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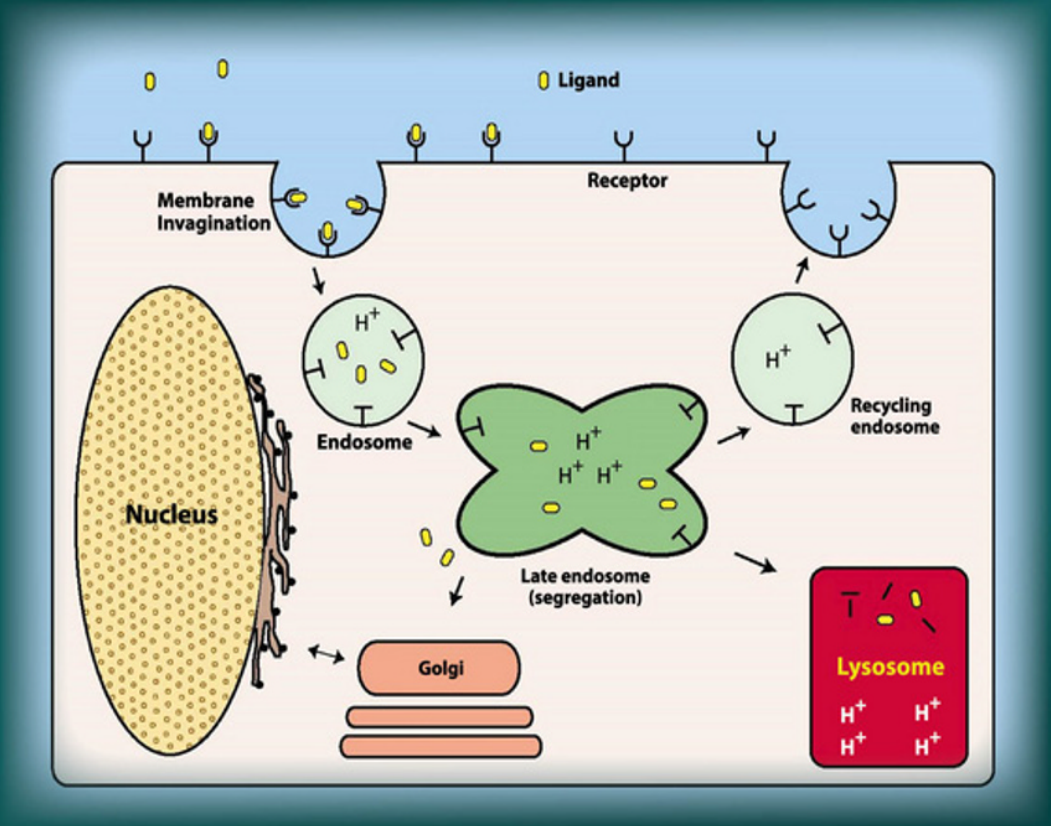
Binghe Wang, Series Editor

Drug Delivery

Principles and Applications

Second Edition



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PREFACE

Drug delivery is an integral part of drug discovery and development. To set the context for the issues related to drug delivery, it is important to look at the big picture as well. It has been 12 years since the last edition of this book was published. As a personal journey, it is amazing to see how different the pharmaceutical industry is now when compared to what it was 12 years ago. Much has changed. In size, the 2003 US pharmaceutical market was $220B (IMS Health, Doug Long); and the 2014 size was $337B (Pharmarceutical Commerce, November 20, 2014). This is an increase of over 50%. While the United States remains the largest market for pharmaceuticals, the rest of the world is changing too, and new and emerging markets are becoming increasingly important. For example, China was ranked no. 10 in pharmaceutical market size in 2003; and in 2013, it had surpassed all European countries in becoming no. 3 behind the United States and Japan (China's Pharmaceutical Industry—Poised for the Giant Leap, KPMG 2011). In 2014, China's pharmaceutical sales were estimated to be about $100B ("The Next Phase: Opportunities in China's Pharmaceuticals Market" by Yvonne Wu, Deloitte, 2011), and China is poised to overtake Japan in 2016 in becoming the no. 2 market with an estimated size of about $150B (Mind power Solutions, March 2012). In terms of business models, the industry is becoming more global. Outsourcing of research work to new and emerging markets such as China and India and going from a model of mostly in-house research and discovery to a heavy emphasis on in-licensing represent a trend. In terms of the science, there is more of an emphasis on biologics with antibodies and antibody conjugates leading the way.

All these changes will affect how we formulate ideas in drug discovery and development, and thus drug delivery issues as a result. For example, the growing weight of the emerging markets means that intellectual property (IP) protection in

countries such as China is becoming increasingly important. In addition, the IP rights of the prodrug in relation to its parent drug may be viewed differently in various countries. With all these changes, some of the drug discovery and development issues have changed too. The "silo" structure, prevalant in the pharmaceutical industry decades ago, is no longer the norm. Team-based discovery and development efforts are more of the norm. The feverish feast with combinatorial chemistry has been replaced by a more rational approach of using diversity in chemistry to address drug discovery problems, with special emphasis on "drug-like" properties. Even with all those changes, some of the drug delivery issues remain the same. Thus the Preface for the first edition is reprinted after this as well. With the new edition, the basic flow of the book has not changed. However, new chapters have been added and old chapters have been updated. Among the new chapters added, there is a special emphasis on delivery to specific organs or sites. For example, we have added chapters on "Intracellular Delivery and Disposition of Small Molecular Weight Drugs" by Jeff Krise and "Intracellular Delivery of Proteins and Peptides" by Can Sarisozen and Vladimir P. Torchilin. These additions largely reflect the tremendous progress made in recent years on the understanding of intracellular trafficking of drugs. We have added chapters on (i) "Transdermal Delivery of Drugs Using Patches and Patchless Delivery Systems" by Bozena Michniak-Kohn and colleagues; (ii) "Nanoparticles as Drug Delivery Vehicles" by Qian Wang and colleagues; (iii) "Evolution of Controlled Drug Delivery Systems" by Xiaoling Li and colleagues, (iv) "Targeted Delivery of Drugs to the Colon" by Anil K. Philip and Sarah K. Zingales; (v) "Protein and Peptide Conjugates for Targeting Therapeutics and Diagnostics to Specific Cells" by Teruna Siahaan and colleagues; (vi) "Drug Delivery to the Lymphatic System" by Qiuhong Yang and Laird Forrest; (vii) "The Development of Cancer Theranostics: a New Emerging Tool towards Personalized Medicine" by Chen and colleagues; (viii) "Vaccine Delivery: Current Routes of Administration and Novel Approaches" by David Volkin and colleagues; and (ix) "Delivery of Genes and Oligonucleotides" by Charles M. Roth. These additions reflect our thinking that specialized delivery to sites beyond the general circulation is a major challenge. The additions of theranostics, nanoparticles in drug delivery, and genes and oligonucleotides largely were based on the tremendous development in these areas since the previous edition.

We hope that this new edition will provide valuable information for students and professionals alike, and welcome suggestions and participations in future revisions.

Binghe Wang, Longqin Hu, and Teruna J. Siahaan
September 2015

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FACTORS THAT IMPACT THE DEVELOPABILITY OF DRUG CANDIDATES

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1.1 CHALLENGES FACING THE PHARMACEUTICAL INDUSTRY

Drug discovery and development is a long, arduous, and expensive journey. It was estimated that the total cost of developing a new drug in the US pharmaceutical industries was well over a billion dollars in the 2000s, and this figure has been increasing [1, 2]. This figure may be slightly better for biotechnology-based research and development (R&D) [1]. The entire process may take up to 14 years [1, 3]! Yet, only 2 out of 10 marketed drugs would return revenues that match or exceed R&D costs according to a recent analysis [4]. There has been a tremendous amount of pressure on the industry to maximize efficiency, shorten development time, and reduce the cost during discovery and development. In order to accomplish such objectives, one needs to analyze the entire drug discovery and development process so as to identify steps where changes can be made to increase efficiency.

The entire endeavor of developing a new drug from an idea to the market is generally divided into several stages: target identification, hit identification/discovery, hits' optimization, lead selection and further optimization, candidate identification, and preclinical and clinical development [5]. Among these, each stage has

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many interrelated aspects and components. A target is identified in early discovery when there is sufficient evidence to suggest a relationship between the intervention of a target and treatment of the disease or conditions. Tens of thousands new molecules are then synthesized and screened against the target to identify a few molecules (hits) with desired biological activities. Analogs of these selected molecules are then made and screened further for improved activities and drug-like properties. Optimization results in identifying a small number of compounds for testing in pharmacological and other models. Those active compounds (leads) are further optimized for their biopharmaceutical properties, and the most drug-like compound(s) (drug candidates, only 1–2 in most cases) are then selected for further preclinical and clinical development. The drug discovery and development path with an emphasis on the discovery stages is schematically illustrated in Figure 1.1.

Having been through the screening and optimization processes, however, of those drug candidates with most drug-like properties, only about 40% successfully make their way into the evaluations in humans (first-in-human or FIH clinical trial) [6]. Unfortunately, data from historical average reveals an almost 90% overall attrition rate in clinical development [6]. In another word, only one molecule successfully makes into the market from 10 compounds tested in humans. Results from another statistical analysis gave a similar success rates for new chemical entities or new molecular entities (NCEs/NMEs) for which an investigational new drug (IND) application or a biologic license application (BLA) was filed in almost four decades [7], and the figure has not changed much [8]. This high attrition rate obviously does not meet the needs of long-term success desired by both the pharmaceutical industry and health care system.

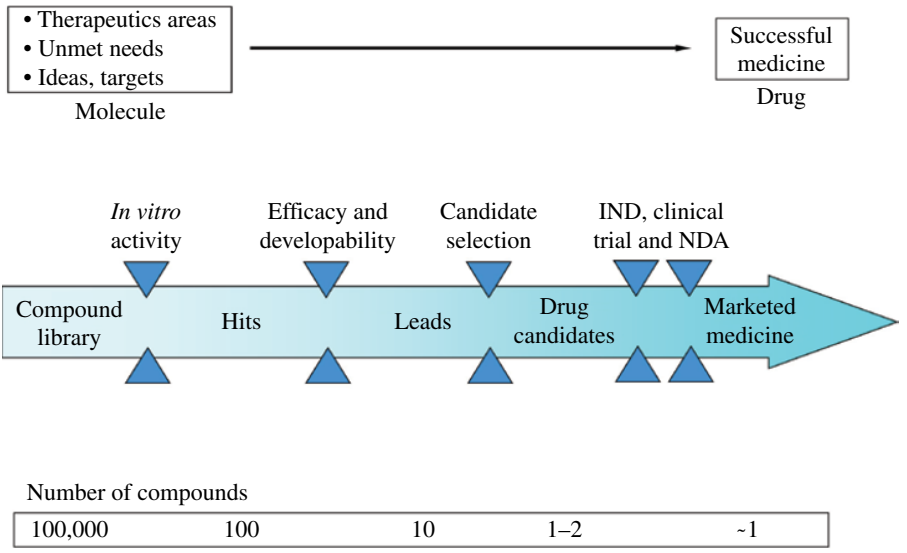

FIGURE 1.1 A schematic illustration of the drug discovery and development process with the estimated number of compounds shown for each step.

Prentis et al. [9] analyzed many factors that potentially were attributable for such a high attrition rate based on the data from seven UK-based pharmaceutical companies from 1964 through 1985. The results from this statistical analysis revealed that a 39% failure was due to poor pharmacokinetic properties in man, 29% was due to a lack of clinical efficacy, 21% was due to toxicity and adverse effects, and about 6% was due to commercial limitations. Although not enough information was available in a great detail, it is believed that some intrinsic relations of these factors existed. For instance, toxicity or lack of efficacy can be precipitated by undesired drug metabolism and pharmacokinetic (DMPK) properties of the molecule. Based on the assumptions that most failures was not due to the lack of "biologic activities" per se as defined by *in vitro* testing, there has been a drive to incorporate the evaluation of drug delivery properties, which may potentially precipitate developmental failures, into the early drug discovery and candidate selection processes with the intention of reducing the proportion of late stage failures, which is obviously most costly.

Rapid development in biology, and in rational and structure-based chemical design in addition to new technologies such as generation of diversity libraries, automation in high throughput screening, and advanced instrumentation in bioanalysis have significantly accelerated lead identification and discovery process [10, 11] for a given target. In light of these scientific and technical advances and under the pressure to reduce the cost and shorten the time of discovery and development, many major organizations in the pharmaceutical industry went through rapid and drastic changes from the late 1990s to early 2000s. A conference entitled "Opportunities for integration of pharmacokinetics, pharmacodynamics, and toxicokinetics in rational drug development" [12] was a landmark of this fundamental change in the pharmaceutical industry [13]. The developability concept was introduced to pharmaceutical R&D with an organizational and functional integration in early drug discovery and development [14]—optimization of DMPK properties of drug candidates in conjunction with toxicology and pharmaceutical development. These changes were successful in addressing some of the specific causes of the attrition. Early investment in optimizing absorption, distribution, metabolism, and elimination (ADME) in drug discovery [15] has successfully reduced attrition rate due to poor human pharmacokinetics from about 39% in the previous survey [9] to approximately 10% in the year of 1991–2000 [16]. A top cause of failures appeared to have shifted to toxicology related. Furthermore, failures due to other reasons, such as the lack of clinical efficacy, remain to be a major issue.

Being encouraged by the successes in addressing ADME issues early on in the discovery and preclinical development, R&D in pharmaceutical industry bolstered the number of drug candidates entering into clinical trials during the early 2000s. Unfortunately, this did not make the expected positive impact on the output in terms of the number of new medicines into the market. The success rate, instead, fell to approximate 5% in the year of 2006–2008 [17]. Thus there is a need of improved understanding of disease mechanism(s) and issues in drug delivery. It shouldn't be forgotten that waves of mergers and acquisitions aim at boosting R&D performance in the pharmaceutical industry apparently failed to effectively address the issues either [18].

