ORAL BIOAVAILABILITY
Basic Principles, Advanced Concepts, and Applications
Edited by Ming Hu and Xiaoling Li
ORAL BIOAVAILABILITY
Wiley Series in Drug Discovery and Development

Binghe Wang, Series Editor

A complete list of the titles in this series appears at the end of this volume.
Dedicated to my dad Zhengye Hu whose inspiration lives on with this book,
to my mom Qihua Chang whose constant love and encouragement persists to this date,
to my wife Yanping Wang whose company endears constant push for perfection, and
to my children Vivian and William whose energy and noise are missed now they are in college.

—Ming Hu

Dedicated to my grandmother Yunzhi Su,
my parents Bailing Li and Jie Hu,
my wife Xinghang, and
my children Richard and Louis
for their unconditional love, encouragement, and understanding.

—Xiaoling Li
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FOREWORD

In Spring of 1983, I took a position at The University of Michigan. There I met my first Chinese student, Ming Hu, from mainland China, and began a personal and professional relationship that has lasted for nearly 30 years. He is now a Professor at the University of Houston and one of the two editors of this book. I am very pleased to have observed his contributions to science and his success as a scientist over the nearly 30 years I have known him and followed his career. It is a pleasure to write this foreword for this book coedited by Ming and his former classmate at Shanghai Medical University, Prof Xiaoling Li at University of the Pacific.

This book has two purposes, to give readers a contemporary understanding of the science of oral bioavailability and to present the state-of-the-art tools that can be used to advance the science of oral bioavailability and solve problems in the development of drug products for oral administration. It presents the advances in the science of oral bioavailability over the last five decades. This multidisciplinary scientific field has steadily progressed from an emphasis on physical sciences such as solubility and solid state properties, to incorporating the significant recent advances in the biological sciences that emphasize transporters, enzymes, and the biological and physiological processes that influence their expression and function.

I will note some of the evolutionary and perhaps revolutionary steps this field of oral bioavailability has taken over the last five decades. In the 1960s and 1970s, application of the physical sciences to the problem of oral drug delivery produced the first wave of major advances that shaped the development of the modern commercial oral dosage form and the science of oral bioavailability. Important physicochemical principles and strategies such as manipulation of dissolution via physical manipulation of the drug and drug product and chemical modification using prodrugs were developed. These approaches are routinely considered and applied in the drug product development process today. The principles governing sustained and controlled release formulations were developed in those “early” years (e.g., Higuchi equation), and have become widely applied in the later decades of the twentieth century. In the 1980s, important progress in the science of oral bioavailability was led by the development of two critical absorption models, rat intestinal segment perfusion model (developed in my laboratory) and Caco-2 cell mono-layer culture model (developed in Dr Ronald T. Borchartt’s lab). Prof Hu studied in both laboratories, and was an early contributor to the development of both of these systems for the study of oral absorption. These methods have since become widely adapted by the pharmaceutical industries. This set the basis for predicting oral absorption and partitioning bioavailability into its component process, dissolution/release, transport/permeation, and metabolism, notability distinguishing absorption and systemic availability. During the 1980s, major advances were also made in the study of metabolism in the intestine as well as the liver, particularly the cytochrome P450s and resultant potential drug–drug interaction mechanisms. In addition to predicting oral absorption, my laboratory also pioneered the concept of exploiting the intestinal mucosal cell peptide transporter (hPEPT1) to improve the oral absorption of polar drugs by making a prodrug, chemically combining the drug and an amino acid with a peptide-bond like structure. This mechanistic concept is the basis for the absorption of many polar drugs and prodrugs. The development of several approved prodrugs including valacyclovir and valganciclovir, while originally empirical, is based on these
transport mechanisms. In the 1990s, I established the concept of the Biopharmaceutical Classification System (BCS), partitioning drugs into classes for drug development and drug product regulation. This BCS approach has found wide use in drug discovery, development as well as regulation. It has been adapted by regulatory authorities and governments around the world as a basis for the regulation of drug product quality.

During this same period, the US Food and Drug Administration began the mandate of requiring studies that predict drug–drug interactions based on the sciences that were developed during the past two decades. Study of efflux transporters began in the 1990s and has exploded in the twenty-first century. While efforts to make an inhibitor of \( p \)-glycoprotein for anticancer application have not produced an approved drug, it is likely that the future will see such a development. The explosion in the study of transporters is ongoing, with the recent addition of efflux transporters such as multidrug resistance-related proteins (MRPs), breast cancer resistant protein (BCRP), and uptake transporters such as organic anion transporting peptides (OATP), organic anion transporters (OATs), and carboxylic acid transporter (CAT). Such advances in our mechanistic understanding of oral bioavailability will most certainly lead to future advances in therapy.

