13

ACCUMULATION OF DRUGS IN TISSUES

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13.1 INTRODUCTION

In order to exert desirable pharmacological action, drugs need to achieve an adequate concentration in their target tissues. The term pharmacokinetics refers to absorption, distribution, metabolism, excretion, and toxicity. This chapter deals, in detail, with the aspects concerned with drug distribution and disposition.

The two fundamental processes that determine the concentration of a drug at any moment and in any region of the body are (1) translocation of the drug molecules and (2) the chemical transformations they undergo due to metabolic action. Drug molecules move around the body in two ways: bulk flow transfer (i.e., in the bloodstream) and diffusional transfer. The chemical nature of the drug makes no difference to its transfer by bulk flow as the cardiovascular system provides a very fast long-distance distribution system. In contrast, diffusional characteristics differ markedly between different drugs. In particular, ability to cross hydrophobic diffusion barriers is strongly influenced by lipid solubility of the drug molecule. Aqueous diffusion is also part of the overall mechanism of drug transport since it is this process that delivers drug molecules to and from the nonaqueous barriers.

Once the drug reaches the bloodstream, it is distributed to all organs including tissues that are not relevant to its pharmacological or therapeutic effect. Thus, after absorption, the drug may not only get reversibly associated with its site of action (receptor) but may get bound to plasma proteins or may accumulate in various storage sites or may enter into tissues that are not involved in its primary action (although they may be involved in metabolism, and excretion). This part of pharmacokinetics, which deals with distribution, metabolism, and excretion, is termed drug disposition because these three phases precisely decide the fate of a drug after absorption. The drug may be evenly, or unevenly, or selectively distributed in different body compartments, which are physiological in character rather than anatomical entities.

13.2 MOVEMENT OF DRUG MOLECULES ACROSS CELL BARRIERS

Plasma membrane limits the boundary of a cell from the rest of the body parts including aqueous compartments. In fact, a single layer of membrane separates intracellular and extracellular compartments. An epithelial barrier such as the gastrointestinal mucosa or renal tubule consists of a layer of cells tightly connected with each other so that molecules traverse at least two cell membranes—inner and outer. Vascular epithelium is still more complicated, with its variation in anatomical disposition and vascular flow between tissues. In some organs, especially the central nervous system and placenta, there are tight junctions between the cells and the endothelium is covered with an impermeable layer of periendothelial cells. In organs such as the liver and spleen, the endothelium is discontinuous, allowing free passage between cells.

There are four main ways by which small molecules cross cell membranes—(1) by diffusing directly through the lipids of the plasma membrane; (2) by diffusing through aqueous pores formed by special proteins that traverse the lipid bilayer; (3) by combining with a transmembrane carrier protein that binds a drug molecule on one side of the membrane, then changes conformation and releases it on the

other; or (4) by pinocytosis. Of these four ways, diffusion through lipid and carrier-mediated transport are very important in relation to pharmacokinetic mechanisms. There is yet another mechanism of transport, by which some drugs are actively transported into the cell by energy-dependent pumps [1]. In some cases, two or more drugs may share the same pump, giving rise to drug–drug interactions A different energy-dependent efflux pump may also pump them out of the cell. It is thought that multidrug resistance (mdr) transporter may play a role in lowering intracellular drug concentrations via an efflux mechanism. Increased expression of P-glycoprotein is often associated with lower intracellular accumulation of certain drugs due to efflux of the drug from the cells [2–5].

13.3 ABSORPTION OF DRUGS

Absorption is defined as the passage of a drug from the site of administration into the plasma. Absorption is therefore important for all routes of administration, except intravenous injection. The main routes of administration are oral, sublingual, rectal, topical application to other epithelial surfaces (e.g., skin, cornea, vagina, and nasal mucosa), inhalation, and injection—subcutaneous, intramuscular, intravenous, and intrathecal [6].

13.3.1 Oral, Sublingual, and Rectal Administration

Oral Administration Although most drugs are swallowed by the oral route, little absorption occurs until the drug reaches the small intestine. The mechanism of drug absorption is the same as for other epithelial barriers, namely, the passive diffusion at a rate determined by the ionization and lipid solubility of the drug molecules. In a few instances intestinal absorption depends on carrier-mediated transport rather than simple lipid diffusion. Examples of the drugs under this category are levodopa and fluorouracil. In general, iron is absorbed via specific carriers in the surface membranes of the jejunal mucosa, and calcium is absorbed by means of vitamin D-dependent carrier systems. The biochemical properties of the drug such as molecular weight, particle size, and pH, along with local factors such as food, intestinal motility, and blood flow, all affect gastrointestinal (GI) absorption. The presence of agents that may affect gastric pH or motility or interact with the drug to produce binding will prevent or slow the absorption. Examples of these agents and the absorption factors affected are antacids (pH), vasodilators (GI perfusion), cathartics (GI motility), and calcium (binding). Some drugs are given to patients as prodrugs since the absorption of the prodrug may be superior to the parent drug (hence increased bioavailability). Examples of prodrugs are levodopa and azarabine, prodrugs of dopamine and azauridine, respectively. The term *bioavailability* indicates the proportion of the drug that passes into the systemic circulation after oral administration, taking into consideration both absorption and metabolic degradation in the gut wall or liver before reaching the systemic circulation.

Sublingual Administration A few drugs are placed under the tongue (taken sublingually) for their direct absorption into the small blood vessels that lie underneath the tongue. The absorption is rapid and the drug enters the systemic circulation

without entering the portal system and so escapes first-pass metabolism. The sublingual route is especially good for nitroglycerin, which is used for treatment of angina.

Rectal Administration Rectal administration is useful for drugs that are required both to produce a local effect (e.g., anti-inflammatory drugs for treating ulcerative colitis) and to produce systemic effects. The spatial positioning of the rectal suppositories avoiding the upper hemorrhoidal vein can avoid the *first-pass effect*, as the encapsulated drug molecules will be directly released into the inferior vena cava. Nevertheless, the popularity of this mode of route has abated because of erratic absorption behavior and also a great deal of advancement in parenteral preparations.

13.3.2 Application to Epithelial Surfaces

Cutaneous Administration Cutaneous administration is used when a local effect on the skin is required (e.g., topically applied steroids). Appreciable absorption may nonetheless occur and lead to systemic effects. Most drugs are absorbed very poorly through unbroken skin. Transdermal dosage forms in which the drug is incorporated as a stick-on patch applied in the area of thin skin are increasingly used. Several drugs are available in this form, for example, estrogen for hormone replacement.

Nasal Sprays Some drugs like peptide hormone analogues (e.g., antidiuretic hormone, gonadotropin-releasing hormone, and calcitonin), if given orally, will be inactive as they will quickly be destroyed in the gastrointestinal tract, and so they are administered in nasal sprays so that adequate absorption takes place through nasal mucosa. Intranasal administration has advantages in several cases. For example, clinical experience suggests that intranasal sumatriptan (a potent serotonin $5HT_{1B/1D}$ agonist widely used in the treatment of migraine) has more advantages over oral and subcutaneous administration, such as more rapid onset of the effect and use in patients with gastrointestinal complaints [7].

Eye Drops Many drugs are applied as eye drops and they get absorbed through the epithelium of the conjunctival sac. Systemic side effects are not caused while desirable local effects are best achieved. One example is dorzolamide (a carbonic anhydrase inhibitor), which is given as eye drops to lower ocular pressure in glaucoma patients. In some cases administration of eye drops may result in unwanted side effects on account of delivery of absorbed material from the eye to the systemic circulation.

13.3.3 Administration by Inhalation

This route is best used for volatile and gaseous anesthetics and the lung serves as the route of administration and elimination. Rapid absorption on account of large surface area and high vascularity of the lung tissue makes it possible to achieve rapid adjustments of plasma concentration of the drugs. Drugs used for their effects on the lung are also given by inhalation, usually as an aerosol. Inhalation therapy

is commonly used in the treatment of asthma as it results in a high concentration of active drug at the site of action. The degree of lung deposition of an inhaled drug is influenced by several factors—the one most important is the inhalation maneuvers. Other possible factors are related to the properties of the formulation, such as particle size and hygroscopic nature. Glucocorticoids (e.g., beclomethasone) and bronchodilators (e.g., salbutamol) are given by this route, which results in high local concentration in the lung while minimizing systemic side effects. Minimizing the systemic absorption by chemical modification of a drug will reduce the side effects. For example, ipatropium, a muscarinic receptor antagonist, a quaternary ammonium ion analogue of atropine, is poorly absorbed into systemic circulation.

13.3.4 Administration by Injection

Intravenous Injection This is the fastest and the most certain route of drug administration. Bolus injection produces a very high concentration of drug first in the right heart and lungs, and then in the systemic circulation. The rate of injection of the drug decides the peak concentration reaching the tissues. Steady intravenous infusion avoids the uncertainties of absorption from other sites while avoiding high peak plasma concentrations resulting from bolus injection. Several antibiotics and anesthetics such as propofol and diazepam, used for treatment of patients with status epilepticus, are administered intravenously for better bioavailability of the drug.

Subcutaneous or Intramuscular Injection Subcutaneous or intramuscular injection of drugs usually produces a faster effect than oral administration, but the rate of absorption depends on the site of injection and on local blood flow. Diffusion through the tissue and removal of the injected drug by local blood flow are the rate-limiting factors.

Intrathecal (Intraspinal) Injection Injection of a drug into the subarachnoid space via a lumbar puncture needle is often used for some specialized purposes. Methotrexate is administered intrathecally in the treatment of certain childhood leukemias. Regional anesthesia can be produced by intrathecal administration of a local anesthetic, such as bupivacaine. Some antibiotics (e.g., aminoglycosides) cross the blood–brain barrier very slowly and in rare cases of nervous system infections they are given intrathecally.

13.4 DRUG DISTRIBUTION

Following absorption from the gastrointestinal tract or direct administration through another route, the drug is distributed to the tissues and organs throughout the body. Most drugs do not localize to one particular site, but are distributed to a number of organs and tissues. Distribution refers to both rate of distribution and extent of distribution. The rate of distribution depends on the membrane permeability and the blood perfusion, while the extent of distribution is influenced by factors such as lipid solubility of the drug, pH–pK_a, plasma protein binding, and intracellular binding [8, 9].

13.4.1 Rate of Distribution

Membrane Permeability Membrane permeability refers to the ability of a drug to cross the cell membranes. This may occur by passive diffusion, where the drug simply moves from an area of higher concentration to one of lower concentration. Non-polar drug molecules dissolve freely in the lipid bilayer of the membrane and are translocated across it by diffusion. The permeability coefficient, P, and the concentration gradient across the membrane will determine the number of drug molecules that will cross per unit area in unit time. In general, two physicochemical factors that significantly contribute to the permeability coefficient are solubility of the drug and its diffusivity across the membrane. Solubility is expressed as a partition coefficient for the substance to be distributed between the membrane phase (lipid environment) and the aqueous environment and the diffusivity is a measure of the mobility of the molecules within membrane lipid and is expressed as a diffusion coefficient. Since the diffusion coefficient varies only slightly among different drug molecules, the partition coefficient remains as the most important variable influencing drug diffusion. Lipid solubility is one of the important determinants of the pharmacokinetic characteristics of a drug. Many properties, such as rate of absorption from the gut, penetration into brain and the other tissues, and the extent of renal elimination, can be predicted from the drug's solubility profile.

Lipid-soluble compounds pass trough the cell membranes very rapidly, whereas water-soluble compounds penetrate more slowly at a rate dependent on their size. Low molecular weight drugs pass through by simple diffusion. The transfer is slow for drugs with molecular diameter above 100 Å. There are two deviations to the typical capillary structure, which result in variation from normal drug permeability. First, the permeability is greatly increased in the renal capillaries by pores in the membrane of the endothelial cells and also in specialized hepatic capillaries (sinusoids), where a complete lining is generally lacking. This results in more extensive transfer of drugs out of the capillary bed. Second, the brain capillaries seem to have impermeable walls restricting the transfer of molecules from blood to brain tissue (blood–brain barrier). Lipid-soluble compounds can readily be transferred but the transfer of polar compounds is severely restricted by the barrier.

The transfer rate of ionizable drugs across the capillary membrane depends on the pK_a of the drug molecule as well as the pH of the blood. Many drugs are weak acids or bases and therefore exist in the un-ionized as well as the ionized form. The ratio of un-ionized to ionized or vice versa will depend on the pH of the environment. The ionized species has very low lipid solubility and is virtually unable to permeate membrane except where a specific transport mechanism exists. The lipid solubility of the un-ionized species will depend on the chemical nature of the drug. Most of the un-ionized drug molecules are adequately lipid soluble. There are, however, exceptions to this rule while dealing with many drug molecules. For example, aminoglycoside antibiotics are sufficiently hydrophilic in the uncharged form, here the presence of hydroxyl groups (electronegative oxygen) in the sugar molecules of the drug that plays an important role in imparting solubility to polar solvents like water.

Ionization affects not only the rate of drug permeation across membranes but also the steady-state distribution of the drug molecules between two or more aqueous compartments like plasma, urine, and gastric juice, if a pH difference exists between

them. Within each compartment the ratio of ionized to un-ionized species of the drug, as determined by the pK_a of the drug and the pH of the environment, will be different and acidic drug will be concentrated in the compartment with high pH and vice versa. This is called ion trapping. The larger the pH difference between two aqueous compartments, the greater will be the concentration gradient produced by ion trapping.

13.4.2 Blood Perfusion

Membrane permeability tends to restrict the transfer and distribution of drugs once they are delivered to the tissue. The other major factor that determines the rate of drug distribution is blood perfusion. The rate at which blood perfuses to different organs varies widely. Total blood flow is greatest to brain, kidneys, liver, and muscle with highest perfusion rates to brain, kidney, liver, and heart. Hence total drug concentration would rise most rapidly in these organs. Highly perfused organs, namely, heart, liver, kidney, and brain, usually receive drug within minutes after its absorption. Distribution to tissues like muscle, fat, and skin may require longer periods of time (minutes to hours). Certain organs such as the adrenals also have large perfusion rates. The relative restriction/contribution of the twin factors of membrane permeability and blood perfusion to drug distribution can better be understood with examples of transfer of thiopental and penicillin to brain and muscle. Thiopental gets into the brain faster than muscle, while penicillin gets into muscle more quickly than it gets into brain. Thiopental is only partly ionized and passes into the brain or muscle easily. But since brain has a perfusion rate approximately 20 times higher than muscle, thiopental transfers in and out of brain more quickly. On the other hand, penicillin, which is quite polar, transfers faster in muscle than brain on account of lesser restriction of muscle capillaries to the drug.

There is growing evidence that the reduction of blood flow in soft tissues is closely associated with the significant impairment of the rate of tissue penetration by antimicrobial agents. For example, in patients with peripheral arterial occlusive disease, it has been demonstrated that the peak concentrations are significantly lower in the interstitium of ischemic soft tissue in comparison with that in healthy and well perfused tissue.

13.4.3 Extent of Drug Distribution

Several physiological barriers influence the extent of drug distribution. Chemicophysical properties, molecular weight, degree of plasma protein binding, and intracellular binding are among the most important intrinsic characteristics that condition the extent of drug distribution in the body [10, 11]. Besides its ability to bind with plasma protein, the size and degree of ionization as well as lipid solubility of a drug molecule specifically regulate its extravascular transfer. Host-related factors such as site of infection, presence of biological barriers (blood–brain barrier, placental barrier, blood–CSF barrier, prostate, ocular membranes), and local pH may contribute to the pharmacological action of drug molecules. Sometimes these factors can inhibit a particular drug molecule as well. Inactivation of the antibacterial agent in the tissue can occur at infection sites by binding to interstitial fluid proteins, soluble intracellular proteins, or subcellular structures (nucleic acids, leukocytic chromatin cell membranes, and mucopolysaccharides).

13.4.4 Physiological Barriers and Reservoirs Influencing Drug Distribution

Blood–Brain Barrier A major problem in drug delivery to brain is the presence of the blood–brain barrier (BBB), which is now well established as a unique membranous barrier that tightly segregates the brain from the blood influx. Endothelial cells of brain capillaries differ from most other capillaries of the body, as they are very tightly joined and lack intracellular pores. In addition, these brain capillaries are also enveloped by less permeable glial cells. Morphologically, this constitutes the *blood–brain barrier* [12, 13]. Anatomically, there exists a dual barrier in the CNS: the blood–brain barrier and blood–CSF barrier (located in the choroid plexus). The BBB places certain constraints on the passage of drugs from the blood to the brain and the CSF. As a rule only lipid-soluble nonionized forms of drugs penetrate more easily into brain as compared to water-soluble ionized forms of drugs [14, 15]. The volatile anesthetics like ether and chloroform, ultrashort acting barbiturates like thiopental, narcotic analgesics like morphine and heroin, dopamine precursor like L-dopa, sympathomimetics like amphetamine and ephedrine, and drugs like diazepam and propranolol can pass through the BBB, while polar compounds like dopamine, serotonin, and streptomycin and quaternary substances like *d*-tubocurarine, hexamethonium, neostigmine, and acetylcholine do not penetrate the BBB. The following five regions of the brain, on account of their relative permeability, do not form part of the BBB—pituitary gland, pineal body, area prostrema near the floor of the fourth ventricle, median eminence, and choroid plexus capillaries. The BBB is further reinforced by a high concentration of P-glycoprotein (P-gp) active drug efflux transporter protein in the luminal membranes of the cerebral capillary endothelium. This efflux transporter actively removes a broad range of drug molecules from the endothelial cell cytoplasm before they cross into the brain parenchyma [13].

Blood–CSF Barrier The second barrier that a systemically administered drug encounters before entering the central nervous system (CNS) is known as the blood–CSF barrier (BCB). Since CSF can exchange molecules with the interstitial fluid of the brain parenchyma, the passage of drug molecules from the blood into the CSF is also carefully regulated by the BCB. Physiologically, the BCB is found in the epithelium of choroid plexus, which are arranged in a manner that limits the passage of molecules and cells into the CSF. The choroid plexus and the arachnoid membrane act together at the barriers between the blood and the CSF. [13]. Only nonionizable lipid-soluble drugs can pass from blood to the CSF as in the case of the BBB where the epithelial cells of choroid plexuses are lined by occluding zonulae. But the brain–CSF barrier is composed of epithelial cells lining the ventricles and occluding zonulae do not connect these. Hence the CSF–brain barrier is extremely permeable to drug molecules from the CSF to brain cells [16]. The significance of this fact is that drugs like penicillin that have poor penetration through the BBB can cross the brain–CSF barrier if given by the intrathecal route and reach the brain tissue in sufficient concentration. In the clinical setting, drug concentrations in CSF are sometimes used as a surrogate for drug concentrations at the target site within the brain. But multiplicity of compartments of the brain and involvement of several factors in the transport of dugs from plasma into the brain and distribution within the brain limit the applicative value of CSF drug levels in prediction of brain target concentrations [17].

Placental Barrier The placental membrane readily allows the transfer of nonpolar lipid-soluble drugs mainly by passive diffusion. However, the other transport mechanisms like active transport and pinocytosis are also operative. Thus, lipid-soluble nonionizable drugs like hypnotics, narcotics, general anesthetics, cardiac glycosides, alcohol, neuroleptics, and certain antibiotics can readily cross the placental barrier, while nonionizable and very high molecular weight substances cannot cross the barrier. Antihypertensive drugs that are lipid soluble bind with proteins to lesser extent, and when in un-ionized form generally pass through the placental barrier with ease [18].

Special Compartments for Drug Distribution Besides plasma proteins, the tissues of the body can also act as reservoirs for drugs, but usually the sites where drugs accumulate are not those where they exert their physiological action. These stored drugs are in equilibrium with the drug in plasma and are only slowly released from these storage sites to provide enough circulating drug to produce biological effects. Consequently, this type of storage, unlike plasma protein binding, represents "a site of loss" rather than "a depot" for continued drug action.

Cellular Reservoir A drug may have a great affinity for plasma proteins, yet primarily be distributed in tissue if the tissue has an even higher affinity for the drug. This affinity could be due to several reasons, for example, binding to tissue proteins (albumin) or top nucleoproteins. Examples are digoxin and emetine to skeletal muscle, heart, liver, kidney (bound to muscle proteins), and retina (nucleoproteins); cadmium, lead, and mercury in kidney (muscle protein, metallothionein); and chlorpromazine in eye (affinity to retinal pigment melanin).

Fat Reservoir Highly lipid-soluble drugs like thiopentone, DDT, and phenoxybenzamine get selectively accumulated in fat and adipose tissue. The fat, however, is a sluggish reservoir due to lesser blood flow; but if body fat starts depleting, as occurs during starvation, the stored drug may be mobilized and toxicity may occur.

Transcellular Reservoir Aqueous humor (e.g., chloramphenicol and prednisolone), CSF (e.g., amino sugars and sucrose), endolymph, joint fluid (e.g., ampicillin), and pleural (e.g., imipramine and methadone), pericardial, and peritoneal sacs can also serve as drug reservoirs.

Bone and Connective Tissue Reservoirs Many drugs and substances like tetracycline, cisplatin, lead, arsenic, and fluorides form a complex with bone salts and get deposited in nails, bones, and teeth. The antifungal drug griseofulvin has an affinity for keratin precursor cells and is selectively accumulated in the skin and fingernails. Bone may become a reservoir for the slow release of toxic substances like lead and arsenic and for the anticancer drug cisplatin into the blood.

Biliary Reservoir The lipophilic drugs with relatively high molecular weight (~325 Da in rats and greater than 500 Da in humans) are generally metabolized in liver and eventually accumulate in bile. Subsequent biliary excretion of such drugs can significantly impact their systemic exposure, which in turn regulates their pharmacological effect on the one hand and their toxicity on the other. Drugs (such as

mycophenolic acid and digoxin) excreted into bile often undergo some degree of reabsorption along the gastrointestinal tract [19, 20]. Enterohepatic recycling is also a very important physiological process for bile salt homeostasis. In humans, more than 95% of the bile salt is reabsorbed in the distal ileum [21]. The biliary clearance of a drug would be extremely valuable in elucidating the potential mechanism operative in hepatobiliary toxicity, predicting drug–drug interaction, and identifying enterohepatic versus enteroenteric recirculation. Several clinical studies have shown that drug–drug interaction can result in changes in drug absorption due to inhibition of transport proteins. For example, inhibition of P-glycoprotein results in increased absorption of talinolol [22]. The liver plays a central role in the biotransformation and removal of xenobiotics and endogenous compounds from the blood; while the majority of small, lipophilic compounds flow through the space of Disse and enter the hepatocyte via the basolateral membrane by simple diffusion. On the other hand, more polar and bulky molecules require transport systems to cross the sinusoidal membrane. After reaching the hepatocyte, drugs can be transported into bile either as an uncharged species or as more hydrophilic metabolites produced after phase I and phase II biotransformation, or they can be excreted into the bloodstream by basolateral transport proteins. In addition to bile salt transport, hepatic transport proteins facilitate excretion of end products of metabolism, such as bilirubin or xenobiotics, into bile for subsequent removal via the intestine.

Plasma Protein Binding as Reservoir Although some drugs are simply dissolved in plasma water, most of them get bound to plasma components, such as albumin, globulin, transferrin, and ceruloplasmin (and also through the BBB), get metabolized, and are excreted in urine, saliva, CSF, and milk. As the free or unbound drug is eliminated from the body, more drug dissociates from the drug–plasma protein complex, glycoproteins, and α- and β-lipoproteins to replenish the free drug levels in plasma. Drugs usually are bound to plasma and cellular proteins in a reversible manner and in dynamic equilibrium, according to the law of mass action [23]. Thus, extensive plasma protein binding may serve as a circulating drug reservoir. But it is only the free fraction of the drug that is pharmacologically active, while the protein bound component is inert. Similarly, it is the free fraction of the drug that diffuses through the capillary wall (and also through the BBB), gets metabolized, and is excreted in urine, saliva, CSF, and milk.

13.5 IMPORTANT PROTEINS THAT CONTRIBUTE TO DRUG BINDING

1. *Albumin.* Of the plasma proteins, albumin is the major drug binding and transport protein of the circulatory system. Drug binding to plasma albumin occurs to a variable extent. It consists of three homologous domains. Each domain consists of two subdomains, A and B. The principal sites of ligand binding to HSA are located in hydrophobic cavities in subdomains IIA and IIIB. HSA is known to undergo different pH-dependent conformational transitions, the N-F transition between pH 5.0 and 3.5, the F-E transition between pH 3.5 and 1.2, and the N-B transition

between pH 7.0 and 9.0. The N-F isomerization involves unfolding and separation of domain III from the rest of the molecule without significantly affecting the rest of the molecule. The I state is characterized by unfolding of domain III together with partial but significant loss of native conformation of domain I. The domain II of HSA remains unaffected in the intermediate state. That the N-B transition has physiological significance is suggested by the fact that under increased Ca^{2+} concentration in blood plasma, the B isomer predominates. Moreover, it is believed that the transport function of albumin is controlled through this transition or akin to it.

Most of the acidic drugs (such as warfarin, penicillin, sulfonamides, phenylbutazone, and salicylic acid), in principle, bind to albumin. Two binding sites for acidic drugs have been identified on human serum albumin. Although less specific, domain I can bind with a variety of structurally unrelated drugs like acenocoumarin, warfarin, dicoumarol, phenylbutazone, phenytoin, valproic acid, sulfonamides, and endogenous substances like bilirubin. On the other hand, site II is more specific. With the exception of benzodiazepines, acidic drugs such as ibuprofen, naproxen, and analogues of probenecid, clofibric acid, cloxacillin, and endogenous substances like tryptophan bind to this site. Some drugs bind to both the sites with different affinities, and examples are salicylic acid, tolubutamide, and indomethacin [24–26].

2. α_2-*Acid Glycoprotein.* While acidic drugs primarily bind to albumin, many lipophilic basic drugs, such as quinidine, imipramine, lidocaine, chlorpromazine, propranolol and its analogues, and spinperidol bind avidly to α_2-acid glycoprotein (AAG). The drug binding to AAG in health and disease has been extensively reviewed by Kremer et al. [27].

3. *Lipoproteins.* The role of lipoproteins as a transport system for drugs has been largely overlooked compared to the extensive reports on drug binding with albumin and AAG. However, there are reports that lipoproteins could be implicated in the binding process; for example, propofol binds extensively to all lipoprotein fractions [28, 29].

4. *Tissue Proteins and Nucleoproteins.* Some drugs, particularly those having high apparent volume of distribution, specifically bind to tissue proteins (e.g., digoxin, emetine, and chloroquine). The steroidal drugs can bind to specific receptor and nuclear proteins present on nucleus.

5. *Miscellaneous Binding Proteins.* Only a few drugs are significantly bound to other special proteins; for example, corticosteroids are bound to steroid binding globulin, transcortin; thyroxine to α-globulin; and antigens to γ-globulin.

13.6 VOLUME OF DISTRIBUTION

The distribution of drugs in the body depends on their lipophilicity and protein binding. Low plasma binding or high tissue binding or high lipophilicity means an extensive tissue distribution. In pharmacokinetics, the distribution is described by the parameter V_d, the apparent volume of distribution [10]. The apparent volume of distribution, V_d, is defined as the volume of fluid required to contain the total

amount, Q, of drug in the body at the same concentration as that present in the plasma, C_p.

$$V_d = Q/C_p$$

Values of V_d for many drugs have been measured. The drugs with high plasma protein binding remain largely restricted to the vascular compartment and tend to have lower volumes of distribution. Some drugs are confined to the plasma compartment. The plasma volume is about 0.05 L/kg body weight. A few drugs such as heparin (V_d of 0.05–0.1 L/kg) are confined to plasma volume because the molecule is so large that it cannot cross the capillary wall easily. More often, the retention of a drug in the plasma following a single dose reflects the strong binding to plasma protein. However, the free drug in the interstitial fluid only exerts a pharmacological effect.

Following multiple dosing, equilibration occurs with an ensuing increase in V_d values. Drugs such as warfarin, sulfamethoxazole, glibenclamide, and atenolol are confined to the plasma compartment. Few drugs are distributed in the extracellular compartment, which has a volume of about 0.2 L/kg and many polar compounds such as vecuronium, gentamicin, and carbenicillin show an approximate V_d of 0.2 L/kg. These drugs cannot easily enter cells because of their low lipid solubility and they do not traverse the blood–brain or placental barriers easily. Drugs like tubocurarine (V_d 0.2–0.4 L/kg) and theophylline (V_d 0.4–0.7 L/kg) are also distributed in the extracellular fluid. Some other drugs are distributed throughout the body water. Total body water represents about 0.55 L/kg, which approximates the distribution volume of relatively lipid-soluble drugs that cross cell membranes, such as phenytoin and ethanol. There are many drugs with the volume of distribution greater than the total body volume on account of binding of drug outside the plasma compartment, or partitioning into body fat. Examples are morphine, tricyclic antidepressants, and haloperidol.