Nevertheless, the march goes on. A fairly recent analysis indicated that the number of approved new drugs from pharmaceutical companies has essentially been relatively constant during the past 60 years [19]. Over a thousand new drugs had been approved by the US Food and Drug Administration (FDA) in this period of time. There is no doubt that these medicines helped enormously in treating diseases, managing health conditions, and improving the quality of life. Indeed, life expectancy and cancer survival rate improved due to new treatments [20, 21]. Death rates in cardiovascular diseases decreased significantly [22]. Average cholesterol level in adults in the United States fell to the ideal level—below 200 mg/dl [23, 24]. The most striking example was the dramatic drop in HIV/AIDS death rate since the approvals of antiretroviral treatments [25]. These testimonial facts are the demonstration of the value of pharmaceutical R&D of new medicine.

Since the first therapeutic monoclonal antibody—muromonab-CD3 (Orthoclone OKT3®)—was approved by the US FDA in 1986 [26], more than 30 therapeutic monoclonal antibodies have been approved, and probably hundreds based on the same platform of therapeutics are under clinical development. This class of molecules mimics the human immune system and very specifically intervene cell membrane-bound or soluble targets by antagonizing (a few agonists too) the pathway or neutralizing the ligand [27]. Monoclonal antibody therapeutics along with other biologics such as recombinant or fusion proteins are commonly referred as large molecule to differentiate from synthetic drugs or small molecules. Based on an analysis [8] of the data up to 2004, clinical approval success rate for large molecule therapeutics more than doubled that for small molecules. An in-depth survey on only monoclonal antibody-based therapeutics reveals similar encouraging trend [28]. The discovery and development of biologics are seeing rapid growth. It is expected that the top list of sales will be dominated by biologics in a few years according to Slatko's analysis based on the observations made in 2010 [29].

Taking advantage of the high specificity of a monoclonal antibody as a guided carrier to deliver chemotherapeutic agent specifically to the tumor cells was truly an innovation in drug delivery. This class of coupled molecules is commonly referred as antibody–drug conjugate (ADC) (for a thorough review, see Zolot et al. [30]). The very first ADC was approved for acute myeloid leukemia in 2000 (although it was withdrawn in 2010 based on US FDA's recommendations) [31]. Shortly in the next few years a CD30-specific ADC, brentuximab vedotin, was approved by the US FDA in 2011. Trastuzumab emtansine was approved in 2013. Through conjugation of anti-human HER2 receptor antibody with mertansine, a tubulin inhibitor, Trastuzumab was created as a unique ADC [32]. Because the monoclonal antibody targets HER2, and HER2 is over-expressed in certain cancer cells, the cytotoxic toxin is delivered specifically to tumor cells such as in breast cancer [33]. It has been proven to be a very successful drug delivery strategy for cancer therapies.

Over the past several decades, the never-ending endeavors conjointly by pharmaceutical, academic, and regulatory scientists and researchers have been devoted to finding more effective and safer medicines for treating variety of diseases. The journey has been focused more closely on understanding the biology, learning the etiology, finding the right

molecule, and delivering the molecule to the right target. Many successful stories and good lessons learned undoubtedly demonstrate that drug delivery has been playing a critical role. The developability of drug candidates is an assemblage of assessments that programmatically ensure and optimize drug delivery. The concept has not changed although the domains of developability have been continuously extending along with the development of technologies and advance in sciences. The evaluation of developability mostly involves the integration of research activities in functional areas such as DMPK, pharmaceutical development, safety assessment, and process chemistry into drug discovery and development process in very early stages of discovery. The inputs from other functional areas as well as those from clinical, regulatory, commercial, and marketing groups in the early stage help to minimize costly mistakes in late stages of development and have become more and more important to the success of the drug discovery and development process. Developability is an overall evaluation of the drug-like properties of an NCE/NME. Many of the changes in the pharmaceutical industry have been driven by the concept of ensuring developability. These changes, in other words, the integration of the sciences and strategies in multifunctional areas in drug discovery and development, are to ensure that the NCEs/NMEs of interest will have the best possibility of success in every step toward the final goal.

In the next few sections, examples of some factors that are often examined for developability and their intrinsic relationship are briefly discussed. This is, of course, not a comprehensive coverage of the developability. However, we do hope that this section will put various chapters in perspective and allow readers to see individual sections in the context of an integrated drug discovery and developmental process.

1.2 FACTORS THAT IMPACT DEVELOPABILITY

In most pharmaceutical companies, many efforts have been made to create a clear framework for selecting compound(s) with minimal ambiguity for further progression. Such a framework is not a simple list of the factors that impact the quality of a drug-like molecule and can vary from company to company [34]. This framework, which is more often referred as "developability criteria," is a comprehensive summary of the characteristics, properties, and qualities of the NCE/NME(s) of interest, which normally consist of preferred profiles with a minimally acceptable range. A preferred profile describes the optimal goal for selection and further progression of a candidate, whereas the minimum range gives the acceptable properties for a compound that is not ideal but may succeed. Molecules that do not meet the criteria will not be further progressed. Such criteria cover all the functional areas in drug development. Some of the major developability considerations are briefly described as examples in the following paragraphs.

1.2.1 Commercial Goal

It is self-evident that we are in a business world! Generally speaking a product needs to bring value to the health care system and be profitable to the manufacturer to be viable. Therefore, early input from commercial, marketing, and medical outcome

professionals is very important for setting up a projective product profile, which profoundly affects the development of the developability criteria for intended therapeutics. In general, this portfolio documents the best possible properties of the product and minimum acceptable ranges that may succeed based on the studies of market desires. These studies should be suggestive based on the results from professional analyses of health care needs, potential market, and existing leading products for the same, similar, or related indications. The following aspects need to be well thought out and fully justified before the commencement of a project: (i) therapeutic strategy, (ii) dose form and regimen, and (iii) the best possible safety profile such as therapeutic window, potential drug–drug interactions, and any other potential adverse effects. Using the development of an anticancer agent, as an example, for therapeutic strategy selection, one may consider the choice of developing a chemotherapeutic (directly attacking the cancer cells) versus anti-angiogentic agent (depriving cancer cells of their nutrients) and in combination versus stand-alone therapy. In deciding the optimal dose form and regimen, one may consider the following: whether an oral or iv or both formulation should be developed, should it be once daily or in a different dose interval, and would projected dose regimen be acceptable or convenient for the patients. The results from such an analysis form the frameworks for developing the developability criteria and become the guidelines of setting up the criterion for each desired property. For example, pharmacokinetic properties such as half-life and oral bioavailability of a drug candidate will have direct impact on developing a drug that is to be administered orally once a day.

1.2.2 The Chemistry Efforts

Medicinal chemistry is always the starting point and a driver of small-molecule drug discovery programs. In a large pharmaceutical R&D organization, early discovery of bioactive compounds (hits) can be carried out either by high throughput screening of compound libraries or by rational design, or a combination of both. Medicinal chemists will then use the structural information of the pharmacophore thus identified to optimize the structures. Chemical tractability needs to be examined carefully at the very beginning when a new chemical series is identified. Chemistry space around the core structure for modification is closely studied. Upon a thorough examination of a small number of compounds, an initial exploratory *structure–activity relationship* (SAR) or quantitative SAR (QSAR) should be developed. Rheault and colleagues [35] described an example of how to establish and explore SAR around a pharmacophore in the discovery of a potent and oral bioavailable BRAF inhibitor. In this example, numerous substructural changes were made leading to the most potent compounds while considering the other properties such as the pharmacokinetics and metabolism. Such efforts are normally made in parallel with several different chemical series. It is important for medicinal chemists that many different SARs are being considered, developed, and integrated into their efforts at the same time, which provide more opportunities to avoid other undesirable properties unrelated to their intended biological activities. Such factors, again, may include potential *CYP*450 inhibition, permeability, selectivity, stability, and solubility, etc.

Structural novelty of the compounds (in other words, can this piece of art be protected in a patent?), complexity of synthetic routes, scalability (can the syntheses be scaled up to industrial production scale?) and the cost of starting materials (cost of goods at the end of the game), potential environmental concerns, and toxic intermediate issues will all need to be closely examined at early stages of the drug discovery and development processes. It is never too early to have those thoughts and to put them into actions.

1.2.3 Biotechnology in the Discovery of Medicine

Comparing to medicinal chemistry efforts in the processes of searching a bioactive molecule, the initiation of a biotechnology-based project is more specific and target driven. The biologic activity of large molecule therapeutics is generally believed to be more specific; therefore, there are fewer unexpected off-target effects and potential toxicity issues, which can be a major advantage. Yet, many different hurdles have to be overcome.

The issues with large-molecule products during early development are similar by nature. Thus monoclonal antibodies are used as an example here. The discovery of hybridoma technology by Köhler and Milstein in 1975 was a milestone in the development of monoclonal antibodies in immunology and biomedicine [36]. The Nobel Prize in Physiology or Medicine in 1984 was awarded jointly to Niels K. Jerne, Georges J.F. Köhler, and César Milstein "for theories concerning the specificity in development and control of the immune system and the discovery of principle for production of monoclonal antibodies" [37]. It is fascinating to see how this discovery has changed the face of immunology and biomedicine nowadays [38].

Monoclonal antibodies can be made fully humanized with current technology. Several molecular and cellular biology techniques have been established to generate human monoclonal antibodies [39]. In addition to affinity maturation, engineering and selection processes for the desired specificity and binding affinity, and protein sequence and amino acid residue that may affect the stability and other physicochemical properties of the molecule are important factors in protein engineering of the molecule. The selection of a production platform and/or cell line for a stable and high-yield production of selected antibody is also a very important developability criterion that has to be considered much early on.

Immunogenicity of protein-based therapeutics has been one of the major safety concerns besides its potentially negative impact on the pharmacokinetics and pharmacodynamics. This aspect has been largely addressed by using fully human products [40]. The immunogenicity of a candidate in animal species used in pharmacology and toxicology models is also a very important factor although the occurrence and its impact are in general not predictable for humans [41]. Successful preclinical pharmacology and toxicology programs are the very first step of preclinical development. The importance of drug delivery has been exhibited even in early preclinical development for large molecule as well. Taking immunogenicity as an example, it may interfere with the investigation of pharmacokinetics and safety assessments in animal species, which may severely hinder the molecule being developed further to FIH.

Antibodies largely undergo protein catabolism leading to their eventual elimination, rather than being metabolized by the *CYPs* or other enzymes. FcRn (neonatal Fc receptor or Brambell [42] receptor) plays an important role in protecting antibodies from proteolysis in the lysosomes. That explains the long half-life of most therapeutic antibodies as well as endogenous ones. Transporters are rarely involved for large molecule's absorption and excretion. It may have less of concerns for drug metabolism-based drug–drug interactions [43]. However, the potential of drug–drug interactions should still be programmatically evaluated [44], especially when a cytokine modulator is being developed since certain soluble cytokines may play a role in regulating the expressions of *CYP* enzymes and transporters. The effects of cytokines, such as interleukin-6 and tumor necrosis factor alpha, on *CYP* modulation and possible mechanisms have been studied [45].

With the introductions to medicinal chemistry- and biotechnology-based drug discovery and early development described already, it should be relatively easy to appreciate the complexities of the factors that may affect drug developability directly and indirectly for ADCs. On top of those factors that have to be considered and evaluated for a small-molecule drug and those for the development of a monoclonal antibody, the linker between the two molecules in terms of chemical type and relative stability in a biological environment is also a key factor that has to be fine-tuned before making an ADC work [46].

1.2.4 Target Validation in Animal Models

Although drug discovery efforts almost always start with *in vitro* testing nowadays, it is well recognized that promising results from *in vitro* testing do not always translate into *in vivo* efficacy. There are numerous reasons that could lead to this discrepancy, some of which are well understood and others are not. Therefore, target validation in animal models before clinical trials in human is a critical step. Before a drug candidate is fully assessed for its safety and brought to a clinical test, demonstration of efficacy of a biologically active compound (e.g., active in an enzyme inhibition assay) in pharmacological models (*in vivo*, if available) is considered as a milestone in the path of discovering a drug candidate. This is sometimes also called proof of mechanism (PoM). Many cases exemplify the challenges and importance of pharmacological models. For example, inhibitors of integrin receptor $\alpha_v\beta_3$ have been shown to inhibit endothelial cell growth, which implies their potential as being clinically beneficial for an anti-angiogenic target for cancer treatment [47]. However, the proposed mechanism did not work in animal models although compounds were found very active *in vitro* [48, 49]. What has been recognized is that integrin receptor $\alpha_v\beta_3$ may not be the exclusive pathway that tumor cell growth depends on. Inhibition of this pathway may induce or shift to a compensatory pathway(s) for angiogenesis.

Advances in mathematical modeling have been providing very useful testing environments and have generated very useful data. Anticancer drugs, for example, may be tested in animal xenograft models. Biomarkers and antitumor efficacy data with the pharmacokinetic information could be modeled for prediction of clinical drug exposure and efficacy [50]. Knowing the limitation of animal models, the information

derived from such *in vitro* and *in vivo* experiments and from mathematical modeling is invaluable for target validation and, furthermore, to provide guidance for dose selections in clinical studies. Also, it should be mentioned here that most biologic therapeutics, such as monoclonal antibodies, are very specific to human target and may not cross-react with that in animal species. This property sometimes paradoxically limits the use of preclinical animal models. Therefore, the availability of directly relevant information from preclinical species may be limited for these types of drug candidates. Nonhuman primates are often used. The development of human transgenic animals has been providing very relevant research tools. For example, hFcRn transgenic mice may predict the pharmacokinetic behavior of human monoclonal antibodies very well [51].

Ideally, an *in vivo* model should comprise all biochemical, cellular, and physiological complexities as in a real-life system, which may predict the behavior of a potential drug candidate in human much better than an *in vitro* system. In order to have a biological hypothesis tested in the system with validity, a molecule has to be evaluated in many other aspects. Knowing the pharmacokinetic parameters such as absorption, distribution, and metabolism in the animal species that is used in the pharmacological model becomes critical. Basic pharmacokinetic parameters will be briefly described in the following paragraph and discussed in detail in several chapters in the book. The importance of drug delivery is demonstrated as early as in an animal model that serves as an early milestone in preclinical drug development.