The advances in the science of oral bioavailability is driven by the needs to develop orally administered drugs, which remains the most acceptable patient compliant means of administering drugs to patients across the globe today. Although the scientific basis was most often the pursuit of industrial scientists, a lack of rapid advancement in the science of oral bioavailability became recognized as a hurdle in the drug development process in the early 1990s as many highly potent compounds (high affinity ligands), for example, HIV \textit{in vitro} were inactive in humans. In a timely or even a watershed event, the National Institute of Health in 1994 organized a conference on “Oral Bioavailability,” where scientists of various backgrounds were organized to address the complex problem facing potent yet poorly bioavailable drug candidates, particularly anti-HIV candidates. Senior managements in many of the major pharmaceutical companies became aware of and recognized the importance of “bioavailability” as the pharmaceutical industry was working hard to fast track the development of anti-HIV drugs. This led to investment by the pharmaceutical industries in the technology and scientists to tackle this oral delivery problem. While actual numbers can be hard to obtain and interpret, my impression is that the attention to bioavailability has led to the decrease in the percentage of clinical trial failures due to oral bioavailability problems. Looking even further into the future, I believe the science of oral bioavailability will be driven by the needs for personalized medicine, individualized treatment plan tailored to patients, and by the commercial need to increase the efficiency and efficacy of oral drug product development. This book provides a comprehensive survey of the modern study of the science of oral bioavailability in the twenty-first century.

GORDON L. AMIDON, Ph.D

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PREFACE

Since the concept of bioavailability has been introduced, significant progress has been made in understanding the science of oral bioavailability and in improving the oral delivery of drugs. Yet, we also find that there is still much to be discovered to have a good handle on oral bioavailability. As a subject, bioavailability encompasses the knowledge and technologies from various disciplines. A pharmaceutical scientist in a specific research area will benefit from a treatise on the topic. Hence, the objective of this book is to provide the framework for fundamental concepts and contemporary practice of bioavailability in pharmaceutical research and drug development.

It is our belief that this book provides both the basic concepts to a novice and the advanced knowledge to veteran pharmaceutical scientists and graduate students in related research fields. Chapter 1 gives a high level summary of this book. The basic concepts of bioavailability are covered in Chapter 2–13. From Chapter 14 to 26, the advanced concepts of bioavailability are discussed in greater depth. Various approaches and methods for improving and studying bioavailability are highlighted in Chapter 27 to 33. The comprehensive coverage of topics on bioavailability in this book offers readers a choice of logically building their knowledge on bioavailability from basic concepts to advanced applications or à la carte based on their specific needs.

A book with such diverse contents requires a multidisciplinary effort. Without the efforts of contributors from different areas, this book would have not been a reality. We would like to personally thank all authors for their contributions and patience during the completion of this book project. Sincere thanks are gratefully extended to Mr Jonathan Rose at John Wiley and Sons, Inc. and Dr Binghe Wang (the book series editor) for their patience, understanding, support, and confidence in us. We would also like to express our appreciations to Mrs. Kathy Kassab for her invaluable secretarial assistance, and to Haseen Khan for her tireless effort in the book production. Finally, we would like to thank the world renowned scientist and leading expert in bioavailability, Prof Gordon L. Amidon for writing an insightful and inspiring forward for this book.

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BARRIERS TO ORAL BIOAVAILABILITY—AN OVERVIEW

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1.1 INTRODUCTION
Oral bioavailability of a drug is a measure of the rate and extent of the drug reaching the systemic circulation and is a key parameter that affects its efficacy and adverse effects. Therefore, study of oral bioavailability has received considerable attention in scientific arena. Unfortunately, we are unable to predict bioavailability as a priori to this date, although we have made significant progress in understanding various components of this complex puzzle, including solubility (e.g., aqueous solubility), partition coefficients (e.g., octanol/water), absorption (e.g., permeability across the Caco-2 cell membrane), metabolism (e.g., microsome-mediated phase I metabolism), and excretion (e.g., efflux via p-glycoprotein). However, understanding a few of these components would not allow us to accurately predict a drug candidate’s bioavailability in humans. Therefore, oral bioavailability remains to be a highly experimental parameter that eludes prediction from modern computational or experimental approaches, although some preliminary progress has been made in recent years. Continued progress to develop a better and more thorough understanding of physicochemical and biochemical profiling of drug or drug-like molecules would be needed to alleviate the problems associated with bioavailability, and some progress has been made in the last decade (Ho and Chien, 2009). Poor oral bioavailability is also one of the leading causes of failures in clinical trials. This is because compounds with low bioavailability would have a highly variable exposure between individuals. If a compound has an average bioavailability of 5%, it would easily vary in the range of 0.5–10%, a 20-fold difference. This difference makes the selection of an appropriate dose particularly difficult since too little may yield no impact and too much could result in toxicity, which is not acceptable for most drugs that desire chronic administration.

