ADVANCED DRUG DELIVERY

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WILEY
I would like to dedicate this book to the pharmaceutical industry.

Ashim K. Mitra

I owe my deepest gratitude to my wife, Dr. Yuyueng Lee, for her love, devotion, and enormous support. I am pleased to mention my children, Eddie and Jason, who have given me encouragement and endless challenge.

Chi H. Lee

I dedicate this book to my parents, Mr. Guangxiong Cheng and Mrs. Pingqing Xu; my wife Lizhi Sun; my children Daniel and Jessica for their love and continuous support; and my mentors who have inspired me to pursue a career in science.

Kun Cheng
CONTENTS

PREFACE xi
ABOUT THE AUTHORS xiii
CONTRIBUTORS xv

PART I  INTRODUCTION AND BASICS OF ADVANCED DRUG DELIVERY 1

1 Physiological Barriers in Advanced Drug Delivery: Gastrointestinal Barrier 3
D. Alexander Oh and Chi H. Lee

2 Solubility and Stability Aspects in Advanced Drug Delivery 21
Hoo-Kyun Choi, Robhash K. Subedi, and Chi H. Lee

3 The Role of Transporters and the Efflux System in Drug Delivery 47
Varun Khurana, Dhananjay Pal, Mukul Minocha, and Ashim K. Mitra

4 Biomaterial in Advanced Drug Delivery 75
Megha Barot, Mitesh Patel, Xiaoyan Yang, Wuchen Wang, and Chi H. Lee

PART II STRATEGIES FOR ADVANCED DRUG DELIVERY 103

5 Strategies of Drug Targeting 105
Ravi S. Shukla, Zhijin Chen, and Kun Cheng

6 Prodrug and Bioconjugation 123
Ranny Krishna Vadlapatla, Sujay Shah, Aswani Dutt Vadlapudi, and Ashim K. Mitra
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Stimuli-Responsive Target Strategies</td>
<td>Chi H. Lee</td>
</tr>
<tr>
<td>9</td>
<td>Implants</td>
<td>Aswani Dutt Vadlapudi, Ashaben Patel, Ramya Krishna Vadlapatla, Durga Paturi, and Ashim K. Mitra</td>
</tr>
<tr>
<td>10</td>
<td>Aptamers in Advanced Drug Delivery</td>
<td>Weiwei Gao, Omid C. Farokhzad, and Nazila Kamaly</td>
</tr>
<tr>
<td>11</td>
<td>Nanofiber</td>
<td>Megha Barot, Mitan R. Gokulgandhi, Animikh Ray, and Ashim K. Mitra</td>
</tr>
<tr>
<td>12</td>
<td>Biomimetic Self-Assembling Nanoparticles</td>
<td>Maxim G. Ryadnov</td>
</tr>
<tr>
<td>13</td>
<td>Protein and Peptide Drug Delivery</td>
<td>Mitesh Patel, Megha Barot, Jwala Remukuntla, and Ashim K. Mitra</td>
</tr>
<tr>
<td>14</td>
<td>Delivery of Nucleic Acids</td>
<td>Shaoying Wang, Bin Qin, and Kun Cheng</td>
</tr>
<tr>
<td>15</td>
<td>Delivery of Vaccines</td>
<td>Hari R. Desu, Rubi Mahato, and Laura A. Thoma</td>
</tr>
<tr>
<td></td>
<td>PART III TRANSLATIONAL RESEARCH OF ADVANCED DRUG DELIVERY</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Regulatory Considerations and Clinical Issues in Advanced Drug Delivery</td>
<td>Mei-Ling Chen</td>
</tr>
<tr>
<td>17</td>
<td>Advanced Drug Delivery in Cancer Therapy</td>
<td>Wanyi Tai and Kun Cheng</td>
</tr>
<tr>
<td>18</td>
<td>Advanced Delivery in Cardiovascular Diseases</td>
<td>Gayathri Acharya, Wuchen Wang, Divya Teja Vavilala, Mridul Mukherji, and Chi H. Lee</td>
</tr>
<tr>
<td>19</td>
<td>Recent Advances in Ocular Drug Delivery</td>
<td>Varun Khurana, Deep Kwatra, Vibhuti Agrahari, and Ashim K. Mitra</td>
</tr>
<tr>
<td>20</td>
<td>Advanced Drug Delivery Against STD</td>
<td>Chi H. Lee</td>
</tr>
<tr>
<td>21</td>
<td>Advanced Drug Delivery to the Brain</td>
<td>Nanda K. Mandava, Mitesh Patel, and Ashim K. Mitra</td>
</tr>
</tbody>
</table>
PART IV  FUTURE APPLICATIONS OF ADVANCED DRUG DELIVERY IN EMERGING RESEARCH AREAS 423