The pharmacokinetics/pharmacodynamics relationship, systemic and tissue levels of drug exposure, frequency of dosing, which allows the drug to demonstrate efficacy, and the strength of efficacy are all very important factors that may affect the future development of an NCE/NME. These are all factors that are directly or indirectly related to the topic and, therefore, have to be fully considered for drug delivery.

1.2.5 Drug Metabolism and Pharmacokinetics

The importance of DMPK in drug discovery and development is reflected in the statistics of attrition rates [9]. Most of the changes in the industry during early 2000s have happened in the areas of DMPK [13] and proven to be effective in reducing attrition [16]. The overall goal of DMPK in drug discovery and development is to predict the pharmacokinetic behaviors of a drug candidate in humans. Nevertheless, the focus could vary at different stages of the process. PK parameters in animal species that will be used in pharmacological (as briefed in the previous paragraphs) and safety assessment models provide very important insights (systemic and tissue exposures) for those studies. The results from pharmacokinetic studies in several animal species generate the data for physiologically-based models or interspecies allometric scaling [52, 53] to predict basic pharmacokinetic behaviors of a product in human. Assays using human tissues, cells, and genetically engineered cell lines provide a tremendous amount of information before a molecule can be tested in clinical studies. Optimizing DMPK developability factors are immensely beneficial for finding the candidate(s) with best the potential for success [54].

Desirable (or undesirable) biological effects of a drug *in vivo* normally are directly related to its exposure. One of the following factors, namely, total systemic exposure, maximum concentration, or duration of the concentration above a certain level, is usually used as a parameter that is correlated with the efficacy and/or adverse effects [55]. The exposure is governed at a given dose by (i) the ability for the body to remove the drug as a xenobiotic and (ii) the route via which the drug is delivered. Blood or plasma clearance (CL) is often used as a measurement of the capability to eliminate a drug molecule from the systemic circulation. Low-to-moderate clearance molecule is desirable in most situations unless a fast-action and short-duration drug is being designed [56]. Biologics such as monoclonal antibodies generally have much lower clearance when compared to small-molecule drugs. Since endosomal proteolysis of monoclonal antibody is protected by its binding to the FcRn receptors [42], the half-life of a therapeutic monoclonal antibody is normally 2–3 weeks. Monthly or even longer dosing interval thus are possible.

A drug can be directly delivered into the systemic circulation by several methods. However, for convenience and many other reasons, oral dosage forms are preferred in many situations. Therefore, oral bioavailability of the compound is one of the very important developability criteria for oral drug delivery. Many factors affect the oral bioavailability of a drug. Orally delivering a biologic therapeutic protein is still quite challenging due to the digestive system. Subcutaneous or intramuscular delivery is the commonly used route of administration in addition to intravenous infusion. The understanding of the mechanisms and factors affecting subcutaneous absorption is still primary. These factors will be discussed in detail in several of the following chapters. In addition to clearance and bioavailability, other major pharmacokinetic parameters that should be evaluated are also discussed in related chapters.

Volume of distribution is a conceptual pharmacokinetic parameter that measures the extent of a drug distributed into tissues. A well-known parameter, elimination half-life, can be derived from clearance and volume of distribution. It is a very important developability criterion, which warrants desired dose regimen. It should be noted here that a discussion of half-life has to be in the context of pharmacologically relevant concentration. A purely mathematically derived half-life is sometimes pharmacologically irrelevant. Some more definitive explanation and comprehensive discussion of the major pharmacokinetic parameters and their biological relevance have been extensively reviewed [57, 58].

These parameters should be examined across several different preclinical species to reliably predict the behavior in human. However, with therapeutic monoclonal antibodies, although available data usually are limited to only one relevant animal species, the predictability has been impressively good and reliable [59]. The pharmacokinetics and pharmacodynamics topics will be discussed in several related chapters in this book.

Inhibition and induction of drug metabolizing enzymes [60], P-glycoprotein (P-gp) substrate property [61, 62], plasma protein binding and binding kinetics [63, 64], metabolic stability in the microsomes or hepatocytes from different species including humans [65], metabolic pathway, and the metabolite(s) identified [66] are all very important developability measurements in the assessment of safety, potential drug–drug interaction,

and predictability. These factors need to be optimized and carefully examined against developability criteria. Drug metabolism-related issues are outlined and discussed in Chapter 9. The impact of transporter including efflux transporter in drug delivery and the models used to study and address these issues are discussed in Chapters 5 and 7.

1.2.6 Preparation for Pharmaceutical Products

Before the early 1990s, the issues of solid state, salt form, aqueous solubility, and dosing formulation for agents used in pharmacological, pharmacokinetic, and toxicological studies have not been brought to full attention. However, an inappropriate salt version or solid form may precipitate potential drug delivery and stability problems (both physicochemically and chemically) during formulation and pharmaceutical engineering. Now it has been realized that the investigation of physicochemical properties of an NCE/NME against developability criteria should start early in the R&D processes. Chapter 3 and several other chapters discuss these physicochemical properties that have major impact on drug delivery.

Aqueous solubility is one of the most important physicochemical properties. It is believed that a drug has to be in solution to be absorbed [67]. From a pharmaceutical development point of view, solid-state form is another important factor that affects solubility and dissolution rate, and eventually the developability. Solid-state form is the determinant of, to some extent, physicochemical stability, intellectual property, and formulation scalability; this factor ought to be carefully examined and optimized. Changes in crystallinity from different chemical processes, in some cases, result in a big difference in bioavailability when the drug is delivered by a solid-dosage formulation.

Many of the earlier-described properties could change when salt version and form change. The salt with the best solubility, dissolution rate (therefore, could result in best bioavailability if by solid dose), stability, and other properties such as moisture absorption should be selected before a molecule enters full development [68]. *In situ* salt screening is a new technology to select the right salt form for drug candidate [69]. For instance, the HCl salt [70, 71] used to be almost the default version for a weak base; however, it has been shown in many cases not to be the best. Application of these screening processes in early drug development is one of the major steps in integrating pharmaceutical development into drug discovery and development.

Preclinical safety assessment (toxicology) is another functional area, which in itself stands to serve as a big milestone in drug discovery and development. NCE/NMEs have to be evaluated for their genetic toxicity as well as acute, short-term, and long-term toxicity when appropriate. The results are crucial for further development of the molecule in FIH clinical study and beyond. Although the principles and importance of toxicology will not be discussed in this book, many efforts in DMPK and pharmaceutics are to assure drug delivery in the animal species used in safety assessment programs. Metabolism profiles of a drug candidate in the species used in the toxicology studies are to be compared with that from human tissues for major difference. The profiles are also examined for potential active/toxic metabolite(s).

It should be noted that process chemistry and biologics production are large functional areas that can have major impacts on a drug's developability, but will not be covered in this book. Although developability criteria in this area will not be discussed here, it is important to point out that it is essential that collaboration with these areas is considered early on in order to define the best strategy for drug delivery.

1.3 REMARKS ON DEVELOPABILITY

The concept of ensuring developability in drug discovery and development represents an integration and synchronization of all functional areas that impact efficiency, and thus the quality and quantity as well as timelines for drug development. Coordination of these multifunctional, interlinked, parallel ongoing scientific and technological research activities is a new challenge to the management of a drug discovery and development enterprise nowadays.

The developability concept was adapted and executed much earlier and more rigorously by larger pharmaceutical companies than their smaller counterparts. However, an analysis by Munos [19] of pharmaceutical innovations in the past 60 years suggested a trend that smaller companies may have outperformed larger companies in their NME/NCE outputs. The underlying reasons for this difference are not clear, especially about whether it was due to a difference in the directions of innovation investments and/or the impact from heightened safety concerns of regulators. There were also not enough data to make the comparison on final approvals. Nonetheless, it was probably more certain that the way in which developability criteria are being adapted and applied was somewhat different. A recent publication clearly indicated varying organization-dependent criteria in different companies [34]. It is reasonable to expect a more focused and objective-driven process in smaller biotechs; wherease larger pharmaceutical companies may use more compartmentalized and criterion-driven development processes. In another word, the question of how we achieve our goals should be asked conjunctively with the questions of how likely it will be to achieve the goals, knowing that the risks, resources, and timelines have to be balanced in practice. Developability is about an in-depth understanding of the molecule regardless of the size of the company or number of molecules in the pipeline.

It is interesting to note some "exceptions" to the commonly accepted developability criteria in the recent history of drug development. In those exceptions the candidate had been successfully developed and even became a blockbuster although the molecule was inborn with some strongly undesired properties. One of the examples would be atorvastatin. The molecule had very limited bioavailability in preclinical species (e.g., ~5% in rats) due to the interplay between transporters and drug metabolizing enzymes in the intestines and liver [72]. Thus, if the preclinical bioavailability criteria used by most preclinical development organizations were applied to atorvastatin, it would not have been selected for development and, therefore, would not have made it to a top-selling drug at all. We learned that in human clinical studies, the bioavailability of atorvastatin was not very high either (14%) [73]. Another story is about a recent-approved cancer drug—dabrafenib (GSK2118436). Inhibition and induction

of major *CYP* enzymes are serious concerns for potential drug–drug interactions. Drug candidates usually are deselected for that reason. If the concerns of *CYP*3A4 induction plus the inhibition of several other *CYPs* (public data in *gsk media*) [74] were used as a litmus test, GSK2118436 would not have been selected for development and, therefore, there would not be the successful story of dabrafenib and trametinib combination therapy for melanoma [75]. The successful stories, or hypothetical arguments if one would, tell us that the developability should never be simply an artificially defined bar for a candidate to jump over. It is a complex process that requires judgment calls based on the full understanding of the properties of a molecule.

1.4 DRUG DELIVERY FACTORS THAT IMPACT DEVELOPABILITY

Delivery of a pharmaceutical agent to the systemic circulation and consequently to the site of action to produce the desired pharmacological effect is the ultimate goal of drug delivery. The developability of a drug candidate from drug delivery perspectives has become the core of developability criterion in drug development. As discussed in the previous sections, many other factors in developability criteria are closely related to drug delivery; these thoughts and practices are applicable from research laboratory all the way to clinical trials and from early discovery to post market development. In order to accomplish the task, one has to overcome numerous barriers that hinder drug delivery.

As the nature of a biological system, multiple or redundant mechanisms may exist to protect the system from exposures to almost any foreign substance while preserving the ability of nutrients uptake. The physiological arrangements and the chemical and biochemical barriers associated with the physiological structures form the first line of defense. Any drug, delivered by any route, will almost certainly encounter some of these barriers before reaching the site of action. These barriers, as well as their physiological and biochemical functions, and their role in drug delivery, will be discussed in detail in Chapter 2. In the first several chapters, general concepts that are directly related to drug delivery, principles in evaluation of drug delivery, along with come common approaches to study drug delivery from anatomical to cellular level are introduced and discussed in sequence from Chapters 3 to 8.

Earlier in this chapter we touched on some conventional routes of drug delivery, such as intravenous injection. Specific factors associated with different routes of drug delivery, such as the first-pass effects following oral administration are discussed in Chapter 9. How a drug molecule interacts with these barriers is very much determined by the intrinsic properties of the molecule. The intrinsic properties are, in another word, the physicochemical and biochemical characteristics of a molecule. In Chapter 3, the physicochemical properties and their implication in formulation and drug delivery will be extensively discussed.

Development of pharmacokinetics and pharmacodynamics relationship by mathematical modeling of the interactions of a drug molecule with the entire biological system is important to the prediction of drug concentrations in the systemic circulation, and, therefore, the pharmacological responses. Better understanding of the system will

allow a pharmaceutical scientist to utilize the system and manipulate the system for the purpose of drug delivery. Chapter 4 discusses the basic principles and topics in pharmacokinetics and pharmacodynamics. Approaches in drug delivery based on the understanding pharmacokinetic principles are essential in pharmaceutical development.

Developability in drug delivery is an overall assessment of all the important factors. For example, in oral drug delivery [76] solubility is important because a drug molecule has to be dissolved to be absorbed. Some lipophilicity is essential for the molecule to cross the cell membrane by diffusion. In order to finally reach systemic circulation, the molecule has to survive various chemical and biochemical attacks in the gastrointestinal system and the liver. A flow chart describing sequentially the factors that can impact drug delivery is illustrated in Figure 1.2. The order in which these factors are listed could also be the order of logical thinking when one plans to tackle an oral drug delivery problem, and could be a reference point for other routes of delivery too.

It is believed that permeability and metabolic stability of a drug molecule are two major factors in drug delivery or in the prediction of a drug's absorption [77] when the molecule is in solution. Permeability can be further divided into passive diffusion and transporter-mediated processes. Metabolism of a drug molecule in the liver and intestine can be evaluated by *in vitro* experimental methods. In many cases, *in vitro* metabolism (intrinsic clearance) can be used to predict *in vivo* metabolic clearance successfully [78]. It is obvious that when efflux transporters, such as P-gp, are

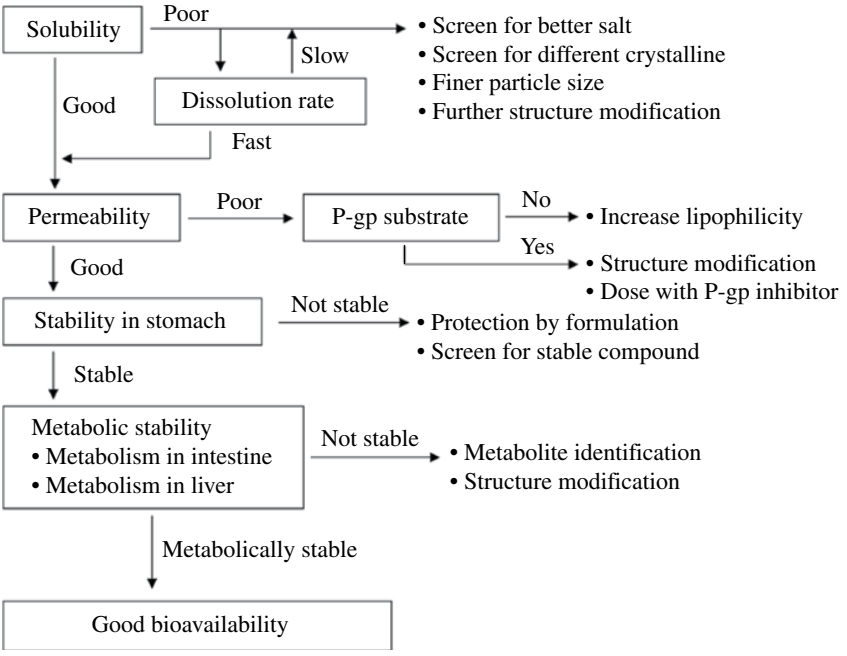

FIGURE 1.2 The evaluation steps of various factors that impact the oral bioavailability of a drug candidate.

involved, the predictability of the *in vivo* clearance using metabolic intrinsic clearance becomes uncertain [79]. A more in-depth understanding of drug transporters and their function in combination with our knowledge on drug metabolism will help predict oral absorption [80, 81]. Transporter-related drug delivery issues as well as *in vivo* and *in vitro* models used to address these issues are discussed in Chapter 5.

