PHARMACEUTICAL FORMULATION DESIGN AND DEVELOPMENT

M-254

Self Learning Material

Directorate of Distance Education

SWAMI VIVEKANAND SUBHARTI UNIVERSITY
MEERUT-250005
UTTAR PRADESH

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Published by : Laxmi Publications Pvt Ltd., 113, Golden House, Daryaganj, New Delhi-110 002.
Tel: 43532500, E-mail: info@laxmipublications.com

DEM-2195-071-PHARMA FORM DESIG DEV M-254 C-3152/011/02
Typeset at: Shubham Composers, Delhi Printed at: Ajit Printers, Delhi

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SYLLABUS

PHARMACEUTICAL FORMULATION DESIGN AND DEVELOPMENT

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THEORY

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   Crystal morphology, crystal optics, polymorphism and purity studies: drug excipient compatibility study.

2. Dissolution Study:
   - Importance, objectives, equipments
   - Biological classification system (BCS); its significance on dissolution study and application in dosage form development.
   - Selection of dissolution medium and conditions.
   - Comparison of dissolution profile by model independent (similarity and dissimilarity factor) and dependent method.
3. Stability studies:
   - Basic concept and objectives of stability study.
   - Order of reaction and their applications in predicting shelf life and half-life of Pharmaceutical formulations.
   - Importance of accelerated stability study.
   - Effect of various environmental / processing on stability of the formulation and techniques for stabilization of products against the same.
   - Regulatory requirements related to stability testing with emphasis on matrixing/bracketing techniques, climatic zone, impurities in stability study photostability testing etc.
   - Application of microcolorimetry in stability study.
4. Polymers – Classification. General methods of synthesis, properties, characterization and evaluation: Biodegradable polymers – Classification – Mechanism

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   Medical prosthetics and packaging.
5. In-vitro In-vivo Correlation (IVIVC)

   Methods of establishing IVIVC

   Factors effecting IVIVC

6. A Brief study on formulation and evaluation of specialized pharmaceutical dosage form Dry syrup, Topical gel, Microemulsion, dry powder, Parenteral
   Emulsion and suspension, occuserts, Clear shampoo, hair cream, Face wash, Sunscreen Lotion, Antiwrinkle cream, Face wash

PRACTICAL

The practical syllabus comprises of the exercises formulated based on the topics mentioned in the Theory syllabus.

BOOKS RECOMMENDED

1. Milo Gibaldi and Donald Perrier, "Pharmacokinetics", Drugs and Pharm. Sci. Series, Vol 15., Marcel Dekker Inc., N.Y.
2. J.C. Wagner, "Fundamentals of Clinical Pharmacokinetics", Drug Intelligence Publications, Hamilton, 1975.
3. Bert N. LaDu, "Fundamentals of Drug Metabolism & Disposition", Waverley Press Inc., Baltimore, 1972.
4. T.Z. Laaky, "Intestinal Absorption & Malabsorption", Raven Press, N.Y., 1975.
5. J.T. Carstensen, "Theory of Pharm. Systems", Vols 1-3, Academic Press, N.Y.
6. U.S. Beans, A.K. Beckett and J.E. Caraless, "Advances in Pharm. Sci.", Vol. 1 to 4.
7. J.T. Carstensen, "Drug Stability: Principles and practices", Drugs and Pharm. Sci. Series, Vol. 43, Marcel Dekker Inc., N.Y.
8. Lisbeth Iliun & Stanley S. Davis: "Polymers in Controlled Drug Delivery", Wright, Bristol (1987).
9. Pharmaceutics "The Science of Dosage form design" by Aulton.
10. Encyclopedia of Pharmaceutical technology Volumes: 1 to 19.
11. Remingtons Pharmaceutical Sciences 19th edition.
12. Pharmaceutical dissolution testing by Banaker.
13. Pharmacokinetics by Welling and Tse.
14. Modern Pharmaceutics by G.S. Banker
15. Clinical Pharmacokinetics, Concepts and applications, by Rowland and Tozer.
16. Biopharmaceutics and Pharmacokinetics – An introduction by Notari.
    Techniques of Solubilization of Drugs by Yalkowsky

UNIT I: PREFORMULATION STUDIES: PERSPECTIVE AND CONCEPT

★ STRUCTURE ★

1.1 LEARNING OBJECTIVES

After going through this unit, students will be able to:

- discuss the introduction of preformulation steadies
- state the drug development drivers challenges, risks and rewards
- explain current for ends in the pharmaceutical industry
- state Lessens learnt and the way forward
- discuss the detailed study of parameters
- elaborate molecular properties
- know about the initial solubility investigations
- state the organic solid state.

1.2 INTRODUCTION

This book is intended to be a practical guide to pharmaceutical preformulation and formulation. It can be used as a reference source and a guidance tool for those working in the pharmaceutical industry or related industries, for example, medical devices and biopharmaceuticals, or anyone wanting an insight into this subject area. The information presented is essentially based on the extensive experiences of the editor and various other contributors who are all actively working in the industry and have learned "best practice" from their experiences.

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There are various excellent books already available that cover the theoretical aspects of different types of pharmaceutical dosage forms and processes. A variety of books are also available that focus on the drug development process, business, and regulatory and project management aspects. The popularity of the first edition of this book, Pharmaceutical Preformulation and Formulation: A Practical Guide from Candidate Drug Selection to Commercial Formulation, confirms my opinion that there is a need for a pragmatic guide to pharmaceutical preformulation and formulation with an emphasis on what practical studies need to be undertaken, for what reasons, and during what key stages of the drug development process. Preformulation, biopharmaceutics, and formulation are all important for candidate drug selection and through the various stages of product development as shown in Fig. 1.3. This book has been written to try and address this need. A logical approach to product development is described in the book, with the key stages identified and the preformulation, biopharmaceuticals, and formulation activities and typical issues at each stage discussed. Wherever possible, the book is illustrated with real or worked examples from contributors who have considerable relevant experience of preformulation, biopharmaceuticals, and formulation development.

Jim Wells' book on preformulation (Wells, 1988) made a strong impact on trainees and pharmaceutical scientists (including myself) working in this field of the pharmaceutical industry when it was introduced two years ago. It describes the important concepts and methods used in preformulation with the underlying theory. To his credit, Wells' book is still useful today, but sadly, the book is now out of print, and existing copies are hard to obtain. It also requires updating to include the abundance of modern preformulation instrumental techniques that have emerged, such as thermogravimetric analysis (TGA), hot-stage microscopy (HSM), X-ray powder diffraction (XRPD), Raman and infrared spectroscopy, and solidstate nuclear magnetic resonance (NMR). These techniques can be used to provide valuable information to characterize the drug substance and aid formulation development using the minimal amounts of compound.

Pharmaceutical Preformulation and Formulation: A Practical Guide from Candidate Drug Selection to Commercial Formulation covers a wider subject area than just preformulation. Topics include biopharmaceutics, drug delivery, formulation, and process development aspects of product development. The book also describes a logical and structured approach to the product development process, recommending at what stages appropriate preformulation, biopharmaceutics, and formulation work are best undertaken.

1.3 DRUG DEVELOPMENT DRIVERS, CHALLENGES, RISKS AND REWARDS

It is important that the reader is aware of the nature of pharmaceutical research and development (R&D) to appreciate the importance of preformulation and formulation in the overall process.

Table 1.1 Major Hurdles to Successful Product Registration and Sale

Activity	*Requirements*
Research	Novel compound (Is it patentable?) Novel biological mechanism (Is it patentable?) Unmet medical needs Potent and selective

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Safety	High margin of safety Nontoxic (not carcinogenic, tetratogenic, mutagenic, etc.)
Clinical	Tolerable side effects profile Efficacious Acceptable duration of action
Drug process	Bulk drug can be synthesized/scaled up
Pharmaceutical	Acceptable formulation/pack (meets customer needs) Drug delivery/product performance acceptable Stable/acceptable shelf life Robust clinical trial process, which can be scaled up and transferred into operations
Regulatory	Quality of data/documentation
Manufacturing	Manufacturable Acceptable cost of goods Able to pass preapproval inspection
Marketing/commercial	Competitive Meets customer needs Value for money Commercial return

In simple terms, the objective of pharmaceutical R&D can be defined as "converting ideas into candidate drugs for development" and the objective of product development as "converting candidate drugs into products for registration and sale." In reality, these goals are extremely challenging and difficult to achieve because of the many significant hurdles a pharmaceutical company has to overcome during the course of drug development. Some of the major hurdles are listed in Table 1.1.

The high risk of failure in drug discovery and development throughout the pharmaceutical industry statistically shows that, on average, only 1 in 5000 compounds screened in research will reach the market. For those that are nominated for development, the failure rate will vary from one in five to one in ten compounds that will achieve registration and reach the marketplace. Most failures in early development are due to drug toxicity or safety issues, whereas a lack of efficacy is the primary reason for late-stage attrition (Lowe, 2008). The relatively high attrition rates of new medicines is a major challenge, particularly when they are expensive phase III clinical failures that have occurred in recent years. Regulators are being more selective in what they approve, and they are demanding more data on efficacy and side effects. Only about 20 new drugs are now approved every year, down from 40 or 50 a decade ago and despite an approximate 70% increase in R&D investment over the last 10 years. On top of this, there is a significant commercial risk from those that are marketed; only 3 out of 10 are likely to achieve a fair return on investment. The products that give poor return on investment are often the result of poor candidate drug selection (the compound does not have the desired properties of safety, selectivity, efficacy, potency, or duration) and/or poor product development (the development program does not establish the value of the product). The latter scenario should, and can be, avoided by careful assessment at the "product design" stage of development.

There has been a recent worrying trend of marketed products being withdrawn a few years after launch. This may be because once it is used by many thousands,

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or even millions, of people, rare but significant side effects can emerge. For example, Merck's blockbuster arthritis drug, Vioxx, was approved in 1999 but withdrawn five years later when linked to increased cardiovascular risks. Another example is the surprise announcement by Pfizer when it withdrew the world's first inhalable insulin product, Exubera, from the market in 2007 following disappointing sales. It would seem that the company had failed to appreciate the customer requirements well enough during the product design phase of development.

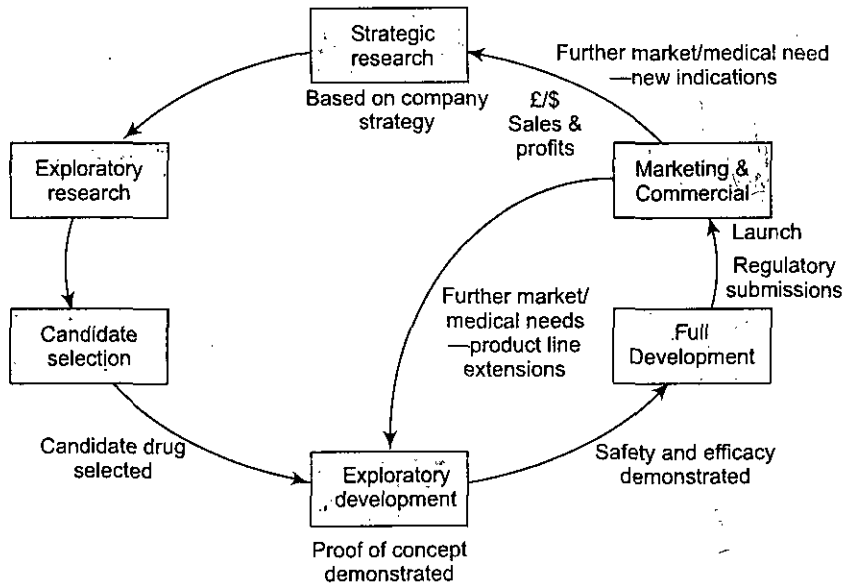

Fig. 1.1 Product life cycle.

To be successful and competitive, research-based pharmaceutical companies must ensure that new discoveries are frequently brought to the market to generate cash flow. This is required to fund the next generation of compounds to meet the therapeutic needs of patients, and of course, to benefit the shareholders. This cycle of events is sometimes referred to as the "product life cycle" and is further illustrated in Figure 1.1.

The overall costs of drug discovery and development to bring a new medicine to the market are increasing at an alarming rate. It is currently estimated that US$1 billion is required to recoup the costs of research, development, manufacturing, distribution, marketing, and sales for a new chemical entity (NCE). Cost estimates are even higher for a new biopharmaceutical product at US$1.2 billion and take longer to develop than a NCE, but tend to enjoy much greater success rates (DiMari and Grabowski, 2007). A significant proportion of this total is for the cost of failures, or in other words, the elimination of unsuccessful compounds. R&D expenditure tends to increase substantially as the compound progresses from drug discovery research through the various clinical trial phases of development. The pivotal phase III patient trials are usually the largest, involving thousands of patients, and hence the most expensive. To reduce development costs, some companies selectively screen and eliminate compounds earlier in the drug development process on the basis of results from small-scale, less expensive studies in human and progress fewer, more certain compounds to later clinical phases.

In spite of the high risks and high costs involved, there is still a huge incentive for pharmaceutical companies to seek the financial rewards from successful marketed

products, especially from the phenomenal success of the rare "blockbuster" (reaching sales of >US$1 billion per year). This can earn the company significant profits to reinvest in research and fund the product development pipeline.

Another factor, the risk of delay to registration and launch, can also have a significant impact on the financial success of a marketed product. McKinsey & Company, a management consultancy, assessed that a product that is six months late to market will miss out on one third of the potential profit over the product's lifetime. In comparison, they found that a development cost overspend of 50% would reduce profits by just 3.5%, and a 9% overspend in production costs would reduce profits by 22% (McKinsey & Co., 1991). The loss of product revenue is often due to competitor companies being first to market, capturing the market share, and dictating the market price, in addition to the loss of effective patent life. Hence, the importance of accelerating and optimizing drug discovery and development, and getting to the market first with a new therapeutic class of medicinal product, cannot be underestimated.

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The second product to market in the same class will usually be compared with the market leader, often unfavourably. The average time from drug discovery to product launch is currently estimated to be 10 to 12 years. Several factors may have contributed to lengthening development times over the years, including an increase in the preclinical phase to select the candidate drug and also an increase in the duration of the clinical and regulatory period required for marketing approval because regulatory agencies are requesting comparator efficacy studies and extensive safety profiling. Benchmarking studies show wide gaps between industry average or worst performance compared with what is achievable as best practice performance (Spence, 1997).

On average, the preclinical phase currently takes four to six years to complete, whereas the time from candidate drug nomination to regulatory submission takes on average six to eight years, and longer for treatments of chronic conditions. Most forward-looking pharmaceutical companies are aiming to reduce these times by reevaluation and subsequently streamlining the development process, for example, by introducing more effective clinical programs and more efficient data reporting systems, forward planning, and conducting multiple activities in parallel. However, this in turn may put formulation development and clinical supplies on the critical path, with pressures to complete these activities in condensed time scales. Suggestions are offered throughout this book on how preformulation, biopharmaceuticals, and formulation can be conducted in the most efficient way to avoid delays in development times. Any reduction in the total time frame of drug discovery to market should improve the company's profitability. In a highly competitive market, product lifetimes are being eroded because of the pace of introduction of competitor products, the rapid introduction of generic products when patents expire and move to "over-the-counter" (OTC) status. Successful pharmaceutical companies are focusing on strategies for optimum "product life cycle management" to maximize the early growth of the product on the market, sustain peak sales for as long as the product is in patent, and delay the post-patent expiry decline for as long as possible. This should maximize the return on investment during a product life cycle to enable the company to recover development costs and make further investments in R&D. Figure 1.2 shows a classic cash flow profile for a new drug product developed and marketed.

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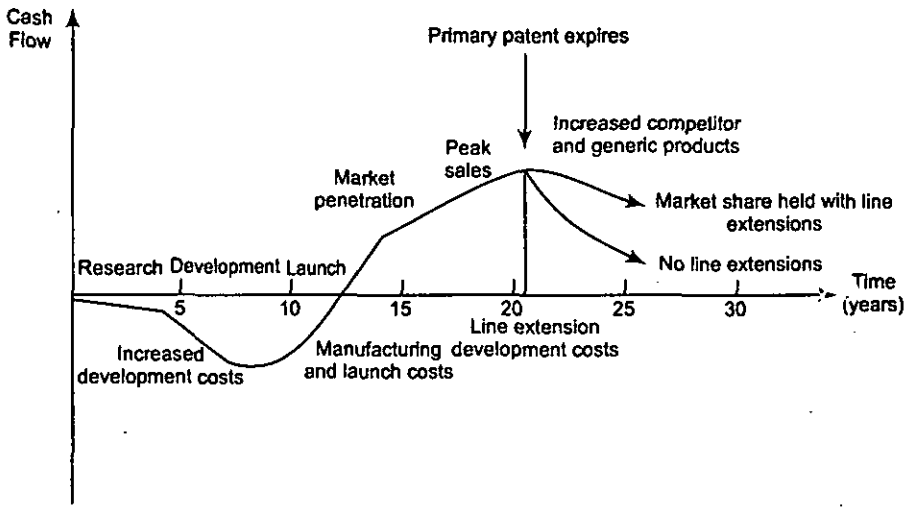

Fig. 1.2 Product life cycle management.

During development there is a negative cash flow, and it may be some time after launch before sales revenue crosses from loss to profit because of manufacturing, distribution, and advertising costs. Profits continue to increase as the market is established to reach peak sales, after which sales decrease, especially after the primary patent expires and generic competition is introduced.

Throughout the life span of a product, it is in a company's interest to ensure the best patent protection to achieve the longest possible market exclusivity. Prior to the primary patent expiring (normally for the chemical drug substance), it is imperative to introduce new indications, formulations, manufacturing processes, devices, and general technology, which are patent protected, to extend the life of the product and maintain revenue. A patent generally has a term of about 20 years, but as development times are getting longer, there will be a limited duration of protection remaining once the product is marketed (the effective patent life). A comparison of effective patent life for pharmaceutical NCEs in various countries around the world shows the same downward trend between the 1960s and the 1980s (Karia et al., 1992; Lis and Walker, 1988). In the EU, products typically enjoy 10 years of patent exclusivity, whereas in the United States, it is typically only 5 years. Getting to the market quickly is a major business-driving force, but this has to be balanced with the development of a product of the appropriate quality. There is a need to generate sufficient information to enable sound decisions on the selection of a candidate drug for development, as well as to develop dosage forms that are "fit for purpose" at the various stages of development. Anything more is wasting precious resources (people and drug substance), adding unnecessary cost to the program, and, more importantly, extending the development time. Perfect quality should not be the target if good quality is sufficient for the intended purpose. This can only be achieved if there is a clear understanding of the customer requirements.

For example, if a simple, non-optimized formulation with a relatively short shelf life is acceptable for phase I clinical studies, any further optimization or stability testing might be considered wasteful, unless the data generated can be used later in the development program. There can be a significant risk associated with doing a minimum development program and cutting corners to fast track to market. Post launch, the cost of a retrospective fix due to poor product/process design and/or development can be extremely high. The additional financial cost from work in product/process redevelopment, manufacturing and validation, technical support,

regulatory submission, and sales and marketing (due to a product recall) can easily wipe out the profit from an early launch. This can have several unpleasant knock-on effects; it may affect the market share and the company's relationship with the regulatory authorities, and its credibility with customers (both externally and internally within the company) may be threatened. These factors need to be taken into account when planning preformulation/formulation studies, which can directly influence the progress of a product to market and final product quality.

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1.4 CURRENT TRENDS IN THE PHARMACEUTICAL INDUSTRY

Increasing competition and threats to the pharmaceutical industry with respect to maintaining continued sales growth and income mean that successful companies going forward will be those that have a portfolio of products capable of showing volume growth. However, to show volume growth, innovative new products are required. The cost of drug discovery and development is escalating because there are no easy targets left and the cost of development and the cost of goods (CoG) sold are increasing. There have been several mergers and acquisitions of research-based pharmaceutical companies since the 1980s, and increased collaborations and inward licensing of products and technologies, in attempts to acquire new leads, to share costs, to reduce the time to license, and to maintain growth. Unfortunately, mergers and acquisitions also result in streamlining and job losses, which improve efficiency and decrease overhead costs at the same time. There is a changing trend in the nature of the candidate drug emerging from pharmaceutical R&D, from a low molecular weight chemical to a more complex macromolecule (biopharmaceuticals). Biopharmaceuticals comprise "biologics" such as vaccines and blood and plasma products, and products derived using biotechnology such as monoclonal antibodies or recombinant proteins that are engineered or derived from mammalian or other cells. Some of these compounds have been derived from biotechnological processes to produce biotechnological medicinal products that fight infection and disease. A typical biotechnology process consists of three major phases to produce the purified bulk active pharmaceutical ingredient (API): (*i*) *fermentation of cells (generally mammalian cell lines for antibody manufacture),* (*ii*) downstream processing to clear up any contamination, and (*iii*) characterization and testing of impurities. The bulk API is then either processed further or just filled in vials or ampoules to produce the drug product.

It is estimated that today there are more than one hundred biotechnological medicinal products on the market, and many more in clinical trials are being developed to treat a wide variety of diseases. Those currently on the market account for 60% of absolute annual sales growth in major pharmaceutical companies, with the remaining 40% being from small molecules (Mudhar, 2006). Biopharmaceuticals possess some advantages over small molecules, for example, some can affect human drug targets, which is not possible with small molecules. They are also difficult to copy when the patent expires, thus keeping the generics at bay. However, there are also some significant disadvantages of using biopharmaceuticals, such as the almost unavoidable loss of any oral dosing route because they tend to be denatured in the gastrointestinal tract or are too large to be absorbed. It can be a major challenge for the formulator to develop self-administered formulations to deliver macromolecules such as proteins and polypeptides into the body. Even if administered by injection, the pharmacokinetics of biopharmaceuticals can be complicated because of built-in clearance mechanisms.

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For both small molecules and biopharmaceuticals, more sophisticated drug delivery systems are being developed to overcome the limitations of conventional forms of drug delivery systems [*e.g.*, tablets and intravenous (IV) solutions], problems of poor drug absorption, noncompliance of patients, and inaccurate targeting of therapeutic agents. One example of emerging drug delivery technology is the use of low-level electrical energy to assist the transport of drugs across the skin in a process known as electrophoresis. This method could be particularly useful for the delivery of peptides and proteins, which are not adequately transported by passive transdermal therapy. The drug absorption rate is very rapid and more controlled compared with passive diffusion across the skin. Another example is the pulmonary delivery of proteins and peptides. The recent successful delivery of insulin using a dry-powder inhaler is impressive since it had to pass so many hurdles including the narrow therapeutic index of insulin and the need for tight particle size control to reach the alveolar surface. This provides encouragement for the delivery of other protein and peptide products delivered by this route. A third example is the use of bioerodable polymers that can be implanted or injected within the body to administer drugs from a matrix, which can be formulated to degrade over a long duration from one day to six months and do not require retrieval. Some of these specific delivery systems are explained in more detail in later chapters on the various dosage forms.

Futuristic drug delivery systems are being developed, which are hoped to facilitate the transport of a drug with a carrier to its intended destination in the body and then release it there. Liposomes, monoclonal antibodies, and modified viruses are being considered to deliver "repair genes" by IV injection to target the respiratory epithelium in the treatment of cystic fibrosis. These novel drug delivery systems not only offer clear medical benefits to the patient, but can also create opportunities for commercial exploitation, especially useful if a drug is approaching the end of its patent life.

There are pressures on the pharmaceutical industry, which affect the way products are being developed. For example, there is a trend for more comprehensive documentation to demonstrate compliance with current good manufacturing practice (cGMP) and good laboratory practice (GLP) and to demonstrate that systems and procedures have been validated. The latest trend is for more information required on the "design space" for the manufacturing process prior to regulatory submission on product optimization. A benefit of doing this is to provide more flexibility for changes to the process within the design space limits once submitted. However, the pressure is for a company to submit early and develop the product "right first time" with a thorough understanding of the product and manufacturing process.

In spite of efforts to harmonize tests, standards, and pharmacopoeias, there is still diversity between the major global markets—Europe, the United States, and Japan-which have to be taken into account in the design of preformulation and formulation programs (Anonymous, 1993). Other pressures facing the pharmaceutical industry are of a political/economical or environmental nature. Some governments are trying to contain healthcare costs by introducing healthcare reforms, which may lead to reduced prices and profit margins for companies, or restricted markets where only certain drugs can be prescribed. Although the beneficial effect of drugs is not questioned in general, the pressure to contain the healthcare costs is acute.

Healthcare costs are increasing partly because people are living longer and more treatments are available. This may influence the commercial price that can be obtained for a new product entering the market and, in turn, the "CoG target."

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The industry average for the CoG target is 5% to 10% of the commercial price, with pressure to keep it as low as possible. This may impact on the choice and cost of raw materials, components and packaging for the product, and the design and cost of manufacturing the drug and product. Environmental pressures are to use environmentally friendly materials in products and processes and to accomplish the reduction of waste emissions from manufacturing processes.

A good example is the replacement of chlorofluorocarbon (CFC) propellants in pressurized metered-dose inhalers (pMDIs) with hydrofluorocarbons (HFAs). The production of CFCs in developed countries was banned by the Montreal Protocol (an international treaty) apart from "essential uses," such as propellants in pMDIs, to reduce the damage to the earth's ozone layer. However, there is increasing pressure to phase out CFCs altogether. The transition from CFC to HFA products involves a massive reformulation exercise with significant technical challenges and costs for pharmaceutical companies involved in developing pMDIs, "Inhalation Dosage Forms." However, this can be turned into a commercial opportunity for some companies, which have developed patent-protected delivery systems to extend the life cycle of their CFC pMDI products.

1.5 LESSONS LEARNT AND THE WAY FORWARD

To achieve the best chance of a fast and efficient development program to bring a candidate drug to market, several important messages can be gleaned from projects that have gone well and from companies with consistently good track records.

There are benefits for pharmaceutical development to get involved early with preclinical research during the candidate drug selection phase. This is to move away from an "over-thewall" handover approach of the candidate drug to be developed from "research" to "development." The drug selection criteria will be primarily based on pharmacological properties such as potency, selectivity, duration of action, and safety/toxicology assessments. However, if all these factors are satisfactory and similar, there may be an important difference between the pharmaceutical properties of candidate drugs. A candidate drug with preferred pharmaceutical properties, for example, good aqueous solubility, crystalline, nonhygroscopic, and good stability, should be selected to minimize the challenges involved in developing a suitable formulation. Another important factor is good long-term planning, ideally from candidate drug nomination to launch, with consideration for the safety, clinical and pharmaceutical development, manufacturing operations, and regulatory strategies involved to develop the product. There is a need for one central, integrated company project plan that has been agreed on by all parties with a vested interest in the project. Needless to say, the plan should contain details of activities, timings, responsibilities, milestones, reviews, and decision points. Reviews and decision points are required at the end of a distinct activity to ensure that the project is still meeting its objectives and should progress to the next stage of development. However, these reviews should not cause any delays to the program, rather, they should ratify what is already progressing. The traditional sequential phases of product development must be overlapped to accelerate the product to market. In reality, plans will inevitably change with time; they should be "living" documents, which are reviewed and updated at regular intervals and then communicated to all parties. There may be several more detailed, lower-level plans focusing on departmental activities, for example, pharmaceutical development, but these plans must be linked to the top-level central project plan.

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Forward planning should provide the opportunity for a well thought out and efficient approach to product development, identifying requirements up front so as to avoid too much deliberation and backtracking along the way. It should also provide a visible communication tool. Good planning is supported by adopting a systematic and structured approach to product development. The development process can be broken down into several key defined stages-product design, process design, product optimization, process optimization, scale-up, and so on. Each stage will have inputs and outputs as shown in Figure 1.3, a simplified framework for product development.

As product development can take several years to complete, it is important to have an effective document management system in place to record the work. The primary reference source for recording experimental work will usually be a laboratory notebook (paper or electronic). The work should be checked, dated, and countersigned to satisfy GLP and intellectual property requirements. Experimental protocols are sometimes useful for defining programs of work, explaining the rationale for the studies, and defining the acceptance criteria. When the studies are completed, the results can be reported with reference to the protocol and acceptance criteria. Laboratory notebooks are referenced in the protocols and reports so that the raw data can be retrieved in the event of an audit.

At the completion of key stages of the work, summary reports can be written, referencing all other protocols and reports relevant to that stage and highlighting the major recommendations and conclusions. In this way, a product development document file can be built up for transfer of information and technology, including the development history and rationale for progression. The file will also be vital for data retrieval in the event of a regulatory inspection.

Finally, successful product development is often associated with good teamwork. The process is multidisciplinary, relying on people with different specialist skills working together to make it happen. This is particularly important at the key interfaces such as preclinical research with pharmaceutical development and pharmaceutical development with manufacturing operations at the final production site. It is therefore useful to have representation on the project teams from all the key specialist functions to ensure buy-in to the plans, strategies, and decisions, and to have a good project management system in place.

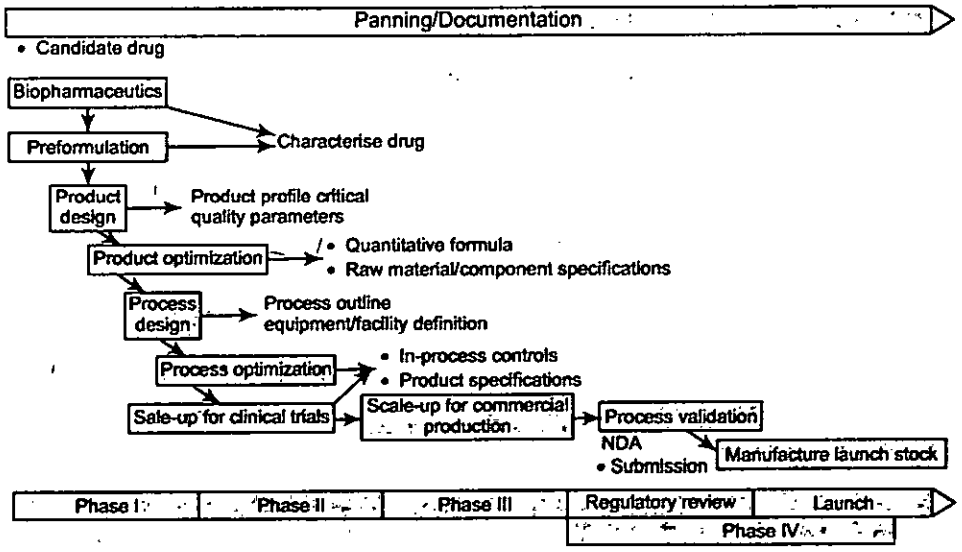

Fig. 1.3 Framework for product development.

1.6 DETAILED STUDY OF PARAMETERS

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Introduction

In recent years, there has been a significant increase in pressure on pharmaceutical companies to discover and develop new medicines ever faster to replace those coming off patent and to counter generic manufacturer competition (Frantz, 2007). Despite the expenditure of many billons of dollars, Joshi (2007) reports that since 1990 an average of only 28 drugs have been approved each year, with the Food and Drug Administration (FDA) approving only 17 new chemical entities (NCEs) in 2002, the lowest number of new drug approvals for the decade leading up to that year (Kola and Landis, 2004). Indeed, the success rate achieved by the industry of bringing a candidate drug (CD) to market is no more than 10% (Schmid and Smith, 2006), and it is estimated that of 30,000 compounds synthesized only 0.003% of discovery compounds will show a satisfactory return on investment (Federsel, 2003). The majority of the attrition occurs in phase II and phase III of development, with approximately 62% of compounds entering phase II undergoing attrition (Kola and Landis, 2004). So, not only does the number of compounds being brought through from discovery phase need to increase, but the amount of effort expended on them needs to reflect the attrition that will occur as they are progressed through early development. One idea being mooted to increase the productivity of the drug discovery process is the concept of lean thinking, which has been used in pharmaceutical manufacturing for process improvement (Petrillo, 2007). Simply put, lean concepts aim to eliminate those steps in the process that do not add value to the process chain. It has been estimated that utilizing lean concepts in the discovery phase, combined with other methods of increasing productivity, would lead to an increase (from 1 in 5 to 1 to 3) in compounds entering clinical trials.

Drug discovery and development is characterized by a number of distinct stages, and typically, the drug discovery process falls into two phases, lead generation (LG) followed by lead optimization (LO) (Davis et al., 2005). The LG period is further subdivided into the activeto-hit (AtH) and the hit-to-lead (HtL) phases (Baxter et al., 2006). The HtL phase utilizes highthroughput screening (HTS) and generates actives, hits, and leads: leads are those compounds that meet predefined chemical and biological criteria to allow selection of the chemistry that provides molecules with drug-like properties (Leeson et al., 2004). Drug-like compounds can be defined as those with pharmacokinetic and pharmacodynamic properties that are independent of the pharmacological target (Vieth et al., 2004). Leeson and Springthorpe (2007) have discussed how drug-like concepts can influence decision making in the medicinal chemistry arena. In this paper, they argue that the wave of molecules presently being synthesized possess significantly different physicochemical properties to those already in clinical development.

One important aspect of the HTS and HtL approach is that it provides multiple chemical series to de-risk future LO work. Thus, the aim of this phase is to increase the drug-like properties (*e.g.*, improve potency, selectivity, pharmacokinetic properties, and decrease toxicity) of lead compounds against a CD target profile (CDTP). During the LO phase, structure-activity relationships (SARs), which correlate molecular properties with biological effects, are derived. When SARs can be measured quantitatively, they become quantitative SARs (QSARs) (Andricopula and

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Montanari, 2005). Two specific examples of LO programs for the systematic optimization of compound series are given by Guile et al. (2006) and Baxter et al. (2006).

The iterative assessment of optimized leads against selection criteria allows identification of the most promising lead candidates. Once the lead candidates have been identified, then assessment of the material characteristics by the development scientists can be initiated (Venkatesh and Lipper, 2000). This phase has traditionally been termed "prenomination" and typically lasts around three to six months. It encompasses investigations into the physicochemical characterization of the solid and solution properties of CD compounds and has been the subject of the books by, for example, Wells (1988) and Carstensen (2002).

Essentially the aim of this phase is to provide an initial evaluation of compounds from a development perspective and support the tolerability studies of compounds. The scope of prenomination and early development studies to be carried out largely depends on the expertise, equipment, and drug substance available, and also on any organizational preferences or restrictions. In some organizations, detailed characterization studies are performed, while other companies prefer to do the minimum amount of work required to progress compounds as quickly as possible into development. There are advantages and disadvantages to both approaches, but an important consideration is to balance the studies that allow an appropriate understanding of the CD with the significant possibility of attrition. However, for the smooth progression of compounds through the preformulation phase, a close interaction between Medicinal Chemistry, Safety Assessment, Pharmaceutical Sciences, Analytical Chemistry, and Process Research and Development departments is essential to assess the physicochemical properties and toxicology of compounds and their progression to the first human dose as quickly as possible (Li, 2004).

If the compound passes these assessments, it can then pass into the late-phase development, which will be dealt with in subsequent chapters. In the case of development studies that can be undertaken to support the nomination of a compound for development, Balbach and Korn (2004) have proposed "the 100 mg approach" for the evaluation of early development CDs. However, as pointed out by Ticehurst and Docherty (2006), if a complete package of work is carried out too early, it may lead to much wasted effort.

On the other hand, if insufficient work is performed, then it may lead to increased pressure to characterize the compound to meet accelerated project demands. Thus, they recommend a "fit for purpose" solid form in the early studies, followed by selection of solid form for a commercial development. For convenience, these phases can be termed early and late development, respectively. The goal of early development can be defined as that to secure a quick, riskmanaged processes for testing the CD in animals and human volunteers for phase I studies. During prenomination, compounds need to be evaluated in animals for exposure/ toxicity purposes [7-day tox and 28-day single and multiple ascending doses (SADs and MADs)] (Kramer et al., 2007). The compound, in a suitable form to ensure systemic exposure (Gardner et al., 2004), needs to be formulated into an appropriate formulation for delivery in the first good laboratory practice (GLP) dose typically as either a suspension or solution.

Reference is made to Chaubal (2004) for a review of this area and Mansky et al. (2007) for a method for rapidly screening preclinical vehicles that enhance the solubility of low solubility compounds. Hitchingham and Thomas (2007) have developed a

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semiautomated system to determine the stability of the dosing formulations. During this stage, there may be a number of compounds with sufficient activity to merit consideration, and so studies must be designed appropriately to allow efficient assessment and selection of suitable compounds for development. Clear differences in in vivo activity may be sufficient to determine which of the candidates are selected. However, other factors that may be important from a pharmaceutical and drug synthesis point of view should also be considered if there is a choice. For example, physicochemical and biopharmaceutical characteristics of the compound(s), ease of scale-up for compound supply, cost of goods, and the nature of the anticipated dosage form should also be part of the decision process.

Ideally, for an oral solid dosage form, a water-soluble, nonhygroscopic, stable, and easily processed crystalline compound is preferred for development purposes; however, other formulation types will have their own specific requirements. For example, inhalation compounds need to be micronized for formulation into a pressurized metered dose or dry powder inhaler. This is an energy-intensive process and can change the crystallinity of compounds, and thus their subsequent interaction with moisture may be important. For a solution formulation, however, the stability of the compound will be paramount, and if instability is a major issue, then alternative measures such as freeze-drying may be required.

Table 1.2 summarizes the prenomination studies that could be carried out on a CD. These are considered to be the minimum tests that should be undertaken, recognizing that during the prenomination phase only a limited quantity of compound, for example, 50 to 100 mg is typically available to the pharmaceutical scientist for characterization. However, it should be emphasized that this is a critical decision period that can profoundly affect the subsequent development of a CD. Thus, the tests shown are considered to be those important for making a rational decision as to which compound, salt, or polymorph to proceed with into development. A poor decision at this point may mean some revisionary work, such as, a change of salt or polymorph being necessary later and a possible delay to the development of the drug for the market.

After first-time-in-human (FTIH) studies in early development, if the compound progresses into full development, a more complete physicochemical characterization of the chosen compound(s), with particular emphasis on the dosage form, should be carried out, thus allowing a rational, stable, and bioavailable formulation to be progressed through to launch. From a development point of view, perhaps the biggest change in the last decade has been the introduction and utilization of HTS technologies, whereby large number of compounds can be assessed in parallel to allow efficient physicochemical profiling as well as salt and polymorph screening (Desrosiers, 2004; Storey et al., 2004; Seadeek et al., 2007; Wyttenbach et al., 2007).

Table 1.2 Suggested Physicochemical Tests Carried Out During Prenomination

Test/activity	*Guidance to amount*	*Timing/comments*
Elemental analysis	4 mg	LO
Initial HPLC methodology	2 mg	LO
NMR spectroscopy	5 mg	LO
Mass spectroscopy	5 mg	LO
General, e.g., MW, structural and empirical formulae	—	LO
1RAJV-visible spectroscopy	5 mg	LO

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Karl Fischer	20 mg	LO
pK_a	10 mg	LO
Log P/log *D*	10 mg	LO
Initial solubility	10 mg	LO/pre nomination
Initial solution stability	*Done on above samples*	LO//prenomination
Crystallinity investigations	20–30 mg	LO/prenomination
Hygroscopicity	5–10 mg	*LO/prenomination*
Initial solid stability	10 mg	Prenomination
Salt selection		
Decide/manufacture salts		Prenomination
Characterize salts—use DVS, X ray, DSC, solubility/stability tests	10–50 mg each salt	Prenomination
Initial polymorphism studies, etc.	100 mg	Prenomination
Investigations of selected salt or neutral compound.		Also included is the propensity of the CD to form hydrates, solvates, and amorphs
Production—use different solvents, cooling rates, precipitation, evaporation techniques, etc		
Polymorphism, etc.		Prenomination
Investigations of selected salt or neutral compound.		
Characterization		
DSCATGA/HSM	2 mg per technique/sample	Prenomination
X-ray powder diffraction,	10 mg/sample,	Prenomination
including temperature and *RH*	0 background holder	Prenomination
FTIR/Raman	2 mg/sample	
Crystal habit-microscopy, light, and SEM	10 mg	*Prenomination*
Stability-stress wrt temperature/humidity	100 mg	Prenomination
Choose polymorph, amorph, or hydrate		Prenomination

Abbreviations: LO, lead optimization; CD, candidate drug; HPLC, high-performance liquid chromatography; NMR, nuclear magnetic resonance; MW, molecular weight; IR/UV, infrared/ultraviolet; DVS, dynamic vapor sorption; DSC, differential scanning calorimetry; TGA, thermogravimetric analysis; HSM, hot-stage microscopy; RH, relative humidity; FTIR, Fourier transform infrared; SEM, scanning electron microscopy; wrt, with respect to.

1.7 MOLECULAR PROPERTIES

Initial Physicochemical Characterization

Initial physicohemical characterization explores the two-dimensional structural properties. Many of the tests carried out, such as proof of structure, are normally performed by the Discovery department, for example, nuclear magnetic resonance (NMR), mass spectra, and elemental analysis. Although important from a physicochemical point of view, these measurements will not be discussed in this chapter. Rather, the text will focus on those tests carried out during prenomination

that will have an important bearing on the selection of a potential CD in relation to the proposed formulation/dosage form.

pK_a Determinations

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Potential CDs that possess ionizable groups, as either weak acids or bases, can be exploited to vary biological and physical properties such as binding to target enzyme or receptor, binding to plasma proteins, gastrointestinal (GI) absorption, central nervous system (CNS) penetration, solubility, and rate of dissolution (as will be discussed later in the chapter). Therefore, one of the most important initial determinations carried out prior to their development is the pK_a or ionization constant(s). Avdeef (2001) and Kerns (2001) have comprehensively reviewed this aspect of discovery work, and the reader is referred to these papers for a detailed account. Strong acids such as HCl are ionized at all relevant pH values, whereas the ionization of weak acids is pH dependent. It is essential to know the extent to which the molecule is ionized at a certain pH, because it affects the properties noted above. The basic theory of the ionization constant is covered by most physical chemistry textbooks, and a most useful text is that by Albert and Sargeant (1984). Fundamental to our appreciation of the determination of this parameter, however, is the Br_nstead and Lowry theory of acids and bases. This states that an acid is a substance that can donate a hydrogen ion, and a base is one that can accept a proton.

For a weak acid, the following equilibrium holds:

$$HA \rightleftharpoons H^+ + A^-$$

For the sake of brevity, a detailed discussion and derivation of equations will be avoided; however, it is important that the well-known Henderson-Hasselbach equation is understood. This equation relates the pK_a to the pH of the solution and the relative concentrations of the dissociated and undissociated parts of a weak acid.

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

where $[A^-]$ is the concentration of the dissociated species and [HA] the concentration of the undissociated species. This equation can be manipulated into the form given by equation given below to yield the percentage of a compound that will be ionized at any particular pH.

$$\%\text{Ionization} = \frac{100}{1+(pH - pK_a)}$$

The Partition Coefficients

It has been shown that many biological phenomena can be correlated with the partition coefficient (log P), such that QSARs can be deduced. These include solubility, absorption potential, membrane permeability, plasma protein binding, volume of distribution, and renal and hepatic clearance. The lipophilicity of an organic compound is usually described in terms of a partition coefficient, log P, which can be defined as the ratio of the concentration of the unionized compound, at equilibrium, between organic and aqueous phases.

$$\log P = \frac{[\text{unionized compound}]_{org}}{[\text{unionized compound}]_{aq}}$$

It is worth noting that this is a logarithmic scale, therefore, a log P ¼ 0 means that the compound is equally soluble in water and the partitioning solvent. If the compound has a log P = 5, then the compound is 100,000 times more soluble in the partitioning solvent. A log P = – 2 means that the compound is 100 times more soluble in water, that is, it is quite hydrophilic.

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Log P values have been studied in approximately 100 organic liquid-water systems. However, since it is virtually impossible to determine log P in a realistic biological medium, the octanol-water system has been widely adopted as a model of the lipid phase (Leo et al., 1971). While there has been much debate about the suitability of this system (Dearden et al., 1988), it is still the most widely used measure of compound lipophilicity in pharmaceutical studies. Octanol and water are immiscible when mixed, but some water does dissolve in octanol in a hydrated state. This hydrated state contains 16 octanol aggregates, with the hydroxyl head groups surrounded by trapped aqueous solution. Lipophilic (unionized) species dissolve in the aliphatic regions of the octanol, while ionized species (see later in the chapter) are drawn to the polar regions (Franks et al., 1993). The partitioning of solutes in different solvent systems has been reported by El-Tayar et al. (1991).

According to Lipinski (1997), log P values of less than 5 are best from a drug-like perspective. Generally compounds with log P values between 1 and 3 show good absorption, whereas those with log P values greater than 6 or less than 3 often have poor transport characteristics. Highly lipophilic molecules have a preference to reside in the lipophilic regions of membranes, and very polar compounds show poor bioavailability because of their inability to penetrate membrane barriers. Thus, there is a parabolic relationship between log P and drug transport such that CDs that exhibit a balance between these two properties will probably show the best oral bioavailability. However, it has been noted that lipophilicity (and molecular weight) increases with time from LG and through LO and accounts for the generic value of log P = 3 stipulated for candidates at the LG stage (Davis et al., 2005). Overall, however, Leeson and Davis (2004) have shown that the log P of compounds has not changed significantly when they reviewed the data for oral compounds gathered over a number of years.

By using data gleaned from the literature, Leeson and Springthorpe (2007) have designated lipophilicity as the most important drug-like physical property, and any increase in this parameter will lead to a lack of selectivity and an increase in attrition. Indeed, they cautioned that although larger lipophilic compounds may exhibit greater binding affinity, they may also show greater binding to, for example, the human ether-a-go-go related gene (HERG) ion channel or cause tissue toxicity by the promotion of cellular phospholipidosis. Thus, by lowering the lipophilicity of compounds, they argue that the attrition rate would be reduced and that even a 5% improvement in attrition would result in a doubling of the number of new medicines.

The partition coefficient refers to the intrinsic lipophilicity of the drug in the context of the equilibrium of unionized drug between the aqueous and organic phases. However, if the drug has more than one ionization center, the distribution of species present will depend on the pH. The concentration of the ionized drug in the aqueous phase will therefore have an effect on the overall observed partition coefficient. This

leads to the definition of the distribution coefficient (log D) of a compound, which takes into account the dissociation of weak acids and bases. For a weak acid this is defined by equation

$$D = \frac{[HA]_{org}}{[HA]_{aq} + [A^-]_{aq}}$$

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1.8 INITIAL SOLUBILITY INVESTIGATIONS

The solubility of a CD may be the critical factor determining its usefulness, since aqueous solubility dictates the amount of compound that will dissolve and, therefore, the amount available for absorption (Bhattachar et al., 2006). If a compound has a low aqueous solubility, it may be subject to dissolution rate-limited absorption within the GI residence time. The importance of solubility, in biopharmaceutical terms, has been highlighted by its use in the biopharmaceutics classification system (BCS) described by Amidon et al. (1995). In this system, compounds are defined in terms of combinations of their solubility and permeability, for example, high solubility and high permeability or low solubility and high permeability. High solubility is defined as the highest dose strength that is soluble in 250 mL or less of aqueous media across the physiological pH range. Poorly soluble drugs can be defined as those with an aqueous solubility of less than 100 mg/mL. If a drug is poorly soluble, then it will only slowly dissolve, perhaps leading to incomplete absorption (Hotter and Dressman, 1997). For further details, the reader may refer to a study by Stegmann et al. (2007) that discusses the importance of solubility in the drug discovery and development arenas.

From a physicochemical perspective, James (1986) has provided some general rules regarding solubility:

1. Electrolytes dissolve in conducting solvents.
2. Solutes containing hydrogen capable of forming hydrogen bonds (H-bonds) dissolve in solvents capable of accepting H-bonds and vice versa.
3. Solutes having significant dipole moments dissolve in solvents having significant dipole moments.
4. Solutes with low or zero dipole moments dissolve in solvents with low or zero dipole moments.

The United States Pharmacopeia (USP) (Table 1.3) gives the following definitions of solubility (extended by Stegmann et al., 2007). Solvents can be classed into various classes, and Table 1.4 gives some examples (Chasette, 1985). For a Lewis acid, the molecule must be electron deficient and, in particular, contain an atom bearing only a sextet of electrons. A Lewis base is where the molecule must have an electron pair for sharing.

Table 1.3 Solubility Definitions

Descriptive term	*Parts of solvent required for 1 part of solute*	*Solubility range (mg/mL)*	*Solubility assigned (mg/mL)*
Very soluble	<1	>1000	1000
Freely soluble	1–10	100–1000	100
Soluble	10–30	33–100	33
Sparingly soluble	30–100	10–33	10

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Slightly soluble	100–1000	1–10	1
Very slightly soluble	1000–10,000	0.1–1	0.1
Practically insoluble or insoluble	≥ 10,000	< 0.1	0.01

Table 1.4 Classification of Solvents

Dipolar aprotic	*Protic*	*Lewis basic*	*Lewis acidic*	*Aromatic*	*Nonpolar*
DMF	Water	Acetone	Chloroform	Toluene	Heptane
DMSO	Ethanol	THF	Dichloromethane-methane	p-Xylene	Hexanes
N-Methyl-2-pyrolidinone	Methanol	Ethyl acetate		Pyridine	Cyclohexane
Acetonitrile	*n*-Butanol	2-Pentanone		Anisole	
	Acetic acid	Methyl-*t*-butyl ether		Ethyl-benzene	
	n-Propanol	Butyl acetate			
	2-Propanol				

Abbreviations: DMF, dimethyl formamide; DMSO, dimethylsulfoxide; THF, tetrahydrofuran.

The properties of solvents can be classified further. For example, Gu et al. (2004) have classified 96 solvents using a number of physicochemical parameters, that is, H-bond acceptor and donor propensity, polarity, dipole moment, dielectric constant, viscosity, surface tension, and cohesive energy density. By using a cluster statistical analysis method, they classified the solvents into 15 groups. Similarly, Xu and Redman-Furey (2007) used a clustering principal components analysis (PCA) technique (on 17 different solvent descriptors) of 57 class 2 and class 3 International Council of Harmonization (ICH) solvents. These were reduced to a set of 20 clusters, with the goal of producing an efficient solid-state screening solvent system.

Solubility Prediction

As is the case with log P, the prediction of solubility is of obvious interest, and various approaches to this problem have been reported by, for example, Chen and Song (2004), Faller and Ertl (2007), and Duchowicz et al. (2007). Faller and Ertl (2007) classified the various solubility prediction methods available as:

1. Fragment-based models
2. Models based on log P
3. Models based on solvation properties
4. Hybrid models

In their paper they posed a very pertinent question, "When can one trust the computed value?" They argued with a variety of physicochemical reasons that since the accuracy of a high-quality solubility assay is within 0.6 log units, it was unrealistic for any computed value to be more accurate than 0.5 log units or a factor of 3 to 5. More recently, Palmer et al. (2008) have adopted an ab initio thermodynamic approach to solubility prediction, and Tsung et al. (2008) have described the prediction of solubility using the nonrandom segment activity coefficient model (NRTL-SAC) and COSMO-SAC methods. Kokitkar et al. (2008) used the NTRL-SAC model for exploring solvent systems for crystallization from which a solvent or mixture of solvents are chosen to carry out the process. While the predicted data are not an exact match to experimental values, they are sufficiently accurate to allow the investigating scientist to move in a particular direction.

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Solubility of a compound in various solvents is also important from a crystallization process point of view and polymorphism screening. By using data extracted from the Cambridge Structural Database (CSD), Hosokawa et al. (2005) attempted to predict the solvents that would be suitable for the crystallization of small molecules. Data collected from 6397 compounds and 15 single solvents that were used to obtain single crystals, were assessed by chemometric analysis to show that ethanol was the best solvent for crystallization (1328 compounds) followed by methanol (1030 compounds).

1.9 THE ORGANIC SOLID STATE

Solid phases or molecular solids are defined in thermodynamic terms as states of matter that are uniform throughout in chemical composition and also in physical state (Wunderlich, 1999).

Molecular solids can exist as crystalline or noncrystalline (amorphous) phases depending on the extent of three-dimensional order and the relative thermodynamic stability hierarchy. Crystalline states can be described as a periodic array of molecules within a three-dimensional framework. Whereas noncrystalline materials (as will be described later in the chapter) lack significant three-dimensional order, but may exhibit lower-dimensional short-range order. Gavazotti (2007) has set a range of criteria by which he judges the solid state of organic compounds. In this paper, a "crystal proper" is defined as one in which the molecule is repeated to 10,000 to 100,000 times its size through a set of translationally periodic symmetry operations.

The crystalline state, as molecular crystals, is a class of solids that are composed of discrete molecules arranged in a structural framework. The structures of molecular crystals are influenced by both intramolecular and intermolecular interactions. Intramolecular forces determine molecular shape, which in turn contributes to the way the molecules pack in the crystal (Wright, 1995). Intermolecular forces are relatively weak, and thus their effect is largely short range. As a consequence of this short-range effect, diversity in the arrangement of molecules within the molecular crystals is brought about, which also gives rise to differences in properties and performance of the molecular crystals. Furthermore, a variation in spatial arrangement can give rise to the enhanced possibility of structural dynamics within molecular crystals, leading to a variation in performance and behaviour of the resultant material. Thus, an understanding of molecular crystals, and in particular the intermolecular interactions driving the molecular packing within the structure, allows an understanding of the material properties.

Any change in the physical or spatial arrangement of the molecules or inclusion of other molecule types (to give a heterogeneous material) results in the formation of different phases termed polymorphs and hydrates/co-crystals, respectively. A significant interest in molecular crystals originates from the ability to use molecular level "crystal engineering" strategies to rationally design crystal packing to control specific physical properties (Ward et al., 1997). The crystal engineering approach utilizes additives and other molecules to direct the self-assembly of the parent molecules to give a desired solid-state motif. Thus, control or understanding of arrangements in molecular crystals leads to control or understanding of various physical properties. In addition to pharmaceuticals, molecular crystals cover a

diverse range of materials used in dyes and speciality chemicals, conductors, nonlinear optical materials, and agrochemicals.