22  Cell-Based Therapeutics  425
Zhaoyang Ye, Yan Zhou, Haibo Cai, and Wen-Song Tan

23  Biomedical Applications and Tissue Engineering of Collagen  445
Chi H. Lee and Yuyung Lee

24  Molecular Imaging of Drug Delivery  469
Zheng-Rong Lu

ANSWERS  489

INDEX  511
PREFACE

During the past four decades, we have witnessed unprecedented breakthroughs in advanced delivery systems for efficient delivery of various therapeutic agents including small molecules as well as macromolecules. The development of advanced drug delivery systems for small-molecule drugs not only improves drug efficacy but also opens up new markets for the pharmaceutical industry. The global market for advanced drug delivery systems is expected to increase to $196.4 billion through 2014. On the other hand, remarkable progresses in molecular biology and biotechnology over the past two decades have not been matched by progresses in efficient delivery systems for the improvement of therapeutic efficacy. Therefore, it is integral to transform our knowledge in molecular biology and biotechnology into the development of effective delivery systems for macromolecular therapeutics.

Advanced Drug Delivery aims to provide up-to-date information of the basics, formulation strategies, and various therapeutic applications of advanced drug delivery. The goal of this book is to teach the philosophy of how to articulate practically the concepts of pharmaceutical sciences, chemistry, and molecular biology in such an integrated way that can ignite novel ideas to design and develop advanced delivery systems against various diseases.

This book is divided into four parts, starting with fundamentals related to physiological barriers, stability, transporters, and biomaterials in drug delivery. Then, it moves on to discuss different strategies that have been used for advanced delivery of small molecules as well as macromolecules. The third part focuses on regulatory considerations and translational applications of various advanced drug delivery systems in the treatment of critical and life-threatening diseases, such as cardiovascular diseases, cancer, sexually transmitted diseases, ophthalmic diseases, and brain diseases. The book ends with the applications of advance drug delivery in emerging research fields, such as stem cell research, cell-based therapeutics, tissue engineering, and molecular imaging. Each chapter provides objectives and assessment questions to facilitate student learning.

According to the report from the American Association of Pharmaceutical Scientists (AAPS), there is a critical shortage of well-trained pharmaceutical scientists in the areas of product development and related pharmaceutical technologies. We hope that this book will serve as a valuable tool not only for pharmacy graduate and undergraduate students but also for those healthcare professionals who have no pharmacy background but are engaged with drug development.

Finally, we would like to express our sincere appreciation and gratitude to all the contributors who spent enormous effort to share their knowledge and expertise in multiple aspects of advanced drug delivery.