In addition to parental delivery of a therapeutic agent, many other routes of drug delivery are developed for convenience, safety, specific targeting, and delivery of special agents. First-pass metabolism is especially applicable to oral drug delivery, and will be discussed in Chapter 9. Several other "unconventional" routes for drug delivery such as pulmonary (Chapter 10) and transdermal absorption (Chapter 11) are discussed together with strategies in development and technical challenges to be considered. Although this book does not cover most routes of delivery individually, the philosophy and logical thinking discussed should be generally applicable to the development of other route of delivery. Figure 1.2 provides, for example, thinking paths in addressing an issue for oral bioavailability. The discussions are further projected into several of later chapters on controlled target-specific drug delivery (Chapters 15–22). Targeting specific organ or tumor tissues through different technologies and potential personalized drug delivery are discussed in Chapters 17–22. Several chapters provided a number of technical approaches to improve drug delivery. Physicochemical approaches by formulation include controlled release (Chapter 15), prodrug approaches (Chapter 12), liposome vehicles (Chapter 13), and nanoparticles (Chapter 14).

It was discussed previously in this chapter that the discovery and development of biologic therapeutics have seen increasing attention and proven to be successful in recent years. Biologic therapeutics are expected to be dominant in the market in the future [29]. Unfortunately, the delivery of biologics had been mostly limited to those by parental injection. A large body of contents related to the delivery of biologic therapeutics or macromolecular drugs are newly added into this edition. Formulations for delivery of vaccines are discussed in Chapter 24. Cutting-edge researches in delivery systems for gene therapy are specifically reviewed and discussed in Chapter 25. It is known that the distribution of large molecules into intracellular space is limited. New developments in sciences and technology focused on intracellular delivery of protein and peptides are introduced in Chapter 23.

The goal of this book is to provide readers with a basic understanding of all the major issues in drug delivery. In this edition, new developments in drug delivery sciences and technology are captured in addition to updates made to those already included in the last edition. A much more detailed examination of various topics can also be found in the references cited in this chapter and the specific discussions in the relevant chapters.

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2

PHYSIOLOGICAL, BIOCHEMICAL, AND CHEMICAL BARRIERS TO ORAL DRUG DELIVERY

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2.1 INTRODUCTION

The development of orally bioavailable drugs is challenging due to the presence of the intestinal mucosa barrier [1–3]. In addition, various potential therapeutic agents derived from biotechnology research (peptidomimetics, peptides, proteins, oligonucleotides) present even bigger challenges than small-molecule drugs. These challenges for oral delivery are due to at least two factors: (1) the presence of physical, physiological, and biochemical barriers of the intestinal mucosa and (2) the unfavorable physicochemical properties of drugs [1, 4]. Oral delivery of small and large molecules has been the focus of many review articles. This chapter focuses on various aspects of the intestinal mucosa barrier and potential solutions to overcome these barriers [1–3].

Many biological barriers protect the human body and segregate each organ according to its functions. These barriers protect organs or tissues from pathogenic invaders, including toxins, viruses, and bacteria. The skin is the largest barrier to protect the body from its surrounding. A more specific protector for the brain is the blood–brain barrier (BBB), which prevents unwanted molecules from entering the brain from the blood stream. Similarly, the intestinal mucosa barrier filters out the unwanted pathogens (i.e., virus and bacteria) and toxins from food and prevents them from getting into the blood stream. Unfortunately, these barriers also become

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barricades for delivering therapeutic agents orally or to the brain to treat brain diseases. Thus, understanding the structure and biological properties of the intestinal mucosa barrier and the physicochemical properties of drugs that can cross the intestinal mucosa barrier are valuable for designing ways to increase the oral bioavailability of the potential drugs. Here, the properties of the intestinal mucosa barrier and types of molecules that can penetrate the barrier will be discussed in greater detail.

Several different obstacles must be overcome for the delivery of drugs through the intestinal mucosa barrier. Although there are differences between the intestinal mucosa barrier and BBB, they have some fundamental similarities. The drug has to have optimal physicochemical properties for it to cross the intestinal mucosa barrier and enter into the systemic circulation. To cross the intestinal mucosa barrier, the drug has to enter the cell cytoplasm via partition into the cell membranes. The presence of efflux pumps can prevent membrane partition of the drug molecules. From the cytoplasm, the drug has to cross basolateral cell membranes before entering the systemic circulation. During this process, the drug also must overcome the biochemical barriers such as metabolizing enzymes that can degrade the drug and prevent it from crossing the basolateral membranes to enter the systematic circulation. To enhance drug delivery to cross these various barriers, many of these factors can be taken into consideration when designing drugs with good intestinal mucosa absorption characteristics.

2.2 PHYSIOLOGICAL BARRIERS TO DRUG DELIVERY

An aqueous mucus layer covers the luminal side of the gastrointestinal tract, and the mucus that is composed of glycoproteins is secreted by the goblet cells. The mucus traps water molecules with a turnover rate of 12–24 hours. A drug molecule has to penetrate the mucus layer with a thickness of 100–150 μm before crossing the epithelial cell layer of the intestinal mucosa barrier [5]. This mucus layer acts as a filter for molecules with molecular weights of 600–800 Da. Drug penetration through this mucus and unstirred water layer is the rate-limiting step before the drug reaches the surface of the epithelial cells of the enterocytes [5, 6].

Under the mucus layer is a single layer of columnar epithelial cells that are joined together by tight intercellular junctions to form a barrier against systemic delivery for orally administered drugs [1, 4, 6]. This layer of cells is composed of enterocytes, goblet cells, endocrine cells, and paneth cells. The amount of goblet cells differs from the small intestine to the distal colon: only 10% of the cells in the small intestine are goblet cells, whereas this percentage increases to 24% in the distal colon. The gastric epithelium from the proximal to distal stomach has four regions including the nonglandular stratified squamous, the cardiac, the glandular proper gastric (fundic), and the pyloric regions. Each region has a different physiological function. The toughness of the stratified squamous region allows it to resist food abrasion, while the cardiac region is responsible for the production of mucus and bicarbonate. Pepsinogen and hydrochloric acid are secreted from the proper gastric region.

The final section is associated with the release of gastrin and pepsinogen [7]. Both villi and crypts are lined with the epithelial cell layer. The microvilli amplify the intestinal surface area for nutrient absorption into the systemic circulation while crypts are responsible for cell renewal [5, 8].

The basolateral side of epithelial cell layer sits on the lumen of the gastrointestinal tract, and the lamina propria supports the epithelial layer [9]. The lamina propria has many components, including smooth muscle cells, nerve cells, lymph vessels, and blood vessels. It serves as a bridge between the food side of the intestinal mucosa and systemic circulation to provide nutrition. The lymphatic system for circulation of immune cells is connected to the intestinal mucosa at the lamina propria. The nerve cells found at the lamina propria link it to the nervous system. The contractility of the intestinal mucosa is regulated by the muscularis mucosa at the mucosa's deepest layer [9].

A drug molecule can cross the intestinal mucosa via several different mechanisms, depending upon its physiochemical properties. Hydrophobic drugs that can partition into the cell membranes are more likely to cross the intestinal mucosa through the transcellular pathway (Fig. 2.1, Pathway A). Drugs that cross

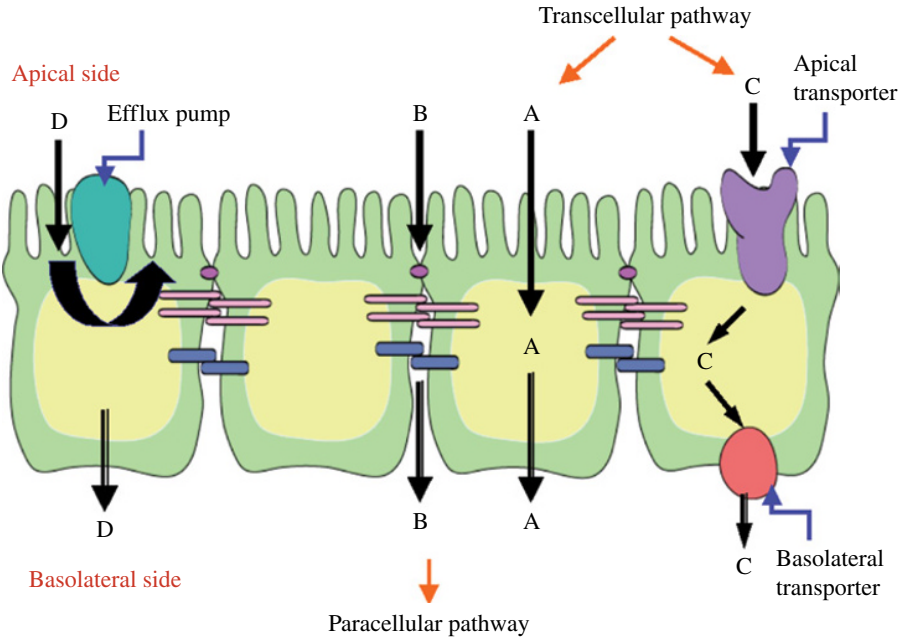

FIGURE 2.1 Several possible mechanisms of drug transport through the intestinal mucosa barrier. Pathway A is the passive transcellular route in which a drug permeates the cell passively by partitioning to cell membranes at both apical (AP) and basolateral (BL) sides. Pathway B is called the paracellular route where the drug passively diffuses between cells at the intercellular junctions. Pathway C is the active transport route where the drug is recognized by transporters, which shuttle the drug from the AP to BL sites. Pathway D is the route where the drug permeation is inhibited by efflux pumps; the efflux pumps expel the drug molecule from the cell membranes during the cell membrane partition process.

via the transcellular pathway normally have a good balance between optimal hydrophobicity and solubility. In contrast, hydrophilic drugs cannot partition into the cell membranes so they cannot cross the intestinal mucosa via Pathway A. For hydrophilic molecules, they must use the paracellular pathway (Fig. 2.1, Pathway B), but only molecules with a hydrodynamic radius less than 11 Å can pass through this pathway [10]. The presence of tight junctions restricts the permeation of molecules through the paracellular pathway [11, 12]. The tight junctions are constructed by protein–protein interactions that connect the membranes of adjacent cells [11, 13–15]. Pathway B is the most likely route of transport for peptides and proteins; however, the large size of peptides and proteins prevents their penetration through the tight junctions. Receptor-mediated endocytosis pathway (Pathway C) is another way for drug molecules to cross the intestinal mucosa barrier. With this pathway, the drug has to be recognized by the transporter on the apical side of the intestinal mucosa barrier. For example, peptide transporters have been shown to improve the oral bioavailability of a drug by conjugating the drug to an amino acid [16–18]. Finally, the intestinal mucosa has efflux pumps (Fig. 2.1, Pathway D) to protect the barrier from unwanted molecules such as toxins to cross this barrier [19]. These pumps recognize molecules with certain features and expel them from the epithelial cell membranes of the intestinal mucosa barrier [20].

2.2.1 Paracellular Pathway

The paracellular pathway is a channel in between cells that is connected with intercellular junctions between neighboring cell membranes (Fig. 2.2) [1, 15, 21, 22]. The tortuous channel of intercellular junctions spans about 80-nm long and runs the entire lateral side of the cells [10]. This pathway allows small molecules and ions to cross the intestinal mucosa barrier. The intercellular junction has three regions: (1) tight junctions (*zonula occludens*), (2) adherens junctions (*zonula adherens*), and (3) desmosomes (*macula adherens*) [1, 15, 21, 22].

2.2.1.1 Tight Junctions The tight junctions (*zonula occludens*) are found at the most apical region of the epithelial cells of the intestinal mucosa barrier. The tight junction region brings the membranes of the opposing cells in close contact, which often is referred to as the "kiss" region. The kiss region can be seen by freeze-fracture electron microscopy as branching fibrils that circumscribe the cells [23]. The tight junction region functions as the gate of the intercellular junctions to prevent molecules from freely passing through the paracellular pathway (Fig. 2.2) [23]. The kiss region is populated with proteins that form cell–cell adhesion among neighboring cells, causing cell surface polarity. This produces the fence function, which restricts free diffusion of lipids and proteins from the apical plasma membrane to the basolateral surface [24, 25]. Thus, paracellular permeation of a drug through the intercellular junctions is regulated by the pore size of the tight junctions. The villus tips have the smallest pores while the crypt regions have the largest pores for the percolation of

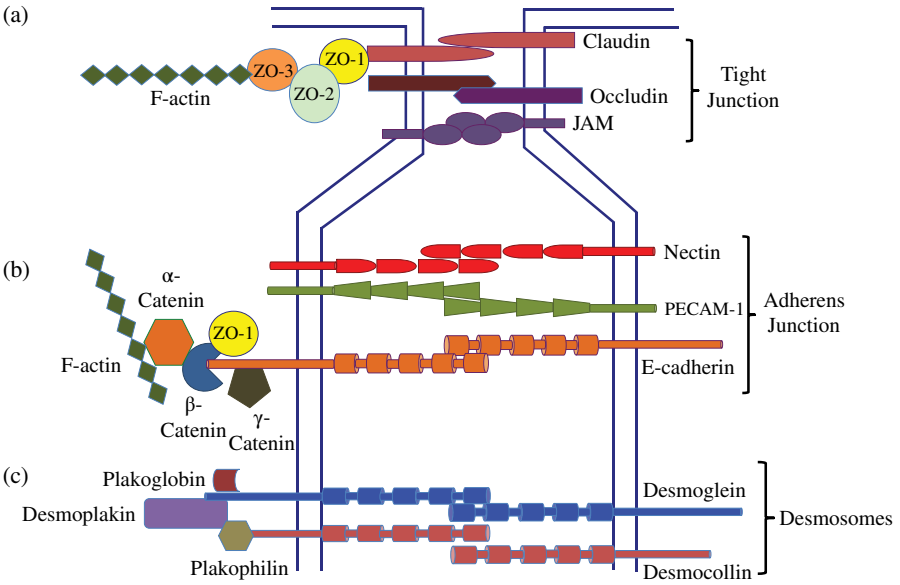

FIGURE 2.2 The intercellular junction is mediated by protein–protein interactions at different regions of intercellular junctions, including (a) tight junction (*zonula occludens*), (b) adherens junction (*zonula adherens*), and (c) desmosomes (*macula adherens*).

molecules through the tight junctions [7]. The integrity of the tight junctions is calcium dependent, and removal of calcium causes a rearrangement of the tight junction proteins [26–28]. It is possible that removal of calcium disrupts the interactions and integrity of calcium-dependent proteins such as cadherins at the adherens junctions. A number of cytokines and growth factors have also been shown to decrease the barrier function of the tight junctions [29].