Crystalline States and Structural Assessment Polymorphism and Related Phenomena

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In 2002, Bernstein pointed out that structural diversity is present in almost every facet of nature, and crystal polymorphism is one manifestation of this diversity. Polymorphism, in a chemical sense, is a solid-state phenomenon where the crystal structures of a chemical entity are different, but correspond to identical liquid and vapor states (McCrone, 1965). A variation in crystal structure is brought about by differences in molecular packing and intermolecular interactions within the three-dimensional framework of the crystalline state. The way the molecules pack is defined in part by the molecular structure itself, and there is also the possibility of forming stable intermolecular interactions such as H-bonds, giving rise to structures with differences in density. Consequently, polymorphs will have different lattice energies, which in turn govern the physical properties and behaviours of the material (Pudipeddi and Serajuddin, 2005). An understanding and control of this phenomenon is of paramount importance in the fields of crystal engineering or material selection, crucial to the pharmaceutical, chemical, food, and agrochemical industries. Figure 1.1 shows the various polymorphs of estrone (Busetta et al., 1973).

Polymorphism is a common phenomenon in small organic molecules, and the occurrence of polymorphs has been documented extensively (Borka and Haleblain, 1990; Byrn et al., 1999; Bernstein, 2002). Specifically, in the area of pharmaceutical material selection, polymorphs are selected on the basis of physical and chemical stability, behaviour to processing and formulation, and biopharmaceutical properties as an assessment of in vivo performance. Knowledge of the relative behaviour of the polymorphs with respect to the properties outlined above allows a rationalized selection. For instance, differences in solubility and dissolution rate between polymorphs can have a pronounced impact on the oral bioavailability (*i.e.*, dissolution and absorption from the GI tract) of pharmaceuticals as exemplified by investigations of formulations of tolbutamide (Kimura et al., 1999). Other differences in properties also include thermodynamic and kinetic variations between polymorphs. Such differences encompass distinctions in reactivity involving both physical (e.g., involving interconversion of a metastable to a stable form) and chemical changes. Physical changes can occur in either the solid state or via a solution-mediated process, but are driven in accordance to Ostwald's law of stages (Threlfall; 2003), which states that a highly metastable form should transform to the most stable form via a series of thermodynamically driven phase transitions.

Differences in chemical reactivity, such as those exemplified by the three physical forms of trans-2-ethoxy cinnamic acid (Cohen and Green, 1973), for which the a and b forms dimerize under UV irradiation, whereas the g form gives no reaction, illustrate the importance of selecting a stable and robust polymorph.

McCrone stated, in 1965, that the number of polymorphs identified is directly proportional to the time and effort spent looking for them. Many approaches can be taken to induce polymorphic changes to explore its occurrence. These include solution-mediated transitions such as recrystallization and solution maturation studies

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(Cardew and Davey, 1985) and thermally induced (Giron, 1995) and mechanical/pressure-induced changes such as those exhibited by chlorpropamide (Wildfong et al., 2005). Other solvent-free methods of isolating polymorphs involve quenching from the molten liquid or gaseous state (sublimation experiments), as are used to isolate polymorphs of venlafaxine hydrochloride (Roy et al., 2005). The occurrence of polymorphism can also be explored using computational methodology (Beyer et al., 2001; Neumann 2008). The basis of these approaches involves in silico generation of all plausible crystal structures, which are subsequently ranked in order of calculated lattice energies. While the applicability has been demonstrated for small rigid structures, there are many limitations in the wider use of this approach-in particular for structures with significant conformational flexibility. Furthermore, the veracity of such approaches depends on the quality of the force fields used to model thermodynamic and kinetic properties satisfactorily (Gavezzotti, 2002), which renders the current approaches applicable only to a small subset of organic structures. In addition to simple variations in hydrogen bonding, polymorphism can also be induced by conformational differences, that is, the existence of different conformers of the same molecule in different polymorphic modifications. When a molecule is conformationally flexible with a number of energetically accessible conformations (typically differing by < 2 kcal/mol), then there is a potential that different crystallization conditions can lead to conformational polymorphism (Nangia, 2008). An example of conformational polymorphism is spiperone (Azibi et al., 1983), and the two conformers are shown in Figure 1.5. Conformational polymorphism has also been reported for ritonavir (Bauer et al., 2001). Two polymorphs have been identified, where the conformers in each form sterically drive the three-dimensional packing and subsequent hydrogen-bonding motif. This in turn resulted in stabilization of the lattice of each polymorph to a differing extent and hence significantly different solubility properties. Furthermore, conformational polymorphism can also result in diversity in bulk properties such as polychromism, as exemplified by the three main polymorphs of 5-XII (an aromatic carbonitrile), each of which exhibits a different colour, red, yellow, and orange (Yu et al., 2000). The polymorphism in this case, and hence the different coloration, was directly due to a variation in the molecular conformation, giving rise to different three-dimensional packing (Yu, 2002).

Bhatt and Desiraju (2007) describe the case of polymorphism of omeprazole, which is due to tautomerism, whereby the crystalline phases of this molecule are solid solutions of the two tautomers existing in a continuous composition range. From a series of experiments where the compositions of the solid solutions were changed, the authors were able to identify the stable form. Furthermore, these investigations identified a number of questions regarding the classification of tautomers as polymorphism or different compounds. It was proposed that distinctions should be made in terms of a structural landscape, which includes a number of solvated and nonsolvated variations of the same molecular species, rather than absolute structural assignments. An interesting extension of tautomerism-induced polymorphism is where two valence tautomers, that is, different electronic structures where non-polar N-atom (sp3 hybridization) and polar structures (sp2 hybridization) were observed in the crystal structure of 7-amino-4-methylcoumarin (Niedzialek and Urbanczyk-Lipowska, 2007).

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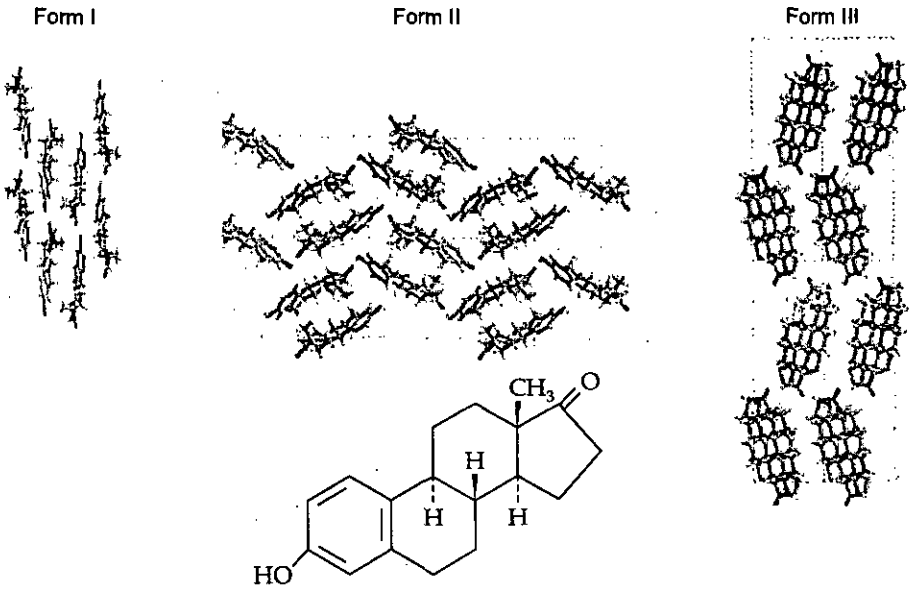

Fig. 1.4 Polymorphs of estrone.

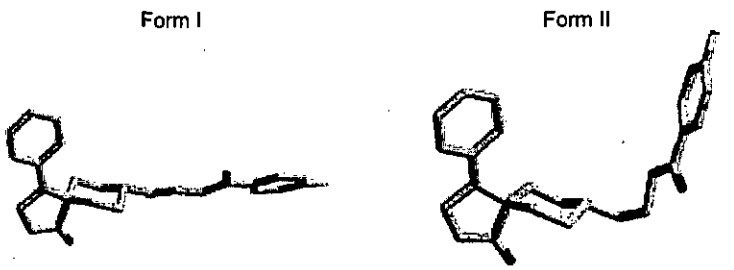

Fig. 1.5 Conformers in spiperone forms I and II.

Polymorph Screening

In the prenomination phase, any polymorphism screen will be somewhat restricted due to the amount of compound that is available at this stage. As such, an appropriate strategy to optimize the scope for assessing the polymorph hypersurface must therefore be employed to reflect this situation. An initial assessment into the propensity for polymorphism is achieved by assessing physical reproducibility of the early batches prepared by medicinal chemists.

Furthermore, the process research and development chemists will also be working on the synthesis and crystallization of the compound (Kim et al., 2005), and physical integrity assessment of these laboratory scale test batches further supplements any polymorphism screens that have or will be conducted. In both these situations, a range of solvents and crystallization conditions may be explored, thus allowing (potentially) a wide area of the polymorphism hypersurface to be evaluated. Moreover, this strategy allows (to a certain degree) an evaluation into the role that impurities play in either templating or prohibiting growth of specific physical forms. Additionally, initial screens, usually using a single largescale batch, are also conducted in parallel on a micro- or semi-microscale. There are a number of literature reports of high-throughput platforms that have been used in academia and industry for solid-form screening (Storey et al., 2004; Hilfiker et al., 2003; Almarsson et al., 2003; Morisette et al., 2003; Florence et al., 2006). Driving the concept to even smaller quantities, Lee et al. (2006) have described the technique of "polymorph farming" on a silicon wafer cast, with chitosan pretreated in different ways to affect the surface

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template properties. Crystallization of solutions of acetaminophen and sulfathiazole gave rise to the different polymorphs, depending upon the level of pretreatment. The obvious attraction of having a high-throughput system is to conduct many hundreds, if not thousands, of crystallization experiments almost in a random fashion, that is, to throw the net far and wide in the hope of scouring as much of the available space as possible. However, a more rationalized and systematic approach will generally lead to a more fruitful search. Indeed, Stahly (2007), in a note of caution, has stated that these high-throughput methods should be used in conjunction with those more systematic methodologies already in place. The rationale being that high-throughput polymorph screening methodologies using evaporation from well plates tend to induce crystallization of a greater number of metastable phases. This was illustrated by Capes and Cameron (2007) who observed that the metastable form of acetaminophen was preferentially nucleated at the edge of the meniscus and was another explanation for the appearance of metastable forms from these experiments.

While increased effort has been expended into exploring the polymorphic hypersurface of compounds, Jarring et al. (2006) indicated that the actual number of solid-state forms is in fact not terribly important from an industrial point of view. Rather, the effort should be focused on finding those physical forms that have an advantage from a performance, formulation, and largescale production perspective. Ultimately, the aimis to identify all development suitable forms and potential near-neighbor polymorphs, with the preference for selecting and progressing the form that is the most thermodynamically stable (Miller et al., 2005).

For polymorph screening purposes, Mirmehrabi and Rohani (2005) have proposed a systematic approach to solvent selection on the basis of the hydrogen-bonding propensities of the solute and the solvent molecules. They were able to calculate a polarity index (PI) for a wide range for ICH class 2 and class 3 solvents (also reporting a very useful table of properties such as dielectric constant, solubility parameter, and dipole moments for the solvents). In this case polymorphism of ranitidine was explored, and they were able to conclude that strong H-bond donor solvents lead to the dominant nitronic acid tautomer of form II, while weak H-bond donors or aprotic solvents favored the enamine tautomer found in form I. This approach therefore allowed easy identification of suitable isolation conditions for these polymorphs.

Interconversion of Polymorphs

In addition to recrystallization, solution maturation or slurry studies can be exploited to induce physical form conversions. These experiments are largely thermodynamically (rather than kinetically) driven and result in the conversion of less stable forms into physical forms that are more thermodynamically stable under the slurry conditions. When a mixture of two or more polymorphs (or hydrate and anhydrate) is slurried, it is sometimes known as a bridging experiment (Ticehurst et al., 2002). The solvents that are used can profoundly affect the rate and extent of conversion. For example, Gu et al. (2001) have studied the influence of the solvent on the rate of solvent-mediated transformations, and Mukuta et al. (2005) have reported the role of impurities, which were found to have a profound impact on the conversion. By studying the polymorphs of sulfamerazine, Gu et al. (2001) found that the rate of transformation was faster in a solvent that afforded high solubilities compared with those in which the solubility was lower. Furthermore, the conversion rate

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was found to be slower in solvents that had a greater H-bond acceptor potential. It was noted that the rate of solution agitation and temperature also affected the speed of the conversion. The more intense the agitation, the quicker the conversion, and since the relationship between these two forms of sulfamerazine is enantiotropic, the rate of conversion to form I was higher at lower temperatures and lower near the transition temperature (508C). This work also suggested that a solubility of at least 8 mM was needed to ensure that the transformation proceeded satisfactorily. The corollary of this is that the solubility should not be too high, for example, greater than 200 mM to avoid using too much of a limited amount of compound (Miller, 2005). As touched upon earlier, the role of impurities can also play a crucial role in the formation or conversion of polymorphic forms. For example, Blagden et al. (1998) have explored the role of related substances (as structurally related impurities) in the case of the disappearing polymorphs of sulfathiazole. These studies showed that a reaction by-product from the final hydrolysis stage could stabilize different polymorphic forms of the compound depending on the concentration of this by-product. By using molecular modeling techniques, they were able to show that the by-product, ethamidosulfathiazole, influenced the hydrogenbonding network and hence polymorphic form and crystal morphology. The presence of impurities can also inhibit solution-mediated phase transformations, and this can be of particular concern for screening for polymorphs at an early stage when perhaps less pure materials are available (Gong et al., 2008). Additionally, changes in the synthetic regime during the progression of the drug compound through development can give rise to significantly different impurities, which even in very minor quantities may affect the appearance or inhibition of specific polymorphs. In this study, it was shown that an acetyl derivative of sulfamerazine inhibited the conversion of form I to form II. An increase in the conversion rate, however, was attributed to a number of factors, namely, (1) increasing the solubility, (2) reducing the level of the impurity causing the problem (possibly by changing the synthetic route), (3) pretreatment of the solid to reach maximum supersaturation, and (4) increased temperature.

While there are a number of ways to screen for polymorphism and related phenomena, Kuhnert-Brandstatter and Gasser (as early as 1971) stated that "investigations of polymorphism can never be considered to be completely exhaustive in that there is always the possibility that with specific seeding a heretofore unknown crystal modification may appear," adding that "there is always the possibility of finding a new modification from some unique solvent and condition." Although Gavezotti and Filippini (1995) agreed that polymorphism of organic crystals was very frequent at room temperature, the appearance of polymorphs was not as common as it sometimes has been portrayed to be. The most recent data gathered from 245 polymorph screens carried at a contract research organization reported that 90% of the compounds exhibited "multiple and noncrystalline forms," of which only 50% were polymorphic (Stahly, 2007).

Dunitz and Bernstein (1995) have reviewed the appearance of and subsequent disappearance of polymorphs. Essentially this describes the scenario whereupon nucleation of a more stable form, the previously isolated, metastable, form could no longer be made. For example, the orthorhombic polymorph of paracetamol previously prepared by crystallization from solution had proved elusive since it was first discovered in 1974. However, crystallizing a supersaturated solution with seeds obtained from melt crystallization gave rise to a suitable laboratory scale

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method to obtain this metastable phase (Nichols and Frampton, 1998). A commentary on this phenomenon by Bernstein and Henck (1998) stated that "we believe that once a particular polymorph has been obtained it is always possible to obtain it again; it is only a matter of finding the right experimental conditions." Yu (2007) has discussed the nucleation behavior of polymorphs and its importance in determining the nature of the polymorph to be isolated, showing that an early nucleating polymorph can generally nucleate a faster-growing polymorph, thus emphasizing the fact that both thermodynamic and kinetic factors have control on the appearance of certain polymorphs. Blagden and Davey (2003) attempted to combine the effect of thermodynamics, kinetics, and nucleation, in conjunction with modeling techniques, to the selection of the polymorphs. However their approach, while showing some success, highlighted the need for an improvement in the prediction of solute-solvent interactions. In most industries in which polymorphism plays an important role in materials and their properties, there are several business drivers for polymorph characterization and selection.

Firstly, there is a need to understand the external effect on structural behaviour, enabling the selection of a robust and stable material that will not interconvert to a less desirable polymorph upon storage or processing. Secondly, it is important to have as much of the polymorph "hypersurface" mapped to ensure that all plausible low-energy structures, which could represent developable forms, are isolated and characterized. Information on structural relationships and the ease of interconversion (exploring both kinetics and mechanisms) allow the selection of the most optimum or developable form. Leading on from this is another business driver, which relates to intellectual property. On identifying all possible developable polymorphs, it is important to have patent coverage to protect intellectual property. In the area of pharmaceuticals there have been several important polymorph litigation cases (Bernstein, 2002; Cabri et al., 2007). As a consequence of the foregoing discussion regarding the physicochemical and biopharmaceutical implications of polymorphism, it can be appreciated that it is an extremely important topic from a drug regulatory perspective (De Camp, 2001).

Polymorph Production Methods

A number of protocols exploring both solvent-mediated and nonsolvent-induced polymorphism can be employed to ensure that as much of the polymorphism hypersurface can be mapped. These are summarized in the following list:

1. Crystallization from different solvents under variable conditions, for example, different agitation speeds and temperatures (as exemplified by Blagden et al., 1998; Threlfall, 2000; Alles_ et al., 2008). Therefore, it is important to screen a variety of solvents that cover a diversity of physicochemical parameters (Table 1.4). Although the solvent of crystallization can be critical in producing a particular polymorph, Getsoian et al. (2008) showed that for carbamazepine varying the crystallization temperature and level of supersaturation was sufficient to produce three of the four known polymorphs from a single solvent.
2. Precipitation by, for example, addition of an antisolvent to a solution containing the drug or by pH adjustment of solutions of weak acids or bases (Bosch et al., 2004). An interesting example of this phenomenon is the quasi-emulsion precipitation of a number of polymorphic compounds using PEG300 as the solvent and water as the antisolvent (Wang et al., 2005). In this system, the intensity of mixing appeared to control the polymorphic form because of increased viscosity of the PEG300-water solution.

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3. Concentration or evaporation. Capes and Cameron (2007) reported that a metastable form was obtained from the periphery of an evaporating solution. The resultant metastable form that was left free of the solvent was unable to transform to the more stable polymorph via a solvent-mediated phase transformation.
4. Formation of polymorphs from solvate desolvation. Nicolai et al. (2007) showed that forms I of spironolactone could be obtained by desolvation of its ethanol solvate.
5. Crystallization from the melt, assuming that melting is not accompanied by thermal degradation. Schmidt et al. (2006) found that modification I of salicaine HCl crystallized from the melt above 1108C and modification II crystallized from the melt below this temperature.
6. Grinding and compression. Trask et al. (2005a) have reported that polymorphic conversions of anthranilic acid (ortho-aminobenzoic acid), for example, could be induced by dry grinding (neat powder) and also in the presence of a small amount of solvent. Linol and Coquerel (2007) have also demonstrated that high-energy milling could be used to accelerate (relative to slurry experiments) the polymorphic conversion between the monoclinic and orthorhombic forms of 5-methyl- (40-methyl phenyl)hydantoin.
7. Lyophilization (freeze-drying) can induce polymorphism, as exemplified by pentamidine isethionate where various polymorphs can be obtained by altering the freeze-drying conditions (Chongprasert et al., 1998). Often, however, the compound is rendered amorphous by the freeze-drying process (Zhu and Sacchetti, 2002).
8. Spray-drying. Amorphous ursodeoxycholic acid can be prepared by spray-drying (Ueno et al., 1998).
9. Crystallization from supercritical fluids. Park et al. (2007) used a supercritical antisolvent (SAS) process to produce different forms of fluconazole.
10. Potentiometric cycling. Llina's et al. (2007) used this method to produce polymorphic forms of sulindac.
11. High pressure. Piracetam has been crystallized in a number of polymorphic forms using the high pressures (0.07-0.4 GPa) generated using a diamond anvil cell Fabbiani et al., 2005).
12. Sublimation. Exploiting the vapor phase as a solvent-free method of crystallizing polymorphs, as shown by Liu et al. (2008).

SUMMARY

- Preformulation, biopharmaceutics, and formulation are all important for candidate drug selection and through the various stages of product development
- Pharmaceutical Preformulation and Formulation: A Practical Guide from Candidate Drug Selection to Commercial Formulation covers a wider subject area than just preformulation. Topics include biopharmaceutics, drug delivery, formulation, and process development aspects of product development.
- Increasing competition and threats to the pharmaceutical industry with respect to maintaining continued sales growth and income mean that successful companies going forward will be those that have a portfolio of products capable of showing volume growth.
- Initial physicochemical characterization explores the two-dimensional structural properties.

- The importance of solubility, in biopharmaceutical terms, has been highlighted by its use in the biopharmaceutics classification system (BCS) described by Amidon et al. (1995).
- Solid phases or molecular solids are defined in thermodynamic terms as states of matter that are uniform throughout in chemical composition and also in physical state.

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REVIEW QUESTIONS

1. Discuss drug development drivers, challenges, risks, and rewards.
2. Discuss the Product life cycle.
3. Discuss the current trends in the pharmaceutical industry.
4. What are the different molecular properties?
5. What are different Initial Physicochemical Characterization?
6. Discuss the Partition Coefficients.
7. Discuss the initial solubility investigations.
8. Discuss the organic solid state.
9. Discuss the Crystalline States and Structural Assessment.

FURTHER READINGS

- Theory and Practice of Social Sciences, Ramakanth Tiwari and Mahesh Dabhade.

UNIT II: DISSOLUTION STUDY

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★ STRUCTURE ★

2.1 LEARNING OBJECTIVES

After going through this unit, students will be able to:

- explain drug dissolution and solubility
- discuss physiological aspects of dissolution and solubility test conditions
- elaborate biological classification system
- state absorption/uptake over the GI membranes
- explain models for studing the absorption potential of drugs.

2.2 DRUG DISSOLUTION AND SOLUBILITY

Drug dissolution is a prerequisite for oral absorption. Thus, a drug that is not fully dissolved cannot be completely absorbed through the GI epithelium. It is thus extremely important to understand drug dissolution and solubility in aqueous media, both in early drug discovery studies and as a prerequisite for the subsequent formulation development. More specifically, drug dissolution/solubility data give important information that provides answers to the following biopharmaceutical questions during the discovery phase:

- Will the drug absorption be limited by the drug dissolution/solubility?
- Will the drug dissolution/solubility limit the bioavailability to an extent that endangers the clinical usefulness of the drug?
- Which types of vehicles are needed in preclinical studies to provide the desired drug exposure?
- Should the substance form be changed to improve dissolution (*e.g.*, salt, polymorph, particle size)?

After the choice of a candidate drug, solubility and dissolution data are used for guidance in the following:

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- Should dissolution rate-enhancing principles be applied in the formulation development (*e.g.*, wetting agents, micronization, solubilizing agents, solid solutions, emulsions, and nanoparticles)?
- In the case of modified release formulations, which formulation principles are suitable and which release mechanisms can be expected?
- Which test conditions should be used for in vitro dissolution testing of solid formulations?

It should be emphasized that dissolution is the dynamic process by which a material is dissolved in a solvent and is characterized by a rate (amount dissolved per time unit), while solubility is the amount of material dissolved per volume unit of a certain solvent. Solubility is often used as a short form for "saturation solubility," which is the maximum amount of drug that can be dissolved at equilibrium conditions. Finally, the term intrinsic solubility is sometimes used as well, which is the solubility of the neutral form of a proteolytic drug. Theoretically, the dissolution rate is most often described by the Noyes-Whitney equation given below:

$$\frac{dm}{dt} = \left(\frac{D \times A}{h}\right) \times \left(C_S - C_1\right) \quad \ldots(1)$$

where D is the diffusion coefficient of the drug substance in a stagnant water layer around each drug particle with a thickness h, A is the drug particle surface area, C_s is the saturation solubility, and C_t is the drug concentration in the bulk solution. If C_t in equation (1) is negligible as compared with C_s, the dissolution rate is not affected by C_t. This state is denoted a "sink condition" and is often assumed to be the case in vivo, owing to the continuous removal of a drug from the intestine due to the absorption over the intestinal wall. A, C_t, and h in equation (1) will be time dependent, whereas the other variables are constants at a certain test condition. The surface area (A) of a dissolving particle will be constantly reduced by time (provided that no precipitation occurs); the thickness of the diffusion layer (h) is dependent on the radius of the particle size; and the bulk solution will increase toward its maximum when the total amount has been dissolved. In addition, no solid drug powder is monodisperse, that is, the starting material will consist of a dispersion of different particle sizes with different surface areas (A). Extensions of equation (1) have therefore been derived that take into account some or all of these factors. A full review of such equations and underlying assumptions and a presentation of some other less used theories for dissolution can be found elsewhere (Abdou, 1989). A modification of equation (1) was recently presented, which takes into account all time-dependent factors that can be useful for predictions of the dissolution rate (Hintz and Johnson, 1989).

The present chapter focuses on aspects of drug solubility/dissolution of specific relevance for biopharmaceutical support in candidate drug selection and preformulation. These aspects include solubility in candidate drug screening, physiological aspects of test media, solubility of amphiphilic drugs, and substance characterization prior to solubility/dissolution tests.

Aspects of Solubility in Candidate Drug Screening

Although drug solubility is an important factor in drug absorption in the GI tract, it has not been extensively screened for as a barrier to absorption. Drug solubility

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should, however, be complementary to models predicting drug permeability through the lipid membrane. Solubility as a high-throughput screening (HTS) parameter has therefore been discussed rather intensively. However, the importance of solubility as a selective tool during early screening of hundreds of compounds, to choose a drug with a potential to be absorbed in vivo in humans has not been fully evaluated. Several drugs that are very useful in the clinical situation have very low water solubility. For example, candesartan cilexetil, an effective and well-tolerated antihypertensive drug, has a water solubility of about 0.1 mg/mL. On the other hand, more soluble drugs will minimize the risk of failure during the subsequent development phase and may avoid delays, increased costs, or discontinuation of the project. Another aspect of solubility is seen during screening for good pharmacokinetic properties of candidate drugs. The HTS systems or in vitro assays are the critical point for most drugs insoluble in water. This means that if the drug is not soluble in the buffer solution used in the in vitro system, it cannot be properly experimentally evaluated. The most common negative effect of this is that the concentration needed to induce transport across the epithelial membrane in the in vitro model is too low to be detected on the receiver side (Table 2.1). For this reason, vehicles known to increase the solubility of sparingly soluble compounds are used (see section "Vehicles for Absorption Studies"). However, since these vehicles are based on surfactant systems, toxic effects may be seen on the membrane (Oberle et al., 1995; Ingels et al., 2007), and the permeability values obtained may be overestimated. New methods are now available for screening large numbers of compounds for determining solubility in small volumes [e.g., the nephelometer (BMG Lab Technologies GmbH)]. This method is based on turbidimetric determinations and is therefore not an exact tool. It can, however, contribute substantially as a first estimate of solubility of sparingly soluble compounds and make it possible to understand the results of the screening methods and to design specific experiments using vehicles.

2.3 PHYSIOLOGICAL ASPECTS OF DISSOLUTION AND SOLUBILITY TEST CONDITIONS

The dissolution of a drug in the gut lumen will depend on luminal conditions, for example, pH of the luminal fluid, volume available, lipids and bile acids, and the hydrodynamic conditions produced from the GI peristaltic movements of the luminal content toward the lower bowel. Such physiological factors influence drug dissolution by controlling the different variables in equation that describe the dissolution rate. This is summarized in Table 2.1 adapted from Dressman et al. (1998).

The test media used for determining solubility and dissolution should therefore ideally reflect the in-vivo situation. The most relevant factors to be considered from an in-vivo perspective are

1. pH (for proteolytic drugs),
2. ionic strength and composition,
3. surface-active agents, and
4. temperature.

Table 2.1 Physicochemical and Physiological Parameters Important to Drug Dissolution in the Gastrointestinal Tract

Factor	*Physicochemical parameter*	*Physiological parameter*
Surface area of drug (A)	Particle size, wettability	Surfactants in gastric juice and bile
Diffusivity of drug (D)	Molecular size	Viscosity of lumenal contents
Boundary layer thickness (h)		Motility patterns and flow rate
Solubility (C_s)	Hydrophilicity, crystal structure, solubilization	pH, buffer capacity, bile food components
Amount of drug already dissolved (C_i)		Permeability
Volume of solvent available (C_l)		Secretions, coadministered fluids

Table 2.2 The pH and Concentration of Most Dominant Ions in Different Parts of the Gastrointestinal Tract in Humans

	pH		*Ionic concentrations (nM)*		
	Fasting	*Fed*	Na^+	HCO_3^-	Cl^-
Stomach	1–2	2–5*	70	<20	100
Upper small intestine	5.5–6.5		140	50–110	130
Lower small intestine	6.5–8				
Colon	5.5–7				

*Dependent on volume, pH, and buffer capacity of the food.

The pH varies in the GI tract from 1 to 8 (Table 2.2), and the dissolution properties should therefore be known over this pH range for orally administered drugs. A more thorough review of intestinal pH conditions can be found elsewhere (Charman et al., 1997; Fallingborg et al., 1989).

It may be argued that, for immediate release formulations intended to quickly dissolve in the stomach, only the more acidic pH levels are of relevance. However, dissolution may occur at higher pH levels for several reasons, for example, concomitant food intake, comedication, diseases, or instant tablet emptying to the small intestine. In addition, since drug absorption over the gastric wall is negligible, the drug will always enter the more neutral conditions in the intestine.

Dissolution studies at pH 1 are generally performed in HCl, which is also present in the stomach. However, to perform studies over the entire pH interval, different buffers are needed to control the pH. In the intestine, pH is controlled by bicarbonate, which is not practical to use in vitro because of the need for continuous bubbling with CO_2. Non-physiological buffer systems, such as phosphates, acetates, or citrates, are therefore often used. It is important to note that the solubility may vary for different buffers at the same pH, because of different "salting in" and "salting out" effects or differences in solubility products when the drug and buffer component are of opposite charges. If such effects occur, the solubility parameters will also be dependent on the concentration of the buffer system, and the influence of the buffer will increase at higher concentrations. Excessive buffer concentrations beyond what is needed to control the pH should therefore be avoided.

The dominant ions in the GI tract are sodium, chloride, and bicarbonate and their concentrations vary between luminal sites along the GI tract (Lindahl et al., 1997). The total concentration of these ions, expressed as ionic strength, has been determined to be 0.10 to 0.16 and 0.12 to 0.19 in the stomach and small

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intestine, respectively. The presence of such ions may affect solubility, especially by the common ion effect. The presence of physiological surface-active agents in the stomach and small intestine will influence the solubility and the dissolution of sparingly soluble drugs by improved wetting of solid particle surface areas and by micellar solubilization. This has been reviewed in more detail by Gibaldi and Feldman (1970) and Charman et al. (1997).

The main endogenous surfactants are the bile acids, which are excreted into the upper jejunum by the bile flow. The bile acids-cholic acid, chenodeoxycholic acid, and deoxycholic acid—are present as conjugates with glycine and taurine as the sodium salts. The total concentrations of bile acids in the upper small intestinal tract are 4 to 6 mM in the fasting state and 10 to 40 mM after ingestion of a meal. The bile acids are reabsorbed in the terminal small intestine (terminal ileum) by active uptake. During normal physiological conditions, micelle formation and solubilization may already occur at the lower bile acid concentrations in the fasting state. The micelles formed not only contain bile acids but are a mixture with endogenous phospholipids excreted by the bile (lecithin) and products from the digestion of dietary fat, such as monoglycerides. The saturation solubility of a sparingly soluble drug has, in some cases, been increased by several orders of magnitude by the addition of physiological amounts of lecithin to a bile salt solution, whereas no solubility improvements are obtained by the formation of mixed micelles for others. It should also be noted that while the solubility of a very sparingly water-soluble drug is increased by the formation of a mixed micelle, the rate of dissolution might be decreased.

Bile acids not only affect the solubility by solubilization of sparingly soluble compounds, they may also decrease the solubility by forming sparingly soluble salts or complexes with drugs. Indications of such phenomena have been shown for a variety of drugs such as pafenolol, tubocurarine, neomycine, kanamycine, nadolol, atenolol, and propranolol (Yamaguchi et al., 1986 a,b,c; Grosvenor and Lo?froth, 1995).

Solubility or dissolution studies in the presence of physiological surfactants may provide important information with respect to the in vivo absorption process of sparingly soluble compounds, although it is hardly possible to reconstitute the full complexity and dynamics of the in vivo situation in an in vitro model. While bile acids and lecithin are available in purified forms, their use is somewhat limited by their high price. Much less well-defined ox bile preparations are also available, which contain a mixture of conjugated bile acids and other bile components. The closest test media to mimic the in vivo lumenal content have been the suggested systems by Dressman et al., the FaSSIF and FeSSIF systems (Dressman et al., 2007, and references therein).

The temperature in dissolution and solubility tests should preferably be identical to the in vivo temperature at the site of administration, since the solubility is dependent on the temperature. The most suitable temperature depends on the intended administration route. For oral administration, testing at 378C is the obvious choice.

2.4 BIOLOGICAL CLASSIFICATION SYSTEM

In the mid-1990s it was realised that a drug's oral absorption was primarily controlled by two features, basic solubility in aqueous media coupled with permeability through the gastrointestinal membrane. It is easy to visualize that if a drug is neither

soluble nor permeable, then no oral absorption will occur after administration of a solid dosage form. For solubility, the aqueous media simulates conditions that the drug would meet on passage through the gastrointestinal tract. Drugs, where the highest administered dose strength dissolves in _250 mL of water between pH 1 and 7.5, are considered to have "high solubility" and those not meeting this specification considered to have "low solubility." Dissolution performance is also considered, and "rapidly dissolving" drugs are defined as those where 85% of the drug dissolves within 30 minutes during a pharmacopoeial dissolution test. This requirement involves the final formulation and therefore will not be applicable during preformulation testing. Permeability is measured using bioavailability with a "high-permeability" drug defined as a ≥ 90% absorption of the administered dose; those compounds not meeting this specification have a "low permeability." During preformulation permeability information can be obtained from in vitro permeation experiments across epithelial cell monolayers.

Drugs are then grouped into classes based on the definitions as in Table 2.3. Although the biopharmaceutics classification system (BCS) is generally for application to finished products, knowledge of a compound's likely classification as early as possible in the development process is useful. Since this can be obtained through solubility and in vitro measurements, it is easy to perform during preformulation. The ideal compounds fall into class I with increasing development issues related to the other classes.

Table 2.3 Biopharmaceutics Classification System

Permeability	*Solubility*	
	High	*Low*
High	**Class I** Compounds well absorbed, good absorption rate, excellent candidates for oral administration, for example, metoprotol	**Class II** Compound absorption limited by solubility, formulations to increase solubility may be required, for example, glibenclamide
Low	**Class III** Compound absorption limited by permeability that can be maximized by maximizing solubility, for example, cimetidine	**Class IV** Compounds with poor and variable oral absorption, for example, hydrochlorothiazide

2.5 ABSORPTION/UPTAKE OVER THE GI MEMBRANES

Mechanisms of Drug Absorption

Several factors originating from the chemical structure and property of the drug molecule and from the physiology within the environment in the GI tract affect the flow of molecules across the intestinal membrane. These factors include solubility, partition coefficient, pK_a, molecular weight, molecular volume, aggregate, particle size, pH in the lumen and at the surface of the membrane, GI secretions, absorptive surface area, blood flow, membrane permeability, and enzymes (for more factors, see Ungell, 1997, and Table 2.4). Complete absorption occurs when the drug has a maximum permeability coefficient and maximum solubility at the site of absorption (Pade and Stavchansky, 1998).

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The uptake of drugs across the intestinal membrane can occur transcellularly across the lipid membrane or paracellularly between the epithelial cells in the tight junctional gap (Ungell, 1997). The transcellular route is generally via carrier proteins or by passive diffusion. In addition, the transport across the cell membrane can be via endocytotic processes. Efflux proteins carrying the drug from the inside back into the lumen (*e.g.*, P-glycoprotein, MRP1-6, etc.) have been proposed to be important for the overall absorption of drugs in the GI tract (Saitoh and Aungst, 1995; Hunter and Hirst, 1997; Makhey et al., 1998; Do¨Ëpenschmitt et al., 1998; Anderle et al., 1998). Models have now been developed for specific studies of the mechanism behind low permeability or active transport via carrier systems, such as oligopeptide transporters, dipeptide transporters, amino acid transporters, and monocarboxylic transporters (Tsuji and Tamai, 1996).

Apart from permeability of the intestine to molecules, the time the molecule spends in the region of absorption, that is, transit time, becomes important. Generally, transit times in humans are seconds in the esophagus, 0.5 to 1.5 hours in the stomach, 3 to 4 hours in the small intestine, and 8 to 72 hours in the colon.

Table 2.4 Physicochemical and Physiological Factors that Influence Drug Bioavailability after Oral Administration

Physicochemical	*Physiological*
Hydrophobicity	Surface area at the site of administration
Molecular size	Transit time and motility
Molecular conformation	pH in the lumen and at surface
pK_a	Intestinal secretions
Chemical stability	Enzymes
Solubility	Membrane permeability
Complexation	Food and food composition
Particle size	Disease state
Crystal form	Pharmacological effect
Aggregation	Mucus and unstirred water layer
Hydrogen bonding	*Water fluxes*
Polar surface area	Blood flow
	Bacteria
	Liver uptake and bile excretion

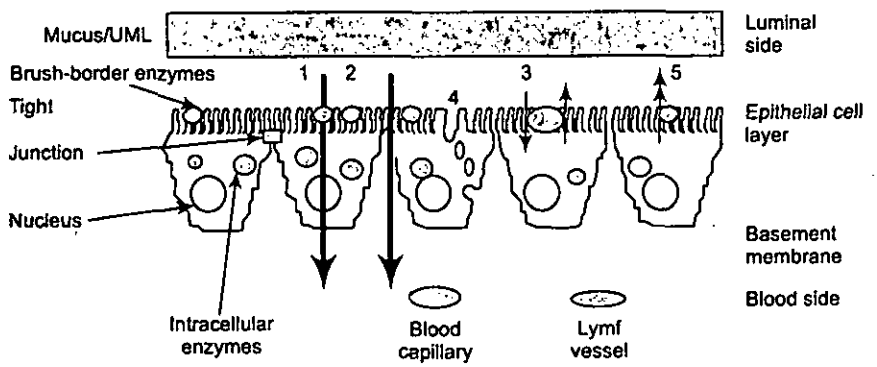

Fig. 2.1 Schematic drawing of the mechanisms and routes of drug absorption across intestinal epithelia. Drugs can be absorbed transcellularly (1) and paracellularly (2) by passive diffusion or transcellularly via carrier-mediated transport (3) or endocytosis (4). Enzymes in the brush-border region or intracellular enzymes and the efflux proteins, for example, P-glycoprotein (5), contribute to the elimination of harmful compounds.

Regionally, the different physiological factors will change, and thereby the potential impact on the drug molecule will also change (Dressman and Yamada, 1991; Horter and Dressman, 1997; Ungell et al., 1997). For developing extended oral drug release dosage forms, knowledge of the regional differences in the absorption pattern becomes very important in evaluation and success (Thomson et al., 1986; Ungell et al., 1997; Kararli, 1995; Pantzar et al., 1993; Narawane et al., 1993). In addition, these mechanisms are also species different (Kararli, 1995) and must be correlated to the human situation. If the regional difference in absorption probability of the drug is known (regional permeability and interactions), increased absorption can be achieved by the use of an absorption window, for example, targeting the drug to a specific region to avoid critical regions of enzymes or low permeability.

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2.6 MODELS FOR STUDYING THE ABSORPTION POTENTIAL OF DRUGS

Models for studying drug absorption that are available in industry and at universities and contract organizations are mainly (Hillgren et al., 1995; Ungell, 1997; Borchardt et al., 1996; Stewart et al., 1995)

- computational methods,
- partitioning between water and oil,
- cell cultures,
- membrane vesicles,
- intestinal rings or sacs,
- excised segments from animals in the Ussing chamber,
- in vitro and in situ intestinal perfusions,
- in vivo cannulated or fistulated animals, and
- in vivo gavaged animals.

All of these models have values that must be correlated to human data, mainly Fa (fraction absorbed) (Ungell, 1997; Lennerna et al., 1997; Artursson et al., 1993; Lennerna et al., 1996; Artursson and Karlsson, 1991; Ungell and Karlsson, 2003) (Figure 2.2).

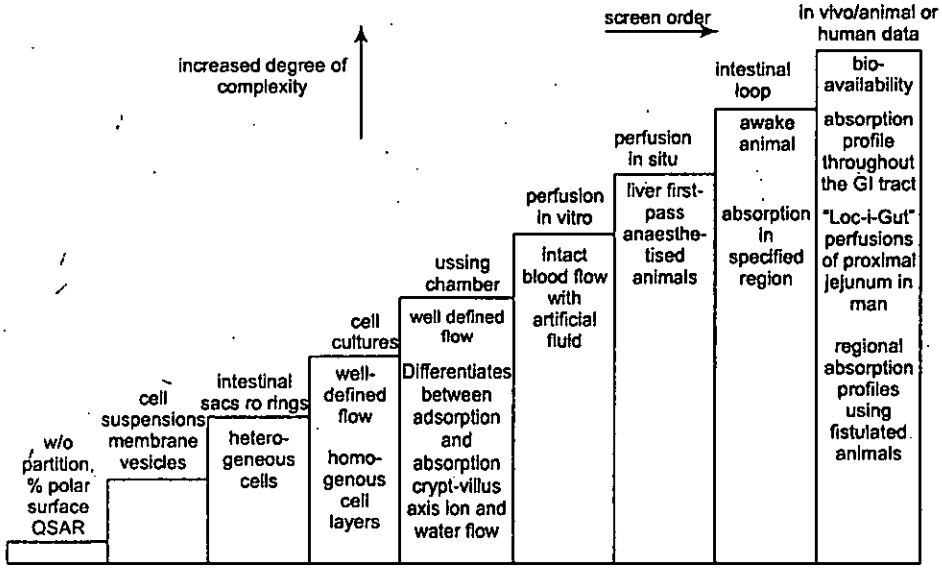

Fig. 2.2 Schematic drawing of a screen ladder. The screen ladder can be used for understanding different complexities in the results, using different screening models. **Source:** From Ungell (1997)

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Correlations have been made in different laboratories using different models (Ussing, perfusion, and Caco-2) (Matthes et al., 1992; Rubas et al., 1993; Tanaka et al., 1995; Lennernaˆs et al., 1997; Fagerholm et al., 1996) and in different laboratories using the same model (Caco-2) (Artursson et al., 1996; Hayeshi et al., 2008).

Methods to describe the process of transport over the GI membrane must describe different mechanisms of absorption for a wide variety of molecules and must be predictive of the absorption process in human. If a new chemical entity (NCE) cannot penetrate the intestinal epithelium, it will not be successfully developed as a pharmaceutical product. This also means that if the rate-limiting step in the absorption process of the drug molecule is not described in the model, the result will be a false positive.

The literature mentions numerous nonbiological (biophysical and computational) and biological in-vitro and in-vivo methods for screening barriers of absorption (for more information see Borchardt et al., 1996; Kararli, 1989; Ungell, 1997; Hillgren et al., 1995; Lipinski et al., 1996; Lundahl and Beigi, 1997; Yang et al., 1997; Hjort-Krarup et al., 1998; Quilianova et al., 1999; Stewart et al., 1997; Lee et al., 1997; Altomare et al., 1997; van der Waterbeemd et al., 1996; Winiwarter et al., 1998; Oprea and Gottfries, 1999). Each method describes a part of the absorption process, mainly the transport through the lipid membrane. However, it is clear that for drug discovery and rational drug development, there is no single ultimate method, but instead, there is a need for more than one of these screening methods. It is also evident from the literature that we need more information regarding the absorption mechanisms of the particular drug entity and its analogues to be able to obtain structure/absorption relationships and to design the most proper method for screening, for example, HTS. Below is a short review of the different methods available for studying drug absorption.

Nonbiological Methods for the Prediction of Oral Drug Absorption

Passive diffusion through the lipid membrane of the GI tract is considered to represent one of the main drug absorption mechanisms. This is a process generally thought to be governed by physicochemical factors of the drug molecule, such as lipophilicity, surface charges, molecular volume/molecular weight, and conformational flexibility (Navia and Chaturvedi, 1996). The size of the molecule and the charge will also govern whether the molecule can passively pass across the epithelium via the paracellular pathway and between the cells via the tight junctional complex. However, it has been argued that the paracellular route is almost nonexistent and seems to be important only for drugs with molecular weights below 200 g/mol and for nonionic or cationic molecules (Karlsson et al., 1999, 1994; Lennernas, 1997). The nonbiological models describing the transmembrane process are very rapid and involve no use of animals. Computer-based models of structure/absorption relationships belong to this group. They have recently become increasingly popular because of the use of chemical libraries and possibilities for testing large sets of biological data, with multivariate analysis models such as partial least squares (PLS) and principal component analysis (PCA) (Eriksson et al., 1999). In fact, the nonbiological methods can, in many cases, replace the biological methods and can be used in an HTS manner. The most challenging ideas for industry today involve trying to avoid time and resource consuming synthesis of structural analogues with no potential of being developed as pharmaceutical products; they therefore focus more on the analogues that have such potential.

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The most widely accepted parameter for predicting drug absorption is the partition coefficient reflecting partitioning of the drug only into a lipid phase (*e.g.*, octanol/water), the log P (or log D) value (see chapter 3), and general rule-of-thumb models such as the Lipinski rule (Lipinski et al., 1996). This rule states that molecules with a molecular weight of less than 500 g/mol, with a clog P of less than 5, with hydrogen donors fewer than 5, and acceptors fewer than 10 will have greater possibilities for being orally available, and violation of at least two of these rules will lower the potential for the drug to be absorbed. This rule is based on historical data (up to 1997) from a vast number of drugs entering the investigational new drug application (IND) phase, including drugs that fulfilled the criteria of pharmacological activity. The rule is also based on several other assumptions: that the transcellular transport is molecular weight dependent, the drug is only absorbed through passive diffusion, the four parameters describe the molecular structure correctly, and the drug is not solubility restricted.

Only the high value of the limit is set; the lower limit of, for instance, clog P is not within the rule. In addition, the parameters in the Lipinski rule describe the two-dimensional structure of the molecule but do not take into account the three-dimensional structure and the true conformation of the molecule. However, regardless of the restrictions of the rule, it can be used as a rule of thumb in the same way as log P or log D is being used, but is a better predictor than lipophilicity alone.

The optimal range in lipophilicity that would reflect a good absorption potential has been suggested to be a log P value between 0 and 3 (Navia and Chaturvedi, 1996) or above 3 (Wils et al., 1994a), depending on the method used. This is a general rule of thumb because it means that very hydrophilic drugs (log $P < 3$) and very lipophilic drugs (log $P > 6$) are often associated with incomplete absorption in-vivo (Navia and Chaturvedi, 1996; Wils et al., 1994a). Drugs with log P values between -3 and 0 and a log P between 3 and 6 often give varying results (Navia and Chaturvedi, 1996). However, the prediction of incomplete absorption for hydrophilic and very lipophilic drugs has been argued. Hydrophilic drugs, such as atenolol and sotalol, are absorbed from the GI tract, although their partition coefficients are low, and very lipophilic drugs, such as fluvastatin, are completely absorbed (Lindahl et al., 1996). The lack of correlation and the varying results obtained by this method are understandable, since lipophilicity is far from being the only determinant of drug absorption.

Today, however, there is a more complex view of the factors governing the partitioning into a lipid phase, for example, multiple molecular structure descriptors, including a number of hydrogen bonds (acceptors and donors) (Burton et al., 1992), polar surface area (Palm et al., 1996), polarity, integy moments, polarizability, and distances between functional groups of importance (Oprea et al., 2000; Zamora et al., 2003; Norinder et al., 1997; Palm et al., 1997; Cruciani et al., 2000; Goodford, 1985). Future quantitative structure activity relationship (QSAR) models will therefore be based more on multivariate analysis, analyzing a complex set of molecular descriptors.

Below is a short description of some of the nonbiological methods that can be used to predict drug absorption. For more detailed information, see, for example, Ungell (1997), Lipinski et al. (1996), Palm et al. (1997), Burton et al. (1992), Nørinder et al. (1997), van der Waterbeemd et al. (1996), and Cruciani et al. (2000).

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Computer-Based Prediction Models

A good relationship has been established between the number of hydrogen bonds of small model peptides and their permeability coefficients, determined using Caco-2 cell monolayers (Burton et al., 1992). The method reflects the ability of the molecule to form hydrogen bonds with the surrounding solvent. The more bonds the molecule forms with water (luminal fluid), the less potential it has to diffuse into a lipid phase of a membrane.

The total number of hydrogen bonds in the molecule can easily be calculated, including the bonds the molecule can form internally. This may be one of the reasons for the lack of correlation seen for the drug fluvastatin, a very lipophilic drug (log P 3.8) that has a total feight hydrogen bonds (Lindahl et al., 1996), whereof several are internal within the drug molecule. The total number of hydrogen bonds that should be the limit is five, according to the Lipinski rule (Lipinski et al., 1996), if the drug is to be completely absorbed in the GI tract in human (Lindahl et al., 1996).

Polar surface area is another important determinant of drug absorption, as first proposed by Palm et al. (1996). The method was described as dynamic molecular surface properties. These were calculated with consideration of all low-energy conformations of some b-blockers, and the water-accessible surface areas, which were calculated and averaged according to a Boltzmann distribution. They found a linear relationship between permeability coefficients, measured both with Caco-2 cells and excised segments from the rat intestine, and percentage polar surface area of b-blockers with different lipophilicity. According to the calculated values of log Doct at pH 7.4, there was not as good a relationship, with some additional impaired ranking order between the substances [calculated according to the method of Hansch and coworkers (Palm et al., 1996)]. Polar surface area has also recently been proposed to explain why drugs with very high log D values are not absorbed (Artursson et al., 1996). The authors suggest that these very lipophilic drugs, instead, show a high degree of polar surface area Biopharmaceutical Support in Candidate Drug Selection 147 toward the environment, which will reduce their ability to diffuse through a lipid phase. This was shown by a bell-shaped correlation between permeability coefficients determined in HT- 29 (18-C) monolayers and log D and, in contrast, a linear relationship between permeability coefficients and calculated polar surface area (Artursson et al., 1996). A good correlation between the fraction absorbed, and the polar surface area of a variety of drugs, as well as for hydrogen acceptors and donors, has been proposed to exist (Palm et al., 1997).

SUMMARY

- Drug dissolution is a prerequisite for oral absorption. Thus, a drug that is not fully dissolved cannot be completely absorbed through the GI epithelium.
- Drug solubility should, however, be complementary to models predicting drug permeability through the lipid membrane. Solubility as a high-throughput screening (HTS) parameter has therefore been discussed rather intensively.
- The dissolution of a drug in the gut lumen will depend on luminal conditions, for example, pH of the luminal fluid, volume available, lipids and bile acids, and the hydrodynamic conditions produced from the GI peristaltic movements of the luminal content toward the lower bowel.

- Although the biopharmaceutics classification system (BCS) is generally for application to finished products, knowledge of a compound's likely classification as early as possible in the development process is useful.
- Several factors originating from the chemical structure and property of the drug molecule and from the physiology within the environment in the GI tract affect the flow of molecules across the intestinal membrane.

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REVIEW QUESTIONS

1. Discuss the drug dissolution and solubility.
2. What are different Aspects of Solubility in Candidate Drug Screening?
3. What are physiological aspects of dissolution and solubility test conditions?
4. Discuss the biological classification system.
5. Discuss the absorption/uptake over the gi membranes.
6. What are different models for studying the absorption potential of drugs?

FURTHER READINGS

- Theory and Practice of Social Sciences, Ramakanth Tiwari and Mahesh Dabhade.

NOTES

UNIT III: STABILITY STUDIES

★ STRUCTURE ★

3.1 LEARNING OBJECTIVES

After going through this unit, students will be able to:

- discuss the introduction of stability of drugs
- explain stability programmer for a new drug
- elaborate stability testing for established drug substances
- state shelf life and recommended storage conditions
- explain effects of various environment on stability
- know about international conference on harmonization (left)
- discuss physical stability, chemical stability and microbiological stability
- explain mechanisms of degradation—hydrolysis oxidation and photolysis.

3.2 INTRODUCTION

When a manufacturer plans to design, manufacture and market a drug product it is his responsibility to provide to the regulatory authorities assurance that the drug product meets with all the labelled claims and is stable in all senses till the expiry date is over. This responsibility implies that he/she undertake stability testing studies in a systematic way right from the Phase-I studies level. Upto the seventies these studies were undertaken by different companies in different manner. But today ICH guidelines help the manufacturers adopt a stability programme that is suitable for them. The final draft the ICH Harmoised Tripartite Guideline "Stability Testing of New Drug Substances and Products was issued by the

International Conference on Harmonization (ICH) Expert Working Group of the ICH on technical requirements for the registration of pharmaceuticals for human use in October 1993. J.T. Cartensen in his book "Drug Stability, Principles and Practices" discussed these guidelines in great detail.