The reasons why oral bioavailability is such a challenge for development of drugs or drug-like substances (e.g., nutraceuticals) are several-fold: first, many physicochemical and biological factors contribute to the bioavailability of a compound; second, many scientific disciplines are involved but few, if any, scientists are good at more than one specific area; third, reliable scaling from animal models to humans is often absent; and fourth, oral bioavailability is often seriously affected by diet and polypharmacy, neither of which can be adequately controlled in a standard clinical trial, considering the diversity of the population—the elderly and seriously ill patients.

In addition, we are normally able to gain access only to limited body fluids such as blood and urine, and fluids surrounding the target tissues/cells are often not accessible. This limitation makes bioavailability, a measure of the extent and rate of absorption and the elimination processes,
Figure 1.1 Organ bioavailability barriers to drugs. The processes that include dissolution from the solids to molecules, transport of the dissolved molecules via passive and carrier-mediated uptake transporters into the cells, and phase I and phase II metabolism inside the enterocytes and beyond are depicted. Drug metabolism mostly occurs in the liver. Drug elimination is mainly via bile and kidney, so other elimination route (e.g., exhalation) is not shown. (See insert for color representation of the figure.)

really representing only systemic blood exposure to drugs (Fig. 1.1). Therefore, it is not surprising that bioavailability would sometimes not satisfactorily correlate with efficacy.

Oral bioavailability remains a major challenge to the development of nutraceuticals and naturally derived chemopreventive agents. For example, many scientists are interested in developing plant-derived polyphenols into chemopreventive agents. Polyphenols are derived from plants and consumed in the form of fruits, vegetables, spices, and herbs. In different regions of the world, a large percentage of dietary polyphenols are consumed in the form of flavonoids from various sources of food intake, although cultural and dietary habit dictates which forms of polyphenols are consumed (Fletcher, 2003; Slavin, 2003; Aggarwal et al., 2007). On the other hand, a large percentage of population do not take sufficient quantities of fruits and vegetables for a variety of reasons (Adhami and Mukhtar, 2006). Therefore, scientists are interested in developing a pill that will mimic the effects of ingesting fruits and vegetables. Yet, today their effort has not produced a single polyphenolic chemopreventive agent; the unsuccessful attempt may be attributed to the poor bioavailability of polyphenols (usually <5%). Poor bioavailability makes the evaluation of a chemopreventive agent a particular challenge, since the clinical trials for chemopreventive agents often involve a large population for a prolonged period and extremely high costs.

When all of the above-mentioned challenges are taken into consideration in the product development of drugs or chemopreventive agents, it is obvious that developing an appropriate oral dosage form for drug candidate or candidate of chemopreventive agent is not a trivial or straightforward task. Although pharmaceutical scientists have great difficulty in predicting and enhancing bioavailability, the reward is also immense as the vast majority of top revenue and prescription leaders are orally administered drugs. Therefore, we devote this chapter to briefly introduce each of the factors that influence bioavailability and guide the readers to the appropriate chapters in this book where they can obtain in-depth contents of each related topic.

As an oral dosage form enters the oral cavity and then the gastrointestinal (GI) tract, several barriers must be overcome before it can reach the systemic circulation and the therapeutic target. On its way to the therapeutic target, a drug in a given dosage form will need to first overcome the preabsorption barrier formed by the hostile acidic and enzymatic environment in the stomach and intestine. Then the drug would encounter the primary barrier formed by the biological membrane, that is, the wall of the GI tract. Once a drug successfully passes the intestinal epithelium barrier, the drug will need to overcome another barrier consisting of transporters and enzymes, which utilize the efflux mechanism to pump the drug back to the intestine and degrade the drug via the first-pass effect. There are
many factors that will affect a drug molecule’s ability to overcome these barriers to reach and remain in the systemic circulation. These factors include the inherent physicochemical properties of the drug molecules, biological characteristics of the GI tract, pathophysiological state, drug–drug or drug–food interactions, etc.

### 1.1.1 Physicochemical Factors

Various physicochemical factors will affect the oral bioavailability of a drug. The importance of physicochemical properties of a drug molecule in drug absorption or permeation was illustrated by Lipinski’s “rule of 5” (Lipinski et al., 2001). Because of the importance of physicochemical properties, a thorough characterization of drug substance would provide fundamental information for drug discovery, as well as for formulation and dosage form development. The characterization of key physicochemical properties of drug substances is described in Chapter 2. One of the key physicochemical properties that play a crucial role in the drug absorption/permeation is solubility. Solubility defines the maximum concentration of a drug available for absorption or permeation, while another important physicochemical property, dissolution rate, controls the rate of the drug available for absorption or permeation. Factors that affect solubility and dissolution rate surely will also influence the bioavailability of the drug. Variation of pH in the GI tract causes drugs to behave differently in terms of solubility and dissolution rate along the GI tract. For an acidic drug, a low solubility and slow dissolution rate in the stomach, where pH is low, can be expected, while for a basic drug, poor solubility owing to precipitation in the intestinal fluids, where pH is high, would happen. An understanding of the basic concept of solubility and dissolution rate forms a solid foundation for comprehending bioavailability. Physicochemical factors also dictate the permeability of drug molecules. Solubility and permeability of a drug are such important factors for drug absorption or bioavailability. The combined effect of these two factors would determine the developability and bioavailability of a compound to a certain extent. Chapters 3 and 4 discuss the two important factors related to drug absorption, namely, solubility and dissolution rate. Chapter 6 provides the fundamentals for drug permeation or absorption. Chapter 7 correlates the physicochemical parameters in vitro and in vivo.

