation; ($\triangle$) Lactose: $r^2 = 0.81$, $p < 0.05$, significant correlation. (Replotted from Ref. 27).

uptake. Figure 7 [27] compares the maximum axial disintegrating pressure and disintegration times of the tablets containing 2% of the disintegrants in a matrix composed of dicalcium phosphate or lactose. As can be seen, a higher disintegration pressure favors rapid disintegration of the dicalcium phosphate-based tablets, but a slower disintegration of lactose-based tablets. Higher initial axial disintegrating pressure rate also yields shorter disintegration times for the dicalcium phosphate-based tablets, but no such correlation is seen with the lactose-based tablets, whose disintegrating pressure rates are much lower than those of the dicalcium phosphate-based tablets (Fig. 8). Maximum water uptake and water uptake rate seem to be poor predictors of disintegration time, as seen in Figs. 9 and 10. However, lactose-based tablets show a trend toward slower disintegration with faster liquid uptake. It was suggested that faster liquid uptake leads to a faster dissolution of lactose and increased porosity to accommodate swelling and/or structural recovery.

Peppas [28] attributes the difference in disintegration rate between soluble and insoluble matrices to two phenomena: an interface-controlled mechanism and a diffusion-controlled mechanism, as represented by Eq. (2),

$$F/F_\infty = 1 - \exp(-kt^n) \tag{2}$$

where F is the disintegration force at time t, F_∞ the maximum force developed, k an expansion rate constant, and n signifies which of the two mechanisms controls the disintegration. The interface-controlled phenomenon involves tablet particles breaking apart from the interface of the tablet and the diffusion-controlled phenomenon involves particles diffusing away. Although it is thought that both happen simultaneously, the degree to which disintegration depends on each system can differ. For example, tablet matrices with a relatively small n of about 0.6 are thought to be dominated by the diffusional mechanism, whereas those with an n higher than 0.9
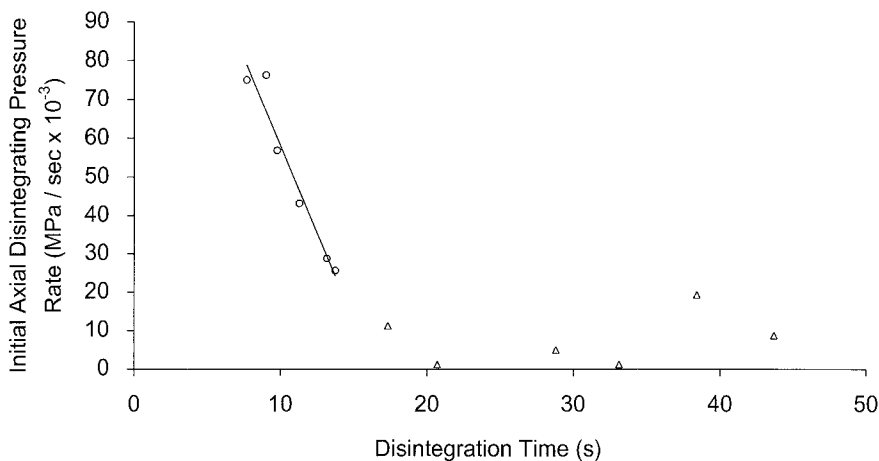
FIG. 8. Initial axial disintegrating pressure rate vs. disintegration time of dicalcium phosphate and lactose tablets containing 2% super disintegrants. (○) Dicalcium phosphate: $r^2 = 0.95$, $p < 0.05$, significant correlation; (△) Lactose: $r^2 = 0.10$, $p < 0.05$, no significant correlation. (Replotted from Ref. 27.)

are thought to be interfacial-mechanism dominant. The value of n would certainly differ based on the solubility of the matrices.