3.3 STABILITY PROGRAMME FOR A NEW DRUG

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These guidelines divide the world into four zones and specify the temperature and relative humidity conditions to be maintained by each zone for stability studies. For example, if a manufacturer plans to sell his products in Zone III he has to submit stability data of his batches of products maintained at the temperature and relative humidity suggested by ICH for Zone III. I will introduce the spirit and the text of the guidelines to you in a very brief manner. Stability testing is done in five different occasion when an NDA is being contemplated.

1. Preformulation and compatibility
2. Preclinical formulation
3. Clinical and NDA formulation
4. Commitment and product monitoring
5. Post NDA change of formulation
6. Preformulation and compatibility

In the early stage of drug designing studies are done to find out what sort of decomposition is possible, what is the mechanism, sensitivity to moisture and oxygen interaction probabilities (compatibilities) optimum pH and polymorphic information. Drug excipient interactions physical as well as chemical are extensively studied.

Preclinical Formulation

Keeping the data from the preformulation studies in mind formulations are designed and manufactured for use in Phase-I trails. More than the one or two formulations being used in Phase-I studies are manufactured and started on stability studies. This is because even a supposedly stable formulation may while in Phase-I use fail with respect to some stability issue, then you must have something to fall back upon.

Clinical Formulation

When a product has passed Phase-I, its dosage level, interactions and stability profile are known to some degree and armed with this knowledge the "Clinical manufacturing group" of the company manufactures several batches of the product and keeps some products from every batch for stability. The required stability aspects of clinical are simply to ascertain that each batch is within specifications during the length of the trail.

Late Clinical and First Pilot Batch

The ICH stability guidelines require that three substantial batches, made in the same type production equipment intended for the final product, be made and that at least 12 months stability be in place at the time of NDA submittal.

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Marketed Product Stability

At the time the NDA is filed, the large clinical and scate-up batches are only about a year old, and the stability data on them is not yet complete. So at this time, the company asks for an expiry date based on extrapolation of the existing stability data. The FDI will take all facts into consideration and grant an expiry date based on a commitment form the company that the company will continue to do stability studies on different batches. The storage requirements and the sampling times are very clearly specified by the ICH guidelines.

A Brief History of ICH

Prior to 1960s there were not many controls over introduction of new drugs and also over the assurance of the quality by the manufacturer over his established drug products. Some stray tragic incidents in some countries like USA and India triggered the introduction of exacting drug laws to ensure the quality, safety and efficacy of the drug. Around 1970s the pharmaceutical industry started getting global but the registration of medicines remained a national responsibility. Although the laws of all the countries were based on the same fundamental obligations to evaluate the quality, safety and efficacy the detailed technical requirements differed from country to country. So the companies had to duplicate many time consuming and expensive test procedures, in order to market new products, internationally. All this resulted in unnecessary expenses and long delays in introducing new drugs. So a necessity to harmonise or make uniform, the testing procedures and regulatory requirements of different countries was felt and the result is the birth of ICH in April 1990.

The birth of ICH took place at a meeting in April 1990, hosted by the EFPIA in Brussels. Representatives of the regulatory agencies and industry associations of Europe, Japan and the USA met primarily to plan an International Conference but the meeting also discussed the wider implications and terms of reference of ICH. The ICH steering committee which was established at that meeting has since met at least twice a year, with the location rotating between the three regions.

The topics first chosen for harmonization were "safety, quality and efficacy and Expert Working Groups were set up to discuss scientific and technical aspects of each harmonization topic.

Steps in ICH Process

The ICH process envisages harmonisation in 5 steps.

Step 1: Concept paper, guideline development through EWG scientific consensus.

Step 2: EWG and steering committee guideline "sign off".

Step 3: Public comments requested and reviewed, drafts revised by regulators.

Step 4: Regulatory parties EWG and steering committee of "sign off" finalized ICH guidelines.

Step 5: Implementation in three regions.

Climatic Zones and Conditions

WHO has issued guidelines, where it is stated that the world is divided into four zones based on the prevailing annual climatic conditions for the purpose of stability testing.

Zone I : temperate

Zone II : subtropical with possible high humidity

Zone III : hot/dry

Zone IV : hot/humid

Tables 3.1 and 3.2 gives some interesting data. Table 3.1 gives the temperatures and relative humidities as recorded in different zones.

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Table 3.1 Mean climatic conditions: measured data in the open air and in the storage room

Climatic Zone	*Measured data in the open air*		*Measured data in storage room*	
	°C	*%RH*	*°C*	*%RH*
I	10.9	75	18.7	45
II	17.0	70	21.1	52
III	24.4	39	26.0	54
IV	26.5	77	28.4	70

RH = relative humidity

Table 3.2

Climatic Zone	*Calculated data*			*Derived storage conditions (for real-time studies)*	
	°C	*°C MKT*[3]	*%RH*	*°C*	*%RH*
I	20.0	20.0	42	21	45
II	21.6	22.0	52	25	60
III	26.4	27.9	35	30	35
IV	26.7	27.4	76	30	70

1. Based on: Grimm W. Storage conditions for stability testing in the EC, Japan and USA; the most important market for drug products. Drug development and industrial pharmacy, 1993, 19:2705-2830.
2. Calculated temperatures are derived from measured temperatures, but all measured temperatures of less than 19°C were set equal to 19°C.
3. MKT = mean kinetic temperature
4. RH = relative humidity

Table 3.2 gives the calculated values of temperature, mean kinetic temperature and relative humidity and also gives derived storage conditions for real time studies. So for example if a manufacturer plans to sell his products in zone-III he/she should do real time studies at 30°C and 35%RH.

What has ICH done so far regarding stability?

It has issued guidelines in six important areas, all of them regarding stability testing of new drugs or dosage forms.

Quality Guidelines Stability=

Q1A : (R2) Stability Testing of New Drug Substances and products (Second Revision)

Q1B : Stability Testing: Photostability Testing of New Drug Substances and Products.

Q1C : Stability Testing for New Dosage Forms

Q1D : Bracketing and Matrixing Designs for Stability Testing of Drug Substances and Products.

Q1E : Evaluation of Stability Data

Q1F : Stability Data Package for Registration Application in Climatic Zones III and IV.

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Q 1 A (R2)

If a manufacturer wants to apply for the registration of a new drug, *i.e.* if he is applying for a (1) Investigative New Drug Application (IND) or (2) New Drug Application (NDA) or (3) New Drug Application (ANDA) then he has to assure the FDA regarding the drug's/drug product's safety, quality and efficacy. For this he has to carry out stability tests and submit stability data. How he should do this is specified by Q1A (R2)?

Selection of Batches

Data from formal stability studies should be provided on at least three primary batches of the drug substance. These batches should be made to a minimum of pilot scale by the same synthetic route as that of the production batches. Specifications which include testing methods and acceptance criteria should be fixed.

Testing frequency:

_____Months_____

Long term : 0, 3, 6, 9, 12, 18, 24

Accelerated storage: 0, 3, 6

Storage conditions recommended

General case

Study	*Storage condition*	*Minimum time period covered by data at submission*
Long term	25°C ± 2°C/60% RH ± 5% RH or 30°C ± 2°C/65% RH ± 5% RH	12 months
Intermediate	30°C ± 2°C/65% RH ± 5% RH	6 months
Accelerated	40°C ± 2°C/75% RH ± 5% RH	6 months

It is up to the applicant to decide whether long term stability studies are performed at 25 + 2°C/60% RH + 5% RH or 30°C + 2°C/65% RH + 5% RH. If 30°C + 2°C/65% RH + 5% RH is the long-term condition, there is no intermediate condition. If long-term studies are conducted at 25°C+ 2°C/60% RH + 5% RH and "significant change" occurs at any time during 6 months' testing at the accelerated storage condition, additional testing at the intermediate storage condition should be conducted and evaluated against significant change criteria. "Significant change" for a drug substance is defined as failure to meet is specification.

Drug substances intended for storage in a refrigerator

Study	*Storage condition*	*Minimum time period covered by data at submission*
Long term	5°C ± 3°C	12 months
Accelerated	25°C ± 2°C/60% RH ± 5% RH	6 months

Drug substances intended for storage in a freezer

Study	*Storage condition*	*Minimum time period covered by data at sub mission*
Long term	–20°C ? 5°C	12 months

On the basis of the testing of all these stored products and analysing them for various stability parameters data is obtained and this data is analysed statistically and a storage statement is made on the label. Almost all conditions are similar for new drug and new drug product. Significant change for a drug substance is defined as failure to meet its specification. Significant change for a drug product is defined as

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1. A 5% change in assay from its initial value; or failure to meet the acceptance criteria for potency when using biological or immunological procedures;
2. Any degradation product's exceeding its acceptance criterion;
3. Failure to meet the acceptance criteria for appearance, physical attributes, and functionality test (*e.g.*, colour, phase separation, resuspendibility, caking, hardness, dose delivery per actuation); however, some changes in physical attributes (*e.g.*, softening of suppositories, melting of creams) may be expected under accelerated conditions; and, as appropriate for the dosage form;
4. Failure to meet the acceptance criterion for pH; or
5. Failure to meet the acceptance criteria for dissolution for 12 dosage units.

3.4 STABILITY TESTING FOR ESTABLISHED DRUG SUBSTANCES

WHO has issued guidelines for stability testing of pharmaceutical products containing well established drug substances in conventional dosage forms. The stability of finished pharmaceutical products depends on environmental factors and on product related factors. So stability considerations should be given, the highest priority in the design and formulation of a product. The shelf life should be established with due regard to the climatic zones. To ensure both patient safety and the rational management of drug supplies, it is important that the expiry date and storage conditions are properly indicated on the label.

Let us look at a few definitions here.

Accelerated stability testing

These are the studies designed to increase the rate of chemical degradation and physical change of a drug by using exaggerated storage conditions as part of the formal stability testing programme. The data thus obtained, in addition to those derived from real-time stability studies, may be used to assess longer-term chemical effects under non-accelerated conditions and to evaluate the impact of short-term excursions outside the label storage conditions, as might occur during shipping. The results of accelerated testing studies are not always predictive of physical changes. These are also known as stress testing studies.

Expiry date

The date given on the individual container of a drug product up to and including which the product is expected to remain within specifications if stored correctly. It is established for each batch by adding the shelf-life period to the date of manufacture.

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Mean Kinetic Temperature

The single test temperature for a drug product corresponding to the effects on chemical reaction kinetics of a given temperature-time distribution. A mean kinetic temperature is calculated for each of the four world climatic zones according to the formula developed by a scientist known as Hayanes. It is normally higher than the arithmetic mean temperature.

Real time (Long term) stability studies

Experiments on the physical, chemical, biological, biopharmaceutical and microbiological characteristics of a drug, during and beyond the expected shelf life and storage periods of samples under the storage conditions expected in the intended market. The results are used to establish the shelf life, to confirm the projected shelf life and to recommend storage conditions.

Stability tests

A series of tests designed to obtain information on the stability of a pharmaceutical product in order to define its shelf-life and utilization period under specified packging and storage conditions. The following table gives the main objectives and uses the different types of stability testing.

Main Objectives of Stability Testing

Objective	*Type of study*	*Use*
To select adequate (from the viewpoint of stability) formulations and container-closure systems	Accelerated	Development of the product
To determine shelf-life and storage conditions	Accelerated and real-time	Development of the product and of the registration dossier
To substantiate the claimed shelf-life	Real-time	Registration dossier
To verify that no changes have been introduced in the formulation or manufacturing process that can adversely affect the stability of the product	Accelerated and real- time	Quality assurance in general, including quality control.

Test Samples

For established products the following schedule is suggested by WHO:

- One batch every other year for formulations considered to be stable, otherwise one batch per year.
- One batch every 3-5 years for formulations for which the stability profile has been established, unless a major change has been made, *e.g.*, in the formulation or the method of manufacture.

Test Conditions

Example of conditions for accelerated stability testing of products containing relatively stable active ingredients

Storage temperature (°C)	*Relative humidity (%)*	*Duration of studies (months)*
40 ± 2	Zone IV - For hot climatic zones or global market	
	75 ± 5	6
40 ± 2	Zone II - For temperate and subtropical climatic zones:	3
	75 ± 5	

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Alternative storage conditions may be observed, in particular, storage for 6 months at a temperature of at least 15°C above the expected actual storage temperature (together with the appropriate relative humidity conditions). Storage at higher temperatures may also be recommended, *e.g.*, 3 months at 45 – 50°C and 75% relative humidity (RH) for zone IV.

3.5 SHELF-LIFE AND RECOMMENDED STORAGE CONDITIONS

The results of stability studies are evaluated with the objective of establishing a tentative shelf life Statistical methods are often used for the interpretation of these results. A tentative shelf - life of 24 months may be established provided the active ingredient is known to be stable.

Products containing less stable active ingredients and formulations not suitable for experimental studies at elevated temperature (*e.g.*, suppositories) will need more extensive real time stability studies. The proposed shelf-life should then not exceed twice the period covered by the real time studies.

After the stability of the product has been evaluated, one of the following recommendations as to storage conditions can be prominently indicated on the label.

- store under normal storage contisions1;
- store between 2 and 8°C (under refrigeration, no freezing);
- store below 8°C (under refrigeration);
- store between – 5 and – 20°C(in a freezer);
- store below – 18°C(in a deep freezer).

Normal storage conditions have been defined by WHO (3) as: "storage in dry, well-ventilated premises at temperatures of 15–25°C or, depending on climatic conditions, upto 30°C. Extraneous odours, contamination, and intense light have to be excluded. Recommended storage conditions must be determined in the light of the conditions prevailing within the country of designated use.

General precautionary statements, such as "protect from light" and/or "store in a dry place", may be included, but should not be used to conceal stability problems. WHO guidelines give a list of essential drugs for which they seek reports from people regarding stability problems. The list consists of 25 drugs and we find it is headed by acetylsalicylic acid and also contains the names of ampicillin, ibuprofen, isosorbide dinitrate, propranolol, spironolactone and warfarin. The stability problems to be reported include pharmacopoeial non-compliance, organoleptic changes and microbial changes.

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Thus, the WHO guidelines help us in carrying out stability studies on established drug substances in conventional dosage forms

Shelf Life Determination Based on Arrhenius Plot

How do we determine the shelf life based on an Arrhenius Plot?

1. We keep several samples of the drug product at at least three temperatures, such as 40°C, 50°C and 60°C.
2. We determine the drug content at all three storage points by taking a number of samples and take the mean drug content. We do this for a few weeks.
3. At each temperature we plot a graph between time and log percent drug remaining. If the decomposition is first order this gives a straight line. If it is zero order, percent drug remaining versus time will give a straight line.
4. Next we take the logK or log of reaction constant on Y-axis and $1/T \times 10^{-3}$ on X-axis and draw a best fit line. This line is the Arrhenius Plot. We extrapolate this line to get K at 25°C and from this we calculate the shelf-life.

If the reaction is following zero-order

Expiration date at 25°C (t_x) = Initial potency – minimum potency/reaction rate at 25°C

$$t_x = Y_o - Y_x / K_o$$

If the reaction is following first order

Expiration date at 25°C (t_x) = Log initial potency – log minimum potency/reaction rate at 25°C

$$t_x = \log Y_o - \log Y_x / K_1$$

where

Y_o = initial potency

Y_x = final potency

K_o = zero order constant

K_1 = first order constant

Shelf Life Determination Based on Real Time Testing

Let me tell you another method which involves real time testing and statistical analysis, followed for determining shelf life.

1. Keep three batches for stability study at least for 1 year at one fixed temperature.
2. Test them at 0, 1, 3, 6, 9 and 12 months for drug content. At each testing time test a number of samples, so that you have a mean and a standard deviation value of the result.
3. Now plot the graph of % drug content on Y-axis and time on X-axis along with confidence intervals. Where the lower 95% confidence curve intersects minimum potency, there you fix the shelf life.

As an example we can see the data and figure given in Tablets, Volume 3, page 355 by Hebet A Lieberman and Leon Lachman. Vitamin Tablets Stability Confidence Intervals at 40°C.

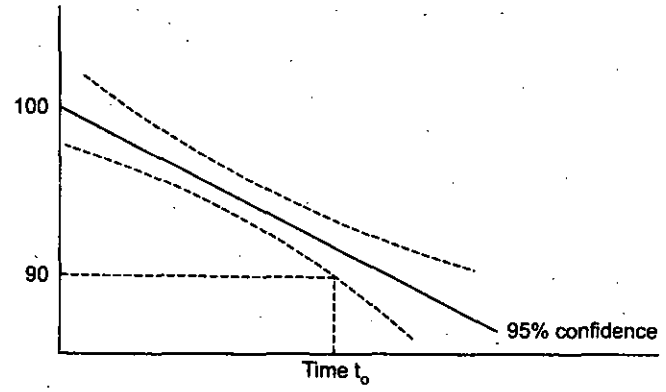

Fig. 3.1 Plot of in potency against time showing 95% confidence limit line

Table 3.3 Vitamin Tablets Stability Confidence Intervals at 40°C

Time (Months)	*Results (mg/tablet)*	*Lower limit*	*Upper limit*
0	100.0	95.2	104.9
1	91.2	88.7	93.8
3	83.1	79.3	87.3
6	75.8	69.8	82.5
9	69.1	61.2	78.2
12	63.0	53.6	74.0

where estimate of the standard error of regression(s)

$$\sqrt{\frac{\sum(y_i - \hat{y}_i)^2}{n-2}}$$

y_i = predicted value at t_1

n = sample size

Sy = standard error of the line

α = 0.1 two-sided 0.05 one-sided

This method also helps formulation scientists in fixing the amount of overages to be added to vitamin products.

3.6 EFFECTS OF VARIOUS ENVIRONMENT ON STABILITY

Introduction

You all must have noticed in daily life, that all objects suffer from what we may call a *'wear and tear'*. All living beings go through a cycle of birth-growth-reproduction and death. All nonliving beings that are generated or grown or manufactured go through a life span in which they influence and are influenced by their environment. Metals corrode and wood is decomposed by the environment. The environment is spoiled by the presence of some chemicals such as benzene, carbon dioxide and methane. So what we may call as a material- environment interaction influences the longevity and purity of all items.

Food as we all know has to be well preserved if we want it to retain its quality. If raw materials such as rice and wheat grains are not stored at the proper temperature and humidity they are spoiled by microbial contamination. Food is spoiled by

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three varieties of decomposition; physical, chemical and microbiological. Have you noticed that rice grains left alone stay fine for years together; rice made into flour is less stable (with time it develops a musty odor and off color) and rice processed into food is stable only for a few hours. My point is "more processing-less stable"; "more contact with water - less stable".

All this is true for drugs also. Pure drugs, solids, liquids, or gases are usually more stable than their formulations. When they are formulated into medicines decomposition happens faster because of the presence of excipients, and moisture and because of processing. So when we say stability, we actually are talking about two varieties of stability; that of the drug and that of the formulation. For example a capsule may become soft, an emulsion may break, a suspension may cake and an ointment may bleed.

Even if solid pure powders of drugs are stored under ideal temperature and humidity conditions -even then there is some degradation. For example consider aspirin. If you take a bottle of an old sample of pure aspirin and smell it you can clearly feel the unmistakable odor of acetic acid. If you granulate aspirin and make it into tablets the rate of decomposition will be faster and if you formulate it into a suspension it will decompose totally into acetic acid and salicylic acid in less than 25 days. This is because the breakdown is a chemical reaction involving the collisions of the molecules, collisions having sufficient energy and the molecules having the proper orientation. This breakdown is affected by various factors such as presence of oxygen, acidity, alkalinity, moisture and light. This breakdown is what we are calling degradation. This is the reason why the manufacturers of medicines are bound by rules to put an expiry date on the medicine. In fact if they cannot put an expiry date then they have to explain reasons for that.

So the researchers in charge of the formulation development do extensive studies to understand the mechanism of degradation and the rate of degradation. Degradation happens because the molecules are hitting one another (like agitated football players in a big field running madly without looking and hitting one another). But there is a method in this madness! And it is a pharmaceutical technologist's job to find the factors that enhance or reduce this madness (light? Heat? Air? Acidity? Alkalinity?). And it is a pharmaceutical chemistry man's job to find the mechanism of this madness- how is it triggered and how is it happening? Oxidation? Hydrolysis? Racemisation? Photolysis?

So then dear students, I would like to bring your attention on to three different angles of stability, physical, chemical and microbiological. It is imperative that the medicine that we are giving, is stable in its entirety from every angle throughout its shelf life period. Often for a new formulation many batches go into the market and a lot of time elapses before all angles of the stability of the product are understood.

The study of the changes that happen in a dosage form as it stands on the shelf of a drug store constitute the contents of the chapter titled as "Drug Stability". Physical stability implies that the formulation is totally unchanged throughout it shelf life and has not suffered any changes by way of appearance, organoleptic properties, hardness, brittleness, particle size etc. The drug release nature (rate and mechanism) should not be altered. Different formulations suffer from different physical challenges and are tested for different stability criteria. Drug release is important from safety and efficacy angle and the physical stability of the

formulation is significant because of its effect on pharmaceutical elegance and on drug content uniformity and drug release rate. Chemical stability implies the lack of any decomposition in the chemical moiety that is incorporated in the formulation as the drug. Chemicals present in the formulation as preservatives or as other excipients may also decompose and their decomposition may influence the physical and chemical stability of the drug. But to test for the chemical stability of a formulation we test for the drug content of the formulation. When we say microbiological stability, we imply that the formulation has not suffered from any microbiological attack and is meeting the standards with respect to lack of contamination/sterility which we have claimed when we kept the product on the shelf.

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3.7 INTERNATIONAL CONFERENCE ON HARMONISATION (ICH)

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is a unique project that brings together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industry in the three regions to discuss scientific and technical aspects of product registration.

The ICH guidelines are submitted to the Committee for Human Medicinal Products (CHMP) for endorsement once they have reached Step 2 or Step 4 of the ICH Process. The CHMP, in consultation with the European Commission decides on the duration for consultation with interested parties (up to 6 months).

The European Agency for the Evaluation of Medicinal Products publishes and distrubutes the Step 2 guidelines for comments. At step 4 the guidelines are endorsed by the CHMP and a timeframe for implementation is established (usually 6 months).

The guidelines are subsequently published by the European Commission in the Rules Governing Medicinal Products in the European Union (http://ec.europa.eu/enterprise/pharmaceuticals/eudralex/index.htm). Step 2 and Step 4 guidelines are available from the European Medicines Agency site on the Internet:

<http://www.emea.europa.eu/htms/human/ich/background.htm>

MHLW

When Step 2 or Step 4 has been reached, the ICH texts are translated into Japanese. Subsequently Pharmaceutical and Medical Safety Bureau (PMSB) Notification for the promulgation or consultation of guidelines written in Japanese is issued with a deadline for comments in the case of consultation drafts, or an implementation date for finalised guidelines. The notifications on guidelines in Japanese and also English attachments (ICH Texts) are available from PMSB or on the Internet by the Pharmaceutical and Medical Devices Agency (PMDA). http://www.pmda.go.jp/ich/ich_index.html

FDA

When Step 2 or Step 4 has been reached, FDA publishes a notice with the full text of the guidance in the Federal Register. Notices for Step 2 guidances include a date for receipt of written comment; Step 4 guidances are available for use on the date they are published in the Federal Register. FDA guidances and guidelines are available on the Internet:

CDER: http://www.fda.gov/cder/guidance/index.htm

CBER: http://www.fda.gov/cber/guidelines.htm

3.8 PHYSICAL STABILITY

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The job of the medicine is to give the therapeutic effect and so the most important thing is that the required drug content is available till the expiry date is over. But the appearance and lack of any change in a physical sense are also important as the patient only observes the exterior and any change from the original position may frighten him or bother him. Some physical changes can have deleterious effects too. An emulsion may crack into two phases and thus different doses drawn from it may have different drug contents. A suspension may have a hard cake as a sediment; as a result of which the supernatant may be bereft of all drug and a dose drawn from the bottom of the bottle may have toxic amounts of drug. A tablet may become soft and ugly or it may become very hard and show very slow dissolution time as a result of which bio-availability may not be good. *Stability is the capacity of a drug product to remain within specifications established to ensure its identity, strength, quality and purity.*

So it is absolutely essential that for all formulations all areas in which instability is likely to occur are understood and stability is tested for. With each formulation the problems are different and their effects and solutions possible are also different. Table 3.4 tries to summarise the instability possibilities in different formulations.

3.9 CHEMICAL STABILITY

Let us try to understand what is known as chemical stability. I said earlier that the rate of decomposition depends on the frequency and intensity of the collisions of the molecules. Let us take off in this class from that point.

When you cut an apple and expose it to the air very quickly it gets deep brown colour—it is getting oxidized. Milk is being turned into curd by a reaction known as fermentation. Every one knows the smell of a poorly stored sample of oil-which is called a rancid oil. What has happened to the oil? It has been hydrolyzed! Lipolytic enzymes in the presence of moisture hydrolyze oils and fats producing glycerol and free fatty acids and thus develop rancidity in fats and oils. So to store oil properly we have to exclude air, light, microorganisms and moisture. In all these reactions; hydrolysis, or oxidation or fermentation a common villain is moisture. All reactions are speeded up in the presence of moisture. Why? **Moisture gives a fertile ground for the growth of microorganisms.** Moisture takes part as a reactant in many chemical reactions and plays the role of a **solvent vector** in many reactions. It has better thermal conductivity than solids and allows better heat transfer-hence molecules have more kinetic energy and you observe more decomposition. So, we can put a **ditto** below foods and write about drugs. All that is true for foods is also true for drugs.

But I want to take you a little deeper first and make you see how a chemical reaction is happening-whether it is oxidation or hydrolysis. Let us try to remember the second law of thermodynamics-a spontaneous reaction always proceeds towards greater entropy (more disorder) and towards loss of free energy. A chemical reaction also tends to occur if it increases the disorder of the system.

Table 3.4

Formulation	*Likely physical instability problems*	*Effects*	*Stability testing*	*Steps to prevent instability*
Oral solutions	1. Loss of flavour 2. Change in taste 3. Presence of off flavours due to interaction with plastic bottle 4. Loss of dye 5. Precipitation 6. Discoloration	Change in smell or feel or taste	A "tester" should smell taste or feel the product and judge it qualitatively and quantitatively. The depth of taste may be judged for example as degree of saltiness or sourness on a scale of 1–5. The depth of flavour may be judged as type of flavour or level of flavour on a scale of 1–5. A colour standard may be used to describe the "intensity" of the discoloration. Clarity should be studied.	Use of proper excipients and suitable packing materials.
Parenteral solutions physical instability occurs due to: 1. Interaction of the contents with the container. 2. Changes in Chemical composition.	1. Discoloration due to photo chemical reaction or oxidation. Ex: thiamine hydrochloride 2. Presence of precipitate due to interaction with container or stopper. 3. Presence of "whiskers". If some small pinholes are present in the ampule due to improper sealing the solution wicks out, the liquid evaporates and the solid settles on the outside. It further helps in wicking out more solution and long lines of crystals form on the outside of the vial which are called whiskers. This may happen due to small hole (< 0.5 µm) going undetected or the crack developing during storage. 4. Clouds: A cloud will appear in the product due to: (*i*) Chemical changes	Change in appearance and in bioavailability. Change in appearance and in bioavailability.	Use of a colour standard to describe the "intensity" of the discoloration. Extent of precipitate may be counted using a Coulter Counter or the number of vials having a precipitate may be counted. Since this precipitate formation is due to a reaction with container or stopper we have to store vials for testing by placing vials in different positions such as (1) upright, (2) on the side and (3) upside down. Periodic observation Study of clarity, pH, sterility, pyrogenicity, volume (for plastic containers) and extractables (for plastic containers) Clouding which may be a prelude to precipitation may occur due to loss of viscosity and this change in viscosity may be followed with a Cup and Bob	Use of antioxidants (0.5%) Acetylcystane or 0.02 – 1% Ascorbic acid) or Chelating agents (0.01 – 0.075 sodium edetale) to prevent discoloration. Change in stopper or material of the container will eliminate the problem. Checking of the manufacturing process Increasing solubility by the use of cosolvents (*e.g.* polyethylene glycol) or by other methods such as micellar approach or complexation will reduce clouding.

	(an ester *e.g.*, polysorbate may hydrolyse producing an acid which is poorly soluble). (*ii*) Solubility product may be exceeded. (*iii*) The original preparation of a supersaturated solution or the use of a metastable form (ex: calcium gluceptate).		Viscometer. Drastic changes in viscosity may happen due to bacterial contamination.	
Suspensions	Settling, creaking, crystal growth	Loss of drug content uniformity in different doses from the bottle and loss of elegance.	1. Subjective tests involving shaking the bottle. 2. Rotating the bottle under reproducible conditions and analyzing the supernatant for drug content. 3. Freeze-thaw testing. 4. X-ray diffraction study 5. Study of sedimentation rate and sedimentation volume 6. Chemical testing for the amount of the preservative 7. Study of pH.	Design of product based on proper pre-formulation studies.
Emulsions	Creaming or cracking	Loss of drug content uniformity in different doses from the bottle and loss of elegance.	1. Study of globule size 2. Study of rheological behaviour with a Cup and Bob Viscometer. 3. Study of pH.	Design of product based on proper pre-formulation studies.
Semisolids (Ointments and suppositories)	1. Changes in: (*a*) Particle size, (*b*) Polymorphic state, or hydration or solvation state (*c*) Consistency (*d*) Drug release rate	Loss of drug content uniformity, loss of elegance and change in drug release rate.	1. Consistency by penetrometer 2. Feel to the touch 3. X-ray diffraction studies. 4. In-vitro drug release study with a diffusion cell.	Design of product based on proper pre-formulation studies.

	2. Caking or coalescence. 3. Bleeding			
Tablets	1. Change in 2. (*a*) Disintegration time (*b*) Dissolution profile (*c*) Hardness (*d*) Appearance	Change in drug release	Testing for hardness, disintegration time, dissolution, appearance, friability, moisture content.	Design of product based on proper pre-formulation studies.
Capsules	Change in (*a*) Appearance (*b*) Dissolution, and (*c*) Strength	Change in drug release	Testing for strength, moisture content, appearance, brittleness and dissolution.	Design of product based on proper pre-formulation studies.

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Unimolecular Reaction

Remember that we are talking about several types of reactions, such as, (1) the splitting up of a molecule into two units, (2) the transformation of an isomer from one form to another, (3) the reaction between two different molecules, and so on. For all these reactions some basic steps are necessary. I will explain a unimolecular reaction first, with the example of the transformation of a *Cis*- dichloroethylene to trans-dichloroethylene.

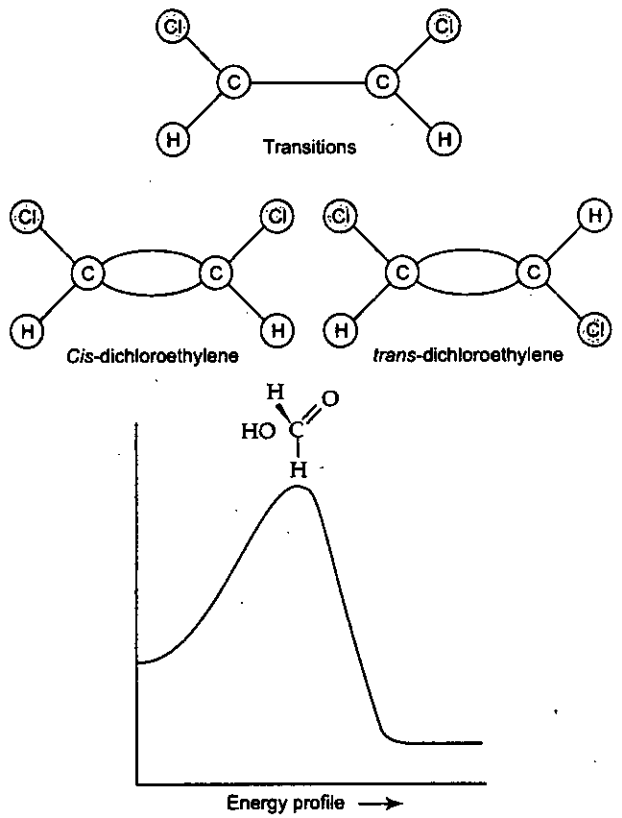

Fig. 3.2 Energy profile for the conversion of *Cis* - to *trans* - dichloroethylene

The energy of a molecule going through this conversion process is represented in Figure 3.2. A molecule of the *cis* compound which is stable and is having low energy, picks up energy as it changes to the transition state configuration and then releases energy as it rolls downhill to the transform. The abscissa of the graph, called the reaction coordinate is a measure of the progress of the reaction. For this reaction to take place, a molecule of *cis* - dichlorothylene must gain an amount of energy shown in Figure 3.2 as E_a, which is called the activation energy for the process. However, all the molecules which have sufficient activation energy do not transform into the product; some of them shed their excess energy and return to the starting state rather than proceeding to form the reaction product. We can write a rate equation as:

$$\text{Rate} = \begin{pmatrix}\text{rate for}\\ \text{energized}\\ \text{molecules}\end{pmatrix} \times \begin{pmatrix}\text{fraction of}\\ \text{molecules}\\ \text{energized}\end{pmatrix} \times \begin{pmatrix}\text{total}\\ \text{number of}\\ \text{molecules}\end{pmatrix}$$

In concentration units

$$\text{Rate} = \begin{pmatrix}\text{rate for}\\ \text{energized}\\ \text{molecules}\end{pmatrix} \times \begin{pmatrix}\text{fraction of}\\ \text{molecules}\\ \text{energized}\end{pmatrix} \times \begin{pmatrix}\text{concentration}\\ \text{molecules}\end{pmatrix}$$

It was established by experimentation that

$$\begin{pmatrix}\text{Fration of molecules having energy}\\ \text{equal to the activation energy}\end{pmatrix} = e^{-(E_a/RT)}$$

where R is the gas constant and T is the absolute temperature

So $$\text{rate} = \begin{pmatrix}\text{Rate for energized}\\ \text{molecules}\end{pmatrix} \times e^{-(E_a/RT)} \times A$$

where [A] = concentration of reactant A

When experiments were done on several chemical reactions, the exponential dependence of rates on temperature was established. The rate for energized molecules is a temperature independent factor and varies over wide limits (10^9 – 10^{16} sec^{-1}) but many results cluster around the value of 10^{13} sec^{-1}. So for a unimolecular reaction

$$\text{Rate} = K\,[A]$$

$$K = \rho\, e^{-(E_a/RT)} \qquad \ldots(1)$$

where ρ = frequency factor

= rate for energized molecules

$$2.303 \log K = 2.303 \log \rho - E_a/RT$$

Bimolecular Reaction

In a bimolecular reaction two molecules react with each other to form products. Molecules must collide to react. These collision rates are much faster and only a fraction of the molecules colliding result in reaction. The rates of these reactions also increase with temperature just as unimolecular reactions do and they also have to reach or attain an activation energy to result in a reaction.

Taking an example of the reaction of a hydroxide ion with methyl iodide,

$$HO^- + CH_3\,I \rightarrow CH_3OH + I^-$$

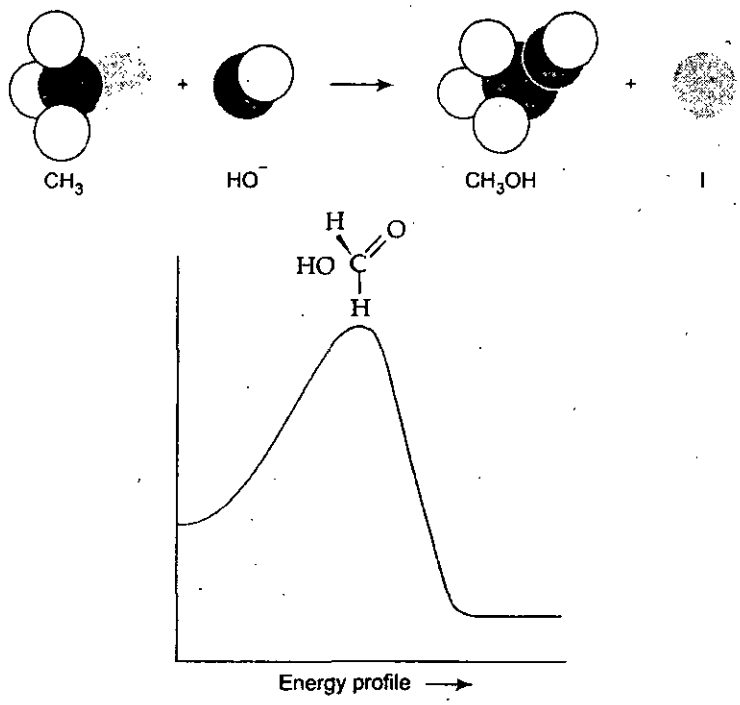

Fig. 3.3 Energy profile for the reaction of hydroxide ion with methyl iodide.

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Just as we have written for the unimolecular reaction let us write a rate expression in terms of molecular collisions for the bimolecular reaction also

$$A + B \rightarrow \text{products}$$

the rate is

$$\text{rate} = \begin{pmatrix} \text{Rate of} \\ \text{collision} \\ \text{between A} \\ \text{and B} \end{pmatrix} \times \begin{pmatrix} \text{fraction of} \\ \text{collisions} \\ \text{with enough} \\ \text{energy} \end{pmatrix} \times \begin{pmatrix} \text{fraction of energetic} \\ \text{collisions resulting} \\ \text{in reaction} \end{pmatrix}$$

The last factor in the rate expression is called the "orientation" factor. It tells us that all the energetic collisions do not result in a reaction. This is because they are not properly oriented to cause a reaction. This term corresponds to the ρ factor in the unimolecular rate expression [eq. (1)]. In this example, collision of a hydroxide ion with the iodine atom of methyl iodide would not result in reaction. Only collisions involving direct contact of the oxygen atom with the carbon atom would be effective in causing reaction to occur. The collision rate is a number that depends on the concentrations of the molecules colliding.

$$\text{rate} = \begin{pmatrix} \text{Collision rate} \\ \text{when [A]=} \\ \text{[B]=IM} \\ . \end{pmatrix} \times \begin{pmatrix} \text{fraction of} \\ \text{collisions} \\ \text{with enough} \\ \text{energy} \end{pmatrix} \times \begin{pmatrix} \text{fraction of} \\ \text{energetic} \\ \text{collisions resulting} \\ \text{in reaction} \end{pmatrix} \times [A] \times [B]$$

or $\text{rate} = \rho e^{-(E_a/RT)} [A] [B]$

The bimolecular rate constant for the reaction

$$A + B \xrightarrow{K} \text{Products}$$

In this model is $K = \pi e^{-(E_a/RT)}$

You can see that the expression for K is the same as that for a unimolecular reaction. But the difference lies in the interpretation and units of ρ. For bimolecular rate processes, ρ has units of litre mole^{-1} sec^{-1}, whereas for unimolecular reactions, ρ is given in sec^{-1}. On the basis of their experience scientists give us these guidelines:

1. A typical reaction will proceed at an appreciable rate at room temperature if E_a is 10 kcal mole^{-1} or less.
2. For such a reaction, the fraction of high energy molecules increases with temperature in such a way that a 10° rise in temperature will roughly double the reaction rate.

Now, I want to compare a unimolecular reaction to a person trying to hit a goal in a game of basketball. The man gathers strength, raises and throws the ball. His attempts are many, every time he gathers sufficient energy but the hits are few and the 'hit' comes only when every move is properly coordinated, *i.e.*, the right orientation is there.

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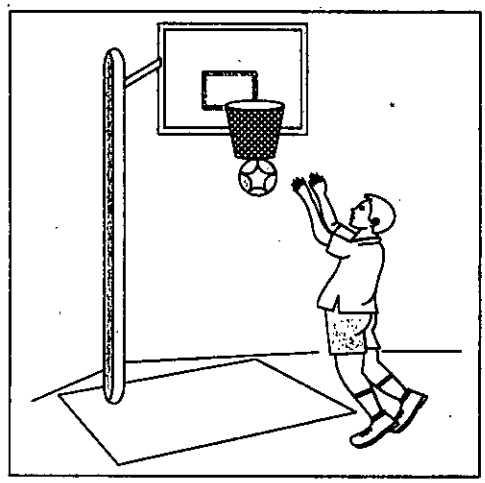

Fig. 3.4

To understand a bimolecular reaction visualize two young girls playing a skipping game with a long rope. One girl swings the rope, starting from below her feet, coming from behind – above her head and in its forward sweep it includes the second girl and comes from beneath her feet by then one swing is over. The second girl jumps into the air as the rope is going below her feet. The girls try a number of times, but perfect smooth circle of the rope is completed only some times, *i.e.*, when both the girls are perfectly oriented to each other and are in perfect coordination.

Fig. 3.5

Degradation in solid dosage forms and solid drugs is usually not much because of one strong reason *i.e.*, the usual range for activation energies for tablet formulation decomposition is about 10 to 20 kcal/mole^{-1} except if diffusion or photolysis is rate determining. Then the rate is about 2 to 3 kcal/mole^{-1} which rarely occurs in tablet degradation. For reactions in which the heat activation energies range is more than 50 k cal/mole^{-1}, the rate of degradation is not of any practical significance at the temperature of shelf life storage of solid formulations.

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I explained all these points before going into degradation mechanisms because I want you to have an idea of how actually a chemical reaction is taking place. So a formulation suffers chemical instability when the drug degrades and the drug content as claimed on the label is not available in it. Other chemicals which are used in the formulation as excipients may also decompose and though their content is not theraupeutically necessary the decomposition is of high pharmaceutical significance. It may alter the appearance as well as the release rate of the drug. Decomposition of the drugs is happening when the drug molecules are energized and are taking a particular orientation, but after taking the orientation or positioning, what exactly is happening? Are the molecules splitting into two? Are they loosing some electrons? Or they gaining some electrons? Are they racemizing? This later step is what we are calling as the pathway or mechanism of degradation. Some well known mechanisms are hydrolysis, oxidation-reduction, racemization, decarboxylation, ring cleavage and photolysis. But the most frequently seen mechanisms are hydrolysis and oxidation reduction. Let us try to understand how these reactions are happening a little later.

3.10 MICROBIOLOGICAL STABILITY

Contamination from microorganisms is a big problem for all formulations containing moisture but it can be a bother in solid dosage forms also if some natural polymers are used because many natural polymers are fertile sources of microorganisms. In the type of hygienic manufacture carried out today where "Quality Assurance" is a prerequisite as per the cGMP procedures, there are definite procedures to prevent microbial contamination in all formulations. But way back in the sixties microbial contamination of pharmaceutical formulations was a big problem. One case of outbreak of Salmonellosis in Sweden attributed to contaminated tablets is discussed in "Pharmaceutics. The Science of Dosage Form Design" by Michael E. Aulton. In this case the infection was traced to the original defatted thyroid powder imported from Hungary which was used to make the tablets. The Pharmaceutical Society of Great Britain set up a working party in 1968 to investigate microbial contamination of pharmaceutical preparations in manufacturing establishments and in hospital and retail pharmacies. This investigation shed light on a number of issues including microbial content of some drugs and medicines and suggested many measures to reduce contamination.

Table 3.5 Sources of Microbial Contamination

Water	Low demand gram-negative groups: Pseudomonas, Xanthamonas, Flavobacterium, Achromobacter
Air	Mould spores: Penicillium, Mucor, Aspergillus Bacterial spores: Bacillus spp. Yeasts
Raw Materials	Micrococci
Earths	Anaerobic spore formers: Clostridium spp
Pigments	Salmonella
Gums	Actinomyces
Animal products	Salmonella, Coliforms
Personnel	Coliforms, Staphylococci, Sterptococci, Coryembacteria

Table 3.5 taken from "Pharmaceutics, The Science of Dosage Form Design" by Michael E. Aulton gives the types of organisms present in different sources.

Extremely hygienic manufacture ensures a product that is free of contamination in the case of all non-sterile preparations and a sterile preparation in the case of all parenterals. There are two strategies followed in the manufacture of microbiologically stable, acceptable pharmaceutical preparations. The first step is to prevent contamination of the product. The second is to formulate the final product so that it is hostile to microorganisms and it is usually done by the addition of preservatives.

For sterile preparations there is either a terminal sterilization process or a closely controlled aseptic manufacturing procedure. In every case the final product is so made to protect the product during storage and minimize contamination while the product is in use. When discussing microbiological stability we have to discuss parenterals as one class and the rest *f* the formulations as one class. Parenterals are either terminally sterilized or manufactured by an aseptic manufacturing procedure. To prevent contamination to the formulation during storage and use many steps are taken such as

1. suitably designing the containers,
2. usually using single dose containers,
3. sticking to proper storage conditions and
4. adding an antimicrobial substance as preservative.

Preservatives used in pharmaceutical preparations

The following Table 3.6 given in "Pharmaceutics The Science of Dosage Form Design" by Michael E. Aulton gives a list of usual preservatives used in pharmaceutical preparations.

Table 3.6

Preparation	*Preservative*	*Concentration % w.v*	*Special factors*
Injections	Phenol Cresol Chlorocresol Phenylmercuric nitrate Benzyl alcohol	0.5 0.3 0.1 0.001 1.0	Not for intraocular, intracardiac or intacisternal or over 15 mil single dose. Closures pretreated.
Eye drops	Phenylmercuric nitrate or Acetate Chlorohexidine Acetate Benzalkonium chloride	0.002 0.01 0.01	Dropper teat pretreated Silicone rubber teats
Mixtures	Chloroform Benzoic acid Methyl paraben Alcohol Sulphur dioxide	0.25 0.1 0.1 12–20 400 parts/10^6	Adsorption. Volatile pH (pKa, 4.2) Adsorption Volatile Volatile
Creams	Parabens Chlorocresol Dichlorobenzyl alcohol Cetyltrimethyl ammonium bromide Phenylmercuric nitrate	0.1–0.2 0.1 0.05–0.2 0.01–0.1 0.001	*$K°_W$ R high $K°_W$ R high $K°_W$ R high

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Tablets	Methylparaben	0.1	
K°_{W} values	**Mineral oil**	**Vegetable oil**	
Chlorocresol	1.5	117	
Methylparaben	0.02	7.5	
Propylparaben	0.5	80.0	

R = ratio of total to free preservative in non-ionic surfactant – water system.

Parenterals and ophthalmic preparations have to be totally free from microorganisms *i.e.*, they have to be sterile. This requirement is met by (1) placing a suitable preservative or combination of preservatives wherever required in the products, (2) storing the products properly, (3) stoppering them properly and by following proper aseptic procedures during administration and during any admixture procedures followed prior to administration. In spite of all these precautions if any microbial growth takes place and is observed the product is condemned and the entire batch from which the product has come is recalled. The storage of these products is done under conditions recommended by WHO which prescribe temperature, humidity, cleanliness as well as colour of the walls of the room.

Non-sterile preparations have less stringent requirements regarding exclusion of microbes. They need not be sterile but it has to be shown that some specifically named organisms are not present in them. Table 3.7 taken from Michael E. Aulton gives the microbial standards for pharmaceutical preparations.

Table 3.7 Microbial standards for pharmaceutical preparations

Requirement	*Authority*
Exclusion	
1. Complete exclusion - sterility Injections, Ophthalmic preparations	BP, EP,
2. Exclusion of named organisms	USP
Raw materials:	
e.g., : Aluminium Hydroxide:	BP
Ps. Aeruginosa/1 g, E. coli/1 g	
Cochineal. Gelatin - E. coli/1 g	
Pancreatin, Thyroid	
– Salmonella/10 g	BP
Maize starch	BP
Tragacanth E. coli/1 g	BP
Oral dosage forms:	
Free from E. coli	USP
Free from E. coli and Salmonella/1 ml.	EP
Topical preparations:	
Free from Ps aeruginosa and S. Aureus	USP
Fee from Enterobacteria, S.aureus and Ps. Aeruginosa	EP
Limit upon number of viable organisms	
Oral dosage froms:	USP
Limit upon total aerobic count of non-specified viable organisms, *e.g.*, Milk of Magnesia 100 cells/ml	
Raw materials:	

• 10^4 aerobic bacteria/ml	EP
• 10^2 yeasts or moulds/ml	
• 10^2 enterobacteria/ml topical preparations	
• 100 organisms/g or ml	EP

BP, British Pharmacopeia 1980; BP Addendum 1986; USP, United States Pharmacopeia XXI; EP, European Pharmacopeia (draft proposals).

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Vulnerability to Microbial Attacks

So dear students, we now understand that different formulations have differing levels of stringency regarding microbial presence. You may be thinking "Drug products are made under such extremely hygienic conditions and are stored under very good conditions. There is no chance for any contamination to enter into them". But however clever we are microorganisms seem to be cleverer still! The risk of contamination is more in non sterile than in sterile products and more in multiple use formulations than in unit dose systems. Most of the raw materials used in the manufacture of medicines support the growth of microbes and so medicines including dry powders and tablets and capsules are susceptible to microbial spoilage. Microbes can survive in a vast range of habitats including volcanic regions and icy regions!. They can use glass and concrete as nutritional sources! So a majority of medicines are a good source of nutrition to the microbes and if the microbe gets access to the medicine it can definitely survive and proliferate. This growth sometimes gives off offensive odours and colours. Thus, we can spot the breakdown of the system due to microbes. Sometimes the microbes grow without any visible sign and this can be really troublesome because then we will administer the medicine without realizing its harmful potential.

Understanding the vulnerability of drug systems to microbes is important. When scientists studied the interaction of microorganisms within foods such as milk and meat products they discovered some curious things. One variety of microbes first attack the medicines and degrade complex nutrients and alter pH levels. Then another variety attacks and thrives on the simple nutrients. For example, syrups may be first contaminated by osmophilic yeasts which can thrive at high sugar concentrations. They then utilize the sugars and create conditions suitable for other microbes. When such syrups are examined there may not be any evidence of the yeasts which started the entire spoilage process.

Effects of Microbial instability

Contamination of a product may sometimes cause a lot of damage and sometimes may not be anything at all. For example, spores of the mould Mucor may be present in a dormant form and never produce spoilage and will not harm the patient who takes the medicine. But if Salmonella enters a medicine, it may not cause any visible damage but would cause a serious health hazard to the patient who consumes it.

If contamination happens in parenterals or in ophthalmic preparations it can be very serious in its effects. But contamination in other nonsterile products is usually not so damaging. It results in general spoilage such as discoloration, breakdown of emulsions and the production of gas and other odours. This is good in one way, if prevents the patient form consuming the medicine. In some cases active

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drugs may be destroyed without any outward signs. Thus, salicylates, phenacetin, paracetamol, atropine, chloramphenicol and hydrocortisone can be degraded to a variety of therapeutically inactive products. Preservatives, especially those that are aromatic in structure can themselves act as a ready source of nutrition to microbes.

Suppose some microorganisms have entered the raw materials used in some parenteral preparation and then are killed in the process of sterilization - still there is some harm. Some toxic substances produced by the bacteria may cause harm. Pyrogens which are the metabolic products of bacterial growth are usually lipo-polysaccharides and they represent a particularly hazardous product released by gramnegative bacteria. If administered inadvertently to a patient they may cause chills and fever.

What to do to prevent microbial spoilage

The proper preservative has to be used. By 'proper' I mean that the preservative must have the required oil/water partition coefficient, it must be non-toxic, odourless, stable and compatible with other formulation components while exerting its effects.

Containers

Traditional glass containers do not interact with the preservatives. If the closure is airtight there is no problem of contamination. But plastic containers pose problems such as permeation through the container or interaction with it. Rubber also reacts with preservatives but it is still used for teats and closures. These teats and closures are treated with the preservatives they are to be in contact with, in order to minimize subsequent uptake during storage. For sterile preparations there is either a terminal sterilization process or a closely controlled aseptic manufacturing procedure. In every case the final product is so made to protect the product during storage and minimize contamination while the product is in use.

3.11 MECHANISMS OF DEGRADATION: HYDROLYSIS, OXIDATION AND PHOTOLYSIS

Let us now see why and how the drugs placed in the products are decomposing. Are they splitting into two? Are they changing into other inactive or toxic forms? Why is this happening and can we prevent it? Degradation happens by a few pathways but the most often seen mechanism is Hydrolysis. Oxidation and photolysis are the next two mechanisms most often seen.

Hydrolysis

The word "hydrolysis" literally means "splitting by water". Before the mechanism of hydrolysis was properly understood scientists thought when some compounds are added to water, the water splits them. For example, a solution of sodium acetate produces acetic acid and hydroxide ions. At one time, the equation for this process would have been written (assuming that there is a chemical reaction between water and sodium acetate).

$$AcONa + H_2O \Leftrightarrow AcOH + NaOH \quad ...(1)$$

But today we know that what is involved is not a reaction with water, in the presence of water sodium acetate is dissociating into acetate ion and sodium ion and the resultant acetate ion is reacting with water to form acetic acid, but this reaction is not fast at all; it happens only to a small extent. So this process is better represented as

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$$AcONa \Leftrightarrow AcO + Na^+ \quad ...(2)$$

$$AcO^- + H_2O \Leftrightarrow AcOH + OH^- \quad ...(3)$$

Equation (2) is essentially complete and the acid base reaction of equation (3) happens only to a small extent. Equation (3) represents "hydrolysis" and we say that the acetate ion is slightly "hydrolyzed" in water to form acetic acid and hydroxide ion. Similarly chemists say that ammonium ion (NH_4^+) is hydrolyzed by water to form ammonia and hydronium ion

$$H_4N^+ Cl^- \rightarrow H_4N^+ + Cl^-$$

$$H_4N^+ + H_2O \Leftrightarrow NH_3 + H_3O^+$$

The term "hydrolyze" is used to include almost any reaction with water. So we must see that though water may be involved in some reactions as the reacting species, in many cases it plays a passive supporting role, that is, it is something like a facilitator. It many times acts as a solvent vector between two reacting species in solution. In solid dosage forms the reactions will be taking place in saturated solutions. Now I want you to observe a few facts regarding hydrolysis:

1. Hydrolysis reactions involve, nucleophilic attack of labile bonds by water on the drug in solution.
2. The reactions involving lactam groups are fastest and are followed by those involving esters, amides and imides in that order.
3. These reactions usually follow first order.
4. If this type of reaction happens due to any other solvent it is called solvolysis.
5. These reactions are catalyzed by H_3O^+ (*c*) presence of divalent metal ions (*d*) ionic hydrolysis (Protolysis) (*e*) heat (*f*) light (*g*) solution and (*h*) high drug concentrations.