Protein–protein interactions at the tight junctions are mediated by occludins, claudins (claudin-3 and -5), and junctional adhesion molecules (i.e., JAM-A, -B, and -C), and these proteins are involved in both the gate and fence functions [11, 12, 30–33]. Occludins and claudins have a similar general structure with some distinct differences. Occludins and claudins have four transmembrane domains, two extracellular domains that form loops (loop-1 and -2), and a cytoplasmic carboxyl tail [34]. The extracellular loops as well as the cytoplasmic domain of claudins and occludins play a vital role in creating cell–cell contact [25]. Both occludins and claudins interact with the cytoplasmic proteins called zonula occludin-1 (ZO-1), ZO-2, or ZO-3 that belong to the membrane-associated guanylate kinase (MAGUK) family. ZO-1 interacts with the C-terminal cytoplasmic domain of occludins via the guanylate kinase-like (GUK) domain while it interacts with claudin via its PDZ1 domain [35, 36]. ZO-1 also interacts with JAM-1 through the PDZ3 domain. ZO-1 stabilizes the tight junction by cross-linking occludins and claudins to the actin cytoskeleton. The phosphorylated occludins and claudins are the main forms located at the tight junctions to maintain tight junction integrity. Dephosphorylation of occludins and

claudins can cause their relocation from the tight junctions' cell surface into the cytoplasmic intracellular compartments [37–40]. This relocation can loosen the tight junctions to increase the penetration of molecules through the tight junctions.

2.2.1.2 Adherens Junctions Adherens junctions are found below the tight junctions and mediate cell–cell adhesion through protein–protein interactions [1, 41]. The adherens junctions are formed prior to the formation of the tight junctions [42]. All three regions of the intercellular junctions work together to make up the transepithelial electrical resistance (TEER) across paracellular pathway [43]. The TEER value is a measure of the integrity of the intercellular junction, and it is a reciprocal of the ability of the ion and small molecule to permeate through the intercellular junctions [44]. The increase in resistance can be correlated to the increase in the number of strands found in the tight junctions of the intestinal mucosa barrier. The TEER values are different in different regions of the human intestine with the jejunum displaying $20\,\Omega\,cm^2$ and the large intestine showing $100\,\Omega\,cm^2$ [45]. Within the adherens junctions, the perijunctional actin–myosin II ring encircles the epithelial cells, impacting the solute permeation in this region [43].

Cell–cell adhesion within the adherens junction is controlled by calcium-dependent E-cadherin and calcium-independent nectin and platelet endothelial cell adhesion molecule-1 (PECAM-1) [1, 15, 46, 47]. E-cadherin is a glycoprotein with an extracellular domain, one single transmembrane domain, and a cytoplasmic domain. The extracellular domain is divided into five repeats (EC1–EC5); each repeat unit has about 110 amino acid residues [48]. Cadherin molecules interact with each other to form cell–cell adhesion in many different ways [15]. For example, E-cadherin protrudes from the cell surface as a dimer (*cis*-dimer), and this *cis*-dimer interacts with another *cis*-dimer from the opposing cell to form a *trans*-dimer. Calcium ions have been shown to be involved in forming a rod-like structure of E-cadherin to induce *trans*-homophilic interactions of E-cadherins. However, structural studies for E- and C-cadherins indicate that there are various ways that cadherins form homophilic interactions [49, 50]. The highly conserved cytoplasmic domain of cadherins is necessary for the adhesion property, and it interacts with α- and β-catenins, which link the cadherins to the actin cytoskeleton [51].

2.2.1.3 Desmosomes The last region of the paracellular pathway is the desmosome, which is located nearest the basolateral membrane surface of the enterocyte. The desmosome is connected by protein–protein interactions of desmocollins (i.e., Dscs: Dsc-1, -2, and -3) and desmogleins (i.e., Dsgs: Dsg-1, -2, -3, and -4), which belong to the calcium-dependent cadherin family such as E- and N-cadherin [52–55]. The difference between Dscs or Dsgs with E-cadherin is in the structure of the cytoplasmic domain. Both Dscs and Dsgs have intracellular cadherin-like sequence (ICS) and intracellular anchor (IA) [52–55]. In addition, Dsgs have proline-rich linker (IPL), repeat unit domain (RUD), and desmoglein terminal domain (DTC). The number of repeats in RUD is different in Dsg-1 (5 repeats) and in Dsg-3 (2 repeats).

2.2.2 Transcellular Pathway

A drug with the appropriate physiochemical characteristics can traverse through the cell by passive diffusion. These optimal characteristics can partly be identified as the rule of five. In the case of peptides, peptidomimetics, and proteins, their physicochemical properties may not be suitable for permeation through the cell membrane via the transcellular pathway. The drug molecules must pass through the lipid bilayers that make up the membranes as the rate-limiting barrier to the passive flow of molecules. The resistance across the transcellular path can be described as resistors in a series arrangement, where the apical and basolateral membranes act as the two resistors [43]. The outer region of the bilayers is surrounded by a large body of water molecules, and it is embedded with proteins (e.g., receptors, enzymes, and transporters) and carbohydrates. The polar head groups of the membranes are in the next region of the cellular barrier, and this region has the highest molecular density, making passive diffusion of drug molecules through the membranes difficult. The third region contains nonpolar tails of phospholipids in the inner membrane region, which have a hydrophobic characteristic [56]. Both bilayer density and hydrophobicity select molecules with optimal physicochemical properties (e.g., size, shape, hydrogen-bonding potential, and hydrophobicity) that can partition into and penetrate the cell membranes.

After the drug partitions into the membranes, it must also enter the cytosol before exiting through basolateral membranes. The cytosol contains various compartments and drug-metabolizing enzymes to trap and/or degrade (metabolize) the drug molecules. It has been shown that basic drugs can be sequestered in the endosomes/lysosomes due to their low pH, and as a result they cannot escape to endosomes to cross the basolateral membranes. Finally, metabolism can change the drug's physicochemical properties from hydrophobic to hydrophilic metabolites that can be trapped in cytosol or lysosomes. Thus, metabolism of the drug can lower the amount of drug molecules that cross basolateral membranes of intestinal mucosa into the systemic circulation.

2.3 BIOCHEMICAL BARRIERS TO DRUG DELIVERY

There is great interindividual variability in the drug metabolism processes as a result of differing enzyme activity (inhibition or induction), genetic polymorphisms, or even disease state [57]. Enzymes found within the intestine are from mammalian and bacteria-associated sources. The mammalian enzymes are located within the lumen and in the enterocytes. The enzymes from microflora are found in the ileum and colon [9]. The focus of this discussion will center only on degradation by the mammalian enzymes.

2.3.1 Metabolizing Enzymes

The first metabolic barrier that drug molecules encounter is a mixture of hydrochloric acid and proteolytic pepsins within the lumen of the stomach. The acidic conditions at pH 2–5 can cause hydrolysis of peptides and proteins, especially when they

contain the aspartic acid residue [5]. The luminal enzymes at the upper small intestine function as the second barrier [5]. In addition, several proteolytic enzymes are found at the lumen of the duodenum, including trypsin, chymotrypsin, elastase, and carboxypeptidase A and B, and their highest activity is found at pH 8. These enzymes degrade 30–40% of large proteins and small peptides within 10 minutes [5].

The major enzymatic barrier is peptidases found within the brush border and in the cytosol of the enterocytes [5]. There is an increase in brush border peptidase activity from the upper duodenum to the lower ileum. These enzymes degrade smaller peptides ranging from di- to tetrapeptides. The brush border peptidases are selective for tripeptides, while the cytosolic proteases have selectivity for dipeptides [5]. The metabolic enzyme activity decreases along the intestine to a nearly negligible rate within the colon, while the drug permeation at the colon epithelium remains good [2]. This indicates that the colon is a good target region for drug delivery to avoid enzyme degradation. The intestinal surface pH on the brush border is 5.5–6.0, and it is more acidic than the lumen pH [58]. The enterocytes have an intercellular pH of 7.0–7.2, and the gastrointestinal pH is also changed during the fasted and fed states [59].

The proximal small intestine shows the greatest metabolic activity due to its large surface area and the plethora of intestinal enzymes and transporters [9]. The intestine has Phase I and II enzymes, and CYP superfamily are the most notable Phase I enzymes. The concentration of P-450 enzymes in the intestinal walls is approximately 20 times less than that found within the liver; however, their drug metabolism activity is comparable to that found in the liver [57, 60]. The highest activity of the P-450 enzymes is displayed in the proximal part of the gastrointestinal tract, and away from the proximal part P-450 enzyme activity decreases [57]. The highest concentration of P-450 enzymes is found in the villus tips of the upper and middle third of the intestine [56]. There is intra- and interindividual variability in an enzyme activity; this is due to the exposure of the enterocytes to external stimuli such as food and drugs that can either induce or inhibit these enzymes. These intestinal P-450 enzymes are more responsive to inducers or stimulators than are their hepatic counterparts [56]. Although the blood flow to the intestine is lower than to the liver, the villus tip has a large surface area where the enzyme can interact freely with its substrate to allow extensive drug metabolisms [61]. Metabolic activity has been shown to be route dependent, and the drug metabolism is normally greater with oral administration than with intravenous administration [57, 62]. In this case, the intestinal metabolism occurs during the initial absorption of the drug across the intestinal barrier, and the metabolism is lower with recirculation of the drug. The major factor that influences route-dependent metabolism is the residence time of the drug within the enterocyte. The residence time can be lengthened when there is drug trapping in cytoplasm and/or lowering of the blood flow. Conversely, the residence time can be shortened due to basolateral clearance by basolateral transporters [57].

The CYP1, CYP2, and CYP3 subfamilies are mostly involved in xenobiotic metabolism. Each subfamily of isoenzymes has its own drug substrate specificity. CYP1A1, CYP2C, CYP2D6, and CYP3A4 enzymes are found within the human small intestine [9]. The characterization of CYP2D6 is difficult because it has

numerous polymorphic forms [63]. The CYP3A4 is the most abundant P-450 enzyme subfamily; it makes up 70% of the CPY in the intestine [63]. There are structural similarities between the intestinal and hepatic CYP enzymes; however, they appear to be independently regulated [9]. Food interactions have been shown to affect the regulation of the intestinal CYP enzymes. Grapefruit juice inhibits CYP3A, while grilled and smoked foods induce CYP1A1 activity [9]. Variations in the enzyme population can also affect the degradation of drug molecules including peptide and protein drugs.

Phase II enzymes are referred to as metabolizing and conjugating enzymes found in the intestine. Phase II enzymes such as glucuronyltransferase, *N*-acetyltransferase, sulfotransferase, and glutathione-*S*-transferase have high activity at the intestinal mucosa barrier [9]. The enzymes can form drug conjugates within the cell to become substrates for the multidrug resistance-associated protein (MRP) family of transporters [64]. The MRP family are ATP-dependent transporters that excrete the substrate into the lumen of the intestinal mucosa.

2.3.2 Transporters and Efflux Pumps

The presence of transporters has been found in the intestinal barrier, and some of these transporters recognize di- and tripeptides. Peptides can be transported through the brush border membrane in a carrier-mediated and pH-dependent fashion [5]. Peptide transport into the cell is energy-dependent and saturable, which are the characteristics of receptor-mediated transport.

Although most transporters are situated on the apical membrane, some of them are also located on the basolateral membrane surface. The Na^+/A amino acid transporter, Na^+/ASC amino acid transporter, GLUT2 hexose transporter, and the Na^+-independent folic acid transporter are examples of such basolateral transporters [56]. PepT1 is an apical H^+/dipeptide transporter, and it is most abundant in the villus tip [18, 65]. The transporter population increases from the duodenum to the ileum, and the expression of this transporter increases during starvation. At the basolateral membrane side, PepT2 transporters act as the H^+/dipeptide transporter to allow the substrates to exit the enterocyte [56, 66].