### 1.1.2 Biological Factors

Oral delivery is a preferred route for the administration of small molecule drugs, because the intestine has a very large surface area, in excess of 200 m², which is the size of a tennis court. Since oral absorption is limited by the drugs with molecular weight <600Da and effective absorption window in the GI tract, permeability of drug through intestinal membrane, physiology of GI tract, and metabolism of drugs in absorption and transport have become important factors with respect to bioavailability. GI tract is not always a hospitable place for drug absorption. Enzymes are secreted in the GI tract at a rate of about 45 g per day in adult humans. Although the primary functions of these enzymes are to digest nutrients such as protein, carbohydrates, and nucleotides, their presence is one of the primary reasons why protein and genetic materials (for gene therapy) cannot be delivered orally, unless special formulation approaches are used. In addition to surviving in the hostile environment, a drug needs to overcome the barriers posted by the intestinal epithelium. Intestinal epithelium is a complex tissue with advanced cellular structures and metabolic functionality. The presence of cellular junctions, especially tight junction, severely impedes the passage of molecules with molecular weight >200 Da via the paracellular route. Therefore, the vast majority of the drug molecules must use the transcellular route. Transcellular route is affected by a myriad of interrelated but sometimes competing biological factors. Although it was always believed that lipophilic molecules have an easy access to the transcellular route, the presence of various efflux transporters that preferentially bind with lipophilic molecules could seriously limit the absorption of lipophilic molecules. In addition, if a molecule is too lipophilic (e.g., log P >5), it may be retained in the cellular membrane. Because intestinal epithelial cells have a functional existence of only three to four days (near or at the tip of the intestinal villus), molecules that bind too tightly will be eventually lost when the epithelial cells slough off. Hydrophobic drug molecules with molecular weight >200 Da cannot penetrate the intestinal epithelium by passive diffusion; they must have special structural motifs that make them attractive for the nutrient transporters such as amino acid transporters (Chapter 17), the small peptide transporter 1 (or PepT1) (Chapter 18), organic ion transporters (Chapter 19), and nucleobases transporters. Assuming drug molecules get into the epithelial cells, there are intestinal first-pass metabolisms capable of further degrading their chance to reach the systemic circulation. These metabolisms are primary phase II metabolism although CYP3A4 is thought to be decently active in the enterocytes. In Chapters 5, 6, 8, and 10, the barriers to oral bioavailability have been described in greater details, with emphasis on GI biology (Chapters 5 and 16), drug absorption (Chapter 6) and metabolism pathways (Chapter 8), and drug excretion by the enterocytes (Chapter 9).

The last major barrier to oral bioavailability, perhaps the most well-known one, is the first-pass metabolism in the liver. Since all drugs absorbed via the GI tract (except the last few centimeters of the rectum) have to enter the portal vein and encounter hepatocytes (each of which can
be called metabolic superstars, escaping liver metabolism is the last step in the oral absorption process.

In addition to these important factors, protein binding, which affects drug distribution and free drug available for metabolism, has also featured in this book (Chapter 11). Lastly, another general factor that affects the systemic exposure, elimination via urine, is discussed in Chapter 12. Taken together, substantial information is provided on the pharmacokinetic behaviors of drugs following oral administration (Chapter 13), many of which can be explained using the information learned from previous chapters.

1.1.3 Diet and Food Effects

Development of drugs is becoming more global and multidimensional. The day where a standard diet is appropriate for clinical trials across the globe is probably over. Traditionally, diet and food effects have focused on the protein content, caloric intake, and fat amounts, and few if any have carefully examined the effects of other more exotic dietary components such as spices. More recently, consumers are taking greater quantities of dietary supplements with increasing frequency and variety. Although we are unable to completely address how these changes in the diet will impact drug bioavailability, various attempts have been made. Chapter 14 has shed some light on this topic.

1.1.4 Drug Interactions

Drug interactions remain a serious concern for the development of new drugs. On the basis of the target patient population, certain types of drug interactions are not acceptable to the manufacturer, FDA (Food and Drug Administration of the United States of America), or both. Traditionally, drug interactions are classified into pharmacodynamic interactions and pharmacokinetic interactions and this book mainly deals with the latter in Chapter 15, since it is a book focused on oral bioavailability.