The molecules having ester or amide functional groups are most susceptible to hydrolysis. Anesthetics, antibiotics, vitamins and barbiturates are examples for drugs that decompose due to hydrolysis. The ester and amide groups have so many similarities that they are called "bioisosteres". Bioisosteres are structures that have similar sizes, shapes and electronic structures.

Let me tell you a few things about local anesthetics so that you will know that we are talking about real drugs. Most of the local anesthetic drugs are either benzoic acid or aniline derivatives. The benzoic acid derivatives are esters developed from cocaine, whereas the aniline derivatives are amides developed from isogramine. These drugs have chemical structures that usually have the following formula:

Lipophilic center → Ester or amide group → Bridge → Hydrophilic center

The lipophilic center helps in the penetration of the cell membrane and the hydrophilic center helps in transportation and in binding to the receptor. When the two centers are in good balance the best local anesthetic action is obtained. So esters or amides are really providing links between two other moieties and as the saying goes "any chain is only as strong as its weakest link". So it is at the point of an ester or an amide that decomposition or splitting usually happens.

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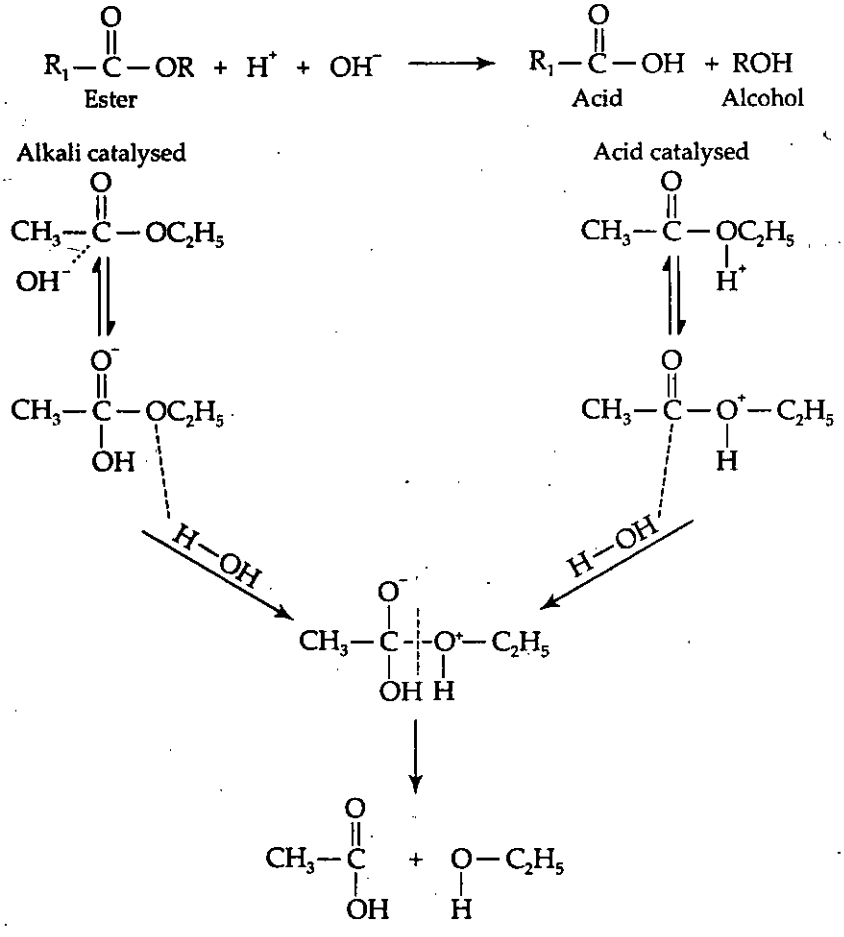
Ester hydrolysis

Esters are compounds having the structure R – C – O – R1 , where R and R1 are carbon groups. An ester can be thought of as being derived by reaction of an alcohol with a carboxylic acid, with the elimination of a molecule of water. The hydrolysis of an ester into a mixture of an acid and alcohol involves the rupture of a covalent bond between a carbon atom and oxygen atom. These reactions usually happen in the presence of water but happen much faster when either an acid or an alkali is present. Acids, alkalies and certain enzymes, which are capable of supplying the hydrogen or hydroxyl ions to the reaction mixture catalyse this hydrolysis. The alkaline hydrolysis of an ester is irreversible and an acid hydrolysis is reversible.

Let us take a brief look at the scheme of ester hydrolysis given by Walters and explained very clearly by Leon Lachman and Patrick De Luca in "Theory and practice of Industrial Pharmacy".

The ester usually is cleaved at the acyl-oxygen linkages, that is between the carbonyl carbon and the oxygen of C_2H_5 ($O^- C_2H_5$). The scheme of ester hydrolysis is this



So the general form of the kinetic equation to express acid or base-catalyzed hydrolysis are as follows. So the general form of the kinetic equation to express acid or base-catalyzed hydrolysis are as follows

$$d(ester)/dt = K\,(ester)\,(H^+)$$

$$d(ester)/dt = K\,(ester)\,(OH^-)$$

So, since the rate seems to depend on the concentrations of two ingredients, it looks as through they are second order equations. But in reality we keep the acid or the alkali in huge excess, so that the small change in the concentration is negligible. Hence the concentration of (H^+) or (OH) is constant throughout the reaction. So we treat it as a pseudo-first order equation and the above two equations reduce to the form.

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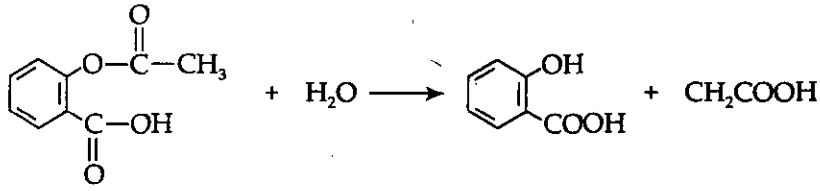
$$d(ester)/dt = - K (ester)$$

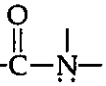
This is an expression for a first order equation. Many drugs have been studied with respect to their decomposition by hydrolysis, but I will take the example of aspirin. Aspirin was most widely studied and a thorough study was performed by Edwards and is discussed by Lachman. The degradation of aspirin in various buffer solutions was studied and the reaction rate was treated as pseudo first - order.



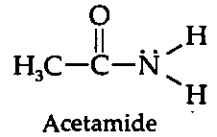
Aspirin Salicylic acid Acetic acid

Aspirin hydrolysis takes place even when the drug is in the solid powder form and if we take an old sample of aspirin and open the lid the unmistakable smell of acetic acid is sure to hit us. The presence of free salicylic acid is recognized and a test for fee salicylic acid is a required test on all samples of aspirin powder. Another advantage in studying the reaction rate of aspirin hydrolysis is that we can follow the concentration of salicylic acid, *i.e.* the product of the reaction and from that calculate the remaining amount of aspirin. We must remember to calculate in molar quantities

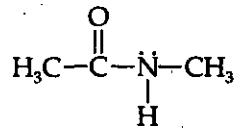
Amide hydrolysis

Amides contain the  group. We can say that they are derived from carboxylic acids and ammonia or amines.

Two examples are:

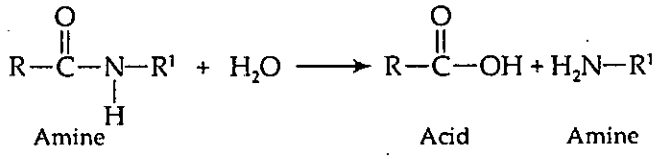


Acetamide



N-methl acetamide

Nylon and a number of other synthetic fibers and films are amides. Proteins are also amides. In all these substances, the constituent units are very large molecules, in which amide groups are the principal building blocks. Hydrolytic cleavage of an amide results in the formation of an acid and an amine.



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Amides are more stable than the esters. Drugs such as niacinamide, phenethicillin, barbiturates, and chloramphenicol have been reported to degrade by amide hydrolysis. Kosky studied the stability of salicylamide and some N-substituted derivatives, and postulated both basic and acid hydrolysis for degradation. Kosky found that in the acid medium, salicylanilide was more stable than salicylamide, which in turn was more stable than benzamide. Aminoalkyl substituents on the nitrogen increased the stability of benzamide. Salicylamide was more stable in basic than in acidic medium, probably due to the protection given by the negative charges on the phenolate ion. The N-alkyl and N-amino alkyl salicylamides were highly resistant to acid and base hydrolysis. This was probably because of the combined steric hindrance by the hydroxyl group in the orthoposition and the alkyl and aminoalkyl group on the nitrogen.

Drugs having ester groups and amide groups in their molecular structure degrade via hydrolysis in the presence of water. Common ester labile bonds are formed between an alcohol and a carboxylic acid. The ester bond is hydrolyzed by hydrogen and hydroxyl ions as shown in the following reactions:

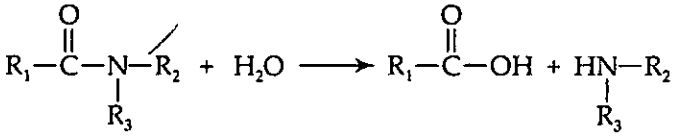
The acyl oxygen in the ester group is protonated and the carboxyl group is further polarized. Nucleophilic attack at the acyl carbon is increased by water. A base, which is a powerful nucleophile, attacks on the acyl carbon and the carbon oxygen bond is broken.

The rate of degradation of an ester labile group is dependent on the characteristics of R_1 and R_2. For a given R_1, the rate of degradation decreases with the higher ackyl group of R_2OH because the higher the alkyl, the fewer electrons are withdrawn whereas for a given R_2 the degrdation rate increases with the increase in electron - withdrawing group (*e.g.*, Cl, NO_2) of R, COOH. The rate of degradation by hydrolysis increases by replacing methyl to ethyl and propyl. The higher alkyl groups possessing the greater electron-donating characteristics increase the electron density at the acyl carbon and thus the attack of OH^- is inhibited. On the contrary electron attracting groups such as chlorine and NO_2 increase the rate of degradation

Substituents can have a dramatic effect on reaction rates. For example, the test - butyl ester of acetic acid is about 120 times more stable than the methyl ester which in turn is approximately 60 times more stable than the vinyl analog.

Amides

An amide is a compound of the type $R-\overset{O}{\overset{\|}{C}}-NH_2$ and is formed by reaction between a carbonylic acid and an amine and is less susceptible than ester groups to hydrolysis. This is because of the lesser electrophilicity of the carbon-nitrogen bond. The amide group is hydrolyzed



The rate of degradation of the amide group by hydrolysis is dependent on the characteristics of the substituents R_1, R_2 and R_3.

Lactam

Antibiotics possessing the β-lactam structure, which is a cyclic amide are hydrolyzed rapidly by ring opening of the β-lactam group. The ring opening of the β-lactam is much faster than that of other amide groups because a four membered ring is joined to a five or six membered ring and a weaker bond exists between carbon and nitrogen of β-lactam.

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The following Table 3.8 taken from page 182 of modern Pharmaceutics (Third Edition) by Gilbert S. Banker and Christopher T. Rhodes gives all the types of drug compounds that are susceptible to hydrolysis and also gives good examples.

Table 3.8. Some Functional Groups Subject to Hydrolysis

Drug type	*Examples*
Esters	Aspirin, alkaloids, Dexmethasne sodium phosphate, Estrone sulfate, Nitroglycerin
Lactones	Pilocarpine, Spironolactone
Amides	Thiacinamide, Chloramphenicol
Lactams	Penicillins, Cephalosporins
Oximes	Steroid oximes
Imides	Glutethimide, Ethosuximide
Malonic ureas	Barbiturates
Nitrogen mustards	Melphalan

Oxidation

Oxidation is the mot important path way of drug decomposition. Oxygen is present everywhere in the atmosphere and exposure to oxygen will decompose drug substances that are not in their most oxidized state through auto oxidation. Oxygen is a diradical and most auto oxidations are free radical reactions. A free radical is a molecule or atom with one or more unpaired electrons. Oxidation/ reduction reactions involve the transfer of electrons or the transfer of oxygen or hydrogen from a substance. Oxidation of inorganic and organic compounds is explained by a loss of electrons and the loss of a molecule of hydrogen, respectively as, Inorganic compounds:

$$(SO_3)^{-2} + 2\ OH \Leftrightarrow (SO_4)^{-2} + H_2O + 2\ e^-$$

Organic Compounds: Loss of hydrogen

When an oxidation reaction involves molecular oxygen, the reaction occurs spontaneously under mild conditions. It is known as auto oxidation. In an auto oxidation process, free radicals formed by thermal or photolytic cleavage of chemical bonds (*e.g.*, peroxide (ROOH) or redox processes with metal ions presenting raw material impurities are involved.

$$Fe^{++} + ROOH \rightarrow Fe^{+++} + OH^- + RO.$$

The free radical formed RO reacts with oxygen to produces a peroxide radical, and the reaction propagates as:

$$RO. + O_2 \rightarrow ROOO.$$

$$ROOO. + RH \rightarrow ROOH + RO.$$

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The free radical reaction continues until all the free radicals are consumed or destroyed. As little as 0.0002 M copper ion will increase the rate of Vitamin C oxidation by a factor of 105. Hydroperoxides contained in polyethylene glycol suppository bases have been implicated in the oxidation of codeine to codeine-N-oxide. Many oxidation reactions are catalyzed by acids and bases. The following table taken Banker gives a list of functional groups susceptible to Auto oxidation.

Table 3.9 Some Functional Groups Subject to Autoxidation

Functional group	*Examples*
Phenols	Phenolsin steroids
Catechols	Catecholamines (dopamine, isoproterenol)
Ethers	Diethylether
Thiols	Dimercaprol (BAL)
Thioethers	Phenothiazines (Chloropromazine)
Carboxylic acids	*Fatty acids*
Nitrites	Amyl nitrite
Aldehydes	Paraldehyde

The products of oxidation are usually electronically more conjugated; thus the appearance of, or a change in, color in a dosage form is suggestive of the occurrence of oxidative degradation.

Photolysis

We have seen in the introductory note on chemical stability that when molecules absorb energy and are energized or activated they go to a high energy state and then release that energy in a chemical reaction and come back to their original position. When this energy of activation is supplied by light and is absorbed by the compound the decomposition reaction is called photolytic. The activated species then returns to ground state by either (*a*) emitting light of a different frequency (this is fluorescene or phosphorescene) or (*b*) decomposition (Photolysis). An excellent example for photolysis is the photolysis is the photo degradation of sodium nitroprusside in aqueous solution. Sodium nitroprusside is administered by intravenous infusion for the management of acute hypertension. If the solution is protected from light, it is stable for at least 1 year; if exposed to normal room light, it has a shelf life of only 4 hours.

The energy associated with the radiation increases as its wavelength decreases, so that the energy of U.V. is greater than that of visible which is greater than that of J.R.Look at the following table.3.10 which I have reproduced from Aulton's book on Pharmaceutics. Relationship between wavelength and associated energy of various forms of light.

Table 3.10

Type of radiation	*Wavelength*	*Energy*
U.V.	50 – 400	kcal mol^{-1}
Visible	400 – 750	287 – 72
		72 – 36
i.r.	750 – 10,000	36 – 1

The higher energy range of visible light and U.V. light cause photolysis. Conventional tungsten filament light bulbs are safe and do not contribute to photolysis. Oxidation to a great extent and hydrolysis to some extent are catalysed by light. Photo-induced reactions are common in steroids; an example is the formation of 2-benzoyl cholestan – 3 – one following is radiation of

Cholest – 2 en – 3 – ol benzoate

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Photolysis is prevented by suitable packing in amber coloured bottles, cardboard outers and in aluminium foil over wraps and blisters. Jen, T. Cartensen in his book on Drug Stability, Principles and Practices gives extensive examples of photolysis. In pharmaceutical systems most reported photolysis reactions have been first order. A good example is the photolysis of cefatoxime. The wavelength of the irradiating light plays an important part in photolysis.

3.12 RATE KINETICS

The rate of decomposition then, depends on the frequency and intensity of the collisions of the molecules. This in turn is dependent on the nature and concentration of the chemicals involved or more specifically on their activation energy, collision frequency and what is called as a orientation factor. They can be predicted because they are happening due to molecular collisions which in turn are dependant on temperature, pH and light. The science that deals with these rates is called "kinetics" and as it describes decomposition it is called the kinetics of decomposition.

When we talk about kinetics, several 'orders' of kinetics can be there, such as zero order, first order, second order and so on, but fortunately for all of us drug decomposition rates do not follow complex orders; they are mostly of zero order or first order.

Rate kinetics is discussed in four different contexts;1. drug stability 2. drug release from dosage forms in dissolution studies 3. absorption, distribution, and elimination studies on the drugs in the body and 4. explaining the drug action at the molecular level when the response is a rate process. In all the cases the concepts are the same and the equations are also same.

At this stage it is imperative for us to get the concepts about zero order and first order because they are very important. These are explained most beautifully by Alfred Martin in his Physical Pharmacy book.

A man called A decides to go by car from a town called Anakapalli to a city called Visakhapatnam. Let us assume they are separated by a distance of 40 km. The man A declares that he will proceed from Anakapalli to Visakhapatmam at a steady speed of 10 km/hr. So if he starts at 10 a.m. in Anakapalli he will reach Visakhapatnam at 2 p.m. In his journey he will neither accelerate nor decelerate. We can say that A is making a journey at a zero order rate.

A man called B also decides to make the same journey by his car; but he declares that his speed at any given time is one fourth of the distance to be covered in km/hr. For the sake of easy calculation let us assume B breaks down his time periods into half hours. Then in the first half hour he travels a distance of 40/8 *i.e.* 5 km; in the second half hour 35/8 *i.e.* 4.375; in the third half hour 30.625/8 *i.e.* 3.828; in the fourth half hour 26.797/8 *i.e.* 3.349 and so on. So as the destination nears his speed will go on decreasing because his speed is one fourth of the distance to be covered. So theoretically speaking B will never reach his destination. B is travelling

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by first order kinetics! You must all have seen a nurse arrange an infusion into a patient's vein at a fixed number of drops per minute; the infusion is flowing by zero order. Suppose you throw a big rock into a stagnant pool of water, suddenly there are waves, and the extent of waves go down with each passing moment, but it will take a lot of time for the pool to become still again. This phenomenon is following a first order kinetics. The rate of decomposition is proportional to some power of the concentration of the reactants and it can be shown like this – $dc/dt = KC^n$ different values to n we will get equations for different orders. If $n = 0$ then $-dc/dt = KC$. If $n = 1$ then $-dCa/dt = KCa$ where Ca is the concentration of the reactant. Let us understand these two orders and let us also learn how to differentiate between these two. For zero order reaction a plot of time vs % drug remaining will be like this

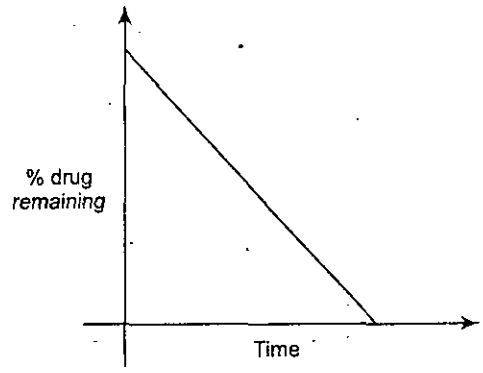

Fig. 3.6

If the drug decomposition is following first order kinetics then plot of time vs % drug remaining will be like this:

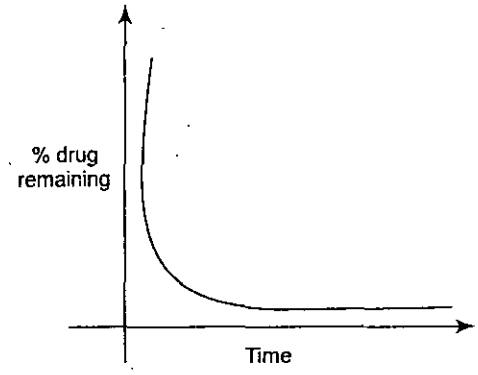

Fig. 3.7

If we plot log % remaining vs time, the plot will be like this,

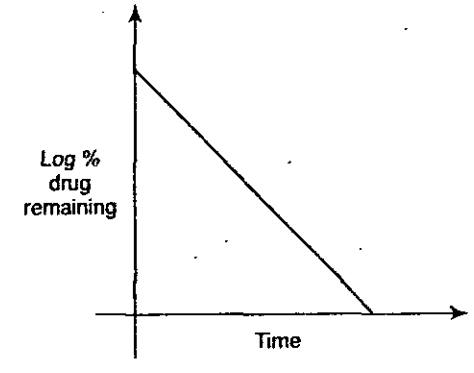

Fig. 3.8

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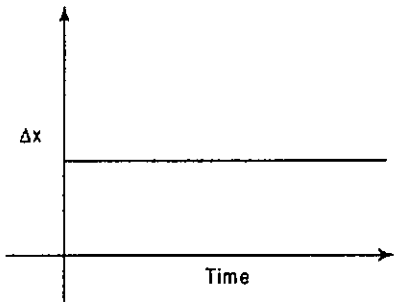
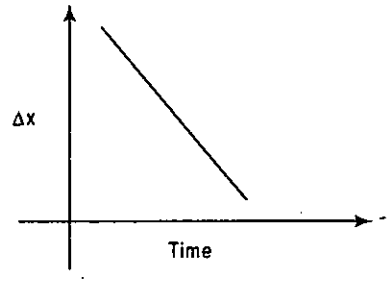
Table 3.11

Zero order	*First order*
1. When the reaction rate is independent of the concentration of the reacting substance, it is dependent on the zero power of the reactant and therefore is considered to be of the zero order reaction. The limiting factor is usually solubility of the drug or absorption of light.	1. When the reaction rate is dependent on the first power of concentration of a single reactant (rate = $k\ C_a$). It is considered to be first order. A reactant decomposes directly into one or more products.
2. Mathematically, $-dCa/dt = K$, $X = Kt +$ Constant or $Ct = C_0 - K_0\ t$ where, C_a = Concentration of reacting material A. K = proportionality factor = reaction rate t = time X = amount reacting C_t = concentration at time 't' C_0 = initial concentration.	2. Mathematically, $-dCa/dt = KCa$ and $-\log Ca = Kt/2.303 +$ Constant or $K = 2.303/t_2 - t_1 \log C_1/C_2$ or $K = 2.303/t \log a/a\text{-}x$ where $a = C_0$, X = amount reacting in time 't' and $a - X$ = the amount remaining after time 't'
3. $t_{1/2} = 0.5\ C_0/K_0$	3. $t_{1/2} = 0.693/K$
4. Slope of the line = K_0	4. slope × 2.303 = K_1
5. Units are : wt/time like mg/hr	5. Units are : hr^{-1}
6. If Δx = differences in amount remaining per unit time then, 	6. If Δx = differences in amount remaining per unit time then, 
7. The reaction comes to a conclusion in a finite time.	7. Theoretically it never comes to a conclusion. But practically it comes so close to completion that it may be considered as complete.
Examples:	Examples:
1. Intravenous infusion.	1. Absorption, distribution, elimination rates.
2. Drug released from transdermal drug delivery systems.	2. Microbial death kinetics.

By looking at all these differences you may feel it is very easy to find the order of the reaction by looking at the data, it is not so easy practically though theoretically it may look like that. The reason is when we actually follow the decomposition of some drugs the data appear as though they are satisfying criteria of both the orders. In such a situation to find the order of the reaction we have to follow the least square method of linear regression.

Second order, third order and more complex reactions are there but drug decomposition usually follows zero order or first order. When we know the order and the reaction rate constant we can predict the expiry date. In the case of vitamins, this data helps in determining the amount of overages we want to add to prolong the shelf life by a particular period of time. By knowing kinetic data and using some statistical methods we can also draw a stability line and 95% and

99% confidence limits to that line. Thus understanding rate kinetics is a key element in understanding the stability of drugs.

3.13 TEMPERATURE

NOTES

Temperature has a high degree of influence on all varieties of chemical reactions and usually they are accelerated by a raise in temperature. This is understandable as we know that with increased temperature the molecules move faster with increased kinetic energy and the rate of collision of molecules increases. Also, a greater available energy causes more molecules to have enough activation energy and the fraction of collisions with enough energy increases. It is said that typically a 10°C increase in temperature produces a 2 – 5 fold increase in decomposition.

The effect of temperature on the rate constant K is indicated by the Arrhenius equation which

$$K = Ae^{-(E_a/RT)}$$

$$\text{Log } K = \text{Log } A - E_a/2.303\ RT$$

where A is a constant that is termed the frequency factor, a is the energy of activation, R is the gas constant and T is the thermodynamic temperature. For drug compounds whose decomposition is log K is temperature, if log K is plotted against 1/T, a straight line is obtained. This is kown as Arrhenius plot. The constants E_a and A may be determined from the slope and intercept of this line, which are equal to $- E_a/2.303R$ and log A respectively. We usually take on the X axis $1/T \times 10^{-3}$ and log K on the *y*-axis. The activation energy E_a is the energy needed to cause reaction and is usually in the range of 15 – 60 k cal mol^{-1} with a mean of 19.8 (Aulton). Values for a wide range of reactions are 10 – 100 k cal mol^{-1}. If we are using E_a in the calculation of shelf life and if we are not sure of this value, we should make a conservative estimate and assume a low value (*e.g.*, 10 k cal mol^{-1}) for it. This assumption gives a shorter shelf life but it is better to avoid any risk to the patient.

Non-Arrhenius behaviour, or decreasing decomposition with temperature has been observed in Pharmaceutical systems as is well explained by Banker. This may be attributed to the possible evaporation of solvent, multiple reaction pathways, change in physical form of the formulation when the temperature of the reaction is changed.

A good example for this is the increased rate of decomposition of ampicillin on freezing. Savello and Shangraw showed that for a 1% sodium ampicillin solution in 5% dextrose, the percentage of degradation at 4 hour is approximately 14% at – 20°C, compared with 6% at 0°C and 10% at 5°C. This decrease in stability in frozen solutions is most frequently observed when the reaction obeys second or higher order kinetics. For example, the formation of nitrosomorpholine from morpholine and nitrite obeys third – order kinetics and the rate of nitrosation is drastically enhanced in frozen solutions.

This behaviour which is contradictory to our basic understanding of reaction mechanisms was reviewed by Pincock and is discussed in Banker. In reactions following second or higher order kinetics, an increase in rate may be brought about by concentration of the reactants in the liquid phase, the solute molecules being excluded from their lattice when the solution freezes. This may also happen due to a change in pH on freezing.

pH

Acidic and alkaline pH influence the rate of decomposition of most drugs. Many drugs are stable between pH 4 and 8. Weekly acidic and basic drugs show good solubility when they are ionized and they also decompose faster when they are ionized. So if the pH of a drug solution has to be adjusted to improve solubility and the resultant pH leads to instability then a way out of this tricky problem is to introduce a water-miscible solvent into the product. It will increase stability by (*a*) suppressing ionization, (*b*) reducing the extreme pH required to achieve solubility, (*c*) enhancing solubility and (*d*) reducing the water activity by reducing the polarity of the solvent. For example, 20% propylene glycol is placed in chlordiazepoxide injection for this purpose.

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Reactions catalysed by pH are monitored by measuring degradation rates against pH, keeping temperature, ionic strength and solvent concentration constant. Some buffers such as acetate, citrate, lactate, phosphate and ascorbate buffers are utilized to prevent drastic change in pH.

Sometimes pH can have a very serious effect on decomposition. As little as 1 pH unit change in pH can cause a change of ten fold in rate constant. So when we are formulating a drug into a solution we should carefully prepare a pH – decomposition profile and then formulate the solution at a pH which is acceptable physiologically and stability-wise also.

3.14 PACKAGING AND STABILITY

Packaging of the drug product is very important when its stability is being considered. The immediate container and closure are particularly important in affecting product stability. Glass, plastic, rubber (natural and synthetic) and metal are the four types of containers commonly utilized for packing drug products.

Glass

Glass is resistant to chemical and physical change and is the most commonly used material, but it has the limitations of :

1. Its alkaline surface may raise the pH of the product.
2. Ionic radicals present in the drug may precipitate insoluble crystals from the glass
3. The clarity of the glass permits the transmission of high energy wavelength of light which may accelerate decomposition.

All these limitations are overcome by the technologists in the following way: (1) the first problem is overcome by the use of Borosilicate glass which contains fewer reactive alkali ions, (2) Treatment of glass with chemicals or the use of buffers helps in overcoming the second problem, (3) Amber coloured glass which transmits light only at wavelengths above 470 nm is used for photolytic drug products.

Plastics

Plastics include a wide range of polymers of varying density and molecular weight, each possessing different physicochemical characteristics. The problems with plastic are:

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1. Migration of the drug through the plastic into the environment.
2. Transfer of environmental moisture, oxygen, and other elements into the pharmaceutical product.
3. Leaching of container ingredients into the drug.
4. Adorption or absorption of the active drug or excipients by the plastic.

For all these problems the solution is to suitably pretreat the plastic chemically. The drug product packed in the final container must be tested for stability.

Metals

Various alloys and aluminium tubes may be utilized as containers for emulsions, ointments, creams and pastes. They may cause corrosion and precipitation in the drug product. Coating the tubes with polymers or epoxy may reduce these tendencies.

Rubber

Rubber also has the problems of extraction of drug ingredients and leaching of container ingredients described for plastics. The use of neoprene, butyl or natural rubber, in combination with certain epoxy, Teflon or varnish coatings reduces drug-container interactions. The pretreatment of rubber vial stoppers and closures with water and steam removes surface blooms and also reduces potential leaching.

SUMMARY

- ICH guidelines help the manufacturers adopt a stability programme that is suitable for them. The final draft the ICH Harmoised Tripartite Guideline "Stability Testing of New Drug Substances and Products was issued by the International Conference on Harmonization (ICH) Expert Working Group of the ICH on technical requirements for the registration of pharmaceuticals for human use in October 1993. J.T.
- WHO has issued guidelines for stability testing of pharmaceutical products containing well established drug substances in conventional dosage forms. The stability of finished pharmaceutical products depends on environmental factors and on product related factors.
- *Pure drugs, solids, liquids, or gases are usually more stable than their formulations.* When they are formulated into medicines decomposition happens faster because of the presence of excipients, and moisture and because of processing.
- The study of the changes that happen in a dosage form as it stands on the shelf of a drug store constitute the contents of the chapter titled as "Drug Stability".
- The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) is a unique project that brings together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industry in the three regions to discuss scientific and technical aspects of product registration.
- Stability is the capacity of a drug product to remain within specifications established to ensure its identity, strength quality and purity.
- when molecules absorb energy and are energized or activated they go to a high energy state and then release that energy in a chemical reaction and come back to their original position. When this energy of activation is supplied by light and is absorbed by the compound the decomposition reaction is called photolytic.

- Temperature has a high degree of influence on all varieties of chemical reactions and usually they are accelerated by a raise in temperature.
- Packaging of the drug product is very important when its stability is being considered. The immediate container and closure are particularly important in affecting product stability. Glass, plastic, rubber (natural and synthetic) and metal are the four types of containers commonly utilized for packing drug products.

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REVIEW QUESTIONS

1. Discuss the Stability programme for a new drug.
2. What is preclinical formulation?
3. What is clinical formulation?
4. What is ICH?
5. Discuss the stability of herbal medicines
6. Discuss the international conference on harmonisation (ICH)
7. Discuss the physical stability of medicine.
8. Discuss the chemical stability of drugs.
9. Discuss the microbiological stability.
10. What is photolysis?

FURTHER READINGS

- Theory and Practice of Social Sciences, Ramakanth Tiwari and Mahesh Dabhade.

UNIT IV: POLYMERS

NOTES

★ STRUCTURE ★

4.1 LEARNING OBJECTIVES

After going through this unit, students will be able to:

- discuss the introduction of polymers and their applications
- describe history of polymers
- explain characteristics and classification of polymers
- state molecular structure of polymers
- discuss properties of polymers
- explain polymer synthesis
- elaborate biodegradable polymers and their practical utility.

4.2 INTRODUCTION

Polymers are substances whose molecules have high molar masses and are composed of a large number of repeating units. There are both naturally occurring and synthetic polymers. Among naturally occurring polymers are proteins, starches, cellulose, and latex. Synthetic polymers are produced commercially on a very large scale and have a wide range of properties and uses. The materials commonly called plastics are all synthetic polymers.

The number of repeating units in one large molecule is called the degree of polymerization. Materials with a very high degree of polymerization are called high polymers. Polymers consisting of only one kind of repeating unit are called homopolymers. Copolymers are formed from several different repeating units. Polymers are formed by chemical reactions in which a large number of molecules called monomers are joined sequentially, forming a chain. In many polymers, only one monomer is used. In others, two or three different monomers may be combined. Polymers are classified by the characteristics of the reactions by which they are formed. If all atoms in the monomers are incorporated into the polymer, the

polymer is called an addition polymer. If some of the atoms of the monomers are released into small molecules, such as water, the polymer is called a condensation polymer. Most addition polymers are made from monomers containing a double bond between carbon atoms. Such monomers are called olefins, and most commercial addition polymers are polyolefins. Condensation polymers are made from monomers that have two different groups of atoms which can join together to form, for example, ester or amide links. Polyesters are an important class of commercial polymers, as are polyamides (nylon).

Most of the organic substances found in living matter, such as protein, wood, chitin, rubber, and resins, are polymers. Many synthetic materials, such as plastics, fibers; (Rayon), adhesives, glass, and porcelain, are also to a large extent polymeric substances.

Protein, enzymes, muscle fibers, polysaccharides and gummy exudates are the natural polymers being used effectively in formulating the variety of pharmaceutical products. The well-known natural polymers used in pharmacy and other fields are chitosan, carrageenan, is paghula, acacia, agar, gelatin, shellac, guar gum and gum karaya[13]. These natural polymers are widely used in pharmaceutical industry as emulsifying agent, adjuvant and adhesive in packaging; and also well suited for pharmaceutical and cosmetic product development. Alginic acid is a natural polymer composed of α-1,4-linked-D-Mannuronic acid and α-1,4-linked L-guluronic acid molecules and is obtained by alkali treatment of seaweeds. It serves as an excellent extragranular disintegrant when it is added before compression.

With the availability of variety of natural polymers, the manufacturers today have achieved a great success in developing the most promising therapeutic systems, namely drug delivery system, which provides an effective therapy to the patients for prolonged periods. Natural polymers obtained from gummy exudates and plant fibres are being discussed here to take a closer look at their applications in pharmacy and other fields.

Xanthan Gum

This gum is produced by a pureculture fermentation of a carbohydrate with ***Xanthomonas campestris*** and purified. It is also known as Corn sugar gum. It is the sodium, potassium or calcium salt of a high molecular weight polysaccharide containing D-glucose, contains not less that 1.5% of pyruvic acid. It is a cream coloured powder, soluble in hot and cold water and neutral to litmus. A 1% solution has viscosity of about 1000 centipoises.

Solutions of xanthan gum demonstrate maximum stability at pH value between 4 and 10. Compared with tragacanth, xanthan gum was found to be easier to use and capable of preparing suspensions of better quality and improved consistency.

Xanthan gum is used as a stabilizer, thickener and emulsifier extensively in pharmaceutical, cosmetic industries and in food industry for dairy products. The pseudo plastic properties of this gum enable toothpastes and ointments both to hold their shape and to spread readily.

The stability was generally good and only a small number of drugs had been found to be incompatible (Amitriptyline, Tamoxifen and Verapamil). For extemporaneous dispensing, a 1% solution of xanthan gum with hydroxy benzoate, prepared in advance, was diluted to 0.5% with water when preparing D-mannose and D-glucuronic acid. It also the suspension.

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Xanthan gum was found to be suitable suspending vehicle for delivering antispasmodics topically along the length of the oesophagus in patients with oesophageal spasm. Coagulation of the gum had been observed when it was used for suspension of certain film coated tablets.

In a recent study the sedimentation volume of suspension with carboxy methyl cellulose and xanthan gum for period of 45 days. Results indicated that xanthan gum in a concentration of 0.2% is superior to carboxy methyl cellulose.

Acacia

The air dried gummy exudates from the stem and branches of *Acacia Senegal* **Willd.** (Family *Mimosaceae*) and other species of Acacia of African origin. It also known as Senegal gum. The tree is known in Kordofan as 'Hashab' and in Senegambia as 'Verek'. The gum, produced in kordofan from tapped trees is considered to be good. The Senegal and Nigerian gum is also of good quality. The Senegal gum is available in the desert areas of India like Rajasthan, Gujarat and Haryana. It is soluble in water leaving only a very small residue of vegetable particles, whereas practically insoluble in alcohol and ether.

Acacia is used as a suspending and emulsifying agent and as a tablet binder. Its demulcent properties are employed in various cough, diarrhoea and throat preparations. The principal use of gum Arabic is in confectionery as an emulsifier, for preserving flavours of soft drinks and also in the manufacture of chewing gums. It is used in the pharmaceutical industry as binding agent in the manufacture of cough pastilles and other medical preparations or as a coating for pills. The gum is also used for hair set and as a suspending agent.

Agar

Agar (or) Agar-Agar, also known as Japanese Isinglass, Chinese-Isinglass or Vegetable Gelatin. It is the dried, hydrophilic and phycocolloidal concentrate from a decoction of various marine red algae, particularly species of ***Gelidium*** (*Gelidaceae*), ***Pterocladi*** (*Gelidaceae*), order Gelidiales and ***Gracilaria*** (*Gracilariaceae*). The dried Agar-Agar usually occurs in bundles comprising thin, membranous, agglutinated strips; or in cut, flaked or granulated forms. It may be week yellowish orange, yellowish grey to pale yellow or colourless. It is tough when damp, brittle when dry, odourless or with a slight odour and has mucilaginous taste. The Agar-Agar is insoluble in cold water, but soluble in boiling water. Agar contain two different polysaccharides named as agarose and agaropectin. Agarose is responsible for gel strength of agar and composed of D-galactose and 3,6-anhydro- L-galactose units. It contains about 3.5% cellulose and 6% of nitrogen containing substance. Agaropectin is responsible for the viscosity of agar solutions.

It is believed to be a sulphonated polysaccharide in which galactose and uronic acid units are partly esterified with sulphuric acid. Agar is used as emulsifying, suspending, stabilizing, thickening or gelling agent and bulk laxative. It is also used in the preparation of jellies, confectionery items, tissue culture studies and in microbiology.

Carrageenan

Carrageenan is the hydrocolloid obtained from red seaweeds by extraction with water or aqueous alkali and recovered by alcoholic precipitation, drum drying or freezing (Class *Rhodophyceae*). It consists of a mixture of the ammonium, calcium,

magnesium, potassium and sodium sulphate esters of galactose and 3,6-anhydro-galactose copolymers. About 30ml water is required to dissolve 1g of it at temperature 80°C. It is widely used as dissolution rate retarding polymer in sustained release dosage form in many pharmaceutical industries.

Solution of carrageenan (1%) was also used to induce inflammation (Paw oedema) for screening of anti-inflammatory activity. Carrageenan is used in pharmacy and food industry as a suspending and gelling agent. Tooth paste, creams, lotions and other cosmetic products are also prepared by using carrageenan. In food industry, it is utilized in milk products, ice creams, chocolate, jams and gels in the concentration of 0.5–1%.

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Sterculia Gum

It is the dried gummy exudates obtained from the tree ***Sterculia urens* Roxb.** (Family – *Sterculiaceae*). It is also known as Sterculia, Karaya, Indian Tragacanth or Bassora Tragacanth gum. It is produced in India, Pakistan and to a small extent in Africa. Karaya also differs from tragacanth in that it contains no starch and stains pink with solution of ruthenium red. It has low water solubility but swells to many times its original volume.

Karaya gum consist of an acetylated, branched heteropolysaccharide with a high component of D-galacturonic acid and D-glucuronic acid residues. The granular grades are used as a bulk laxative, being only next to psyllium seed in use for this purpose. The powdered gum is used in lozenges, pastes and dental fixative powders and it has proved particularly useful as an adhesive for stoma appliances. It also acts as stimulant. It is available, with frangula, as granules. The cross linked Tragacanth (Epichlorhydrin) exhibits superior wicking and swelling action and hence can be used as a potential disintegrant.

Gelatin

Gelatin is a product obtained by partial hydrolysis of collagen derived from skin, white connective tissue and bones of animals. The process converts insoluble collagens into soluble gelatin, the solution of which is then purified and concentrated to a solid form. It is soluble in a hot mixture of glycerol and water and in 6N acetic acid, whereas it is practically insoluble in alcohol, chloroform, fixed oils, volatile oils and ether. Gelatin is used in the preparation of pastes, pastilles, suppositories, coating of tablets and manufacturing of hard and soft capsule shells. It is also used for the microencapsulation of drugs and other industrial materials. Specially purified and pyrogen free gelatins are available for intravenous injection and a grade with big 'Bloom strength' is used for making gelatin capsules and for bacteriological culture media.

Chitosan

Chitosan is a natural polymer obtained by deacetylation of chitin. It is present in shell fish. Chitosan is a linear polymer of β(1-4) linked 2-amino-2- deoxy-D-glucopyranose. Chitin is isolated from the exoskeleton of crustaceans such as crabs, krill and shrimps. It gives no reactions for cellulose or lignin. When heated with 50% potash at 160–170° C for one hour, it is converted into chitosan, $C_{14}H_{26}O_{16}N_2$, ammonia and acids such as acetic and oxalic. Chitosan with a concentration of 1.25% in dilute acetic acid has very high viscosity, *i.e.*, 120 cps. Its molecular weight is 1,43,000

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to 2,10,000. It is a cationic polysaccharide and contains approximately 6.5% of nitrogen.

Chitosan is a novel drug carrier material and it improves the dissolution rate of controlled release matrix tablets. The additional uses of chitosan are as coating agent, gel former, and to induce desirable properties such as mucoadhesion and permeation enhancement to improve oral bioavailability of a drug. Microcapsules were prepared from Gum Karaya and Chitosan using the principle of complex coacervation for the first time with a continuous oil-phase and they were also evaluated for their *in vivo* performance.

Ispaghula

Ispaghula husk consist of dried seeds of the plant ***Plantago ovata* Forsk.** (Family – *Plantaginaceae*) commonly known as *Isabgol* or *Ispaghula* or Spogel seeds. It contains mucilage, which is present in the epidermis of seeds.

It contains no toxic principles and when taken with water or milk most of it pass out of gastro-intestinal tract in 6 to 12 hours. Larger doses are essential as their action is produced partly by lubricating action of mucilage and partly by the increase in bulk of intestinal contents, which mechanically stimulates the intestinal peristalsis.

Mucilage is used as binding agent in the granulation of material for preparation of compressed tablets. It is used as a suspending and thickening agent due to its high swelling factor and ability to give a uniform viscous solution. It is much sought in pharmaceutical industry as enteric coating material, tablet disintegrator and also used in sustained release drug formulations.

4.3 HISTORY OF POLYMERS

Polymers were discovered long before anyone understood what they were. It wasn't until 1920 that German chemist Hermann Staudinger (1881–1965) made his macromolecular hypothesis, suggesting that polymers are actually giant molecules formed by the permanent attachment of countless smaller molecules. Through careful experiments, he proved his hypothesis to be correct and was rewarded with the 1953 Nobel Prize in Chemistry.

4.4 CHARACTERISTICS OF POLYMERS

1. Low density
2. Low coefficient of friction
3. Good corrosion resistance
4. Good mouldability
5. Excellent surface finish can be obtained
6. Can be produced with close dimensional tolerances
7. Economical
8. Poor tensile strength
9. Low mechanical properties
10. Poor temperature resistance
11. Can be produced transparent or in different colours

4.5 CLASSIFICATION OF POLYMERS

Polymers may be classified as follows, according to the mechanical response at elevated temperatures

1. Thermoplasts 2. Thermosets

(*a*) **Thermoplasts:**

- Thermoset polymers soften when heated and harden when cooled. Simultaneous application of heat and pressure is required to fabricate these materials.
- On the molecular level, when the temperature is raised, secondary bonding forces are diminished so that the relative movement of adjacent chains is facilitated when a stress is applied.
- Most Linear polymers and those having branched structures with flexible chains are thermoplastics.
- Thermoplastics are very soft and ductile.

The commercial available thermoplasts are

- Polyvinyl Chloride (PVC) and Polystyrene
- Polymethyl methacrylate
- Polystyrene

(*b*) **Thermosets:**

- Thermosetting polymers become soft during their first heating and become permanently hard when cooled. They do not soften during subsequent heating. Hence, they cannot be remolded/reshaped by subsequent heating.
- In thermosets, during the initial heating, covalent cross-links are formed between adjacent molecular chain. These bonds anchor the chains together to resist the vibration and rotational chain motions at high temperatures. Cross linking is usually extensive in that 10 to 15% of the chain mer units are cross linked. Only heating to excessive temperatures will cause severance of these crosslink bonds and polymer degradation.
- Thermoset polymers are harder, stronger, more brittle than thermoplastics and have better dimensional stability.
- They are more usable in processes requiring high temperatures
- Most of the cross linked and network polymers which include
  * Vulcanized rubbers
  * Epoxies
  * Phenolic
  * Polyester resins

  are thermosetting.
- Thermosets cannot be recycle, do not melt, are usable at higher temperatures than thermoplastics, and are more chemically inert.

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4.6 MOLECULAR STRUCTURE OF POLYMERS

The physical characteristics of a polymer depends on its:

- Molecular weight
- Shape
- Structure

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1. **Molecular Weight:**
   * Extremely large molecular weights are to be found in polymers with very long chains.
2. **Molecular Shape:**
   * Polymer chain molecules are strictly straight that the zig-zag arrangement of the backbone atoms is disregarded. Single chain bonds are capable of rotation and bending in three dimensions.
   * Some polymer consists of large number of molecular chains, each of which are may bend, coil and kink, leading to extensive intertwining and entanglement of neighboring chain molecules. These random coils and molecular entanglements are responsible for a number of important characteristics of polymers.
   * Some of the mechanical and thermal characteristics of polymers are a function of the ability of chain segments to experience rotation in response to applied stresses or thermal vibrations.
3. **Molecular Structures:**
   * Linear Polymers: Linear polymers are those in which the mer (the group of atoms that constitutes a polymer chain repeat unit) units are joined together end to end in single chain. These long chains are flexible. For linear polymers, there may be extensive van der Waals bonding between the chains. Some of the common polymers that form with linear structures are polyethylene, polyvinyl chloride, polystyrene, polymethyl merthacrylate, nylon and the fluorocarbons
   * Branched Polymers: Polymers may be synthesized in which side-branch chains are connected to the main ones. These are called branched polymers. The branches, considered to be the part of the main-chain molecules result from side reactions that occur during the synthesis of the polymer. The chin packing efficiency is reduced with the formation of side branches, which results in a lowering of the polymer density
   * Cross linked Polymers: In cross linked polymers, adjacent linear chains are joined one to another at various positions by covalent bonds. The process of cross linking is achieved either during synthesis or by a non-reversible chemical reaction that is usually carried out at an elevated temperature. This cross linking is accomplished by additive atoms or molecules that are covalently bonded to the chains. Many of the rubber elastic materials are cross linked. In case of rubbers, it is called vulcanization
   * Network Polymers: Trifunctional mer units, having three active covalent bonds, form three-dimensional networks instead of the linear chain framework assumed by bifunctional mers. Polymers composed of a trifunctional units are termed network polymers. A polymer that is highly cross linked may be classified as a network polymer. These materials have distinctive mechanical and thermal properties. The epoxies and phenol-formaldehyde belong to this group.

4.7 PROPERTIES OF POLYMERS

The physical properties of a polymer, such as its strength and flexibility depend on:

1. **Chain length** - in general, the longer the chains the stronger the polymer;
2. **Side groups** - polar side groups give stronger attraction between polymer chains, making the polymer stronger;
3. **Branching** - straight, unbranched chains can pack together more closely than highly branched chains, giving polymers that are more crystalline and therefore stronger;
4. **Cross-linking** - if polymer chains are linked together extensively by covalent bonds, the polymer is harder and more difficult to melt.

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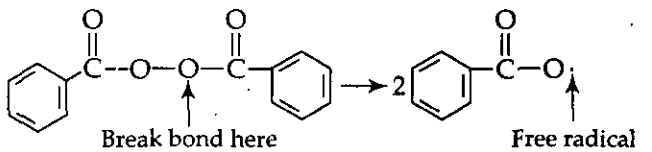
4.8 POLYMER SYNTHESIS

The study of polymer science begins with understanding the methods in which these materials are synthesized. Polymer synthesis is a complex procedure and can take place in a variety of ways. **Addition polymerization** describes the method where monomers are added one by one to an active site on the growing chain.

Addition Polymerization

The most common type of addition polymerization is free radical polymerization. A *free radical* is simply a molecule with an unpaired electron. The tendency for this free radical to gain an additional electron in order to form a pair makes it highly reactive so that it breaks the bond on another molecule by stealing an electron, learing that molecule with an unpaired election (which is another free radical). Free radicals are often created by the division of a molecule (known as an *initiator*) into two fragments along a single bond. The following diagram shows the formation of a radical from its initiator, in this case benzoyl peroxide.



The *stability* of a radical refers to the molecule's tendency to react with other compounds. An unstable radical will readily combine with many different molecules. However a stable radical will not easily interact with other chemical substances. The stability of free radicals can vary widely depending on the properties of the molecule. The *active center* is the location of the unpaired electron on the radical because this is where the reaction takes place. In free radical polymerization, the radical attacks one monomer, and the electron migrates to another part of the molecule. This newly formed radical attacks another monomer and the process is repeated. Thus the active center moves down the chain as the polymerization occurs.

There are three significant reactions that take place in addition polymerization: *initiation* (birth), *propagation* (growth), and *termination* (death). These separate steps are explained below.

Initiation Reaction

The first step in producing polymers by free radical polymerization is initiation. This step begins when an *initiator* decomposes into free radicals in the presence of monomers. The instability of carbon-carbon double bonds in the monomer makes

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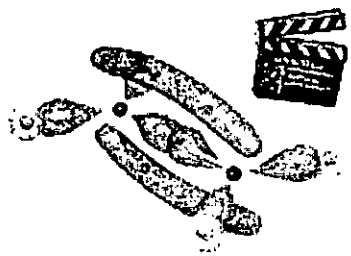
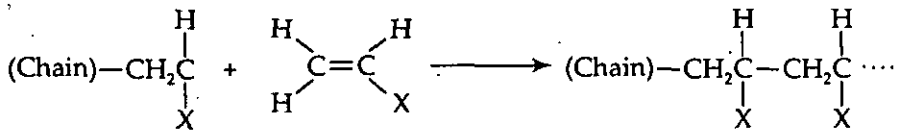
them susceptible to reaction with the unpaired electrons in the radical. In this reaction, the active center of the radical "grabs" one of the electrons from the double bond of the monomer, leaving an unpaired electron to appear as a new active center at the end of the chain. Addition can occur at either end of the monomer. This process is illustrated in the following animation in which a chlorine atom possessing an unpaired electron (often indicated as $Cl^{\cdot}$) initiates the reaction. As it collides with an ethylene molecule, it attracts one of the ethylene's pair of pi bonded electrons in forming a bond with one of the carbons. The other pi electron becomes the active center able to repeat this process with another ethylene molecule. The sigma bond between the carbons of the ethylene is not disturbed. (Note that a molecular orbital model is employed here in describing this process. See any introductory college chemistry text for further discussion)



In a typical synthesis, between 60% and 100% of the free radicals undergo an initiation reaction with a monomer. The remaining radicals may join with each other or with an impurity instead of with a monomer. "Self destruction" of free radicals is a major hindrance to the initiation reaction. By controlling the monomer to radical ratio, this problem can be reduced.

Propagation Reaction

After a synthesis reaction has been initiated, the propagation reaction takes over. In the propagation stage, the process of electron transfer and consequent motion of the active center down the chain proceeds. In this diagram, (chain) refers to a chain of connected monomers, and X refers to a substituent group (a molecular fragment) specific to the monomer. For example, if X were a methyl group, the monomer would be propylene and the polymer, polypropylene.

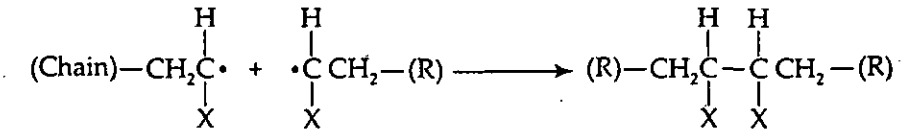


In free radical polymerization, the entire propagation reaction usually takes place within a fraction of a second. Thousands of monomers are added to the chain within this time. The entire process stops when the termination reaction occurs.

Termination Reaction

In theory, the propagation reaction could continue until the supply of monomers is exhausted. However, this outcome is very unlikely. Most often the growth of a polymer chain is halted by the termination reaction. Termination typically occurs in two ways: combination and *disproportionate.*

Combination occurs when the polymer's growth is stopped by free electrons from two growing chains that join and form a single chain. The following diagram depicts combination, with the symbol (R) representing the rest of the chain.



Disproportionation halts the propagation reaction when a free radical strips a hydrogen atom from an active chain. A carbon-carbon double bond takes the place of the missing hydrogen. Termination by disproportionation is shown in the diagram.