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ABOUT THE AUTHORS

Ashim K. Mitra  received his Ph.D. in pharmaceutical chemistry in 1983 from the University of Kansas. He joined the University of Missouri—Kansas City (UMKC) in 1994 as chairman of the Pharmaceutical Sciences Department. He is currently the Vice Provost for Interdisciplinary Research, the UMKC Curators’ Professor of Pharmacy, and a co-director of the Vision Research Center, UMKC School of Medicine. He has more than 30 years of experience in the field of ocular drug delivery and disposition. He has authored and co-authored over 280 refereed articles and 60 book chapters in the area of formulation development and ocular drug delivery; he has been awarded 9 patents and has presented (along with his research group) well over 500 presentations/abstracts at national and international scientific meetings. Prof. Mitra’s work has attracted over US$6 million in funding from government agencies such as the National Institutes of Health (NIH), Department of Defense (DOD), and pharmaceutical companies. He is the recipient of numerous research awards from NIH, AAPS, AACP, ARVO, and pharmaceutical organizations.

Chi H. Lee  received his B.S. degree in pharmacy from Seoul National University, South Korea. After getting his M.S. degree at the University of Washington, Seattle, he attended Rutgers University, North Brunswick, NJ, where he earned his Ph.D. degree. He completed his postdoctoral training at the University of Michigan Medical Center, Ann Arbor.

Prof. Lee was previously a member of the faculty at the University of Louisiana, Monroe, College of Pharmacy, before he moved to the University of Missouri—Kansas City, where his responsibilities include teaching undergraduate and graduate pharmacy students.

Prof. Lee has been actively involved in pharmaceutical research for more than three decades and has a special interest in the areas of formulation development and pathological mechanisms on microbicidal and cardiovascular devices and polymer-based systems. He has authored more than 55 articles and three book chapters on those subjects, and he has delivered more than 200 scientific presentations at local, national, and international symposia.

Prof. Lee has received grants from various funding agencies including the National Institutes of Health (NIH) and the American Heart Association. He has served as a member of the American Association of Pharmaceutical Scientists, Society for Biomaterials, American Association of College of Pharmacy, Controlled Release Society, and American Heart Association.

Kun Cheng  is an associate professor of pharmaceutical sciences at the University of Missouri—Kansas City (UMKC). He received his B.S. and M.S. degrees in pharmaceutical sciences from China Pharmaceutical University. He also received an M.S. degree in pharmacy from the National University of Singapore. He worked at the Bright Future Pharmaceutical Company in Hong Kong prior to joining the University of Tennessee Health Science Center, where he received his Ph.D. in pharmaceutical sciences. His current research focuses on the development of novel drug delivery systems for siRNA and small-molecule drugs.
Much of the effort from his laboratory has dealt with the therapeutic exploration of macromolecular agents, which have poor stability and inefficient cellular uptake.

Prof. Cheng has been actively engaged in extramural professional activities and in teaching graduate and PharmD students. He has edited one book titled *Advanced Delivery and Therapeutic Applications of RNAi* and two theme issues for the journals *Molecular Pharmaceutics* and *Pharmaceutical Research*. He is the recipient of the 2011 American Association of Pharmaceutical Scientists (AAPS) New Investigator Grant Award in Pharmaceutics and Pharmaceutical Technologies.
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PART I

INTRODUCTION AND BASICS OF ADVANCED DRUG DELIVERY
1

PHYSIOLOGICAL BARRIERS IN ADVANCED DRUG DELIVERY: GASTROINTESTINAL BARRIER

D. ALEXANDER OH AND CHI H. LEE

1.1 Chapter objectives
1.2 Introduction
1.3 Physiological factors influencing drug absorption
1.4 Physicochemical factors influencing drug absorption
1.5 Strategies to overcome gastrointestinal barriers in drug delivery
1.6 Summary
Assessment questions
References

1.1 CHAPTER OBJECTIVES

- To outline gastrointestinal anatomy and physiology impacting advanced oral drug delivery systems.
- To review key physiological and physicochemical factors influencing drug absorption.
- To illustrate efficient strategies for overcoming gastrointestinal barriers in drug delivery.