Since super disintegrants are highly hydrophilic yet insoluble in water, they would be expected to be more effective in breaking the tablet apart interfacially than controlling the diffusion per se. Indeed, Caramella et al. [29] observed that disintegration occurred readily for tablets containing insoluble calcium phosphate, whereas tablets containing highly soluble β-lactose disintegrated slowly. Such phenomena were explained by a lower value of n for the system containing β-lactose. In other words,
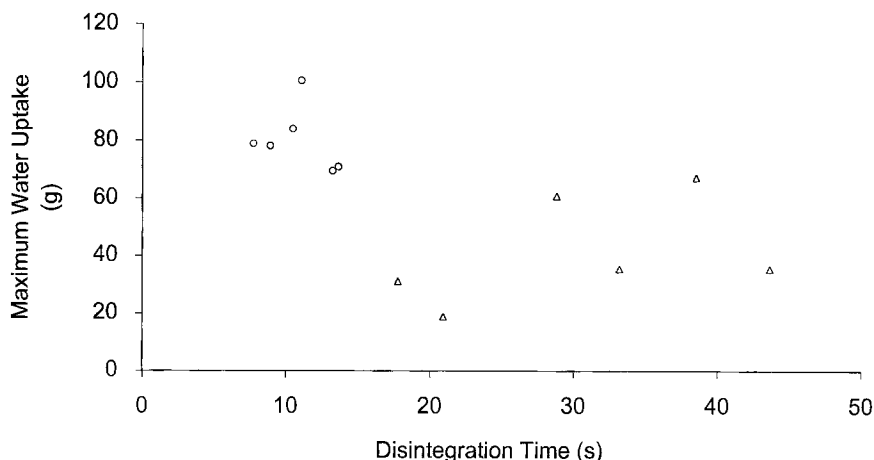
FIG. 9. Maximum water uptake vs. disintegration time of dicalcium phosphate and lactose tablets containing 2% super disintegrants. (○) Dicalcium phosphate: no significant correlation; (△) Lactose: no significant correlation. (Replotted from Ref. 27.)
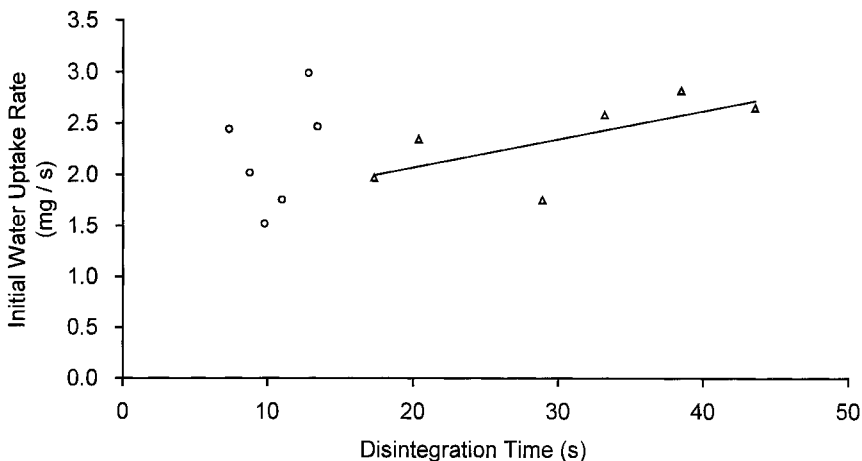
FIG. 10.   Initial water uptake rate vs. disintegration time of dicalcium phosphate and lactose tablets containing 2% super disintegrants. (○) Dicalcium phosphate: no significant correlation; (△) Lactose: $r^2 = 0.47$, $p < 0.05$, no significant correlation. (Replotted from Ref. 27).

the interface-controlled mechanism of super disintegrants could not overcome the diffusion-controlled mechanism of the β-lactose [29].