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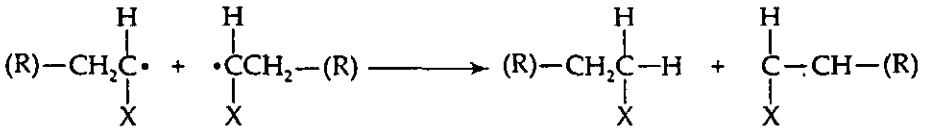

Disproportionation can also occur when the radical reacts with an impurity. This is why it is so important that polymerization be carried out under very clean conditions.

Living Polymerization

There exists a type of addition polymerization that does not undergo a termination reaction. This so-called "living polymerization" continues until the monomer supply has been exhausted. When this happens, the free radicals become less active due to interactions with solvent molecules. If more monomers are added to the solution, the polymerization will resume.

Uniform molecular weights (low *polydispersity*) are characteristic of living polymerization. Because the supply of monomers is controlled, the chain length can be manipulated to serve the needs of a specific application. This assumes that the *initiator* is 100% efficient.

4.9 BIODEGRADABLE POLYMERS AND THEIR PRACTICAL UTILITY

The environmental impact of persistent plastic wastes is raising general global concern, and disposal methods are limited. Incineration may generate toxic air pollution, satisfactory landfill sites are limited, and recycling methods for commingled waste are expensive and often energy - intensive. In addition, petroleum resources are finite and are becoming limited. It will be important to find durable plastic substitutes, especially in short - term packaging and disposable applications. The continuously growing public concern in the problem has stimulated research interest in biodegradable polymers as alternatives to conventional nondegradable polymers such as polyethylene and polystyrene etc.

Several concerns must be addressed prior to commercial use of biobased primary packaging materials. These concerns include degradation rates under various conditions, changes in mechanical properties during storage, potential for microbial growth, and release of harmful compounds into packaged food products. Furthermore, the biopackaging must function as food packaging and meet the requirements of particular food products.

In Europe, the biopackaging field is regulated primarily by two EU directives: "Plastic Materials and Articles Intended to Come into Contact with Foodstuffs" (90/128/EEC), with later amendments, and "Packaging and Packaging Waste Directive"

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(94/62/EEC). Biopackaging often has difficulties in complying with the migration requirements of the directive on "Plastic Materials and Articles Intended to Come into Contact with Foodstuffs". Furthermore, several of the raw materials and additives used to produce biopackaging materials are not included in the list of approved components. Polymers derived from renewable resources ("biopolymers") are broadly classified according to the method of production. This gives the following three main categories:

- Polymers directly extracted/removed from natural materials (mainly plants): Examples are polysaccharides such as starch and cellulose and proteins such as casein and wheat gluten.
- Polymers produced by "classical" chemical synthesis from renewable bio–derived monomers: A good example is polylactate, a biopolyester polymerized from lactic acid monomers. The monomer itself is produced by fermentation of carbohydrate feedstock.
- **Polymers produced by microorganisms or genetically transformed bacteria:** The best known biopolymer types are the polyhydroxyalkanoates, mainly poly(hydroxybutyrates) and copolymers of hydroxybutyrate (HB) and hydroxyvalerate (HV). Such copolymers are produced by Monsanto and are better known by the generic trade name "Biopol2". Polyhydroxyalkanoates function in microorganisms as energy substrates and for carbon storage.

Most commonly available natural polymers (category 1 above) are extracted from agricultural or forest plants and trees. Examples are cellulose, starch, pectins, and proteins. These are cell - wall, plant - storage (starch), or structural polymers. All are by nature hydrophilic and somewhat crystalline; all factors may cause processing and performance problems. Starch may offer a substitute for petroleum - based plastics. A renewable degradable carbohydrate biopolymer that can be purified from various sources by environmentally sound processes, starch, by itself, has severe limitations due to its water solubility. Articles made from starch will swell and deform upon exposure to moisture. To improve some of its properties, in the past decades a number of researchers have often blended starch with hydrophobic polymers in the form of petroleum polymers, both to increase biodegradability and to reduce the usage of petroleum polymer.

Fully biodegradable synthetic polymers, such as poly(lactic acid) (PLA), polycaprolactone (PCL), and poly(hydroxybutyrate - valerate) (PHBV), have been commercially available since 1990. However, these synthetic polymers are usually more expensive than petroleum - based polymers and also have slow degradability. Blending starch with these degradable synthetic polymers has recently become a focus of researchers. Advanced research results obtained by many scientists have established that blending of starch with poly(vinyl alcohol) and ethylene vinyl alcohol can be used for production of degradable films, and that biodegradable plastic substitutes can be produced by blending of starch with degradable poly(hydroxybutyrate - valerate) (PHBV). Preparation of new degradable polymers by blending of starch with degradable polycaprolactone (PCL) was the base for commercial trials. Unfortunately the mechanical strength properties of these blends were very limited. Of these biopolymers, because of its biodegradability and tissue compatibility, PLA has been extensively studied in medical implants, suture, and drug delivery systems since the 1980s. PLA is attractive for disposable and biodegradable plastic substitutes, due to its better mechanical properties, although it is still more expensive than conventional plastics. Also, its degradation rate is still low in relation to the waste accumulation rate.

Natural Polymers

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Biopolymers are defined as polymers formed under natural conditions during the growth cycles of all organisms. Therefore they are also named natural polymers. They are formed within cells by complex metabolic processes. For materials applications, cellulose and starch are most interesting. However, there is an increasing attention in more complex hydrocarbon polymers produced by bacteria and fungi, particularly in polysaccharides such as xanthene, curdlan, pullulan, and hyduromic acid.

Starch is a polymer of hexacarbon monosaccharide - D - glucose. It is extremely abundant in corn seeds, potato tubers, and the roots and stems of other plants. The D - glucose structure can exist both in open - chain and in ring forms; the ring configuration is ascribed to D - glucopyranose. The pyranose ring is a more thermodynamically stable structure and it constitutes the sugar structure in the solutions.

Starch is mainly composed of D - glucopyranosis polymers bound by α - 1,4 – and α - 1,6 - glycoside links. These links are formed between the first carbon atom (C1) of one molecule and the fourth (C4) or sixth (C6) of the second one. As the aldehyde group on one end of a starch polymer is always free, these starch polymers always possess at least one reducing tip. The other end of the polymer is an irreducible tip. Depending on the degree of polymer branching occurring in a starch molecule, there may be great numbers of irreducible tips. The formation of α links in a starch molecule enables some parts of starch polymers to generate helix structures; this is determined by the orientations of hydroxy (– OH) groups on the first carbon atom (C1) and the pyranose ring. Studies on starch ' s chemical properties and structure have established that it is composed of two components, both also polysaccharides: amylose (20 – 35%) and amylopectin. The ratio of these components varies, subject to the source of origin. Amylose is a linear polymer, whereas the amylopectin molecule is substantially bigger and branched. These structural differences cause marked differences in starch's characteristics and functions. Starch appears in plants as granules (reserve material), the sizes, shapes, and structures of which depend on their sources of origin.

Although the main components of all kinds of starch are the polymers amylose and amylopectin, there is considerable recorded diversity in the structures and characteristics of the natural starch granules [4]. The granule diameters vary from under 1 μm up to over 100 μm and their shapes may be regular (round, oval, angular) or totally irregular. Potato starch obtained from potato sprout tubers *Solanum tuberosum* L. has granules of varied size (from 10 up to 100 μm) and of different shapes (round, oval, oviform, oblong, shell - shaped, and other irregular forms). Starch is employed in the cosmetics and pharmaceutical industries for producing dusting powders and powders and as a filler. In addition, it serves as a means to obtain glucose, ethyl alcohol, and dextrins, as well as for stiffening and binding in these industries. Wheat starch from wheat grains (*Triticum vulgare* Villars) exists as single granules of two types: large ovals of 15 – 45 μm in diameter and smaller, more rounded forms of 2 – 7 μm in diameter. This type of starch is applied as a neutral dusting powder or as an ingredient in pharmaceutical preparations. In some plants - in oats or rice, for example - complex starch granules develop through binding of single molecules in an organized way.

The distribution of amylose and amylopectin inside a starch granule is well ordered. However, during heating in the presence of water, the packing of the two polymers becomes chaotic. This loss of internal order occurs at different temperatures, depen-

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ding on the starch type. With persistent warming in water, the natural granules swell and finally their structure gets destroyed. The polymers are then released into the water surroundings.

The starch degradation process proceeds very slowly: first dextrins are formed, and these in turn undergo hydrolysis to maltose disaccharide, to be eventually broken down into two glucose molecules.

Starch is a strongly hygroscopic, chemically neutral substance. It swells greatly in water, due to penetration of water molecules into its branched structure. As mentioned above, long boiling makes it dissolve in water or in weak acids, as well as in solutions with hydroxides of potassium, rubidium, cesium, or francium and concentrated solutions of chloral hydrate. Soluble starch (*Amylum solubile)* is obtained as a result of long boiling of starch with water or weak acid; link cleavage at the amylopectin chain branching sites is then observed, and eventually a water - soluble product is formed. It is employed as an indicator in chemical analysis (iodometry).

Studies on starch include examination of: water absorption, chemical modification of molecules, behavior under agitation, and high - temperature, thermomechanical abrasion resistance. Although starch is a polymer, its strength under stress appears to be low. At temperature above 150°C, the glycoside bonds start cracking and over 250°C starch granules subside endothermally. At low temperatures, however, some reorganization of hydrogen bonds is observed together with straightening of the molecule chains during the cooling process (retrogradation).

In some extreme cases, under 10 °C, precipitation is reported. Starch may be hot - water - soluble and formed in thin films; its molecular orientation causes brittleness in both foils and solid packages produced in this way. Both amylose and amylopectin consist of glucopyranosis molecules, yet the structural differences between these two polymers determine their different properties. Amylose is mostly a linear polymeric molecule, consisting of α - 1,4 - linked D - glucopyranose (Figure 4.1). The molecular weight of amylose varies from 500 anhydroglucose units (AGU) in high - amylose maize starch to more than 6000 AGU in potato starch. Recent research, though, suggests that amylose also contains some branchings. For purposes of simplification, the polymer structure is presented as a normal chain, but amylose is often characterized with a helix structure.

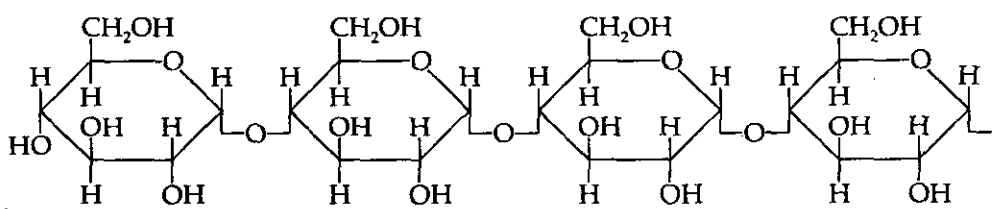

Fig. 4.1 Amylose structure

The helix structure contains C–H bonds , due to which it is hydrophobic, allowing a type of additive complexes with free fatty acids, fatty acid glycerides, some alcohols, and iodine to be generated. Iodine addition proves to be an important diagnostic method for starch characterization. Amylose absorbs up to 20% iodine and stains blue. Bonding with lipids, especially mono - and diglycerides, is a well - known property of amylose helix. The configuration and structural indivisibility of amylose – lipid complexes are affected by numerous factors such as temperature, pH, fatty acid structure, or glyceride, as well as by the contact time and/or agitation time between an amylose " carrier " and a linked molecule. A developing complex can change the features of starch.

Bonding of amylose to fats or to food emulsifiers such as mono - and diglycerides can change the starch gelatinization temperature or the textural and viscous profiles of the formed mass and can impede the retrogradation process.

After starch granules have been boiled, amylose possesses a gel formation capacity that allows rebinding of the dissolved amylose polymers. This property is noticeable in the behavior of some kinds of amylose - rich starch (wheat, rice, and high - amylose maize).

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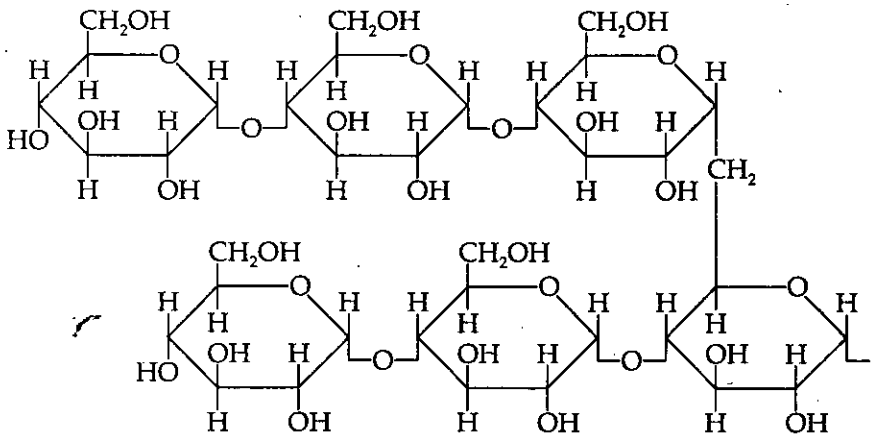

Fig. 4.2 Structure of amylopectin

Amylopectin, dominant in most starch kinds, is a branched polymer of substantially larger size than amylose. Amylopectin consists of α - 1,4 - bonded glucose segments, linked by α - 1,6 bonds at the branching sites (Figure 4.2). Estimates are that around 4 – 6% of bonds in a standard amylopectin molecule appear to be α - 1,6 links, which results in over 20,000 branchings in a molecule, although the branchings are not large. Studies suggest a bimodal size distribution of polymer chains: namely small and large chains. Small chains have a average degree of polymerization (DP) of about 15, whereas the bigger chains have DPs of around 45. This unique configuration contributes to the crystalline nature of amylopectin and to ordered arrangements of amylopectin molecules within the starch granule. The branched chains of amylopectin behave just like those of amylose, but in the case of amylopectin whole chains - or more often their fragments - can be twisted spirally.

Owing to this strongly branched structure of amylopectin, its properties differ from those of amylose: because of the large size of the amylopectin molecules and their structure, for example, retrogradation proceeds more slowly than in amylase and gel formation is inhibited. Starches consisting mainly of amylopectin (wax starches) are considered not to be gelating, but they usually show compact and rubbery textures. Amylopectin heated in water swells and forms a paste, it absorbs iodine poorly (around 0.6%), and stains violet or red - brown.

Amylose from different botanic sources shows varying degrees of polymerization (DPs), about 1500 – 6000, whereas the considerably bigger amylopectin molecules exhibit DPs from around 300 000 – 3 000 000. From these figures and from the molecular weight (MW) of anhydrous glucose (162), the MW of amylose can range from 243 000 up to 972 000. Reports say, however, that amylose from potato starch is of 1 000 000 MW, but its mean molecular weight is usually under 500 000. The MW of amylopectin varies between 10 000 000 and 500 000 000. The differences in the MWs of amylose and amylopectin are directly connected with their plant origins, methods of polymer isolation, and MW determination method.

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Cellulose was isolated for the first time around 150 years ago. It is different from other polysaccharides produced by plants because the molecular chains forming it are very long and are made up of a single repeating unit. This structure is observed in the crystalline state. Isolated from the cell walls in microfibrils by chemical extraction, cellulose in all forms is a highly crystalline polymer of high molecular mass and is infusible and insoluble. As a result of this it is usually converted into derivative substances to make it easier for processing.

Chitin and chitosan : Chitin is a skeletal polysaccharide making up a basic shell constituent of crabs, lobsters, shrimps, and insects. Chitin can be degraded by chitinase. It is insoluble in its native form, although chitosan, a partly deacetylated form of chitin, is water - soluble. The materials are biocompatible and demonstrate antimicrobial activity as well as heavy metal absorptivity. They are widely used in the cosmetics industry, due to their water - retaining and moisturizing capacities. Used as carriers, chitin and chitosan allow the synthesis of water - soluble prodrugs. Chitinous fibers serve in the manufacture of artificial skin and absorbable sutures.

Proteins, used as materials, are mostly insoluble and infusible without prior modification, and so are used in natural form. This description is especially true for the fibrous proteins wool, silk, and collagen. All proteins are specific copolymers with regular arrangements of different kinds of α - amino acids; protein biosynthesis is thus an extremely complex process demanding many enzymes of different types.

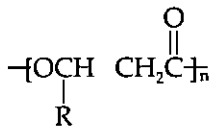

Fig. 4.3 Structure of bacterial polyester (R = – $(CH_2)_x$ – CH_3, x = 0 – 8 or more).

Gelatin , animal protein, consists of 19 amino acids joined by peptide linkages. It can be broken up by a variety of proteolytic enzymes to obtain its constituent amino acids or peptide components. Gelatin is a water - soluble, biodegradable polymer with wide industrial, pharmaceutical, and biomedical applications. In addition, it is also used for production of coatings and for microencapsulating various drugs and biodegradable hydrogels.

A method for gelatin application to produce thin flexible artificial skin adherent to an open wound to protect it from infection and fluid loss has been developed. This material was obtained as a blend of commercial gelatin and polyglycerol, either natural or after its epoxidation with epichlorohydrin, formed into thin films by casting on trays covered with Teflon. The films were tough and spontaneously adhered to open wounds. The films can contain bioactive molecules such as growth factors or antibiotics that will be released for a couple of days. The skin substitute prepared in this way could be sterilized with γ - rays or produced under sterile conditions.

In research into biodegradable materials, increasing interest has been reported in natural polyesters generated by various bacteria as reserve materials, due to fact that they are melt - processable polymers obtained from some renewable sources. The members of this thermoplastic biopolymer family, the general structure of which is shown in Figure 4.3, exhibit variation in their material properties from rigid brittle plastics through flexible to hard elastomers, subject to the alkyl group R and the polymer composition.

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SUMMARY

- Polymers are substances whose molecules have high molar masses and are composed of a large number of repeating units. There are both naturally occurring and synthetic polymers. Among naturally occurring polymers are proteins, starches, cellulose, and latex. Synthetic polymers are produced commercially on a very large scale and have a wide range of properties and uses. The materials commonly called plastics are all synthetic polymers.
- With the availability of variety of natural polymers, the manufacturers today have achieved a great success in developing the most promising therapeutic systems, namely drug delivery system, which provides an effective therapy to the patients for prolonged periods.
- **Addition polymerization** describes the method where monomers are added one by one to an active site on the growing chain.
- There are three significant reactions that take place in addition polymerization: *initiation* (birth), *propagation* (growth), and *termination* (death).
- The first step in producing polymers by free radical polymerization is initiation. This step begins when an *initiator* decomposes into free radicals in the presence of monomers.
- The continuously growing public concern in the problem has stimulated research interest in biodegradable polymers as alternatives to conventional nondegradable polymers such as polyethylene and polystyrene etc.
- Biopolymers are defined as polymers formed under natural conditions during the growth cycles of all organisms.

REVIEW QUESTIONS

1. Discuss natural polymers and their applications.
2. What are Characteristics of Polymers?
3. Discuss they Classification of Polymers.
4. Discuss the Molecular Structure of Polymers.
5. Discuss the Properties of Polymers.

FURTHER READINGS

- Theory and Practice of Social Sciences, Ramakanth Tiwari and Mahesh Dabhade.

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UNIT V: IN VITRO - IN VIVO CORRELATION: FROM THEORY TO APPLICATIONS

★ STRUCTURE ★

5.1 LEARNING OBJECTIVES

After going through this unit, students will be able to:

- discuss the introduction of in vitro in vivo correlation
- explain correlation levels of in vitro in vivo
- justify the systematic development of a correlation
- explain the important considerations in developing a correlation
- describe Biopharmaceutics classification system (BCS)
- elaborate Bioavailability studies for developing of IVIVC
- discuss the application of an IVIVC

5.2 INTRODUCTION

A key goal in pharmaceutical development of dosage forms is a good understanding of the in-vitro and in-vivo performance of the dosage forms. One of the challenges of biopharmaceutics research is correlating in-vitro drug release information of various drug formulations to the in-vivo drug profiles (IVIVC). Thus the need for a tool to reliably correlate in-vitro and in-vivo drug release data has exceedingly increased. Such a tool shortens the drug development period, economizes the

resources and leads to improved product quality. Increased activity in developing IVIVCs indicates the value of IVIVCs to the pharmaceutical industry. IVIVC can be used in the development of new pharmaceuticals to reduce the number of human studies during the formulation development as the main objective of an IVIVC is to serve as a surrogate for in vivo bioavailability and to support biowaivers. It supports and/or validates the use of dissolution methods and specification settings. This is because the IVIVC includes in vivo relevance to in vitro dissolution specifications. It can also assist in quality control for certain scale-up and post-approval changes (SUPAC). With the proliferation of modified-release products, it becomes necessary to examine the concept of IVIVC in greater depth. Investigations of IVIVC are increasingly becoming an integral part of extended release drug development. There must be some in vitro means of assuring that each batch of the same product will perform identically in vivo. This chapter represents the FDA guidance, development, evaluation, and validation of an IVIVC to grant biowaivers, and to set dissolution specifications for oral dosage forms, biopharmaceutics classification systems (BCS), BCS biowaivers, application of BCS in IVIVC development and concept of mapping. The importance of dissolution media and methodology and pharmacokinetic studies in the context of IVIVC has been highlighted. The chapter also covers the literature examples of IVIVCs regarding internal and external validation, compendial dissolution assessment, formulation dependency of IVIVCs, and IVIVCs of pure enantiomers versus racemate drugs. The same principles of IVIVC used for oral extended release products may be applied for non-oral products such as parenteral depot formulations and novel drug delivery systems as well.

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In recent years, the concept and application of the in vitro-in vivo correlation (IVIVC) for pharmaceutical dosage forms have been a main focus of attention of pharmaceutical industry, academia, and regulatory sectors. Development and optimization of formulation is an integral part of manufacturing and marketing of any therapeutic agent which is indeed a time consuming and costly process. Optimization process may require alteration in formulation composition, manufacturing process, equipment and batch sizes. If these types of changes are applied to a formulation, studies in human healthy volunteers may be required to prove that the new formulation is bioequivalent with the old one. Certainly, implementation of these requirements not only halts the marketing of the new formulation but also increases the cost of the optimization processes. It would be, desirable, therefore, to develop in vitro tests that reflect bioavailability data. A regulatory guidance for both immediate- and modified-release dosage forms has been, therefore, developed by the FDA to minimize the need for bioavailability studies as part of the formulation design and optimization. IVIVC procedures are specific to certain countries but could be adopted or used as the background for regulatory recommendations by other countries. IVIVC can be used in the development of new pharmaceuticals to reduce the number of human studies during the formulation development. The main objective of an IVIVC is to serve as a surrogate for in vivo bioavailability and to support biowaivers. IVIVCs could also be employed to establish dissolution specifications and to support and/or validate the use of dissolution methods.

This is because the IVIVC includes in vivo relevance to in vitro dissolution specifications. It can also assist in quality control for certain scaleup and post-approval changes, for instance, to improve formulations or to change production processes. There must be some in vitro means of assuring that each batch of the same product will perform identically in vivo. With the proliferation of modified-release products, it is essential to examine the concept of IVIVC in greater depth.

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Therefore, a more detailed article covering various aspects of an IVIVC study including complete process of developing the correlation with high quality, accurate and precise predictability, and identifying specific applications for such correlations might be of importance. Although the focus of discussion, in this review, will primarily be centered on modified-release formulations for which IVIVC is believed to be more defined, various aspects of the IVIVC of immediate-release dosage forms are also discussed.

Definitions

The term correlation is frequently employed within the pharmaceutical and related sciences to describe the relationship that exists between variables. Mathematically, the term correlation means interdependence between quantitative or qualitative data or relationship between measurable variables and ranks (1). From biopharmaceutical standpoint, correlation could be referred to as the relationship between appropriate in vitro release characteristics and in vivo bioavailability parameters. Two definitions of IVIVC have been proposed by the USP and by the FDA.

United State Pharmacopoeia (USP) definition

The establishment of a rational relationship between a biological property, or a parameter derived from a biological property produced by a dosage form, and a physicochemical property or characteristic of the same dosage form.

Food and Drug Administration (FDA) definition

IVIVC is a predictive mathematical model describing the relationship between an in vitro property of a dosage form and a relevant in vivo response. Generally, the in vitro property is the rate or extent of drug dissolution or release while the in vivo response is the plasma drug concentration or amount of drug absorbed .

5.3 CORRELATION LEVELS

Five correlation levels have been defined in the IVIVC FDA guidance. The concept of correlation level is based upon the ability of the correlation to reflect the complete plasma drug level-time profile which will result from administration of the given dosage form (2).

Level A Correlation

This level of correlation is the highest category of correlation and represents a point-to-point relationship between in vitro dissolution rate and in vivo input rate of the drug from the dosage form (2). Generally, percent of drug absorbed may be calculated by means of model dependent techniques such as Wagner-Nelson procedure or Loo-Riegelman method or by model-independent numerical deconvolution (2). These techniques represent a major advance over the single-point approach in that these methodologies utilize all of the dissolution and plasma level data available to develop the correlations (2) and will be discussed more in detail later in this chapter. The purpose of Level A correlation is to define a direct relationship between in vivo data such that measurement of in vitro dissolution rate alone is sufficient to determine the biopharmaceutical rate of the dosage form. In the case of a level A correlation, an in vitro dissolution curve can serve as a surrogate for in vivo

performance. Therefore, a change in manufacturing site, method of manufacture, raw material supplies, minor formulation modification, and even product strength using the same formulation can be justified without the need for additional human studies. It is an excellent quality control procedure since it is predictive of the dosage form's in vivo performance.

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Level B Correlation

A level B IVIVC utilizes the principles of statistical moment analysis. In this level of correlation, the mean in vitro dissolution time (MDTvitro) of the product is compared to either mean in vivo residence time (MRT) or the mean in vivo dissolution time (MDTvivo). MRT, MDTvitro and MDTvivo will be defined throughout the manuscript where appropriate. Although a level B correlation uses all of the in vitro and in-vivo data, it is not considered to be a point-to point correlation, since there are a number of different in-vivo curves that will produce similar mean residence time values. A level B correlation does not uniquely reflect the actual in-vivo plasma level curves. Therefore, one can not rely upon a level B correlation alone to justify formulation modification, manufacturing site change, excipient source change, etc. In addition in-vitro data from such a correlation could not be used to justify the extremes of quality control standards.

Level C Correlation

In this level of correlation, one dissolution time point (t50%, t90%, etc.) is compared to one mean pharmacokinetic parameter such as AUC, tmax or Cmax. Therefore, it represents a single point correlation and doses not reflect the entire shape of the plasma drug concentration curve, which is indeed a crucial factor that is a good indicative of the performance of modified-release products. This is the weakest level of correlation as partial relationship between absorption and dissolution is established. Due to its obvious limitations, the usefulness of a level C correlation is limited in predicting in vivo drug performance. The usefulness of this correlation level is subject to the same caveats as a level B correlation in its ability to support product and site changes as well as justification of quality control standard extremes. Level C correlations can be useful in the early stages of formulation development when pilot formulations are being selected. While the information may be useful in formulation development, waiver of an in vivo bioequivalance study (biowaiver) is generally not possible.

Multiple-level C Correlation

A multiple level C correlation relates one or several pharmacokinetic parameters of interest (Cmax, AUC, or any other suitable parameters) to the amount of drug dissolved at several time points of the dissolution profile. A multiple point level C correlation may be used to justify a biowaiver, provided that the correlation has been established over the entire dissolution profile with one or more pharmacokinetic parameters of interest. A relationship should be demonstrated at each time point at the same parameter such that the effect on the in-vivo performance of any change in dissolution can be assessed. If such a multiple level C correlation is achievable, then the development of a level A correlation is also likely. A multiple level C correlation should be based on at least three dissolution time points covering the early, middle, and late stages of the dissolution profile.

Level D Correlation

Level D correlation is a rank order and qualitative analysis and is not considered useful for regulatory purposes. It is not a formal correlation but serves as an aid in the development of a formulation or processing procedure.

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5.4 SYSTEMATIC DEVELOPMENT OF A CORRELATION

Any well designed and scientifically sound approach would be acceptable for establishment of an IVIV correlation. As the development of an IVIVC is a dynamic process starting from the very early stages of development program through the final step, the following practical and detailed approach with industrial application is summarized from reference number 5 without modifications.

"To understand how an IVIVR is used throughout the product development cycle, it is useful to become familiar with the following terms as they relate to a typical product development cycle for oral extended-release product (Figure 5.1). An ***assumed IVIVR*** is essentially one that provides the initial guidance and direction for the early formulation development activity. Thus, during stage 1 and with a particular product concept in mind, appropriate in vitro targets are established to meet the desired in vivo profile specification. This assumed model can be the subject of revision as prototype formulations are developed and characterized in vivo, with the results often leading to a further cycle of prototype formulation and in vivo characterization. Out of this cycle and in vivo characterization and, of course, extensive in vitro testing is often developed what can be referred to as ***retrospective IVIVR***. With a defined formulation that meets the in vivo specification, Stage 2 commences. At this stage based on a greater understanding and appreciation of defined formulation and its characteristics, a ***prospective IVIVR*** is established through a well defined prospective IVIVR study. Once the IVIVR is established and defined it can be then used to guide the final cycle of formulation and process optimization leading into Stage 3 activities of scale-up, pivotal batch manufacture, and process validation culminating in registration, approval and subsequent post-approval scale-up and other changes. Thus rather than viewing the IVIVR as a single exercise at a given point in a development program, one should view it as a parallel development in itself starting at the initial assumed level and being built on and modified through experience and leading ultimately to a prospective IVIVR".

"Stage 1: To undertake the development of an oral extended-release product, stage 1 targets first must be defined. The target in vivo profile needs to be first established, based on, if possible, pharmacokinetic/pharmacodynamic models. Clearly, as described in the pioneering work of Amidon in relation to the original biopharmaceutic drug classification and the work of Corrigan relating to extended release product, characterizing the permeability properties of a drug substance is a key element both in establishing the initial feasibility of any formulation program and in the subsequent interpretation of the observed in vivo absorption characteristics of a given dosage form. The physicochemical characteristics of the drug substance itself, in the context of how these affect the formulation approach and in the context of relevance to dissolution at distal sites in the gastro-intestinal tract, need to be taken into account. Based on this information a priori in vitro methods are usually

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then developed and a theoretical in vitro target is established, which should achieve the desired absorption profile. Essentially at this stage a level A correlation is assumed and the formulation strategy is initiated with the objective of achieving the target in vitro profile. The prototype formulation program itself is normally initiated with some knowledge or expectation of what technologies and/or mechanism of release are particularly suited to meet the desired targets. This work is usually done at a laboratory level of manufacture with the simplest dissolution methodology that seems appropriate. Prototypes that meet the target in vitro profile are then selected involving one or, very often, more than one technology or formulation approach. At least one, but usually more than one prototype within each technology or formulation approach is tested. More extended in vitro characterization, which looks at the robustness of these prototypes across dissolution conditions such as pH, medium, agitation speed and apparatus type, is routine at this point. Certainly, stage 1 activity should culminate in a pilot PK study. This is typically a four or five-arm cross-over study. The size of this pilot pharmacokinetic study will vary depending on the inherent variability of the drug itself but typically range from 6 to 10 subjects. The results of this pilot PK study provide the basis for establishing what has been referred to as a retrospective IVIVR. In other words, a number of different prototypes with some level of variation in release rate have now been characterized both in vitro and in vivo. This information first allows a reality check on both the in vivo and assumed IVIVR, either matching expectation or often causing a fundamental shift in the assumed IVIVR. After the results of the in vivo study are known, there is often a phase of significant revision of the in vitro methods, sometimes driven by the need to detect an in vitro difference that was observed in vivo but that had not been detected using the original in vitro methods. This work sometimes results in revised in vitro targets and reformulation strategy and the same cycle of activity again".

"*Stage 2:* By this stage of the development process, a defined formulation that meets the in vivo targets has been achieved. The aim is to progress through the normal formulation process optimization steps ultimately into scale-up, registration, and approval. In stage 2, a defined formulation and ideally a good understanding of the mechanism of release of this formulation has been established. Based on this a priori understanding, and from a sort of retrospective data generated from stage 1, an empirical basis exist for determining the primary formulation related rate controlling variables. For extended release products, this a priori understanding is usually more obvious than might be the case for immediate-release products. Based on this information, a number of products with different release rates are usually manufactured by varying the primary rate controlling variable but within the same qualitative formulation. Extensive in vitro characterization is again performed across pH, media and apparatus, but the stage 1 work is also taken into account. This leads to execution of a prospective IVIVR study. The IVIVR is developed and defined after an analysis of the result of that prospective in vivo study. It can often involved further in vitro method development in the context of the observed results, but clearly with the objective of establishing a definitive IVIVR. This ideally is a level A IVIVC but, in particular, multiple-level C IVIVC continues to be both an acceptable and useful IVIVR. This work should also result in the definitive in vitro method that has been shown to be correlated with in vivo performance and sensitive to the specific formulation variables.

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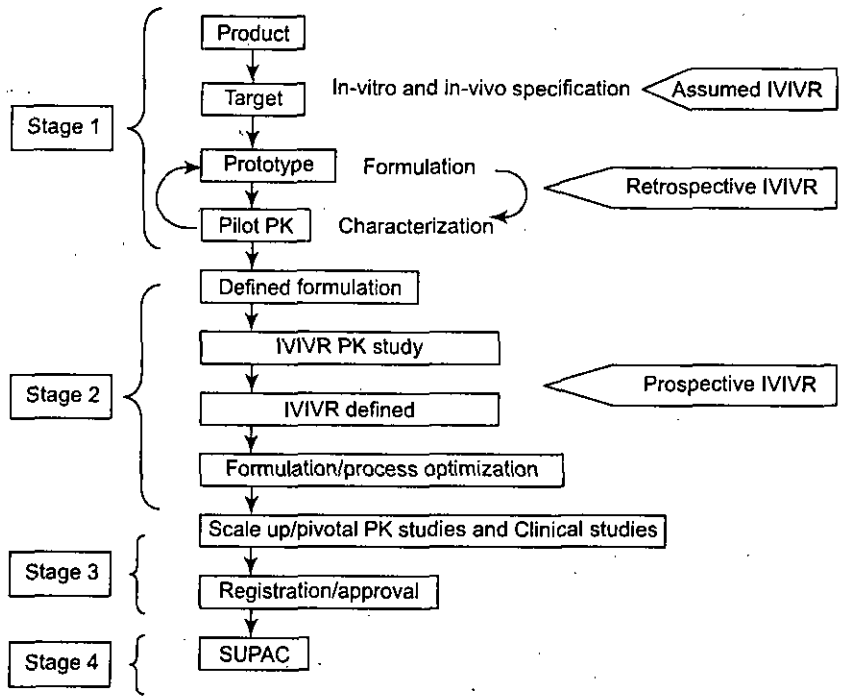

Fig. 5.1 The product development process for extended-release products

Once the IVIVR is established, it is routinely used in the completion of the formulation/process optimization program using statistically based experimental design studies looking at critical formulation and process variables and their interactions. By now with a correlated in vitro method, the robustness of the formulation and process can be established. This information can also be used to establish appropriate in-process and finished-product specification, of course, the appropriate targets for scale-up".

Development of in-vitro in-vivo correlation and validation using in vitro dissolution and in vivo time course is also illustrated in Figure 5.1.

5.5 IMPORTANT CONSIDERATIONS IN DEVELOPING A CORRELATION

When the dissolution is not influenced by factors such as pH, surfactants, osmotic pressure, mixing intensity, enzyme, ionic strength, a set of dissolution data obtained from one formulation is correlated with a deconvoluted plasma concentration-time data set. To demonstrate a correlation, fraction absorbed in-vivo should be plotted against the fraction released in vitro. If this relationship becomes linear with a slope of 1, then curves are superimposable, and there is a 1:1 relationship which is defined as point-to-point or level A correlation. Under these circumstances, the correlation is considered general and could be extrapolated within a reasonable range for that formulation of the active drug entity. In a linear correlation, the in-vitro dissolution and in-vivo input curves may be directly superimposable or may be made to be superimposable by the use of appropriate scaling factor (time corrections). Time scaling factor should be the same for all formulations and different time scales for each formulation indicate absence of an IVIVC.

Non-linear correlation may also be appropriate. In cases where, the dissolution rate depends on the experimental factors mentioned above, the deconvoluted plasma

concentration-time curves constructed following administration of batches of product with different dissolution rates (at least two formulations having significantly different behaviour) are correlated with dissolution data obtained under the same dissolution condition. If there is no one-to-one correlation other levels of correlation could be evaluated.

If one or more of the formulations (highest or lowest release rate formulations) may not illustrate the same relationship between in vitro performance and in-vivo profiles compared with the other formulations, the correlation is still valid within the range of release rates covered by the remaining formulations. The in-vitro dissolution methodology should be able to adequately discriminate between the study formulations. Once a system with most suitable discrimination is developed, dissolution conditions should be the same for all formulations tested in the biostudy for development of the correlation.

During the early stages of correlation development, dissolution conditions may be altered to attempt to develop a one-to-one correlation between the in vitro dissolution profile and the in vivo dissolution profile. An established correlation is valid only for a specific type of pharmaceutical dosage form (tablets, gelatin capsules, etc.) with a particular release mechanism (matrix, osmotic system, etc.) and particular main excipients and additives. The correlation is true and predictive only if modifications of this dosage form remain within certain limits, consistent with the release mechanism and excipients involved in it. Extrapolation of IVIVC established in healthy subjects to patients has to be taken into account. Drugs are often taken just before, with or after meal. All these factors may increase variability. A posterior correlation might be established using the patients' data only to increase the knowledge of the drug. The release rates, as measured by percent dissolved, for each formulation studied, should differ adequately (e.g., by 10%). This should result in vivo profiles that show a comparable difference, for example, a 10% difference in the pharmacokinetic parameters of interest (C_{max} or AUC) between each formulation.

5.6 BIOPHARMACEUTICS CLASSIFICATION SYSTEM (BCS)

The Biopharmaceutics Classification System (BCS) is a drug development tool that allows estimation of the contribution of three fundamental factors including dissolution, solubility and intestinal permeability, which govern the rate and extent of drug absorption from solid oral dosage forms (6). Drug dissolution is the process by which the drug is released, dissolved and becomes ready for absorption. Permeability is referred to the ability of the drug molecule to permeate through a membrane in to the systemic circulation. BCS is also a fundamental guideline for determining the conditions under which IVIVCs are expected. It is also used as a tool for developing the in-vitro dissolution specification. The classification is dealing with drug dissolution and absorption model, which considers the key parameters controlling drug dissolution and absorption as a set of dimensionless numbers: the absorption number, the dissolution number, and the dose number.

Absorption Number (A_n)

The Absorption Number (A_n) is the ratio of the Mean Residence Time (T_{res}) to the Mean Absorption Time (T_{abs}) and is calculated by equation (1).

$$A_n = T_{res}/T_{abs} = (\pi R^2 L/Q)/(R/P_{eff}) \quad ...(1)$$

Dissolution Number (D_n)

The Dissolution Number (D_n) is the ratio of T_{res} to Mean Dissolution Time (T_{diss}) and could be estimated using equation (2).

$$D_n = T_{res}/T_{diss} = (\pi R^2 L/Q)/(\rho r_0^2/3DC_s^{min}) \quad ...(2)$$

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Dose Number (D_o)

The Dose Number (D_o) is calculated using equation (3).

$$D_o = \text{Dose}/(V_o \times C_s^{min}) \quad ...(3)$$

where L = tube length, R = tube radius, π = 3.14, Q = fluid flow rate, r_o = initial particle radius, D = particle acceleration, ρ = particle density, P_{eff} = effective permeability, V_o is the initial gastric volume equal to 250 ml which is derived from typical bioequivalence study protocols that prescribe administration of a drug product to fasting human volunteers with a glass of water at the time of drug administration and C_s^{min} is minimum aqueous solubility in the physiological pH range of 1–8.

The dose, dose number, solubility, and estimated dissolution number for a number of drugs are listed in Table 5.1. The fraction dose absorbed could be estimated using these three major dimensionless parameters. However, the extent of solubilization and potential particle aggregation in the small intestine is unknown and therefore, the solubility, dose and dissolution number of a drug in-vivo is difficult to estimate precisely. As the drug dissolution and intestinal permeability are the fundamental parameters governing rate and extent of drug absorption, drugs could be categorized into high/low solubility and permeability classes. Thus, the expectations regarding IVIVC could be stated more clearly as are summarized in Table 5.1.

Table 5.1 IVIVC expectations for immediate release products based on BCS (from ref. 6 with modification)

Class	*Solubility*	*Permeability*	*Absorption rate control*	*IVIVC expectations for Immediate release product*
I	High	High	Gastric emptying	IVIVC expected, if dissolution rate is slower than gastric emptying rate, otherwise limited or no correlations
II	Low	High	Dissolution	IVIVC expected, if in-vitro dissolution rate is similar to in-vivo dissolution rate, unless does is very high
III	High	Low	Permeability	Absorption (permeability) is rate determining and limited or no IVIVC with dissolution
IV	Low	Low	Case by case	Limited or no IVIVC is expected

Class I compounds such as metoprolol exhibit a high absorption (An) and a high Dissolution (Dn) number. The rate-limiting step to drug absorption is drug dissolution or gastric emptying rate if dissolution is very rapid. This group of drugs is expected to be well absorbed unless they are unstable, form insoluble complexes, are secreted directly from gut wall, or undergo first pass metabolism. For immediate release products that release their content very rapidly the absorption rate will be controlled by the gastric emptying rate and no correlation of in-vivo data with

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dissolution rate is expected. Dissolution test for immediate release formulations of class I drugs, therefore, need only to verify that the drug indeed is rapidly released from the dosage form under mild aqueous conditions. A dissolution specification of 85% dissolution of drug contained in immediate release in 15 minutes may insure bioequivalance. The FIP consider a formulation as a very fast releasing when at least 80% of the drug substance is dissolved in about 20–30 minutes under reasonable and justified test conditions. The aforementioned dissolution time limits are based on typical gastric emptying times for water in the fasted state.

When a class I drug is formulated as an extended release product in which the release profile controls the rate of absorption, and the solubility and permeability of the drug is site independent, a level A correlation is most likely. However, once the permeability is site dependent a level C correlation is expected.

Class II drugs such as phenytoin has a high absorption number, An, but a low dissolution number, Dn. In vivo drug dissolution for Class II drugs is, therefore, a ratelimiting factor in drug absorption (except at very high dose number, Do) and consequently absorption is usually slower than Class I and takes place over a longer period of time. The limitation can be *equilibrium* or *kinetic* in nature. In the case of an *equilibrium* problem enough fluid is not available in the GI tract to dissolve the dose. For example, 33.3 liters (Table 5.1) of fluid are required to dissolve one dose of griseofulvin. As the total volume of fluid entering the GI tract within 24 hrs period is only about 5 to 10 liters, insufficient fluid would be available at any given time to dissolve the entire dose of griseofulvin. Griseofulvin exhibits a high dosing number (D_o) and a low dissolution number (Dn). Bioavailability and the fraction of the dose absorbed can be improved by decreasing Do by reducing the dose, by taking more water with the administered dose or by increasing drug solubility. The dose of a drug is determined on the basis of pharmacokinetic/ pharmacodynamic considerations and could not be altered. The volume of water initially is taken with dosage form will be limited by patient compliance and anatomical and physiological capacity of the stomach. For griseofulvin, therefore, only enhancement of the drug solubility through appropriate formulation approach (i.e. solid dispersion) can lead to reduced Dn considerably and to increase drug bioavailability. In the case of *kinetic* problem, the entire dose of the drug dissolves too slowly. For example a typical dose of digoxin is 0.5 mg and has a V sol of 20.8 ml which results in a small Do. In spite of the small volume of fluid required to dissolve 0.5 mg of the drug, it is shown that bioavailability of digoxin depends on the particle size. Digoxin exhibits dissolution rate limited absorption (D_n = 0.52) at particle sizes of greater than 10 μ in diameter. These comply with the reports indicating that digoxin, in micronized form, and griseofulvin, in ultramicronized form, was almost completely absorbed . For class II drugs, therefore, a strong correlation between dissolution rate and the in vivo performance could be established . As pointed out earlier, the appropriate design of in vitro dissolution tests such that discriminate between formulations with different bioavailabilities plays a major role in the ability of the IVIVC predictability. Therefore, it is essential that in vitro dissolution tests reflect in vivo situations when it is used to establish an IVIVC. Dissolution media and methods that reflect the in vivo controlling process are particularly important in this case if good IVIV correlations are to be obtained. The dissolution profile for class II drugs requires multiple sampling times and the use of more than one dissolution medium. Addition of surfactant to simulate in vivo environment might be required.

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When a class II drug is formulated as an extended release product and the solubility and permeability of the drug is site independent, a level A correlation is expected. However, once the permeability is site dependent little or no IVIV correlation is expected. BCS classifications in conjunction with the numerous of compendial and physiological media available could be employed as a fundamental guidances for designing appropriate biorelevant dissolution conditions leading to a more meaningful prediction of in vivo performances. For class I drugs, simple and mild aqueous dissolution media such as SGF without pepsin is suitable, while milk as dissolution medium might be appropriate for specific food/formulation interaction. For neutral class II drugs, the fluid simulating conditions in the proximal intestine in the fasted state (FaSSIF) reflects the dissolution in the upper GI tract under fasted state conditions. If a class II drug is a weak base, SGFsp could be used to assess the dissolution of the drug in the stomach under fasted state conditions. To verify the possibility of drug precipitation under intestinal conditions, performing dissolution in fasted state intestinal conditions (FaSSIF) may be appropriate. Comparison of dissolution results obtained under fasted conditions to those of FeSSIF could be a good indicative of whether the formulation should be administered before or after meals.

In the case of class II weak acids, dissolution could be performed in FaSSIF as a suitable representative of intestinal fasted state conditions. Milk with its composition of lipids and proteins or FeSSIF containing high bile salt/lecithin levels can be employed to simulate the fed state conditions. Class III drugs, such as cimetidine, are rapidly dissolving and permeability is the ratecontrolling step in drug absorption. Rapid dissolution is particularly desirable in order to maximize the contact time between the dissolved drug and absorption mucosa.

Therefore, the duration of dissolution should be at least as stringent for class III drugs as for class I drugs . Furthermore, Class III drugs exhibit a high variability in rate and extent of absorption, but if dissolution is fast such that 85% of drug dissolves in 15 minutes, the variation could be attributed to GI transit, luminal contents, and membrane permeation rather than dosage form factors. As drug permeation is rate controlling, limited or no IVIV correlation is expected.

Class IV drugs are low solubility and low permeability drugs. This class of drugs exhibit significant problems for effective oral delivery. It is anticipated that inappropriate formulation of drugs fall in class IV, as in the case of class II drugs, could have an additional negative influence on both the rate and extent of drug absorption. Thus for all categories, it is anticipated that well-designed dissolution tests can be a key prognostic tool in the assessment of both the drugs potential for oral absorption and of the bioequivalance of its formulations.

5.7 DISSOLUTION MEDIA AND METHODOLOGY

Drug absorption from a solid dosage form following oral administration depends on the release of the drug substance from the drug product, the dissolution or solubilization of the drug under physiological conditions, and the permeability across the gastrointestinal tract. Because of the critical nature of the first two of these steps, in vitro dissolution may be relevant to the prediction of in vivo performance. The Comparison between dissolution profiles could be achieved using a difference factor (f_1) and a similarity factor (f_2) which originates from simple model

independent approach. The difference factor calculates the percent difference between the two curves at each time point and is a measurement of the relative error between the two curves:

$$f_1 = \left\{\left[\sum_{t=1}^{n}|R_t - T_t|\right] \Big/ \left[\sum_{t=1}^{n} R_t\right]\right\} * 100 \qquad ...(4)$$

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where, n is the number of time points, R_t is the dissolution value of the reference batch at time t, and T_t is the dissolution value of the test batch at time t.

The similarity factor is a logarithmic reciprocal square root transformation of the sum squared error and is a measurement of the similarity in the percent dissolution between the two curves.

$$f_2 = 50 * \log\left\{\left[1 + (1/n)\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} * 100\right\} \qquad ...(5)$$

Generally, f_1 values up to 15 (0–15) and f_2 values greater than 50 (50–100) ensure sameness or equivalence of the two curves. The mean in vitro dissolution time (MDT_{vitro}) is the mean time for the drug to dissolve under in vitro dissolution conditions. This is calculated using the following equation (3)

$$MDT_{vitro} = \int_0^{\infty} (M_{\infty} - M(t))dt / M_{\infty} \qquad ...(6)$$

5.8 BIOAVAILABILITY STUDIES FOR DEVELOPMENT OF IVIVC

A bioavailability study should be performed to characterize the plasma concentration versus time profile for each of the formulation. Bioavailability studies for IVIVC development should be performed with sufficient number of subjects to characterize adequately the performance of the drug product under study. In prior acceptable data sets, the number of subjects has ranged from 6 to 36. Although crossover studies are preferred, parallel studies or crossstudy analyses may be acceptable. The latter may involve normalization with a common reference treatment. The reference product in developing an IVIVC may be an intravenous solution, an aqueous oral solution, or an immediate release product. IVIVCs are usually developed in the fasted state. When a drug is not tolerated in the fasted state, studies may be conducted in the fed state. Drug absorption from GI tract following ingestion of an oral dosage form could be influenced by a number of in vivo variables. For the determination of reproducible in vivo parameters and consequently useful in vitro in vivo relationship, it is imperative that such variables be identified. As a result, the study should be designed appropriately that as many variables as possible be eliminated or controlled to prevent or minimize their disturbance of IVIVC. Control or standardization of a number of variables including subject selection criteria such as age, gender, physical condition, etc., and the abstinence by the subject from coffee and other xanthenes containing beverages or food, alcohol, irregular diets and smoking before and during the study should be taken in to consideration. Food, posture and exercise may influence hepatic blood flow which in turn may substantially affect the absorption of drugs possessing high hepatic extraction ratio. As pointed out earlier, one method to develop level A correlation is to estimate the in-vivo absor-

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ption or dissolution time course using an appropriate deconvolution techniques such as Wagner-Nelson procedure or Loo-Riegelman method or numerical deconvolution for each formulation and subject. Wagner-Nelson and Loo-Riegelman methods are both model dependent in which the former is used for a onecompartment model and the latter is for multicompartment system. The Wagner-Nelson is less complicated than the Loo-Riegelman as there is no requirement for intravenous data.

However, misinterpretation on the terminal phase of the plasma profile may be possible in the occurrence of a flip-flop phenomenon in which the rate of absorption is slower than the rate of elimination. According to Wagner-Nelson method, the cumulative fraction of drug absorbed at time t is calculated from Equation 7 as follows:

$$F_T = \frac{C_T + K_E \int_0^T C\,dt}{K_E \int_0^\infty C\,dt} \qquad ...(7)$$

where, C_T is plasma concentration at time T and K_E is elimination rate constant. The apparent absorption rate constant (K_a) could be obtained from the least square fitted log-linear plot of the percent unabsorbed versus time. The absorption half-life ($t_1/2a$) is calculated as $0.693/K_a$. The Loo-Riegelman method requires drug concentration time data after both oral and intravenous administration of the drug to the same subject and the fraction absorbed at any time t is given by:

$$F_T = \frac{C_T + K_{10} \int_0^T C\,dt + (X_p)_T / V_c}{K_{10} \int_0^\infty C\,dt} \qquad ...(8)$$

where, in addition to symbols defined previously, $(X_p)_T$ is the amount of drug in the peripheral compartment as a function of time after oral administration and V_c is the apparent volume of the central compartment. K_{10}, the apparent first order elimination rate constant of drug from the central compartment, is estimated from a previous or subsequent intravenous study of the same subject. $(X_p)_T/V_c$ can be estimated by a rather complicated approximation procedure requiring both oral and intravenous data.

Deconvolution is a numerical method used to estimate the time course of drug input using a mathematical model based on the convolution integral. For example the absorption rate time course (rabs) that results in plasma concentration (c_t) may be estimated by solving the convolution integral equation for rabs.

$$c(t) = \int_0^t c_\delta (t-u) r_{abs}(u)\,du \qquad ...(9)$$

where, c_δ represents the concentration time profile resulting from an instantaneous absorption of a unit amount of drug which is typically from bolus intravenous injection or reference oral solution data, $c(t)$ is the plasma concentration versus time profiles of the tested formulations, rabs is the input rate of the oral solid dosage form in to the body and u is the variable of integration. Deconvolution method requires no assumptions regarding of the number of compartments in the model or the kinetics of absorption. Linear distribution and elimination are assumed. Like the Loo-Riegelman method, deconvolution requires data obtained after both oral and intravenous administration in the same subject and assumes no differences

in the pharmacokinetics of drug distribution and elimination from one study to the other. Drug concentrations must be measured at the same times following both oral and intravenous administration during the time that drug is absorbed after oral administration. Mean residence time is the mean time that the drug resides in the body and is calculated by following equation:

$$MRT = AUMC/AUC \quad ...(10)$$

Mean in vivo dissolution time reflects the mean time for drug to dissolve in vivo from a solid dosage form and is estimated as:

$$MDT_{solid} = MRT_{solid} - MRT_{solution} \quad ...(11)$$

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5.9 EVALUATION OF PREDICTABILITY OF IVIVC

An IVIVC should be evaluated to demonstrate that predictability of in-vivo performance of a drug product from its in vitro dissolution characteristics is maintained over a range of in vitro dissolution release rates and manufacturing changes (Figure 5.2). Since the objective of developing an IVIVC is to establish a predictive mathematical model describing the relationship between an in-vitro property and a relevant in-vivo response, the proposed evaluation approaches focus on the estimation of predictive performance or, conversely, prediction error.