1.2 INTRODUCTION

Drug delivery through oral administration is a complicated process. A drug must withstand the digestive processes and penetrate through the gastrointestinal (GI) barrier into the bloodstream. Drugs absorbed from the GI tract travel through portal veins to the liver, and then they are subjected to first-pass metabolism by the hepatic enzymes before entering the systemic circulation [1]. The oral route of drug administration is traditionally known as the most preferred route for systemic drug delivery, even though there are disadvantages, such as unpredictable and erratic absorption, gastrointestinal intolerance, incomplete absorption, degradation of drug in GI contents, and presystemic metabolism, mostly resulting in reduced bioavailability.

The primary functions of the GI tract are absorption and digestion of food, as well as secretion of various enzymes or fluids [2]. The gastrointestinal mucosa forms a barrier between the body and a luminal environment that contains not only nutrients but also potentially hostile microorganisms and toxins. The normal function of the GI barrier, which is referred to the properties of the gastric and intestinal mucosa, is essential for disease prevention and overall maintenance of health. The major challenge in drug delivery through the GI tract is to achieve efficient transport of nutrients and drugs across the epithelium while rigorously excluding passage of harmful molecules and organisms into the body.

The performance of GI barriers to drug transport may largely depend on the physicochemical characteristics of drugs. Water-soluble small molecules may not be easily absorbed unless a specific transporter to those molecules is present, while lipophilic drugs can be relatively well absorbed through GI barriers. Mucosal transporters include PEPT, OATP, OCT, MCT, ASBT, MDR1, MRP, and BCRP among others [3] as shown in Figure 1.1. Large-molecule drugs, such as antibodies and proteins, may suffer extensive enzymatic degradation in the GI tract [4].

In this chapter, gastrointestinal mucous membranes and gut physiology will be intensively covered from the perspective of physiological barriers, which will lead to thorough understanding of key obstacles to advanced oral drug delivery.
1.2.1 Anatomy of Gastrointestinal Tract

1.2.1.1 Gastrointestinal Anatomy The major components of the gastrointestinal tract are the stomach, small intestine, and large intestine. The small intestine with a length of about 6 m includes the duodenum, jejunum, and ileum [5]. The stomach is a pouch-like structure lined with a relatively smooth epithelial surface. Extensive absorption of numerous weakly acidic or nonionized drugs and certain weakly basic drugs were demonstrated in the stomach under varying experimental conditions [2, 6, 7].

The small intestine is the most important site for drug absorption in the gastrointestinal tract. The epithelial surface area through which absorption of drug takes place in the small intestine is enormously large because of the presence of villi and microvilli, finger-like projections arising from and forming folds in the intestinal mucosa as shown in Figure 1.2 [8]. The surface area decreases sharply from proximal to distal small intestine and was estimated to range from 80-cm²/cm serosal length just beyond the duodeno-jejunal flexure to about 20-cm²/cm serosal length just before the ileo-cecal valve in humans [9]. The total surface area of the human small intestine is about 200 to 500 m² [6, 7]. The small intestine is made up of various types of epithelial cells, i.e., absorptive cells (enterocytes), undifferentiated crypt cells, goblet cells, endocrine cells, paneth cells, and M cells. There is also a progressive decrease in the average size of aqueous pores from proximal to distal small intestine and colon [10, 11].

The small intestine is the most involved region for carrier-mediated transport of endogenous and exogenous compounds. The proximal small intestine is the major area for absorption of dietary constituents including monosaccharides, amino acids, vitamins, and minerals. Both vitamin B₁₂ and bile salts appear to have specific absorption sites in the ileum [2]. The large intestine has a considerably less irregular mucosa than the small intestine.

1.2.1.2 Pores The aqueous pores render the epithelial membranes freely permeable to water, monovalent ions and hydrophilic solutes with a smaller molecular size [2, 6, 7]. It was estimated that the hypothetical pores in the proximal intestine have an average radius of 7.5 Å, and those in distal intestine (ileum) have that of about 3.5 Å [10]. The pore sizes of the aqueous pathway for buccal and sublingual mucosa in pigs were estimated as 18–22 and 30–53 Å, respectively [12]. Since the molecular size of most drug molecules are larger than a pore size in the membrane, drug transport through pores seems to be of minor importance in drug absorption. However, some larger polar compounds with molecular weights up to several hundreds are still absorbed through active participation of the membrane components.