Factors Affecting Disintegrant Activity

Particle Size

Both the rate and force of disintegrant action may be dependent upon the particle size of the disintegrant. Smallenbroek et al. [30] found that starch grains with relatively large particle size were more efficient than the smaller particle size grades. This is probably because the continuous hydrophilic network of disintegrants is more efficiently built up by the bigger particles. Rudnic et al. [8] also found that coarser grades of crospovidone (50–100 μm, Grade B; 50–300 μm, Grade C) were more efficient than the finer particles (<15 μm, Grade A). The differences in disintegration efficiency between Grades B and C were not clear, however. When List and Muazzam [24] evaluated two different grades of crospovidone particles (100-200 μm and >315 μm), the efficiencies between the two grades were similar (Table 2). Results for the other disintegrants, Amberlite IRP88 and potato starch, support that coarser particle sizes allow more efficient disintegration than finer particles. For disintegrants that swell extensively, this can be explained by the observed force development. Indeed, larger particles of sodium starch glycolate swelled more rapidly and to a greater extent than the smaller particles [6].

Molecular Structure

Disintegrants can vary in molecular structure based on how they are manufactured or processed. Corn starch, for example, contains different proportions of two sugar

TABLE 2 Effect of Particle Size and Compression Pressure on Swelling Pressure and Disintegration Time[a]

Disintegrant[b]	Compression Pressure, (MPa)	ParticleSize (µm)	Swelling Pressure (kPa)	Disintegration Time (s)
Amberlite IRP88	62.5	<50	66.0	84
	156.0	<50	112.1	30
Amberlite IRP88	62.5	100–200	108.3	52
	156.0	100–200	226.2	22
Potato Starch	62.5	<50	16.5	254
	156.0	<50	31.0	164
Potato Starch	62.5	80–100	23.4	160
	156.0	80–100	44.5	77
Polyplasdone XL	62.5	100–200	89.8	31
	156.0	100–200	177.2	14
Polyplasdone XL	62.5	>315	76.0	42
	156.0	>315	148.0	17

[a] From Ref. 24.
[b] Tablet composition: 2.5% disintegrant, 1% magnesium stearate, and Emcompress.

fractions, amylose and amylopectin. Schwartz and Zelinski [31] concluded that the linear polymer amylose was responsible for the disintegrant properties associated with starch, whereas the branched polymer amylopectin was responsible for the gummy property. Varying the amylose to amylopectin ratio did not affect the porosities of the resulting tablets. Rudnic et al. [6] evaluated the effects of cross-linking and carboxymethyl substitution in sodium starch glycolate and concluded that the swelling of the disintegrant was largely inversely proportional to the degree of cross-linkage. Swelling also was inversely proportional to the level of substitution, but to a lesser degree. Shah et al. [32] found that carboxymethylcellulose, having high molecular weight and low levels of carboxymethylation, was best for tablet disintegration.

Effect of Compression Force

Compression force affects disintegration time in different ways. First, it governs the penetration of dissolution fluids into the matrix by controlling the porosity of the compact. Low compression force can lead to relatively high tablet porosity and rapid penetration of water. However, it has often been observed that tablets containing starch exhibit disintegration times that tend to pass through a minimum as compression force increases [21]. At low compression forces, any possible swelling or deformation recovery that may take place may be more or less accommodated by the porosity, whereas at intermediate compression forces a maximal disintegrating effect may develop. At high compression forces, fluid penetration may be impeded by a further reduction of porosity while particle deformation of the disintegrants becomes more important. In general, List and Muazzam [14] found increased swelling pressures at higher compression forces when various amberlite resins, starches, and crospovidones were used at 2.5% concentration in dicalcium phosphate matrix tablets (Table 2). Similar findings were reported by Fassihi [21] and Brzeczko [27].

In two different studies [33,34], Khan and Rhodes observed that tablets containing sodium starch glycolate disintegrate relatively slowly at low compression force, fast at intermediate compression force, and slowly again at high compression force. However, the effects of compression force on the disintegration time of other types of disintegrants, such as cation exchange resin, calcium sodium alginate, and various forms of starches, varied widely. Perhaps the effect of compression force on the disintegration time depends on the nature of the disintegrants, such as their mechanism of disintegration and deformation characteristics.