Methodology for the evaluation of IVIVC predictability is an active area of investigation and a variety of methods are possible and potentially acceptable. A correlation should predict in vivo performance accurately and consistently. Depending on the intended application of an IVIVC and the therapeutic index of the drug, evaluation of prediction error internally and/or externally may be appropriate. Evaluation of internal predictability is based on the initial data used to define the IVIVC model (Figure 5.2).

Evaluation of external predictability is based on additional test data sets. Internal predictability is applied to IVIVC established using formulations with three or more release rates for non-narrow therapeutic index drugs exhibiting conclusive prediction error. If two formulations with different release rates are used to develop IVIVC, then the application of IVIVC would be limited to specified categories. Under these circumstances, for complete evaluation and subsequent full application of the IVIVC, prediction of error externally is recommended.

External predictability evaluation is not necessary unless the drug is a narrow therapeutic index, or only two release rates were used to develop the IVIVC, or, if the internal predictability criteria are not met *i.e.*, prediction error internally is inconclusive. However, since the IVIVC will potentially be used to predict the in vivo performance for future changes, it is of value to evaluate external predictability when additional data are available.

The objective of IVIVC evaluation is to estimate the magnitude of the error in predicting the in vivo bioavailability results from in vitro dissolution data (Figure 5.2). This objective should guide the choice and interpretation of evaluation methods. Any appropriate approach related to this objective may be used for evaluation of predictability.

Internal predictability

All IVIVCs should be studied regarding internal predictability. One recommended approach involves the use of the IVIVC model to predict each formulation's plasma

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concentration profile (or C_{max} and/or AUC for a multiple Level C IVIVC) from each respective formulation's dissolution data. This is performed for each formulation used to develop the IVIVC model (Figure 5.2). Practically, in vitro dissolution rates is first estimated from dissolution data and is converted to in vivo dissolution rates by using the IVIVC model generated slope and intercept. If the cumulative drug release profile is sigmoid, then the Hill equation could be used to parameterize the in vitro drug release.

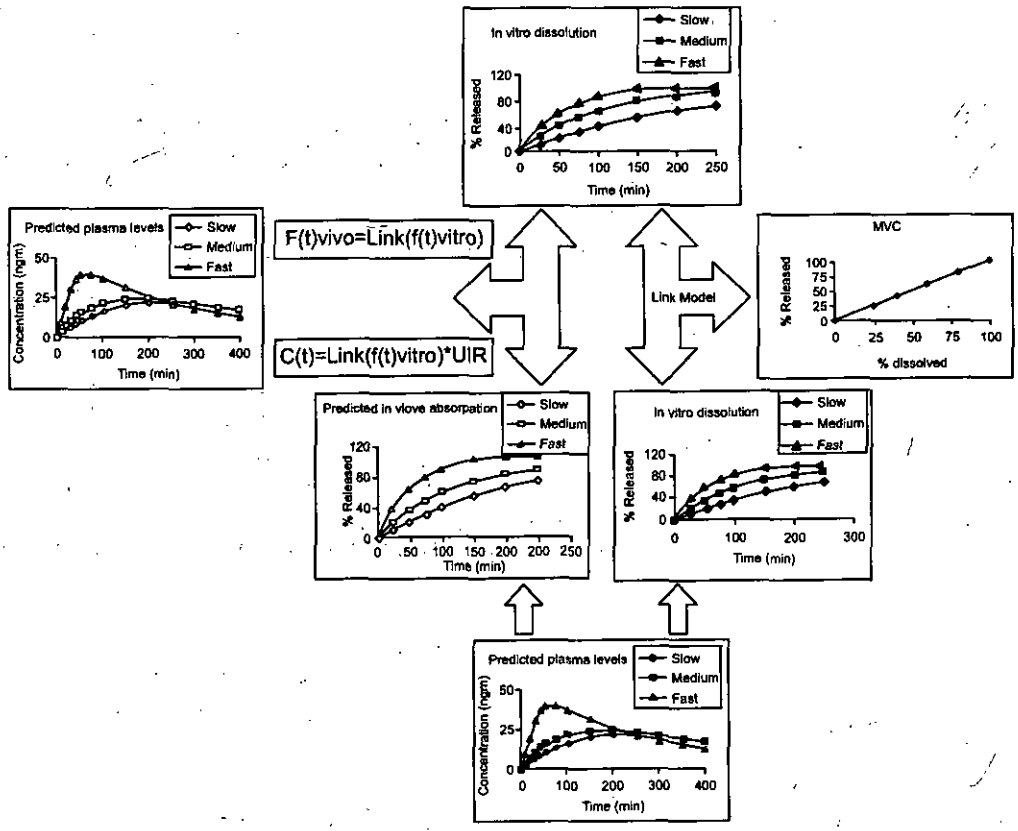

Fig. 5.2 *Representative observed and predicted dissolution and plasma profiles which routinely used to develop and validate an in vitro in vivo correlation*

$$\%\text{Dissolved} = D_{max} \times t^{\gamma} / D_{50}^{\gamma} \times t^{\gamma} \quad ...(12)$$

where, %D = the percent drug dissolved at time t, D_{max} = the maximum % drug dissolved, D_{50} = the time required for 50% of the drug to dissolve, t = time and γ = the sigmoidicity factor. In vitro release rates can be calculated by taking the first derivative of the Hill equation as listed below:

$$R_{dis} = \frac{d\%D}{dt} = \frac{\gamma D_{max} D_{50}^{\gamma} t^{\gamma-1}}{(D_{50}^{\gamma} + t^{\gamma})^2} \quad ...(13)$$

The prediction of the plasma concentrations from the corresponding in vivo dissolution profiles is then accomplished by convolution of the in vivo dissolution rates and the pharmacokinetic model for the so called unit impulse response result from i.v. bolus data, oral solution or rapidly releasing (in vivo) immediate release dosage forms using equation (9). In this equation symbols are as previously mentioned. The model predicted bioavailability is then compared to the observed bioavailability for each formulation. The percent prediction errors for C_t, C_{max} or AUC could be determined as follows:

$$\%PE = \frac{(\text{Observed Parameter} - \text{Predicted Parameter}) \times 100}{\text{Predicted Parameter}} \quad ...(14)$$

Average absolute percent prediction error (% PE) of 10% or less for Cmax and AUC establishes the predictability of the IVIVC. In addition, the % PE for each formulation should not exceed 15%. If these criteria are not met, that is, if the internal predictability of the IVIVC is inconclusive, evaluation of external predictability of the IVIVC should be performed as a final determination of the ability of the IVIVC to be used as a surrogate for bioequivalence.

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External predictability

Most important when using an IVIVC as a surrogate for bioequivalence is confidence that the IVIVC can predict in vivo performance of subsequent lots of the drug product. Therefore, it may be important to establish the external predictability of the IVIVC. This involves using the IVIVC to predict the in vivo performance for a formulation with known bioavailability that was not used in developing the IVIVC model. % PE of 10% or less for C and AUC establishes the external predictability of an IVIVC. % PE between 10 – 20% indicates inconclusive predictability and the need for further study using additional data sets. Results of estimation of PE from all such data sets should be evaluated for consistency of predictability. % PE greater than 20% generally indicates inadequate predictability, unless otherwise justified:

5.10 APPLICATION OF AN IVIVC

Biowaivers

The FDA guidance outlines five categories of biowaivers: (1) biowaivers without an IVIVC, (2) biowaivers using an IVIVC: non-narrow therapeutic index drugs, (3) biowaivers using an IVIVC: narrow therapeutic index drugs, (4) biowaivers when in vitro dissolution is independent of dissolution test conditions and (5) situations for which an IVIVC is not recommended for biowaivers .

Biowaivers may be granted for manufacturing site changes, equipment changes, manufacturing process changes, and formulation composition changes according to a predictive and reliable IVIVC. The changes may range from minor changes that are not significant to alter product performance to major ones where an IVIVC is not sufficient to justify the change for regulatory decision.

Establishment of Dissolution Specifications

It is relatively easy to establish a multipoint dissolution specification for modified-release dosage forms. The dissolution behavior of the biobatch maybe used to define the amount to be released at each time point. However, the difficulty arises in the variation to be allowed around each time point. The FDA guidance describes the procedures of setting dissolution specifications in cases of level A, multiple level C, and level C correlation and where there is no IVIV correlation.

Once an IVIVC developed, IVIVC should be used to set specifications in such a way that the fastest and lowest release rates allowed by the upper and lower dissolution specifications result in a maximum difference of 20% in the predicted C_{max} and AUC. Predicted plasma concentration and consequent AUC and C_{max} could be

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calculated using convolution or any other appropriate modeling techniques. In the case of multiple level C correlation, the last time point should be the time point where at least 80% of drug has dissolved. For level C correlation, reasonable deviations from ±10 % may be acceptable if the range at any time point does not exceed 25%. When there is no IVIVC, the tolerance limits may be derived from the spread of in vitro dissolution data of batches with demonstrated acceptable in vivo performance (biobatch), or by demonstrating bioequivalence between batches at the proposed upper and lower limit of the dissolution range (the so called *side batch concept*). Variability in release at each time point is recommended not to exceed a total numerical difference of ±10% (a total of 20%) or less of the labelled claim. In certain cases, deviations from this criterion can be acceptable up to a maximum range of 25%. Beyond this range, the specification should be supported by bioequivalence studies.

Concept of Mapping

Mapping is a process which relates Critical Manufacturing Variables (CMV), including formulation, processes, and equipment variables that can significantly affect drug release from the product, to a response surface derived from an in vitro dissolution curve and an in vivo bioavailability data. The mapping process defines boundaries of in vitro dissolution profiles on the basis of acceptable bioequivalency criteria. The goal is to develop product specifications that will ensure bioequivalance of future batches prepared within the limits of acceptable dissolution specifications. Dissolution specifications based on mapping would increase the credibility of dissolution as a bioequivalency surrogate marker and will provide continuous assurance and predictability of the product performance. Figure 5.3 shows the mapping provides for the employment of a dissolution method correlated to the rate and extent of drug bioavailability, which has also been optimized to be sensitive to CMV.

Mapping

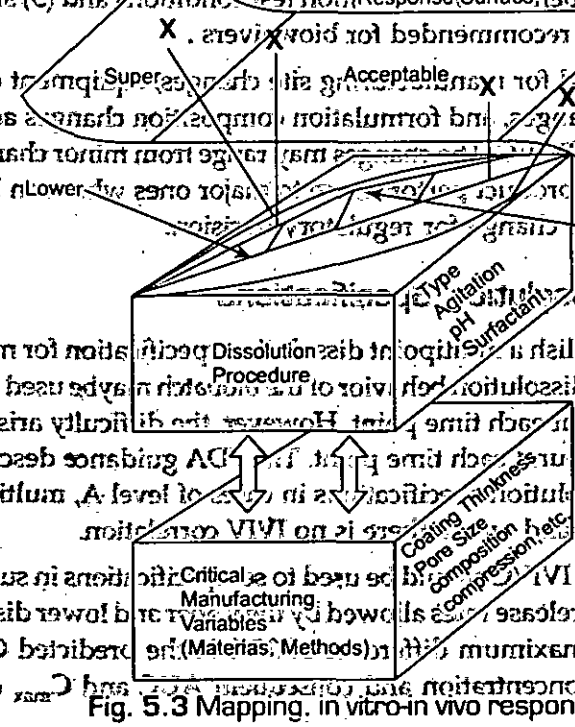

Fig. 5.3 Mapping. In vitro-In vivo response surface.

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SUMMARY

- A key goal in pharmaceutical development of dosage forms is a good understanding of the in vitro and in vivo performance of the dosage forms. One of the challenges of biopharmaceutics research is correlating in vitro drug release information of various drug formulations to the in vivo drug profiles (IVIVC).
- IVIVC is a predictive mathematical model describing the relationship between an in vitro property of a dosage form and a relevant in vivo response.
- This level of correlation is the highest category of correlation and represents a point-to-point relationship between in vitro dissolution rate and in vivo input rate of the drug from the dosage form (2).
- During the early stages of correlation development, dissolution conditions may be altered to attempt to develop a one-to-one correlation between the in vitro dissolution profile and the in vivo dissolution profile.
- The Biopharmaceutics Classification System (BCS) is a drug development tool that allows estimation of the contribution of three fundamental factors including dissolution, solubility and intestinal permeability, which govern the rate and extent of drug absorption from solid oral dosage forms (6).
- A bioavailability study should be performed to characterize the plasma concentration versus time profile for each of the formulation. Bioavailability studies for IVIVC development should be performed with sufficient number of subjects to characterize adequately the performance of the drug product under study.
- Methodology for the evaluation of IVIVC predictability is an active area of investigation and a variety of methods are possible and potentially acceptable.

REVIEW QUESTIONS

1. Discuss in vitro - in vivo correlation.
2. Discuss the definitions of in vitro - in vivo correlation.
3. Discuss the correlation levels in vitro - in vivo correlation.
4. What is the systematic development of a correlation?
5. What is the Bioavailability studies for development of IVIVC?

FURTHER READINGS

- Theory and Practice of Social Sciences, Ramakanth Tiwari and Mahesh Dabhade.

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UNIT VI: DOSAGE FORM DESIGN: PHARMACEUTICAL AND FORMULATION CONSIDERATIONS

★ STRUCTURE ★

6.1 LEARNING OBJECTIVES

After going through this unit, students will be able to:

- discuss the need for dosage forms
- describe general considerations in dosage form design
- state preformulation studies
- explain pharmaceutical ingredients and excipients definitions and types
- discuss the harmonization of standards
- state preservatives of pharmaceutical preparation.

Drug substances are seldom administered alone; rather they are given as part of a formulation in combination with one or more nonmedicinal agents that serve varied and specialized pharmaceutical functions. Selective use of these nonmedicinal agents, referred to as pharmaceutical ingredients or excipients, produces dosage forms of various types. The pharmaceutical ingredients solubilize, suspend, thicken, dilute, emulsify, stabilize, preserve, color, flavor, and fashion medicinal agents into efficacious and appealing dosage forms. Each type of dosage form is unique in its physical and pharmaceutical characteristics. These varied preparations provide the manufacturing and compounding pharmacist with the challenges of formulation

and the physician with the choice of drug and delivery system to prescribe. The general area of study concerned with the formulation, manufacture, stability, and effectiveness of pharmaceutical dosage forms is termed pharmaceutics.

The proper design and formulation of a dosage form requires consideration of the physical, chemical, and biologic characteristics of all of the drug substances and pharmaceutical ingredients to be used in fabricating the product. The drug and pharmaceutical materials must be compatible with one another to produce a drug product that is stable, efficacious, attractive, easy to administer, and safe. The product should be manufactured with appropriate measures of quality control and packaged in containers that keep the product stable. The product should be labelled to promote correct use and be stored under conditions that contribute to maximum shelf life.

Methods for the preparation of specific types of dosage forms and drug delivery systems are described in subsequent chapters. This chapter presents some general considerations regarding physical pharmacy, drug product formulation, and pharmaceutical ingredients.

6.2 THE NEED FOR DOSAGE FORMS

The potent nature and low dosage of most of the drugs in use today precludes any expectation that the general public could safely obtain the appropriate dose of a drug from the bulk material. Most drug substances are administered in milligram quantities, much too small to be weighed on anything but a sensitive prescription or electronic analytical balance. For instance, how could the lay person accurately obtain from a bulk supply the 325 mg of aspirin found in the common tablet? Not possible. Yet compared with many other drugs, the dose of aspirin is formidable (Table 6.1). For example, the dose of ethinyl estradiol, 0.05 mg, is 1/6,500 the amount of aspirin in an aspirin tablet. To put it another way, 6,500 ethinyl estradiol tablets, each containing 0.05 mg of drug, could be made from an amount of ethinyl estradiol equal to the amount of aspirin in just one standard tablet.

When the dose of the drug is minute, as with ethinyl estradiol, solid dosage forms such as tablets and capsules must be prepared with fillers or diluents so that the dosage unit is large enough to pick up with the fingertips. Besides providing the mechanism for the safe and convenient delivery of accurate dosage, dosage forms are needed for additional reasons:

- To protect the drug substance from the destructive influences of atmospheric oxygen or humidity (coated tablets; sealed ampuls)

Table 6.1 Some Drugs with Relatively Low Usual Doses

Drug	*Usual Dose(MG)*	*Category*
Betaxolol HCl	10.00	Antianginal
Clotrimoxazole	10.00	Antifungal
Methylphenidate HCl	10.00	CNS stimulant
Medroxyprogesterone acetate	10.00	Progestin
Mesoridazine besylate	10.00	Antipsychotic
Morphine sulfate	10.00	Narcotic analgesic
Nifedipine	10.00	Coronary vasodilator
Omeprazole	10.00	Antiulcerative
Quinapril HCl	10.00	Antihypertensive
Chlorazepate dipotassium	7.50	Tranquilizer
Buspirone HCl	5.00	Antianxiety

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Enalapril maleate	5.00	Antihypertensive
Hydrocodone	5.00	Narcotic analgesic
Prednisolone	5.00	Adrenocortical steroid
Albuterol sulfate	4.00	Bronchodilator
Chlorpheniramine maleate	4.00	Antihistaminic
Felodipine	2.50	Vasodilator
Glyburide	2.50	Antidiabetic
Doxazosin mesylate	2.00	Antihypertensive
Levorphanol tartrate	2.00	Narcotic analgesic
Prazosin HCl	2.00	Antihypertensive
Risperidone	2.00	Antipsychotic
Estropipate	1.25	Estrogen
Bumetanide	1.00	Diuretic
Clonazepam	1.00	Anticonvulsant
Ergoloid mesylates	1.00	Cognitive adjuvant
Alprazolam	0.50	Antianxiety
Colchicine	0.50	Gout suppressant
Nitroglycerin	0.40	Antianginal
Digoxin	0.25	Cardiotonic (maintenance)
Levothyroxine	0.10	Thyroid
Misoprostol	0.10	Antiulcerative, abortifacient
Ethinyl estradiol	0.05	Estrogen

- To protect the drug substance from the destructive influence of gastric acid after oral administration (enteric-coated tablets)
- To conceal the bitter, salty, or offensive taste or odor of a drug substance (capsules, coated tablets, flavored syrups)
- To provide liquid preparations of substances that are either insoluble or unstable in the desired vehicle (suspensions)
- To provide clear liquid dosage forms of substances (syrups, solutions)
- To provide rate-controlled drug action (various controlled-release tablets, capsules, and suspensions)
- To provide optimal drug action from topical administration sites (ointments, creams, transdermal patches, and ophthalmic, ear, and nasal preparations)
- To provide for insertion of a drug into one of the body's orifices (rectal or vaginal suppositories)
- To provide for placement of drugs directly in the bloodstream or body tissues (injections)
- To provide optimal drug action through inhalation therapy (inhalants and inhalation aerosols)

6.3 GENERAL CONSIDERATIONS IN DOSAGE FORM DESIGN

Before formulating a drug substance into a dosage form, the desired product type must be determined insofar as possible to establish the framework for product development. Then, various initial formulations of the product are developed and examined for desired features (*e.g.*, drug release profile, bioavailability, clinical effectiveness) and for pilot plant studies and production scale-up. The formulation that best meets the goals for the product is selected to be its *master formula*. Each batch

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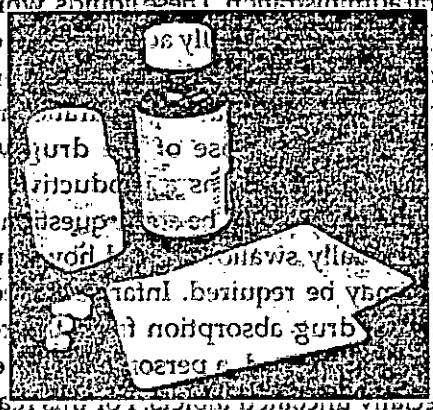

Fig. 6.1 Various forms of a drug substance marketed by a Pharmaceutical Company to meet the special requirements of the patient.

There are many different forms into which a medicinal agent may be placed for the convenient and efficacious treatment of disease. Most commonly, a manufacturer prepares a drug substance in several dosage forms and strengths for the efficacious and convenient treatment of disease (Figure 6.1). Before a medicinal agent is formulated into one or more dosage forms, among the factors considered are such therapeutic matters as the nature of the illness, the manner in which it is treated (locally or through systemic action), and the age and anticipated condition of the patient.

If the medication is intended for systemic use and oral administration is desired, tablets and/or capsules are usually prepared because they are easily handled by the patient and are most convenient in the self-administration of medication. If a drug substance has application in an emergency in which the patient may be comatose or unable to take oral medication, an injectable form of the medication may also be prepared. Many other examples of therapeutic situations affecting dosage form design could be cited, including motion sickness, nausea, and vomiting, for which tablets and skin patches are used for prevention and suppositories and injections for treatment.

Fig. 6.2 Oral dosage devices to assist in measuring doses for children

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The age of the intended patient also plays a role in dosage form design. For infants and children younger than 5 years of age, pharmaceutical liquids rather than solid forms are preferred for oral administration. These liquids, which are flavored aqueous solutions, syrups, or suspensions, are usually administered directly into the infant's or child's mouth by drop, spoon, or oral dispenser (Figure 6.2) or incorporated into the child's food. A single liquid pediatric preparation may be used for infants and children of all ages, with the dose of the drug varied by the volume administered. When a young patient has a productive cough or is vomiting, gagging, or simply rebellious, there may be some question as to how much of the medicine administered is actually swallowed and how much is expectorated. In such instances, injections may be required. Infant-size rectal suppositories may also be employed, although drug absorption from the rectum is often erratic. During childhood and even adulthood, a person may have difficulty swallowing solid dosage forms, especially uncoated tablets. For this reason, some medications are formulated as chewable tablets. Many of these tablets are comparable in texture to an after-dinner mint and break down into a pleasant-tasting creamy material.

Newly available tablets dissolve in the mouth in about 10 to 15 seconds; this allows the patient to take a tablet but actually swallow a liquid. Capsules have been found by many to be more easily swallowed than whole tablets. If a capsule is moistened in the mouth before it is swallowed, it becomes slippery and readily slides down the throat with water. Also, a teaspoonful of gelatin dessert, liquid candy, or syrup placed in the mouth and partially swallowed before placing the solid dosage form in the mouth aids in swallowing them. Also, if a person has difficulty swallowing a capsule, the contents may be emptied into a spoon, mixed with jam, honey, or other similar food to mask the taste of the medication and swallowed. Medications intended for the elderly are commonly formulated into oral liquids or may be extemporaneously prepared into an oral liquid by the pharmacist. However, certain tablets and capsules that are designed for controlled release should not be crushed or chewed, because that would interfere with their integrity and intended performance.

Many patients, particularly the elderly, take multiple medications daily. The more distinctive the size, shape, and color of solid dosage forms, the easier is proper identification of the medications. Errors in taking medications among the elderly occur frequently because of their multiple drug therapy and impaired eyesight. Dosage forms that allow reduced frequency of administration without sacrifice of efficiency are particularly advantageous.

In dealing with the problem of formulating a drug substance into a proper dosage form, research pharmacists employ knowledge gained through experience with other chemically similar drugs and through the proper use of the physical, chemical, biologic, and pharmaceutical sciences. The early stages of any new formulation include studies to collect basic information on the physical and chemical characteristics of the drug substance. These basic studies are the *preformulation* work needed before actual product formulation begins.

6.4 PREFORMULATION STUDIES

Before the formulation of a drug substance into a dosage form, it is essential that it be chemically and physically characterized. The following *preformulation studies* (1) and others provide the type of information needed to define the nature of the drug

substance. This information provides the framework for the drug's combination with pharmaceutical ingredients in the fabrication of a dosage form.

Physical Description

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It is important to understand the physical description of a drug substance prior to dosage form development. Most drug substances in use today are solid materials, pure chemical compounds of either crystalline or amorphous constitution. The purity of the chemical substance is essential for its identification and for evaluation of its chemical, physical, and biologic properties. Chemical properties include structure, form, and reactivity. Physical properties include such characteristics as its physical description, particle size, crystalline structure, melting point, and solubility. Biologic properties relate to its ability to get to a site of action and elicit a biologic response.

Drugs can be used therapeutically as solids, liquids, and gases. Liquid drugs are used to a much lesser extent than solid drugs; gases, even less frequently. Liquid drugs pose an interesting problem in the design of dosage forms and delivery systems. Many liquids are volatile and must be physically sealed from the atmosphere to prevent evaporation loss. Amyl nitrite, for example, is a clear yellowish liquid that is volatile even at low temperatures and is also highly flammable. It is kept for medicinal purposes in small sealed glass cylinders wrapped with gauze or another suitable material. When amyl nitrite is administered, the glass is broken between the fingertips, and the liquid wets the gauze covering, producing vapors that are inhaled by the patient requiring vasodilation. Propylhexedrine is another volatile liquid that must be contained in a closed system.

This drug is used as a nasal inhalant for its vasoconstrictor action. A cylindrical roll of fibrous material is impregnated with propylhexedrine, and the saturated cylinder is placed in a suitable, usually plastic, sealed nasal inhaler. The inhaler's cap must be securely tightened each time it is used. Even then, the inhaler maintains its effectiveness for only a limited time because of the volatility of the drug.

Another problem associated with liquid drugs is that those intended for oral administration cannot generally be formulated into tablet form, the most popular form of oral medication, without chemical modification. An exception to this is the liquid drug nitroglycerin, which is formulated into sublingual tablets that disintegrate within seconds after placement under the tongue. However, because the drug is volatile, it has a tendency to escape from the tablets during storage, and it is critical that the tablets be stored in a tightly sealed glass container. For the most part, when a liquid drug is to be administered orally and a solid dosage form is desired, one of two approaches is used. First, the liquid substance may be sealed in a soft gelatin capsule. Vitamins A, D, and E, cyclosporin (Neoral, Sandimmune), and ergoloid mesylates (Hydergine LC) are liquids commercially available in capsule form. Second, the liquid drug may be developed into a solid ester or salt form that will be suitable for tablets or drug capsules. For instance, scopolamine hydrobromide is a solid salt of the liquid drug scopolamine and is easily pressed into tablets. Another approach to formulate liquids into solids is by mixing the drug with a solid or melted semisolid material, such as a high-molecular-weight polyethylene glycol. The melted mixture is poured into hard gelatin capsules to harden and the capsules sealed. For certain liquid drugs, especially those taken orally in large doses or applied topically, their liquid nature may have some advantage in therapy. For example, 15 mL doses of mineral oil may be administered conveniently as such.

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Also, the liquid nature of undecylenic acid certainly does not hinder but rather enhances its use topically in the treatment of fungus infections of the skin. However, for the most part, pharmacists prefer solid materials in formulation work because they can easily form them into tablets and capsules. Formulation and stability difficulties arise less frequently with solid dosage forms than with liquid preparations, and for this reason many new drugs first reach the market as tablets or dryfilled capsules. Later, when the pharmaceutical problems are resolved, a liquid form of the same drug may be marketed. This procedure is doubly advantageous, because for the most part physicians and patients alike prefer small, generally tasteless, accurately dosed tablets or capsules to the analogous liquid forms. Therefore, marketing a drug in solid form first is more practical for the manufacturer and suits most patients. It is estimated that tablets and capsules constitute the dosage form dispensed 70% of the time by community pharmacists, with tablets dispensed twice as frequently as capsules.

Microscopic Examination

Microscopic examination of the raw drug substance is an important step in preformulation work. It gives an indication of particle size and size range of the raw material along with the crystal structures. Photomicrographs of the initial and subsequent batch lots of the drug substance can provide important information in case of problems in formulation processing attributable to changes in particle or crystal characteristics of the drug. During some processing procedures, the solid drug powders must flow freely and not become entangled. Spherical and oval powders flow more easily than needle-shaped powders and make processing easier.

Heat of Vaporization

The use of vapor pressure is important in the operation of implantable pumps delivering medications as well as in aerosol dosage forms. Another application is the use of nasal inhalants (propylhexedrine with menthol and lavender oil: Benzedrex) for treating nasal congestion. In this latter dosage form, the quantity of drug required for effectiveness and a reasonable estimate of time of usefulness can be determined. Also, in the case of spills in inaccessible places, the time to evaporation of a substance can also be calculated. Some volatile drugs can even migrate within a tablet dosage form so the distribution may not be uniform any longer. This may have an impact in tablets that are scored for dosing where the drug in one portion may be higher or lower than in the other portion. Exposure of personnel to hazardous drugs due to handling, spilling, or aerosolizing of drugs that may vaporize (oncology agents) is another application as the increase in mobility of the hazardous drug molecules may be related to temperature of the environment. Some drugs, such as carmustine, experience greater vapor pressures with increased temperature as compared to cyclophosphamide, etoposide, cisplatin, and 5-fluorouracil, as illustrated in Physical Pharmacy Capsule 1, Heat of Vaporization.

Melting Point Depression

A characteristic of a pure substance is a defined melting point or melting range. If not pure, the substance will exhibit a change in melting point. This phenomenon is commonly used to determine the purity of a drug substance and in some cases the compatibility of various substances before inclusion in the same dosage form.

This characteristic is further described in Physical Pharmacy Capsule 2, Melting Point Depression.

The Phase Rule

Phase diagrams are often constructed to provide a visual picture of the existence and extent of the presence of solid and liquid phases in binary, ternary, and other mixtures. Phase diagrams are normally two-component (binary) representations, as shown in Physical Pharmacy Capsule 3, The Phase Rule, but can also be three-component representations, as shown in Physical Pharmacy Capsule 4, Triangular Phase Diagram.

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Particle Size

Certain physical and chemical properties of drug substances, including dissolution rate, bioavailability, content uniformity, taste, texture, color, and stability, are affected by the particle size distribution. In addition, flow characteristics and sedimentation rates, among other properties, are important factors related to particle size. It is essential to establish as early as possible how the particle size of the drug substance may affect formulation and efficacy. Of special interest is the effect of particle size on absorption. Particle size significantly influences the oral absorption profiles of certain drugs, including griseofulvin, nitrofurantoin, spironolactone, and procaine penicillin. Also, satisfactory content uniformity in solid dosage forms depends to a large degree on particle size and the equal distribution of the active ingredient throughout the formulation. Figure 6.3 shows a particle size analyzer.

CAPSULE 1

Heat of Vaporization

The amount of heat absorbed when 1 g of a liquid vaporizes is known as the heat of vaporization of that liquid and is measured in calories. The heat of vaporization of water at 100°C is 540 cal/g or about 9.720 cal/mofle. This is the same quantity of heat energy that is released when 1 g of steam condenses to water at 100°C. This energy exchange is important in processes like steam sterilization as it is this energy transfer that results in death of microorganisms.

The movement of molecules varies with temperature. In liquids, this results in a tendency of the molecules to escape the liquid environment into a gaseous environment and possibly loss of the liquid. In the case of solids that sublime, the movement of the molecules is from the solid state to the vapor state. As an example, if one looks at an older bottle containing aspirin, there may be crystals of aspirin on the inside walls of the container. With ibuprofen, the walls of the container may become cloudy as the ibuprofen sublimes.

The use of vapor pressure is important in the operation of implantable pumps delivering medications as well as in aerosol dosage forms. Exposure of personnel to hazardous drugs due to handling, spilling, or aerosolizing of drugs that may vaporize (oncology agents) is another application as the increase in mobility of the hazardous drug molecules may be related to temperature of the environment. Some drugs, such as carmustine, experience greater vapor pressures with increased

temperature as compared to cyclophosphamide, etoposide, cisplatn and 5-fluorouracil, as illustrated in the table below. Particle size affects vapor pressure; the smaller the particle size, the greater the vapor pressure. This demonstrates the importance of personnel protection with working with micronized hazardous powders. The time to evaporation of a substance can also be calculated.

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PHYSICAL PHARMACY CAPSULE 6.1 CONT.

The variation of vapor pressure with temperature is described by the form of the Clausius-Clapeyron equation, as follows:

$$\frac{d \ln p}{dT} = \frac{\Delta H_{vap}}{RT^2}$$

assuming that δH_{vap} is constant, integration of the equation gives:

$$\log P = \frac{-\Delta H_{vap}}{2.303\,RT} + \text{constant}$$

A plot of the log of the vapor pressure versus 1 /T should be linear and the slope will equal $-\Delta H_{vap}/2.303$ R from which the enthalpy of vaporization can be calculated. With data obtained from Kiffmever TK, Kube C, Opiolka S, et al. Pharm J 2002;268:331, the following table can be constructed:

	Measured Vapor Pressure (Pa)	
Compound	*20°C*	*40°C*
Carmustine	0.019	0.530
Cisplatin	0.0018	0.0031
Cyclophosphamide	0.0033	0.0090
Etoposide	0.0026	0.0038
Fluorouracil	0.0014	0.0039

CAPSULE 2

Melting Point Depression

The *melting point,* or *freezing point,* of a pure crystalline solid is defined as the temperature at which the pure liquid and solid exist in equilibrium. Drugs with a low melting point may soften during a processing step in which heat is generated, such as particle size reduction, compression, sintering, and so on. Also, the melting point or range of a drug can be used as an indicator of purity of chemical substances (a pure substance is ordinarily characterized by a very sharp melting peak). An altered peak or a peak at a different temperature may indicate an adulterated or impure drug. This is explained as follows:

The *latent heat of fusion* is the quantity of heat absorbed when 1 g of a solid melts; the molar heat of fusion (ΔH_f) is the quantity of heat absorbed when 1 mole of a solid melts. High-melting-point substances have high heat of fusion, and low-melting-point substances have low heat of fusion. These characteristics are related to the types of bonding in the specific substance. For example, ionic materials have high heats of fusion (NaCl melts at 801°C with a heat of fusion of 124 cal/g),

and those with weaker van der Waals forces have low heats of fusion (paraffin melts at 52°C with a heat of fusion of 35.1 cal/g). Ice, with weaker hydrogen bonding, has a melting point of 0°C and a heat of fusion of 80 cal/g.

The addition of a second component to a pure compound (A), resulting in a mixture, will result in a melting point that is lower than that of the pure compound. The degree to which the melting point is lowered is proportional to the mole fraction (N_A) of the second component that is added. This can be expressed thus:

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$$\Delta T = \frac{2.303\, RTT_0}{\Delta H_f} \log N_A$$

where ΔH_f is the molar heat of fusion,
T is the absolute equilibrium temperature,
T_0 *is the melting point of pure A, and*
R is the gas constant.

PHYSICAL PHARMACY CAPSULE 6.2 CONT.

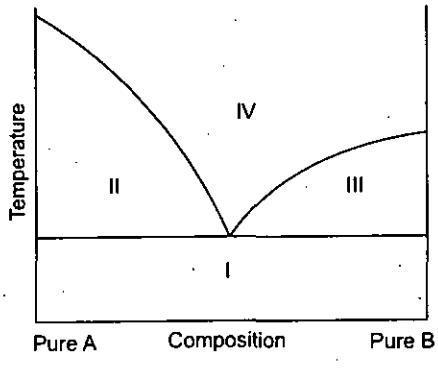
Two noteworthy things contribute to the extent of lowering of the melting point:

1. Evident from this relationship is the inverse proportion between the melting point and the heat of fusion. When a second ingredient is added to a compound with a low molar heat of fusion, a large lowering of the melting point is observed; substances with a high molar heat of fusion will show little change in melting point with the addition of a second component.
2. The extent of lowering of the melting point is also related to the melting point itself. Compounds with low melting points are affected to a greater extent than compounds with high melting points upon the addition of a second component (*i.e.*, low-melting-point compounds will result in a greater lowering of the melting point than those with high melting points).

CAPSULE 3

The Phase Rule

A phase diagram, or temperature-composition diagram, represents the melting point as a fun-ction of composition of two or three component systems. The figure is an example of such a rep-resentation for a two-component mixture.



This phase diagram depicts a two-component mixture in which the components are completely miscible in the molten state and no solid solution or addition compound is formed in the solid state. As is evident, starting from the extremes of either pure component A or pure component B, as the second component is added, the melting point of the pure component decreases. There is a point on this phase diagram at which a minimum melting point occurs (*i.e.*, the eutectic

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point). As is evident, four regions, or phases, in this diagram, represent the following:

I Solid A + solid B

II Solid A + melt

III Solid B + melt

IV Melt

Each phase is a homogenous part of the system, physically separated by distinct boundaries.

A description of the conditions under which these phases can exist is called the *Phase Rule*, which can be presented thus:

$$F = C - P + X$$

where F is the number of degrees of freedom,

C is the number of components,

P is the number of phases, and

X is a variable dependent upon selected considerations of the phase diagram (1, 2, or 3).

C describes the minimum number of chemical components to be specified to define the phases. F is the number of independent variables that must be specified to define the complete system (*e.g.*, temperature, pressure, concentration).

Example 1. In a mixture of menthol and thymol, a phase diagram similar to that illustrated can be obtained. To describe the number of degrees of freedom in the part of the graph moving from the curved line starting at pure A, progressing downward to the eutectic point, and then following an increasing melting point to pure B, it is evident from this presentation that either temperature or composition will describe this system, since it is assumed in this instance that pressure is constant. Therefore, the number of degrees of freedom to describe this portion of the phase diagram is given thus:

$$F = 2 - 2 + 1 = 1$$

In other words, along this line either temperature or composition will describe the system.

Example 2. When in the area of a single phase of the diagram, such as the melt (IV), the system can be described thus:

$$F = 2 - 1 + 1 = 2$$

In this portion of the phase diagram, two factors, temperature and composition, can be varied without a change in the number of phases in the system.

Example 3. At the eutectic point,

$$F = 2 - 3 + 1 = 0$$

and any change in the concentration or temperature may cause disappearance of one of the two solid phases or the liquid phase.

Phase diagrams are valuable for interpreting interactions between two or more components, relating not only to melting point depression and possible liquefaction at room temperature but also the formation of solid solutions, coprecipitates, and other solid-state interactions.

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CAPSULE 4

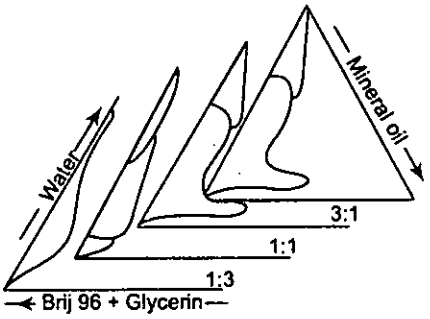
Triangular (Three-component) Phase Diagram

A three-component phase diagram has four degrees of freedom: F = 3 – 1 + 2 = 4. In this case, temperature and pressure are two of the conditions and the concentrations of two of the three components make up the rest. Only two concentrations are required because the third will be the difference between 100% and the sum of the other two components.

These systems are used for determining miscibility/solubility, coacervation regions, gel-forming regions for multicomponent mixtures, etc. To read a 3-phase diagram, each of the three corners of the triangle represent 100% by weight of one of the components (A, B, C) and 0% by weight of the other two (A, B, C). The lines joining the corner points forming the triangle each represent two component mixtures of the three possible combinations (AB, BC, and CA). If two of the components are known, the third is known by difference. Any combination of the three components is described by a single point on the diagram. Combining different proportions of the three components and observing for an end point (solubility, gel-formation, haziness, etc.), the phase differences can be visualized, as follows.

PHYSICAL PHARMACY CAPSULE 6.4 CONT.

The following is a stack of four separate pseudoternary phase diagrams for a quaternary system composed of Brij 96, glycerin, mineral oil, and water. The Brij 96:glycerin ratio is noted on the diagram and is considered one of three components. The shaded regions represent gel systems while the clear regions represent fluid systems.



In addition to observing the phase changes in a single plane, the use of stacked ternary phase diagrams enables one to visualize the change using different ratios of one of the components (in this case, the Brij 96:glycerin ratios). Constructions like this enable a pharmaceutical scientist to select the best ratios and combinations of components for a formulation.

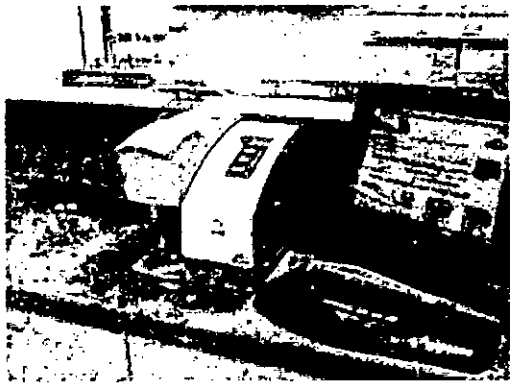


Fig. 6.3 Mastersizer 2000E particle size analyzer.

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Polymorphism

An important factor on formulation is the crystal or amorphous form of the drug substance. Polymorphic forms usually exhibit different physicochemical properties, including melting point and solubility. Polymorphic forms in drugs are relatively common. It has been estimated that at least one third of all organic compounds exhibit polymorphism.

In addition to polymorphic forms, compounds may occur in noncrystalline or amorphous forms. The energy required for a molecule of drug to escape from a crystal is much greater than is required to escape from an amorphous powder. Therefore, the amorphous form of a compound is always more soluble than a corresponding crystal form.

Evaluation of crystal structure, polymorphism, and solvate form is an important preformulation activity. The changes in crystal characteristics can influence bioavailability and chemical and physical stability and can have important implications in dosage form process functions. For example, it can be a significant factor relating to tablet formation because of flow and compaction behaviors, among others. Various techniques are used to determine crystal properties. The most widely used methods are hot stage microscopy, thermal analysis, infrared spectroscopy, and X-ray diffraction.

Solubility

An important physicochemical property of a drug substance is solubility, especially aqueous system solubility. A drug must possess some aqueous solubility for therapeutic efficacy. For a drug to enter the systemic circulation and exert a therapeutic effect, it must first be in solution. Relatively insoluble compounds often exhibit incomplete or erratic absorption. If the solubility of the drug substance is less than desirable, consideration must be given to improve its solubility.

The methods to accomplish this depend on the chemical nature of the drug and the type of drug product under consideration. Chemical modification of the drug into salt or ester forms is frequently used to increase solubility.

A drug's solubility is usually determined by the equilibrium solubility method, by which an excess of the drug is placed in a solvent and shaken at a constant temperature over a long period until equilibrium is obtained. Chemical analysis of the drug content in solution is performed to determine degree of solubility.

Solubility and Particle Size

Although solubility is normally considered a physicochemical constant, small increases in solubility can be accomplished by particle size reduction as described in the Physical Pharmacy Capsule 5, Solubility and Particle Size.

Solubility and pH

Another technique, if the drug is to be formulated into a liquid product, is adjustment of the pH of the solvent to enhance solubility. However, for many drug substances pH adjustment is not an effective means of improving solubility. Weak acidic or basic drugs may require extremes in pH that are outside accepted physiologic limits or that may cause stability problems with formulation ingredients. Adjustment of pH usually has little effect on the solubility of substances other than electrolytes. In many cases, it is desirable to use cosolvents or other techniques such as complexation,

micronization, or solid dispersion to improve aqueous solubility. A review of pH is provided in Physical Pharmacy Capsule 6, Principles of pH. The effect of pH on solubility is illustrated in Physical Pharmacy Capsule 7, Solubility and pH.

In recent years, more and more physicochemical information on drugs is being made available to pharmacists in routinely used reference books. This type of information is important for pharmacists in different types of practice, especially those involved in compounding and pharmacokinetic monitoring.

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CAPSULE 5

Solubility and Particle Size

The particle size and surface area of a drug exposed to a medium can affect actual solubility within reason, for example, in the following relationship:

$$\log \frac{S}{S_0} = \frac{2\gamma V}{2.303\,RTr}$$

where S is the solubility of the small particles,
S_0 is the solubility of the large particles,
γ is the surface tension,
V is the molar volume,
R is the gas constant,
T is the absolute temperature, and
r is the radius of the small particles.

The equation can be used to estimate the decrease in particle size required to increase solubility. For example, a desired increase in solubility of 5% would require an increase in the S/S_0 ratio to 1.05; that is, the left term in the equation would become log 1.05. If a powder has a surface tension of 125 dynes per centimetre, molar volume of 45 cm^3, and temperature of 27°C, what is the particle size required to obtain the 5% increase in solubility?

$$\log 1.05 = \frac{(2)(125)(45)}{(2.303)(8.314 \times 10^7)(300)r}$$

$$r = 9.238 \times 10^{-6} \text{ cm or } 0.09238\ \mu$$

A number of factors are involved in actual solubility enhancement, and this is only an introduction to the general effects of particle size reduction.

Dissolution

Variations in the biologic activity of a drug substance may be brought about by the rate at which it becomes available to the organism. In many instances, dissolution rate, or the time it takes for the drug to dissolve in the fluids at the absorption site, is the rate-limiting step in absorption. This is true for drugs administered orally in solid forms such as tablets, capsules, or suspensions, and for those administered intramuscularly. When the dissolution rate is the rate-limiting step, anything that affects it will also affect absorption. Consequently, dissolution rate can affect the onset, intensity, and duration of response and control the overall bioavailability of the drug from the dosage form, as discussed in the previous chapter.

The dissolution rate of drugs may be increased by decreasing the drug's particle size. It may also be increased by increasing its solubility in the diffusion layer. The most effective means of obtaining higher dissolution rates is to use a highly water-soluble salt of the parent substance. Although a soluble salt of a weak acid will precipitate as the free acid in the bulk phase of an acidic solution, such as gastric fluid, it will do so in the form of fine particles with a large surface area.

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CAPSULE 6

Principles of pH

pH is a critical variable in pharmaceutics, and a basic understanding of its principles and measurement are important. Let's begin with a definition of the term pH. The p comes from the word power. The H, of course, is the symbol for hydrogen. Together, the term pH means the hydrogen ion exponent.

The pH of a substance is a measure of its acidity, just as a degree is a measure of temperature. A specific pH value tells the exact acidity. Rather than stating general ideas, such as cherry syrup is acidic or the water is hot, a specific pH value gives the same relative point of reference, thus providing more exact communication. "The cherry juice has a pH of 3.5" or "the water is at 80°C". provides an exact common language.

pH is defined in terms of the hydrogen ion activity:

$$pH = -\log_{10} a_{H+} \text{ or } 10^{-pH} = a_{H+}$$

pH equals the negative logarithm of the hydrogen ion activity, or the activity of the hydrogen ion is 10 raised to the exponent – pH. The latter expression renders the use of the p exponent more obvious. The activity is the effective concentration of the hydrogen ion in solution. The difference between effective and actual concentration decreases as one moves toward more dilute solutions, in which ionic interaction becomes progressively less important.

Normally, reference is made to the hydrogen ion when reference should be made to the hydronium ion (H_3O^+). It is a matter of convenience and brevity that only the hydrogen ion is mentioned, even though it is normally in its solvated form:

$$H^+ + H_2O = H_3O^+$$

The complexing of the hydrogen ion by water affects activity and applies to other ions, which partially complex or establish an equilibrium with the hydrogen ion. In other words, equilibrium such as

$$H_2CO_3 = H^+ + HCO_3^-$$
$$HC_2H_3O_2 = H^+ + C_2H_3O_2^-$$

complexes the hydrogen ion so that it is not sensed by the pH measuring system. This is why an acid-base titration is performed if the total concentration of acid (H^+) is needed. These effects on hydrogen ion activity are obvious, but other more subtle effects are involved in the correlation of activity and concentration. The activity of the hydrogen ion can be defined by its relation to concentration {C_H^+, molality} and the activity coefficient f_H^+:

$$aH^+ = f_H + C_H^+$$

If the activity coefficient is unity, activity is equal to concentration. This is nearly the case in dilute solutions, whose ionic strength is low. Since the objective of most

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pH measurements is to find a stable and reproducible reading that can be correlated to the results of some process, it is important to know what influences the activity coefficient and therefore the pH measurement.

The factors that affect the activity coefficient are the temperature (T), the ionic strength (μ), the dielectric constant (ε), the ion charge (Z_j), the size of the ion in angstroms (Å), and the density of the solvent (d), All of these factors are characteristics of the solution that relate the activity to the concentration by two main effects: the salt effect and the medium effect; the latter relates the influence that the solvent can have on the hydrogen ion activity. Thus, hydrogen activity is related to concentration through a salt effect and a solvent effect. Because of these influences, a sample pH value cannot be extrapolated to another temperature or dilution. If the pH value of a particular solution is known at 40°C, it is not automatically known at 25°C.

The pH Scale

In pure water, hydrogen and hydroxyl ion concentrations are equal at 10^{-7} M at 25°C. This is a neutral solution. Since most samples encountered have less than 1 M H^+ or OH^-, the extremes of pH 0 for acids and pH 14 for bases are established. Of course, with strong acids or bases, pH values below 0 and above 14 are possible but infrequently measured.

Measurement of pH

The activity of the hydrogen ion in solution is measured with a glass electrode, a reference electrode, and a pH meter.

Combination Electrodes

A combination electrode is a combination of the glass and reference electrodes into a single probe. The main advantage in using a combination electrode is with the measurement of small volume samples or samples in limited-access containers.

CAPSULE 7

Solubility and pH

pH is one of the most important factors in the formulation process. Two areas of critical importance are the effects of pH on solubility and stability. The effect of pH on solubility is critical in the formulation of liquid dosage forms, from oral and topical solutions to intravenous solutions and admixtures.

The solubility of a weak acid or base is often pH dependent. The total quantity of a monoprotic weak acid (HA) in solution at a specific pH is the sum of the concentrations of both the free acid and salt (A^-) forms. If excess drug is present, the quantity of free acid in solution is maximized and constant because of its saturation solubility. As the pH of the solution increases, the quantity of drug in solution increases because the water-soluble ionizable salt is formed. The expression is

$$HA \overset{K_a}{\leftrightarrow} H^+ + A^-$$

where K_a is the dissociation constant.

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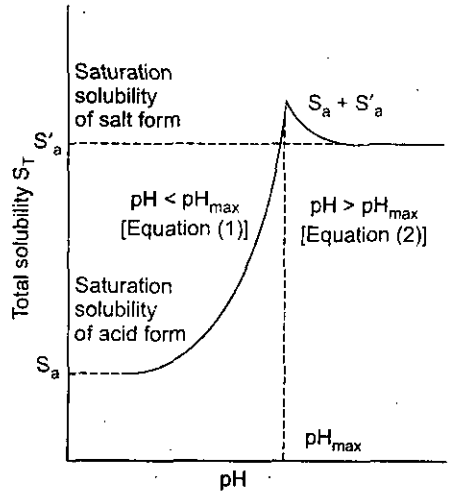
There may be a certain pH level reached where the total solubility (S_T) of the drug solution is saturated with respect to both the salt and acid forms of the drug, that is, the pH_{max}. The solution can be saturated with respect to the salt at pH values higher than this, but not with respect to the acid. Also, at pH values less than this, the solution can be saturated with respect to the acid but not to the salt. This is illustrated in the accompanying figure.



To calculate the total quantity of drug that can be maintained in solution at a selected pH, either of two equations can be used, depending on whether the product is to be in a pH region above or below the pH_{max}. The following equation is used when below the pH_{max} :

$$S_T = S_a\left(1+\frac{K_a}{[H^+]}\right) \qquad \ldots(1)$$

The next equation is used when above the pH_{max}

$$S_T = S'_a\left(1+\frac{K_a}{[H^+]}\right) \qquad \ldots(2)$$

where S_a is the saturation solubility of the free acid and
S'_a is the saturation solubility of the salt form.

Example

A pharmacist prepares a 3.0% solution of an antibiotic as an ophthalmic solution and dispenses it to a patient. A few days later the patient returns the eye drops to the pharmacist because the product contains a precipitate. The pharmacist, checking the pH of the solution and finding it to be 6.0, reasons that the problem may be pH related. The physicochemical information of interest on the antibiotic includes the following:

Molecular weight	285 (salt) 263 (free acid)
3.0% solution of the drug	0.1053 M solution
Acid form solubility (S_a)	3.1 mg/mL(0.0118 M)
K_a	5.86×10^{-6}

Using equation (1), the pharmacist calculates the quantity of the antibiotic in solution at a pH of 6.0 (**Note:** pH of 6.0 = $[H^+]$ of 1×10^{-6})

$$S_T = 0.0118\ [1^+] = 0.0809 \text{ molar}$$

From this the pharmacist knows that at a pH of 6.0, a 0.0809 M solution can be prepared. However, the concentration that was to be prepared was a 0.1053 M solution; consequently, the drug will not be in solution at that pH. The pH may have been all right initially but shifted to a lower pH over time, resulting in precipitation of the drug. The question is at what pH (hydrogen ion concentration) the drug will remain in solution. This can be calculated using the same equation and information. The S_T value is 0.1053 M.

$$0.1053 = 0.0118\left[1 + \frac{5.86 \times 10^{-6}}{[H^+]}\right]$$

$$[H^+] = 7.333 \times 10^{-7}, \text{ or a pH of } 6.135$$

The pharmacist prepares a solution of the antibiotic, adjusting the pH to above about 6.2, using a suitable buffer system, and dispenses the solution to the patient—with positive results.

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An interesting phenomenon concerns the close relationship of pH to solubility. At a pH of 6.0, only a 0.0809 M solution could be prepared, but at a pH of 6.13 a 0.1053 M solution could be prepared. In other words, a difference of 0.13 pH units resulted in

$$\frac{0.1053 - 0.0809}{0.0809} = 30.1\%$$

more drug going into solution at the higher pH than at the lower pH. In other words, a very small change in pH resulted in about 30% more drug going into solution. According to the figure, the slope of the curve would be very steep for this example drug, and a small change in pH (x-axis) results in a large change in solubility (y-axis). From this, it can be reasoned that if one observes the pH-solubility profile of a drug, it is possible to predict the magnitude of the pH change on its solubility.