1.2.1.3 Tight Junctions Tight junctions are closely associated areas of two cells whose membranes join together, forming a virtually impermeable barrier to fluid. Tight
junctions are composed of the structural proteins (occludin and claudins), the scaffold proteins (ZO-1, ZO-2, fodrin, cingulin, symplekin, 7H6, and p130), and the actin cytoskeleton [13]. Paracellular transport of drugs mostly occurs via tight junctions (Figure 1.3). The permeability of intestinal epithelium to large molecules or ionized drugs depends on the combination of transcellular transport via adsorptive endocytosis and paracellular transport through tight junctions.

Tight junctions were conceptualized a century ago as secreted extracellular cement, forming an absolute and unregulated barrier within the paracellular space [2, 6, 7]. The contribution of the paracellular pathway of the gastrointestinal tract to the general correlation between environment and host molecular interaction was considered to be negligible. It is apparent that tight junctions have extremely dynamic structures engaged with developmental, physiological, and pathological situations.

To date, particular attention has been placed on the role of tight junction dysfunction in the pathogenesis of several diseases, particularly autoimmune diseases with viral etiology [2, 6, 7]. Pathophysiological regulation of tight junctions is influenced by various factors including secretory

FIGURE 1.2 Gastrointestinal anatomy [8].
IgA, enzymes, neuropeptides, neurotransmitters, dietary peptides and lectins, yeast, aerobic and anaerobic bacteria, parasites, proinflammatory cytokines, free radicals, and regulatory T-cell dysfunction [2].

1.2.2 Gastrointestinal Physiology

A comprehensive review of the physiological parameters that affect oral absorption in the context of formulation performance and drug dissolution was recently published [14]. This physiologically relevant information to oral human drug delivery could serve as a basis for the design of advanced drug delivery systems.

1.2.2.1 Gastrointestinal Components

(i) **Bile Salts.** Bile salts are known to enhance the absorption of hydrophobic drugs by enhancing their wettability. The absorption rates of such drugs as griseofulvin can be facilitated when they are taken after meals as a result of the increase of bile salts excretion that promotes their dissolution rates [2]. On the contrary, bile salts reduced the absorption of certain drugs, such as aminoglycosides, neomycin, and nystatin, through the formation of non-absorbable complexes with bile salts [15].

(ii) **Mucin.** Mucin is a viscous muco-polysaccharaide (glycoprotein) that lines the gastrointestinal mucosa for protection and lubrication purposes [16]. Mucin has a negative anionic charge, so it may form a nonabsorbable complex with some drugs, such as aminoglycosides and quaternary ammonium compounds, subsequently affecting their absorption.

(iii) **Enzymes.** Since GIT fluid contains high concentrations of enzymes needed for food digestion, some enzymes may act on drugs. For example, esterases secreted by pancreas affect the metabolic process of ester derivative drugs including aspirin and proproxyphe through the hydrolysis process in the intestine [17]. In epithelial cells, the partial location of the enzyme on the basolateral pole underlies the vectoral transport of salts, water, and organic solutes (e.g., bile salts) across the tissue, whereas in nonepithelial cells, such as fibroblasts, the enzyme is evenly distributed on the cell surface [4].

1.2.2.2 Gastrointestinal Blood Flow

About 28% of cardiac output is supplied to the gastrointestinal tract by blood capillaries [2, 6, 7]. The blood perfusion of the gastrointestinal tract plays a critical role in drug absorption by continuously preserving the concentration gradient across the epithelial membrane. Polar molecules that are slowly absorbed exhibit no significant dependence on blood flow, but lipid-soluble molecules and molecules that are small enough to penetrate easily through the aqueous pores are greatly affected by the rate of blood flow. In general, the drug absorption rate is not significantly affected by physiological variability in mesenteric blood flow because blood flow is rarely a rate-limiting step in the drug absorption process through the gastrointestinal tract.