P-glycoprotein (Pgp) serves as an efflux pump found at the brush borders of the villus tips of the small and large intestine [67–69]. The efflux pump can prevent the membrane partition of small molecules (e.g., natural products, fluorescent dyes, and anticancer drugs) and peptides. The hydrophobic aromatic and tertiary amine serve as signature recognition for this efflux pump in drug molecules. The expression of Pgp increases from the stomach to the colon [7]. Pgp has broad substrate recognition with a wide range of molecular structures, and the substrate affinity varies as a function of intestinal region [56, 68]. A common feature of the substrates is hydrophobicity. As mentioned previously, the efflux pumps assist the intestinal metabolism by returning the drug to the lumen, allowing the metabolizing enzymes to work on the drug another time as well as preventing product inhibition by removing primary metabolites that have been formed [60, 68]. This interaction is enhanced due to the colocalization of the CYP3A enzymes and Pgp on the apical membrane as well

as the overlap in substrate specificities and shared inducers and inhibitors [60, 68]. There are several inhibitors of Pgp including GF-120918, cyclosporine A (CsA), and PCS833, and these inhibitors can enhance the biological barrier transport of drugs that are substrates for Pgp. Grapefruit juice also interferes with the transport mediated by Pgp; however, not all substrates for the CYP3A enzyme behave as substrates for Pgp [67, 70, 71]. Pgp receptors are also expressed in other biological barriers (i.e., liver, kidney, pancreas, and capillary endothelium of the brain) and function as a defense mechanism against xenobiotics [72].

2.4 CHEMICAL BARRIERS TO DRUG DELIVERY

The chemical structure of a drug determines its solubility and barrier permeability profiles and, in turn, the effective concentration at the intestinal lumen influences the rate and extent of intestinal absorption [73]. Unfavorable physiochemical properties have been shown to be the limiting factors in oral absorption of peptides and peptidomimetics [74–76]. As an example, the structural factors that affect the permeation of peptides will be described here.

2.4.1 Hydrogen-Bonding Potential

Hydrogen-bonding potential to water molecules has been shown to be an important factor in the permeation of peptides. Studies *in vivo* and in various cell culture models of the intestinal mucosa and BBB indicate that desolvation or hydrogen-bonding potential regulates the permeation of peptides [73, 76–78]. The energy needed to desolvate the polar amide bonds in the peptide to allow it to enter and traverse the cell membrane is the principle behind the concept of hydrogen-bonding potential. For small organic molecules, the octanol-water partition coefficient is the best predictor of cell membrane permeation with a sigmoidal relationship [77]. However, this is not the case with peptides; the desolvation energy or hydrogen-bonding potential is a better predictor for membrane permeation of peptides. Burton et al. have reported partition coefficients of model peptides in *n*-octanol/Hanks' balanced salt solution (HBSS), isooctane/HBSS, and heptane/ethylene glycol systems [79]. It was found that measuring the partition coefficient of peptide in heptane/ethylene glycol correlates well with the hydrogen-bonding potential. This method is simpler and more direct than the method that uses the difference in partition coefficients between octanol/HBSS and isooctane/HBSS [79].

The predictive rule of five by Lipinski has been used to predict the transport of a molecule through membranes of biological barriers; this rule is based on the H-bond potential, Log *P* value, and molecular weight [80, 81]. Molecules with lower potential H-bonds (e.g., 2 H-bonds) to water have higher membrane permeation than those with higher H-bonds (e.g., 8 H-bonds). A drug molecule with a molecular weight higher than 500 Da with more than five hydrogen bond donors and ten hydrogen bond acceptors is less likely to cross the biological barriers. It is predicted, that even if it is a hydrophobic molecule with a molecular weight higher than 500 Da (e.g., >800 Da), it will have difficulty in crossing the biological membranes of the intestinal mucosa.

2.4.2 Other Properties

In the case of peptides, other properties such as size, charge, and hydrophilicity influence the peptide membrane partition and permeation [74, 76]. A change in hydrophilicity of a peptide may alter its route of permeation; as the lipophilicity increases, the peptide permeation shifts from paracellular to transcellular pathways. Studies using Caco-2 cell monolayers confirm that drug permeation via the paracellular path is size dependent, and this highlights the sieving abilities of the intercellular junctions [76]. Although the paracellular path is negatively charged, the effect of charge on paracellular permeation of molecules is not well understood. One study suggests that a positive net charge on a peptide produces the best paracellular permeation, but another study suggests that a −1 or −2 charge is most effective in paracellular transport [74]. It has also been suggested that the effect of charge is negligible as the molecular size of the peptide increases [74].

2.5 DRUG MODIFICATIONS TO ENHANCE TRANSPORT ACROSS BIOLOGICAL BARRIERS

Several methods have been explored to improve drug permeation across biological barriers [1, 18, 76, 82–86]. One method is by chemical modification of drug entities such as prodrug and peptidomimetic. Another method is designing a formulation that enhances the drug permeation through the biological barriers.

2.5.1 Prodrugs and Structural Modifications

A prodrug approach has been utilized to optimize drug solubility and transport as discussed in more detail in Chapter 12 [87, 88]. A prodrug is defined as a chemical derivative that is inactive pharmacologically until it is converted *in vivo* to the active drug moiety [87, 88]. A targeted prodrug design has emerged in which prodrugs have been used to target membrane transporters or enzymes [83, 84]. This method improves the oral drug absorption or site-specific drug delivery. Extensive knowledge about the structure, distribution within biological barriers, and substrate specificities of the transporter is needed for using it as a target for drug delivery.

Prodrug strategies have been very successful with small molecules; however, their use in peptides has not been widely implemented. The cyclic peptide prodrug approach has been shown to improve peptide membrane permeation [76]. In this method, the N- and C-termini of the peptide are connected via a linker to form a cyclic peptide. The linker can be cleaved by esterase to release the linear peptide. The formation of a cyclic peptide prodrug increases the conformational rigidity, improves the intramolecular hydrogen bonding, and lowers the hydrogen-bonding potential to water molecules as a solvent. In addition, the lipophilicity of the cyclic prodrug increases, which shifts its transport from paracellular to transcellular [75]. It has also been reported that cyclic peptides are less susceptible to amino- and carboxy-peptidases than linear peptides because the amino and carboxy terminals are protected from these enzymes [76].

Peptide structural modification has been applied to improve peptide membrane permeation. Metabolism of peptides can occur in various regions along the route to oral absorption, and inhibition of this degradation is advantageous in enhancing drug delivery. To improve enzymatic stability, peptides have been converted to peptidomimetics. In this case, the peptide bond is converted to its bioisostere that is stable to proteolytic enzymes [86]. Other structural modification strategies to improve membrane permeation of peptides include lipidization, halogenation, glycosylation, cationization, and conjugation to polymers [85].

2.5.2 Formulations

Intestinal absorption of drug molecules can be improved by designing an optimal formulation [86, 89]. For peptides, several methods to enhance drug absorption have been suggested, including addition of ion-pairing and complexation molecules, nonsurfactant membrane permeation enhancers, surfactant adjuvants, or combinations of these additives [86]. Addition of perturbants of tight junctions such as cytoskeletal agents, oxidants, hormones, calcium chelators, and bacterial toxins into the formulation has been investigated to improve drug permeation [89]. Another novel delivery system involves the use of mucoadhesives to enhance drug delivery because of their long retention time at the targeted mucosal membrane; lectins have been identified as potential carriers for peptides in an oral mucoadhesive system [4]. For peptides, their coadministration with inhibitors of metabolizing enzymes has also been suggested to increase oral absorption [86, 90, 91].

Modulations of protein–protein interactions in the intercellular junctions have been shown to increase paracellular permeation of molecules through the biological barriers [1]. Peptides derived from tight junction proteins such as occludin and claudin can modulate the intercellular junctions of the biological barriers. A 29-mer peptide (C1C2) derived from claudin-1 can lower the TEER values of cell monolayers as well as increase the permeation of paracellular permeation of 10 kDa protein, which was labeled with fluorescein isothiocyanate (FITC) [92]. This peptide enhances the brain delivery of opioids such as tetrodotoxin and opioid peptide *in vivo* [92]. A claudin hexapeptide (DFNYNP) can disrupt and weaken the tight junctions of cell monolayers. This disruption is because of internalization of claudins into the vehicles in cell cytoplasmic domain [93]. An occludin peptide called OCC1 can lower the TEER values of Caco-2 cell monolayers, and it increases permeation of ^{14}C-mannitol across the cell monolayers [94]. HAV- and ADT-peptides derived from the EC1 domain of E-cadherin can also modulate the intercellular junctions of MDCK cell monolayers [95, 96]. These peptides lowered the TEER values of MDCK cell monolayers and also enhanced the ^{14}C-mannitol transport across the monolayers. The HAV hexapeptide (Ac-SHAVSS-NH_2) can improve brain delivery of ^{14}C-mannitol, ^{3}H-daunomycin (anticancer agent), and Gd-DTPA (MRI contrast agent) using the *in-situ* rat brain perfusion model [97]. These peptides can also increase the brain delivery of Gd-DTPA, a near IR dye R800, and an 800cw polyethylene glycol (25 kDa) through the BBB in *in vivo* balb/c

mice [98]. These results indicate the possibility of delivering drug and diagnostic molecules through the biological barriers *in vivo* by modulating the cell–cell adhesion at the intercellular junctions.

2.6 CONCLUSIONS

The absorption of orally administered drug molecules depends on the successful passage of the molecules through several barriers to drug delivery. The gastrointestinal epithelial layer is a formidable obstacle to the passage of drugs. The drug molecules can pass either between the cells or through the cells, depending on their physiochemical properties. Recent studies have shown that metabolism within the intestine forms a major obstruction to drug absorption. The concerted activity of these drug-metabolizing enzymes and efflux systems confounds the problem. Although many challenges exist for traversing the intestinal epithelial layer, pharmaceutical scientists and medicinal chemists are overcoming them with innovative methods to optimize pharmacological activity and enhance drug delivery.

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3

PHYSICOCHEMICAL PROPERTIES, FORMULATION, AND DRUG DELIVERY

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3.1 INTRODUCTION

The goal of drug formulation and delivery is to administer a drug at a therapeutic concentration to a particular site of action for a specified period of time. The design of the final formulated product for drug delivery depends upon several factors. First, the drug must be administered using a narrow set of parameters that are defined by the therapeutic action of the drug. Some of the parameters include the site of action (either targeted to a specific region of the body or systemic), the concentration of the drug at the time of administration, the duration that the drug must remain at a therapeutic concentration, and initial release rate of the drug for oral/controlled release systems. Second, the drug must remain physically and chemically stable in the formulation for at least 2 years. Third, the choice of delivery method must reflect the preferred administration route for the drug, such as oral, parenteral, and transdermal.

A complete knowledge of the relevant therapeutic and physicochemical properties of the drug is required to determine the proper formulation and delivery method of a drug. For example, the physicochemical properties of the drug strongly influence the choice of delivery methods. Figure 3.1 shows the interdependence of the three main

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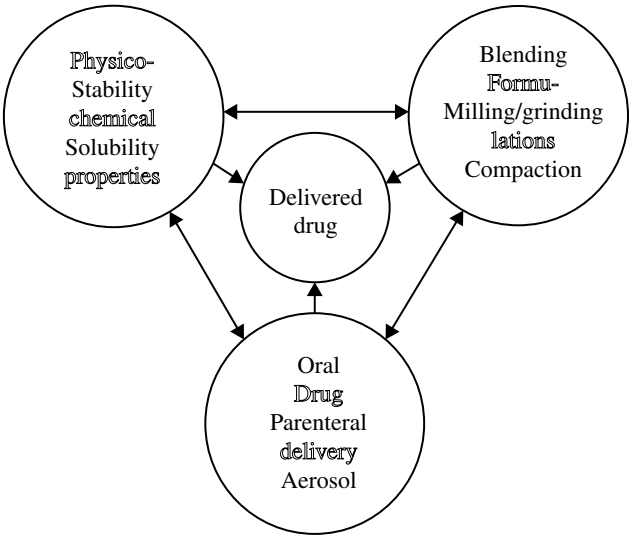

FIGURE 3.1 Schematic diagram showing the interdependence of physicochemical properties, formulation, and drug delivery.

topics covered in this chapter. This creates a problem in dividing this chapter into specific sections, as a discussion of the important physicochemical properties of a drug will be different for oral administration of a solid tablet compared with parenteral administration of a drug in solution. For this reason, we have chosen to take a broad approach in the physicochemical properties section of discussing the basic physicochemical properties that are determined for almost all drugs. A similar approach has been taken in the formulation and delivery sections.

This chapter is divided into three sections. In the physicochemical section, the two most relevant physicochemical properties to drug delivery—solubility and stability—are discussed. In addition to providing a basic understanding of the importance of solubility and stability to drug delivery, methods to enhance solubility and physical and chemical stability are described. The second section focuses on the processes required for the proper drug formulation. Since most drugs are administered in the solid state, the formulation process for tablets is described in detail. The last section is a discussion of some of the basic drug delivery methods, with an emphasis on the physicochemical properties that impact those methods.

3.2 PHYSICOCHEMICAL PROPERTIES

The most important goal in the delivery of a drug is to bring the drug concentration to a specific level and maintain it at that level for a specific duration of time. Stability and solubility are two key physicochemical properties that must be considered when designing a successful drug formulation. Many challenges must be overcome to formulate a product that has sufficient chemical and physical stability to avoid

degradation during the shelf life of the product, yet has sufficient solubility (and dissolution rate) to reach the required therapeutic level.

The physicochemical properties of the drug in both solution and solid state play a critical role in drug formulation. The solid-state form of the drug is often preferred, because it is often more chemically stable, easier to process, and more convenient to administer than liquid formulations. However, if the drug is in the solid state, it must dissolve before it can be therapeutically active, and once it is in solution, it must be both sufficiently soluble and chemically stable. For these reasons it is critical to determine the physicochemical properties of the drug both in solution and in the solid state.

There are several parameters that affect the solubility and chemical stability of a drug in solution. The pH of the solution can dramatically affect both the solubility and chemical stability of the drug. Buffer concentration/composition and ionic strength can also have an effect, especially on chemical stability. The hydrophobic/hydrophilic nature of the drug influences solubility. A typical characterization of a drug will start with a study of the chemical stability of the drug as a function of pH. The structure of the degradation products will be characterized to determine the mechanism of the degradation reaction.

In the solid state, the form of the drug will affect both the solubility and the physicochemical stability of the drug. A full characterization of the drug in the solid state will often include a determination of the melting point and heat of fusion using differential scanning calorimetry, loss of solvent upon heating using thermogravimetric analysis, and a characterization of the molecular state of the solid using diffraction and spectroscopic techniques.

In the following two sections, solubility and stability will be discussed as they relate to drug formulation. In the solubility section, the emphasis is on methods to increase solubility. In the stability section, the emphasis is on describing the types of reactions that lead to decreased stability.