Classical pharmacokinetic drug interactions typically involve phase I metabolic enzymes, and clinical examples of this type of interactions are well documented in the literature. From a pharmacokinetic point of view, drug interaction may cause a rise or a fall in body exposure of drug, that is, change in $C_{\text{max}}$ (maximal drug concentration) and/or AUC (area under the curve) values. From a mechanistic point of view, a rise in exposure is typically related to inhibition of enzyme activities or down regulation of relevant metabolic enzymes, whereas a fall in exposure is typically related to activation of dormant enzymes or induction of relevant metabolic enzymes.

More recently, FDA is contemplating the inclusion of efflux transporters into the drug interaction universe, and provisional guidance has been issued. This could further complicate the drug development process and increase the complexity and cost of development. The reasons are several-fold. First, many drugs undergo efflux and phase I metabolism simultaneously and therefore it is difficult to sort out the precise mechanisms of drug interactions. Second, there are few demonstrated clinical cases where interactions with efflux transporters have been confirmed as the sole source of drug interactions. Third, metabolic enzymes may develop significant interplay with the efflux transporters such that it would be necessary to interact with both components of the disposition in order to display clinically significant effects. Many of these are discussed in Chapter 26.

1.1.5 Formulation Factors

Based on the physicochemical and biological factors that affect the bioavailability, we can use different strategies to overcome the barriers for bioavailability (Chaubal, 2004). One can design a dosage form that can avoid the harsh environment in the stomach or optimally utilize the absorption window. For example, an enteric-coated dosage form will not dissolve until it reaches the intestine while a gastroretentive drug delivery system can prolong the resident time of dosage forms in the GI tract. Oral dosage forms can be coated with rate-limiting membranes that can control the rate of drug release from the dosage form. Increased solubility and dissolution rate are effective ways to improve bioavailability. One can create an effective dissolution rate that supplies the proper amount of soluble drug for absorption. Nanoparticles are increasingly becoming an important part of modern drug dosage form design as incorporation of nanoparticles can often alleviate challenges associated with poor solubility. Varieties of pharmaceutical technologies and drug delivery approaches have been used to improve the physicochemical properties of the drugs. Approaches for various dosage form design and solubility/dissolution enhancement can be found in Chapters 20 and 21. Enhancement of solubility and dissolution rate allows formulation scientists to manipulate the factors related to drug substances for improving bioavailability. To improve bioavailability, one can also modulate the permeability of drug across the intestinal epithelium. Chapters 22 and 23 represent some of the attempts in this direction.

1.2 SCIENTIFIC DISCIPLINES INVOLVED

It was once thought that the intestinal tract is a very accommodating organ for drug absorption because this organ is built to absorb nutrients. If drugs are good for us, should the intestinal tract be there to do what benefits us? It was not until 1990s that the myth—if medicinal chemists can develop active compounds in vitro, formulation scientists can make a finished product to deliver them in vivo—was found to be untrue. Development of two classes of
compounds, renin inhibitors and HIV protease inhibitors, convinced drug development scientists and senior management in pharmaceutical companies that oral bioavailability matters because the intestine is not just an absorption organ.

Scientists with various training and education backgrounds are involved in the development of orally administered drugs. Aside from classical biologists and medicinal chemists that are involved in the drug discovery phase, more preclinical ADME (absorption, distribution, metabolism, and excretion) works are now integrated into the drug discovery area. Once a candidate is selected, additional ADME work plus toxicology will be needed to further advance the candidate into clinical trials. Then, physician researchers, nurse practitioners, biostatisticians, marketing professionals, and pharmaceutical economists become involved in the clinical studies. At the same time, a different group of scientists, many with engineering background, are making decisions on the manufacturing and processing parameters. Therefore, it is not entirely unexpected that scientists in different groups do not always have the proper background to fully understand each other. One of the purposes of this book is to provide an easy-to-understand section for the scientists in different areas to understand ADME and their terminologies.

One of the important goals of this book is to give a practical guide to the use of several state-of-the-art technologies and methodologies to readers who may or may not be familiar with these techniques in order to gain a basic understanding and knowledge for practical approaches and/or methods. The readers can dive into the contents ranging from advanced reviews on various important efflux transporters that affect drug absorption and excretion (Chapter 24), to the coupling between efflux transporters and enzymes (Chapter 25), to computational methods and approaches to predict bioavailability (Chapter 33). For the technology and methodology, the readers can find a detailed description on the Caco-2 cell culture model (Chapter 27), MDCK (Madin Darby canine kidney and other related cell culture models (Chapter 28), intestinal perfusion (Chapter 29), liver perfusion (Chapter 30), primary hepatocytes (Chapter 30), in vivo pharmacokinetics (Chapter 31), and methods to determine regulation of enzymes and transporters (Chapter 32).