Munoz et al. [35] found that the effect of compression pressure on disintegration time depended on the concentration of the super disintegrant Explotab used. Figure 11 shows that the shortest disintegration time could be achieved at ca 7% disintegrant concentration. At this concentration, compression force has little effect on disintegration time. The disintegration time was more affected by compression force at low disintegrant concentration, being shortest at intermediate compression force. This type of biphasic effect of compression force on disintegration time also was observed for AcDiSol [36] with a surface-response curve similar to that of Explotab. When disintegration times were studied at 5 and 10% disintegrant, 5% AcDiSol yielded the lowest porosity, lowest yield pressure in Heckel analysis, and shortest disintegration time. At 10% disintegrant concentration, the tablets showed a slight expansion after compression, which could explain a slightly increased disintegration time compared to the 5% concentration.

The effect that compression force can have on the disintegration efficiency seems, therefore, largely dependent on the mechanism of the disintegrant action. The effectiveness of swelling or structure recovery may well be dependent on attaining a compression force that achieves a critical porosity in the matrix. On the other hand, the capillary uptake of liquid, which is a necessary precursor to these mechanisms could be compromised if the tablet matrix is compressed to a porosity too low.
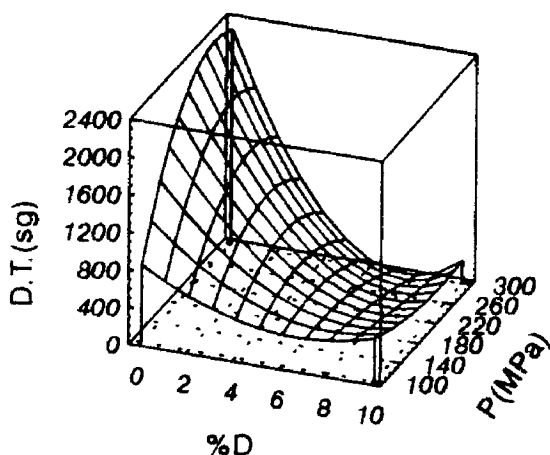
FIG. 11. Surface response of disintegration time as functions of compression pressure and percentage disintegrant. (From Ref. 35, reproduced with permission from the copyright owner.)

Matrix Solubility

The disintegrant mechanism seems to depend not only on the disintegrant itself but also on the matrix. Disintegrants work most effectively in insoluble matrices [27]. Insoluble matrices, such as those containing calcium phosphate do not disintegrate adequately without disintegrants. On the other hand, tablets and capsules that primarily consist of water-soluble fillers or drugs tend to dissolve rather than to disintegrate, even in the presence of disintegrating agents. It has been suggested that during the dissolving process, the water acts as a plasticizer [37], which can potentially reduce the development of disintegrating force. In addition, soluble materials that tend to swell can form viscous plugs which may impede further penetration of moisture into the matrix. However, the addition of disintegrants almost predictably shortens disintegration time, despite the solubility of the matrix.

Incorporation in Granulation

The method of incorporating disintegrants in granulation has been controversial. Should the disintegrant be all extragranular, all intragranular, or divided between these two locations? Shotton and Leonard [38] reported that maize starch, sodium calcium alginate, alginic acid, and other disintegrants gave more rapid disintegration when incorporated extragranularly than intragranularly in a sulfadiazine granulation. They also reported that the latter method gave a finer dispersion and they concluded that the best compromise was to use both intra and extragranular disintegrants.