1.2.2.3 Luminual pH

The pH of gastric fluid varies considerably according to the sites and contents. Gastric secretions have a pH of less than 1, but the pH of gastric contents is usually between 1 and 3 as a result of dilution and diet [2, 6, 7]. The pH of the stomach contents is briefly but distinctly elevated after a meal; thus, pH values of 5 or even greater are not unusual. Fasting tends to decrease the pH of gastric fluids and subsequently influences the pH of the stomach.

The luminal pH in the small intestine is about 6 to 7 [18], and large intestines have a similar luminal pH as described in Table 1.1. The acidic microclimate pH in the human jejunum was elevated in disease states and contributed to the deviation of the absorption profiles of weak electrolytes from the pH-partition hypothesis [19].

The pH at the absorption site is an integral factor in drug absorption because most drugs are either weak organic acids or bases [2, 6, 7]. Since the gastrointestinal barrier is highly permeable to uncharged and lipid-soluble solutes, a drug may be well absorbed from the segment of the gastrointestinal tract where a favorable pH exists but poorly absorbed from other segments where a less favorable pH exists. Weakly acidic drugs rapidly dissolve in alkaline pH, while basic drugs are more soluble in acidic pH. In addition, disintegration of certain pharmaceutical dosage forms is pH dependent; for example, enteric coated tablets dissolve only in alkaline pH. Luminal pH can influence the stability of some drugs including erythromycin, which is rapidly degraded in the acidic pH [20, 21].
1.2.2.4 Gastric Emptying and Gastrointestinal Motility

The volume of gastric contents greatly influences the concentration of a drug in the stomach. The rate of gastric emptying is governed by the volume of gastric contents and has a direct impact on the chemical and physical properties of chyme in the duodenum and jejunum [6]. Standard low bulk meals and liquids are transferred from the stomach to the duodenum in an apparent first-order fashion with a half-life of 20 to 60 minutes in healthy adults [6]. In addition, numerous factors as described in Table 1.2 can influence the rate of the gastric emptying process.

Gastric emptying is the major factor that greatly contributes to unusually large intersubject variability in the absorption of drugs released from enteric-coated tablets [22]. Gastric emptying is retarded by fats and fatty acids in the diet, high concentrations of electrolytes or hydrogen ion, high viscosity, mental depression, lying on the left side, and diseases, such as gastroenteritis and gastric ulcer [23]. Gastric emptying of liquids is much faster than that of solid food or solid dosage forms. Gastric emptying is promoted at low stomach pH and retarded at alkaline pH. Various drugs including atropine and narcotic analgesics, amitriptyline, propantheline, and imipramine can also retard gastric emptying [24, 25].

The gastrointestinal tract during the fasting state undergoes the characteristic sequences of motion (i.e., waves of activity) known as the interdigestive myoelectric complex or migrating motility complex [6]. The motility of the small intestine called the small bowel transit time also plays an integral role in drug absorption. The mean transit time of unabsorbed food residues or insoluble granules through the human small intestine is estimated to be about 4 hours [26].

Apart from dissolution of a drug and its permeation through the GI membrane, gastric emptying can also serve as a rate-limiting step in the drug absorption from the intestine. It is generally accepted that fast gastric emptying increases the bioavailability of most drugs. For example, a good correlation was found between stomach emptying time and peak plasma concentration for acetaminophen [27]. The rapid gastric emptying is desirable, when a fast onset of drug action is required (i.e., pain killers), when dissolution of a drug occurs in the intestine, when a drug is not stable in the gastric media, or when a drug is best absorbed from the distal part of the small intestine (e.g., vitamin B12) [2]. Delayed gastric emptying may be preferred, if the food and/or gastric juice promote the disintegration and dissolution of a drug, if

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<thead>
<tr>
<th>TABLE 1.2 Factors Affecting Gastric Emptying</th>
</tr>
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<tr>
<td><strong>Gastric Emptying</strong></td>
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<tr>
<td>Increase</td>
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Source: Adapted from Refs. 1, 14, 82, and 90.
a drug like griseofulvin dissolves slowly, if a drug irritates the gastric mucosa (e.g., aspirin), or if a drug is absorbed from the proximal part of the intestines and prolonged duration of action is needed (e.g., vitamin C) [21, 28]. Delayed intestinal transit time may be suitable for enteric coated formulations, sustained release dosage forms, and drugs with site-specific absorption in the intestines.