Rapid advances in the human genomics and proteomics promise to better predict factors determining human responses to drugs. Although the price of sequencing the whole human genome remains out of the practical range at present, rapid advances in this area are expected to make the practice an economic reality in the not so distant future. Recent passage of a law by the Congress of the United States of America to ban discrimination based on genetic information should provide the legal framework to protect an individual’s right to utilize his/her genetic information for better health care. This law and progress in the economics of human genome sequencing will mean that, in the near future, we could develop criteria that will dose patients according to his/her genetic makeup—a radical progress in the field of individualized pharmacy. We will all welcome the day that geneticists become active participants in the drug development process, instead of limiting their participation only in the drug discovery process.

1.3 SUMMARY AND OUTLOOK

Oral bioavailability remains a big challenge for small molecules, and an even bigger challenge for macromolecular drugs such as protein. Despite decades of effort, there is no product for oral insulin. This book devotes itself to the study of various biological and physicochemical principles and methodologies that can be used to understand the oral bioavailability problems and to devise strategies that can be used to overcome these problems. Although we still cannot predict bioavailability as a priori at this time, it is getting closer to the moment when we would be able to do so for the small molecular drugs. Efforts undertaken by various drug delivery companies are on the brink of achieving oral delivery of active insulin. Therefore, the science of oral bioavailability is closer than ever in the history of drug development to become an enabler of drug development, instead of an obstacle to drug development. Together with the advent of individualized genomic information, we are heading to a day when each of the patients could receive drug according to his/her conditions. We are all very hopeful that this day is within our grasp in the near future.

REFERENCES

PHYSICOCHEMICAL CHARACTERIZATION OF PHARMACEUTICAL SOLIDS

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2.1 INTRODUCTION

Solid dosage forms, such as tablets and capsules, are a common means of administration of pharmaceutically active ingredient (API) in humans (Gennaro, 1985; Brittain et al., 1991; Brittain, 1995). They are manufactured by processing a number of powdered solids together, most commonly, blending or mixing of multiple components, milling or size reduction, granulation which may be done either using a granulating fluid or in the dry state (roller compaction), compression into tablets and coating (Lachman et al., 1986). All these processes may be influenced by the physical properties of the solids, and, thus, their importance is being increasingly recognized. A number of tests have been included in the United States Pharmacopeia (USP) to characterize these physical properties of powdered solids (The United States Pharmacopeia and National Formulary, 2002). It is important to have a comprehensive understanding of the physical characteristics earlier during product development to prevent future problems such as was observed with ritonavir. In this case, a new, more thermodynamically stable, less soluble polymorph was being formed during bulk drug manufacturing after the product was launched in the market. As a consequence, stability and bioavailability of the product were at risk (Bauer et al., 2001).

The API and excipients used in formulations can exist in various physical phases that can impact processability,
stability, and performance of the formulation, which have been briefly summarized below.

2.1.1 Crystalline and Amorphous Phases

Crystalline materials are characterized by a regular, well-defined, and long-range (\(-20 \text{ Å}\)) periodicity in the arrangement of the constituent atoms, ions, or molecules. They exhibit sharp melting points and characteristic X-ray powder patterns. Amorphous materials lack long-range order in their molecular arrangement. They do not have melting points and their X-ray patterns show a broad halo. The most common route of obtaining amorphous solids is by rapid cooling of a melt below its melting point where the structural characteristics of the liquid are maintained but the viscosity is much higher. This is considered as a “supercooled liquid.” On further cooling, a characteristic temperature known as the glass transition temperature ($T_g$) is observed below which the solid is “kinetically frozen” into an unstable glassy state with properties different from both the supercooled liquid and crystalline form. Amorphous solids possess higher free energy than their crystalline counterparts due to which they have higher apparent solubilities, dissolution rates, and enhanced chemical reactivities (Hancock and Zografi, 1997). For example, the amorphous form of sulfapyridine was found to have a higher apparent solubility and dissolution rate than its crystalline counterpart (Gouda et al., 1977). When the same dose was administered to dogs, therapeutically adequate concentrations were obtained with amorphous novobiocin while the crystalline form was not absorbed at all. This difference in bioavailability was attributed to the differences in apparent solubility and dissolution rates between the amorphous and crystalline phases (Mullins and Macek, 1960).