3.2.1 Solubility

A drug must be maintained at a specific concentration to be therapeutically active. In many cases the drug solubility is lower than the required concentration, in which case the drug is no longer effective [1]. There is a trend in new drug molecules toward larger molecular weights, which often leads to lower solubility. The ability to formulate a soluble form of a drug is becoming both more important and more challenging. This has resulted in an extensive research on methods to increase drug solubility.

Solubility is affected by many factors. One of the most important factors is pH. Other factors that affect the solubility of the drug include temperature, hydrophobicity of the drug, solid form of the drug, and the presence of complexing agents in solution.

For drugs with low solubility, special efforts must be made to bring the concentration into the therapeutically active range. In this section some of the common methods to increase solubility will be discussed: salt versus free form, inclusion compounds, prodrugs, solid form selection, and dissolution rate. It should be noted that efforts to increase solubility also have an influence (often negative) on the stability of a compound. For this reason the most soluble form is often not the first choice when formulating the drug.

3.2.1.1 Salt versus Free Forms One of the easiest ways to increase the solubility of a therapeutic agent is to make a corresponding salt form of the drug. The salt form must be made from either the free acid or free base. Carboxylic acids are the most common acidic functional groups found in drug molecules, while amines are the most common basic groups. An important consideration in the choice of salt versus free forms of a drug is that the pH changes depending upon the location in the intestinal tract. In the stomach, the pH is typically 1–3 and changes to 6–8 in the small intestine. Since the majority of adsorption occurs in the small intestine, it is often desirable to have the maximum solubility at neutral to basic pH values. In general, the acid form of a drug will be ionized at intestinal pH values and therefore more soluble, whereas the basic form will be unionized and less soluble. Salts are typically more soluble than the free forms, although this often comes with increased hydrophilicity and possible decrease in chemical stability due to increased moisture sorption.

Usually the choice of salt versus free forms is made based upon the physicochemical properties of each individual compound. However, some generalizations can be made. Free acid forms of a drug usually have adequate solubility and dissolution rates at pH values found in the intestine, and salts of weak bases may be preferred to the free forms because of greater solubility and dissolution rates. It should also be noted that the counter ion can have a dramatic effect upon the solubility and/or stability of the drug. Salt form screening is routinely performed on compounds to determine the counter ion that possesses the best combination of solubility and stability.

3.2.1.2 Inclusion Compounds Another method for improving solubility is to create an inclusion compound between the drug molecule and a host molecule. To be effective, the host/guest inclusion compound must have a higher solubility than the individual drug molecule. An inclusion complex of a drug is usually not crystalline and thus should have a higher solubility than a crystalline material. Cyclodextrins complexed to drugs are an example of inclusion compounds commonly used in pharmaceutics.

Cyclodextrins are nonreducing cyclic oligosaccharides made up of six to eight glucopyranose molecules. This class of molecules has a unique structure that is often represented as a tapered doughnut (with the opening at one side larger than the other). The guest molecule then fits inside this cavity and is much less likely to crystallize. Such complexes are also used to improve drug stability by reducing interactions between the drug and its environment. Chemically modified cyclodextrins, which exhibit different stabilizing effects than the natural forms, are also used. They also increase the solubility of insoluble drugs by complexing the drug with the cyclodextrin, generating a metastable form of the drug. Two examples of drugs whose solubility is enhanced by cyclodextrins are prednisolone [2] and prostaglandin E_1 [3]. Figure 3.2 shows an example demonstrating the improvement in solubility provided by sulfobutyl ether-β-cyclodextrin (Captisol™) for prednisolone [4].

3.2.1.3 Prodrugs Prodrugs are chemically modified forms of the drug that commonly contain an additional functional group (e.g., an ester group) designed to enhance solubility, stability, and/or transport across a biological membrane. Once the

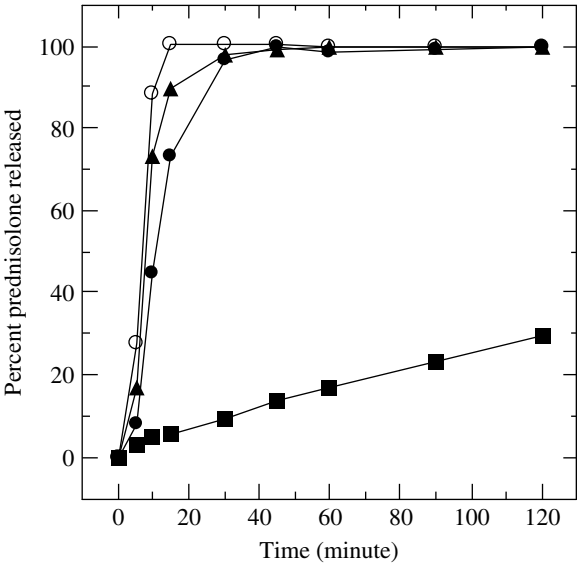

FIGURE 3.2 Plot of percent prednisolone released versus time for different complexes of cyclodextrin and prednisolone. Prednisolone : $(SBE)_{7m}$-β-CD at a 1 : 1 molar ratio (●), Prednisolone : $(SBE)_{7m}$-β-CD at a 1 : 2 molar ratio (○), Prednisolone : HP-β-CD at a 1 : 2 molar ratio (▲), Prednisolone : sugar (■). Okimoto et al. [4], pp. 1562–1568, figure 2. Reproduced with permission of Springer.

prodrug is inside the body, the additional functional group is cleaved off, either hydrolytically or enzymatically, leaving the drug so that it may fulfill its therapeutic function. Examples of prodrugs (given as prodrug[drug]) that improve solubility include fosphenytoin[phenytoin] [5–7], valacyclovir[acyclovir] [8–10], and capecitabine[5-fluorouracil] [11]. More discussion of prodrugs can be found in Chapter 12.

3.2.1.4 Solid Form Selection A drug can exist in multiple forms in the solid state. If the two forms have the same molecular structure but different crystal packing, then they are polymorphs. Pseudopolymorphs (or solvatomorphs) differ in the level of hydration/solvation between forms. Polymorphs and pseudopolymorphs in principle will have a different solubility, melting point, dissolution rate, etc. While less thermodynamically stable polymorphs have higher solubilities, they also have the potential to convert to the more thermodynamically stable form. This form conversion can lead to reduced solubilities for the formulated product. One example is the case of Ritonavir, a protease inhibitor compound used to treat AIDS. Marketed by Abbott Labs as Norvir, this compound began production in a semisolid form and an oral liquid form. In July 1998, dissolution tests of several new batches of the product failed. The problem was traced to the appearance of a previously unknown polymorph (Form II) of the compound. This form is thermodynamically more stable than Form I and, therefore, less soluble. In this case the solubility is at least a factor of two

below that of Form I [12]. The discovery of this new polymorph ultimately led to a temporary withdrawal of the solid form of Norvir from the market and a search for a new formulation.

3.2.1.5 Dissolution Rate While not directly related to solubility, the ability to rapidly reach the therapeutic concentration may be useful for fast-acting therapeutic agents, and compensate for drugs that may have sufficient solubility but are metabolized/excreted too quickly to reach the desired concentration. An example of a method to enhance dissolution is the WOWTAB® technology by Astellas Pharma, Japan [13].

3.2.2 Stability

Formulation scientists must consider two types of stability: chemical and physical. Physical stability is the change in the physical form of the drug, for example, an amorphous form changing into a crystalline form. The chemical composition remains the same as it was prior to crystallization, but the drug now has different physical properties. Chemical stability is a change in the molecular structure through a chemical reaction. Hydrolysis and oxidation are two common chemical degradation pathways.

3.2.2.1 Physical Stability Physical stability can refer to molecular-level changes, such as polymorphic changes, or macroscopic changes, such as dissolution rate or tablet hardness. At the molecular level, form changes include amorphous to crystalline, changes in crystalline form (polymorphism), and changes in solvation state (solvatomorphism). The impact of polymorphic changes on the solubility of Ritonavir was discussed in the previous section. In general, a metastable solid form may convert to a more thermodynamically stable form, and it is usually desirable to market the most stable form if possible to avoid such transformations. The presence of seed crystals of the more stable form may initiate or accelerate the conversion from the metastable form to the more stable form. In addition, the presence of solvents, especially water, may cause formation of a solvate with significantly different physicochemical properties. Desolvation is also a possible reaction. For drug formulations, the choice of salt forms (hydrates, solvates, and polymorphs) plays a role in identifying the most suitable form for the pharmaceutical product. Polymorphism in drug formulations makes the characterization of polymorphic forms very important. This is most commonly done with X-ray powder diffraction or solid-state NMR spectroscopy.

When improvements in physical stability of a product are needed, choices must be made based upon the nature of the problem and the desired goal. One of the first choices made is to use the most stable polymorph of the drug. This may involve an extensive polymorph screening effort to attempt to find the most stable polymorph. If the most stable polymorph is undesirable for some reason (e.g., solubility issues), then avoiding contamination of the desired polymorph with seeds of the most stable polymorph becomes very important. In a product that uses an amorphous form of a drug, it is critical to inhibit crystallization to avoid dramatic changes in stability and solubility.

3.2.2.2 Chemical Stability Chemical degradation of the drug includes reactions such as hydrolysis, dehydration, oxidation, photochemical degradation, or reaction with excipients. The constant presence of water and oxygen in our environment means that exposure to moisture or oxygen can affect the chemical stability of a compound. Chemical stability is very important not only because a sufficient amount of the drug is needed at the time of administration for therapeutic purposes but also because chemical degradation products may adversely affect the properties of the formulated product, and may even be toxic.

Determining how a drug degrades and what factors affect degradation is very important in pharmaceutical product development. The importance of reaching (or avoiding) the activation barrier of a particular chemical process makes temperature one of the most important variables in this area [14]. A second factor in drug degradation is pH. The degradation rate depends on the pH of the formulation and/or the compartments of the body in which the drug is present. Many drug degradation pathways are catalyzed by either hydronium or hydroxide ions, reiterating the important role of water [14]. Described below (with an example or two) are several degradation reactions including hydrolysis, dehydration, oxidation, photodegradation, isomerization, racemization, decarboxylation, and elimination.

Hydrolysis is one of the most common drug degradation reactions. In hydrolysis reactions the drug reacts with water to form two degradation products. The two most common types of hydrolysis reactions encountered in pharmaceutical chemistry are the hydrolysis of ester or amide functional groups. Esters hydrolyze to form carboxylic acids and alcohols, while amides form carboxylic acids and amines. For example, the ester bond in aspirin is hydrolyzed to produce salicylic acid and acetic acid, while the amide bond is hydrolyzed in acetaminophen [15–17].

Dehydration reactions are another common degradation pathway. Ring closures are a fairly common type of dehydration, as is seen for both lactose [18, 19] and glucose [20–22]. Both of these compounds dehydrate to form 5-(hydroxymethyl)-2-furfural. Batanopride is another example of a compound that can undergo a dehydration reaction [23].

Elimination degradation pathways are also possible. Decarboxylation, in which a carboxylic acid releases a molecule of CO_2, occurs for p-aminosalicylic acid [24]. Oxidation is very common as well, largely due to the presence of oxygen during manufacture and/or storage. Several examples can be found in Yoshioka and Stella [14]. Isomerization and racemization reactions are other degradation pathways. Some examples of compounds that undergo isomerization reactions are amphotericin B [25] and tirilazad [26].

Photodegradation of pharmaceuticals has been known for decades. A complication encountered when studying photodegradation reactions is that there are many degradation pathways that each have the potential to yield different products. When oxidizers are present, photodegradation can accompany oxidation.

There are several options available to improve the stability of drugs. One is the use of cyclodextrins, in which the formation of the inclusion complex produces a more stable form of the drug. Examples of cyclodextrins inhibiting drug degradation include tauromustine [27], mitomycin C [28], and thymoxamine [29]. Another

possibility is to generate a prodrug that has increased stability compared to the parent compound. Examples of prodrugs that enhance stability include [prodrug (drug)] enalapril (enalaprilat) [30–32] and dipivefrin (epinephrine) [33].

3.3 FORMULATIONS

Formulation is the stage of product manufacturing in which the drug is combined with various excipients to prepare a dosage form for delivery of the drug to the patient. Excipients are defined by IPEC-Americas [34] as "… substances other than the pharmacologically active drug or prodrug which are included in the manufacturing process or are contained in a finished pharmaceutical product dosage form." These include binders to form a tablet, aggregates to keep the tablet together, disintegrants to aid dissolution once the drug is administered, and coloring or flavoring agents. Excipients help keep the drug in the desired form until administration, aid in delivering the drug, control the release rate of the drug, or make the product more appealing in some way to the patient.

Formulation is dictated by the physicochemical properties of the drug and excipients. Each drug delivery method has specific formulation issues. As previously mentioned, the solid dosage form is the most convenient and most preferred means of administering drugs, and therefore the discussion here will focus on solid dosage forms. The vast majority of solid dosage forms are tablets, which are produced by compression or molding. Powders are the most common form of both the drug and the excipients prior to processing. The process of creating tablets from bulk materials has a number of steps. Some of these are discussed later.

3.3.1 Processing Steps

First, milling is often used to ensure that the particle size distribution is adequate for mixing. Milling both reduces the particle size and produces size and shape uniformity. There are several milling options, though perhaps the most common is the ball mill, in which balls are placed inside a hard cylindrical container along with the bulk drug. The cylinder is then turned horizontally along its long axis to cause the balls to repeatedly tumble over one another, thereby breaking the drug particles into smaller pieces.

Next, the drug and excipients must be blended or mixed together. It is very important at this stage that the bulk properties of the materials be conducive to mixing. This means that the materials must have good flowability characteristics. Lubricants such as magnesium stearate may be added to improve the flowability of the formulation.

Once the formulation has been blended, it must then be compressed into a tablet. Flowability remains important at this stage of the processing because a uniform dose of the blended ingredient mixture must be delivered to the tableting machine. Poor flowability results in poor tablet weight reproducibility. Lubricants are needed to ensure that the tablet can be removed intact from the die once it has been compressed. Finally, the tablet may require a coating. This could be as simple as a flavor coating,

or it could be an enteric coating designed to avoid an upset stomach by delaying dissolution until the tablet enters the small intestine.