13.7 INFLUENCE OF LIPOPHILICITY AND LYSOSOMAL ACCUMULATION ON TISSUE DRUG DISTRIBUTION

Distribution of a drug in the body is dependent on the permeation properties, the blood flow rates in various tissues, and plasma and tissue uptake. The distribution of drugs *in vivo* is largely determined by uptake competition among individual tissues [30, 31]. The lipophilic drugs are characterized by extensive accumulation in tissues, which leads to a high volume of distribution. Nonspecific binding to cellular membrane (phospholipids) and uptake by acidic compartments (mainly lysosomes) are responsible for such a distribution pattern [32]. Lysosomal trapping is an important mechanism of distribution of basic psychotropic drugs [33]. Lysosomal trapping has been reported to contribute to the uptake of despiramine and chloroquine in various tissues [34]. Basic drugs are distributed widely in various tissues in the following order: lung, fat, heart, kidney, brain, gut, muscle, and bone. The fat volume in the body influences the deposition kinetics. There is a good correlation in various tissues between the tissue–plasma concentration ratio and the octanol–water coefficient among various drugs.

13.8 PARTITION OF THE DRUG INTO FAT AND OTHER TISSUES

Drug accumulation or concentration in a tissue or biological fluid occurs when the influx of a drug is greater than the efflux. However, concentration gradients do not govern the rate of active transport. Thus, active transport can play a part in accumulation. Fat represents a large nonpolar compartment. In practice, this is important only for a few drugs mainly because the effective fat–water partition coefficient is relatively low for most drugs. For example, sequestration of morphine by body fat is of little importance as the drug's lipid–water partition coefficient is only 0.4, although the drug is sufficiently lipophilic to cross the blood–brain barrier. On the other hand, a drug such as thiopental, with a fat–water partition coefficient of about 10, accumulates substantially in body fat. The second factor that limits the sequestration of a drug in body fat is the amount of blood supply. Since fat receives less than 2% of cardiac output, drugs are delivered to body fat rather slowly. But some drugs like clofazimine (a riminophenazine with anti-inflammatory properties), used in the treatment of leprosy and other mycobacterial infections, extensively accumulates in fatty tissues and organs with in the reticuloendothelial system and the drug is retained for a long time even after stopping administration of the drug [35]. Sequestration also involves either drug ionization or drug binding to proteins and other macromoleules, preventing efflux by diffusion or active transport.

13.9 SPECIAL DRUG DELIVERY SYSTEMS

Several approaches are being explored to improve drug delivery. Some of these are polymeric carriers [36], biologically erodable microspheres [37], nanoparticles [38, 39], prodrugs [40, 41], antibody–drug conjugates, liposomes, and coated implantable devices [42, 43].

Many drugs can be formulated using simple vehicles; however, a large number of chemical entities pose problems because of the dose and poor solubility in simple solvents and cosolvent systems. As a result, there is a need for advanced formulations that will permit the administration of challenging drugs efficiently. For that purpose, drugs can be attached to the polymeric carriers or dispersed in suitable (colloidal) vehicles. In the past, surfactant systems that solubilized the drug and thereby provided a transparent product for injection were in use; for example, cremophor was a material of choice—unfortunately, it has been associated with anaphylactic reactions. Workers in the field hence sought alternative lipid carriers such as liposomes and lipid emulsions. Although fat emulsions have been in trend for decades for parenteral nutrition and these systems have been used with success as drug carriers, yet they are avoided, as such preparations allowed the drugs to rapidly exit the system and reach the bloodstream, hence limiting the possibilities of drug targeting [44]. Successful homing of drugs to the desired biological compartment of the host depends on the intrinsic properties of the drug, or it can also be achieved by manipulation of the carrier/delivery system, as little can be done to influence the target and its surroundings. Liposomes have been developed as carriers for the delivery of therapeutic and diagnostic agents against various infectious diseases. Incidentally, the particulate nature of the vesicles may facilitate passive homing of the entrapped drug molecules to the macrophages, which harbor many of the important pathogens in

their intracellular compartments such as *Mycobacterium, Leishmania*, and *Candida* species, belonging to three different major classes of microbes, namely, bacteria, protozoans, and fungi, respectively. Moreover, macrophages upon interaction with particulate drug delivery vehicles may act as the secondary depot for them, thereby helping in targeted delivery of entrapped drug molecules.

Particulate novel drug delivery vehicles such as liposomes, microspheres, and niosomes deliver entrapped material to macrophages and dendritic cells mainly. Liposomes have been documented as microscopic vesicles composed of phospholipid bilayers surrounding aqueous compartments. Liposomes have been used extensively as drug carriers. Potential applications of this delivery system include cancer chemotherapy, enzyme therapy, immunomodulation, antimicrobial therapy, metal detoxification, diagnostics, and topical therapy [45–49].

Injecting drugs encapsulated in liposomes has an advantage: since they are colloidal in nature, they are recognized as foreign particles and can readily be taken up by phagocytic cells of the immune system (cf. macrophages); thus, the drugs rapidly accumulate in the mononuclear phagocyte system (MPS). The inherent tendency of the liposomes to concentrate in MPS can be exploited in enhancing the nonspecific host defense against various intracellular pathogens by entrapping chemotactic or immunomodulatory chemical entities in them besides using them as carriers of antibiotics against various intracellular infections. Liposomal systems have been optimistically considered as "magic bullets" for more than three decades. Drug delivery with liposomes as the carrier system provides options and opportunities for designing biostable and/or site-specific drug therapy. Engineered or tailored versions of liposomes that offer the potentials of exquisite levels of specificity for drug targets are receiving much attention these days. Depending on the site of targeting, liposomes may be modified by either grafting chemotactic ligands such as peptides, polysaccharides, or affinity ligands like antibodies to the liposomal surface directly or may be coated via poly(ethylene glycol) anchors [50–52].

The delivery of diagnostic, therapeutic, and immunomodulatory agents to lymph nodes is mandatory for treatment of diseases with lymphatic involvement [53–55]. Saccharide modified liposomes showed enhanced absorption from the injection site and enhanced lymph node uptake compared to control liposomes [56]. Liposomes coated with nonspecific human antibodies injected subcutaneously showed a modestly increased lymphatic absorption and lymph node uptake compared to liposomes without the coating [57]. For example, administration of anti-HLA-DR bearing immunoliposomes resulted in a threefold higher accumulation in regional lymph nodes [58]. Poly(ethylene glycol) (PEG) coated liposomes were found to avoid uptake by the lymphatic system as compared to the plain liposomes [59, 60]. A novel method to enhance lymph node uptake of biotin-coated liposomes has been explored to facilitate the uptake of the drug-bearing liposomes [61].

The amino-phospholipids are generally confined to the inner leaflet of the plasma membrane. Translocation of phosphatidylserine (PS) to the outer leaflet serves as a signal for triggering recognition by macrophages [62]. Studies on the interaction between liposomes and macrophages have shown that larger particles may be phagocytosed more efficiently by macrophages than smaller liposomes [63]. Similarly, grafting of tetrapeptide tuftsin on the liposome surface would therefore enable us not only to "home" the liposomized drug to macrophages but also to stimulate these cells nonspecifically against infections [64–69].

Analysis of the intracellular trafficking patterns of the liposomal antigens reveals that after being phagocytosed by macrophages, liposomal antigen readily escapes from the endosomes into the cytoplasm of the macrophages [70]. Moreover, liposomes made up of lipids with fusogenic properties have been shown to deliver their entrapped molecules in the cytosol of the target cells more efficiently than conventional forms of liposomes. The membrane lipid composition of microorganisms exhibits a large amount of anionic phospholipids that play a pivotal role in membrane–membrane fusion [71]. Liposomes made up of lipids from various microorganisms have been used for targeting to macrophages as well as dendritic cells [72–75]. Recently, we have demonstrated that antigen entrapped in liposomes made up of lipids from *Escherichia coli* and edible yeast generate strong humoral as well as cell-mediated responses far better then conventional egg PC liposomes [76–78]. Moreover, sterically stabilized liposomes have been found to possess increased stability and to play a major role in $CD8^+$ T-cell response by targeting mature as well as immature dendritic cells. In a more recent study, PorA, a major antigen of *Neisseria meningitidis*, was purified and reconstituted in different types of targeted liposomes, by using mannose or phosphatidylserine as targeting moieties, or with positively charged liposomes for targeting dendritic cells (DCs). The use of targeted PorA liposomes resulted in an improved uptake by and activation of DCs and an increased localization in draining lymph nodes [79].

The interaction of liposomes with phagocytic cells is followed by either stable adsorption to the cell surface or cellular uptake of intact vesicles by an energy-dependent mechanism leading to lysosomal degradation of the liposomes and their contents. Liposome adsorption to the cell surface seems to be the rate-limiting step, since it can be assumed that stably adsorbed vesicles are more susceptible to subsequent uptake than vesicles that are loosely attached to the cell surface. Several prerequisites have to be successfully met that eventually enable liposomes to deliver biologically active agents to macrophages. Essentially, liposomes must readily bind to and be phagocytosed by free and fixed phagocytes; they must prevent degradation of entrapped drug; they must retain the encapsulated agent for delivery to the intracellular compartment of RES cells; and they must localize to macrophages in organs where metastasis or macrophage-associated disorders occur. Moreover, it has been shown that liposome with specific lipid composition or those harboring fusogenic peptide on their surface can specifically fuse with the target cells.

In general, liposomes are the most widely studied carrier in drug targeting to macrophages. However, the extent of liposome binding and subsequent ingestion by macrophages depends on a number of features of the lipid vesicles. These include composition, type, size, and surface properties of liposomes. For example, PS containing negatively charged liposomes can associate more effectively and deliver the liposomal content more efficiently to the macrophages than PS containing neutral liposomes [80–83]. Similarly, smaller liposomes deliver drugs more effectively than larger ones; presumably they are internalized more efficiently [84]. Some studies implied that positively charged and large sized liposomes could, however, improve liposome uptake in comparison with their counterparts [85, 86].

It was also observed that liposome uptake increased linearly with the incubation time and concentration. Higher uptake was observed with smaller and negatively charged liposomes. Inclusion of increasing amounts of cholesterol and sphingomyelin resulted in a decrement in the uptake by macrophages. The same study also

revealed that the uptake of liposomes differed considerably in different mouse strains [80–83].

The fatty acyl chains of the phospholipids used in the preparation of liposomes can regulate their *in vivo* stability. The efficacy of a liposomal formulation of a drug can be affected by the composition of liposomes [87]. Encapsulation of ampicillin in vesicles of solid liposomes—prepared from distearoylphosphatidylcholine (DSPC) and dipalmitoylphosphatidylglycerol (DPPG)—as compared with fluid type liposomes (composed of cholesterol, PC, and PS) resulted in delay of intracellular killing of *Listeria monocytogenes*. Besides charge, size and lamellarity of the liposomes are important characteristics that regulate their uptake by macrophages. Systematic evaluation of multilamellar vesicles (MLVs) with different phospholipid composition reveals that certain classes of phospholipids are recognized preferentially by macrophages. Inclusion of negatively charged phospholipids, such as phosphatidylserine (PS) or phosphatidylglycerol, in MLVs consisting of phosphatidylcholine (PC) greatly enhances their binding to and phagocytosis by macrophages. In contrast, neutral MLVs composed exclusively of PC are not efficiently taken up by macrophages. Such enhancement of phagocytosis has been shown to occur in mouse peritoneal macrophages, mouse Kupffer cells, rodent alveolar macrophages, human peripheral blood monocytes, and human alveolar macrophages [88]. Normal rat alveolar macrophages were incubated with two different types of liposomes—MLV (<5 lamellae) and REV (reverse evaporation vesides) (<5 lamellae)—and adjusted to contain equal amounts of lipid and entrapped muramyldipeptide. Similarly, it was found that the extent of tumoricidal activity was higher in the case of MLV than that of REV. Recently developed poly(ethylene glycol)-coated liposomes, known as Stealth liposomes, are not readily taken up by the macrophages in the reticuloendothelial system; however, they do stay in the circulation for a relatively long period of time [89–91]. It is simply because of the fact that long PEG chains impart steric hindrance in the interaction of liposomes with lipoproteins. In addition, it has been shown that incorporation of different polymers into liposomes can enhance liposome circulation time in a concentration-dependent manner. On the other hand, peptide-grafted liposomes were found to be more leishmanicidal than its ungrafted counterpart. This is because the peptide-grafted liposomes are phagocytosed more efficiently by macrophages [92]. Recently, it has been demonstrated that incorporation of a peptide from HIV-1 can also facilitate intracellular delivery of protein drugs [93].

Macrophages possess various receptors such as Fc receptors, complement, fibronectin lipoprotein, mannosyl, galactosyl, and many other receptors. These macrophage surface receptors determine the control of activities such as activation, recognition, endocytosis, and secretion. A useful approach for promoting the uptake of liposomal content by macrophages is to incorporate ligands capable of interacting with macrophage surface receptors [94]. Various research groups showed that uptake of ligand-incorporatcd liposomes was significantly higher than liposomes without ligands [95–101]. For example, mannose receptors on the macrophage surface have been exploited by developing neoglycoprotein and mannose residue on the surface of interacting drug carriers [101–103]. Studies on the interaction of mannosylated liposomes with macrophages indicate that targeted liposomes present much higher affinity for those cells than the unconjugated vesicles.

Liposomes composed of mannosylated *myo*-inositol (extracted from the cell wall of microorganisms) have been shown to be taken up by peritoneal macrophages.

Liposomes were composed of egg phosphatidylcholine, cholesterol, and dicetylphosphate with or without monoarachidic acid esters (MAEs). The liposomes with MAEs were rapidly eliminated from blood. More than 60% of the eliminated liposomes were recovered in the liver and spleen [98]. The modification did not, however, affect the distribution to the other tissues. The modified liposomes are taken up by Kupffer cells of the liver and resident macrophages of the spleen via mannose receptor-mediated binding endocytosis. Moreover, both *in vitro* and *in vivo* uptake of liposomes have been increased extraordinarily by targeting these carriers through Fc surface receptors of macrophages. Coupling of rabbit immunoglobulin with liposomes increases the uptake by rat liver macrophages more than five times compared with control liposomes [103–105]. *In vivo* study demonstrated that 80–85% of the coupled liposomes were accumulated in the liver within 1 h of the injection. This was because of the presence of the Fc moiety in immunoglobulin-coupled liposomes. Mouse monoclonal antibody modified with W-succinimidyl-3-(2-pyridyldithio) propionate in order to conjugate with liposomes containing dideoxycytidine triphosphate by covalent bonding was found to home entrapped material to the macrophages. The uptake of antibody-conjugated liposomes was four to six times higher than that of plain liposomes. This increase in uptake was thought to be due to the Fc receptor-mediated binding [105].

In a manner similar to liposomes, size, surface property, composition, concentration, and hydrophilicity or lipophilicity of microspheres and nanoparticles play a significant role in the uptake by macrophages. The events of phagocytosis include contact with pseudopods of macrophages followed by their engulfment into the cytoplasm by lamellipods [106]. Hydrophobic and relatively large microspheres are more susceptible to phagocytosis than their hydrophilic counterparts. Likewise, nanoparticles with lipophilic coating are taken up by macophages more readily as compared to their hydrophilic counterparts. Coating the particle surface with opsonic materials and activating macrophages with various activating factors can improve the extent of phagocytosis. Incubation time and dose of the vehicles can also control the process of phagocytosis.

The influence of surface charge and size of microspheres on their phagocytosis by mouse peritoneal macrophages was studied by using polystyrene and phenylated polyacrolein microspheres of different diameter as well as modified cellulose microspheres with different surface charge [107]. It was found that efficiency of uptake was maximum when size range of microsphere was on the order of 1–2 μm. For both negatively and positively charged particles, the extent of phagocytosis was increased with increasing zeta potentials, and was the lowest when zeta potential was zero [108]. However, there was no significant difference in the phagocytosis between the cationic and anionic surfaces when compared at a zeta potential of the same absolute value. Modified cellulose microspheres were allowed to incubate with macrophages in order to study the influence of the hydrophobicity on macrophage phagocytosis. Hydrophobic microspheres prepared from benzoyl cellulose were the most susceptible to phagocytosis and the nonionic hydrophilic ones were the least susceptible. It was observed that an optimal surface hydrophobicity is necessary for the microspheres to be phagocytosed [109].

Similarly, nanoparticles made from polyalkylcyanoacrylate and polymethylmethacrylate and human serum albumin microspheres have been used to study the influence of various parameters that can regulate uptake by human macrophages. The incorporation of lipophilic polymethylmethacrylate in nanoparticles was found to

result in better phagocytosis, when compared with polyalkylcyanoacrylate nanoparticles of similar size range [109]. Polybutylcyanoacrylate nanoparticles coated with lipophilic Pluronic F68—a biocompatible poloxamer—increased phagocytosis by nearly 50%, while Pluronic F108 had no influence. Nanoparticles of the same material were phagocytosed to a larger extent if they were of larger diameter. For example, phagocytosis of nanoparticles made from human serum albumin of 1.5 μm in diameter was higher than that of 200 nm diameter nanoparticles. Recently, it was found that poly(ethylene glycol)-distearate-incorporated microspheres can modify the extent of the phagocytosis depending on the concentration of the excipient used in their preparation [110]. It seems that microsphere association or uptake by macrophages might be a saturable process. For a given cell density, uptake of particles into macrophages was also dose dependent. In one study, the most avid uptake was observed with a microsphere dose of 1 mg/L; however, there was a gradual reduction in the uptake as microsphere dose was increased further [111].

In a manner that is typical of a particulate delivery system, coating of microspheres with opsonic materials and amphiphiles can modify significantly the extent of phagocytosis by macrophages, depending on the state of macrophage activation. Several proteins such as γ-globulin, human fibronectin, bovine tuftsin, and gelatin enhance the phagocytosis, while bovine serum albumin reduces the phagocytosis of cellulose microspheres [112]. It was also observed that the presence of fetal calf serum increases the phagocytosis of gelatin-grafted cellulose microspheres, while showing no effect on other protein-grafted microspheres. The influence of crosslinking and concentration of gelatin microspheres on the phagocytosis by mouse peritoneal macrophages has also been studied. Glutaraldehyde-mediated crosslinking of gelatin microspheres was found to facilitate interferon targeting to macrophages [113]. The study showed that phagocytosis of microspheres decreased with decreasing concentration of gelatin and glutaraldehyde and was proportional to the amount of microspheres added, until a saturation of phagocytosis was observed at higher doses of microspheres. It was also demonstrated that precoating or surface immobilization with gelatin was the most effective method to enhance the phagocytosis among all other opsonic proteins. Phagocytic uptake of poly styrene microspheres, coated with a series of perfluoroalkylated amphiphiles derived from phosphocholine and poly(ethylene glycol), was studied in the presence or absence of serum and peritoneal macrophages were used as phagocytic cells [114]. Phagocytic uptake tests carried out at 37 °C showed that microspheres coated with any surfactants cause a decrement in the phagocytosis under both conditions—either in the presence or absence of serum. However, the extent of decrease varied among the surfactants, and in most instances, the presence of serum had no influence on the phagocytosis when microspheres were coated with the same surfactant.' Microspheres from some biodegradable substances such as copolymers of polylactic acid and polyglycolic acid, cross-linked potato starch, hydroxyethyl starch, dextran, lichenan, and mannan are found to be successfully phagocytosed by macrophages [115–119].

When encapsulation materials produced from nanoparticles in the 1–100 nm size range have a larger surface area for the same volume, smaller pore size improved both solubility and structural properties. Hence, the drug nanoparticles, irrespective of the method of production, represent a technology that may overcome solubility problems and bioavailability problems of all poorly soluble drugs. The transforma-

tion of any drug into drug nanoparticles, leading to an increase in saturation solubility and dissolution velocity and providing the general feature of an increased adhesiveness to surfaces, is one of the most important achievements. Surface modification of the drug nanocrystals can further increase the benefits, by producing mucoadhesive nanosuspensions for oral application (e.g., targeting to the stomach or colon) or surface-modified site-specific nanoparticles for intravenous injection (e.g., targeting to the brain or bone marrow). A fusion of the novel nanosuspension technology with the traditional dosage forms (e.g., incorporating drug nanoparticles into pellets or tablets for oral delivery) is also a noteworthy advantage.

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14

SALT AND COCRYSTAL FORM SELECTION

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14.1 INTRODUCTION

Form selection is critical to the successful development of an active pharmaceutical ingredient (API) [1–5]. A number of physical properties vary with the solid form (a partial list is given in Table 14.1) and several properties such as crystallinity, solubility, and stability need to be measured and evaluated to determine whether a candidate will be acceptable for development [6, 7]. If other options are needed, a different crystalline form, a different salt, or a cocrystal may be explored. It has been estimated that approximately half of all administered drugs are formulated as salts [8]. With more poorly soluble drugs being found and developed, form selection can

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TABLE 14.1 Properties that Can Vary with Solid Form

Bioavailability
Hygroscopicity
Dissolution rate
Solubility
Physical stability
Chemical stability
Compressibility
Chargeability
Filterability
Flow
Millability
Melting point
Color
Excipient compatibility
Morphology
Density
Taste
Toxicology
Levels of gastric irritation
Clinical results

improve properties, such as solubility and ultimately bioavailability, and increase the potential viability of a candidate. Form selection is therefore an integral part of the drug development process.

Various approaches to form selection exist during development. One general approach is to perform sufficient work initially in order to choose a good candidate early (before the Investigational New Drug (IND) application is filed and clinical trials are started). This approach is believed to reduce the time to market. Another approach is to initially limit the time and money spent choosing a form early in development since many compounds will be eliminated. For this latter scenario, a form is chosen that is believed to be adequate for early trials, knowing that changing forms later will have the added cost and time commitment for bridging studies. In practice, the actual approach will be some combination of these two extremes.

Clearly, the risk assessment for each compound must be evaluated based on the information available for the drug being developed. The dosage form to be developed also needs to be considered when choosing the form, since the desired properties for an oral dosage form will be significantly different from those needed for a suspension or an IV formulation. For most compounds, a complete picture for form selection and an understanding of the solid-state properties should be developed and presented at the end of Phase II clinical trials.

It should be noted that form selection also influences intellectual property position. Crystalline forms including salts and cocrystals are patentable. Additional form selection studies may be carried out late in development for intellectual property protection on improved products. Understanding the solid-state properties of the API is critical to these endeavors.

This chapter discusses solid form screening and the selection process for pharmaceutical compounds with the main focus on salts and cocrystals. Information on

counterion/guest selection, preparation, characterization, and property evaluation are also presented. The need for polymorph screening as part of the selection process is included, and the role of quantitative methods to determine the solid forms present in API and drug product is also discussed.

14.2 SALTS AND COCRYSTALS

When ionizable groups are present in an API molecule, a salt can be formed which often provides better physical properties, such as increased water solubility or melting point. In addition, nonionizable compounds and salts may form cocrystals with the desired properties. Working definitions for these materials are given below and the concepts are shown schematically in Fig. 14.1.

Salt. Acid/base chemistry creates a new species; a free acid or free base is needed for salt formation. An acid–base complex is formed that contains the API along with additional nontoxic molecular species (counterion) in the same crystal structure.

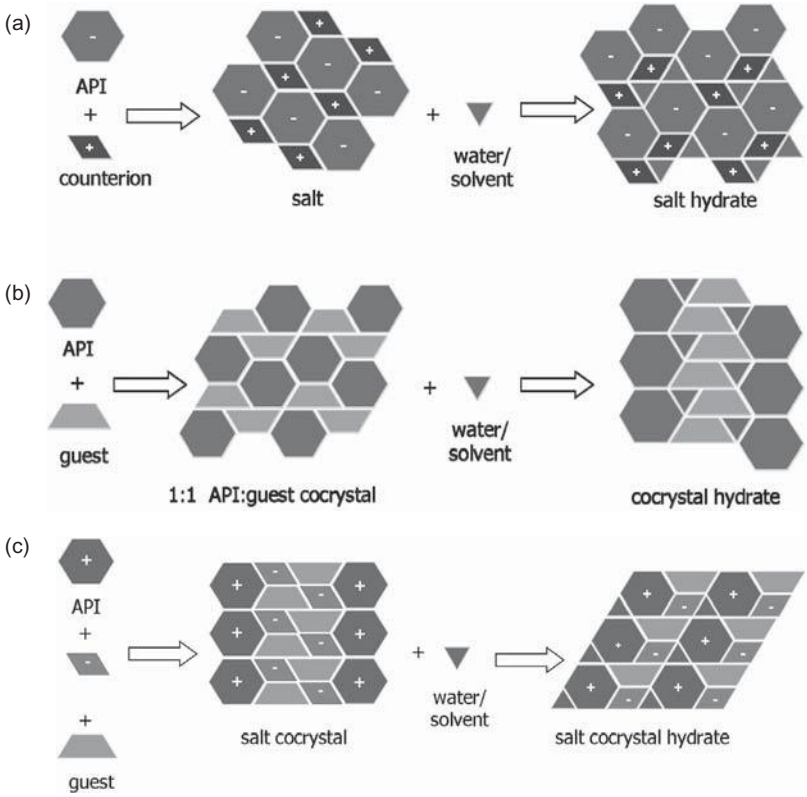


FIGURE 14.1 Schematic of salt and cocrystal formation: (a) salt and salt hydrate/solvate, (b) cocrystal and cocrystal hydrate/solvate, and (c) salt cocrystal and salt cocrystal hydrate/solvate.

Cocrystal. A crystalline molecular complex of an API or a salt of an API; it consists of two or more components, with each component being an atom, ionic compound, or molecule.

Salts are commonly produced and used as drug substances in the pharmaceutical industry, and numerous studies of salts and salt selections have been reported [9–13]. Cocrystals are comparatively new to the pharmaceutical industry but have been studied and used in other industries for years [14]. As a solid form, cocrystals perform essentially the same function as salts according to the regulatory definition of an active moiety [15]: "the molecule or ion, excluding those appended portions of the molecule that cause the drug to be an ester, salt (including a salt with hydrogen or coordination bonds), or other noncovalent derivative (such as a complex, chelate, or clathrate) of the molecule, responsible for the physiological or pharmacological action of the drug substance." For all compounds, including cocrystals, the regulatory requirement is not the categorization of the particular form; rather, it is to adequately and accurately characterize the system, to understand scientifically the form that is being developed, and to transfer this information to the regulatory agency. From this perspective, salts, hydrates, and cocrystals are identical in that they all contain the essential active moiety plus additional ions or molecules that are present primarily to impart specific physical properties on the solid form containing the active moiety.

A number of variables need to be considered when selecting a form, such as target solubility, dosage form to be developed, loading in the dosage form, physical and chemical stability, and amount of material available. It should be emphasized that there is more than one way to select a salt or cocrystal since numerous factors should be considered in the selection process. A tiered approach to salt/cocrystal selection is helpful when screening a number of possible candidates. Common properties used to evaluate materials include crystallinity, hygroscopicity, solubility, melting point, density, and stability. An example of a decision tree designed to select a material for an oral dosage form is given in Fig. 14.2a. The variables included in this selection include crystallinity, hygroscopicity, solubility, and polymorphism. These decision trees can be modified for different situations. Properties needed for an injectable formulation are usually different from those required for an oral dosage form, as shown in Fig. 14.2b. Because the properties needed for the selection process cover a broad range, it is imperative that a multidisciplinary approach be used to choose the solid form. The three main departments that deal directly with the solid form issues are chemical development (to produce the API with the desired properties), analytical (for testing of materials), and pharmaceutics (for preformulation and formulation development). Other groups, such as discovery, toxicology, and pharmacokinetics may also provide useful information during the selection process.

Salt/cocrystal selection involves experimentally identifying and characterizing a variety of different multicomponent solids by incorporating a number of secondary molecular species into the lattice with the active moiety. By taking a broader view concerning the types of secondary molecules that are experimentally investigated and not limiting the counterions tested based on pK_a, a wider variety of solid forms with potentially useful properties can be considered in the process of form selection. The cocrystal discussion in this chapter is intended to act as a guide in the expansion of the targeted multicomponent solids that are investigated during form selection.