In recent years, more and more physicochemical information on drugs is being made available to pharmacists in routinely used reference books. This type of information is important for pharmacists in different types of practice, especially those who compound and do pharmacokinetic monitoring.

The dissolution rates of chemical compounds are determined by two methods: the constant surface method, which provides the intrinsic dissolution rate of the agent, and particulate dissolution, in which a suspension of the agent is added to a fixed amount of solvent without exact control of surface area. The constant-surface method uses a compressed disc of known area. This method eliminates surface area and surface electrical charges as dissolution variables. The dissolution rate obtained by this method, the *intrinsic dissolution rate*, is characteristic of each solid compound and a given solvent in the fixed experimental conditions. The value is expressed as milligrams dissolved per minute per centimetres squared. It has been suggested that this value is useful in predicting probable absorption problems due to dissolution rate. In particulate dissolution, a weighed amount of powdered sample is added to the dissolution medium in a constant agitation system. This method is frequently used to study the influence of particle size, surface area, and excipients upon the active agent. Occasionally, the surface properties of the drug produce an inverse relationship of particle size to dissolution. In these instances, surface charge and/or agglomeration results in the reduced particle size form of the drug presenting a lower effective surface area to the solvent due to incomplete wetting or agglomeration. Fick's laws describe the relationship of diffusion and dissolution of the active drug in the dosage form and when administered in the body, as shown in Physical Pharmacy Capsule 8, Fick's Laws of Diffusion and the Noyes-Whitney Equation. Early formulation studies should include the effects of pharmaceutical ingredients on the dissolution characteristics of the drug substance.

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Membrane Permeability

Modern preformulation studies include an early assessment of passage of drug molecules across biologic membranes. To produce a biologic response, the drug molecule must first cross a biologic membrane. The biologic membrane acts as a lipid barrier to most drugs and permits the absorption of lipid-soluble substances by passive diffusion, while lipid-insoluble.

CAPSULE 8

Fick's Laws of Diffusion and the Noyes-Whitney Equation

All drugs must diffuse through various barriers when administered to the body. For example, some drugs must diffuse through the skin, gastric mucosa, or some other barrier to gain access to the interior of the body. Parenteral drugs must diffuse through muscle, connective tissue, and so on, to get to the site of action, even intravenous drugs must diffuse from the blood to the site of action. Drugs must also diffuse through various barriers for metabolism and excretion.

Considering all the diffusion processes that occur in the body (passive, active, and facilitated), it is not surprising that the laws governing diffusion are important to drug delivery systems. In fact, diffusion is important not only in the body but also in some quality control procedures used to determine batch-to-batch uniformity of products (dissolution test for tablets based on the Noyes-Whitney equation, which can be derived from Fick's law).

When individual molecules move within a substance, diffusion is said to occur. This may occur as the result of a concentration gradient or by random molecular motion.

Probably the most widely used laws of diffusion are known as Fick's first and second laws. Fick's first law involving steady-state diffusion (where dc/dx does not change) is derived from the following expression for the quantity of material (M) flowing through a cross section of a barrier (S) in unit time (t) expressed as the flux (J):

$$J = dM/(Sdt)$$

Under a concentration gradient (dc/dx), Fick's first law can be expressed thus:

$$J = D[(C_1 - C_2)/h] \text{ or } j = -D(dC/dx)$$

where J is the flux of a component across a plane of unit area,
C_1 and C_2 are the concentrations in the donor and receptor compartments,
h is the membrane thickness, and
D is the diffusion coefficient (or diffusivity).

The sign is negative, denoting that the flux is in the direction of decreasing concentration. The units of J are grams per square centimetre; C, grams per cubic centimetre; M, grams or moles; S, square centimetres; x, centimetres; and D, square centimetres per second.

D is appropriately called a diffusion coefficient, not a diffusion constant, as it is subject to change. D may change in value with increased concentrations. Also, D can be affected by temperature, pressure, solvent properties, and the chemical nature of the drug itself. To study the rate of change of the drug in the system, one needs

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an expression that relates the change in concentration with time at a definite location in place of the mass of drug diffusing across a unit area of barrier in unit time; this expression is known as Fick's second law. This law can be summarized as stating that the change in concentration in a particular place with time is proportional to the change in concentration gradient at that particular place in the system.

In summary, Fick's first law relates to a steady-state flow, whereas Fick's second law relates to a change in concentration of drug with time, at any distance, or an unsteady state of flow.

The diffusion coefficients ($D \times 10^{-6}$) of various compounds in water (25°C) and other media have been determined as follows: ethanol, 12.5 cm^2 per second; glycine, 10.6 cm^2 per second; sodium lauryl sulfate, 6.2 cm^2 per second; glucose, 6.8 cm^2 per second.

The concentration of drug in the membrane can be calculated using the partition coefficient (K) and the concentration in the donor and receptor compartments.

$$K = (C_1/C_d) = (C_2/C_r)'$$

where C_1 and C_d are the concentrations in the donor compartment (g/cm^3)

and C_2 and C_r are the concentrations in the receptor compartment (g/cm^3).

K is the partition coefficient of the drug between the solution and the mem-brane. It can be estimated using the oil solubility of the drug versus the water solubility of the drug. Usually, the higher the partition coefficient, the more the drug will be soluble in a lipophilic substance. We can now write the expression:

$$dM/dt = [DSK(C_d - C_r)]/h$$

or in sink conditions,

$$dM/dt = DSKC_d/h = PSC_d$$

The permeability coefficient (centimetres per second) can be obtained by rearranging to:

$$P = DK/h$$

Example 1. A drug passing through a 1 mm thick membrane has a diffusion coefficient of 4.23×10^{-7} cm^2 per second and art oil-water partition coefficient of 2.03. The radius of the area exposed to the solution is 2 cm, and the concentration of the drug in the donor compartment is 0.5 mg/mL. Calculate the permeability and the diffusion rate of the drug.

$h = 1$ mm $= 0.1$ cm

$D = 4.23 \times 10^{-7} cm^2$/second

$K = 2.03$

$r = 2$ cm, $S = \pi(2 \text{ cm})^2 = 12.57$ cm^2

$C_d = 0.5$ mg/mL

$P = [(4.23 \times 10^{-7} \text{ cm}^2\text{/second}) (2.03)]/0.1 \text{ cm} = 8.59 \times 10^{-5}$ mg/second

$dM/dt = (8.59 \times 10^{-6} \text{ cm/second}) (12.57 \text{ cm}^2) (0.5 \text{ mg/mL}) = 5.40 \times 10^{-5}$ mg/second

$(5.40 \times 10^{-5}$ mg/second) (3600 second/hour) = 0.19 mg/hour

In the dissolution of particles of drug, the dissolved molecules diffuse away from the individual particle body. An expression to describe this, derived from Fick's equations, is known as the Noyes and Whitney expression, proposed in 1897. It can be written as follows:

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$$dC/dt = (DS/Vh)(C_s - C)$$

where C is the concentration of drug dissolved at time t,
D is the diffusion coefficient of the solute in solution,
S is the surface area of the exposed solid,
V is the volume of solution,
h is the thickness of the diffusion layer,
C_s is the saturation solubility of the drug, and
C is the concentration of solute in the bulk phase at a specific time, t.

It is common practice to use sink conditions in which C does not exceed about 20% of the solubility of the drug being investigated. Under these conditions, the expression simplifies to

$$dC/dt = (DSC_s/Vh)$$

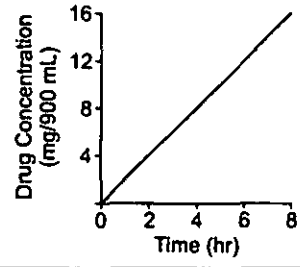
and incorporating the volume of solution (V), the thickness of the diffusion layer (h), and the diffusivity coefficient (D) into a coefficient k (to take into account the various factors in the system), the expression becomes

$$dC/dt = kSC_s$$

As the factors are held constant, it becomes apparent that the dissolution rate of a drug can be proportional to the surface area exposed to the dissolution medium. A number of other expressions have been derived for specific application to various situations and conditions.

These relationships expressed as Pick's first and seconwd laws and the Noyes-Whitney equation have great importance and relevance in pharmaceutical systems.

Example 2. The following information was obtained using the USP 32–NF 27 dissolution apparatus 1. The drug is soluble at 1 g in 3 mL of water, so sink conditions were maintained; the surface area of the tablet exposed was 1.5 cm^2 (obtained by placing the tablet in a special holder exposing only one side to the dissolution medium); and the dosage form studied was a 16 mg sustained-release tablet; the release pattern should be zero order. What is the rate of release of drug?

Time (Hours)	*Drug Concentration (mg/900 mL of Solution)*	*Graph of Release Profile*
0.0	0.0	
0.5	1.0	
1.0	1.9	
2.0	4.1	
4.0	8.0	
6.0	11.8	
8.0	15.9	

In this problem, since the surface area (S) was maintained constant at 1.5 cm^2 and the solubility (C_s) of the drug is constant at 1 g in 3 mL of water, the plot of concentration versus time (t) yields a slope with a value of kSC_s, or k_2, expressing the rate of release of the drug as

$$dC/dt = kSC_s$$

The slope of the line $= \Delta y/\Delta x - (y_2 - y_1)/(x_2 - x_1)$
$= (1\ 5.9\ mg - 0\ mg)/(8.0\ h - 0\ h)$
$= 15.9/8 = 1.99\ mg/h$

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Therefore, the rate of release of the sustained-release preparation is 1.99, or approximately 2 mg per hour. From this, the quantity of drug released at any time (t) can be calculated.

Substances can diffuse across the barrier only with considerable difficulty if at all. The interrelationship of the dissociation constant, lipid solubility, and pH at the absorption site with the absorption characteristics of various drugs are the basis of the pH partition theory. Data obtained from the basic physicochemical studies, specifically, pK_a, solubility, and dissolution rate, provide an indication of absorption. To enhance these data, a technique using the everted intestinal sac may be used to evaluate absorption characteristics of drug substances. In this method, a piece of intestine is removed from an intact animal, is everted, and is filled with a solution of the drug substance, and the degree and rate of passage of the drug through the membrane sac are determined. This method allows evaluation of both passive and active transport. In the latter stages of preformulation testing or early formulation studies, animals and humans must be studied to assess the absorption efficiency and pharmacokinetic parameters and to establish possible in-vitro and in-vivo correlation for dissolution and bioavailability.

Partition Coefficient

The use of the partition coefficient is described in some detail in Physical Pharmacy Capsule 9, Partition Coefficient. Inherent in this procedure is the selection of appropriate extraction solvents, drug stability, use of salting-out additives, and environmental concerns. The octanol-water partition coefficient is commonly used in formulation development. Following the illustrations provided earlier, it is defined as

$$P = \frac{\text{(Conc. of drug in octanol)}}{\text{(Conc. of drug in water)}}$$

P depends on the drug concentration only if the drug molecules have a tendency to associate in solution. For an ionizable drug, the following equation is applicable:

$$P = \frac{\text{(Conc. of drug in octanol)}}{[1 - \alpha]\text{(Conc. of drug in water)}}$$

where α equals the degree of ionization.

pK_a/Dissociation Constants

Among the physicochemical characteristics of interest is the extent of dissociation or ionization of drug substances. This is important because the extent of ionization has an important effect on the formulation and pharmacokinetic parameters of the drug. The extent of dissociation or ionization in many cases is highly dependent on the pH of the medium containing the drug. In formulation, often the vehicle is adjusted to a certain pH to obtain a certain level of ionization of the drug for solubility and stability. In the pharmacokinetic area, the extent of ionization of a drug has a strong effect on its extent of absorption, distribution, and elimination. The dissociation constant, or pK_a, is usually determined by potentiometric titration. For the practicing pharmacist, it is important in predicting precipitation in admixtures and in calculating the solubility of drugs at certain pH values. Physical Pharmacy Capsule 10, pK_a/Dissociation Constants, presents a brief summary of dissociation and ionization concepts.

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Drug and Drug Product Stability

One of the most important activities of preformulation work is evaluation of the physical and chemical stability of the pure drug substance. It is essential that these initial studies be conducted using drug samples of known purity. The presence of impurities can lead to erroneous conclusions in such evaluations. Stability studies conducted in the preformulation phase include solid-state stability of the drug alone, solution phase stability, and stability in the presence of expected excipients. Initial investigation begins with knowledge of the drug's chemical structure, which allows the preformulation scientist to anticipate the possible degradation reactions.

Drug Stability: Mechanisms of Degradation

Chemical instability of medicinal agents may take many forms because the drugs in use today are of such diverse chemical constitution. Chemically, drug substances are alcohols, phenols, aldehydes, ketones, esters, ethers, acids, salts, alkaloids, glycosides, and others, each with reactive chemical groups having different susceptibilities to chemical instability. Chemically, the most frequently encountered destructive processes are hydrolysis and oxidation. Hydrolysis is a solvolysis process in which (drug) molecules interact with water molecules to yield breakdown products. For example, aspirin, or acetylsalicylic acid, combines with a water molecule and hydrolyzes into one molecule of salicylic acid and one molecule of acetic acid.

CAPSULE 9

Partition Coefficient

The oil-water partition coefficient is a measure of a molecule's lipophilic character; that is, its preference for the hydrophilic or lipophilic phase. If a solute is added to a mixture of two immiscible liquids, it will distribute between the two phases and reach an equilibrium at a constant temperature. The distribution of the solute (unaggregated and undissociated) between the two immiscible layers can be described thus:

$$K = C_U/C_L$$

where K is the distribution constant or partition constant,
C_U is the concentration of the drug in the upper phase, and
C_L is the concentration of the drug in the lower phase.

This information can be effectively used in the

1. Extraction of crude drugs.
2. Recovery of antibiotics from fermentation broth.
3. Recovery of biotechnology-derived drugs from bacterial cultures.
4. Extraction of drugs from biologic fluids for therapeutic drug monitoring.
5. Absorption of drugs from dosage forms (ointments, suppositories, transdermal patches).
6. Study of the distribution of flavoring oil between oil and water phases of emulsions.
7. In other applications.

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This basic relationship can be used to calculate the quantity of drug extracted from or remaining behind in a given layer and to calculate the number of extractions required to remove a drug from a mixture.

The concentration of drug found in the upper layer (U) of two immiscible layers is given thus:

$$U = Kr/(Kr + 1)$$

where K is the distribution partition constant and

r is V_u/V_1, or the ratio of the volume of upper and lower phases.

The concentration of drug remaining in the lower layer (L) is given thus:

$$L = 1/(Kr + 1)$$

If the lower phase is successively extracted again with n equal volumes of the upper layer, each upper (U) contains the following fraction of the drug:

$$U_n = Kr/(Kr + 1)^n$$

where U_n is the fraction contained in the nth extraction and

n is the nth successive volume.

The fraction of solute remaining in the lower layer (L_n) is given thus:

$$L_n = 1/(Kr + 1)^n$$

More efficient extractions are obtained using successive small volumes of the extraction solvent than single larger volumes. This can be calculated as follows when the same volume of extracting solvent is used in divided portions. For example, the fraction L_n remaining after the nth extraction:

$$L_n = \frac{1}{\left(\frac{Kr}{n} + 1\right)^n}$$

Example 1. At 25°C and pH 6.8, the K for a second generation cephalosporin is 0.7 between equal volumes of butanol and the fermentation broth. Calculate the U, L, and L_n (using the same volume divided into fourths).

U = 0.7/(0.7 + 1) = 0.41, the fraction of drug extracted into the upper layer

L = 1/(0.7 + 1) = 0.59, the fraction of drug remaining in the lower layer

The total of the fractions in the U and L = 0.41 + 0.59 = 1.

If the fermentation broth is extracted with four successive extractions accomplished by dividing the quantity of butanol used into fourths, the quantity of drug remaining after the fourth extraction is

$$L_{4\,th} = \frac{1}{\left(\frac{0.7 \times 1}{4} + 1\right)} = 0.525$$

From this, the quantity remaining after a single volume, single extraction is 0.59, but when the single volume is divided into fourths and four successive extractions are done, the quantity remaining is 0.525; therefore, more was extracted using divided portions of the extracting solvent. Inherent in this procedure is the selection of appropriate extraction solvents, drug stability, use of salting-out additives, and environmental concerns.

CAPSULE 10

pK_a/Dissociation Constants

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The dissociation of a weak acid in water is given by this expression:

$$HA \leftrightarrow H^+ + A^-$$

$$K_1[HA] \leftrightarrow K_2[H^+]\,[A^-]$$

At equilibrium, the reaction rate constants K_1 and K_2 are equal. This can be rearranged, and the dissociation constant defined as

$$K_a = \frac{K_1}{K_2} = \frac{[H^+][A^+]}{[HA]}$$

where K*a* is the acid dissociation constant.

For the dissociation of a weak base that does not contain a hydroxyl group, the following relationship can be used:

$$BH^+ \leftrightarrow H^+ + B$$

The dissociation constant is described by

$$K_a = \frac{[H^+][B^+]}{[BH^+]}$$

The dissociation of a hydroxyl-containing weak base,

$$B + H_2O \leftrightarrow OH^- + BH^+$$

The dissociation constant is described by

$$K_b = \frac{[OH^-][BH^+]}{[B]}$$

The hydrogen ion concentrations can be calculated for the solution of a weak acid using

$$[H^+] = \sqrt{K_aC}$$

Similarly, the hydroxyl ion concentration for a solution of a weak base is approximated by

$$[OH^-] = \sqrt{K_bC}$$

Some practical applications of these equations are as follows.

Example 1. The K_a of lactic acid is 1.387×10^{-4} at 25°C. What is the hydrogen ion concentration of a 0.02 M solution?

$$[H^+] = \sqrt{1.387 \times 10^{-4} \times 0.02} = 1.665 \times 10^{-3} \text{ G- ion/L}$$

Example 2. The K_b of morphine is 7.4×10^{-7}. What is the hydroxyl ion concentration of a 0.02 M solution?

$$[OH^-] = \sqrt{7.4 \times 10^{-7} \times 0.02} = 1.216 \times 10^{-4} \text{ G- ion/L}$$

Hydrolysis is probably the most important single cause of drug decomposition, mainly because a great number of medicinal agents are esters or contain such other groupings as substituted amides, lactones, and lactams, which are susceptible to the hydrolytic process.

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Another destructive process is oxidation, which destroys many drug types, including aldehydes, alcohols, phenols, sugars, alkaloids, and unsaturated fats and oils. Chemically, oxidation is loss of electrons from an atom or a molecule. Each electron lost is accepted by some other atom or molecule, reducing the recipient. In inorganic chemistry, oxidation is accompanied by an increase in the positive valence of an element, for example, ferrous (+2) oxidizing to ferric (+3).

In organic chemistry, oxidation is frequently considered synonymous with the loss of hydrogen (dehydrogenation) from a molecule. Oxidation frequently involves free chemical radicals, which are molecules or atoms containing one or more unpaired electrons, such as molecular (atmospheric) oxygen (•O—O•) and free hydroxyl (•OH). These radicals tend to take electrons from other chemicals, thereby oxidizing the donor.

Many of the oxidative changes in pharmaceutical preparations have the character of autoxidations. Autoxidations occur spontaneously under the initial influence of atmospheric oxygen and proceed slowly at first and then more rapidly. The process has been described as a type of chain reaction commencing with the union of oxygen with the drug molecule and continuing with a free radical of this oxidized molecule participating in the destruction of other drug molecules and so forth.

In drug product formulation work, steps are taken to reduce or prevent deterioration due to hydrolysis, oxidation, and other processes. These techniques are discussed later.

Drug and Drug Product Stability: Kinetics and Shelf Life

Stability is the extent to which a product retains within specified limits and throughout its period of storage and use (*i.e.*, its shelf life) the same properties and characteristics that it possessed at the time of its manufacture. Five types of stability concern pharmacists:

1. *Chemical*: Each active ingredient retains its chemical integrity and labelled potency within the specified limits.
2. *Physical*: The original physical properties, including appearance, palatability, uniformity, dissolution, and suspendability are retained.
3. *Microbiologic*: Sterility or resistance to microbial growth is retained according to the specified requirements. Antimicrobial agents retain effectiveness within specified limits.
4. *Therapeutic*: The therapeutic effect remains unchanged.
5. *Toxicologic*: No significant increase in toxicity occurs.

Chemical stability is important for selecting storage conditions (temperature, light, humidity), selecting the proper container for dispensing (glass vs plastic, clear vs amber or opaque, cap liners), and anticipating interactions when mixing drugs and dosage forms. Stability and expiration dating are based on reaction kinetics, that is, the study of the rate of chemical change and the way this rate is influenced by concentration of reactants, products, and other chemical species and by factors such as solvent, pressure, and temperature. In considering chemical stability of a pharmaceutical, one must know the reaction order and reaction rate. The reaction order may be the overall order (the sum of the exponents of the concentration terms of the rate expression), or the order with respect to each reactant (the exponent of the individual concentration term in the rate expression).

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Rate Reactions

The reaction rate is a description of the drug concentration with respect to time. Most commonly, zero-order and first-order reactions are encountered in pharmacy. These are presented in Physical Pharmacy Capsule. 11, Rate Reactions, along with some appropriate examples.

Q_{10} Method of Shelf Life Estimation

The Q_{10} method of shelf life estimation lets the pharmacist estimate shelf life for a product that has been stored or is going to be stored under a different set of conditions. It is explained in Physical Pharmacy Capsule 12, Q_{10} Method of Shelf Life Estimation.

Enhancing Stability of Drug Products

Many pharmaceutical ingredients may be used to prepare the desired dosage form of a drug substance. Some of these agents may be used to achieve the desired physical and chemical characteristics of the product or to enhance its appearance, odor, and taste. Other substances may be used to increase the stability of the drug substance, particularly against hydrolysis and oxidation. In each instance, the added pharmaceutical ingredient must be compatible with and must not detract from the stability of the drug substance.

There are several approaches to the stabilization of pharmaceutical preparations containing drugs subject to hydrolysis. Perhaps the most obvious is the reduction or elimination of water from the pharmaceutical system. Even solid dosage forms containing water-labile drugs must be protected from humidity in the atmosphere.

This may be accomplished by applying a waterproof protective coating over tablets or by keeping the drug in a tightly closed container. It is fairly common to detect hydrolyzed aspirin by noticing an odor of acetic acid upon opening a bottle of aspirin tablets. In liquid preparations, water can frequently be replaced or reduced in the formulation through the use of substitute liquids such as glycerin, propylene glycol, and alcohol. In certain injectable products, anhydrous vegetable oils may be used as the drug's solvent to reduce the chance of hydrolytic decomposition.

Decomposition by hydrolysis may be prevented in other liquid drugs by suspending them in a nonaqueous vehicle rather than dissolving them in an aqueous solvent. In still other instances, particularly for certain unstable antibiotic drugs, when an aqueous preparation is desired, the drug may be supplied to the pharmacist in a dry form for *reconstitution* by adding a specified volume of purified water just before dispensing. The dry powder is actually a mixture of the antibiotic, suspending agents, flavorants, and colorants; when reconstituted by the pharmacist, it remains stable for the period over which the preparation is normally consumed. Refrigeration is advisable for most preparations considered subject to hydrolysis.

CAPSULE 11

Rate Reactions

Zero-Order Rate Reactions

If the loss of drug is independent of the concentration of the reactants and constant with respect to time (*i.e.*, 1 mg/mL/hour), the rate is called zero order. The mathematical expression is

$$\frac{-dC}{dt} = k_0$$

where k_0 is the zero-order rate constant [concentration(C)/time(t)].

The integrated and more useful form of the equation:

$$C = -k_0 t + C_0$$

where C_0 is the initial concentration of the drug.

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The units for a zero rate constant k_0 are concentration per unit time, such as moles per litre-second or milligrams per millilitre per minute,

It is meaningless to attempt to describe the time required *for all* material in a reaction to decompose, that is, infinity. Therefore, reaction rates are commonly described by k or by their half-life, $t_{1/2}$.

The half-life equation for a zero-order reaction:

$$t_{1/2} = (1/2)(C_0/k_0)$$

If the C_0 changes, the $t_{1/2}$, changes. There is an inverse relationship between the $t_{1/2}$ and k.

Example 1. Adrug suspension (125 mg/mL) decays by zero-order kinetics with a reaction rate constant of 0.5 mg/mL/hour. What is the concentration of intact drug remaining after 3 days (72 hours), and what is its $t_{1/2}$?

$$C = -(0.5 \text{ mg/mL/hour})(72 \text{ hour}) + 125 \text{ mg/mL}$$

$$C = 89 \text{ mg/mL after 3 days}$$

$$t_{1/2} = 1/2(125 \text{ mg/mL})/(0.5 \text{ mg/mL/hour})$$

$$t_{1/2} = 125 \text{ hours}$$

Example 2. How long will it take for the suspension to reach 90% of its original concentration?

$$90\% \times 125 \text{ mg/mL} = 112.5 \text{ mg/mL}$$

$$t = \frac{C - C_0}{-k_0} - \frac{112.5 \text{ mg/mL} - 125 \text{ mg/mL}}{-0.5 \text{ mg/mL/hour}} = 25 \text{ hours}$$

Drug suspensions are examples of pharmaceuticals that ordinarily follow zero-order kinetics for degradation.

First-Order Rate Reactions

If the loss of drug is directly proportional to the concentration remaining with respect to time, it is called a first-order reaction and has the units of reciprocal time, that is, time^{-1}. The mathematical expression is

$$\frac{-dC}{dt} = kC$$

where C is the concentration of intact drug remaining,

t is time,

(dC/dt) is the rate at which the intact drug degrades, and

k is the specific reaction rate constant.

The integrated and more useful form of the equation:

$$\log C = \frac{-kt}{2.303} + \log C_0$$

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where C_0 is the initial concentration of the drug. In natural log form, the equation is

$$\ln C = -kt + \ln C_0$$

The units of k for a first-order reaction are per unit of time, such as per second.

The half-life equation for a first-order reaction is

$$t_{1/2} = 0.693/k$$

and can be easily derived from the first-order equation by substituting values of C = 50% and C_0 = 100%, representing a decrease in concentration by 50%.

Example 3. An ophthalmic solution of a mydriatic drug at 5 mg/mL exhibits first-order degradation with a rate of 0.0005/day. How much drug will remain after 120 days, and what is its half-life?

$$\ln C = -(0.0005/\text{day})(120) + \ln(5\ \text{mg/mL})$$
$$\ln C = -0.06 + 1.609$$
$$\ln C = 1.549$$
$$C = 4.71\ \text{mg/mL}$$
$$t_{1/2} = 0.693/0.0005/\text{day}$$
$$t_{1/2} = 1.386\ \text{days}$$

Example 4. In Example 3, how long will it take for the drug to degrade to 90% of its original concentration?

$$90\%\ \text{of}\ 5\ \text{mg/mL} = 4.5\ \text{mg/mL}$$
$$\ln 4.5\ \text{mg/mL} = -(0.0005/\text{day})^t + \ln(5\ \text{mg/mL})$$
$$t = \frac{\ln 4.5\ \text{mg/mL} - \ln 5\ \text{mg/mL}}{-0.0005/\text{day}}$$
$$t = 210\ \text{days}$$

Energy of Activation: Arrhenius Equation

Stability projections for shelf life (t_{90}, or the time required for 10% of the drug to degrade with 90% of the intact drug remaining) are commonly based on the Arrhenius equation:

$$\log = \frac{k_2}{k_1} = \frac{Ea(T_2 - T_1)}{2.3RT_1T_2}$$

which relates the reaction rate constants (k) to temperatures (T) with the gas constant (R) and the energy of activation (Ea).

The relationship of the reaction rate constants at two different temperatures provides the energy of activation for the degradation. By performing the reactions at elevated temperatures instead of allowing the process to proceed slowly at room temperature, the Ea can be calculated and a k value for room temperature determined with the Arrhenius equation.

Example 5. The degradation of a new cancer drug follows first-order kinetics and has first-order degradation rate constants of 0.0001 per hour at 60°C and 0.0009 at 80°C. What is its Ea?

$$\log = \frac{(0.0009)}{(0.0001)} = \frac{Ea(353 - 333)}{(2.3)(1.987)(353)(333)}$$
$$Ea = 25{,}651\ \text{kcal/mol}$$

CAPSULE 12

Q_{10} Method of Shelf Life Estimation

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The Q_{10} approach, based on Ea, which is independent of reaction order, is described as

$$Q_{10} = e^{[(Ea/R)\,[(1/T+10)-(1/T)]]}$$

where Ea is the energy of activation,
R is the gas constant, and
T is the absolute temperature.

In usable terms, Q_{10}, the ratio of two different reaction rate constants, is defined thus:

$$Q_{10} = \frac{K_{(T+10)}}{K_T}$$

The commonly used Q values of 2, 3, and 4 relate to the energies of activations of the reactions for temperatures around room temperature (25°C). For example, a Q value of 2 corresponds to an Ea (kcal/mol) of 12.2, a Q value of 3 corresponds to an Ea of 19.4, and a Q value of 4 corresponds to an Ea of 24.5. Reasonable estimates can often be made using the value of 3.

The equation for Q_{10} shelf life estimates is

$$t_{90}(T_2) = \frac{t_{90}(T_1)}{Q_{10}^{(\Delta T/10)}}$$

where t_{90} T_2 is the estimated shelf life,
t_{90} T_1 is the given shelf life at a given temperature, and
ΔT is the difference in the temperatures T_1 and T_2.

As is evident from this relationship, an increase in ΔT will decrease the shelf life and a decrease in ΔT will increase shelf life. This is the same as saying that storing at a warmer temperature will shorten the life of the drug and storing at a cooler temperature will increase the life of the drug.

Example 1. An antibiotic solution has a shelf life of 48 hours in the refrigerator (5°C). What is its estimated shelf life at room temperature (25°C)?

Using a Q value of 3, we set up the relationship as follows:

$$t_{90}(T_2) = \frac{t_{90}(T_1)}{Q_{10}^{(\Delta T/10)}} = \frac{48}{e^{[(25-5)/10]}} = \frac{48}{3^2} = 5.33 \text{ hours}$$

Example 2. An ophthalmic solution has a shelf life of 6 hours at room temperature (25°C). What is the estimated shelf life in a refrigerator at 5°C? (**Note:** Since the temperature is decreasing, ΔT will be negative.)

$$t_{90}(T_2) = \frac{6}{e^{[(5-25)/10]}} = \frac{6}{3^{-2}} = 6 \times 3^2 = 54 \text{ hours}$$

These are estimates, and actual energies of activation can often be obtained from the iterature for more exact calculations.

Together with temperature, pH is a major determinant of the stability of a drug prone to hydrolytic decomposition. Hydrolysis of most drugs depends on the relative concentrations of the hydroxyl and hydronium ions, and a pH at which each

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drug is optimally stable can be easily determined. For most hydrolyzable drugs, optimum stability is on the acid side, somewhere between pH 5 and 6.

Therefore, through judicious use of buffering agents, the stability of otherwise unstable compounds can be increased. Buffers are used to maintain a certain pH, as described in Physical Pharmacy Capsule .13, Buffer Capacity. Pharmaceutically, oxidation of a susceptible drug substance is most likely to occur when it is not kept dry in the presence of oxygen or when it is exposed to light or combined with other chemical agents without proper regard to their influence on oxidation. Oxidation of a chemical in a pharmaceutical preparation is usually accompanied by an alteration in the color of that preparation.

It may also result in precipitation or a change in odor. The oxidative process is diverted and the stability of the drug is preserved by agents called *antioxidants,* which react with one or more compounds in the drug to prevent progress of the chain reaction. In general, antioxidants act by providing electrons and easily available hydrogen atoms that are accepted more readily by the free radicals than are those of the drug being protected. Various antioxidants are employed in pharmacy. Among those most frequently used in aqueous preparations are sodium sulphite (Na_2SO_3, at high pH values), sodium bisulphite ($NaHSO_3$, at intermediate pH values), sodium metabisulphite ($Na_2S_2O_5$ at low pH values), hypophosphorus acid (H_3PO_2), and ascorbic acid. In oleaginous (oily or unctuous) preparations, alpha-tocopherol, butyl hydroxy anisole, and ascorbyl palmitate find application.

In June 1987, U.S. Food and Drug Administration (FDA) labelling regulations went into effect requiring a warning about possible allergic- type reactions, including anaphyl axis, in the package insert for prescription drugs to whose final dosage form sulphites have been added. Sulphites are used as preservatives in many injectable drugs, such as antibiotics and local anesthetics. Some inhalants and ophthalmic preparations also contain sulphites, but relatively few oral drugs contain these chemicals. The purpose of the regulation is to protect the estimated 0.2% of the population who are subject to allergic reactions to the chemicals. Many sulphite-sensitive persons have asthma or other allergic conditions. Previous to the regulations dealing with prescription medication, the FDA issued regulations for the use of sulphites in food.

Asthmatics and other patients who may be sulphite sensitive should be reminded to read the labels of packaged foods and medications to check for the presence of these agents. Sulphite agents covered by the regulations are potassium bisulphite, potassium metabisulphite, sodium bisulphite, sodium metabisulphite, sodium sulphite, and sulfur dioxide. The FDA permits the use of sulphites in prescription products, with the proper labelling, because there are no generally suitable substitutes for sulphites to maintain potency in certain medications. Some but not all epinephrine injections contain sulphites.

The proper use of antioxidants permits their specific application only after appropriate biomedical and pharmaceutical studies. In certain instances, other pharmaceutical additives can inactivate a given antioxidant. In other cases, certain antioxidants can react chemically with the drugs they were intended to stabilize without a noticeable change in the appearance of the preparation. Because oxygen may adversely affect their stability, certain pharmaceuticals require an oxygen-free atmosphere during preparation and storage. Oxygen may be present in pharmaceutical liquids in the airspace within the container or may be dissolved in the liquid vehicle. To avoid these

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exposures, oxygen-sensitive drugs may be prepared in the dry state and packaged in sealed containers with the air replaced by an inert gas such as nitrogen, as may liquid preparations. This is common practice in commercial production of vials and ampuls of easily oxidizable preparations intended for parenteral use.

Trace metals originating in the drug, solvent, container, or stopper are a constant source of difficulty in preparing stable solutions of oxidizable drugs. The rate of formation of color in epinephrine solutions, for instance, is greatly increased by the presence of ferric, ferrous, cupric, and chromic ions. Great care must be taken to eliminate these trace metals from labile preparations by through purification of the source of the contaminant or by chemically complexing or binding the metal through the use of specialized agents that make it chemically unavailable for participation in the oxidative process. These chelating agents are exemplified by calcium disodium edetate and ethylenediaminetetraacetic acid.

Light can also act as a catalyst to oxidation reactions, transferring its energy (photons) to drug molecules, making the latter more reactive through increased energy capability. As a precaution against acceleration of oxidation, sensitive preparations are packaged in light-resistant or opaque containers. Because most drug degradations proceed more rapidly as temperature increases, it is also advisable to maintain oxidizable drugs in a cool place. Another factor that can affect the stability of an oxidizable drug in solution is the pH of the preparation. Each drug must be maintained in solution at the pH most favorable to its stability. This varies from preparation to preparation and must be determined on an individual basis for the drug in question.

CAPSULE 13

Buffer Capacity

pH, buffers, and buffer capacity are especially important in drug product formulation, since they affect the drug's solubility, activity, absorption, and stability and the patient's comfort.

A buffer is a system, usually an aqueous solution that can resist changes in pH upon addition of an acid or base. Buffers are composed of a weak acid and its conjugate base or a weak base and its conjugate acid. Buffers are prepared by one of these processes:

1. Mixing a weak acid and its conjugate base or a weak base and its conjugate acid
2. Mixing a weak acid and a strong base to form the conjugate base or a weak base and a strong acid to form the conjugate acid

Using the Henderson-Hasselbach equation:

$$pH = pK_a + \log (\text{base/acid})$$

Remember that the acid is the proton donor and the base is the proton acceptor.

Example 1. A buffer is prepared by mixing 100 mL of 0.2 M phosphoric acid with 200 mL of 0.08 M sodium phosphate monobasic. What is the pH of this buffer? (K_a of phosphoric acid = 7.5×10^{-3})

Moles acid = (0.2 mol/1000 mL)(100 mL) = 0.02 mol; (0.02 mol)/(0.3 L) = 0.067 M

Moles base = (0.08 mol/1000 mL)(200 mL) = 0.016 mol; (0.016 mol)/(0.3 L) = 0.053 M

$pK_a = -\log 7.5 \times 10^{-3} = 2.125$

pH = 2.125 + log (0.016 mol/0.02 mol) = 2.028

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Example 2. Determine the pH of the buffer prepared as shown:

Sodium acetate 50 g

Conc. HCl 10 mL

Water q.s. 2 L

Helpful numbers:

pK_a acetic acid = 4.76

m.w. sodium acetate = 82,08

m.w. acetic acid = 60.05

m.w. HCl =36.45

Conc. HCl, 44% HCl w/v

$NaAc + HCl \rightarrow NaCl + HAc + NaAc$

(0.609 mol) (0.121 mol) (0.121 mol) (0.121 mol) (0.488 mol)

HCl: {(10 mL)[(44 g)/(100 mL)] (1 mol)/(36.45 g)} = 0.121 mol

NaAc: (50 g)[(1 mol)/(82.08 g] = 0.609 mol (0.609 mol) – (0.121 mol) = 0.488 mol

pH = 4.76 + log (0.488 mol)/(0.121 mol) = 5.367

The ability of a buffer solution to resist changes in pH upon the addition of an acid or a base is called buffer capacity (β) and is defined thus:

$$\beta = \Delta B/\Delta pH$$

where ΔB is molar concentration of acid or base added,
ΔpH is change in pH due to addition of acid or base, and
ΔpH can be determined experimentally or calculated using the Henderson-Hasselbach equation.

Example 3. If 0.2 mole of HCl is added to a 0.015 M solution of ammonium hydroxide and the pH falls from 9.5 to 8.9, what is the buffer capacity?

$$\Delta pH = 9.5 - 8.9 = 0.6$$

$$\Delta B = 0.2 \text{ mol/L} = 0.2 \text{ M}$$

$$\beta = 0.2 \text{ M}/0.6 = 0.33 \text{ M}$$

Example 4. If 0.002 mole of HCl is added to the buffer in Example 1, what is its buffer capacity? After adding 0.002 mole HCl:

H_3PO_4: 0.02 mol + 0.002 mol = 0.022 mol

NaH_2PO_4: 0.016 mol – 0.002 mL = 0.014 mol

$$pH = 2.125 + \log (0.014 \text{ mol}/0.022 \text{ mol}) = 1.929$$

$$\Delta pH = 2.028 - 1.929 = 0.099$$

$$\Delta AB = 0.002 \text{ mol}/0.3 \text{ L} = 0.0067 \text{ M}$$

$$\beta = 0.0067 \text{ M}/0.099 = 0.067 \text{ M}$$

Another approach to calculating buffer capacity involves the use of Van Slyke's equation:

$$\beta = 2.3\ C\ \{K_a[H^+]/(K_a[H^+])^2\}$$

where C is the sum of the molar concentrations of the acid and base, and $[H^+] = 10^{-pH}$.

Example 5. What is the Van Slyke buffer capacity of the buffer prepared in Example 1?

$$C = 0.0067\ M + 0.0053\ M = 0.12\ M$$

$$K_a = 7.5 + 10^{-3}$$

$$[H^+] = 10^{-2.028} = 9.38 \times 10^{-3}\ M$$

$$\beta = 2.3\ (0.12\ M)\ \{[(7.5 \times 10^{-3}\ M)\ (9.38 - 10^{-3}\ M)]/[(7.5 \times 10^{-3}\ M)/(9.38 \times 10^{-3}\ M^2)]\} = 0.68\ M$$

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Table 6.2 Some USP Drugs and Preparations Especially Subject to Chemical or Physical Deterioration

Preparation	*Category*	*Monograph or Label Warning*
Epinephrine bitartrate ophthalmic solution	Adrenergic	Do not use inhalation, injection, nasal, or ophthalmic solution if it is brown or contains a precipitate
Epinephrine inhalation solution Epinephrine injection Epinephrine nasal solution Epinephrine ophthalmic solution		
Isoproterenol sulphate inhalation, solution	Adrenergic (bronchodilator)	Do not use inhalation or injection if it is pink to brown or contains a precipitate
Isoproterenol inhalation solution	Antianginal	To prevent loss of potency, keep in original container or supplemental container specifically labelled suitable for nitroglycerin tablets
Nitroglycerin tablets		
Paraldehyde	Hypnotic	Subject to oxidation to form acetic acid

Statements in the United States Pharmacopeia (USP), as with those in Table 6.2, warn of the oxidative decomposition of drugs and preparations. In some instances, the specific agent to employ as a stabilizer is mentioned in the monograph, and in others the term "suitable stabilizer" is used. An example in which a particular agent is designated for use is in the monograph for potassium iodide oral solution, USP. Potassium iodide in solution is prone to photocatalyzed oxidation and the release of free iodine, with a resultant yellow-to-brown discoloration of the solution. The use of light-resistant containers is essential to its stability. As a further precaution against decomposition if the solution is not to be used within a short time, the USP recommends the addition of 0.5 mg of sodium thiosulphate for each gram of potassium iodide. In the event free iodine is released during storage, the sodium thiosulphate converts it to colorless and soluble sodium iodide:

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$

In summary, for easily oxidizable drugs, the formulation pharmacist may stabilize the preparation by the selective exclusion from the system of oxygen, oxidizing agents, trace metals, light, heat, and other chemical catalysts to the oxidation process. Antioxidants, chelating agents, and buffering agents may be added to create and maintain a favorable pH. In addition to oxidation and hydrolysis, destructive processes include polymerization, chemical decarboxylation, and deamination. However, these processes occur less frequently and are peculiar to only small groups of chemical substances. Polymerization is a reaction between two or more identical molecules that forms a new and generally larger molecule.

Formaldehyde is an example of a drug capable of polymerization. In solution it may polymerize to paraformaldehyde $(CH_2O)n$, a slowly soluble white crystalline substance that may cloud the solution. The formation of paraformaldehyde is enhanced by cool storage, especially in solutions with high concentrations of formaldehyde.

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The official formaldehyde solution contains approximately 37% formaldehyde and according to the USP, should be stored at temperatures not below 15°C (59°F). If the solution becomes cloudy upon standing in a cool place, it usually may be cleared by gentle warming. Formaldehyde is prepared by the limited oxidation of methanol (methyl alcohol), and the USP permits a residual amount of this material to remain in the final product, since it can retard the formation of paraformaldehyde. Formaldehyde solution must be maintained in a tight container because oxidation of the formaldehyde yields formic acid.

$$\underset{\text{Methanol}}{CH_3OH} \xrightarrow{(O)} \underset{\text{Formaldehyde}}{HCHO} \xrightarrow{(O)} \underset{\text{Formic acid}}{HCOOH}$$

Other organic drug molecules may be degraded through processes in which one or more of their active chemical groups are removed. These processes may involve various catalysts, including light and enzymes. Decarboxylation and deamination are examples of such processes; the former is decomposition of an organic acid (R•COOH) and release of carbon dioxide gas, and the latter is removal of the nitrogen-containing group from an organic amine. For example, insulin, a protein, deteriorates rapidly in acid solutions as a result of extensive deamination. Thus, most preparations of insulin are neutralized to reduce the rate of decomposition.

Stability Testing

FDA's Current Good Manufacturing Practice regulations include sections on stability and stability testing of pharmaceutical components and finished pharmaceutical products. In addition, FDA and International Conference on Harmonization guidelines and guidances provide working recommendations to support the regulatory requirements. Among these are the following:

- "Stability Testing of New Drug Substances and Products"
- "Quality of Biotechnological Products: Stability Testing of Biotechnology/ Biological Drug Products"
- "Photostability Testing of New Drug Substances and Products"
- "Stability Testing of New Dosage Forms"

Drug and drug product stability testing during every stage of development is critical to the quality of the product. Drug stability is important during preclinical testing and in clinical (human) trials to obtain a true and accurate assessment of the product being evaluated. For a marketed drug product, assurance of stability is vital to its safety and effectiveness during the course of its shelf life and use.

The FDA-required demonstration of drug stability is necessarily different for each stage of drug development, such as for a 2-week preclinical study, an early Phase I study, a limited Phase II trial, a pivotal Phase III clinical study, or for a new drug application. As a drug development program progresses, so do the requisite data to demonstrate and document the product's stability profile. Before approval for marketing, a product's stability must be assessed with regard to its formulation; the influence of its pharmaceutical ingredients; the influence of the container and closure; the manufacturing and processing conditions (*e.g.*, heat); packaging components; conditions of storage; anticipated conditions of shipping, temperature, light, and humidity; and anticipated duration and conditions of pharmacy shelf life and patient use. Holding intermediate product components (such as drug granulations for tablets) for long periods before processing into finished pharmaceutical products can affect the stability of both the intermediate component and

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the finished product. Therefore, in-process stability testing, including retesting of intermediate components, is important.

Product containers, closures, and other packaging features must be considered in stability testing. For instance, tablets or capsules packaged in glass or plastic bottles require different stability test protocols from those for blister packs or strip packaging. Drugs particularly subject to hydrolysis or oxidative decomposition must be evaluated accordingly. And sterile products must meet sterility test standards to ensure protection against microbial contamination. All preservatives must be tested for effectiveness in the finished product.

As noted elsewhere in this section, drug products must meet stability standards for long-term storage at room temperature and relative humidity. Products are also subjected to accelerated stability studies as an indication of shelf life stability. It is an FDA requirement that if the data are not submitted in the approved application, the first three post approval production batches of a drug substance must be subjected to longterm stability studies and the first three postapproval production batches of drug product must be subjected to both long-term and accelerated stability studies.

Drug instability in pharmaceutical formulations may be detected in some instances by a change in the physical appearance, color, odor, taste, or texture of the formulation, whereas in other instances chemical changes may not be self-evident and may be ascertained only through chemical analysis. Scientific data pertaining to the stability of a formulation can lead to prediction of the expected shelf life of the proposed product, and when necessary to redesign of the drug (*e.g.*, into more stable salt or ester form) and to reformulation of the dosage form. Obviously, the rate at which a drug product degrades is of prime importance. The study of the rate of chemical change and the way it is influenced by such factors as the concentration of the drug or reactant, the solvent, temperature and pressure, and other chemical agents in the formulation is reaction kinetics.

In general, a kinetic study begins by measuring the concentration of the drug at given intervals under a specific set of conditions including temperature, pH, ionic strength, light intensity, and drug concentration. The measurement of the drug's concentration at the various times reveals the stability or instability of the drug under the specified conditions with the passage of time. From this starting point, each of the original conditions may be varied to determine the influence of such changes on the drug's stability. For example, the pH of the solution may be changed while the temperature, light intensity, and original drug concentration are held constant.

The findings may be presented graphically, by plotting the drug concentration as a function of time. From the experimental data, the reaction rate may be determined and a rate constant and half-life calculated. The use of exaggerated conditions of temperature, humidity, light, and others to test the stability of drug formulations is termed accelerated stability testing. Accelerated temperature stability studies, for example, may be conducted for 6 months at 40°C with 75% relative humidity. If a significant change in the product occurs under these conditions, lesser temperature and humidity may be used, such as 30°C and 60% relative humidity. Short-term accelerated studies are used to determine the most stable of the proposed formulations for a drug product. In stress testing, temperature elevations in 10°C increments higher than used in accelerated studies are employed until chemical or physical degradation. Once the most stable formulation is ascertained, its long-term stability is predicted

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from the data generated from continuing stability studies. Depending on the types and severity of conditions employed, it is fairly common to maintain samples under exaggerated conditions of both temperature and varying humidity for 6 to 12 months. Such studies lead to the prediction of shelf life for a drug product. In addition to the accelerated stability studies, drug products are subjected to long-term stability studies under the usual conditions of transport and storage expected during product distribution. In conducting these studies, the different national and international climate zones to which the product may be subjected must be borne in mind and expected variances in conditions of temperature and humidity included in the study design. Geographic regions are defined by zones: zone I, temperate; zone II, subtropical; zone III, hot and dry; and zone IV, hot and humid. A given drug product may encounter more than a single zone of temperature and humidity variations during its production and shelf life. Furthermore, it may be warehoused, transported, placed on a pharmacy's shelf, and subsequently in the patient's medicine cabinet, over a varying time course and at a wide range of temperature and humidity. In general, however, the long-term (12 months minimum) testing of new drug entities is conducted at 25°C ± 2°C and at a relative humidity of 60% ± 5%.

Samples maintained under these conditions may be retained for 5 years or longer, during which time they are observed for physical signs of deterioration and chemically assayed. These studies, considered with the accelerated stability studies previously performed, lead to a more precise determination of drug product stability, actual shelf life, and the possible extension of expiration dating. When chemical degradation products are detected, the FDA requires the manufacturer to report their chemical identity, including structure, mechanism of formation, physical and chemical properties, procedures for isolation and purification, specifications and directions for determination at levels expected to be present in the pharmaceutical product, and their pharmacologic action and biologic significance, if any. Physical Pharmacy Capsule. 14 Analytical Methods and Standard Curves discusses some analytical methods and standard curve construction used in studies of this type.

In addition, signs of degradation of the specific dosage forms must be observed and reported. For the various dosage forms, this includes the following :

Tablets: Appearance (cracking, chipping, mottling), friability, hardness, color, odor, moisture content, clumping, disintegration, and dissolution.

Capsules: Moisture tackiness, color, appearance, shape, brittleness, and dissolution.

Oral solutions and suspensions: Appearance, precipitation, pH, color, odor, redispersibility (suspensions), and clarity (solutions).

Oral powders: Appearance, color, odor, and moisture.

Metered-dose inhalation aerosols: Delivered dose per actuation, number of metered doses, color, particle size distribution, loss of propellant, pressure, valve corrosion, spray pattern, and absence of pathogenic microorganisms.

Topical nonmetered aerosols: Appearance, odor, pressure, weight loss, net weight dispensed, delivery rate, and spray pattern.

Topical creams, ointments, lotions, solutions, and gels: Appearance, color, homogeneity, odor, pH, resuspend ability (lotions), consistency, particle-size distribution, strength, and weight loss.

Ophthalmic and nasal and oral inhalation preparations: Appearance, color, consistency, pH, clarity (solutions), particle size and resuspendability (suspensions, ointments), strength, and sterility.

Small-volume parenterals: Appearance, color, particulate matter, dispersibility (suspensions), pH, sterility, pyrogenicity, and closure integrity.

Large-volume parenterals: Appearance, color, clarity, particulate matter, pH, volume and extractables (when plastic containers are used), sterility, pyrogenicity, and closure integrity.

Suppositories: Softening range, appearance, and melting.

Emulsions: Appearance (such as phase separation), color, odor, pH, and viscosity.

Controlled-release membrane drug delivery systems: Seal strength of the drug reservoir, decomposition products, membrane integrity, drug strength, and drug release rate.

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Under usual circumstances, most manufactured products must have a shelf life of 2 or more years to ensure stability at the time of consumption. Commercial products must bear an appropriate expiration date that sets out the time during which the product may be expected to maintain its potency and remain stable under the designated storage conditions. The expiration date limits the time during which the product may be dispensed by the pharmacist or used by the patient.

Prescriptions requiring extemporaneous compounding by the pharmacist do not require the extended shelf life that commercially manufactured and distributed products do because they are intended to be used immediately on receipt by the patient and used only during the immediate course of the prescribed treatment. However, these compounded prescriptions must remain stable and efficacious during the course of use, and the compounding pharmacist must employ formulative components and techniques that will result in a stable product.

In years past, pharmacists were confronted primarily with innocuous topical prescriptions that required extemporaneous formulation. However, in recent years there has been a need to compound other drug delivery systems, for example, progesterone vaginal suppositories and oral suspensions, from tablets or capsules. When presented with a prescription that requires extemporaneous compounding, the pharmacist is confronted with a difficult situation, because the potency and stability of these prescriptions is a serious matter. Occasionally, the results of compatibility and stability studies on such prescriptions are published in scientific and professional journals. These are very useful; however, for some prescriptions stability and compatibility information is not readily available. In these instances, it behooves the pharmacist to contact the manufacturer of the active ingredient or ingredients to solicit stability information.

Also, a compilation of published stability information is included in *Trissel's Stability of Compounded Formulations*. The published stability data are applicable only to products that are prepared identically to the products that are reported.

USP guidelines on stability of extemporaneous compounded formulations state that in the absence of stability information applicable to a specific drug and preparation, the following guidelines can be used: nonaqueous liquids and solid formulations in which the manufactured drug is the source of the active ingredient, not later than 25% of the time remaining until the product's expiration date or 6 months, whichever is earlier; nonaqueous liquids and solid formulations in which a USP or National Formulary (NF) substance is the source of active ingredient, a beyond-use date of 6 months; for water-containing formulations prepared from ingredients in solid form, a beyond-use date not later than 14 days in storage at cold temperatures; for all other formulations, a beyond-use date of the intended duration of therapy or 30 days, whichever is earlier.

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CAPSULE 14

Analytical Methods and Standard Curves

Any study involving concentration of a drug requires an analytical method and the development of stan-dard curves. There are numerous analytical methods used in pharmacy. It is important for pharmacists to have a basic understanding of pharmaceutical analysis to ensure that valid results are obtained when tests are being conducted. It is important to know (*a*) when to test, (*b*) what to test, (*c*) what method(s) to use, (*d*) how to interpret the results, (*e*) the limits of the test, and (*f*) the importance of analytical testing in the overall quality program in pharmacy.

The goal in analytical testing is to produce results as accurately, efficiently, and quickly as possible. Any analytical method used should have accuracy, speed, reproducibility, and specificity. No single analytical method is ideally suited for all drugs; each method has its own strengths and weaknesses, and there are a number of factors that determine the validity and reliability of results.