1.2.3 Gastrointestinal Barrier

1.2.3.1 Barrier to Bioavailability  Bioavailability is traditionally defined as a ratio of drug amount at the systemic circulation to the amount taken into gut lumen, which is equivalent to an extent of absorption. The whole absorption process is a sequential event and includes dissolution and precipitation in the gut, enzymatic and chemical degradation, permeation through epithelial membrane, and metabolism at both the intestinal wall and the liver. It also includes other physiologic conditions, such as blood flow and transit time as described above, which are all together considered as barriers against complete absorption. The other element of the absorption process is the rate of absorption, which is governed by a series of time-dependent steps, such as dissolution, gastric emptying, intestinal transit time, and membrane permeability, at the brush border epithelial cells [28].

For a drug administered orally, the two most common reasons for its poor bioavailability are the decreased absorption and presystemic first-pass effects [28]. Low solubility and/or less optimal membrane permeability are key parameters for decreased absorption [29]. Before a drug reaches the blood circulation, it has to pass for the first time through organs of elimination, namely, the GI tract and the liver. The loss of drug through biotransformation by such eliminating organs during its passage to systemic circulation is called first-pass or presystemic metabolism. The low drug concentration or complete absence of the drug in plasma after oral administration is indicative of first-pass effects.

The presystemic metabolization of a drug is influenced by luminal, gut wall, bacterial, and hepatic enzymes [28]. Luminal enzymes are the enzymes present in the gut fluids as well as those from intestinal and pancreatic secretions. The enzymes from pancreatic secretions include hydrolases that metabolize ester drugs like cholanephilicol and peptides, split the amide linkages through hydrolysis, and ultimately inactivate the protein and polypeptides drugs [2]. Gut wall enzymes also known as mucosal enzymes are mostly present in stomach mucosa. Intestinal mucosa has both phase I and phase II enzymes, e.g., CYP3A4/5, CYP2C9, CYP2C19, CYP2D6, UGT, GST, and SULT as shown in Figure 1.1 [3]. The activity of phase I enzymes in the gut wall is the highest at the duodenum and decreases distally. The highest expression of phase I enzymes was found between villous tips and midvilli. The GI microbes are rich in colon, whereas they are poorly present in stomach and small intestines [2]. Thus, many orally administered drugs remain unaffected by bacterial enzymes.

The colonic microbes generally render a drug more potent or toxic on biotransformation. For example, sulfasalazine, a drug used in ulcerative colitis, is hydrolyzed to sulfapyridine and 5-amino-salicylic acid by the microbial enzymes of the colon. Hepatic enzymes play a major role in biotransformation of most drugs going through the first-pass effect before they reach the systemic circulation. Liver has both phase I (oxidation, reduction, hydrolysis) and phase II (glucuronidation, sulfation, methylation, acetylation) enzymes [2, 6]. Among them, cytochrome P450 enzymes are responsible for metabolism and bioactivation of about 75% of all drugs [30].

In the lumen of the small intestine, dietary fat is hydrolyzed to its components, monoacylglycerol (MG) and free fatty acids (FAs), and subsequently dispersed in bile acids. The pH in close proximity to the enterocyte surface is lower than other sites, causing protonation of the fatty acids. Free FAs then dissociate from the bile salt micelles and either passively diffuse or are transported across the brush border membrane by protein-mediated transporters like cluster determinant 36 (CD36), fatty acid binding protein (FABPpm), and fatty acid transport protein family members (FATPs) [31]. Both CD36 and FABPpm are found to reside in specialized microdomains known as lipid rafts [32].