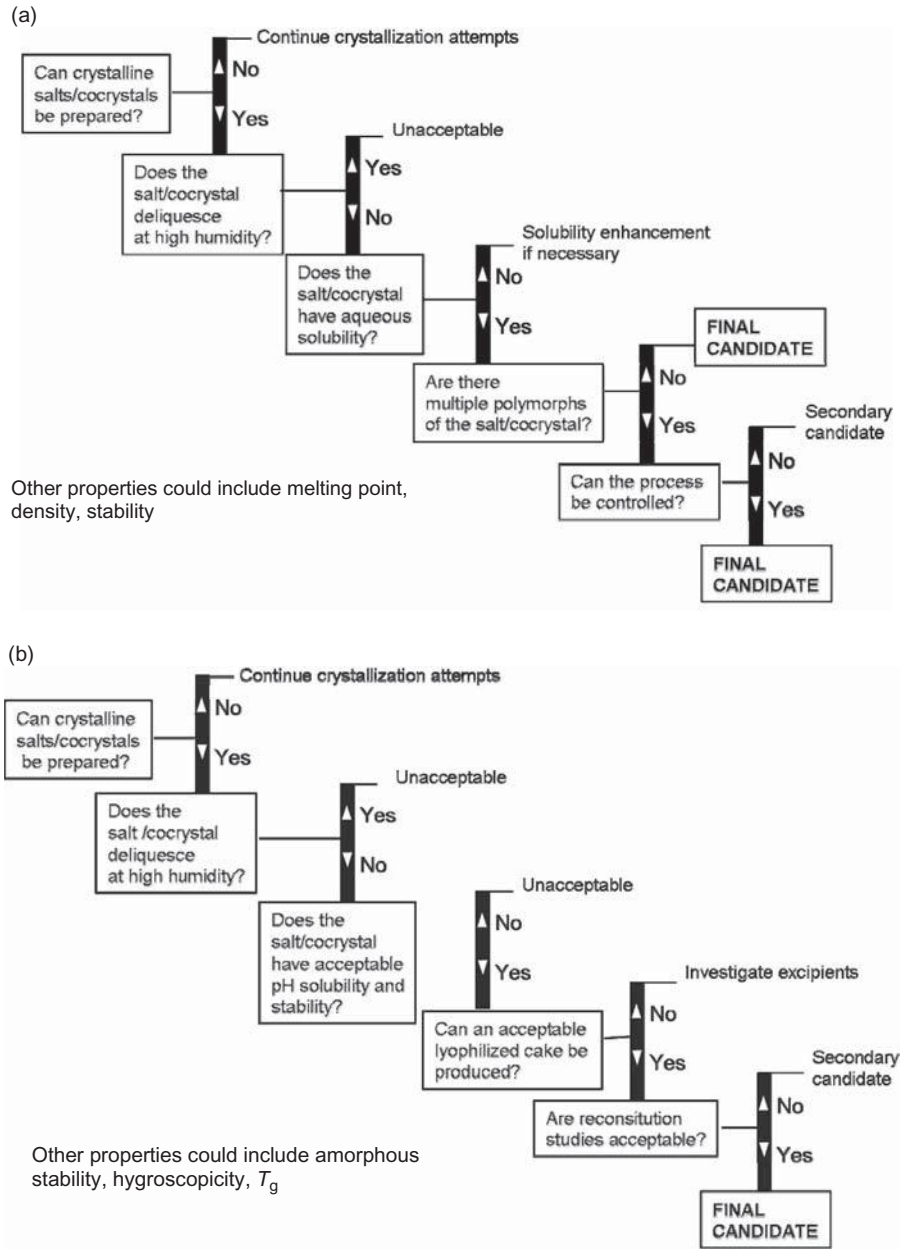


FIGURE 14.2 Decision trees for salt/cocrystal selection: (a) example for an oral dosage form and (b) example for an injectable formulation.

14.3 FORM SELECTION PROCESS

The form selection process is divided into five sections: counterion/guest selection, formation, characterization, evaluation, and polymorph screening. Each section provides an overview of common methodologies used.

14.3.1 Counterion/Guest Selection

Pharmaceutically acceptable counterions and cocrystal guests are often used to simplify the regulatory concerns surrounding the product, since information about the materials is already known and has been filed previously with the regulatory agency. The list of pharmaceutically acceptable compounds typically includes molecules that are readily found in marketed products. Figure 14.3 shows the most common counterions found in marketed products based on databases compiled in our laboratory; the results are similar to those reported elsewhere [8]. If acceptable toxicology can be demonstrated for a new entity and it provides significant property improvement, a strong case can be made for using a new substance as a guest or counterion.

There are other considerations when determining a list of possible counterions and guests. For instance, the loading in the drug product may make a high molecular weight counterion or guest unacceptable. Commonly, sodium or potassium are used as counterions for acidic APIs when high loading is a factor, but small organic species may still be preferable over inorganic ions. The type of dosage form also needs to be considered since there will be limitations for injectable, ocular, and inhalation formulations that may be different from those encountered with oral

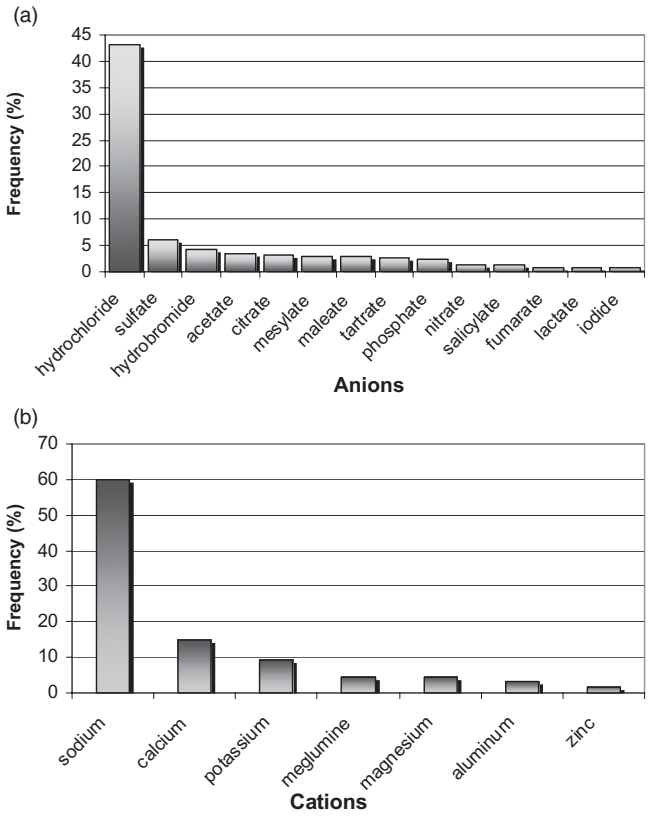


FIGURE 14.3 Common counterions found in marketed pharmaceutical products: (a) anions and (b) cations.

formulations [8]. The indication expected for the API may also have limitations regarding other components that can be present.

For salts, the pK_a of the relevant functional groups on the drug molecule is considered initially. A two to three unit separation between the pK_a values of the functional groups on the API and the counterion is commonly invoked when making salts [16]. This is fine as a guideline, but salts and cocrystals can be formed outside this range based on the decreased solubility of the salt formed. Hydrochloride (HCl) salts are commonly produced for pharmaceutical bases, but there are issues that should be considered when using HCl [8, 17]. Some issues to consider for an HCl salt include the common ion effect *in vivo*, corrosive properties during production, and loss of HCl during processing. Many companies have a list of acceptable counterions that are used for every salt selection. Although this is an acceptable place to start, many of the counterions on the list may not be ideal for the molecule being developed. It is important to look at the chemistry and the solid-state aspects of salts and cocrystals while compiling a list of possible counterions and guests that are to be empirically evaluated.

A cocrystal screen is an empirical exercise but can be guided by an analysis of the API in order to determine the potential for cocrystallization and to gather information to assist in the selection of the most appropriate guest molecules. The number and arrangement of the hydrogen bond donors and acceptors, salt forming ability, size and conformational flexibility, stability of the homomeric structure, physical properties, and solubility requirements are all considered during the initial evaluation. Crystal engineering also relies heavily on information contained in the Cambridge Structural Database [18]. The crystal engineering approach used to design an effective cocrystal screen is based on academic studies [19–21] and the application of this methodology to pharmaceuticals has generated a number of recent successes [22].

The ability of the selected guests to form hydrogen bonded synthons with the API that are energetically more favorable than the homomeric intermolecular interactions can be an important consideration in guest selection [23]. This is consistent with the observation that after the intramolecular hydrogen bonds form, the strongest remaining hydrogen bond donors typically interact with the best acceptor sites [24]. If these groups are on the same molecule, then a homomeric form is favored; however, by choosing API : guest combinations that place the strongest donor and acceptor on different molecular species, the potential for cocrystallization is increased.

It should be noted that many of the compounds used as counterions in salt selections can also be used as guests for cocrystals. We find that pharmaceutically acceptable, weak carboxylic acids typically used in salt screens are particularly useful guest molecules. One of the most effective ways for a company to initiate cocrystal screening is by using these carboxylic acids to extend the range of a traditional salt screen of a basic API. By removing the traditional limitations on counterion selection based on pK_a values, the potential for finding stable solid forms that meet the physical property requirements is increased. The concern over whether a solid is a cocrystal or a salt should be secondary to the consideration of the properties of the solid form.

Pharmaceutically “unacceptable” compounds may be employed if cocrystal formation is used initially as a purification or isolation step. The cocrystal can then be

broken to isolate the API [25]. The API can be used upon isolation/crystallization or a cocrystal or salt with a pharmaceutically acceptable guest could be produced for development.

Although finding cocrystals can be challenging, the unique benefits of cocrystals outweigh the possible difficulties because they provide a screening option for APIs that cannot form salts. Until now, nonionizable or poor salt-forming APIs typically have had no recourse for crystalline solid form variation other than the adventitious formation of hydrates or solvates. Cocrystallization provides a systematic approach to form selection for these APIs.

14.3.2 Salt/Cocrystal Formation

After selecting a list of possible counterions and guests, the crystallization experiments to be performed need to be planned. There are a number of issues to consider when producing salts and cocrystals. This section outlines some of these issues, such as solvent choice, solution crystallization experiments, nonsolvent options, and possible stoichiometries.

One of the first considerations in salt or cocrystal formation from solvents is the solubility of the API as well as the counterions or guests. A common solvent or miscible solvent system with acceptable solubility must be found for the experiments. This appears to be straightforward, but it can become a major issue for poorly soluble drugs. This is especially problematic for cocrystal formation. When a strong salt forms, charge balance requirements necessitate the crystallization of the anion and cation together and the effects of solubility of either of the individual ions (and conjugate acid/base) are minimized compared to the analogous situation of cocrystallizing two independent nonionized components. It has been shown that the phase diagram for a cocrystal is related to the solubility of the individual components [26]. For example, experiments that involve solvent systems in which the guest is very soluble and the API has very low solubility are less likely to produce a solid phase containing both components.

Other considerations are methods of preparation and the type of screening methods used. Automated methods using 96 well plates, for example, screen a large number of counterions or guests with less material [27, 28]. The amount of each sample produced is determined by the solubilities of the components and is typically small (1–3 mg). Due to the small sample size, characterization is usually limited to optical microscopy, X-ray powder diffraction (XRPD), and/or Raman spectroscopy. Crystallization conditions are limited to solvent based experiments, such as evaporations or cooling when solubility permits, and a limited temperature range is available for most plates. If solids are not formed initially, recrystallization attempts may be more difficult because of experimental limitations with the plate. Crystalline materials that are produced in the plates need to be produced on a larger scale for further characterization to confirm stoichiometry and volatile content. Traditional (manual) preparation methods typically screen a smaller number of counterions or guests and will use more material per experiment. These methods produce adequate material for preliminary characterization or additional recrystallization attempts if necessary. The use of automated versus manual techniques is a decision that is usually based on material availability and known properties.

Numerous solvent methods have been used to produce salts and cocrystals. Commonly, both the API and counterion/guest are dissolved and the solutions added together. Parameters such as the order of acid/base addition, temperature, or rate of addition can all play an important role in salt or cocrystal formation. For cocrystal experiments, the solubility of the API is often lower than that of the guest compounds being screened. This can lead to the preferential nucleation of a homomeric API solid phase instead of cocrystal formation. To reduce the effects of solubility differences, yet still retain the advantages of high throughput methods, slurry experiments are often employed in the screening process.

Salt exchanges in solution or with the aid of a resin are also possible choices depending on the properties of the API [29]. Gases, such as HCl or HBr (hydrogen bromide), have also been bubbled into API solutions to produce salts [30]. Preparation techniques such as precipitation and evaporation are commonly used to produce the solid. If precipitation does not readily occur once the components are mixed, other methods may be needed to produce solids, such as concentration of the solution, antisolvent addition, lower temperatures, or a combination of these procedures.

Nonsolvent methods can also be used. Fusion methods based on Kofler techniques have been reported for a variety of systems [31]. More recently, solid-state grinding, with or without small amounts of solvent, has been used successfully for cocrystal and salt formation [32]. Even though these, or even some solvent based methods, may not be suitable for a large scale process, the seeds obtained from these experiments can be used to produce larger amounts of the desired material.

Stoichiometry is another consideration in salt and cocrystal formation. More than one acidic or basic site may be present for the API. If more than one site is available on the API, control of stoichiometry may be an issue if the pK_a values are similar. Diacid counterions, such as fumarate and malate, can also be used to investigate different stoichiometries in salts or cocrystals. An example of a system with two basic sites with similar pK_a values is given in Table 14.2. Two different stoichiometries of a hydrobromide (HBr) salt were targeted. Based on the yield and elemental data, it was obvious that the disalt was formed in both cases, regardless of the targeted stoichiometry. Additional XRPD data showed that both experiments produced the same crystal form of the disalt.

In general, there are common solvents and procedures, such as evaporation, that are used for salt and cocrystal formation, but they will not work in every case. It is

TABLE 14.2 Characterization Data on a Hydrobromide Salt

Assay	1:1 HBr:API	2:1 HBr:API	Expected Values for Disalt
C	49.77	49.92	49.48
H	4.05	3.97	3.97
N	14.83	14.91	15.05
Br	28.86	29.20	28.63
Yield	Poor yield	Good yield	—
XRPD	Form A	Form A	—

important to consider the properties of the API and design experiments that are appropriate and have the greatest chance of success. A variety of methods will also produce a wider range of crystalline solids to choose from during form selection and development.

14.3.3 Characterization

Characterization is essential for identification of the solid produced during the screen and provides the information necessary to determine if the new material can easily be developed. Information from several techniques is often required to confirm that a salt or cocrystal was prepared and to determine the chemical and physical properties. Numerous references are available that describe solid-state characterization techniques for new salts and cocrystals [1, 12, 13, 33, 34]. The theory and instrumentation of commonly used techniques are not reviewed in this section, but these techniques are discussed in terms of the information provided on new salts/cocrystals.

X-ray powder diffraction (XRPD) is often used to determine if the material is crystalline and to identify the solid form. A unique XRPD pattern does not always indicate that a new salt or cocrystal was formed and the data should be compared to the XRPD patterns of known forms of the API and the counterion/guest. As shown in Fig. 14.4, an HCl salt preparation experiment (diethyl ether fast evaporation) using Form A of the free amine resulted in the formation of a new solid form of the free amine (Form B). The HCl salt was produced from acetone using a combination of slow cool and slow evaporation techniques. The presence, or absence, of the HCl was confirmed using elemental analysis.

Raman spectroscopy is another fast and efficient tool used to determine salt or cocrystal formation [35]. Unique spectra obtained on any new material should be compared to the spectra obtained on the initial components, as shown in Fig. 14.5. In this case, the Raman spectrum indicates that a cocrystal of theophylline and salicylic acid was formed and was confirmed by other methods of analysis. Infrared (IR) spectroscopy can also be used to determine salt formation. Shifts in the carbonyl bands will indicate salt formation but can also indicate a different crystal form, so comparison with known forms is critical. The presence of NH_2 peaks in an IR spectrum can confirm that the ionized species is present in the new material. Amorphous materials usually exhibit broad IR or Raman peaks, but they can also exhibit a unique spectrum that can make interpretation more difficult.

Solid-state nuclear magnetic resonance (ssNMR) spectroscopy is a powerful tool for studying solid forms of salts or cocrystals [36, 37]. Solid-state NMR can confirm that a salt or cocrystal was formed and provide structural or conformational information [38, 39]. Solvated forms can be identified as well. In some cases, ssNMR can distinguish solid forms that are indistinguishable by other techniques [40].

Solution nuclear magnetic resonance (NMR) spectroscopy can provide a variety of information on new solids. In many cases, a solution proton NMR can reveal if a salt is formed, even if the counterion or guest is not directly observable. The chemical shift of protons that are at or near the site of charge transfer or complex formation will change compared to the chemical shift of those protons in the neutral or uncom-

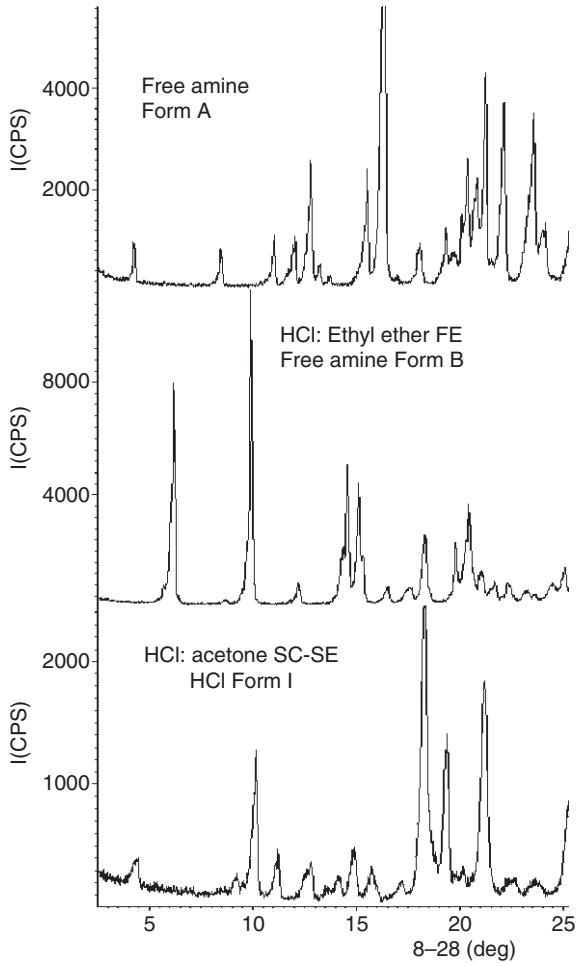


FIGURE 14.4 XRPD data for HCl salt attempts: (top) free amine Form A, (middle) HCl salt attempt from ethyl ether fast evaporation that resulted in free amine Form B, and (bottom) HCl salt attempt from acetone slow cool/slow evaporation that resulted in HCl salt Form I. The presence or absence of HCl was confirmed by other methods.

plexed species. An example is given in Fig. 14.6, which compares the spectral region of interest for an acetate salt and the free base. The NH resonance of the secondary amine at approximately 1.9 ppm in the free base spectrum is shifted to approximately 9.5 ppm (inset) as the protons of the ammonium salt. In addition, methylene proton resonances alpha to the ammonium ion have shifted approximately 0.2 ppm downfield compared to the position of those resonances in the free base.

The stoichiometry of an organic counterion or guest can be determined as well. A stoichiometry of approximately 1 : 1 acetate : API was determined from the peak integration for the acetate salt (Fig. 14.6). If an organic solvent is present, the amount can be determined and indicates whether the material is a solvate or not. This type of information is crucial in determining the developability of the new solid and can easily be obtained on a few milligrams of material.

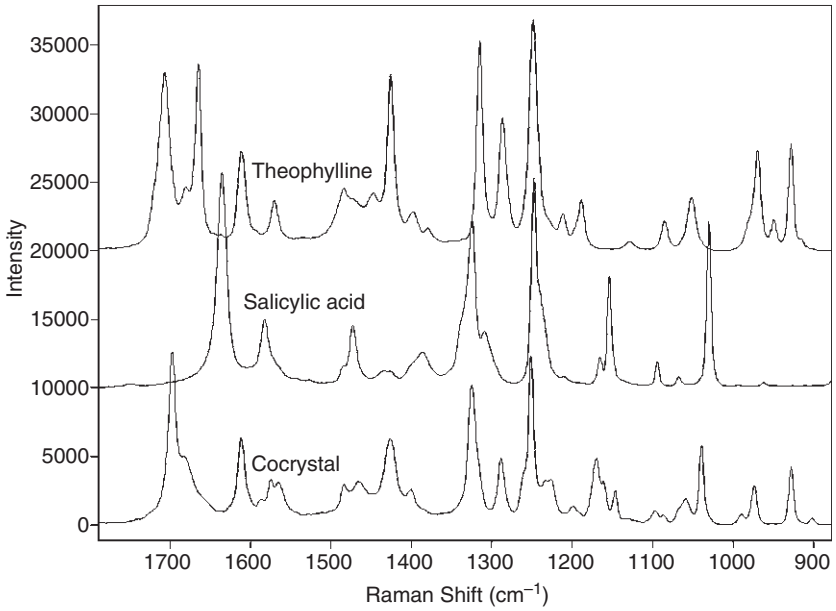


FIGURE 14.5 Raman spectra of (top) theophylline, (middle) salicylic acid, and (bottom) theophylline : salicylic cocrystal.

Differential scanning calorimetry (DSC) can also be used for the characterization of new solids if the free base or acid has a distinct thermal curve [11, 12]. DSC can be very sensitive to small amounts of the initial material depending on the strength of the melting endotherm. An example is given in Fig. 14.7. The free base exhibits a melting endotherm at approximately 228 °C while the salt exhibits a melting endotherm at approximately 250 °C. There is an additional broader endotherm observed for the salt at approximately 149 °C, which later proved to be due to the volatization of solvent from the crystalline material.

Other characterization can also be performed depending on the amount of material available. Confirming the stoichiometry with elemental analysis is a common practice. Measuring the chemical purity with HPLC is important for many API materials that may be prone to degradation under acidic or basic conditions. Determining the solvent content using NMR, gas chromatography (GC), thermogravimetry (TG), TG-IR, KF, or other methods is critical in assessing the developability of a new salt or cocrystal.

14.3.4 Evaluation

Once acceptable salts or cocrystals are found based on the characterization data, other properties need to be evaluated to choose the best compound for development. This assessment is performed as the latter part of the tiered flowchart, as shown in the examples in Fig. 14.2. Any number of factors can be investigated during this period and they should be tailored to the known issues of the API as well as the anticipated dosage form.

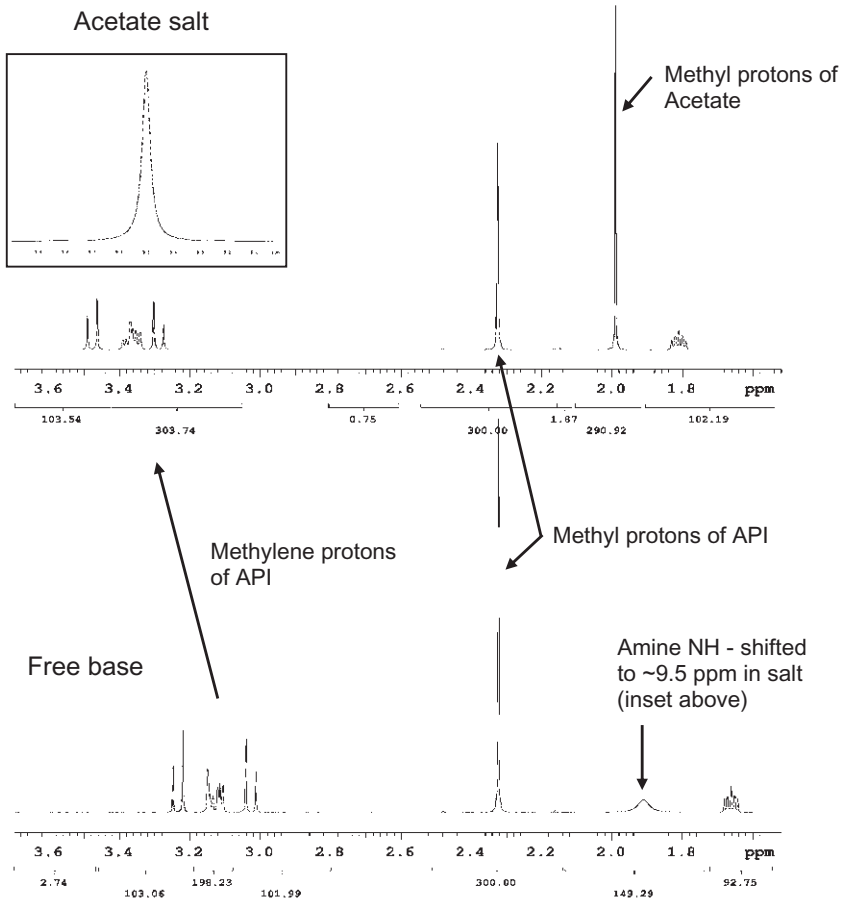


FIGURE 14.6 Solution proton NMR spectra of (top) acetate salt and (bottom) free base. Shifting observed for methylene and amine protons in acetate salt spectrum is due to salt formation. Stoichiometry of the acetate salt can also be estimated by integration of the methyl proton resonances for the acetate and the API.

A wide range of acceptable values are possible for the properties identified as important, and they depend on the development plan and dosage form [9]. In general, a solubility of 0.1–10 mg/mL is targeted for a solid oral dosage form and greater than 10 mg/mL for parenteral formulations. A pH range of 3–10 for an aqueous solution is targeted for intravenous delivery. A melting point of greater than 100 °C is usually desired for acceptable processing, especially if drying or grinding steps are anticipated. Hygroscopicity can be acceptable if the material does not deliquesce at 60–75% RH or if minimal water uptake occurs during normal processing relative humidity conditions (typically 30–75% RH). Hygroscopicity is commonly evaluated by the amount of water sorbed by the material, but other changes, such as hydrate formation, should also be investigated when assessing

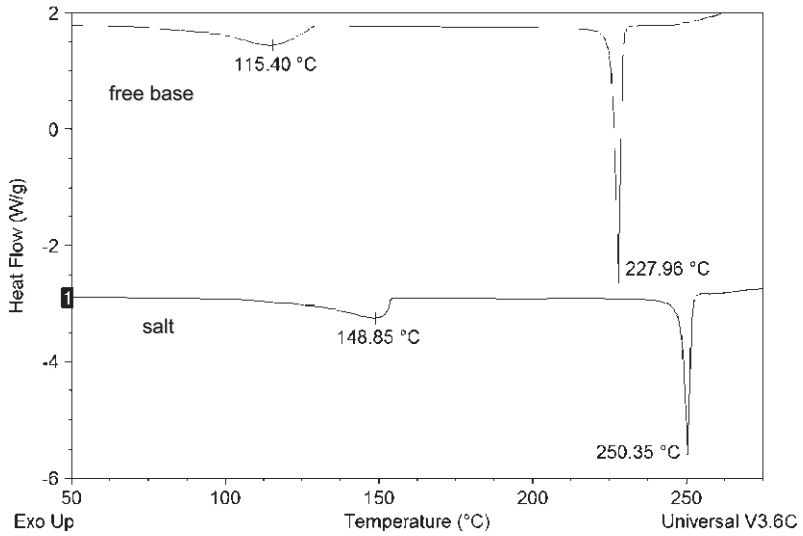


FIGURE 14.7 DSC curves of a pharmaceutical (top) free base and (bottom) salt. High temperature peaks are melting endotherms and low temperature broad endotherms are due to volatilization.

hygroscopicity [41]. Chemical and physical stability can be determined using accelerated stability conditions, such as 40 °C/75% RH over 1–2 weeks. Chemical stability can be evaluated by HPLC determination of impurities and physical stability can be evaluated by XRPD to determine a change in crystal structure. Crystal size and shape can be important to handling properties in processing and manufacturing, and an early assessment of these properties can save significant development time. The propensity of a salt or cocrystal to exist as multiple crystal forms can be evaluated at this stage with an abbreviated polymorph screen. Information on the formation of crystalline solvates from certain solvents can be used as an early guide for the crystallization step and will narrow the possible solvent choices. It should be noted that even salts or cocrystals with numerous forms can easily be developed once a controlled process is in place to produce the desired form.