Van Kamp et al. [39] evaluated the method of incorporation of Primojel, AcDiSol, and Polyplasdone XL in prednisone tablets formed from lactose granules. Whether the incorporation of the super disintegrant was intragranular, extragranular, or evenly distributed in both sites, they found little or no difference in disintegration time, crushing strength, or dissolution of prednisone. Interestingly, their results with potato starch showed discrepancies with the earlier work of Shotton and Leonard [38] in that intragranular starch was more effective than extragranular starch (Table 3). Gordon et al. [40] reported that naproxen, a poorly soluble drug at gastric pH, dissolved faster when AcDiSol was incorporated intragranularly, compared to extragranularly or evenly distributed between the intra and extragranular portions. More recently, a study reported by Khattab et al. [41] showed that the combined incorporation of

TABLE 3 Effect of Disintegrant Incorporation in Granules on Tablet Properties.[a]

Disintegrant	Crushing Strength (kgf)[b]			Disintegration Time (s)		
	Intra	Equal	Extra	Intra	Equal	Extra
Control		6.5			664	
4% Primojel	5.3	5.0	5.8	38	41	49
4% Ac-Di-Sol	3.8	4.8	5.7	110	126	148
4% Nymcel zsd 16	4.0	4.3	6.5	499	540	488
4% Polyplasdone XL	5.8	6.0	6.1	31	40	43
20% Potato Starch	3.3	3.4	2.1	69	80	110

[a] From Ref. 39.
[b] 1 kgf = 9.8 N.

intra and extragranular disintegrating agents (sodium starch glycolate, croscarmellose sodium, or crospovidone) in a paracetamol granulation resulted in faster disintegration and dissolution than extragranular or intragranular incorporation alone.

More studies are necessary to elucidate the effect of other factors, such as the type of binder, the type of filler, and the solubility of the matrix, which may significantly influence the effectiveness of disintegrants in different modes of incorporation. For example, Becker et al. [42] found that extragranular crospovidone was more effective in an acetaminophen tablet with a binder of maltodextrin (Licab DSH), pregelatinized maze starch (Lycab PGS), or low-substituted hydroxypropyl cellulose (L-HPC) than with a polyvinylpyrrolidone or hydroxypropyl methylcellulose binder. In addition, the difference seen in the effectiveness of starch in different modes of incorporation between the Shotton [38] and the Van Kamp [39] studies may be related to the absence or presence of lactose, a soluble filler. Unlike Shotton, Van Kamp et al. used lactose as a soluble filler, which might have reduced the relative effectiveness of extragranular starch, making the intragranular incorporation more favorable.

The observations summarized in Table 4 make it difficult to generalize that one method of incorporation of disintegrant in granulation is better than another. However, when all of the data are taken together, it would appear that the combined addition of disintegrants both extragranularly and intragranularly would provide the best opportunity for optimal disintegrant effectiveness.

Effect of Reworking

The effect of recompressing a wet-massed microcrystalline cellulose matrix containing super disintegrants on swelling force kinetics also has been investigated [43]. When the disintegrants were placed extragranularly, only Explotab among those considered retained good efficiency after reworking. When placed intragranularly, all disintegrants had reworking efficiencies equivalent to that of the nondisintegrant control. Adding 2% disintegrant extragranularly prior to the second compression restored disintegrant activity for Polyplasdone XL, but only partially for AcDiSol. In further work [44], reworked tablets containing 2% disintegrant extragranularly were studied. The data in Table 5 illustrate that maximal swelling forces were reduced in all cases, but there was no correlation with tablet disintegration time.

Incorporation in Hard Gelatin Capsules

The utility and performance of super disintegrants in direct-fill powder formulations for hard-shell capsules filled on tamping machines are roughly analogous to those of direct compression tablet formulation. In a study where capsules were filled under controlled tamping force conditions using an instrumented Zanasi LZ 64 dosator machine, dicalcium phosphate-based formulations containing hydrochlorothiazide and different super disintegrants were tested for dissolution times [45]. The croscarmelloses were found to be more effective than sodium starch glycolate in promoting hydrochlorothiazide dissolution, whereas crospovidone gave the poorest results. In a follow-up multifactorial study [46] all main parameters, including disintegrant type, compression force, level of lubricant, and filler type, were found to have significant effects on dissolution (Figs. 12, 13). At lower disintegrant concentration, increasing

TABLE 4 Effect of Disintegrant Incorporation in Granules on the Disintegration Efficiency of Tablets