2.1.2 Polymorphic Forms

Polymorphism is the ability of a compound to crystallize as two or more phases having different arrangements and/or molecular conformations in the crystal lattice (Brittain, 1999). Polymorphs of a given compound are chemically identical but, in general, different in structure and properties including dissolution rates, melting point, density, hardness, and crystal shape. The choice of the polymorphic form may determine the physical and chemical stability, compressibility, and bioavailability of the drug substance (Haleblian et al., 1971). A compound can exist in a number of polymorphic forms but only one form is thermodynamically stable at a given temperature and pressure, while the others are metastable. The metastable polymorphs have higher free energies, apparent solubilities, and dissolution rates than their stable counterparts. The larger the free energy difference between the stable and metastable polymorphs, the higher is the expected difference in solubility (Brittain, 1999). For example, when different polymorphs of tolbutamide (forms I, II, III, and IV) were administered to dogs, forms II and IV showed higher bioavailabilities than forms I and III (Kimura et al., 1999). While it is generally preferred to formulate the most thermodynamically stable form, under some circumstances, it may be desirable to use the metastable forms in formulations because of their higher dissolution rates. However, owing to higher reactivity of metastable phases, their physical, as well as chemical, stability needs to be carefully monitored during processing and storage.

2.1.3 Solvates

Solvates are adducts or molecular complexes containing solvent molecules within the crystal structure in either stoichiometric or nonstoichiometric proportions. If the incorporated solvent molecule is water, the solvate is referred to as a hydrate (Vippagunta et al., 2001). The incorporation of the solvent molecule in the crystal lattice results in differences in physical and pharmaceutical properties. Differences in solubility of the hydrated and anhydrous phases may result in a difference in bioavailability. When anhydrous ampicillin and ampicillin trihydrate were administered to dogs, the peak serum levels following administration of the anhydrate were higher and occurred earlier (Poole et al., 1968). Similar results were observed after their oral administration to humans (Ali and Farouk, 1981). Similarly, differences in the solubility of the anhydrous and the dehydrate phases of carbamazepine resulted in differences in bioavailability when administered to dogs. The bioavailability of carbamazepine dehydrate was lower than that of the corresponding anhydrate forms (Kobayashi et al., 2000).

The discussion above indicates that it is important to characterize materials to select and control the desirable form in formulations. Brittain et al. (1991) have defined a systematic approach for physical characterization of pharmaceutical solids where the material properties were classified as molecular, particulate, and bulk properties. The objective of this chapter is to summarize the techniques used for physicochemical characterization of solids based on this classification. This chapter provides only a brief outline and the reader is encouraged to go through the references for a deeper understanding of the subject.

2.2 MOLECULAR LEVEL PROPERTIES

These are defined as those characteristics that could be measured at a molecular level. They include spectroscopic techniques and are based on properties such as molecular interactions and molecular bond energies. These studies
can be performed at a very early stage with the advantage of using minimum amount of material and providing information regarding polymorphic form, solvate phase, and crystallinity.

2.2.1 Ultraviolet/Visible (UV/vis) Diffuse Reflectance Spectroscopy

Although UV/vis techniques are widely used for the analysis of solutions, it can be adapted for the characterization of solids. This is performed by using diffuse reflectance techniques instead of in the transmission mode. It is associated with the fraction of radiation that penetrates into the bulk and then emerges (Brittain, 1995). The instrumentation consists of a light source, a monochromator, an integrating sphere, and a detector. The instrument and sample preparation may be optimized to minimize undesirable specular reflectance (surface reflectance of the incident beam). Several diffuse reflectance theories have been proposed but the Kubelka–Munk theory is the most generally accepted. Diffuse reflectance can be expressed as

\[ \frac{k}{s} = \frac{(1 - R_\infty)^2}{2R_\infty} \]

where \( k \) and \( s \) = the molar absorption and the scattering coefficients, respectively, and \( R_\infty \) = the reflectivity of an infinitely thick sample.

The equation is valid in weakly absorbing systems with a small particle size (∼1 μm) without significant contribution from specular reflectance (Kubelka, 1948).

UV/vis diffuse reflectance spectroscopy has been used to evaluate solid–solid interactions in formulations. The effect of formulation composition and processing variables on the microenvironment in solid dosage forms was evaluated using indicator probes providing a measure of the physicochemical nature of the formulation in the solid state (Govindarajan et al., 2006a). The technique has also been used to correlate pH of the solution before freeze-drying and chemical reactivity of the freeze-dried material (Govindarajan et al., 2006b), and evaluation of the Maillard reaction between a primary amine and lactose (Wu et al., 1970).

2.2.2 Vibrational Spectroscopy

Infrared (IR) and Raman spectroscopies are complementary techniques widely used for the characterization of pharmaceutical solids. The IR region in the electromagnetic spectrum can be divided into three regions: the near-IR (4000–14,000 cm\(^{-1}\)), mid-IR (400–4000 cm\(^{-1}\)), and far-IR (100–400 cm\(^{-1}\)) with the near- and mid-IR generally being used for analysis. When a sample is irradiated, absorption of IR energy results in transitions between molecular vibrational and rotational energy levels (either of single pairs of atoms or groups of atoms). The molecular vibrations depend on the structure of the analyte and thus can be used for the identification of a molecular identity. IR and Raman spectroscopies complement each other by evaluating various functional groups. While polar groups such as C=O and NH are likely to be IR active, bonds such as C=C and SS are more likely to be Raman active (Brittain, 1995).