This type of tiered approach was reported for BMS-180431 [11]. Seven crystalline salts were initially prepared: sodium, calcium, zinc, magnesium, potassium, lysine, and arginine. Tier 1 tested the hygroscopicity and three salts moved on to the next tier (magnesium, lysine, and arginine). Tier 2 encompassed solubility and crystal form changes and two salts, arginine and lysine, exhibited acceptable properties. The last tier tested stability (4 week evaluation) and excipient compatibility. The arginine salt was found to be the best candidate with the lysine salt as the alternate choice. The entire process was completed in approximately 6 weeks.

The evaluation process can be as simple or as complex as needed for the development of a particular compound. Numerous factors need to be considered when choosing the best salt or cocrystal for development, and it needs to be tailored to the compound. In some cases, there may not be a salt or cocrystal that exhibits all the desired attributes. When this occurs, a risk assessment must be performed to determine what extra considerations may be needed to develop the compound. If

a compound is hygroscopic above 30% RH but the other attributes are acceptable, a decision needs to be made whether controlling manufacturing conditions below this level is feasible and cost effective. These types of decisions require a multidisciplinary approach among numerous groups.

14.3.5 Polymorph Screen

Once a salt or cocrystal is chosen, a more extensive polymorph screen should be performed to find the best crystal form for development [42]. Based on screens performed in our lab, it was found that 87% of the compounds investigated resulted in more than one form including amorphous material (Fig. 14.8a) [43]. Multiple polymorphs, defined as forms having the same chemical composition, were found in 51% of the cases, and hydrates were found in 37% of the screens. As shown in Fig. 14.8b, two to four forms were found for the largest number of compounds, but many cases exist where significantly more forms were obtained.

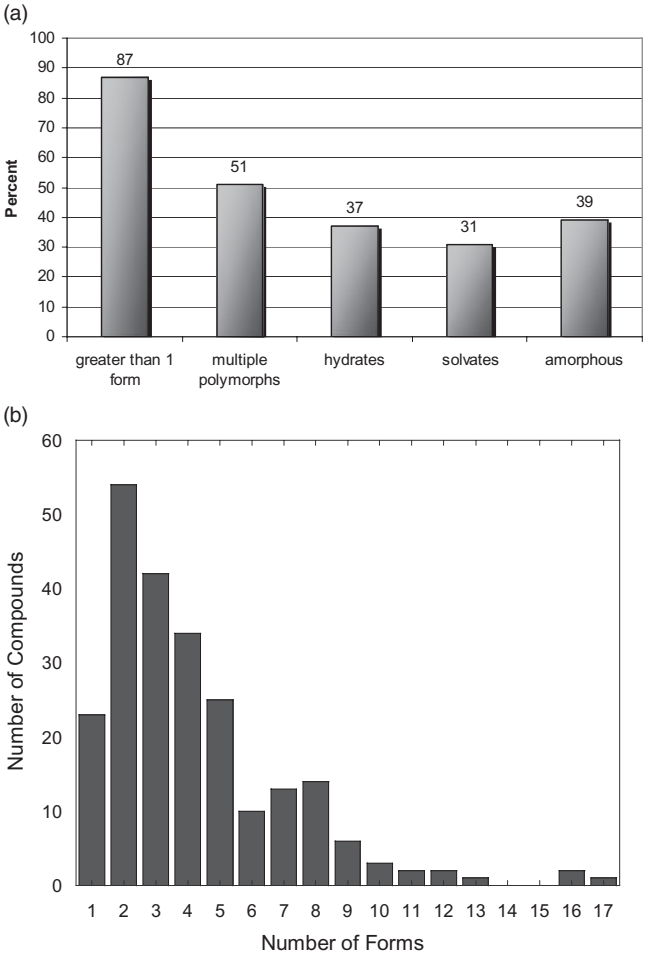


FIGURE 14.8 Forms observed in polymorph screens.

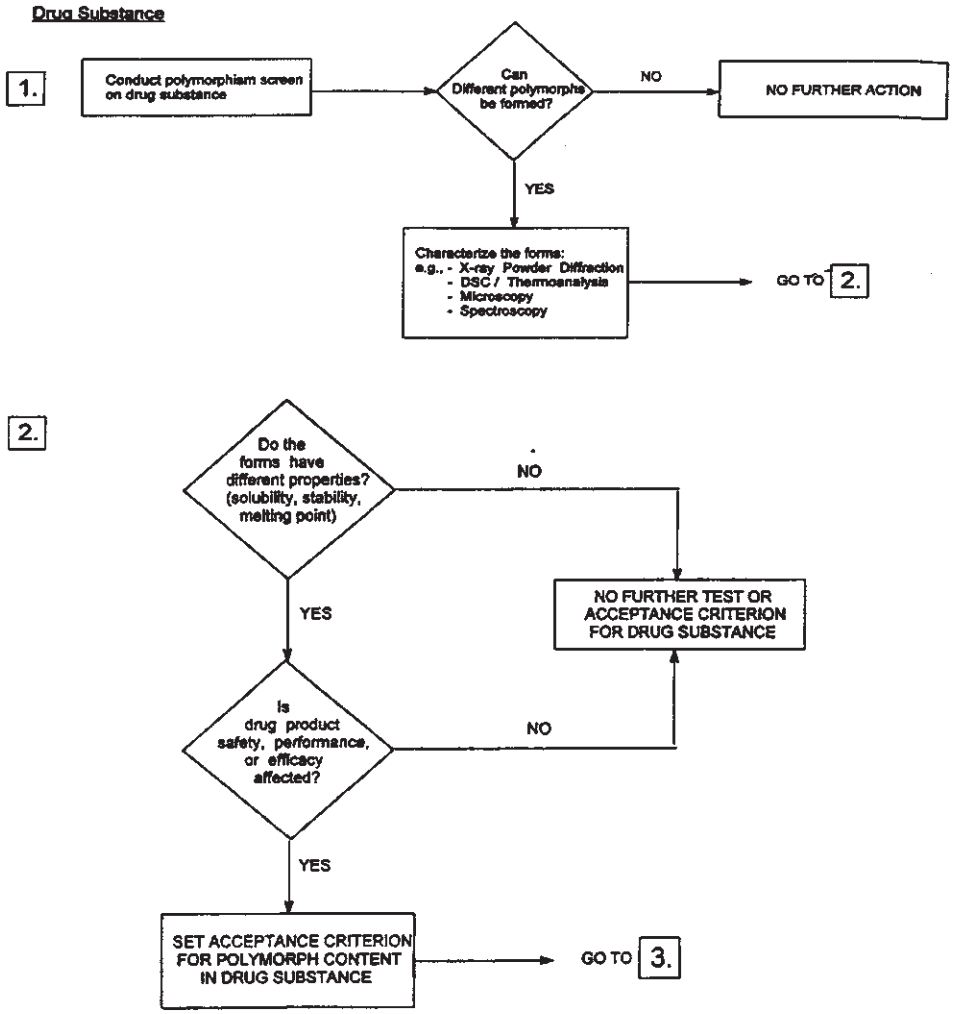


FIGURE 14.9 Decision trees for polymorph screening and property determination [44, 45].

A polymorph screen is recommended by regulatory agencies. The U.S. Food and Drug Administration (FDA) has recommended guidelines for polymorph screens [44, 45] and these decision trees are presented in Fig. 14.9. The purpose of a polymorph screen is to find multiple forms of a compound and it can be considered as a search for seeds. The decision trees are available to help guide the development of an API that can exist as more than one form. Issues such as stability, solubility, and performance in the dosage form need to be addressed for different forms of an API.

Polymorph screens can and should be performed at various stages of the development process, depending on the type of information that is required [46]. One automated screen early in development is not always sufficient to find the relevant forms for development. Possible screens and when they could be performed are summarized in Table 14.3 and Fig. 14.10. The screen should be tailored to the type

TABLE 14.3 Possible Polymorph Screens During Development

Type of Screen	Goal
Early	• Propensity for polymorphism • Indication of most stable forms • Indication of stable hydrates
Full	• Most stable form (enantiotropy) • Most stable hydrate • Other forms
Focused	• Selected form studied under GMP manufacturing conditions
Comprehensive	• Increase certainty of knowing most stable form • Find form giving improved drug product • Patent protection of all forms • Find noninfringing forms • Reduce patent costs by finding new forms at one time

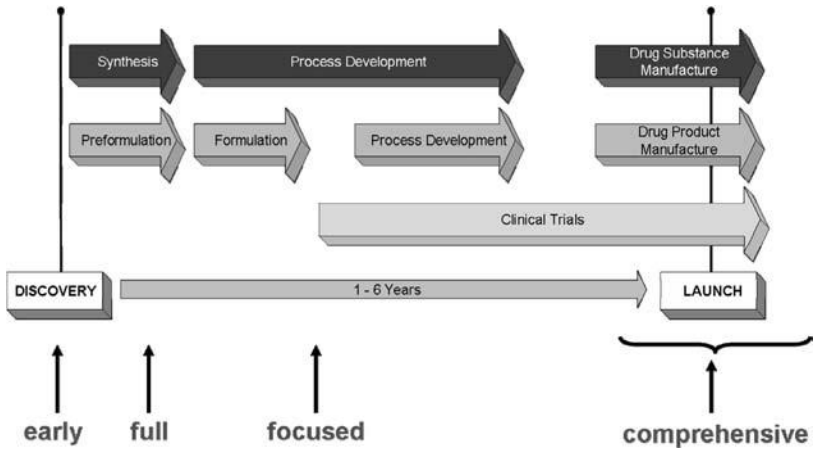


FIGURE 14.10 Types of polymorph screens at various stages of development.

of information required at that stage of development and, as the API progresses, additional information may be needed. The number and type of screens performed for an API will be determined based on the properties of the drug substance, the dosage form, and special issues that may come up during development.

Common steps in a polymorph screen are:

1. Characterize starting material.
2. Generate solid samples.
3. Collect XRPD data on solids produced.
4. Classify the XRPD patterns.
5. Scale up if needed.
6. Characterize each form.
7. Compare properties.
8. Determine relative thermodynamic stability.
9. Select form.

A wide range of crystallization techniques used in polymorph screens have been reported and reviewed [47, 48]. The majority of them are solvent based. A variety of factors are considered when choosing solvent systems for a screen, including solubility of the API, solvents to be used in manufacturing, a range of polarities and functionalities, and a range of water activities. Choices should not be limited to Class 3 solvents at this stage of the selection process. Crystallization experiments can include evaporations, slurries, cools, diffusion (vapor or liquid), solvent drop grinding, and antisolvent addition. The solubility of the API in organic solvents can help guide the type of crystallization experiments to perform. Material that is poorly soluble in most solvents would lend itself to slurry experiments or elevated temperature experiments with subsequent cooling. Material that is highly soluble may oil out at concentrations that are too high. Crystallizations covering both thermodynamic and kinetic conditions should be included since manufacturing processes commonly employ kinetic crystallization conditions (such as antisolvent addition). Experiments intended to influence the nucleation event should also be considered [49]. Nonsolvent based experiments, such as thermal treatment, sublimation, grinding, and compression [50], are also valuable and should be included whenever possible. Crystallization conditions used in a screen do not have to mimic manufacturing conditions. Seeds produced from small scale experiments can commonly be used to make larger amounts of desired forms.

The number and types of experiments should vary from compound to compound since crystallization conditions will change with the properties of the API. The choice of manual versus automated experiments also needs to be determined. A combination of solvent based experiments (automated and manual) along with nonsolvent based crystallizations will provide a more comprehensive screen and cover more of the crystallization space for the compound of interest. It is not necessary, and is sometimes counterproductive, to perform thousands of the same type of experiment, which will cover a limited portion of the crystallization space.

Analysis of the solids produced during the screen is the next step. A variety of techniques are used to characterize new materials, such as XRPD, thermal methods, and spectroscopic methods. A multidisciplinary approach is needed to understand the polymorphic system of an API. One technique will not provide all the necessary information on new crystal forms of the material and how they relate to one another. Numerous references are available that describe characterization of new forms [13, 32, 42, 46]. Analytical techniques in this section are discussed in terms of the information provided on new solids obtained during a polymorph screen.

XRPD is commonly used to determine if the samples are crystalline and it is a direct measurement of the crystal form. Once data are collected, the patterns are classified into different groups that may represent different forms of the material. At this stage, a different pattern may represent a pure material, preferred orientation, or a mixture of forms. Additional characterization information is needed on a representative sample to determine if a pure phase has been produced.

Once crystallinity has been established, determining the presence of solvents is important. Crystal forms can be solvated or unsolvated. In many cases, solvated forms are dismissed in a screen because they cannot readily be used as an API. However, solvates can play an important role in processing and crystallization development and can help explain the formation of one form over another from a certain solvent or process. Solvates would usually not be chosen for development,

but upon drying a new unsolvated form may be produced that has all the properties required for the targeted product. Hydrates can be considered a subset of solvates, with water being the solvent incorporated into the lattice. Various types of hydrates have been characterized and discussed [51]. Two common types are stoichiometric and variable hydrates. Stoichiometric hydrates contain water in molar ratios. Those that are stable over a wide range of relative humidity conditions are possible candidates for development. Others may be unstable and unsuitable for further consideration. Variable hydrates typically contain water in nonstoichiometric amounts and the amount of water readily changes with relative humidity conditions. These types of hydrates are difficult to characterize, control, and develop.

Melting point can be a factor during development and should be determined for the various forms. DSC, hot-stage microscopy, or a melting point apparatus can be used to measure this property. A higher melting solid, generally greater than 100 °C, is desirable. A low melting material is more difficult to dry, mill, or compress. DSC data can also provide information on high temperature crystal form changes that may impact development [42], as well as information on physical purity of the sample. Other data can be invaluable in determining if a sample is a pure form or a physical mixture of two or more forms. Solid-state NMR has been used to characterize many pharmaceutical materials and can commonly provide information on mixtures that is difficult to obtain by other methods [35, 36]. IR spectroscopy and Raman spectroscopy are also commonly used to differentiate crystalline forms [32].

Once the forms have been characterized, it is important to understand the relationship between the forms. A schematic, such as the one given in Fig. 14.11 [52], can be constructed to visualize changes in the forms during development. An early

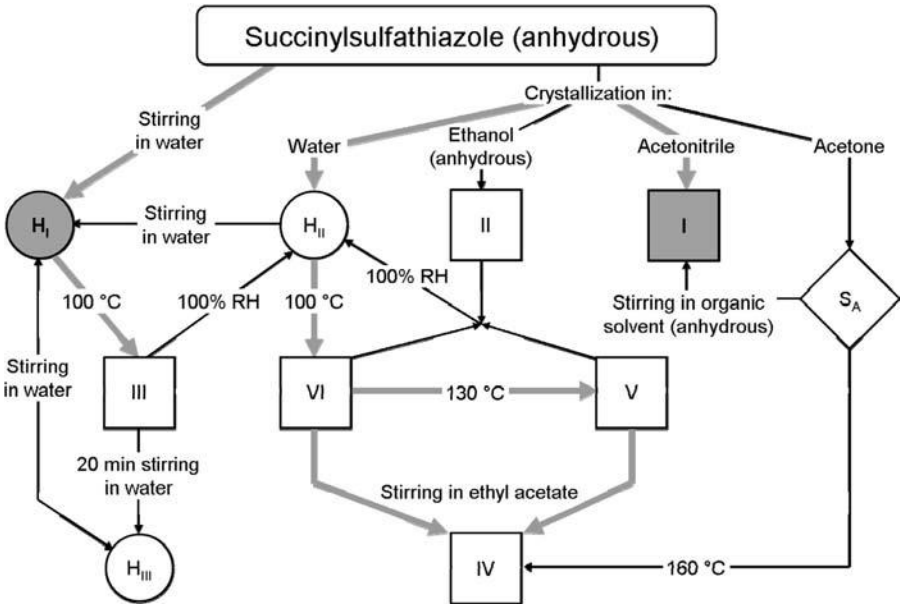


FIGURE 14.11 Schematic of succinylsulfathiazole forms produced under various conditions [52].

understanding of possible form changes during processing, such as upon drying or exposure to water, will help to design acceptable processes that will minimize form changes [53, 54].

The thermodynamically most stable form should be identified if more than one acceptable unsolvated form is found in the screen [46, 55, 56]. The most stable crystalline form is defined as that with the lowest free energy and it will also exhibit the lowest solubility. In a monotropic system, the relative free energy for the two forms remains the same along the entire temperature range, as shown in the energy–temperature diagram in Fig. 14.12a. The heat of fusion curves show that the more stable form has the higher melting point and the greater heat of fusion. In an enan-

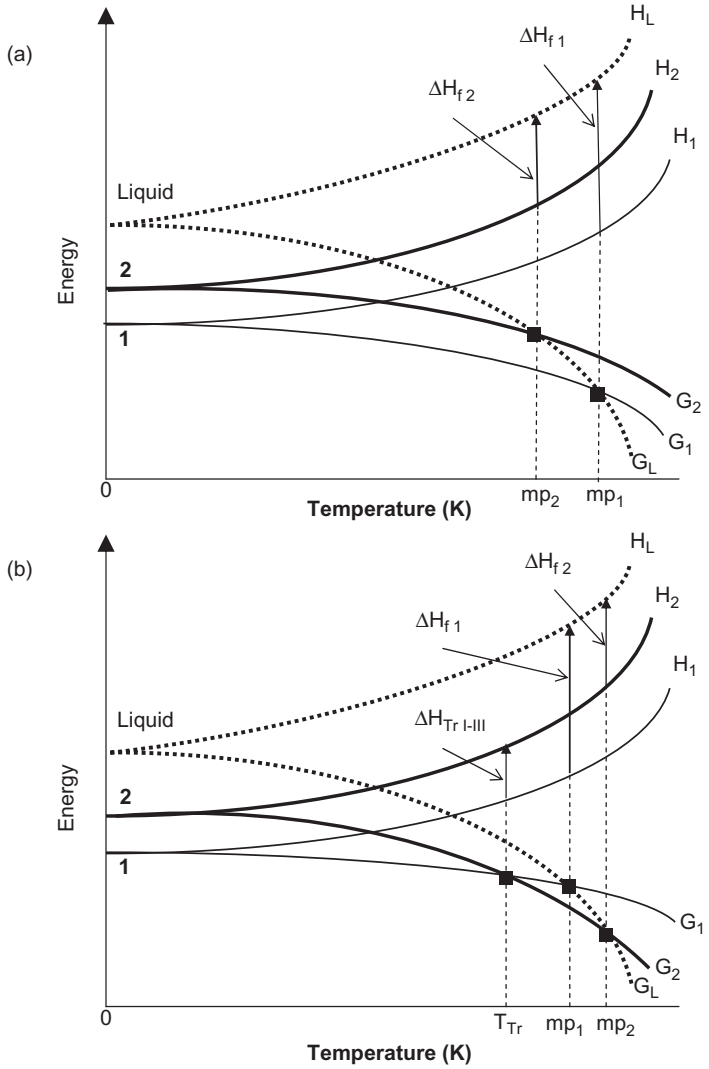


FIGURE 14.12 Energy–temperature diagrams for (a) monotropic and (b) enantiotropic systems.

TABLE 14.4 Burger and Ramburger Rules to Determine the Thermodynamically Stable Form [57]

Rule	Description
Heat of transition rule	If an endothermic transition is observed at some temperature below the melting point, it may be assumed that there are two forms related enantiotropically.
	If an exothermic transition is observed below the melting point, it may be assumed that there are two forms related monotropically or the transition temperature is higher.
Heat of fusion rule	If the higher melting form has the lower heat of fusion, the two forms are usually enantiotropic; otherwise they are monotropic.
IR rule	If the first absorption band in the infrared spectrum of a hydrogen-bonded molecular crystal is higher for one modification than for the other, that form may be assumed to have the larger entropy.
Density rule	If one modification of a molecular crystal has a lower density than the other, it may be assumed to be less stable at absolute zero.

tiotropic system, the relative stabilities change at a temperature defined as the transition temperature, as shown in Fig. 14.12b. In energy–temperature diagrams, the free energy curves can cross, but the enthalpy curves do not. Based on this, the more stable form below the transition temperature in an enantiotropic system will exhibit the lower melting point and the higher heat of fusion. There are four rules proposed by Burger and Ramburger to determine the type of relationship between forms [57] and these are summarized in Table 14.4. Other methods to determine the stability include plotting solubility data at different temperatures in a van't Hoff plot [58] as well as eutectic mixtures [59]. This information is important for crystallization processes since crystallization above the transition temperature will favor one form and below the transition temperature will favor the other. It is also critical for long term stability since the metastable form may transform to the more stable form over time. A metastable form with sufficient stability to provide an acceptable shelf life is another option to consider.

At this stage, other factors need to be considered in the form selection process. Information on solubility, intrinsic solubility, modified absorption potential [60], and chemical and physical stability will help in selection of the best candidate for development.

14.3.6 Crystallization and Quantitation

Once a crystal form is found, crystallization method development will be needed to determine the best conditions to produce material with optimal characteristics, with crystal form being only one consideration. Crystallization conditions to produce the salt or cocrystal directly are the best option. However, a recrystallization step may be needed to produce the desired crystal form with acceptable purity and yield. Numerous factors, such as crystallization equipment, solvent, temperature, cooling rate, antisolvent addition, and other parameters, will need to be investigated [61].

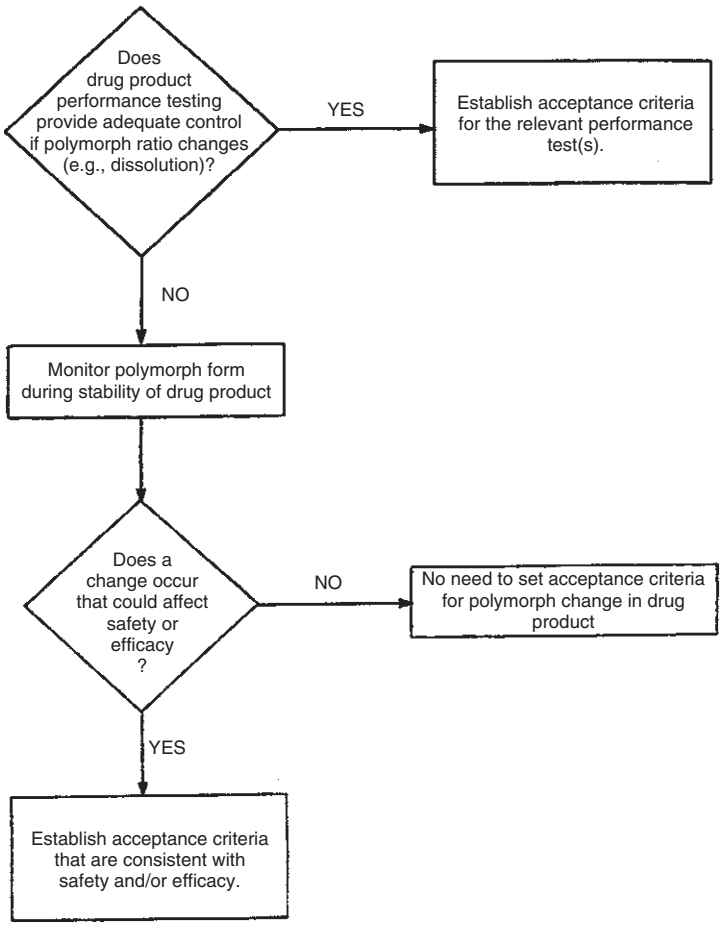


FIGURE 14.13 Decision tree for polymorphic form in drug product [44, 45].

Seeding is also a good option for many systems. Knowing the transition temperature for an enantiotropic pair will be necessary to determine the best crystallization conditions.

When multiple forms are known, the process needs to be controlled to make the desired form of the API consistently. An assay, qualitative or quantitative, may be needed for confirmation, as outlined in Fig. 14.9. Quantitation of forms may also need to be addressed for the drug product as shown in the flowchart in Fig. 14.13. Common analytical techniques used to quantitate the forms in solid samples include XRPD, DSC, IR and Raman spectroscopy, and NMR. Moisture balance data can also be used to quantitate amorphous materials in crystalline solids. Specificity needs to be determined for the technique being used for method development. Guidelines for quantitative method development have been reported [62] and the parameters to be investigated include specificity, linearity, range, accuracy, precision, detection limit, and quantitation limit. Different types of quantitative methods can be developed, as summarized in Table 14.5. Different assays can be used at various stages of development and to obtain suitable data for specific situations. An assay

TABLE 14.5 Summary of Solid-State Quantitative Assays

Quantitative Method	Scope of Assay	Result
Limit test	A limit of detection for a technique is determined (such as 2%)	Result is specified as "less than 2% Form B present"
Specification assay	An assay limit is determined for a technique (such as 95%)	Result is specified as "greater than 95% Form A present"
Full quantitative method	A minimum quantifiable limit (MQL) (such as 2%) and linear range (such as 2–25%) are determined	"X% of Form B" if within the quantitation range of 2–25%, or "less than 2% Form B" if below MQL, or "greater than 25% Form B" if above linear range

may be needed to show that a new process is producing the desired form and may eventually be sunsetted after control is established over a period of time.

14.4 CONCLUSION

Significant information is needed to select a form for development. Making salts and cocrystals of the API for evaluation is a small part of the form selection process. Characterization of physical properties and understanding the interaction of various forms in a solid system are critical for the success of a formulated product and can significantly reduce time to market. A qualitative or quantitative assay may be needed for the API and/or drug product if multiple forms are found. The information obtained during form selection can be used for regulatory and intellectual property documentation and can help extend the life of an important franchise.

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15

DISSOLUTION

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15.1 INTRODUCTION

Dissolution of the active ingredients is of paramount importance for the successful performance of a dosage form. Dissolution testing is a convenient tool for ensuring intra- and interbatch performance compliance of dosage forms. Furthermore, the release profiles obtained from *in vitro* dissolution tests can be used for predicting *in vitro–in vivo* correlation models. Thus, carefully conducted dissolution tests are useful for ensuring dosage form functionality.

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The physicochemical characteristics of a drug and its site of action/absorption together dictate the type of dosage form to be developed. The plethora of additives and technologies available in the hands of the product development scientist make it possible to design a dosage form possessing the predetermined drug release attributes. Great strides in the excipient and technology arena, however, make it imperative to critically evaluate their influence on *in vitro* drug dissolution during the preformulation phase. This involves proper selection of dissolution media, dissolution apparatus, operation variables, and interpretation of generated data. Although a preliminary estimate of the dosage form's *in vivo* performance can be gauged by applying *in vitro–in vivo* correlation models, it is essential to test the dosage form in animals to demonstrate its efficacy and safety. Therefore, the functionality of dosage form is first demonstrated in appropriate animal model(s) during the preclinical phase before testing it in human beings. Figure 15.1 gives a schematic representation of the steps to be followed during the course of dosage form development with respect to dissolution testing of a dosage form.

15.2 FACTORS INFLUENCING DRUG DISSOLUTION FROM DOSAGE FORMS

Dissolution of drug from a dosage form is unequivocally the most important indicator of its anticipated *in vivo* performance. Both nature of formulation ingredients and methods of incorporating them influence drug release from dosage forms. Therefore, formulation and process variables demand careful evaluation during the initial product development phase in order to achieve the predetermined drug dissolution profile.

15.2.1 Formulation Variables

A variety of excipients are available for use in solid dosage forms. Functional differences in excipients are known to exist depending on the material and usage and it is difficult to generalize findings for all the drugs. Therefore, the potential of these excipients to modify drug release from dosage forms should be critically evaluated during formulation. Although a detailed discussion on all excipients would not be possible, a few categories needing special attention are discussed next.

Diluents Diluents are used in solid dosage forms as main bulk forming agents. Being present in large quantities, they can exert profound influence on the stability of the active ingredient, ease of manufacturing, drug release, and bioavailability profile. Table 15.1 lists important diluents used for direct compression and wet granulation. It is evident from this table that an excipient influences compression kinetics, tablet properties, and eventually functionality of the dosage form.