3.3.2 Influence of Physicochemical Properties on Drugs in Formulations

Most of the processing steps depend at least indirectly upon the physicochemical properties of the drug. Particle size, shape, and morphology often are determined by the solid form of the drug and the conditions from which the drug is crystallized. Aspirin, for example, can have multiple crystal morphologies depending upon the conditions of recrystallization [35]. Processing can also result in changes in the form of the drug. Amorphous drug formation, changes in the polymorphic form of the drug, or the production of crystal defects can all have a negative effect upon the solubility and stability of the drug [36]. Drug–excipient interactions can affect both solubility and stability. These interactions impact the physical properties of the drug by altering its chemical nature by reactions such as desolvation, or the Maillard reaction (also known as the browning reaction based on the color of the products).

Physicochemical changes in the form of the drug at the formulation and processing stages are almost always undesirable. Such changes can be very costly if found only toward the end of product development. Thus, many times it is desirable to perform preformulation studies to determine the optimum form for delivery [14, 37].

3.3.3 Other Issues

New excipients are needed in the industry, as not all formulation needs are satisfied by currently known excipients. This situation is likely to worsen over time as new products, each with potentially unique requirements, are brought to the development stage. Despite this need, the introduction of new excipients is becoming more difficult [38] because new excipients face similar regulatory requirements as new drugs themselves. Difficulty in satisfying different nations' regulatory requirements on excipients sometimes makes it more difficult for companies to make a single product that can be marketed in different countries.

3.4 DRUG DELIVERY

For many drugs, the therapeutic nature of the drug dictates the method of administration. For example, oral drug delivery may be the most logical choice for gastrointestinal diseases. If drug release is systemic, then the choice of method often relies on the physicochemical and therapeutic properties of the drug. Transdermal drug delivery, although having the advantage of being noninvasive, has several criteria that must be met by the drug in order to be delivered properly, such as high potency, ready permeability through the stratum corneum, and non-irritation.

In drug delivery, the three most important questions are as follows: When is the drug delivered? Where is the drug delivered? How is the drug delivered? For this reason the rest of the section is divided into three parts that address the when, where, and how of drug delivery.

3.4.1 Duration of Release

The goal of drug delivery is to maintain the drug at the appropriate therapeutic level for a specified period of time. There are several methods to achieve this goal, some of which are demonstrated in Figure 3.3. The first is the administration of a single dose, with immediate release of drug to the site of action. This method is useful for acute therapeutic treatment requiring a short period of action. For chronic problems, the goal is to maintain the drug at the therapeutic level for a sustained period of time. Multiple-dose administration is one method for providing sustained therapeutic levels of drug. However, there are many disadvantages to multiple-dose therapy, including variations in drug levels during the treatment period and requiring patient compliance with dosage regimen requirements. To avoid this problem, non-immediate release devices are used to deliver the drug over an extended period of time. Non-immediate release devices have three types of release mechanisms: delayed release, prolonged release, and controlled release. Delayed release allows multiple doses to be incorporated into a single dosage form, alleviating the problem of frequent dosing and patient noncompliance. The prolonged release device extends the release of the drug, for example, by slowing the dissolution rate of the drug compared with an immediate release device. The controlled release device meters out the drug to maintain a constant release rate throughout the desired dosage period. In the prolonged and controlled release dose, there is usually an initial release of drug to bring the drug into the therapeutic window, followed by additional drug that is released over a longer period of time. Nonimmediate release devices maintain a more consistent level of drug than multiple doses while retaining the advantage of requiring fewer doses, which helps with patient compliance. The disadvantage of non-immediate

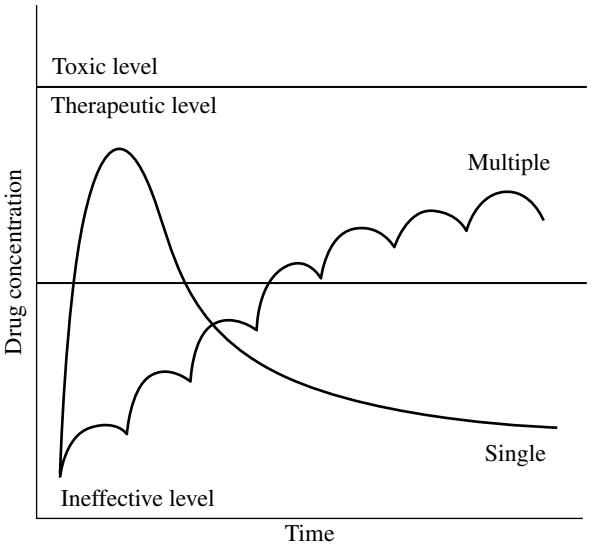

FIGURE 3.3 Plot of drug concentration versus time for single- and multiple-dose therapy.

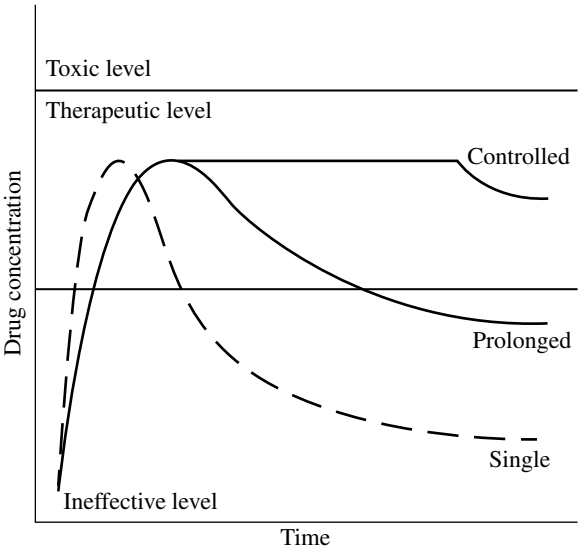

FIGURE 3.4 Plot of drug concentration versus time for nonimmediate release systems.

release delivery devices is the inability to stop delivery if adverse reactions are observed in the patient. The concentration characteristics of different non-immediate release systems are shown in Figure 3.4.

Non-immediate or sustained release devices can be divided into two categories. The first is a reservoir device, where the drug is loaded into the reservoir as either a solid or a liquid. Drug release occurs by diffusion through either a semipermeable membrane or a small orifice. Lasers are commonly used to generate uniform orifices through which the drug will diffuse. Osmotic pressure is commonly used to provide the driving force for drug dispersion. The second is a matrix diffusion device, where the drug is dispersed evenly in a solid matrix. Polymers are commonly used as the matrix. Drug delivery is accomplished by either dissolution of the matrix, with corresponding release of drug, or diffusion of the drug from the insoluble matrix.

The physicochemical properties of the drug are critical in the design of the dosage form. Solubility, stability, and pH can strongly affect whether a drug can be delivered effectively from a controlled delivery device. Because sustained release devices often contain multiple doses that if released immediately would reach toxic levels, the physicochemical properties and formulation process may have to be more tightly controlled compared with immediate release systems.

3.4.2 Site of Administration

Targeted drug delivery is often used if the desired site of action is located in a diseased organ or tissue, and release of the drug systemically would produce toxic or deleterious effects. One approach to targeted drug delivery is to place the delivery device adjacent to the site of action, which is especially applicable if the device is

controlled release. The other approach is to design the drug so that it has a particular receptor that is found only within the targeted tissue.

3.4.3 Methods of Administration

There is an increasing number of delivery systems that are available for drug delivery. The drug delivery method is chosen based upon the physicochemical properties of the drug, the desired site of action, the duration of action, and the biological barriers (including rapid drug metabolism) that must be overcome to deliver the drug. Some of the most common delivery methods are tablets (oral), parenteral, transdermal, and aerosol. The advantages and disadvantages of each of these methods are described in the text that follows.

3.4.3.1 Oral Administration The oral drug delivery method is the most common and usually the most preferred drug delivery method by both the formulator and the patient for reasons discussed earlier in this chapter. If the oral delivery method is not chosen, it is primarily due to incompatibilities with the physicochemical properties, site of action, or a biological barrier. Disadvantages of oral drug administration form include the low pH of the gastric juices, the first-pass effect of the liver, oral metabolism, and that some patients may have difficulty swallowing the dosage form.

3.4.3.2 Parenteral Administration Parenteral dosage forms include a wide variety of delivery routes, including injections, implants, and liposomes. The advantages of parenteral delivery systems are that they avoid first-pass effects, oral metabolism, and the harsh chemical environment of the stomach's gastric juices. The disadvantage is that the delivery mechanism is invasive.

3.4.3.3 Transdermal Administration Transdermal drug delivery systems have several advantages over other drug delivery methods. These include avoiding gastrointestinal drug adsorption, first-pass effects, replacement of oral administration, and oral metabolism. It also provides for multiday therapy from a single dose, quick termination of drug administration, and rapid identification of the medication. The biggest disadvantage of transdermal delivery systems is that only relatively potent drugs are suitable for administration in this manner. Other disadvantages include drug irritation of the skin and adhering the system to the skin.

3.4.3.4 Aerosol Administration Aerosols can be used for nasal, oral, and topical drug delivery. For topical delivery, aerosols have the advantages of convenient use, protection from air and moisture, and maintaining sterility of the dosage form. Metered dose inhalers (MDIs) are used for oral and nasal delivery of drugs. MDIs are used most effectively for the treatment of asthma and are being developed for the delivery of insulin [39]. They have the advantages of avoiding first-pass effects and degradation within the GI tract, and rapid onset of action. Some of the disadvantages of aerosol delivery systems for oral and nasal delivery include particle size uniformity of drug for proper delivery.

3.4.3.5 Other Delivery Methods In addition to the methods described earlier, suspensions, emulsions, ointments, and suppositories are all effective drug delivery methods. New delivery methods are continually being developed as many of the new drugs have low solubilities and stabilities, requiring improved methods for delivery of these drugs. Improvements in the delivery methods for peptides and proteins are necessary as they continue to be developed as drugs.

3.5 CONCLUSION

Designing a successful drug delivery method for a new therapeutic agent requires a thorough understanding of the physicochemical properties of the drug. If all of the relevant physicochemical properties are not determined, the drug may not be correctly formulated, resulting in product failure at scale up, or even after the drug is on the market. In this chapter we have tried to explain some of the relevant physicochemical properties that must be considered in the proper formulation of a drug method.

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4

TARGETED BIOAVAILABILITY: A FRESH LOOK AT PHARMACOKINETIC AND PHARMACODYNAMIC ISSUES IN DRUG DISCOVERY AND DEVELOPMENT

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4.1 INTRODUCTION

Drug delivery strategies, including the design and choice of target and biological molecular platforms are intended to improve drug efficacy and safety to enhance the overall therapeutic index of new or existing drug. Pharmacokinetic (PK) and pharmacodynamic (PD) properties of drugs play an important role in the drug delivery; these properties are a critical part of drug discovery and development process. It has been increasingly recognized that early optimization for key parameters such as absorption, distribution, metabolism, and excretion (ADME) of a drug candidate during the drug discovery process is important to reduce the failure rate during the development stage. With the rapid increases in cost and duration for drug discovery and development, the critical decisions are being made at every stage of drug development. PK–PD evaluations and analyses can identify the key

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"drug-like" ADME properties and establish the PK–PD relationship of drug efficacy and safety, which help in decision making.

4.2 TARGET BIOAVAILABILITY

In assessing pharmacokinetic (PK) and pharmacodynamic (PD) issues in drug delivery, it is necessary to carefully consider the definition of bioavailability. The concept of "target bioavailability," a term that extends the idea that the true bioavailability of a drug, is the fraction of the administered dose that reached the site of action.

One way of viewing the many processes involved in the delivery of a drug from its site of administration to its site of action is to consider each barrier along the delivery path. One or more mechanism(s) may be affecting the rate and extent of drug that reaches the site of action during these processes (Fig. 4.1). The process depicted in Figure 4.1 is a representative example for oral administration and is not meant to be exhaustive. The potential barriers that a compound must pass to reach the systemic circulation are traditionally thought to contribute to the bioavailability of a compound (Fig. 4.1). Since the target bioavailability is the fraction of the administered dose that reached the site of action. Additional barriers need to be overcome after the drug leaves the bloodstream to reach the site of action (Fig. 4.1). The importance of various barriers depends on the site of administration and the physicochemical characteristics of the drug.

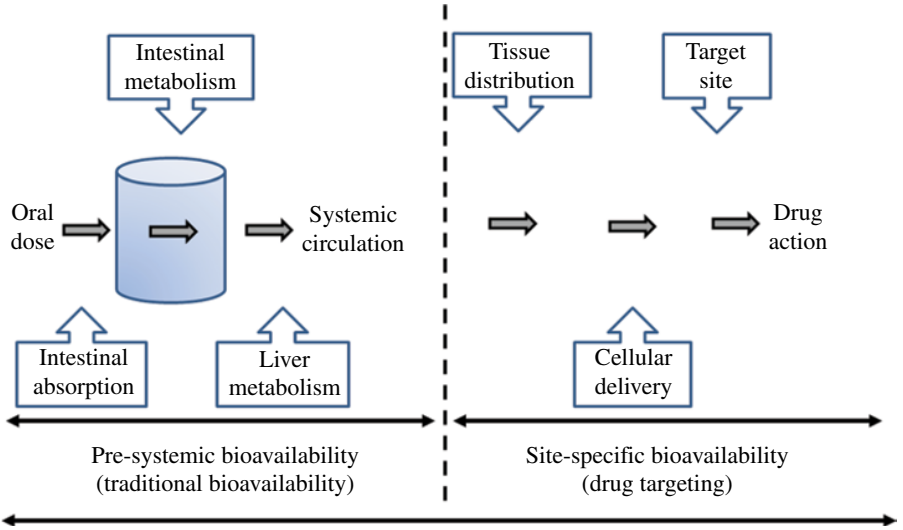

FIGURE 4.1 A schematic representation of the potential biological barriers that an orally administered compound must pass before reaching the site of action.