Investigators	Drug (D), Binder (B), Filler	Disintegrants	Order of Disintegration Efficiency	Comments
Shotton et al. [38]	Sulfadiazine (D) Povidone (B) No filler	Maize starch Na calcium alginate Alginic acid Microcrystalline cellulose Colloidal aluminum silicate	Extra > intra	Extragranular incorporation yielded fastest disintegration, but intragranular incorporation yielded finer particles. Equal distribution of disintegrants is recommended.
Van Kamp et al. [39]	Prednisone (D) Gelatin (B) Lactose (F)	Potato starch Primojel Polyplasdone XL Nymcel	Intra > extra Intra > equal > extra Intra > equal > extra Extra > intra > equal	Small difference between the modes of incorporation for super disintegrants (Table 3).
Khattab et al. [40]	Paracetamol (D) Polyvinylpyrrollidone (B) No filler	Croscarmellose Na Na starch glycolate Crospovidone	Equal > extra > intra Equal > intra or extra Equal > intra > extra	Overall the equal distribution of disintegrants yielded the fastest disintegration and dissolution.

TABLE 5 Rework Efficiency (% RE) of Super Disintegrants[a]

Disintegrant (2%)	Relative F_s[b]		% RE[c]
	35% porosity	40% porosity	
Control	0.842	0.848	45
Polyplasdone XL	0.941	0.926	64
Explotab	0.737	0.863	86
AcDiSol	1.045	0.951	45

[a] From Ref. 44.

[b]Rel. $F_s = \dfrac{\text{Maximum swelling force (1st compression)}}{\text{Maximum swelling force (2nd compression)}}$

[c]%RE $= \dfrac{\text{AUC (1st compression)}}{\text{AUC (2nd compression)}} \times 100$

The AUC is the area under the curve of the disintegration time vs. compression pressure graphs.

the tamping force improved the dissolution of hydrochlorothiazide, most likely due to reduced porosity. When the lactose filler was replaced by dicalcium phosphate, the magnitude and order of effectiveness of the disintegrants changed.

Like with tablets, the effect of disintegrants in rapidly soluble capsule matrices is lower than in water-insoluble matrices. Perhaps doubling the concentration normally required for tablets is needed for efficient disintegration and significantly increases dissolution. This need for higher disintegrant concentration is reflected in the higher porosity of capsule plugs compared to compressed tablets. At equivalent concentrations in model lactose or dicalcium phosphate-based systems, sodium starch glycolate and croscarmellose sodium were more effective than crospovidone in promoting dissolution of hydrochlorothiazide from capsules manufactured with the same tamping force [47]. For either filler, disintegration times and swelling correlated well with dissolution.
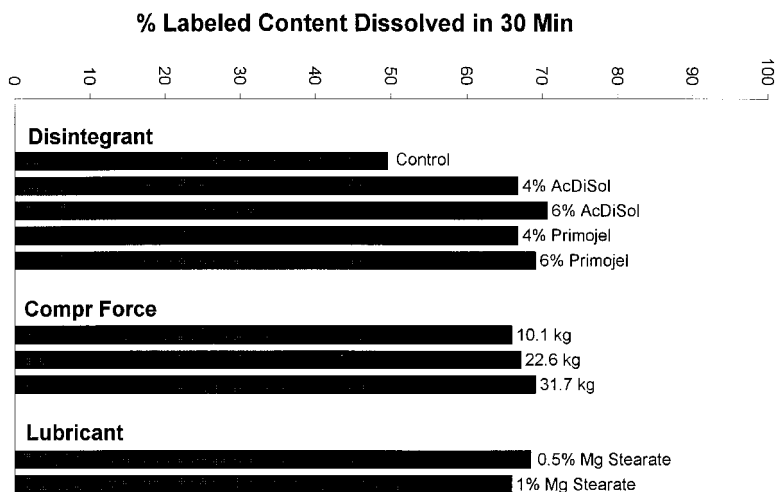
FIG. 12. The averaged effect of disintegrant, compression force, and lubricant on the release of hydrochlorothiazide from anhydrous lactose-based capsules. Control = 0% disintegrant. (Replotted from Ref. 47.)