Furthermore, the effect of precompression, which is often used for reworking tablet/capsule granules, on drug release should be evaluated especially while attempting dry granulation. The quality of tablets containing a combination of microcrystalline cellulose and lactose was found to increase after reworking when the proportion of spray dried lactose was increased. However, the tablet weight uniformity decreased [5]. Generally, recompression reduces tablet strength and this

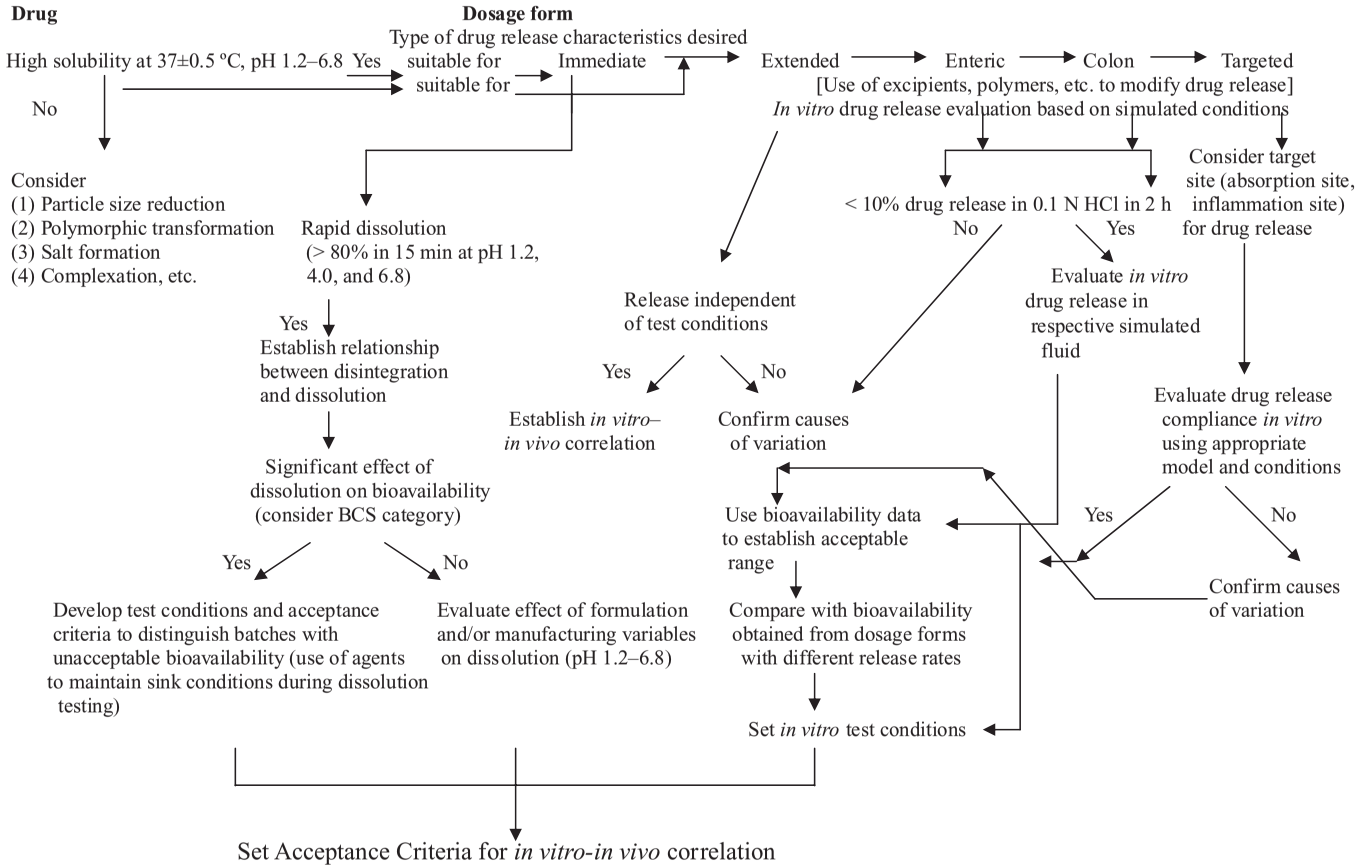


FIGURE 15.1 Schematic representation of steps to be followed during dosage form development.

TABLE 15.1 A Few Important Diluents and Their Properties

Excipient (Trade Name)	Properties		Suitable for	Effect on Drug Dissolution	References
	Advantages	Disadvantages			
Microcrystalline cellulose (Avicel, PH-101)	Good compressibility, forms hard tablets at low pressure, fast tablet disintegration, available in different particle sizes for use in different types or tablets	Fair flowability, costly	• Direct compression • Wet granulation • With nonaqueous binding system • Higher carrying capacity • Hard tablet at low applied pressure • Controlled release when combined with lactose, starch, etc.	Capillarity explains the penetration of water into tablet, thereby destroying cohesive bonds between particles	1–3
Powdered cellulose	Little inherent lubrication property, inexpensive	Poor flowability, poor compressibility, poor binding, high dilution potential	• Wet granulation	Tablets often disintegrate readily; but chances of physical entrapment are also there, which may lead to delayed dissolution	1, 2
Dicalcium phosphate	Fairly good compressibility, excellent flow property	Soluble in acidic pH of stomach	• Direct compression • Wet granulation with nonaqueous binding system	Negative effect on dissolution by using increased proportions with microcrystalline cellulose; because of hydrophobic nature, may limit the dispersion of drug particles, hence may lead to slow dissolution	1, 2

Spray dried lactose	Good compressibility, excellent flow property, low hygroscopicity, low reactivity with active ingredient; excellent physical, chemical, and microbiological stability	Browning due to the presence of 5-hydroxy methyl 2-furaldehyde when combined with amines, moisture, lactates, phosphates; has relatively poor dilution potential	• Direct compression • Wet granulation with nonaqueous binding system	Aids in dissolution	1, 2, 4
Starch directly compressible (Sta-Rx-1500)	Acts as local desiccant to help stabilize moisture sensitive drugs, free flowing, self lubricating	Tablets with high content are soft and may be difficult to dry, susceptible to softening when combined with 0.5% magnesium stearate	• Direct compression	Starch granules swell in dissolution media forming a viscous matrix that retards dissolution medium penetration and hence decrease the dissolution	1, 2
Compressible sugar (invert sugar, malt dextrins)	Highly compressible, inexpensive nonhygroscopic (invert sugar is hygroscopic at elevated temperature and high humidity)	Readily soluble, calorigenic, not acceptable for diabetes	• Tablet at high moisture content requires high ejection force • Low lubricant requirement		1, 2

TABLE 15.1 *Continued*

Excipient (Trade Name)	Properties		Suitable for	Effect on Drug Dissolution	References
	Advantages	Disadvantages			
Mannitol	Nonhygroscopic, needs combination with other directly compressible excipients for good compression, relatively inert with high heat stability	Poor flowability and requires high lubricant addition, expensive	• Mouth dissolving • Buccal tablet • Chewable tablet • Generally wet granulated using auxillary binder to produce compressible granules	Not relevant	1, 2
Ethyl cellulose (TOEC-Aqualon)	High ethoxyl content and low viscosity, excellent flowability, drug release triggered by circumferential failure of the coat, greater lag time (3–16 h) by increasing coating compression force (5-L5KN) than other grades	Relatively expensive	• Compression coating	May have a serious retadant effect on disintegration and drug dissolution release	1, 2
Pregelatinized starch (PCS PC-10)	Free flowing, good binding ability, good disintegrating capacity, high dilution potential, some lubricant action, good oil absorption capacity	Requires high pressure to produce hard table; binding gets reduced by lubricants; magnesium stearate softens the tablet	• Direct compression	Effect is concentration dependent, generally improves dissolution	1, 2

reduction becomes more significant when initial compression is carried out at higher pressure. Hence, these tablets can be expected to exhibit faster drug release.

Directly Compressible Vehicles Direct compression is preferred over wet granulation as it requires fewer unit operations and less space, saves time, and labor costs, and hence is more economical. Tablets prepared by direct compression exhibit less variation in dissolution profile after storage [6]. In addition, as directly compressed tablets disintegrate, discrete drug particles are released—unlike granules from wet granulation—which ensures faster as well as complete drug dissolution. It is well known that if fragmentation predominates during compression, an increase in specific surface area leads to enhanced dissolution. However, if consolidation is the predominating phenomenon, an increase in compression pressure decreases dissolution. Therefore, it is essential to evaluate the compression behavior of compressible vehicles before using them as diluents in solid dosage forms.

LACTOSE Lactose exhibits polymorphism depending on crystallization conditions. Crystalline lactose undergoes fragmentation while amorphous lactose undergoes plastic deformation during compression. Tablets prepared from amorphous lactose show greater hardness with increasing water content [7].

α-Lactose monohydrate (100#) is generally used in direct compression due to its good flow property. It undergoes fragmentation during compression and exhibits poor binding [8] and the tablet strength decreases with increase in particle size [9]. Tablets prepared by combining microcrystalline cellulose and α-lactose exhibit greater hardness with an increase in proportion of the former [9]. α-Lactose monohydrate exhibits good flowability, compressibility, and hardness when mixed with polyethylene glycol 6000 [10]. A multifunctional coprocessed, directly compressible diluent containing lactose, polyvinyl pyrrolidone, and croscarmellose sodium was found to exhibit better flow properties, compressibility, and disintegration than lactose monohydrate [11]. The binding capacity of α-lactose monohydrate increases considerably after dehydration, wherein it changes from single crystals to aggregates of anhydrous α-lactose particles [12]. Tablets containing anhydrous α-lactose show slow disintegration and dissolve during disintegration [5]. Anhydrous β-lactose monohydrate is 10-fold more soluble than α-lactose monohydrate. The slowing effect is suggested to be due to the presence of small pore diameters and precipitation of anhydrous α-lactose as α-lactose monohydrate in these pores. The smaller initial solubility of α-lactose monohydrate produces this effect and the tablets exhibit higher dissolution [13].

Spray dried lactose contains a mixture of crystals of lactose monohydrate and spherical agglomerates of small crystals bound together by glassy or amorphous material. The hardness of tablets was found to increase with decrease in particle size [14]. Furthermore, disintegration time is reported to increase with an increase in compression force [15]. Addition of lubricant to spray dried lactose prolongs the disintegration time and drug dissolution [16].

The granulated form of α-lactose monohydrate (agglomerated lactose) possesses good flow and binding property but not as good as spray dried lactose. The presence of increasing amounts of β-lactose in agglomerated lactose is suggested to impart strong intergranular cohesion [17].

CELLULOSE DERIVATIVES Microcrystalline cellulose (MCC) finds wide application in the formulation of tablets by both direct compression and wet granulation. MCC possesses good flow property, hardness, and friability. It is available in different grades depending on physical state, powder (PH-101) and granular (PH-102) as well as particle size. It can be used for controlling the release of both water-soluble and water-insoluble drugs when combined with other diluents like lactose, starch, and dibasic calcium phosphate [18]. The granular form (PH-102) is reported to exhibit better flow [15]. The denser versions of PH-101 or PH-102—PH-301 and PH-302, respectively—have improved flowability but reduced compressibility [19]. Tablets containing higher proportion of PH-101 were found to exhibit higher crushing strength and lower disintegration time, while those containing PH-102 and PH-200 exhibited lower crushing strength and shorter disintegration time [20]. An increase in the ratio of length to diameter of particles (Celous KR 801) yields tablets with higher tensile strength [21]. Similarly, presence of cellulose II polymorph in Avicel PH-102 gives more elasticity to particles than Avicel PH-102 that contains cellulose I polymorph [22].

SUGARS Sucrose is widely used in the manufacture of chewable tablets [23]. However, its use is restricted due to concerns regarding microbial growth, high calorie contribution, and moisture sensitivity. Di-Pac consists of 97% sucrose and 3% modified dextrin [24]. It is directly compressible but tends to cake and lose its fluidity at high moisture level. Due to cocrystallization of sucrose and dextrin, Di-Pac undergoes plastic deformation and produces harder tablets that dissolve slower than those obtained from sucrose alone [25]. Mannitol is water soluble and nonhygroscopic and is an important ingredient in chewable tablets due to its negative heat of solution that produces a cooling effect in the buccal cavity. Emdex is produced by hydrolysis of starch and consists of aggregates of dextrose microcrystals intermixed with higher molecular weight sugars. Theophylline tablets prepared with emdex were found to exhibit higher mechanical strength, rapid disintegration, and faster drug release than the tablets prepared with maltodextrin M 450 [26]. The rate of release of water-insoluble drug was found to be significantly affected by addition of lubricants in tablets prepared from maltodextrin (M 700, M 500, and M 150) while the release of water-soluble drug remained unaffected [27].

Starch may be obtained from corn, wheat, potatoes, tapioca, or rice. Tablets containing a high concentration of starch are usually soft. An increase in starch content and precompression force and a decrease in granule size was found to enhance the dissolution rate of salicylic acid tablets [28]. Presence of high initial moisture content in starch often leads to poor tablet properties. Therefore, directly compressible varieties are used for improving processing characteristics.

Sta-Rx-1500, a partially gelatinized cornstarch, is free flowing and directly compressible. If compressed alone, it is self-lubricating. However, on addition of 5–10% ingredient that does not possess lubricating property, it requires additional lubricant, usually a glidant, such as colloidal silicone. It softens on mixing with magnesium stearate [29]. Compressible starch gives faster dissolution as compared to other normal starches [20, 30]. Rice starch (Era Tab) produced tablets of terfenadine with higher crushing strength and lower friability than those produced from Sta-Rx-1500, dicalcium phosphate, or Avicel PH-101 [31]. Uni-pure, a fully gelatinized maize starch, was found to possess strong binding properties with faster dissolution and disintegration [32].

DICALCIUM PHOSPHATE Dicalcium phosphate dihydrate is the most common inorganic material used as a diluent in direct compression. The slightly alkaline nature (pH 7.0–7.4) makes it less suitable for use with drugs that are prone to alkali degradation [33]. Emcompress, containing aggregates of dicalcium phosphate, is directly compressible. However, it requires addition of a large quantity of lubricant for easy ejection from dies. Because Emcompress undergoes fragmentation during consolidation, the added lubricant significantly reduces dissolution of drugs [34].

Coprocessed Diluents for Direct Compression Coprocessing of well established diluents is a method that is used for obtaining an integrated product, which possesses superior functionality. A few well known coprocessed, directly compressible diluents are summarized in Table 15.2. Although improved functionality depends on the specific purpose related to drug delivery, all directly compressible diluents, in general, need careful evaluation with respect to flowability, tablet characteristics, and reworkability [35].

COPROCESSED LACTOSE Ludipress consists of α-lactose monohydrate (93.4%), polyvinyl pyrrolidone (3.2%), and crospovidone (3.4%). In spite of the presence of disintegrant, tablets containing Ludipress take more time to disintegrate than those containing α-lactose monohydrate, Tablettose, or anhydrous β-lactose [36]. Although addition of lubricant is essential, the mixing time was not found to influence the crushing strength of tablets [37]. The disintegration time of tablets compressed using Ludipress at 100 MPa pressure did not change, whereas that for Cellactose tablets got prolonged [38, 39]. Cellactose contains α-lactose monohydrate (75%) and cellulose (25%). Good compressibility is attributed to synergistic effect by fragmentation of lactose and plastic deformation of cellulose [40, 41]. Investigators have reported better compressibility of Cellactose as compared to Ludipress, Fast Flo Lactose, Tablettose, Di-Pac, anhydrous lactose [37, 42], and a mixture of Avicel PH-101 and Tablettose [43]. Reduction in compression pressure, which does not eliminate macropores, was reported to decrease the disintegration time of Cellactose tablets [44]. Tablets prepared from granules containing lactose coprocessed with microcrystalline cellulose and dextrin [45], dicalcium phosphate, and microcrystalline cellulose [46] were found to exhibit satisfactory properties.

Pharmatose DCL 40 contains β-lactose (95%) and anhydrous lacitol (5%). It is spherical in shape, exhibits good flowability, and is comparatively stable on exposure to high humidity [23].

StarLac is produced by spray drying of α-lactose monohydrate and maize starch [47]. Its tablets exhibited very good flow behavior, acceptable crushing force due to presence of lactose, rapid disintegration due to starch, and rapid release of theophylline [48].

COPROCESSED MICROCRYSTALLINE CELLULOSE A codried mixture of microcrystalline cellulose and β-cyclodextrin was found to exhibit improved flowability and compressibility [49]. However, coprocessing microcrystalline cellulose with sodium lauryl sulfate by ultrasonic homogenization followed by spray drying yielded a product that was inferior to microcrystalline cellulose for tableting paracetamol [50]. The PH-M series of microcrystalline cellulose is reported to be suitable for rapidly disintegrating tablets of acetaminophen and ascorbic acid [51]. The tensile strength of tablets prepared from coprocessed granules containing microcrystalline cellulose

TABLE 15.2 A Few Important Commercially Available Coprocessed Diluents and Their Functionality

Brand Name	Adjuvants	Properties
Cellactose	Coprocessed lactose and microcrystalline cellulose (MCC)	Among directly compressible fillers, MCC is the most compressible and has the highest dilution potential because of its high binding and good disintegrating properties. MCC is of interest when combined with other less compressible excipients as an added component in coprocessed direct tableting excipients.
XyliTab	Directly compressible xylitol and sodium CMC	Xylitab granulates provide the convenience of direct compression. Use of Xylitab avoids the need for lengthy traditional granulation processes and allows dry mixing of tablet components.
Ludipress	β-Lactose and crospovidone, PVP	Direct compression auxiliary with medium porosity and good flow properties.
Starlac	Lactose and maize starch	Direct compression excipient, improved flowability, rapid disintegration of tablets, increases crushing strength.
Pharmatose DCL 11	Anhydrous lactose, maize starch	Excellent flow properties and porous surface structure ensure excellent mixing properties as a consequence; DCL 11 permits very low tablet weight variation and high drug content uniformity; DCL 11 is only slightly affected by the level of lubrication.
Pharmatose DCL 14	Mixture of fine crystals of lactose monohydrate and amorphous lactose	Directly compressible product, better compaction properties, uniform particle size, high degree of flowability.
Avicel CE 15	MCC and guar gum	Incorporates particle-engineered products and process technology; provides excellent control of sensory factors that affect palatability; imparts such pleasing sensory features to chewable tablets that the perceived taste of drugs is remarkably altered; produces comparably softer tablets that are less friable and disintegrate rapidly.
Carbofarma GS 12	Calcium carbonate (90%) and pregelatinized starch (10%)	High fluidity (easy workability at high speeds), high density (allows high calcium dosage in small tablets), excellent palatability (no awful feeling especially in chewable formulations).
Carbofarma GM 11	Calcium carbonate (90%) and maltodextrin (10%)	High fluidity, high density, and excellent palatability.
Plasdone S-630	Vinyl acetate and vinyl pyrrolidone	Versatile wet granulation tablet binder, viscosity modifier, crystal growth inhibitor, and solubilizer in liquid dosage forms; available in pyrogen-free "C" grade suitable for parenteral and ophthalmic applications; soluble in water and all pharmaceutically acceptable solvents; protective colloid/stabilizer, demulcent, and lubricant in ophthalmic solutions, solubilizer by complexation.
BarcroftTM CS90	Spray dried calcium carbonate and starch	Highly compressible and free flowing. Simply adjusting the compression force used can allow one to make both chewable and conventional tablets.
Di-Pac	Sucrose and dextrin	Excellent flowability, high compressibility, low hygroscopicity, sweet and nonreactive.
Advantose FS 95	Codried fructose	Turns fructose into an excellent excipient for pharmaceutical, nutraceuticals, and chewable vitamin tablets, improved flow properties, excellent compressibility.

and calcium carbonate decreases with an increase in the amount of calcium carbonate [52].

Prosolv contains microcrystalline cellulose (98%) coprocessed with colloidal silicone dioxide (2%). It has better flowability and compressibility [19, 53] and the tablets maintain tensile strength even in the presence of magnesium stearate, unlike microcrystalline cellulose [54]. A marked enhancement in disintegration time was observed in tablets coated with Prosolv as compared to Avicel [55].

Low crystallinity cellulose is prepared by reacting cellulose with 85% w/w phosphoric acid fumes at room temperature for 1 h, then at 50 °C until a viscous solution is formed. This is poured into water to obtain a fine powder [56–58]. Kumar et al. [59] found that tablets obtained by compressing low crystallinity cellulose (LCPC) were intact even after 6 h, whereas those obtained by compressing LCPC-2000 disintegrated in 4 min. LCPC-4000 was found to be the most ductile material and exhibited the highest compression and compaction characteristics [59].

Lubricants Lubricants are used for reducing the friction between granules and the die wall during compression. Antiadherents prevent sticking to the punch, while glidants improve the flowability of granules. They act by either fluid (hydrodynamics) lubrication by providing a continuous layer of fluid lubricant or by boundary lubrication that results from adherence of polar portions of molecules with carbon chains to metallic surfaces. Additional mechanisms by which antiadherents and glidants improve granule properties relate to electrostatic charge, gas adsorption, and van der waals forces [60, 61].

Lubricants are hydrophobic in nature. Their presence on the granule/tablet surface may decrease the area of contact between the particles and interfere in particle–particle bonding, thereby providing a less cohesive tablet [62]. Furthermore, the coating of lubricant on particles renders the surface hydrophobic and may increase the disintegration time while decreasing the dissolution rate.

An increase in concentration of calcium stearate, glyceryl monostearate, magnesium stearate, or stearic acid from 0.1% to 5% was observed to decrease the dissolution rate of salicylic acid and aspirin, whereas no effect was observed when talc was used [63]. It is important to ascertain whether the lubricant exhibits interaction with excipients or not. Although magnesium stearate possesses higher specific surface area than sodium stearyl fumarate, its adhesion to drug–crospovidone agglomerates results in a decrease in dissolution rate [64]. The negative effect of lubricants on dissolution can be counteracted by choosing diluents that possess both high solubility and fragmentation [65]. The deleterious effect of magnesium stearate (0.5%) on dissolution of ketorolac tromethamine was alleviated by hydrophobic anionic surfactants (sodium *N*-lauroyl sarcosinate, sodium stearoyl 2-lactylate, and sodium stearate), nonionic surfactants (polaxmer 188), and cationic surfactant (cetyl pyridinium chloride). However, lipophilic surfactant (glyceryl monostearate) was ineffective in counteracting the effect of magnesium stearate [66]. The deleterious effect of lubricant on drug dissolution can also be overcome by including superdisintegrants in the formulation [67].

The effect of lubricant on dissolution depends on the aqueous solubility of the drug. Magnesium stearate did not influence the dissolution rate of hydrochlorothiazide, sorivudine, and aztreonam capsules stored at 50 °C for six months. Although caking was observed in capsules of all the drugs on storage at 40 °C/75% RH, the

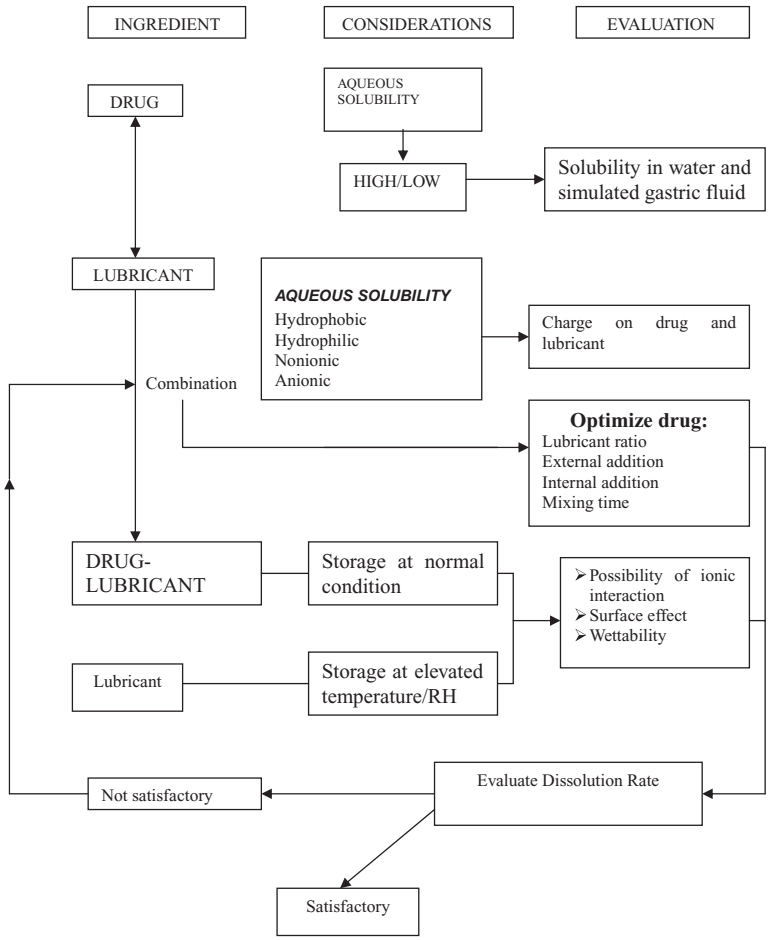


FIGURE 15.2 Evaluating the influence of lubricant on drug dissolution.

decrease in dissolution rate of only sorivudine could be attributed to this effect. On the other hand, aztreonam, due to its inherent high aqueous solubility, did not exhibit any discernible effect on dissolution [68].

The addition of lubricants is known to decrease tablet hardness and results in incomplete drug dissolution [64, 69–72]. External addition of magnesium stearate was found to produce tablets that disintegrated immediately and rapidly released trypsin as compared to those containing internally added lubricant. This was ascribed to higher water penetration rate in tablets prepared by external addition of magnesium stearate [73]. This aspect becomes important when a high dose of poorly wettable drug has to be incorporated in tablet formulation [74]. A decision tree for evaluating the influence of lubricants on drug dissolution during preformulation phase is depicted in Fig. 15.2.

Disintegrants The dissolution of a solid dosage form depends to a large extent on its mode of disintegration. The properties of disintegrant and diluent together

dictate the disintegration as well dissolution of a solid dosage form. Disintegrants acting by swelling mechanism generally increase the dissolution efficiency with an increase in compression pressure [74]. The fragmentation at high compression pressure was suggested to be responsible for increasing dissolution rate of tablets containing starch [75]. Slightly swelling disintegrant (potato starch) containing diazepam tablets were found to exhibit more disintegration time with an increase in mixing time with magnesium stearate. On the other hand, tablets containing strongly swelling disintegrant (sodium starch glycolate) were not significantly affected. Furthermore, *in vitro* dissolution of potato starch containing tablets could be correlated with *in vivo* absorption by employing a high stirring rate [76]. This suggests an overwhelming role of swelling capacity of disintegrant in influencing drug dissolution from dosage forms.

Systems containing high quantity of hydrophobic drug possess an area with pronounced hydrophobicity where wetting acts as the rate-limiting step in dissolution. In such systems, the incorporation of disintegrant does not affect drug dissolution [77]. An increase in hydrophobicity of tablet formulation decreases the efficiency of superdisintegrants [78]. Storage of wet granulated tablets containing superdisintegrants revealed a decrease in dissolution efficiency, with the effect being more pronounced for croscarmellose than for crospovidone or sodium starch glycolate [79]. This is due to slow loss of swelling capacity of disintegrants upon storage of wet granulated tablets.

Incorporation of a large concentration of superdisintegrant reduces the release of poorly soluble drugs due to formation of a viscous layer around the granules during the dissolution process [80]. Lundqvist et al. [81] reported a reduction in disintegration and an increase in friability time when the amount of disintegrant pellets compressed into tablets was increased. However, disintegrants possessing a higher true particle density enhance the disintegration time of pellets. Furthermore, although barium sulfate or calcium carbonate were more effective than magnesium oxide or ferric oxide, use of higher concentration produced an adverse effect due to the large increase in pellet density [81]. Therefore, both the type and concentration of superdisintegrant seem to be critical for obtaining optimum drug release characteristics.

Furthermore, the performance of a disintegrant varies with the pH of the medium. Significant differences in dissolution of acetaminophen from tablets containing sucrose and Primogel or Ac-Di-Sol are attributed to the depressed functioning of Primogel coupled with stronger binding of sucrose in acidic medium than that in neutral medium [82]. Similarly, tablets of terfenadine showed improved disintegration/dissolution with superdisintegrants in the order Crospovidone > Ac-Di-Sol > Primogel > low substituted hydroxypropyl cellulose. Starch decreases dissolution rate by forming a barrier in the form of swollen grains around the particles [83].

It is important to note that the solubility of the major component (diluent) in a tablet formulation can significantly affect disintegration and dissolution. Water-soluble materials tend to dissolve rather than disintegrate, while insoluble materials produce a rapidly disintegrating tablet if an appropriate quantity of disintegrant is included in the formulation [78]. For soluble formulations, the development of disintegration force could be hindered [84]. In addition, the role of soluble excipients like lactose that possibly act passively by hydrogen bond annihilation cannot be ruled out when present in tablet formulations [5]. As the quantity of water

penetrating into a tablet is limited, the soluble excipients can be expected to partially consume the available water, leaving only a part of it for development of disintegration force. Lopez-Solis and Villafuerte-Robles [85] found greater dissolution improvement of norfloxacin when tablets contained a dissolving diluent, Pharmatose DCL 11 and less hygroscopic disintegrant PVP XL 10, as compared to tablets containing more hygroscopic disintegrant like croscarmellose or starch 1500. Both sodium starch glycolate and croscarmellose sodium showed significantly reduced water uptake than crospovidone in 0.1 N HCl. Tablets containing the former disintegrant revealed significant increase in disintegration time for slowly disintegrating (lactose based) tablets as compared to rapidly disintegrating (dicalcium phosphate based) tablets. The dependence of dissolution rate of hydrochlorothiazide on both water uptake and solubility of ingredients in the testing medium suggests the importance of swelling capacity of disintegrants in determining drug release from tablets [86].