Spectrometers usually consist of an electromagnetic source, a sample chamber, and a detector. For IR analysis, sample preparation can be carried out using different methods including (i) alkali halide pellet, where the analyte is pulverized with either KBr or KCl and compressed into discs; (ii) mull preparation, where the analyte is mixed with ~1 mg of mineral oil; (iii) use of a neat powdered sample in diffuse reflectance infrared Fourier transform spectroscopic technique (DRIFTS), it is noninvasive but is particle size dependent; (iv) attenuated total reflectance (ATR), where the sample is placed in contact with an IR transmitting crystal with a high refractive index through which the IR beam is directed and then penetrates a few micrometers into the sample; and (v) photoacoustic spectroscopy—modulated IR radiation is selectively absorbed by the sample. Similar to DRIFTS, no sample preparation is required for Raman spectroscopy where analysis can be carried out on small samples of the neat material. Both IR and Raman spectroscopies can be combined with an optical microscope enabling analysis of a few crystals (Bugay, 2001).

Since IR and Raman techniques are based on the molecular structure of materials, they can be used for the analysis of polymorphs and solvates. Five forms of tranilast (three polymorphs and two solvates) were characterized by using IR and Raman spectroscopies. While form II was determined to be a conformationally distinctive polymorph, form III had a different packing with weaker intermolecular hydrogen bonds. Owing to its ease and rapidity, IR spectroscopy was used to determine polymorph contamination during process development (Vogt et al., 2005). Some authors have reported the use of these techniques for the analysis of materials with different degrees of crystallinity (Okumura and Otsuka, 2005) and solid dispersions (Konno and Taylor, 2006). Raman spectroscopy was used to detect amorphous indomethacin at a level of 2% w/w using a calibration curve of indomethacin with various degrees of crystallinity. A growing application of NIR spectroscopy is in the field of process analytical technologies (PATs) such as real-time monitoring of blending processes (El-Hagrasy and Drennen, 2005).
2.2.3 Solid-State Nuclear Magnetic Resonance (SSNMR)

Nuclear magnetic resonance (NMR) spectroscopy has been extensively used to analyze molecules in the solution phase. The use of NMR for the study of solids is now being widely used for the characterization of pharmaceutical solids. There are several interactions that a nucleus with a magnetic moment undergoes when placed in a magnetic field. Some of these include Zeeman interactions (interaction between magnetic moment of the nucleus and the external magnetic field), dipole–dipole interaction (magnetic coupling of two nuclei through space), chemical shift (magnetic shielding by surrounding electrons), and spin–spin couplings (indirect coupling between two spins). A thorough discussion of all these interactions is outside the scope of this chapter; therefore, references are provided (Brittain, 1995; Tishmack et al., 2003).

There are, however, some differences in the analysis of solutions and solids. The strong dipolar interactions and chemical shift anisotropy (CSA) in solids lead to broad peaks in the spectrum. These are not observed in solutions because of the fast random motions of the samples. CSA occurs in solids since the orientation of the molecules is fixed with respect to the magnetic field. These issues can be overcome by probes that can be subjected to high decoupling power and by rapidly spinning the sample. Magic angle spinning (MAS) can also be used wherein the line width can be minimized by spinning the sample at an angle of 54.7° with the magnetic field (Brittain, 1995; Tishmack et al., 2003).

Solid-state nuclear magnetic resonance (SSNMR) has been used for the qualitative and quantitative characterization of solids that can exist as polymorphs, solvates, or in the amorphous state. MAS NMR was used for the evaluation of several polymorphs and hydrates of olanzapine (Fig. 2.1) (Reutzel-Edens et al., 2003). The spectra were characterized by highly resolved, sharp resonances. The unique chemical shifts reflected the presence of the carbon nuclei in different polymorphic forms and states of hydration. Presence of form II in form III as an impurity was observed using X-ray diffraction (XRD) and this was confirmed by using SSNMR (Reutzel-Edens et al., 2003). In a review by Shah et al. (2006), several examples are discussed where SSNMR was used for the evaluation of amorphous forms of drugs.

2.2.4 Solubility

Solubility is defined as the concentration of the dissolved solid in a solvent medium in a saturated solution in equilibrium with the solid at a defined temperature and pressure (Martin et al., 1983). The time dependence of this solubilization process is often measured, which is referred to as dissolution testing (Grant and Higuchi, 1990) (see Chapter 4 for details). Various dissolution testing methods of dosage forms have been reported in the USP. Some of the factors affecting solubility include the physical form of the solid, the nature and composition of the solvent, temperature, and pressure (The United States Pharmacopeia and National Formulary, 2002). Solubility is the topic of discussion in Chapter 3, and therefore has not been discussed in detail here.