Selection of an Analytical Method

One general consideration in analytical method selection is the type of information that is needed; quanti-tative (potency, concentration), semiquantitative {where a "cutoff" level is involved, as in endotoxin levels) or qualitative (yes/no type of testing, including substance identification, sterility). Another consideration involves the physical and chemical characteristics ofthe analyte, including its solubility, partition co-efficient, dissociation constant (pK_a), volatility, binding, and the quantity present.

One must consider the degree of quantitative measurement in the validation process, for example accuracy, repeatability/reproducibility, and precision are required; generally, the greater the level that is required, the more sophisticated and expensive the analytical methods that must be used. This is also governed by the types of instrumentation that are on hand or available and the standards available for comparison.

Factors Involved in Methods Selection

The ultimate analytical method selected depends upon a number of factors, including sample requirements, sample handling/preparation/purification requirements, type of data needed, and levels of specificity and accuracy required.

Sampling Requirements

In any analytical method, there may be certain sample requirements that impact one's choice, such as the number of samples needed, the difficulty in obtaining a representative sample, the physical state of the sample (solid, liquid or gas), the type of container required for collection and storage of the sample (some analytes may sorb to the walls or cap liner of the sample containers), and leaching of the container material into the sample, if a liquid, may occur. All these may cause problems in analysis. In the event of sorption, siliconization of the sample vials may sometimes help.

The storage requirements for the sample after collection must be specified (type of container, material used, UV protection, latex contamination, etc.). The effects of air, such as oxidation of the sample ingredients, the presence of carbon dioxide

and the formation of insoluble carbonates, pH changes, free versus bound drug, etc., must be considered. The sample must be stored at the proper temperature (refrigerated, frozen or controlled room) prior to shipment and during shipment. Procedures to follow if the sample is accidentally frozen or experiences a freeze-thaw cycle should be detailed.

In considering the chemical and physical stability of the sample, the effects of water must also be considered. If the sample must be maintained in a dry environment, including a desiccant, this should be detailed. The stability of the sample during storage, extraction, and preparation must be determined. The potential for enzymatic breakdown, or other adverse effects of pH, temperature, solvents, bacterial growth, etc., must be addressed. If volatile solvents are required, special handling must be implemented to prevent evaporation because if some of the solvent is allowed to evaporate, the resulting concentration may be falsely elevated.

The sample matrix effects must be determined. Any effects caused by sample viscosity (pipetting, aspiration), ionic strength (immunoassays, dialysis), buffers (ionized/unionized ratio can alter the extraction efficiency of an analyte prior to analysis), and vapor pressure, where drug can be lost must be considered. If any sample pretreatment is required prior to shipment or working in-house, consider any inaccuracies that may occur from pipetting, which is one of the most common sources of analytical errors when working with small volumes.

There must be a consideration of any physical methods of separation and purification that might be used. Most analytical methods require some degree of sample pretreatment to prepare it for analysis. These may include crystallization from solution, distillation, sublimation, solvent extraction, solid-phase extraction, chromatography, centrifugation; the proper choice of separation and purification depends upon the physical and chemical properties of the sample, including its solubility, volatility, binding, quantity present, etc. The effect of any substances in the formulation that may interfere or alter the results must be known beforehand.

Data Interpretation Requirements

The collection of raw data from the analytical process must be done appropriately. One must ensure that appropriate and valid descriptive statistics are used to analyze the data, and that the operating parameters of the analytical instruments are well established. Reference values, if available, should be provided with the analytical results. A description of the analytical controls used by the laboratory is important for documentation, as well as the source of reference standards used to establish standard curves.

Analytical Methods

In pharmaceutical analysis, analytical methods can be generally divided into physical testing methods. Methods that interact with electromagnetic radiation, conductometric techniques, immunoassay methods, separation techniques and others.

Nonspecific methods generally include melting, freezing and boiling points, density, refractive index, polarimetry, ultraviolet/visible spectroscopy, and pH. Methods that are somewhat more specific include infrared spectroscopic, mass spectroscopy, ion selective electrodes, immunoassay methods, and chromatographic methods (high performance liquid chromatography [HPLC] and gas chromatography [GC]), provided proper standards are used.

Methods that can be routinely used for testing incoming bulk materials, whether active or excipients, include melting, freezing and boiling points, density, refractive index, UV/Visible spectroscopy, infrared spectroscopy, polarimetry, pH, and the separation methods. Final products may generally require a method such as HPLC or GC. A classification of analytical methods follows along with suggested analytical methods that can be used for different dosage forms.

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Classification of Analytical and Microbiological Methods

Physical testing procedures
- Melting point
- Freezing point
- Boiling point
- Density
- Refractive index
- Optical rotation (Polarimetry)
- Thermal analysis
- Color change
- Precipitate formation
- Viscosity change

Interaction of electromagnetic radiation
- Ultraviolet/Visible spectroscopy
- Infrared spectroscopy
- Fluorescence/Phosphorescence spectroscopy
- Raman spectroscopy
- X-ray spectroscopy
- Flame emission and Atomic absorption spectroscopy
- Polarimetry
- Refractometry
- Interferometry

Conductance methods
- pH
- Ion selective electrodes
- Polarography

Immunoassay
- Radioimmunosassay
- Enzyme multiplied immunoassay technique
- Enzyme linked immunosorbent assay
- Fluorescent immunoassay
- HPLC
- GC
- Thin layer chromatography
- Paper chromatography
- Column chromatography

Gravimetric
- Balance

Others

Osmolality Microbiological methods

Sterility testing

Endotoxin testing

Preservative effectiveness testing

Suggested analytical methods for various dosage forms, depending upon the active drug:

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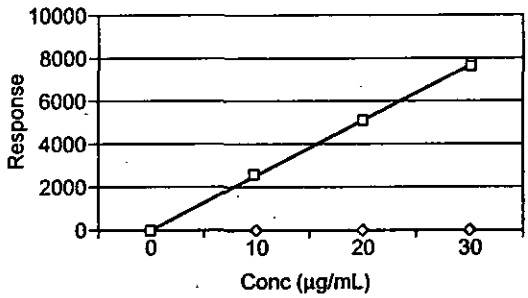
Dosage Form	*Analytical Method*												
	Wt	*Vol*	*pH*	*Osm*	*RI*	*Sp Gr*	*MP*	*UV/vis*	*HPLC*	*GC*	*IR*	*Steril*	*Endo*
Bulk substances	—	—	*	—	*	—	*	*	*	*	*	—	—
Powders	*	—	—	—	—	—	—	—	*	*	—	—	—
Capsules	*	—	—	—	—	—	—	—	*	*	—	—	—
Tablets		*	—	—	—	—	—	—	—	*	*	—	—
Lozenges	*	—	—	—	—	—	—	—	*	*	—	—	—
Suppositories	*	—	—	—	—	*	*	—	*	*	—	—	—
Sticks	*	—	—	—	—	*	*	—	*	*	—	—	—
Solutions	*	*	*	*	*	*	—	*	*	*	—	—	—
Suspensions	*	*	*	—	—	*	—	—	*	*	—	—	—
Emulsions	*	*	*	—	—	*	—	—	*	*	—	—	—
Semisolids	*	—	—	—	—	*	*	—	*	*	—	—	—
Gels	*	*	*	—	*	*	—	—	*	*	—	—	—
Ophthalmics, Otics & Nasals	*	*	*	*	*	*	—	*	*	*	—	*(Oph. Only)	—
Inhalations	*	*	*	*	*	—	—	*	*	*	—	*	—
Injections	*	*	*	*	*	*	—	*	*	*	—	*	*

Construction of a Standard Curve

A standard curve is constructed by analyzing samples (standards) of known composition, generally in increasing concentrations. As each standard is analyzed, an instrumental response (Absorbance, Peak Height, Peak Area, Other Numerical Value) will be obtained. The standard concentrations are plotted as the "*x*" axis on a graph and the instrumental responses are plotted on the "*y*" axis. As an example, The following table represents the results from an HPLC analytical method of methotrexate.

Concentration (µg/mL)	0	10	20	30
Response (Peak Height in units)	0	2600	5190	7780

When plotted on a graph, one obtains the following:



The next step involves analyzing the unknown sample to obtain a response from the instrument. For example, if the unknown sample provided an instrumental response of 3895, checking that value on the *y*-axis and moving toward the right on the graph until it intersects the plotted line and dropping down to the *x*-axis, we can read a value of 15 µg/mL of the methotrexate. As an

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option, the equation of the line can be calculated and the concentration determined by substituting the values of "y" and "b" with the slope of the line to obtain the drug concentration, as follows:

$$m = \Delta y/\Delta x = (7780 - 0)/(30 - 0) = 7780/30 = 259.3$$
$$y = mx + b$$
$$3895 = 259.3\ x + 0$$
$$x = 15.02\ \mu g/mL$$

Thus, if an oral aqueous liquid preparation is made from a tablet or capsule formulation, the pharmacist should make up only at most 14 days' supply, and it must be stored in a refrigerator. Further more, the pharmacist must dispense the medication in a container conducive to stability and use and must advise the patient of the proper method of use and conditions of storage of the medication.

Finally, when compounding on the basis of extrapolated or less than concrete information, the pharmacist is well advised to keep the formulation simple and not to shortcut but use the necessary pharmaceutical adjuvants to prepare the prescription.

6.5 PHARMACEUTICAL INGREDIENTS AND EXCIPIENTS—DEFINITIONS AND TYPES

To produce a drug substance in a final dosage form requires pharmaceutical ingredients. For example, in the preparation of solutions, one or more *solvents* are used to dissolve the drug substance, *flavors* and *sweeteners* are used to make the product more palatable, *colorants* are added to enhance appeal, *preservatives* may be added to prevent microbial growth, and *stabilizers*, such as antioxidants and chelating agents, may be used to prevent decomposition, as previously discussed. In the preparation of tablets, *diluents* or *fillers* are commonly added to increase the bulk of the formulation, *binders* to cause adhesion of the powdered drug and pharmaceutical substances, *antiadherents* or *lubricants* to assist smooth tablet formation, *disintegrating agents* to promote tablet breakup after administration, and coatings to improve stability, control disintegration, or enhance appearance. Ointments, creams, and suppositories acquire their characteristic features from their pharmaceutical bases. Thus, for each dosage form, the pharmaceutical ingredients establish the primary features of the product and contribute to the physical form, texture, stability, taste, and overall appearance.

Table 6.3 presents the principal categories of pharmaceutical ingredients, listing some of the official and commercial agents in use. Additional discussion of many ingredients may be found in the chapters where they are most relevant.

6.6 HANDBOOK OF PHARMACEUTICAL EXCIPIENTS AND FOOD AND CHEMICALS CODEX

The *Handbook of Pharmaceutical Excipients* presents monographs on more than 250 excipients used in dosage form preparation. Each monograph includes such information as nonproprietary, chemical, and commercial names; empirical and chemical formulas and molecular weight; pharmaceutical specifications and chemical and physical properties; incompatibilities and interactions with other excipients and drug substances; regulatory status; and applications in pharmaceutical formulation

or technology. Additional excipients commonly used are listed in the Food Chemicals Codex (FCC), now owned and published by the USP. The Codex contains information on general provisions and requirements applying to specifications, tests and assays of the FCC, monograph specifications, flavor chemicals, infrared spectra, and general tests and assays.

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6.7 HARMONIZATION OF STANDARDS

There is great interest in the international harmonization of standards applicable to pharmaceutical excipients. This is because the pharmaceutical industry is multinational, with major companies having facilities in more than a single country, with products sold in markets worldwide, and with regulatory approval for these products required in each country. Standards for each drug substance and excipient used in pharmaceuticals are contained in pharmacopeias-or for new agents, in an application for regulatory approval by the relevant governing authority. The four pharmacopeias with the largest international use are the *United States Pharmacopeia-National Formulary* (USP-NF), *British Pharmacopeia, European Pharmacopeia,* and *Japanese Pharmacopeia.* Uniform standards for excipients in these and other pharmacopeias would facilitate production efficiency, enable the marketing of a single formulation of a product internationally, and enhance regulatory approval of pharmaceutical products worldwide. The goal of harmonization is an ongoing effort by corporate representatives and international regulatory authorities. A few of the more common and widely used pharmaceutical excipients, including sweeteners, flavors, colors, and preservatives, are discussed here.

6.8 APPEARANCE AND PALATABILITY

Although most drug substances in use today are unpalatable and unattractive in their natural state, their preparations present them to the patient as colorful, flavorful formulations attractive to the sight, smell, and taste. These qualities, which are the rule rather than the exception, have virtually eliminated the natural reluctance of many patients to take medications because of disagreeable odor or taste. In fact, the inherent attractiveness of today's pharmaceuticals has caused them to acquire the dubious distinction of being a source of accidental poisonings in the home, particularly among children who are lured by their organoleptic appeal.

Table 6.3 Examples of Pharmaceutical Ingredients

Ingredient type	*Definition*	*Examples*
Acidifying agent	Used in liquid preparations to provide acidic medium for product stability	Citric acid Acetic acid Fumaric acid Hydrochloric acid Nitric acid
Alkalinizing agent	Used in liquid preparations to provide alkaline medium for product stability	Ammonia solution Ammonium carbonate Diethanolamine Monoethanolamine Potassium hydroxide Sodium bicarbonate

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		Sodium borate Sodium carbonate Sodium hydroxide Trolamine
Adsorbent	An agent capable of holding other molecules onto its surface by physical or chemical (chemisorption) means	Powdered cellulose Activated charcoal
Aerosol propellant	Agent responsible for developing the pressure within an aerosol container and expelling the product when the valve is opened	Carbon dioxide Dichlorodifluoromethane Dichlorotetrafluoroethane Trichloromonofluoromethane
Air displacement	Agent employed to displace air in a hermetically sealed container to enhance product stability	Nitrogen Carbon dioxide
Antifungal preservative	Used in liquid and semisolid preparations to prevent growth of fungi. Effectiveness of parabens is usually enhanced by use in combination	Butylparaben Ethylparaben Methylparaben Benzoic acid Propylparaben Sodium benzoate Sodium propionate
Antimicrobial preservative	Used in liquid and semisolid preparations to prevent growth of microorganisms	Benzalkonium chloride
Antioxidant	Used to prevent deterioration of preparations by oxidation	Ascorbic acid Ascorbyl palmitate Butylated hydroxyanisole Butylated hydroxytoluene Hypophosphorus acid Monothioglycerol Propyl gallate Sodium ascorbate Sodium bisulphite Sodium formaldehyde Sulfoxylate Sodium metabisulphite
Buffering agent	Used to resist change in pH upon dilution or addition of acid or alkali	Potassium metaphosphate Potassium phosphate, monobasic Sodium acetate Sodium citrate, anhydrous and dihydrate
Chelating agent	Substance that forms stable water-soluble complexes (chelates) with metals; used in some liquid pharmaceuticals as stabilizers to complex heavy metals that might promote instability. In such use, they are also called *sequestering* agents	Edetic acid Edetate disodium
Tablet coating agent	Used to coat a tablet to protect against decomposition by atmospheric oxygen or humidity, to provide a desired release pattern, to mask taste or odor, or for aesthetic purposes. Coating may be	

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	sugar, film, or thick covering around a tablet. Sugar-coated tablets generally start to break up in the stomach. Film forms a thin cover around a formed tablet or bead. Unless it is enteric, film dissolves in the stomach. Enteric coating passes through the stomach to break up in the intestines. Some water-insoluble coatings (*e.g.*, ethylcellulose) are used to slow the release of drug in the gastrointestinal tract	
Sugar coating		Liquid glucose Sucrose
Film coating		Hydroxyethyl cellulose Hydroxypropyl cellulose Hydroxypropyl methylcellulose Methyl cellulose (*e.g.*, Methocel) Ethylcellulose (*e.g.*, Ethocel)
Enteric coating		Cellulose acetate phthalate Shellac (35% in alcohol, pharmaceutical glaze)
Tablet direct compression *excipient*	Used in direct compression tablet formulations	Dibasic calcium phosphate (*e.g.*, Ditab)
Tablet disintegrant	Used in solid forms to promote disruption of the mass into smaller particles more readily dispersed or dissolved	Alginic acid Polacrilin potassium (*e.g.*, Amberlite) Sodium alginate Sodium starch glycolate Starch
Tablet glidant	Used in tablet and capsule formulations to improve flow properties of the powder mixture	Colloidal silica Cornstarch Talc
Tablet lubricant	Used in tablet formulations to reduce friction during tablet compression	Calcium stearate Magnesium stearate Mineral oil Stearic acid Zinc stearate
Tablet or capsule opaquant	Used to render a coating opaque. May be used alone or with a colorant	Titanium dioxide
Tablet polishing agent	Used to impart an attractive sheen to coated tablets	Carnauba wax White wax
Tonicity agent	Used to render solution similar in osmotic-dextrose characteristics to physiologic fluids, *e.g.*, in ophthalmic, parenteral, and irrigation fluids	Sodium chloride
Vehicle	Carrying agent used in formulating a variety of liquids for oral and parenteral administration Generally, oral liquids are aqueous (*e.g.*, syrups) or hydroalcoholic (*e.g.*, elixirs). Solutions for intravenous use are aqueous,	

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	whereas intramuscular injections may be aqueous or oleaginous	
Stiffening agent	Used to increase thickness or hardness of a preparation, usually an ointment	Cetyl alcohol Cetyl esters wax Microcrystalline wax Paraffin Stearyl alcohol White wax Yellow wax
Suppository base	Vehicle for suppositories	Cocoa butter Polyethylene glycols (mixtures) PEG 3350
Surfactant (surface active agent)	Substances that absorb to surfaces or interfaces to reduce surface or interfacial tension. May be used as wetting agents, detergents, or emulsifying agents	Benzalkonium chloride Nonoxynol 10 Octoxynol 9 Polysorbate 80 Sodium lauryl sulphate Sorbitan monopalmitate
Suspending agent	Viscosity-increasing agent used to reduce sedimen-tation rate of particles in a vehicle in which they are not soluble; suspension may be formulated for oral, parenteral, ophthalmic, topical, or other route	Agar Bentonite Carbomer (*e.g.*, Carbopol) Carboxymethylcellulose sodium Hydroxyethyl cellulose Hydroxypropyl cellulose Hydroxypropyl methylcellulose Kaolin Methylcellulose Tragacanth Veegum
Sweetening agent	Used to impart sweetness to a preparation	Aspartame Dextrose Glycerin Mannitol Saccharin sodium Sorbitol Sucrose
Tablet antiadherents	Prevent tablet ingredients from sticking to punches and dies during production	Magnesium stearate
Tablet binders	Substances used to cause adhesion of powder particles in tablet granulations	Acacia Alginic acid Carboxymethylcellulose sodium Compressible sugar (*e.g.*, Nu-Tab) Ethylcellulose Gelatin Liquid glucose Methylcellulose Povidone Pregelatinized starch
Tablet and capsule diluent	Inert filler to create desired bulk, flow properties, and compression characteristics of tablets and capsules	Dibasic calcium phosphate Kaolin Lactose Mannitol Microcrystalline cellulose

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		Powdered cellulose Precipitated calcium carbonate Sorbitol Starch
Colorant	Used to impart color to liquid and solid (*e.g.*, tablets and capsules) preparations	FD and C Red No. 3 FD and C Red No. 20 FD and C Yellow No. 6 FD and C Blue No. 2 D and C Green No. 5 D and C Orange No. 5 D and C Red No. 5 Caramel Ferric oxide, red
Clarifying agent	Used as a filtering aid for its adsorbent qualities	Bentonite
Emulsifying agent	Used to promote and maintain dispersion of finely subdivided particles of liquid in a vehicle in which it is immiscible. End product may be a liquid emulsion or semisolid emulsion (*e.g.*, a cream)	Acacia Cetomacrogol Cetyl alcohol Glyceryl monostearate Sorbitan monooleate Polyoxyethylene 50 stearate
Encapsulating agent	Used to form thin shells to enclose a drug for ease of administration	Gelatin
Flavorant	Used to impart a pleasant flavor and often odor to a preparation. In addition to the natural flavorants listed, many synthetic ones are used	Anise oil Cinnamon oil Cocoa Menthol Orange oil Peppermint oil Vanillin
Humectant	Used to prevent drying of preparations, particularly ointments and creams	Glycerin Propylene glycol Sorbitol
Levigating agent	Liquid used as an intervening agent to reduce the particle size of a powder by grinding, usually in a mortar	Mineral oil Glycerin Propylene glycol
Ointment base	Semisolid vehicle for medicated ointments	Lanolin Hydrophilic ointment Polyethylene glycol ointment Petrolatum Hydrophilic petrolatum White ointment Yellow ointment Rose water ointment
Plasticizer	Component of film-coating solutions to make film more pliable, enhance spread of coat over tablets, beads, and granules	Diethyl phthalate Glycerin
Solvent	Used to dissolve another substance in preparation of a solution; may be aqueous or not (*e.g.*, oleaginous). Cosolvents, such as water and alcohol (hydroalcoholic) and water and glycerin, may be used when needed.	Alcohol Corn oil Cottonseed oil Glycerin Isopropyl alcohol Mineral oil

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	Sterile solvents are used in certain preparations (*e.g.*, injections)	Oleic acid Peanut oil Purified water Water for injection Sterile water for injection Sterile water for irrigation
Flavored, sweetened		Acacia syrup Aromatic syrup Aromatic elixir Cherry syrup Cocoa syrup Orange syrup Syrup
Oleaginous		Corn oil Mineral oil Peanut oil Sesame oil
Sterile		Bacteriostatic sodium chloride injection
Viscosity-increasing agent	Used to render preparations more resistant to flow. Used in suspensions to deter sedimentation, in ophthalmic solutions to enhance contact time (*e.g.*, methylcellulose), to thicken topical creams, etc.	Alginic acid Bentonite Carbomer Carboxymethylcellulose Sodium Methylcellulose Povidone Sodium alginate Tragacanth

There is some psychologic basis to drug therapy, and the odor, taste, and color of a pharmaceutical preparation can play a part. An appropriate drug has its most beneficial effect when it is accepted and taken properly by the patient. The proper combination of flavor, fragrance, and color in a pharmaceutical product contributes to its acceptance.

An "electronic tongue" is used to aid in providing a global "taste fingerprint" during formulation development. It provides information on bitterness levels and the stability of flavors in terms of taste (Figure 4.4).

Flavoring Pharmaceuticals

The flavoring of pharmaceuticals applies primarily to liquids intended for oral administration. The 10,000 taste buds on the tongue, roof of the mouth, cheeks, and throat have 60 to 100 receptor cells each (10). These receptor cells interact with molecules dissolved in the saliva and produce a positive or negative taste sensation. Medication in liquid form comes into immediate and direct contact with these taste buds. The addition of flavoring agents to liquid medication can mask the disagreeable taste. Drugs placed in capsules or prepared as coated tablets may be easily swallowed with no contact between the drug and the taste buds. Tablets containing drugs that are not especially distasteful may remain uncoated and unflavored. Swallowing them with water usually is sufficient to avoid undesirable taste sensations. However, chewable tablets, such as certain antacid and vitamin products, usually are sweetened and flavored to improve acceptance.

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The flavor sensation of a food or pharmaceutical is actually a complex blend of taste and smell, with lesser influences of texture, temperature, and even sight. In flavor-formulating a pharmaceutical product, the pharmacist must give consideration to the color, odor, texture, and taste of the preparation. It would be incongruous, for example, to color a liquid pharmaceutical red and give it a banana taste and a mint odor.

The color of a pharmaceutical must have a psychogenic balance with the taste, and the odor must also enhance that taste. Odor greatly affects the flavor of a preparation or foodstuff. If one's sense of smell is impaired, as during a head cold, the usual flavor sensation of food is similarly diminished.

The medicinal chemist and the formulation pharmacist are well acquainted with the taste characteristics of certain chemical types of drugs and strive to mask the unwanted taste through the appropriate use of flavoring agents. Although there are no rules for unerringly predicting the taste sensation of a drug based on its chemical constitution, experience permits the presentation of several observations. For instance, although we recognize and assume the salty taste of sodium chloride, the formulation pharmacist knows that not all salts are salty but that their taste is a function of both cation and anion. Whereas salty tastes are evoked by chlorides of sodium, potassium, and ammonium and by sodium bromide, bromides of potassium and ammonium elicit bitter and salty sensations, and potassium iodide and magnesium sulfate (epsom salt) are predominantly bitter. In general, low-molecular-weight salts are salty, and high-molecular-weight salts are bitter.

Fig. 6.4 Electronic tongue to assist in formulation development

With organic compounds, an increase in the number of hydroxyl groups (–OH) seems to increase the sweetness of the compound. Sucrose, which has eight hydroxyl groups, is sweeter than glycerin, another pharmaceutical sweetener, which has but three hydroxyl groups. In general, the organic esters, alcohols, and aldehydes are pleasant to the taste, and since many of them are volatile, they also contribute to the odor and thus the flavor of preparations in which they are used. Many nitrogen-containing compounds, especially the plant alkaloids (*e.g.*, quinine) are extremely bitter, but certain other nitrogen-containing compounds (*e.g.*, aspartame) are extremely sweet. The medicinal chemist recognizes that even the most simple structural change in an organic compound can alter its taste. *d*-Glucose is sweet, but *l*-glucose has a slightly salty taste; saccharin is very sweet, but *N*-methyl-saccharin is tasteless.

Thus, prediction of the taste characteristics of a new drug is only speculative. However, it is soon learned and the formulation pharmacist is then put to the task of increasing the drug's palatability in the environment of other formulative agents. The selection of an appropriate flavoring agent depends on several factors, primarily the taste of the drug substance itself. Certain flavoring materials are more effective than others

in masking or disguising the particular bitter, salty, sour, or otherwise undesirable taste of medicinal agents. Although individuals' tastes and flavor preferences differ, cocoa-flavored vehicles are considered effective for masking the taste of bitter drugs. Fruit or citrus flavors are frequently used to combat sour or acid-tasting drugs, and cinnamon, orange, raspberry, and other flavors have been successfully used to make preparations of salty drugs more palatable.

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The age of the intended patient should also be considered in the selection of the flavoring agent, because certain age groups seem to prefer certain flavors. Children prefer sweet candy-like preparations with fruity flavors, but adults seem to prefer less sweet preparations with a tart rather than a fruit flavor. Flavors can consist of oil—or water—soluble liquids and dry powders; most are diluted in carriers. Oil-soluble carriers include soybean and other edible oils; water-soluble carriers include water, ethanol, propylene glycol, glycerin, and emulsifiers. Dry carriers include maltodextrins, corn syrup solids, modified starches, gum arabic, salt, sugars, and whey protein. Flavors can degrade as a result of exposure to light, temperature, head space oxygen, water, enzymes, contaminants, and other product components, so they must be carefully selected and checked for stability. The different types of flavors include natural, artificial, and spice:

Natural flavor: Essential oil, oleoresin, essence or extractive, protein hydrolysate, distillate, or any product of roasting, heating, or enzymolysis, which contains the flavoring constituents derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, seafood, poultry, eggs, dairy products, or fermentation products thereof whose significant function in food is flavoring rather than nutritional. [CFR 101.22(a)(3)] In "all natural" flavors, one doesn't necessarily know the exact chemical composition.

Artificial flavor: Any substance used to impart flavor that is not derived from a spice, fruit or fruit juice, vegetable or vegetable juice, edible yeast, herb, bark, bud, root, leaf or similar plant material, meat, fish, poultry, eggs, dairy products, or fermentation products thereof. [CFR 101.22(a)(1)]

Spice: Any aromatic vegetable substance in whole, broken, or ground form, except substances traditionally regarded as foods, such as onions, garlic, and celery; whose significant function in food is seasoning rather than nutritional; that is true to name; and from which no portion of any volatile oil or other flavoring principle has been removed. [CFR 101.22(a)(2)]

In addition to the types of flavors, you should be aware of commercial flavor designations, including the following (**Note:** ABCD would be the flavor name, *e.g.*, cherry):

Natural ABCD flavor	All components derived from ABCD
ABCD flavor, natural	At least one component and artificial-derived from ABCD. No definition of natural to artificial ratio.
ABCD flavor, WONP	All components natural. At least one component derived from ABCD.
Natural flavor, ABCD type	All components natural. No components derived from ABCD.
ABCD flavor, artificial	All components are artificial.
Conceptual flavors	May contain artificial flavors. No reference point. May only have to declare in ingredient declaration.

A general guide to using flavors is to start as follows (keep in mind it is usually possible to add more flavor, but once it is added, it is too late to remove it).

Water-soluble flavors	Generally start at 0.2% for artificial and 1% – 2% for natural flavors.
Oil-soluble flavors	Generally start at 0.1% in finished product for artificial flavors and 0.2% for natural flavors.
Powdered flavors	Generally start at 0.1% in finished product for artificial flavors and 0.75% for natural flavors.

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Sweetening Pharmaceuticals

In addition to sucrose, a number of artificial sweetening agents have been used in foods and pharmaceuticals over the years. Some of these, including aspartame, saccharin, and cyclamate, have faced challenges over their safety by the FDA and restrictions to their use and sale; in fact, in 1969, FDA banned cyclamates from use in the United States.

The introduction of diet soft drinks in the 1950s provided the spark for the widespread use of artificial sweeteners today. Besides dieters, patients with diabetes are regular users of artificial sweeteners. Over the years, each of the artificial sweeteners has undergone long periods of review and debate. Critical to the evaluation of food additives are issues of metabolism and toxicity. For example, almost none of the saccharin a person consumes is metabolized; it is excreted by the kidneys virtually unchanged. Cyclamate, on the other hand, is metabolized, or processed, in the digestive tract, and its by-products are excreted by the kidneys. Aspartame breaks down in the body into three basic components: the amino acids phenylalanine and aspartic acid, and methanol. These three components, which also occur naturally in various foods, are in turn metabolized through regular pathways in the body. Because of its metabolism to phenylalanine, the use of aspartame by persons with phenylketonuria (PKU) is discouraged, and diet foods and drinks must bear an appropriate label warning indicating that the particular foodstuff not be consumed by such individuals. They cannot metabolize phenylalanine adequately, so they undergo an increase in the serum levels of the amino acid (hyperphenylalaninemia). This can result in mental retardation and can affect the fetus of a pregnant woman who has PKU. Passage in 1958 of the Food Additives Amendment to the Food, Drug, and Cosmetic Act produced a major change in how the federal government regulates food additives. For one thing, no new food additive may be used if animal feeding studies or other appropriate tests showed that it caused cancer. This is the famous Delaney Clause. The *amount* of the substance one would have to consume to induce cancer is not significant under the Delaney Clause. Another critical feature of the 1958 amendment was that it did not apply to additives that were generally recognized by experts as safe for their intended uses. Saccharin, cyclamate, and a long list of other substances were being used in foods before the amendment's passage and were "generally recognized as safe"-or what is known today as GRAS. Aspartame, on the other hand, was the first artificial sweetener to fall under the 1958 amendment's requirement for premarketing proof of safety, because the first petition to FDA for its approval was filed in 1973. In 1968, the Committee on Food Protection of the National Academy of Sciences issued an interim report on the safety of nonnutritive sweeteners, including saccharin. In the early 1970s, FDA began a major review of hundreds of food additives on the GRAS list to determine whether current studies justified their safe status. In 1972, with new studies under way, FDA decided to take saccharin off the GRAS and establish interim limits that would permit its continued use until additional studies were completed. (Previous studies indicated that male and female rats fed doses of saccharin developed a signif-

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cant incidence of bladder tumors.) In November 1977, Congress passed the Saccharin Study and Labeling Act, which permitted saccharin's continued availability while mandating that warning labels be used to advise consumers that saccharin caused cancer in animals. The law also directed FDA to arrange further studies of carcinogens and toxic substances in foods.

Cyclamate was introduced into beverages and foods in the 1950s and dominated the artificial sweetener market in the 1960s. After much controversy regarding its safety, the FDA issued a final ruling in 1980 stating that safety has not been demonstrated. Since that date, scientific studies have continued the search for conclusive support or rejection of the FDA decision. At question is cyclamate's possible carcinogenicity and its possible causation of genetic damage and testicular atrophy. See the indicated references for a review of the recent history of sweeteners, including saccharin, cyclamate, fructose, polyalcohols, sucrose, and aspartame.

Acesulfame potassium, a nonnutritive sweetener discovered in 1967, was approved in 1992 by the FDA. It previously was used in a number of other countries. Structurally similar to saccharin, it is 130 times as sweet as sucrose and is excreted unchanged in the urine. Acesulfame is more stable than aspartame at elevated temperatures and FDA initially approved it for use in candy, chewing gum, confectionery, and instant coffee and tea.

A relatively new sweetening agent in U.S. commerce is Stevia powder, the extract from the leaves of the plant *Stevia rebaudiana bertoni*. It is natural, nontoxic, safe, and about 30 times as sweet as cane sugar, or sucrose. It can be used in both hot and cold preparations. Table 6.4 compares three of the most commonly used sweeteners in the food and drug industry. Most large pharmaceutical manufacturers have special laboratories for taste-testing proposed formulations of their products. Panels of employees or interested community participants participate in evaluating the various formulations, and their assessments become the basis for the firm's flavoring decisions. The flavoring agent in liquid pharmaceutical products is added to the solvent or vehicle component of the formulation in which it is most soluble or miscible. That is, water-soluble flavorants are added to the aqueous component of a formulation and poorly water-soluble flavorants are added to the alcoholic or other nonaqueous solvent component of the formulation. In a hydroalcoholic or other multisolvent system, care must be exercised to maintain the flavorant in solution. This is accomplished by maintaining a sufficient level of the flavorant's solvent.

Table 6.4 Comparison of Sweeteners

Source	*Sucrose*	*Saccharin*	*Aspartame*
	Sugar cane; Sugar beet	Chemical synthesis; phthalic anhydride, a petroleum product	Chemical synthesis; methyl ester dipeptide of phenylalanine and aspartic acid
Relative	1	300	180–200 sweetness
Bitterness	None	Moderate to strong	None
Aftertaste	None	Moderate to strong sometimes metallic or bitter	None
Calories	4/g	0	4/g
Acid stability	Good	Excellent	Fair
Heat stability	Good	Excellent	Poor

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Coloring Pharmaceuticals

Coloring agents are used in pharmaceutical preparations for esthetics. A distinction should be made between agents that have inherent colour and those that are employed as colorants. Certain agents-sulfur (yellow), riboflavin (yellow), cupric sulfate (blue), ferrous sulphate (bluish green), cyanocobalamin (red), and red mercuric iodide (vivid red)—have inherent color and are not thought of as pharmaceutical colorants in the usual sense of the term.

Although most pharmaceutical colorants in use today are synthetic, a few are obtained from natural mineral and plant sources. For example, red ferric oxide is mixed in small proportions with zinc oxide powder to give calamine its characteristic pink color, which is intended to match the skin tone upon application.

The synthetic coloring agents used in pharmaceutical products were first prepared in the middle of the 19th century from principles of coal tar. Coal tar (*pix carbonis*), a thick, black viscid liquid, is a by-product of the destructive distillation of coal. Its composition is extremely complex, and many of its constituents may be separated by fractional distillation. Among its products are anthracene, benzene, naphtha, creosote, phenol, and pitch. About 90% of the dyes used in the products FDA regulates are synthesized from a single colorless derivative of benzene called aniline. These aniline dyes are also known as synthetic organic dyes or as coal tar dyes, since aniline was originally obtained from bituminous coal. Aniline dyes today come mainly from petroleum.

Many coal tar dyes were originally used indiscriminately in foods and beverages to enhance their appeal without regard to their toxic potential. It was only after careful scrutiny that some dyes were found to be hazardous to health because of either their own chemical nature or the impurities they carried. As more dyestuffs became available, some expert guidance and regulation were needed to ensure the safety of the public. After passage of the Food and Drug Act in 1906, the U.S. Department of Agriculture established regulations by which a few colorants were *permitted* or *certified* for use in certain products. Today, the FDA regulates the use of color additives in foods, drugs, and cosmetics through the provisions of the Federal Food, Drug, and Cosmetic Act of 1938, as amended in 1960 with the Color Additive Amendments. Lists of color additives *exempt* from certification and those *subject* to certification are codified into law and regulated by the FDA. Certified color additives are classified according to their approved use: (*a*) FD and C color additives, which may be used in foods, drugs, and cosmetics; (*b*) D and C color additives, some of which are approved for use in drugs, some in cosmetics, and some in medical devices; and (*c*) external D and C color additives, the use of which is restricted to external parts of the body, not including the lips or any other body surface covered by mucous membrane. Each certification category has a variety of basic colors and shades for coloring pharma-ceuticals. One may select from a variety of FD and C, D and C, and external D and C reds, yellows, oranges, greens, blues, and violets. By selective combinations of the colorants one can create distinctive colors (Table 6.5).

As a part of the National Toxicology Program of the U.S. Department of Health and Human Services, various substances, including color additives, are studied for toxicity and carcinogenesis. For color additives, the study protocols usually call for a 2-year study in which groups of male and female mice and rats are fed diets containing various quantities of the colorant. The killed and surviving animals

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are examined for evidence of long-term toxicity and carcinogenesis. Five categories of evidence of carcinogenic activity are used in reporting observations: (*a*) "clear evidence" of carcinogenic activity; (*b*) "some evidence"; (*c*) "equivocal evidence," indicating uncertainty; (*d*) "no evidence," indicating no observable effect; and (*e*) "inadequate study," for studies that cannot be evaluated because of major flaws.

The certification status of the colorants is continually reviewed, and changes are made in the list of certified colors in accordance with toxicology findings. These changes may be (*a*) the withdrawal of certification, (*b*) the transfer of a colorant from one certification category to another, or (*c*) the addition of new colors to the list. Before gaining certification, a color additive must be demonstrated to be safe. In the case of pharmaceutical preparations, color additives, as with all additives, must not interfere with therapeutic efficacy, nor may they interfere with the prescribed assay procedure for the preparation.

Table 6.5 Examples of Color Formulations

Shade or Color	*FD and C DYE*	*% of Blend*
Orange	Yellow No. 6	100
	or	
	Yellow No. 5	95
	Red No. 40	5
Cherry	Red No. 40	100
	or	
	Red No. 40	99
	Blue No. 1	1
Strawberry	Red No. 40	100
	or	
	Red No. 40	95
	Red No. 3	5
Lemon	Yellow No. 5	100
Lime	Yellow No. 5	95
	Blue No. 1	5
Grape	Red No. 40	80
	Blue No. 1	20
Raspberry	Red No. 3	75
	Yellow No. 6	20
	Blue No. 1	5
Butterscotch	Yellow No. 5	74
	Red No. 40	24
	Blue No. 1	2
Chocolate	Red No. 40	52
	Yellow No. 5	40
	Blue No. 1	8
Caramel	Yellow No. 5	64
	Red No. 3	21
	Yellow No. 6	9
	Blue No. 1	6
Cinnamon	Yellow No. 5	60
	Red No. 40	35
	Blue No. 1	5

From literature of Wamer-Jenkinson Co., St. Louis, Mo.

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In the 1970s, concern and scientific questioning of the safety of some color additives heightened. A color that drew particular attention was FD&C Red No. 2, because of its extensive use in foods, drugs, and cosmetics. Researchers in Russia reported that this color, also known as amaranth, caused cancer in rats. Although the FDA was never able to determine the purity of the amaranth tested in Russia, these reports led to FDA investigations and a series of tests that eventually resulted in withdrawal of FD&C Red No. 2 from the FDA certified list in 1976 because its sponsors were unable to prove safety. That year, FDA also terminated approval for use of FD&C Red No. 4 in maraschino cherries and ingested drugs because of unresolved safety questions. FD&C Red No. 4 is now permitted only in externally applied drugs and cosmetics.

FD&C Yellow No. 5 (also known as tartrazine) causes allergic-type reactions in many people. People who are allergic to aspirin are also likely to be allergic to this dye. As a result, the FDA requires listing of this dye by name on the labels of foods (*e.g.*, butter, cheese, ice cream) and ingested drugs containing it. A colorant becomes an integral part of a pharmaceutical formulation, and its exact quantitative amount must be reproducible each time the formulation is prepared, or else the preparation would have a different appearance from batch to batch. This requires a high degree of skill, for the amount of colorant generally added to liquid preparations ranges from 0.0005% to 0.001% depending upon the colorant and the depth of color desired. Because of their color potency, dyes generally are added to pharmaceutical preparations in the form of diluted solutions rather than as concentrated dry powders. This permits greater accuracy in measurement and more consistent color production.

In addition to liquid dyes in the coloring of pharmaceuticals, lake pigments may also be used. Whereas a chemical material exhibits coloring power or tinctorial strength when dissolved, pigment is an insoluble material that colors by dispersion. An FD&C lake is a pigment consisting of a substratum of alumina hydrate on which the dye is adsorbed or precipitated. Having aluminum hydroxide as the substrate, the lakes are insoluble in nearly all solvents. FD and C lakes are subject to certification and must be made from certified dyes. Lakes do not have a specified dye content; they range from 10% to 40% pure dye. By their nature, lakes are suitable for coloring products in which the moisture levels are low. Lakes in pharmaceuticals are commonly used in the form of fine dispersions or suspensions. The pigment particles may range in size from less than 1 μm up to 30 μm. The finer the particle, the less chance for color speckling in the finished product. Blends of various lake pigments may be used to achieve a variety of colors, and various vehicles, such as glycerin, propylene glycol, and sucrose-based syrup, may be employed to disperse the colorants. Colored empty gelatin capsule shells may be used to hold a powdered drug mixture. Many commercial capsules are prepared with a capsule body of one color and a cap of a different color, resulting in a two-colored capsule. This makes certain commercial products more readily identifiable than solid-colored capsules. For powdered drugs dispensed as such or compressed into tablets, a generally larger proportion of dye is required (about 0.1%) to achieve the desired hue than with liquid preparations.

Both dyes and lakes are used to color sugarcoated tablets, film-coated tablets, directc ompression tablets, pharmaceutical suspensions, and other dosage forms (17). Traditionally, sugar-coated tablets have been colored with syrup solutions containing varying amounts of the water-soluble dyes, starting with very dilute solutions, working up to concentrated color syrup solutions. As many as 30 to 60 coats are common. With the lakes, fewer color coats are used. Appealing tablets have been

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made with as few as 8 to 12 coats using lakes dispersed in syrup. Water-soluble dyes in aqueous vehicles or lakes dispersed in organic solvents may be effectively sprayed on tablets to produce attractive film coatings. There is continued interest today in chewable tablets, because of the availability of many direct-compression materials such as dextrose, sucrose, mannitol, sorbitol, and spraydried lactose. The direct-compression colored chewable tablets may be prepared with 1 lb of lake per 1,000 lb of tablet mix. For aqueous suspensions, FD&C water-soluble colors or lakes may be satisfactory. In other suspensions, FD&C lakes are necessary. The lakes, added to either the aqueous or nonaqueous phase, generally at a level of 1 lb of color per 1,000 lb of suspension, require homogenization or mechanical blending to achieve uniform coloring.

For the most part, ointments, suppositories, and ophthalmic and parenteral products assume the color of their ingredients and do not contain color additives. Should a dye lose the certification status it held when a product was first formulated, manufactured, and marketed, the manufacturer must reformulate within a reasonable length of time, using only color additives certified at the new date of manufacture. In addition to esthetics and the certification status of a dye, a formulation pharmacist must select the dyes to be used in a particular formula on the basis of their physical and chemical properties. Of prime importance is the solubility of a prospective dye in the vehicle to be used for a liquid formulation or in a solvent to be employed during a pharmaceutical process, as when the dye is sprayed on a batch of tablets. In general, most dyes are broadly grouped into those that are water soluble and those that are oil soluble; few if any dyes are both. Usually, a water-soluble dye is also adequately soluble in commonly used pharmaceutical liquids like glycerin, alcohol, and glycol ethers. Oil-soluble dyes may also be soluble to some extent in these solvents and in liquid petrolatum (mineral oil), fatty acids, fixed oils, and waxes. No great deal of solubility is required, since the concentration of dye in a given preparation is minimal.

Another important consideration when selecting a dye for use in a liquid pharmaceutical is the pH and pH stability of the preparation to be colored. Dyes can change color with a change in pH, and the dye must be selected so that no anticipated pH change will alter the color during the usual shelf life. The dye also must be chemically stable in the presence of the other formulative ingredients and must not interfere with the stability of the other agents. To maintain their original colors, FD&C dyes must be protected from oxidizing agents, reducing agents (especially metals, including iron, aluminum, zinc, and tin), strong acids and alkalis, and excessive heating. Dyes must also be reasonably photostable; that is, they must not change color when exposed to light of anticipated intensities and wavelengths under the usual conditions of shelf storage. Certain medicinal agents, particularly those prepared in liquid form, must be protected from light to maintain their chemical stability and their therapeutic effectiveness. These preparations are generally kept in dark amber or opaque containers. For solid dosage forms of photolabile drugs, a colored or opaque capsule shell may enhance the drug's stability by shielding out light rays.

6.9 PRESERVATIVES

In addition to the stabilization of pharmaceutical preparations against chemical and physical degradation due to changed environmental conditions within a form-

ulation, certain liquid and semisolid preparations must be preserved against microbial contamination.

Sterilization and Preservation

Although some types of pharmaceutical products, for example, ophthalmic and injectable preparations, are sterilized by physical methods (autoclaving for 20 minutes at 15 lb pressure and 121°C, dry heat at 180°C for 1 hour, or bacterial filtration) during manufacture, many of them also require an antimicrobial preservative to maintain their aseptic condition throughout storage and use. Other types of preparations that are not sterilized during their preparation but are particularly susceptible to microbial growth because of the nature of their ingredients are protected by the addition of an antimicrobial preservative. Preparations that provide excellent growth media for microbes are most aqueous preparations, especially syrups, emulsions, suspensions, and some semisolid preparations, particularly creams. Certain hydroalcoholic and most alcoholic preparations may not require the addition of a chemical preservative when the alcoholic content is sufficient to prevent microbial growth. Generally, 15% V/V alcohol will prevent microbial growth in acid media and 18% V/V in alkaline media. Most alcohol-containing pharmaceuticals, such as elixirs, spirits, and tinctures, are self-sterilizing and do not require additional preservation. The same applies to other individual pharmaceuticals that by virtue of their vehicle or other formulative agents may not permit the growth of micro-organisms.

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Preservative Selection

When experience or shelf storage experiments indicate that a preservative is required in a pharmaceutical preparation, its selection is based on many considerations, including some of the following:

- The preservative prevents the growth of the type of microorganisms considered the most likely contaminants of the preparation.
- The preservative is soluble enough in water to achieve adequate concentrations in the aqueous phase of a system with two or more phases.
- The proportion of preservative remaining undissociated at the pH of the preparation makes it capable of penetrating the microorganism and destroying its integrity.
- The required concentration of the preservative does not affect the safety or comfort of the patient when the pharmaceutical preparation is administered by the usual or intended route; that is, it is nonirritating, nonsensitizing, and nontoxic.
- The preservative has adequate stability and will not be reduced in concentration by chemical decomposition or volatilization during the desired shelf life of the preparation.
- The preservative is completely compatible with all other formulative ingredients and does not interfere with them, nor do they interfere with the effectiveness of the preservative agent.
- The preservative does not adversely affect the preparation's container or closure.

General Preservative Considerations

Microorganisms include molds, yeasts, and bacteria, with bacteria generally favoring a slightly alkaline medium and the others an acid medium. Although few micro-

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organisms can grow below pH 3 or above pH 9, most aqueous pharmaceutical preparations are within the favorable pH range and therefore must be protected against microbial growth. To be effective, a preservative agent must be dissolved in sufficient concentration in the aqueous phase of a preparation. Furthermore, only the undissociated fraction or molecular form of a preservative possesses preservative capability, because the ionized portion is incapable of penetrating the microorganism. Thus, the preservative selected must be largely undissociated at the pH of the formulation being prepared. Acidic preservatives like benzoic, boric, and sorbic acids are more undissociated and thus more effective as the medium is made more acid.

Conversely, alkaline preservatives are less effective in acid or neutral media and more effective in alkaline media. Thus, it is meaningless to suggest preservative effectiveness at specific concentrations unless the pH of the system is mentioned and the undissociated concentration of the agent is calculated or otherwise determined. Also, if formulative materials interfere with the solubility or availability of the preservative agent, its chemical concentration may be misleading, because it may not be a true measure of the effective concentration. Many incompatible combinations of preservative agents and other pharmaceutical adjuncts have been discovered in recent years, and undoubtedly many more will be uncovered in the future as new preservatives, pharmaceutical adjuncts, and therapeutic agents are combined for the first time. Many of the recognized incompatible combinations that inactivate the preservative contain macromolecules, including various cellulose derivatives, polyethylene glycols, and natural gums. These include tragacanth, which can attract and hold preservative agents, such as the parabens and phenolic compounds, rendering them unavailable for their preservative function. It is essential for the research pharmacist to examine all formulative ingredients as one affects the other to ensure that each agent is free to do its job. In addition, the preservative must not interact with a container, such as a metal ointment tube or a plastic medication bottle, or with an enclosure, such as a rubber or plastic cap or liner. Such an interaction could result in decomposition of the preservative or the container closure or both, causing decomposition and contamination. Appropriate tests should be devised and conducted to prevent this type of preservative interaction.

Mode of Action

Preservatives interfere with microbial growth, multiplication, and metabolism through one or more of the following mechanisms:

- Modification of cell membrane permeability and leakage of cell constituents (partial lysis)
- Lysis and cytoplasmic leakage
- Irreversible coagulation of cytoplasmic constituents (*e.g.*, protein precipitation)
- Inhibition of cellular metabolism, such as by interfering with enzyme systems or inhibition of cell wall synthesis
- Oxidation of cellular constituents
- Hydrolysis

A few of the commonly used pharmaceutical preservatives and their probable modes of action are presented in Table 6.6.

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Table 6.6 Probable Modes of Action of Some Preservatives

Preservative	*Probable Modes of Action*
Benzoic acid, boric acid, *p*-hydroxybenzoates	Denaturation of proteins
Phenols and chlorinated phenolic compounds	Lytic and denaturation action on cytoplasmic membranes and for chlorinated preservatives, also by oxidation of enzymes
Alcohols	Lytic and denaturation action on membranes
Quatemary compounds	Lytic action on membranes
Mercurials	Denaturation of enzymes by combining with thiol (–SH) groups

Preservative Utilization

Suitable substances may be added to a pharmaceutical preparation to enhance its permanency or usefulness. Such additives are suitable only if they are nontoxic and harmless in the amounts administered and do not interfere with the therapeutic efficacy or tests or assays of the preparation. Certain intravenous preparations given in large volumes as blood replenishers or as nutrients are not permitted to contain bacteriostatic additives, because the amounts required to preserve such large volumes would constitute a health hazard when administered to the patient. Thus preparations like dextrose injection, USP, and others commonly given as fluid and nutrient replenishers by intravenous injections in amounts of 500 to 1,000 mL may not contain antibacterial preservatives. On the other hand, injectable preparations given in small volumes-for example, morphine sulfate injection, USP, which provides a therapeutic amount of morphine sulfate in approximately a 1 mL volume—can be preserved with a suitable preservative without the danger of the patient receiving an excessive amount of the preservative. Examples of the preservatives and their concentrations commonly employed in pharmaceutical preparations are benzoic acid (0.1% to 0.2%), sodium benzoate (0.1% to 0.2%), alcohol (15% to 20%), phenylmercuric nitrate and acetate (0.002% to 0.01%), phenol (0.1% to 0.5%), cresol (0.1% to 0.5%), chlorobutanol (0.5%), benzalkonium chloride (0.002% to 0.01%), and combinations of methylparaben and propylparaben (0.1% to 0.2%), the latter being especially good against fungus. The required proportion varies with the pH, dissociation, and other factors already indicated as well with the presence of other formulative ingredients with inherent preservative capabilities.

For each type of preparation to be preserved, the research pharmacist must consider the influence of the preservative on the comfort of the patient. For instance, a preservative in an ophthalmic preparation must have an extremely low degree of irritant qualities, which is characteristic of chlorobutanol, benzalkonium chloride, and phenylmercuric nitrate, frequently used in ophthalmic preparations. In all instances, the preserved preparation must be biologically tested to determine its safety and efficacy and shelf-tested to determine its stability for the intended shelf life of the product.

SUMMARY

- The proper design and formulation of a dosage form requires consideration of the physical, chemical, and biologic characteristics of all of the drug substances and pharmaceutical ingredients to be used in fabricating the product.

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- If the medication is intended for systemic use and oral administration is desired, tablets and/or capsules are usually prepared because they are easily handled by the patient and are most convenient in the self-administration of medication.
- In dealing with the problem of formulating a drug substance into a proper dosage form, research pharmacists employ knowledge gained through experience with other chemically similar drugs and through the proper use of the physical, chemical, biologic, and pharmaceutical sciences.
- Microscopic examination of the raw drug substance is an important step in preformulation work. It gives an indication of particle size and size range of the raw material along with the crystal structure.
- Drug and drug product stability testing during every stage of development is critical to the quality of the product. Drug stability is important during preclinical testing and in clinical (human) trials to obtain a true and accurate assessment of the product being evaluated.
- The goal of harmonization is an ongoing effort by corporate representatives and international regulatory authorities.

REVIEW QUESTIONS

1. Discuss the need for dosage forms.
2. Discuss the general considerations in dosage form design.
3. Discuss preformulation studies.
4. Explain pharmaceutical ingredients and Excipients-Definitions and Types.
5. What is Flavouring Pharmaceuticals?
6. Define coloring Pharmaceuticals.
7. What is Preservatives in Pharmaceutical point of viewed?

FURTHER READINGS

- Theory and Practice of Social Sciences, Ramakanth Tiwari and Mahesh Dabhade.