Furthermore, the surface area of disintegrated agglomerates during dissolution needs to be critically considered while judging drug dissolution from tablets. Difference in dissolution rate of aspirin from tablets containing different superdisintegrants was attributed to their behavior in promoting disintegration. Video images of the dynamic process indicated Ac-Di-Sol, Primogel, or Polyplasdone XL 10 promote, respectively, rapid disintegration, slow disintegration, and rapid disintegration but into large agglomerates [87].

Therefore, drug release from tablets is intimately connected with the performance of disintegrant present in the formulation. Solubility of diluent, water uptake, swelling behavior, development of disintegration force, and disintegration behavior of disintegrant are critical parameters to be considered while optimizing a tablet dosage form with respect to drug dissolution.

Release Modifiers Polymers are used for modifying the release of drugs from solid dosage forms. They may be employed in matrix form or as a coating for this purpose. Furthermore, the physical form (tablets, granules, beads, pellets, etc.) of the dosage form may vary. Figure 15.3 gives an overview of the decision making process while selecting a release modifier for solid dosage forms. The choice of polymer(s) for modifying drug release depends on many factors, as summarized in Table 15.3. The drug release characteristics from dosage forms become more complex when combinations of release modifiers are used. It would not be possible to discuss all the release modifiers here with respect to their mechanism of drug release. Hence, a few important factors that may influence drug dissolution in the presence of release modifiers are discussed next.

Polymer Characteristics The desired drug release profile governs the choice of release modifier(s). Polymers possessing pH-independent solubility (Carbopol and HPC) provide continuous drug release in acidic as well as alkaline buffer media. Polymers that have pH-dependent solubility (Eudragits) are suitable for achieving drug release at a particular pH or site in the gastrointestinal tract. Polymers that are insoluble at all pH values (ethyl cellulose) need to be combined with other polymers to obtain the desirable drug release profile. Such polymers may be combined with pH-sensitive polymers to obtain drug release at specific pH values [88] or with polysaccharides to obtain drug release in the colon [89].

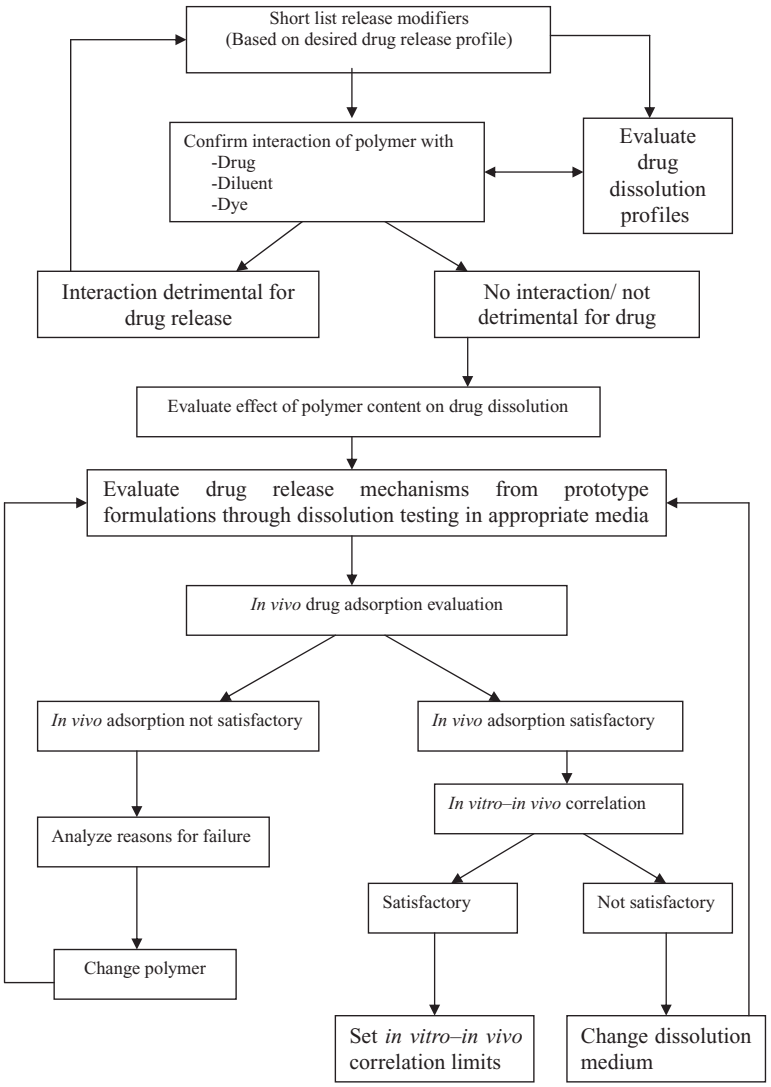


FIGURE 15.3 Decision tree for evaluating the influence of release modifiers on drug dissolution.

Polymer Content The selected polymer or polymer combination is developed into the prototype formulation and evaluated for drug release profile through dissolution testing. Usually, preparation of a series of formulations with different polymer contents exhibiting different dissolution profiles [90] helps in elucidating the drug release mechanism(s). The drug release rate from a tablet formulated using polymer(s) depends on the molar ratio of polymer(s) [91, 92]. Chiao and Price [93] observed that release rate of propranolol hydrochloride from cellulose acetate butyrate microspheres increased with higher drug to polymer ratio and decreased with increasing diameter.

TABLE 15.3 Dissolution as a Tool for Accessing Functionality of Release Modifiers

Purpose (Dosage Form)	Polymer Characteristic Desired	Dissolution Behavior Desired	Correlation with Absorption[a]
Release in stomach	Good solubility in 0.1 N HCl	Drug level detectable immediately. Peak level corresponds to disintegration.	Drug detectable in plasma soon after ingestion. Concentration falls depending on t_{50}
Continuous release in stomach (floating)	Restricted solubility in 0.1 N HCl; swells in acidic medium, thereby delaying drug release.	Drug released with little delay; concentration rises continuously.	Drug detectable with little delay and with continuous rise for 3–5 h (fed state trial essential)
Sustained release	pH-independent solubility. Continuous release over extended time period irrespective of pH.	Drug released slowly with delay. Release continues at constant rate in pH-progression media.	Drug detectable in blood with little delay. Constant concentration maintained over prolonged period.
Colon release	Not soluble in both acidic and alkaline pH; degradation due to bacteroids present in cecal contents.	No/little (<10%) drug released on sequential exposure to 0.1 N HCl (2 h) and pH 7.5 (3 h). Total release in pH 6.8 containing cecal contents.	No drug detectable until 2 h after oral ingestion; abrupt rise in concentration after 5 h. (Assurance of no effect of pH on drug release essential.)
Site-specific release	Soluble at pH of the site.	Drug released only after exposure to particular pH.	No drug detected until dosage form reaches the desired site.
Time release	Slow erosion over the desired time interval.	Very little drug released over a particular time interval; abrupt release after that.	Negligible drug in blood until a few hours after ingestion; abrupt increase thereafter. (Assurance of no effect of pH, gastric emptying, and dietary factors essential.)

[a]Assuming high solubility, high gastrointestinal permeability, and passive absorption.

The absolute amount of polymers also influences drug release. Extended release tablets of cephalexin showed reduced release in the presence of higher amounts of HPMC [94]. Release of ascorbic acid from tablets revealed that t_{50} and t_{90} was delayed from 1 h to 1.4 h and from 4.55 h to 7.2 h, respectively, when the total polymer content was increased to 60 g/500 g in comparison to 45 g/500 g. Similar

results were obtained when Kollicoat SR 30D was used in formulating ibuprofen granules [95]. However, slight modification in HPMC concentration does not significantly affect drug release rate [96].

Polymer–Drug Interaction Various interactions taking place between the drugs and polymers may lead to change in drug release pattern, where drug solubility in external medium as well as its diffusion capacity within the polymer network play an important role. The ability of Eudragit polymers to adsorb acidic drugs from a solution at pH 7.4 is well known. The ionized acid molecules carry a negative charge to ammonium groups linked to polymer backbone. This allows electrostatic binding to take place [97, 98]. This phenomenon occurs when the pH of the dissolution medium is higher than the pK_a of the drug [97]. In the case of tris-HCl buffer (pH 7.4), chloride ions compete with drug molecule anions for active sites in the polymer backbone, thus resulting in a lower adsorption rate of drugs [99].

Many acids have been used to prepare chitosan based controlled release drug delivery systems. Such conditions may not be desirable for some drugs, especially for an acid labile drug [100]. It was further confirmed by Nunthanid et al. [101] that the drug release behavior was sustained from high molecular weight chitosan acetate films. In addition, they observed sustained release of theophylline from tablets prepared with spray dried chitosan and as its amount increased, the drug release occurred at a much slower rate [101].

Polymer–Diluent Interaction Like other factors, polymer–diluent interaction may also affect drug release. Microcrystalline cellulose allowed rapid penetration of aqueous medium, resulting in rapid erosion of polymer matrices. Lactose played an important role in imparting a physical barrier affecting diclofenac release kinetics by reducing tortuosity of diffusion pathway [102]. Anionic dyes like erythrosine, panceau 4 R, sunset yellow, and tartrazine are immiscible with chitosan citrate. But brilliant blue and green FS at concentrations of 0.02–1.0% w/w are miscible with chitosan. This could be due to reduced interaction because of the presence of positive charge on these dye molecules. Drug dissolution with these coated tablets was pH dependent and also corresponded to the ability of chitosan to protonate in the medium. Faster drug dissolution occurs from coated tablets colored with brilliant blue than from those colored with green FS. This is due to the fact that brilliant blue exhibits more water solubility due to the presence of more SO_3H groups [103]. In another study, the miscibility of dye and chitosan was found to depend on the molecular configuration and ionic group of dye molecule. Drug dissolution from tablets coated with green FS is slightly slower than that from tablets coated with plain films and core tablet, respectively [104].

Polymer–Excipient Interaction Drug–excipient, excipient–excipient, and polymer–excipient interactions are of major preformulation consideration in the pharmaceutical industry and usually they can be predicted at early stages of product development. Interactions in the liquid state are more complicated than in the solid state, as sometimes predictions can be complicated by the dispersion medium, change in pH of the medium, ionic strength, a difference in the solubility parameter of the drug, or temperature fluctuations [98]. It is reported that PEG 6000 and Eudragit L-30 D have similar solubility parameters. This increases their affinity

toward each other [105]. Chances of interaction of Eudragit L-30D with PEG 6000 increase in an acidic medium due to hydrogen bonding between the ether oxygen of the oxyethylene groups of PEG 6000, resulting in faster dissolution [98]. PEG usually interacts with molecules that have hydrogen bonding functions as polymethacrylic acid polymers [105, 106].

Physicochemical Characteristics of Drug The physicochemical properties of pharmaceuticals, including solubility and dissolution rate, can be influenced by the degree of crystallinity, solvation rate, crystal form, particle size/surface area, and so on. The solubility of a drug is influenced by many factors, which in turn affect the dissolution behavior. The rate of dissolution assumes great importance if it is lower than the rate of diffusion to the site of absorption or the rate of absorption of the drug. In such cases, there is a need for manipulating the drug characteristics in order to achieve the required dissolution profile.

Decreasing the particle size is the most common approach for increasing the solubility of a drug. However, the role of particle size should be considered for drugs exhibiting solubility of 0.1 mg/mL or less. This is because the chances of toxic effects associated with faster dissolution of a drug often increase with an increase in the drug solubility [107]. It is important to note that decreasing the particle size of a hydrophobic drug, phenytoin, actually decreases the proportion of particles in contact with dissolution medium. Furthermore, the small particles have air adsorbed on their surface due to which they float on the surface of the dissolution medium, thereby exhibiting lower dissolution [108]. The stability of different salts/coprecipitates in the gastro intestinal fluids needs to be critically evaluated. The most soluble sodium salt of penicillin is the least bioavailable form as it is most unstable in the digestive fluids [109].

Deaggregation of the drug particles is necessary to aid faster dissolution as evidenced by significantly different plasma concentrations obtained after administration of different brands of chloramphenicol capsules [108].

The availability of the weakly acidic drug para amino-salicylic acid is only 77% of that of its salt forms. A correlation in the rank order of availability of its potassium, calcium, or sodium salt forms indicated the pivotal role of their respective drug solubility in influencing the absorption of para amino-salicylic acid [108].

The beneficial effect of modifying the nonionized drugs into salt forms was reported for tetracycline and tolbutamide. The dissolution rate of weakly basic drug (tetracycline) decreases, whereas for the weakly acidic drug (tolbutamide) it increases as the pH of the dissolution medium is increased. However, the dissolution of salt forms of aluminum acetyl salicylate, sodium warfarin, or pamoate salt of benzamphetamine was found to be lower than that of their nonionized forms. This was suggested to be due to precipitation of an insoluble particle or film on the surface of the tablet rather than in bulk solution, which decreased the effective surface area by preventing deaggregation of the particles. Furthermore, the properties of the inactive ingredients present in the dosage form may also be influenced by the pH of the dissolution medium and may exert profound influence on dissolution characteristics of drugs (phenytoin, nitrofurantoin) [108]. Table 15.4 lists a few drugs whose dissolution is known to be influenced by physicochemical properties. In view of the above, it seems essential to compare the solubility and dissolution profile of the active ingredient/salt form with that of the dosage form in an

TABLE 15.4 Influence of Physicochemical Factors on Dissolution Rate of Drugs

Drug	Characteristic Feature Influencing Dissolution	Remarks	References
Folic acid	Particle size	Formulations made from 9.0 μm particles release higher amount at 60 min as compared to formulations containing 24.4 μm sizes. Difference not statistically significant.	110
Griseofulvin	Particle size	Unmodified griseofulvin is required to dissolve NLT 70% and micronized griseofulvin is required to dissolve NLT 85% of stated amount in presence of 4% or 0.54% w/v SLS.	111
Nitrofurantoin	Particle size	Particles smaller than 10 μm are more rapidly and completely absorbed from tablet dosage form than macro-crystalline form (74–177 μm) from capsule.	108
Phenacetin	Particle size	Dissolution rate increased with increasing size. Addition of Tween-80 to dissolution medium increases the dissolution rate as particle size is decreased.	108
Para amino-salicylic acid (PAS)	Salt form	Solubility of nonionized PAS = 1 g/600 mL Potassium PAS = 1 g/10 mL Calcium PAS = 1 g/7 mL Sodium PAS = 1 g/2 mL Salt forms are more rapidly available than nonionized drug.	108
Tolbutamide	pH effect	As pH increases, dissolution rate increases.	108
Tetracycline	pH effect	As pH increases, dissolution rate decreases.	108
Ampicillin, glutethemide, theophylline	Anhydrous forms (solvate form)	At room temperature anhydrous forms exhibit higher dissolution rate than their corresponding hydrates due to difference in free energy of hydration.	112
Azetazolamide, carbamazepine, indomethacin	Metastable forms (phase transformations)	Because of the higher free energy they dissolve more rapidly than their corresponding stable forms.	113
Diclofenac sodium	Solid-solid transition	Anhydrous form dissolves faster than hydrated form. Storage under uncontrolled environment conditions (RH ≤ 60%, 25 °C) transforms the anhydrous form to hydrous form; adverse effect is anticipated during dosage form manufacturing too.	114
Chloramphenicol palmitate (polymorphic forms: A and B)	Polymorphism	As percentage of polymorph B increases, extent and rate of availability also increase due to increase in rate of dissolution. But since polymorph B is less stable, the less soluble polymorphic form A is used in formulations.	108
Diclofenac	Complexation (with cyclodextrin)	Time for dissolving 50% of drug is decreased. Dissolution efficiency of complex is 40.31 as compared to 1.21 of drug alone.	115
Triamterene	Complexation (with cyclodextrin)	95% of drug dissolves in 2 h, whereas complexed drug dissolves within 10 min.	116

appropriate medium in order to discern the effect of factors other than the inherent characteristics of the drug molecule.

15.2.2 Process Variables

Granulation Procedure

Wet/Dry Granulation The type of granulation process can influence the dissolution rate of tablets [117]. A wet granulation process imparts hydrophilic properties to granules and hence is known to improve the dissolution rate of poorly soluble drugs [118]. Tablets of cephalexin formulated by a dry granulation process exhibited slower dissolution than those formulated by using a wet granulation technique. This was attributed to slow penetration of dissolution medium into tablets prepared by dry granulation [94]. On the other hand, dissolution of naproxen sodium tablets was fastest when they were manufactured by a direct compression method. The exothermic reaction during wet granulation produced the hydrate form of naproxen, which dissolved at a slower rate [119].

HPMC swells in contact with dissolution medium [120, 121] and forms a thick gel, which in turn may decrease the pore size and hence reduce drug release. However, in the presence of PVP (highly water-soluble additive) tablets prepared by wet granulation may undergo faster dissolution due to contact of the dissolution medium inside the swollen HPMC [94]. The dissolution rate of ticlopidine hydrochloride was improved by incorporating cornstarch in wet granulated tablets as compared to dry granulation. This could be attributed to better bonding and better homogeneity of starch disintegrant within the tablets [122]. Phenylpropanolamine tablets prepared with different granulation techniques resulted in different dissolution profiles. Partial melt and melt granulated tablets resulted in slower drug release than that obtained after dry blending or wet grinding. This was perhaps due to formation of different matrix structures from different methods. Furthermore, insufficient heat generated during compression does not seem enough to melt the wax and does not form internal structure similar to that produced in melt granulated and partial melt granulated tablets [123]. Faster dissolution of phenobarbital sodium was obtained from tablets prepared by compression with Avicel than that from tablets prepared by wet granulation with gelatin [124]. Tablets prepared by wet granulation using ethyl cellulose and lactose, acacia mucilage and lactose, or starch paste and lactose released the drug in the order: acacia mucilage and lactose = starch paste and lactose > acacia mucilage and lactose [117]. Therefore, the type of additive seems to play an important role in influencing drug dissolution besides the method of granulation.

Granulating Solvent Ghorab and Adeyeye [125] granulated ibuprofen with β-cyclodextrin using water, ethanol (95%), and isopropanol as the granulating solvents. Isopropanol and water, as granulating solvents, enhanced the dissolution of the oven-dried batches more than ethanol [125].

Drying Process A significant effect on particle size as well as on release rate of ibuprofen from microspheres was observed when the cooling time from 65 °C to room temperature was varied between 2 min and 100 min. The drug release was

maximum when drying was rapid. Granules prepared by a faster cooling process seemed to be more porous and had a less smooth surface [126].

Granule Size Granule size is not of considerable importance while studying dissolution rate of a drug, especially when the granules are softer [127, 128]. The effects of granule size and concentration of magnesium stearate as lubricant on the dissolution rate of paracetamol tablets were studied. The results obtained show that dissolution rate was increased as the granule size of the tablet was increased for tablets prepared with 1.5% magnesium stearate as lubricant. However, tablets prepared with different granule size exhibited no pronounced effect on the dissolution characteristics when the concentration of magnesium stearate used was 0.75% [129].

Mixing Some physical interactions between drug and excipient can take place during the dry blending phase of powders/granules. Blending with lubricants/glidants/antiadherents results in their absorption on particle surfaces, thereby increasing the hydrophobicity of drug particles. This decreases drug dissolution [2]. The dissolution rate of prednisolone was found to depend on the length of time for which ingredients were mixed with magnesium stearate [130].

Compression Force The relationships between compression force and dissolution can be of four types:

1. Dissolution rate is increased as compression force is increased (e.g., use of starch paste as granulating agent).
2. Dissolution is slowed as the compressional force is increased (e.g., use of methylcellulose as granulating agent).
3. Dissolution rate is faster as compression force is increased to a maximum value. After this, the dissolution rate decreases with increase in compression force (e.g., use of gelatin solution as granulating agent).
4. Dissolution rate is slowed down as compression force is increased. However, further increase in compression pressure leads to an increase in dissolution rate [131].

Generally, an increase in compression force should decrease the bonding of particles because of crushing or cleavage of particles that in turn may increase the dissolution rate. Investigations on spironolactone tablets employed a granulated mixture of calcium carbonate and starch. The effect of manufacturing variables like compression force, crushing strength, or friability was studied by using tablets having the same composition but prepared under different conditions. The tablets compressed from each mixture exhibited a significant relationship between compression force and crushing strength or friability. Tablet strength increased linearly with increase in applied pressure. However, tablets prepared from large granulates showed an increase in disintegration time with increase in compression force, whereas those prepared from smaller granulates exhibited no dependence on compression force. In addition, disintegration times were shorter for the latter type of tablets. Furthermore, tablets prepared from smaller granulates required less time to dissolve [132].

Compression alters the size of drug particles, which in turn can affect the dissolution rate [133]. In a study conducted by Ganderton et al. [134], the dissolution rate of prednisolone tablets was examined at different compressional pressures. The dissolution rate of prednisolone from tablets prepared at low pressure was less compared to tablets manufactured at high pressures. With further increase in pressure, depressed dissolution rates were observed. This could be attributed to reduced breakage of tablets compressed at low pressure and release of stress along with loss of small air bubbles leading to disruption in tablets compressed at very high pressure. However, rebonding of material in tablets compressed at very high pressure produced stronger and denser tablets, which were not readily penetrated by dissolution medium [134]. A difference in pellet density of 19% was observed after compression at different pressures. The dissolution rate for the lot with lowest density made at lowest compression was significantly greater than other lots. This could be due to "channeling" that occurs within the pellet, thereby increasing the effective dissolving surface [135]. A study conducted by Chiao and Price [93] revealed faster release of propranolol hydrochloride from compressed tablets than from uncompressed microspheres. This may be attributed to rupture of microspheres at higher compression pressure. A compression force $<200\,kg/cm^2$ to the inner core tablet influenced the release behavior of drug but a force $>200\,kg/cm^2$ delayed the lag time [136]. Hence, it could be concluded that the effect of compression pressure is dependent on the pressure range evaluated and on the properties of drug, filler, binder, and so on. Therefore, it is rather difficult to predict the effect of compression pressure on the dissolution rate. Nevertheless, compression does influence drug release and this could be evaluated in relation to the properties of other formulation components.

15.3 DISSOLUTION TEST DESIGNING

Drug dissolution testing is a fundamental part of drug product development and manufacturing and is also employed as a quality control tool to monitor batch-to-batch consistency of drug release from a product. The dissolution test has extensively been used as a test to reflect the bioavailability of a drug product in humans. The advancements made for obtaining better correlation between dissolution and systemic availability include use of different media, evaluation of entire drug release profile, use of dialysis membrane, and flow through cell.

Drug classification based on permeability as well as solubility is gaining recognition and may be extremely useful during the early stages of dissolution methodology development. Changing the dissolution medium allows for testing the dosage form under conditions similar to the gastrointestinal tract. For a commercial product, dissolution testing is primarily used (1) to confirm manufacturing and product consistency, (2) to evaluate product quality during its shelf life, (3) to assess post-approval changes, and (4) to minimize the need for bioequivalency studies [137].

While developing a dissolution test for poorly soluble compounds during the early drug development phase, the process should focus on assessing relevant physical and chemical properties and dosage form design, as these play a major role in selecting the dissolution medium and apparatus. However, the strategy for designing dissolution tests depends on availability of additional data as well.

15.3.1 Media Selection

The choice of medium depends on the purpose of dissolution test. For batch-to-batch quality testing, selection of dissolution medium is based partially on the solubility data and the dose range of drug in the product to ensure that sink conditions may be justifiable, if it is shown to be more discriminating or if it provides reliable data, which otherwise can only be obtained with the addition of surfactants. On the other hand, when a dissolution test is used for evaluating the biopharmaceutical properties of a dosage form, it is more important that the proposed biorelevant test closely simulates the gastrointestinal environment rather than necessarily producing sink conditions [138].

The dissolution characteristics of oral conventional formulations should first be evaluated using test media within the physiological pH range (pH 1.2–6.8) and between pH 1.2 and 7.5 for modified release formulations. During method development, it may be useful to measure the pH of a test medium before and after the run to observe whether the pH changes during the test. An aqueous medium without surfactants is preferred, but an aqueous medium with surfactants may be used either to increase the probability of establishing an *in vivo* relationship or to increase the drug solubility in case of low solubility compounds [139, 140]. Table 15.5 highlights this aspect because different pharmacopoeias utilize different surfactants for testing drug release. The need for surfactants as well as their type and concentration need to be justified [143, 144]. The amount of surfactant needed depends on the critical micelle concentration of surfactant and the degree to which the compound partitions into the surfactant micelles. Furthermore, the critical micelle concentration of

TABLE 15.5 Use of Different Surfactants at Various Concentrations During Dissolution Testing of Tablets [111, 141, 142]

Surfactant	Concentration	Drug Product (Tablet)	Official in
Sodium lauryl sulfate	0.5% w/v	Medroxypyrogesterone acetate	USP
	1% w/v	Megestrol	USP
	0.09% w/v	Norethindone and ethinyl estradiol	USP
	0.09% w/v	Norethindone and mestranol	USP
	0.02% w/v	Norethindone acetate	USP
	0.05% w/v	Penicillamine	USP
	0.2% w/v	Praziquantel	USP
	0.1% w/v	Spironolactone	USP
	0.1% w/v	Spironolactone and hydrochlorothiazide	USP
	4% w/v	Griseofulvin	USP, IP
	1% w/v	Carbamazepine	USP
	0.3% w/v	Estradiol	USP
	0.54% w/v	Ultramicrosize griseofulvin	USP
Polysorbate 80	5 ppm in water	Levonorgesterol and ethinyl estradiol	USP
Polysorbate 20	1% v/v	Lacidipine	BP
Sodium dodecyl sulfate	0.07% w/v	Cyproterone	BP
	1.5% w/v	Griseofulvin	BP
	0.1% w/v	Spironolactone	BP
	0.3% w/v	Nimodipine	BP

surfactant depends on the characteristics of the surfactants and the ionic strength of the dissolution medium.

Development of medium for colon-specific drug delivery systems is a great challenge. This is due to the diversity in rationale behind developing a delivery system. In addition, inadequate understanding of the colon's hydrodynamics and motility complicates the development of *in vitro* evaluation. USP dissolution methods using multiple-pH media are employed for such products [145].

Simple aqueous media such as simulated gastric fluid without pepsin (SGFsp) can be satisfactorily used for BCS-I (high solubility, high permeability) drugs. Milk as a biorelevant medium may be useful for detecting special food-formulation interactions. However, for BCS-II drugs (low solubility, high permeability), the choice of dissolution medium shall depend on the ionization behavior of the drug. For neutral BCS-II drugs, the presence of solubility enhancers seems to be necessary. However, for weakly basic BCS-II compounds, whose dissolution in stomach is of prime importance, the use of SGFsp medium seems to be important while assessing the initial dissolution. Furthermore, comparison of dissolution behavior in fluids that simulate conditions in the proximal small intestine in the fed state and also in fluids that simulates condition in the proximal small intestine in the fasted state is needed to establish the effect of meals on absorption of these drugs [146].

15.3.2 Apparatus Selection

Physical and chemical properties of a drug as well as formulation play an important role in selection of dissolution test apparatus, especially in the case of poorly soluble compounds. Sometimes, the existing dissolution apparatus may need modifications to accommodate new release mechanisms; for example, in the case of a nondisintegrating dosage form, there is a requirement of delivery orifice. On the other hand, a disintegrating delivery system faces problems as there is the requirement of transferring the dosage form (without losing any portion) to a different medium. In the case of modified release delivery systems, the challenging task is to change the medium to obtain a pH gradient or simulation of fed and fasted conditions. Both USP apparatus III (reciprocating cylinder) and apparatus IV (flow through cell) are useful when the drug release from the dosage form is to be tested in different media [111, 147, 148]. However, it is important to note that both type and purpose of using a particular dissolution apparatus are often different in USP, BP, and IP (Table 15.6). Despite these differences, each pharmacopoeia aims to closely mimic the *in vivo* conditions. Still, endeavors are needed to achieve similarity in dissolution testing apparatus for various dosage forms among different pharmacopoeias.