% Labeled Content Dissolved in 30 Min
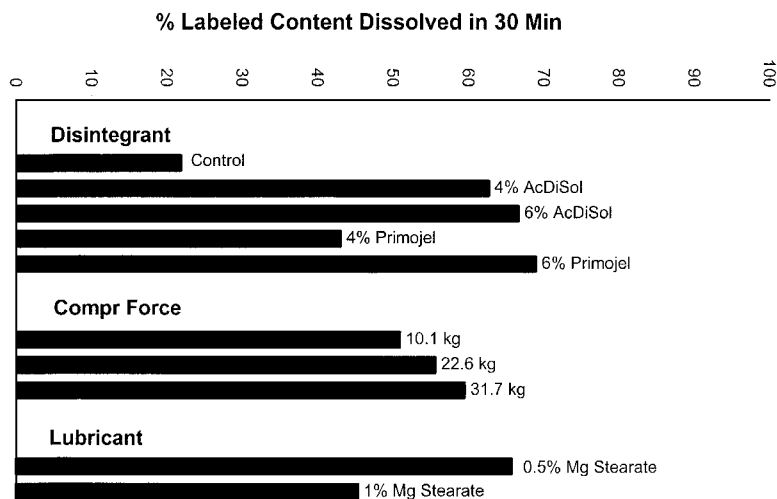
FIG. 13. The averaged effect of disintegrant, compression force, and diluent on the release of hydrochlorothiazide from dicalcium phosphate based capsules. Control = 0% disintegrant. (Replotted from Ref. 47.)

New Disintegrants

Gellan gum and Xanthan SM appear to have performance characteristics similar to those of super disintegrants. Gellan gum, an anionic polysaccharide of linear tetrasaccharides, is derived from *Pseudomonas elodea* [48]. It is a food-grade substance which is used as a gelling or suspending agent. Interestingly, when 4% gellan gum was incorporated in ibuprofen tablets, disintegration time was 4 min, which was much faster than that obtained using dried starch or Avicel PH 102 (>15 min), and comparable to those of Explotab, AcDiSol, and Kollidon CL (4–7 min).

Xanthan SM is a new USP xanthan derivative with higher hydrophilicity and lower gelling tendency [49]. In aspirin tablets with 3% Xanthan SM, disintegration time was about 10 min. Increasing the concentration of disintegrant above 3% did not improve the disintegration time, whereas the most effective concentration of AcDiSol was 5%, yielding a disintegration time of less than 5 min. Xanthan SM, like AcDiSol, is poorly soluble in water, but is reported to swell extensively. The use of these gums and their derivatives as disintegrants should be further explored.

Summary

Super disintegrants are excipients used to promote rapid breakdown of oral solid dosage forms to aid dissolution in vivo. Commonly used super disintegrants include sodium starch glycolate, croscarmellose sodium, and crospovidone. Super disintegrants differ from traditional starch in that they are effective at much lower concentrations. This provides formulation scientists greater flexibility, particularly in designing direct-compression tablets. However, the effectiveness of both starch and super disin-

tegrants depends heavily upon the composition of the tablet matrix, compression pressure, and in the case of granulation, the method of incorporation.

Because of the complexities involved, the mechanism of action of super disintegrants is not well understood. Some of the proposed mechanisms include water wicking, swelling, deformation recovery, particle repulsion, and heat of wetting. Water uptake is a necessary precursor to all other mechanisms. Not all mechanisms are well supported by research. Disintegrants appear to function by multiple mechanisms, but a predominant mechanism seems to be characteristic of each disintegrant type. Regardless of their validity, all proposed mechanisms have the potential to at least generate a disintegrating force within the matrix and this appears to be a unifying concept.

Acknowledgment

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