In addition, modifications in existing apparatus can be done depending on its applicability to the type of dosage forms. Burns et al. [149] found the modified paddle (BP type II paddle) method best for studying the dissolution release profile of floating propanolol capsules. In this apparatus paddle blades were set at the surface of the dissolution medium [149].

The surface area of a pure drug may be held constant in order to evaluate the dissolution rate. Agitation intensity is another alternative, where alteration of stationary film thickness surrounding the particles is reflected in a change in dissolution rate constant and should be carefully controlled.

TABLE 15.6 Critical Differences in Dissolution Rate Test Apparatus Used in Various Pharmacopoeias [111, 141, 142]

Dissolution Apparatus	Used for Dosage Form			Critical Differences		
	USP	BP	IP	USP	BP	IP
1	Coated tablet Uncoated tablet Delayed release Extended release	Conventional	Conventional Coated Uncoated	Basket	Basket	Paddle
2	Conventional Enteric coated Extended release	Conventional Extended release Delayed release	Conventional Coated Uncoated	Paddle	Paddle	Basket
3	Extended release Modified release dosage form Bead type	Modified release dosage form	(Not official)	Reciprocating cylinder	Flow through cell apparatus	(Not official)
4	Modified release (especially when active ingredient has limited solubility/soft gelatin, bead products, suppositories/ poorly soluble drugs)	(Not official)	(Not official)	Flow through cell apparatus	(Not official)	(Not official)
5	Transdermal	(Not official)	(Not official)	Paddle over disk	(Not official)	(Not official)
6	Transdermal	(Not official)	(Not official)	Cylinder	(Not official)	(Not official)
7	Non disintegrating modified release dosage form Transdermal	(Not official)	(Not official)	Reciprocating holder	(Not official)	(Not official)

15.3.3 Dissolution Apparatus Operating Parameters

The operating parameters of dissolution testing apparatus also need to be evaluated critically before finalizing the dissolution conditions for a drug product. These parameters include medium volume, temperature, aeration/deaeration, sinker evaluation, sampling time point specification (single point or multiple point), and agitation speed.

Volume of Dissolution Medium Table 15.7 lists pharmacopoeial dissolution testing requirements of a few drugs representing the biopharmaceutical classification scheme (Classes I–IV).

It is evident from this table that the recommended volume of dissolution medium varies from 500 to 1000 mL. A volume of 900 mL is the most common volume, while using apparatus 1 and apparatus 2 according to USP, BP, and IP specifications. The volume can be increased to 2–4 L in special cases (e.g., in the case of low solubility compounds) and then the standard vessels are rejected and large vessels are used for conducting dissolution testing. The volume can be decreased to 100–250 mL in the case of highly potent and low dose dosage forms [150, 151]. Hence, in both cases, proper justification is required for increasing/decreasing the volume of the testing medium.

Temperature of Dissolution Medium The standard temperature conditions for dissolution testing of conventional oral (to be swallowed) dosage forms is $37 \pm 0.5\,^{\circ}C$ [111, 141, 142]. Drug solubility is temperature dependent. Hence, control of temperature during the dissolution testing is very crucial. However, for products that are intended to be dissolved/dispersed in water and then administered orally, a temperature of $17.5 \pm 2.5\,^{\circ}C$ needs to be employed [111]. A comparison of dissolution profiles of HALO™ at $36.5\,^{\circ}C$ and $37\,^{\circ}C$ indicated a considerable decrease in release (54% after 60 min at $36.5\,^{\circ}C$, 63% after 60 min at $37\,^{\circ}C$). Similarly, release of propranolol was observed to be 73% at $37 \pm 0.2\,^{\circ}C$ as compared to 84% at $37 \pm 0.5\,^{\circ}C$ after 300 min. Therefore, proper control of temperature during dissolution testing seems to be important [149].

Aeration/Deaeration The dissolved gas/air in the dissolution medium can influence dissolution of drug particles. Dissolved air can lead to a change in the pH of the dissolution medium. Furthermore, air bubbles may act as a barrier for dissolution if they are present on the dosage form or in the mesh of the basket. In addition, aeration can retard dissolution as it can push the particles away from the center and toward the walls of the dissolution vessel [152, 153].

Sinker Selection USP recommends some nonreactive material wire when the dosage forms tend to float [111]. The sinker should be evaluated for its ability to maintain the dosage form at the bottom of the vessel without inhibiting drug release. Paddle apparatus tablet sinkers were used by Cappola to prevent tablets from sticking to the bottom of the dissolution vessel. Overall, dissolution for all tablets with sinkers showed more rapid and complete drug release than tablets without sinkers [154].

TABLE 15.7 Pharmacopoeial Dissolution Requirements of a Few Drug Products Belonging to the Biopharmaceutical Classification Scheme (Classes I–IV)

Drug product	Pharmacopoeial Requirements		
	USP	BP	IP
	BCS Class I		
Aspirin (acetyl salicylic acid)	0.05 M acetate buffer (pH 4.5), 500 mL, apparatus 1 (50 rpm), NLT 80% of Q[a] dissolved in 30 min.	Acetate buffer (pH 4.5), apparatus 1 (50 rpm), NLT 70% of Q dissolved in 45 min.	Not recommended.
Aspirin tablet (delayed release)	Apparatus 1 (100 rpm), 90 min (buffer stage).	0.1 N HCl, 1000 mL, apparatus 1 (100 rpm), NLT 5% of Q in 2 h; mixed phosphate buffer (pH 6.8), 900 mL, NLT 70% of Q in 45 min.	Not recommended.
Digoxin tablet	0.1 N HCl, 500 mL, apparatus 1 (120 rpm), NLT 805 of Q dissolved in 60 min (average of 12 tablets has LT 75% of Q in 60 min). If amount dissolved in 60 min is MT 95% for any individual tablet, amount dissolved in 15 min is NMT 90% for each tablet.	Water, 600 mL, 120 rpm, basket apparatus, NLT 75% of Q is dissolved in 60 min.	Water, 600 mL, 120 rpm, basket apparatus, NLT 75% of Q is dissolved in 60 min.
Prednisolone tablet	Water, 900 mL, apparatus 2 (50 rpm), NLT 70% of Q is dissolved in 30 min.	Water, 900 mL, apparatus 2 (50 rpm), NLT 70% of Q is dissolved in 45 min.	Water, 900 mL, paddle (50 rpm), NLT 70% of Q is dissolved in 30 min.
Quinine SO_4 tablet	0.1 N HCl, 900 mL, apparatus 1 (100 rpm), NLT 85% of Q is dissolved in 30 min.	0.1 N HCl, 900 mL, basket apparatus (100 rpm), NLT 70% of Q is dissolved in 45 min.	0.1 N HCl, 900 mL, apparatus 2 (100 rpm), NLT 70% of Q is dissolved in 30 min.
Quinine SO_4 ER tablet	0.1 N HCl (900 mL), apparatus 1 (100 rpm) 20–50% dissolved in 0.125 D h; 43–73% dissolved in 0.5 D hNLT 70% dissolved in 1.5 D h	Not recommended.	Not recommended.
Quinine $BiSO_4$ ER tablet	Not recommended.	0.1 N HCl (900 mL), basket (100 rpm), NLT 70% of Q is dissolved in 45 min.	Not recommended.
Warfarin (sodium) tablet	Water, 900 mL, apparatus 2 (50 rpm), NLT 80% of Q is dissolved in 30 min.	0.68% w/v potassium dihydrogen orthophosphate (pH 6.8), 900 mL, basket (100 rpm), NLT 70% of Q is dissolved in 45 min.	0.68% w/v potassium dihydrogen orthophosphate (pH 6.8), 900 mL, paddle (100 rpm), NLT 70% of Q is dissolved in specific time.

TABLE 15.7 ***Continued***

Drug product	Pharmacopoeial Requirements		
	USP	BP	IP
Levodopa tablet	0.1 N HCl, 900 mL, apparatus 1 (100 rpm), NLT 75% of Q is dissolved in 30 min.	0.1 N HCl, 900 mL, apparatus 1 (100 rpm), NLT 70% of Q is dissolved in 45 min.	0.1 N HCl, 900 mL, apparatus 2 (75 rpm), NLT 75% of Q is dissolved in 30 min.
Lithium carbonate tablet	Water, 900 mL, apparatus 1 (100 rpm), NLT 60% of Q is dissolved in 30 min.	Not recommended.	Water, 900 mL, paddle (100 rpm), NLT 60% of Q is dissolved in 30 min.
Paracetamol tablet	Not recommended.	Phosphate buffer (pH 5.8), 900 mL, apparatus 2 (50 rpm), NLT 70% of Q is dissolved in 45 min.	Phosphate buffer (pH 7.6), 900 mL, paddle (50 rpm), NLT 80% of Q is dissolved in 30 min.
Diazepam tablet	0.1 N HCl, 900 mL, apparatus 1 (100 rpm), NLT 85% of Q is dissolved in 30 min.	0.1 N HCl (900 ml), apparatus 1 (100 rpm), NLT 70% of Q is dissolved in 45 min.	Water (900 mL), paddle apparatus (100 rpm), NLT 85% of Q is dissolved in 45 min.
Theophylline tablet	Water, 900 mL, apparatus 2 (50 rpm), NLT 80% of Q is dissolved in 45 min.	Not recommended.	Not recommended.
		BCS Class II	
Griseofulvin tablet	Water, 4% sodium lauryl sulfate (SLS), 1000 mL, apparatus 2 (100 rpm), NLT 70% of Q is dissolved in 60 min.	Water, 1.5% sodium dodecyl sulfate, 1000 mL, apparatus 2 (100 rpm), NLT 70% of Q is dissolved in 45 min.	Water, 4% SLS, 900 mL, paddle apparatus (100 rpm), NLT 70% of Q is dissolved in 60 min.
Dapsone tablet	Dilute HCl (2 in 100), 1000 mL, apparatus 1 (100 rpm), NLT 70% of Q is dissolved in 60 min.	0.1 M HCl, 900 mL, apparatus 1 (100 rpm), NLT 70% of Q is dissolved in 45 min.	2% v/v HCl, 900 mL, paddle apparatus (100 rpm), NLT 70% of Q is dissolved in 60 min.
Ibuprofen	Phosphate buffer (pH 7.2), 900 mL, apparatus 1 (150 rpm), NLT 75% of Q is dissolved in 30 min.	Not recommended.	Phosphate buffer (pH 7.2), 900 mL, paddle apparatus (100 rpm), NLT 50% of Q is dissolved in 30 min.
Naproxen tablet	Phosphate buffer (pH 7.4), 900 mL, apparatus 2 (50 rpm), NLT 80% of Q is dissolved in 45 min.	Phosphate buffer (pH 7.4), 900 mL, apparatus 2 (50 rpm), NLT 70% of Q is dissolved in 45 min.	Not recommended.
Naproxen enteric coated	Not recommended.	0.1 N HCl, 900 mL, apparatus 2 (50 rpm), 2 h in acid stage, NLT 5% of Q in acid stage. Buffer stage: 900 mL, 45 min, NLT 70% of Q in buffer stage.	Not recommended.

Trimethoprim	0.01 N HCl, 900 mL, apparatus 2 (50 rpm), NLT 75% of Q is dissolved in 45 min.	Not recommended.	Not recommended.
		BCS Class III	
Acyclovir tablet	Not recommended	0.1 M HCl, 900 ml, apparatus 2 (50 rpm), NLT 70% of Q is dissolved in 45 min.	Not recommended.
Chlorpromazine HCl tablet	0.1 N HCl, 900 mL, apparatus 1 (50 rpm), NLT 80% of Q is dissolved in 30 min.	0.1 M HCl, 900 mL, apparatus 2 (50 rpm), NLT 70% of Q is dissolved in 45 min.	Not recommended.
Rythromycin stearate tablet	Phosphate buffer 0.05 M (pH 6.8), 900 mL, apparatus 2 (100 rpm), NLT 75% of Q is dissolved in 120 min.	2.722% Sodium acetate (pH 5.0), 900 mL, apparatus 2 (50 rpm), NLT 70% of Q is dissolved in 45 min.	Not recommended.
Hydrochlorothiazide tablet	0.1 N HCl, 900 mL, apparatus 1 (100 rpm), NLT 60% of Q is dissolved in 60 min.	Not recommended.	0.1 M HCl, 900 mL, paddle apparatus (100 rpm), NLT 60% of Q is dissolved in 45 min.
		BCS Class IV	
Acetazolamide tablet	0.1 N HCl, 900 mL, apparatus 1 (100 rpm), NLT 75% of Q is dissolved in 60 min.	Not recommended.	Not recommended.
Cefixime tablet	Potassium phosphate buffer 0.05 M (pH 7.2) 900 mL, apparatus 1 (100 rpm), NLT 75% of Q is dissolved in 45 min.	Not recommended.	Not recommended.
Furosemide	Phosphate buffer (pH 5.8), 900 mL, apparatus 2 (50 rpm), NLT 80% of Q is dissolved in 60 min.	Phosphate buffer (pH 5.8), 900 mL, apparatus 2 (50 rpm), NLT 80% of Q is dissolved in 45 min.	Not recommended.
Hydralazine tablet	0.1 N HCl, 900 ml, apparatus 1 (100 rpm), NLT 60 % of Q is dissolved in 30 min.	Not recommended.	Not recommended.
Sulfasalazine tablet	Phosphate buffer (pH 7.5), 900 mL, apparatus 1 (100 rpm), NLT 85% of Q is dissolved in 60 min.	Not recommended.	Not recommended.

[a]Q, stated/labeled amount.

Sampling Time Point Specification Sampling at early time points is used to show that there is little/no probability of dose dumping/accumulation. Drug release during the initial period should not be more than the specified amount. An intermediate time point is chosen to evaluate the *in vitro* drug release profile. Lastly, the final time point is used to explain almost complete release of drug from the respective dosage form. In the case of products with more than one active ingredient, drug release is determined at different time intervals for different active ingredients of the drug product [152]. Single point sampling during dissolution does not yield an estimate of the dissolution pattern of drug from a dosage form. Table 15.8 summarizes a few important drug products that are required to undergo single/multiple time point sampling. It is evident that multiple sampling assumes more importance while testing a modified release dosage form because it becomes essential to evaluate the entire dissolution profile rather than only the extent of drug dissolved.

Rotational Speed Variation in the amount of drug dissolved can be due to variation of the hydrodynamics in the vicinity of a tablet. Fluctuations in flow can affect shearing of the tablet surface, deagglomeration of particles, mass transfer from solid to liquid, and mixing of tablet fragments and thus eventually influencing the hydrodynamics. The literature reveals that a change in rotational speed can alter the dissolution rate [28, 155]. Kukura et al. [156] showed that increasing the stirring from 50 to 100 rpm increased the intensity of shear force exerted by the fluid but did not improve the homogeneity of the spatial distribution of shear. Dissolution testing of carbamazepine tablets using the paddle method in hydroalcoholic medium was conducted at 100 rpm and 75 rpm. The results showed a decrease in the amount of carbamazepine dissolved at lower rotation speed [157]. An increase in rate and extent of ranitidine dissolved was observed along with less individual tablet variability when the basket method at 30 rpm was used instead of the paddle method at 500 rpm [154].

Altered Dissolution Environment To study the impact of slight variations in vessel dimensions, a metal strip was placed to simulate altered dissolution environment. During testing, the metal strip is expected to force the product/product-deaggregates to remain off center, resulting in a large surface area for dissolution. In addition, the contact of dissolution fluid with product should also increase as the distance from the center increases. However, results with or without a metal strip were observed to be dependent, which could be due to product sensitivity to the density of disintegrated particles or gradual change in available surface of the dissolution vessel. Nondisintegrating tablets showed higher dissolution than disintegrating tablets with a metal strip in place due to production and settling of denser particles at the bottom [158]. Hence, slight modifications in the dimensions/shape of dissolution vessels do not seem to exert a remarkable influence on drug release rate provided there is no appreciable change in the hydrodynamics inside the vessel.

Viscosity of Medium Dissolution rate decreases with an increase in the viscosity of the dissolution medium especially when the dissolution process is diffusion controlled [159].

TABLE 15.8 A Few Examples of Single Point and Multiple Point Correlation

Drug Product	Dissolution Condition	Recommended Units Sampling		Official in
Aspirin tablet	0.05 M acetate buffer, 500 mL, pH 4.5, apparatus 1, 50 rpm, 30 min.	NLT 80% of Q is dissolved in 30 min.		USP
Buffered aspirin tablet	0.05 M acetate buffer, 500 mL, pH 4.5, apparatus 2, 75 rpm, 30 min.	NLT 80% of Q is dissolved in 30 min.		USP
Aspirin ER tablet	Test 1: 0.1 N HCl, 900 mL, apparatus 2, 60 rpm, time 1 h; 4 h.	Tolerance time	Amount dissolved	USP
		1 h	20–55%	
		4 h	NLT 80%	
Diazepam capsule	0.1 N HCl, 900 mL, apparatus 1, 100 rpm, time 45 min.	NLT 85% of Q is dissolved in 45 min.		USP
Diazepam ER capsule	Simulated gastric fluid TS (without enzymes), 900 mL, apparatus 1, 100 rpm, 0.042 D h, 0.167 D h, 0.333 D h, 0.5 D h.	Time (h)	Amount dissolved	USP
		0.042 D	15–27%	
		0.167 D	49–66%	
		0.333 D	76–96%	
		0.5 D	85–115%	
Methylphenidate HCl tablet	Water, 900 mL, apparatus 1, 100 rpm, 45 min.	NLT 755 of Q is dissolved in 45 min.		USP
Methylphenidate HCl ER tablet	Water, 500 mL, apparatus 2, 50 rpm 0.125 D h, 0.25 D h, 0.438 D h, 0.625 D h, 0.875 D h.	Time (h)	Amount dissolved	
		0.125	20% and 50%	
		0.25	35% and 70%	
		0.438	53% and 83%	
		0.625	70% and 95%	
		0.875	NLT 80% amount dissolved	

TABLE 15.8 ***Continued***

Drug Product	Dissolution Condition	Recommended Units Sampling		Official in
Oxprenolol HCl tablet	0.1 N HCl, 900 mL, apparatus 1, 100 rpm, 30 min.	NLT 80% of Q is dissolved in 30 min.		USP
Oxprenolol HCl ER tablet	Acid medium (0.1 N HCl), 900 mL. Dissolution medium (simulated intestinal fluid Ts: without enzyme), 900 mL, apparatus 1, 100 rpm, 1 h in acid.	Time	Amount dissolved	USP
		1 (acid medium)	Between 15% and 45%	
		1 (dissolution medium)	Between 30% and 60%	
		3 (dissolution medium)	Between 50% and 80%	
		7 (dissolution medium)	NLT 75%	
Procainamide HCl tablet	0.1 N HCl, 900 mL, apparatus 1, 100 rpm, 75 min.	NLT 80% of Q in 75 min.		USP
Procainamide HCl ER tablet	Test 1: 0.1 N HCl, 900 mL, apparatus 2, 50 rpm, time 1, 4, 6 hr	Time	Amount dissolved	USP
		1	Between 30% and 60%	
		4	Between 60% and 90%	
		6	NLT 75%	
	Test 2: acid stage (1 h), buffer stage (0.05 M, pH 7.5, PO_4 buffer), NLT 8 h	Time	Amount dissolved	
		1	Between 30% and 60%	
		4	Between 60% and 90%	
		8	NLT 80%	
	Test 3: medium same as in test 2; apparatus 2, 50 rpm; time 1, 3, 6, 8 h	Time	Amount dissolved	
		1	Between 25% and 50%	
		3	Between 40% and 75%	
		6	Between 65% and 90%	
		8	NLT 80%	

Ionic Strength Ionic strength of the dissolution medium plays an important role in the dissolution process [160]. Dissolution behavior of extended release dosage forms that use hydrophilic gel forming polymers seems to be significantly affected by small changes in the ionic strength of the dissolution medium. Certain ionic salts and drugs are reported to cause failure of HPMC based extended release products [161]. HPMC based extended release diclofenac sodium tablets had significantly different release profiles in dissolution media of the same pH but different ionic strengths. The dissolution rate of theophylline from extended release granules prepared using HPMC as a retarding agent was observed to increase with increasing ionic strength of the dissolution medium whereas an initial decrease followed by an increase in the dissolution rate of theophylline was observed from commercially available extended release matrix tablet formulation with an increase in the ionic strength of the dissolution medium [162, 163]. Alterations in dissolution profiles may be due to changes in thermal gelation point (critical temperature that determines the sol to gel transition of polymers in aqueous media) of the polymers [161], increased surface erosion leading to dissolution of polymer chains, increased osmotic pressure [161, 162], and/or lower polymer solubility [164].

pH of Medium pH of the dissolution medium influences solubility of the drug. In addition, it can affect the solubility/stability of the excipients. Both factors may produce an entirely different dissolution profile if the pH of the dissolution medium is not selected carefully. Furosemide tablets were observed to dissolve faster as the pH of the medium was increased. The tablet brand that dissolved poorly at pH 4.6 also exhibited poor bioavailability [165]. The intrinsic dissolution rate of piroxicam increased with an increase in pH of the medium [144]. Thus, careful selection of pH and buffers composing the dissolution medium seems to be essential for *in vitro* dissolution studies as well as for prediction of bioavailability.

15.4 ANALYSIS OF DISSOLUTION DATA

Whenever a new solid dosage form is developed or produced, it is necessary to ensure that drug dissolution occurs in an appropriate manner. In addition, for a pharmaceutical company to obtain approval for a generic product, there are a series of criteria that must be addressed to demonstrate that the product is bioequivalent to a proprietary counterpart. Under certain conditions, one of these criteria is to demonstrate that the dissolution curves of the two products are similar. The *in vivo* bioavailability study investigates the rate and extent of drug absorption in humans. The fact that drug absorption depends on the dissolved state of the drug has encouraged pharmaceutical companies to explore the relationship between *in vivo* drug bioavailability and *in vitro* dissolution. It has been suggested that *in vitro* dissolution testing can be used as a surrogate for *in vivo* bioequivalence studies to assess equivalence between the test and reference formulations, and for postapproval changes. If dissolution profile similarity is demonstrated for the formulations before and after the changes, then expensive *in vivo* bioequivalence testing can be waived off. Various procedures have been proposed to compare dissolution profile data (Fig. 15.4). The utility of these methods is briefly discussed next.

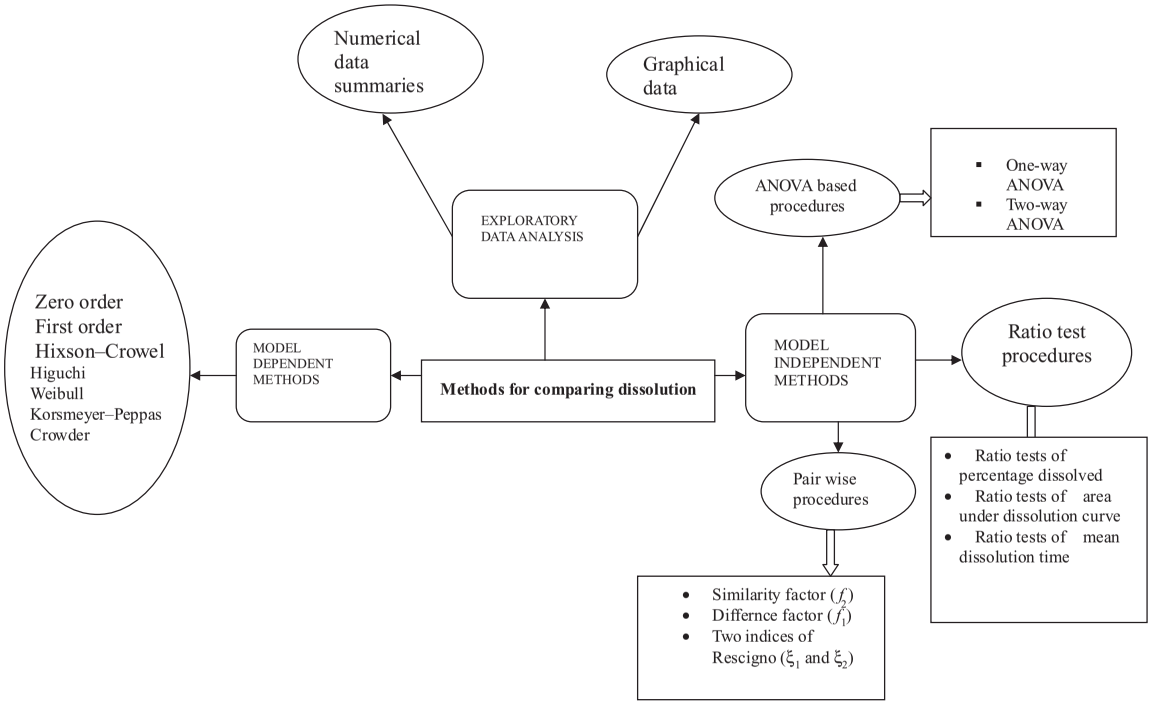


FIGURE 15.4 Various methods used for evaluating dissolution data.

Exploratory Data Analysis As a first step, it is useful to perform exploratory data analysis to compare dissolution profile data. The U.S. FDA does not currently endorse these methods. In this method the dissolution profile data are illustrated graphically by plotting mean dissolution profile data for each formulation with error bars extending to two standard errors at each dissolution time point. Then the data are summarized numerically and 95% confidence intervals are evaluated in mean dissolution profiles at each dissolution time point. This method is useful in obtaining an improved understanding of dissolution data. It can also be used as a first step to compare the dissolution profile data (in a graphical as well as numerical manner). However, the analysis of dissolution profiles becomes difficult if the error bars for two formulations overlap (graphically as well as numerically) [166, 167].

Statistical Methods (Model-Independent Methods) Using one-way ANOVA, statistical comparisons of the mean dissolution data at each dissolution time point are performed. One-way ANOVA is equivalent to the *t*-test in the case where the dissolution profile data of two formulations is compared. This method takes the variability in the dissolution profile data into account while comparing each time point. Two-way ANOVA is used in data analysis when both formulation and time are class variables (in the case of immediate release data when one-way ANOVA is not applicable). But the major limitation is that this method ignores the correlation between the dissolution time points [168, 169].

Multivariate Approach (MANOVA) Methods These are based on repeated measures, where time is the repeated factor and percent drug dissolved is the dependent variable. The method is more precise as it takes into account the variability as well as the correlation structure in data. However, this method may not be too informative, because it doesn't contain information about the nature of differences between mean dissolution profiles. In addition, it is difficult to implement this method tactically [170].

Chow–Ki Method This is another statistical method, which is comparable to the approach used for assessing the average bioequivalence of two formulations. Equivalence limits for similarity at each dissolution time point are derived from Q (as defined in USP). It uses the concept of local and global similarity to assess closeness of test to reference dissolution profiles. This method is relatively easy to implement. Correlation between successive time points is accounted for by using an autoregressive time series model. Power and Type I error of this method are unknown at this stage. Type I error is the probability of concluding that the test and reference mean profiles are different when in fact they are similar. Type II error is the probability of concluding that the test and reference mean profiles are the same when they are actually different [168].

Model-Independent Method This uses the dissolution data in their native form. Two mathematical methods are described in this method. The first is the difference factor equation, which is also called the f_1 equation, and the second is the similarity factor, also called the f_2 equation. The FDA approves both equations for dissolution profile comparison but the similarity equation is preferred.

$$f_1 = \left(\frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \right) \times 100$$

$$f_2 = 50 \log_{10} \left\{ \left(1 + \frac{1}{n} \sum_{t=1}^{n} w_t (R_t - T_t)^2 \right)^{-0.5} \times 100 \right\}$$

where n is the number of dissolution sample time points; w_t is the optional weighting factor, and R_t and T_t are the mean percent drug dissolved at each time point, respectively, for each reference and test product. Evaluation requires the use of a minimum of three time points (except zero), not less than 12 individual values for every time point for each formulation, and the standard deviation should not be more than 10% from the second to the last time point. When the calculated values of f_1 lie between 0 and 15 and those of f_2 lie above 50, the dissolution profiles are interpreted to be similar. In f_2 estimation, bias could take place due to the contribution of variances in the percentage of drug dissolved in test and reference preparations measured at a particular time point. Unbiased similarity factor (f_2^*) is calculated to determine the effect of variances of the percentage of drug dissolved from test and reference products at particular time points on f_2:

$$f_2^* = 50 \left(\log_{10} \left\{ 1 + \left(\frac{1}{n} \right) \left[\sum_{t=1}^{n} (R_t - T_t)^2 - \sum_{t=1}^{n} (S_r^2 - S_t^2)/N \right] \right\}^{-0.5} \times 100 \right)$$

where $S_r^2 - S_t^2$ is the variance in the percentage of drug dissolved measured at any time point of reference and test preparations and N is the number of units on which dissolution is conducted. By this method it is easy to compare the data. In addition, it also provides a single number to describe the comparison of dissolution profile data. However, it is not possible to know the Type I and Type II errors [171–173].

Rescigno's Indices Rescigno's indices have been developed for comparing blood-plasma concentration functions. ξ_i and ξ_2 are considered analogous to f_1 and f_2:

$$\xi_i = \left[\frac{\int_0^{t_n} |R_t - T_t|^i dt}{\int_0^{t_n} |R_t + T_t|^i dt} \right]^{1/i}$$

where R_t and T_t are the mean percentages of drug dissolved, respectively, for reference and test formulations at each time point and t_n is the final dissolution time point. ξ_i can be thought of as a function of the weighted average of the vertical distances between test and reference mean profiles at each time point. More precisely, ξ_i is the absolute value of vertical distance and ξ_2 is the square of vertical distance.

The denominator of ξ_i is a scaling factor. The main advantage of this method is that, unlike f_1, the value of ξ_i remains unchanged if reference and test are interchanged. But this method does not take into account variability and correlation structure data like f_1 and f_2 equations, and also it is difficult to calculate Rescigno's indices. Moreover, there is no definite criterion for concluding difference or similarity data profile [174].

Korsmeyer–Peppas Model This is a simple, semiempirical model relating exponentially the drug release to the elapsed time. This model uses the following equation for evaluation of dissolution data:

$$f_t = at^n$$

where a is a constant incorporating structural and geometric characteristics of the dosage form and n is a release exponent indicative of the drug release mechanism. This equation can be written

$$f_t = \frac{M_t}{M_\infty} \quad \text{(fractional release of drug)}$$

where M_t/M_∞ is a function of t (fractional release of drug). Modifying the above equation to accommodate lag time generates the following equation:

$$\frac{M(t-l)}{M_\infty} = a(t-l)^n$$

where l is the lag time.

In the case of a burst effect, the equation becomes

$$\frac{M_t}{M_\infty} = at^n + b$$

In the absence of lag time or burst effect, at^n is used and the resulting model, also known as the power law, has been used, very frequently, to describe the drug release from several modified release dosage forms [175, 176].

It is a simple and empirical model and describes drug release from modified release dosage forms [175, 177, 178]. This model assumes that the rate-limiting step to drug release is erosion of matrix. The time-dependent diffusional resistance to eroding matrix is hypothesized not to influence drug release [179].

Crowder Model This is a mechanistic model. It uses repeated measures regression analysis techniques for comparing data from different formulations. It is considered superior to other modeling based approaches and takes covariance structure into account while statistically comparing the data. However, its practical utility is limited because it may be difficult to analyze the data using conventional statistical packages [180].

Baker–Lonsdale Model This has been developed from the Higuchi model:

$$f_t = \frac{3}{2}\left[1-\left(1-\frac{M_t}{M_\infty}\right)^{2/3}\right]-\frac{M_t}{M_\infty} = Kt$$

where K is the release rate constant (slope), and M_t; and M_∞ are the amounts of drug released, respectively, at time t and infinite period. This model has been utilized for linearizing release data from several formulations of microcapsules or microspheres [181–183].

The Hopfenberg model utilizes the following equation to describe the dissolution data:

$$\frac{M_t}{M_\infty} = 1-\left(1-\frac{K_0 t}{c_0 a_0}\right)^n$$

where M_t is the amount of drug dissolved in time t; M_∞ is the total amount of drug dissolved when dosage form is exhausted; M_t/M_∞ is the fraction of dose dissolved; K_0 is the erosion rate constant; C_0 is the initial drug concentration in matrix; and A_0 is the initial radius of spheres/cylinder/half-thickness of the slab, where on A value of 1, 2, or 3 is used for a slab, a cylinder, or a sphere, respectively. This model is generally useful while studying release from surface eroding devices (slab, cylinder, sphere) [184].

Higuchi Model Higuchi developed several theoretical models to study the release of water-soluble and poorly soluble drugs incorporated in semisolid and/or solid matrixes. Generally, a simplified equation is used for analyzing dissolution data:

$$Q_t = K_{\mathrm{H}}\mathrm{t}$$

where K_{H} is the Higuchi dissolution constant. This model is widely used to describe drug dissolution from modified release dosage forms [185–187].

Hixson–Crowell Model This has been used to describe the release profile by considering the diminishing surface of the drug particles during dissolution:

$$Q_0^{1/3} - Q_t^{1/3} = K_{\mathrm{s}}t$$

where K_{s} is the constant incorporating the surface–volume relationship; Q_0 and Q_t are, respectively, the initial amount of drug and the remaining amount of drug in the dosage form at time t. Upon simplification, the equation can be written

$$(1-f_t)^{1/3} = 1-K_\beta t$$

where $f_t = 1-(Q_t/Q_0)$, and f_t represents the rug-dissolved fraction at time t and K_β is the release constant. This model is applied to dosage forms (e.g., tablets) where the dissolution occurs in planes that are parallel to the drug surface—that is, if tablet

dimensions diminish proportionally in such a way that the geometrical form remains constant all the time. The major assumption made is that the release rate is limited by the drug particles' dissolution rate and not by diffusion through the polymeric matrix [188].

Weibull Function This is a modeling based method used for evaluating the dissolution data profiles:

$$m = 1 - \exp\left(\frac{-(t - Ti)^b}{a}\right)$$

where a defines the time scale of the process, T_i is the lag time before the onset of the dissolution or release process (in most cases zero), b is the shape parameter, m is the accumulated fraction of drug, and t is the solution time of the drug.

Upon rearranging, this equation becomes

$$\log[-\ln(1-m)] = b\log(t - T_i) - \log a$$

On the basis of this equation, the plot between log of dissolved amount of drug versus log of time will be linear for dosage forms. The main limitation of this model is the lack of a kinetic basis for its use due to the nonphysical nature of the parameters. In addition, it cannot characterize the dissolution kinetic properties of a drug. Furthermore, there is no single parameter related to the intrinsic dissolution rate of the drug. Lastly, it is of limited use for establishing *in vitro–in vivo* (IVIV) correlation [189–191].

Order of Drug Release Analysis Zero order and first order kinetics and ratio test procedures are modeling based methods. Drug dissolution from dosage forms that do not disaggregate and release the drug slowly is characterized by zero order kinetics (assuming that area doesn't change):

$$Q_t = Q_0 + K_0 t$$

where Q_t is the amount of drug dissolved in time t, Q_0 is the initial amount of drug in solution, and K_0 is the zero order release rate constant. This method can be used to describe drug dissolution of modified release dosage forms [192, 193].

First order drug release can be represented by

$$\ln Q_t = \ln Q_0 + K_1 t$$

where K_1 is the first order release constant. This equation is modified to yield the following equation:

$$\ln Q_t = \ln Q_0 + K_1 tS$$

where S is the solid area accessible to dissolution [192].

Ratio Test Procedures These compare dissolution profiles of two formulations at a particular time point. Ratio tests of percentage dissolved can be performed to give a 90% confidence interval for the mean ratio of percentage dissolved [194].

15.5 *IN VITRO–IN VIVO* CORRELATION

Ethical constraints make it imperative to generate pharmacological and toxicological data in laboratory animals and *in vitro* systems before human testing can begin. Two fundamental challenges faced by pharmaceutical scientists pertain to scaling up of pharmacokinetic data from animals to humans and extrapolation of the *in vitro* data to the *in vivo* situation.

15.5.1 Physiological Concerns

Drug absorption is influenced by physicochemical characteristics of the drug as well as physiological factors. While physicochemical factors are species independent, physiological factors are species dependent. Therefore, it is essential to examine the bioavailability of a drug in animals with respect to the physiological differences with human beings. Often, the knowledge of different absorption or first-pass metabolism in animals helps in reasonably extrapolating the absorption of drugs to that in human beings (Table 15.9). Furthermore, differences in the gastrointestinal physiology of monkeys and dogs with respect to human beings play a significant role in addressing the differences observed in dosage form performance (Table 15.10). It is evident from Table 15.11 that solution is emptied from the stomach within 1 h and it is only slightly influenced by feeding. t_{lag}, t_{max}, and MAT of tablet were much longer than those of solution. The gastric emptying of tablets in the fed state is delayed more than the solution dosage forms. It is noteworthy that agitation intensity in the gastrointestinal tract of monkeys is closer to that in humans than that in dogs. This leads to approximately similar gastric emptying time of solid dosage forms in monkeys and humans [207].

The rate of distribution of a drug to the organs or tissues is determined by the rate of blood flow and ease with which the drug molecules cross the capillary wall and penetrate the cells. Like most physiological parameters, blood flow and circulation time can be extrapolated across species by use of an allometric equation [210, 211]:

$$y = aw^b$$

where y is the blood flow, a is the allometric coefficient, b is the allometric exponent, and w is the body weight. The allometric relationship between blood circulation time (seconds) and total body weight is $21\,w^{0.21}$ [212].

The smaller animal species deliver drugs faster and more frequently to organs of elimination. This is expected to result in rapid elimination of drugs in smaller animals than in humans [210, 211]. Furthermore, differences in amino acid sequences and the presence of different isoforms and enzymes in different species may lead to significantly different patterns of drug metabolism (Table 15.12). Drugs and their metabolites are usually eliminated from the body via urine and bile. The relative contribution of biliary and urinary excretion to overall elimination of drugs depends

TABLE 15.9 A Few Examples of Interspecies Differences in Drug Bioavailability

Drug	Bioavailability				Remarks	References
	Dog	Rat	Monkey	Human		
Indinavir (solution)	72%	24%	19%	40–60%	Decreased bioavailability in rat and monkey due to extensive hepatic first-pass metabolism.	195
L-365,260: cholecystokinin receptor antagonist (suspension)	9%	14%	—	—	Limited bioavailability was attributed to poor absorption as a result of low aqueous solubility.	196
L-365,260: cholecystokinin receptor antagonist (solution in PEG 600)	70%	50%	—	—	Improved bioavailability due to greater amount of drug soluble in PEG 6000.	196
Melatonin (oral dose 10 mg/kg) Intraperitoneal dose	100% 74%	53.5%	100%	—	Suggested lack of substantial hepatic first-pass of melatonin in rats. However, the oral bioavailability of melatonin in dogs decreased to 16.9% following a 1 mg/kg oral dose, indicating dose-dependent bioavailability in dogs.	197

on the nature of the drugs and is significantly affected for molecules possessing molecular weight greater than 7500 kDa [220]. Many drugs are excreted unchanged by the kidneys. The rate of renal excretion depends on renal blood flow, glomerular filteration rate (GFR), tubular secretion, and reabsorption [201]. Both GFR and number of nephrons show a good allometric relationship. Like GFR, renal excretion of drugs also shows a good allometric relationship across species. Thus, the renal clearance of drugs in humans can be extrapolated from animal data by use of an allometric approach. But this approach requires at least four or five animal species in order to obtain a proper allometric relationship. An alternative to predict human renal clearance is to use the ratio of GFR between rats and humans. The ratio of renal clearance of various drugs between rats and humans is roughly equal to the ratio of GFR between these two species [221].

TABLE 15.10 Summary of Physiological Parameters that May Influence Drug Bioavailability in Different Species

Species	Biliary Excretion Rate (of Organic Chemicals)	Plasma Volume (L/kg)	Body Weight (kg)	Maximum Life Period (years)	Relative Length of Small Intestine (%)	Average Absolute Length of Small Intestine (m)	pH of Small Intestine	Transit Time	Glomerular Filteration Rate (mL/min/kg)	References
Rabbit	Relatively poor	0.0314	3.0	8.0	60	1.51	6–8	*a*	4.8	198–200
Pig	*a*	*a*	*a*	*a*	78	14.16–18.29	6–7.5 (fasted)	*a*	*a*	198, 199
Mouse	Good	*a*	0.02	2.7	*a*	*a*	*a*	*a*	10	201
Monkey	Relatively poor	0.0448	3.5	20	*a*	*a*	5.6–9 (fasted)	*a*	*a*	199, 201, 202
Dog	Good	0.0515	12.5	20	85	2.48–4.14	6.2–7.5, 4.5–7.5 (fasted)	0.5–2 h	4.0	198, 199, 203, 204
Rat	Good	0.0313	0.25	4.7	64	0.82	6.5–7.1	1.5 h	8.7	198, 203, 205
Human	Relatively poor	0.0436	70	93	79	6.25	6.8–8.6, 5–7 (fasted)	2.7–8.5 h	1.8	198, 201, 202, 205, 206

[a]Not found.

TABLE 15.11 Gastric Emptying Time of Dosage Forms Under Fasted and Fed State in Animals and Humans

Type of Dosage Form	Parameter	Human		Monkeys		Dog		References
		Fasting	Fed	Fasting	Fed	Fasting	Fed	
Solution	MAT (h)	1.3 ± 0.3	2.6 ± 0.2	0.8 ± 0.4	1.8 ± 1.4	0.7 ± 0.4	1.3 ± 0.6	207, 208
	*t*lag (h)	0.1 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.2 ± 0.2	0.3 ± 0.1	
	*t*max (h)	1.0	3.0	1.0 ± 0.2	1.8 ± 1.3	1.0	1.0	
Controlled release tablet	MAT (h)	3.4 ± 0.5	6.0 ± 1.6	3.6 ± 2.0	Plasma concentration rose rapidly, *t*lag was delayed	2.1 ± 0.6	Not calculated, delayed compared to human	207, 208
	*t*lag (h)	1.7 ± 0.6	3.3 ± 0.8	2.3 ± 1.5		1.2 ± 0.5		
	*t*max (h)	2.0	6.0	3.7 ± 2.1		3.0		
Coumarin (solution)	*t*max (h)	0.2	—	0.21 ± 0.03	—	—	—	209
Rolipram (suspension)	*t*max (h)	0.38 ± 0.22	—	1.7 ± 0.9	—	—	—	209
Zomepira (solution)	*t*max (h)	0.6 ± 0.3	—	1.2 ± 0.8	—	—	—	209

TABLE 15.12 Summary of Drug Metabolizing Enzymes Present in Liver of Different Species

Species	Gender/Strain	Liver Weight	Enzyme	References
Dog	Mixed/beagle	360 g (sd = 20)	Cytochrome P450 (CYP) CYP1A1/1A2; ethoxyresorufin O-deethylation CYP1A2; phenacetin O-deethylation CYP2A6; coumarin 7-hydroxylation CYP2B6; pentoxyresorufin O-dealkylation CYP2C9; phenytoin p-hydroxylation CYP3A4; human 3A4 antibodies CYP2E1; human 2E1 antibodies CYP2C9; human 2C9 antibodies CYP3A/2B; ethylmorphine N-demethylation	213–216
Rat	Female and male/Sprague–Dawley	10.6 g (sd = 3)	Cytochrome P450 (CYP) CYP3A/2B; ethylmorphine N-demethylation CYP3A4/5; nifedipine oxidation CYP3A4/5; erythromycin N-demethylation CYP3A4; benzphetamine N-demethylation CYP2E1; aniline p-hydroxylation CYP2D6; bufuralol 1-hydroxylation CYP2C19; S-mephenytoin 4′-hydroxylation CYP2C9; phenytoin p-hydroxylation CYP2B6; pentoxyresorufin O-dealkylation CYP2A6; coumarin 7-hydroxylation CYP1A2; phenacetin O-deethylation	216

Mouse	Mixed/ICR	Not found	GST; chlorodinitrobenzene glutathione transferase	217
Human	Mixed	1700 g	Cytochrome P450 (CYP) t	213–216, 218
			CYP1A1/1A2; ethoxyresorufin O-deethylation	
			CYP1A2; phenacetin O-deethylation	
			CYP2A6; coumarin 7-hydroxylation	
			CYP2B6; pentoxyresorufin O-dealkylation	
			CYP2C8/2C9; tolbutamide hydroxylation	
			CYP2C9; phenytoin p-hydroxylation	
			CYP2C19; S-mephenytoin 4′-hydroxylation	
			CYP2D6; bufuralol 1′-hydroxylation	
			CYP2E1; aniline p-hydroxylation	
			CYP3A; chlorpromazine S-oxygenation	
			CYP3A4; benzphetamine N-demethylation	
			CYP3A4/5; nifedipine oxidation	
			CYP3A/2B; ethylmorphine N-demethylation	
Rabbit	Mixed/New Zealand white	Not found	GST; chlorodinitrobenzene glutathione transferase	217, 219
			GST; dichloronitrobenzene glutathione transferase	
			GST; ethacrynic acid glutathione transferase	
Monkey	Male/ cynomolgus	62.5 g	Cytochrome P450 (CYP) content	213
			CYP1A2; phenacetin O-deethylation	
			CYP2A6; coumarin 7-hydroxylation	
			CYP2D6; dextromethorphan O-demethylation	
			CYP2E1; chlozoxazone 6-hydroxylation	
			CYP2E1; ethoxycoumarin O-deethylation	

15.5.2 Correlation

The development and subsequent validation of an *in vitro–in vivo* correlation (IVIVC) is an increasingly important component of dosage form optimization. An IVIVC is a relationship (preferably linear) between a biological parameter (C_{max}, t_{max}, or AUC) produced by a dosage form and an *in vitro* characteristic (e.g., *in vitro* dissolution data). The IVIVC guidance developed by the U.S. FDA states that the main objective of developing and evaluating an IVIVC is to enable the dissolution test to serve as a surrogate for *in vivo* bioavailability studies. An IVIVC has been defined as "a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form and an *in vivo* response." Generally, rate or extent of drug dissolution is the *in vitro* property while plasma drug concentration/amount of drug absorbed is the *in vivo* property.

The development of correlation usually involves three steps: (1) formulation development with different release rates, (2) obtaining *in vitro* dissolution profiles and *in vivo* plasma concentration profiles for these formulations, and (3) estimation of *in vivo* absorption or *in vitro* dissolution time course using an appropriate technique for each formulation.

Level A correlations are the most common type of correlation submitted to the FDA. It is usually estimated by a two-stage procedure comprising deconvolution followed by comparison of the fraction of drug absorbed to the fraction of drug dissolved. A correlation of this type is generally linear and represents a point-to-point relationship between the *in vitro* and *in vivo* input rate. In a linear correlation, the *in vitro* and *in vivo* data curves are superimposable or may be made superimposable with the help of a scaling factor. One of the alternative approaches to develop Level A correlation is a convolution procedure that models the relationship between *in vitro* dissolution and plasma concentration in a single step.

Level B correlation uses the principles of statistical moment analysis. The mean *in vitro* dissolution time is compared to either the mean residence time or the mean *in vivo* dissolution time. It also uses all of the *in vitro* and *in vivo* data, but it is not considered to be a point-to-point correlation. Level B correlation does not reflect the actual *in vivo* plasma level curve.

Level C correlation establishes a single point relationship between a dissolution parameter like t_{50} and a pharmacokinetic parameter like AUC. It does not reflect the complete shape of the plasma concentration time curve, which is an essential factor.

Multiple Level C correlates one or several pharmacokinetic parameters ofinterest to the amount of drug dissolved at several time points of the dissolution profile [222, 223].

The USP recognizes these levels of IVIVC. However, the applicability of IVIVC depends on the inherent permeability of the molecules across the gastrointestinal membrane. Table 15.13 summarizes the possibility of IVIVC for immediate as well as extended release oral products on the basis of the biopharmaceutical classification scheme (BCS).

A successful IVIVC utilizes (1) *in vitro* values to evaluate different batches of a pharmaceutical product as a quality control check to ensure the desired physiologic performance, and (2) *in vitro* values as a tool for developing a series of dosage forms to obtain a desired *in vivo* performance. These objectives can only be fulfilled if a

TABLE 15.13 Possibility of IVIVC of IR and ER Dosage Forms According to BCS [223]

Type of Formulation	Class	Solubility	Permeability	IVIVC (Possible/ Not Possible)
Immediate release (IR)	I	High	High	May not be possible
	II	Low	High	May not be possible
	III	High	Low	May not be possible
	IV	Low	Low	May be possible
Extended release (ER)	Ia	High + site dependent	High + site dependent	Level A
	Ib	High + site dependent	Site dependent with narrow absorption window	Level C
	IIa	Low + site dependent	High + site dependent	Level A
	IIb	Low + site dependent	Site dependent with narrow absorption window	May not be possible
	Va	Variable	Variable	May not be possible
	Vb	Variable	Variable	Level A

relationship between *in vitro* and *in vivo* parameters is confirmed. The approaches for IVIVC are summarized in Fig. 15.5.

IVIVCs can be divided into nonquantitative and quantitative correlations. In the case of nonquantitative correlations, the two variables are not related to each other mathematically as observed in rank order correlation (not popular now) [224–226]. In the case of quantitative correlations, the variables correlate with each other through a linear or nonlinear equation. A quantitative correlation can be established by using well known estimated values of important *in vitro* dissolution processes and *in vivo* parameters; for instance, amount dissolved at a specific time point can be correlated with fraction absorbed or absorption rate constant. These correlations can be categorized as single point correlations/or multiple point correlations.

Single point correlations are not very informative and sometimes they are not easy to apply practically [227, 228]. On the other hand, point-to-point or multiple point correlations are informative and are established in two ways. In the first case, a relationship is established between the actual time course of the *in vitro* dissolution and the time course of dissolution in the lumen or arrival in the general circulation, which is estimated by using a deconvolution method (observed concentration in the bloodstream versus time profile). In the second case, the relationship between actual plasma concentrations versus time profile is evaluated. In both cases, there is a requirement of intravenous or oral solution data or in the case of extended release product of high solubility, oral data from immediate release solid dosage form is required. In the case of multiple point correlation, *in vitro* dissolution

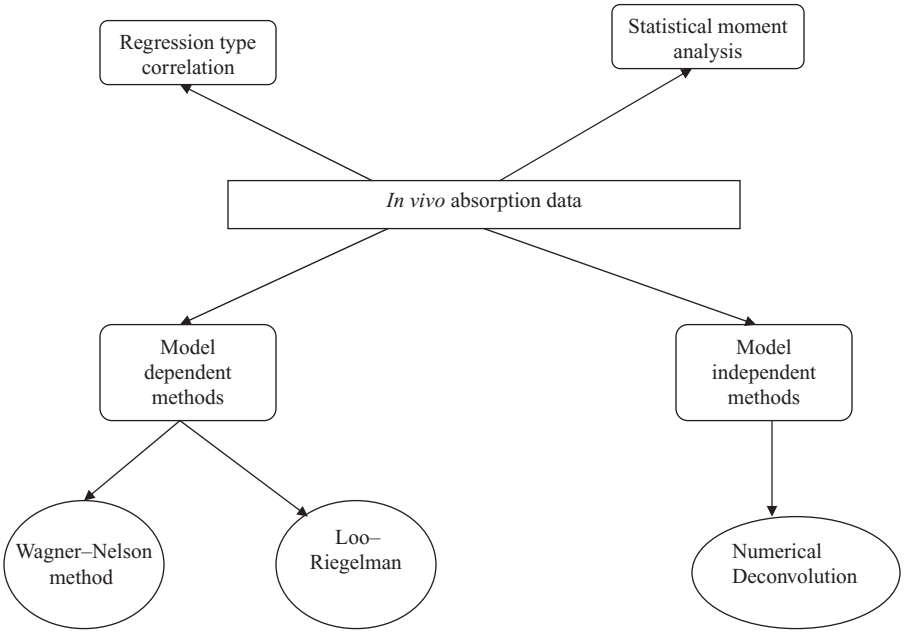


FIGURE 15.5 Approaches for *in vivo* data analysis.

datasets must be compared with each other at the time of evaluation as well as application [222, 229]. In addition to the well recognized methods of IVIVC, a few useful methods are summarized in Table 15.14.

15.5.3 Biowaivers for Changes in the Manufacture of a Drug Product

1. *Biowaivers Without an IVIVC.* For formulations consisting of beads in capsules, with the only difference between strengths being the number of beads, approval of lower strengths without an IVIVC is possible, provided bioavailability data are available for the highest strength.

2. *Biowaivers Using an IVIVC.* Nonnarrow therapeutic Index Drugs: If an IVIVC is developed with the highest strength, any lower strengths may be granted approval if these strengths are proportional or qualitatively the same. Furthermore, the *in vitro* dissolution profiles of all strengths should be similar, and all strengths should have the same release mechanisms. In addition, changes in release controlling excipients in the formulations should be within the range of release controlling excipients of established correlations.

3. *Biowaivers Using an IVIVC.* Narrow therapeutic Index Drugs: If external predictability of IVIVC is established, following waivers are likely to be granted, if the release rate of at least two formulations have been studied for IVIVC development. If an IVIVC is developed with the highest strength, any lower strength may be granted approval if the strengths are proportional or qualitatively the same. Furthermore, the *in vitro* dissolution profiles of all strengths should be similar and

TABLE 15.14 A Few Methods of IVIVC with Respect to Time Profile

Correlation Method	Remarks	References
Characterization of time profile		
Distribution functions (e.g., Weibull function)	Represented by *in vitro* and *in vivo* time profiles, for example, (a) *in vitro* release profile corresponds to distribution of drug released at time t and the corresponding probability distribution function profile characterizes the release rate; (b) plasma concentration profile represents drug distribution in plasma at any time and its cumulative distribution function represents the drug absorbed and already eliminated.	230–232
Semi-invariants (moments)	Area (K_0), mean (K_1), variance (K_2), skewness (K_3), and kurtosis (K_4) are semi-invariants. All semi-invariants are defined in terms of integrals of time profile between $t = 0$ and $t = \infty$. Variance, skewness, and kurtosis are called higher rate semi-invariants.	230–232
Comparison of time profiles		
General aspects	Model-independent techniques compare data pairs observed at corresponding time values,	230–232
Model-dependent/-independent comparison	where time is only a class effect (e.g., paired t-test, ANOVA). Model-dependent techniques are better as they assume that observed data pairs belong to a general distribution function.	
Horizontal/vertical comparison	Vertical comparison represents the value obtained at a given time (i.e., extent characteristics of the process) and horizontal comparison represents the time required to reach a certain ordinate value (i.e., rate aspect of process).	230–232
Comparison by semi-invariants	For the comparison of two differential plasma concentration profiles the index of bioequivalence is used (discussed in data analysis).	230–232
Rescigno's indices (ξ_1 and ξ_2)		
Model-independent indices		230–232
Moore–flanner index (f_1)	$f_1 = \dfrac{\sum \lvert R - T \rvert}{\sum R}$	
Moore–flanner index (f_2)	$f_2 = 50 \log \left\{ \dfrac{100}{\left(\sqrt{1 + \sum (R^{\wedge} - T^{\wedge})2}\right)/N} \right\}$	
Statistical consideration	Statistical comparisons are performed in terms of dissimilarity rather than similarity. If computed statistics or indices exceed a predefined decision limit, both samples are considered as different.	230–232
Decision intervals and limits	The Chow–Ki model, multivariate aspects, and bootstrap techniques are used.	

all strengths should have the same release mechanism. Biowaiver for the approval of new strengths is applicable to strengths lower than the highest strength, within the dosing range that has been established to be safe and effective, provided that the new strengths are proportional or qualitatively the same, have the same release mechanisms, have similar *in vitro* dissolution profiles, and are manufactured using the same type of equipment and the same process at the same site as other strengths that have bioavailability data available. The generic products can also qualify for this biowaiver (a) if bioequivalence has been established for all strengths of reference listed products, (b) if dose proportionality has been established for reference listed products, and (c) if bioequivalence is established between the generic product and reference listed products and all strengths are compositionally proportional or qualitatively the same and have a similar release mechanism as well as *in vitro* dissolution profiles.

4. *Biowaivers When in Vitro Dissolution Is Independent of Dissolution Test Conditions.* Narrow and nonnarrow therapeutic Index Drugs: Biowaivers may be granted if dissolution data are submitted in compendial medium and in three other media (water, 0.1 N HCl, USP buffer pH 6.8). In addition, if *in vitro* dissolution should be shown to be independent of dissolution test conditions after change is made in drug product manufacturing, biowaivers may be granted.

5. *Situations for Which IVIVC Is Not Recommended.* IVIVC is not recommended (a) for approval of a new formulation (extended release) of an approved drug product when the new formulation has a different release mechanism, (b) for approval of a dosage strength higher/lower than doses that have been shown to be safe and effective in clinical trials, (c) for approval of another sponsor's product, even with the same release controlling mechanism, and (d) for approval of a formulation change involving a nonrelease controlling excipient in the drug product that may significantly affect drug absorption [222, 233, 234].

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