Like dietary fat and cholesterol, lipid-soluble drugs are absorbed by the fat absorption pathways [2]. The oral absorption rates of griseofulvin and vitamin D were enhanced by certain oily formulations including the bile salt micelles that were transferred to blood circulation via the intestinal lymph system. The process of lipid absorption is classified into 3 steps: 1) the uptake, 2) assembled into lipoproteins, and 3) secretion of lipid into the lymphatic circulation. Each step in lipid absorption may be subjected to the pathway-involved regulation.

1.2.3.2 Barrier to Immunity  The intestinal epithelium is the largest mucosal surface, providing an interface between the external environment and the mammalian host. The intestinal mucosa is continuously exposed to an immense load of antigens from ingested food, resident bacteria, and invading viruses [33]. The single-cell epithelial layer lining the gut lumen (Table 1.1, surface area ~2.1 to 5.9 × 10^6 cm²) has biphasic functions, playing a major role in the digestion and absorption of nutrients and simultaneously constituting the organism’s most important barrier between the internal and external environments. Epithelial permeability to nutrients depends on the regulation of intercellular tight junctions (TJs) as well as the activity of transcellular transport via endocytosis [13]. Epithelium has its ability to regulate the trafficking of macromolecules between the host organ and its environment through barrier properties. Intact macromolecules can be absorbed either via the
transcellular or the paracellular pathway. For the transcellular pathway, the uptake of macromolecules occurs through the endocytosis process, followed by fusion with lysosomes (phagolysosomes) with potential degradation of the macromolecules before being delivered into the submucosa. In contrast, macromolecules penetrate the intestinal epithelium through the paracellular pathway, reaching the submucosa mostly in an intact form.

Paracellular passage of macromolecules under either physiological or pathological conditions is safeguarded by gut-associated lymphoid tissue (GALT) [34]. GALT serves as a containment system that prevents potentially harmful intestinal antigens from accessing the systemic circulation and induces systemic tolerance against luminal antigens through the processes involved with polymeric Ig A secretion and induction of T-regulatory-cell activity and immune tolerance [34]. Macrophages, leukocytes, and mucosal mast cells (MMCs) release various preformed mediators that alter gut function. MMCs release various preformed mediators, such as histamine, serotonin, and mast-cell proteases, as well as newly synthesized mediators including leukotrienes, prostaglandins, platelet-activating factor, interleukin-4, and TNF-α. Most of these mediators affect epithelial permeability, which might explain, in part, enhanced intestinal permeability featured in both T helper 1 (Th1)-mediated and Th2-mediated pathologies [34].

In disease conditions including inflammatory bowel disease, excessive penetration of antigens through the epithelial layer may result in inappropriate immune stimulation, leading to chronic gastrointestinal inflammation [33]. The permeability of substrates through the intestinal epithelium depends on the regulation process of the mucosal immune system and intercellular tight junctions. Serum immunoglobulin to food antigen macromolecules were found in patients with inflammatory bowel disease and celiac disease, indicating that an enhanced amount of these proteins permeate through the intestinal epithelium, and trigger a systemic immune response. The permeability of substrates through the intestinal barrier increased in such disease conditions as food sensitivity, intestinal diseases [35], acute gastroenteritis, chronic intestinal infections, surgery, exercise, stress, extensive burns, malnutrition, secretory IgA deficiency, anti-inflammatory drugs, and viral infections [33].

1.2.3.3 Barrier to Microorganisms The acidic pH of the stomach and the antibacterial activity of pancreatic enzymes, bile, and intestinal secretions have also served as GI tract barriers [36]. Peristalsis and the natural loss of epithelial cells remove microorganisms. If peristalsis is slowed, the removal process of microorganisms is delayed, producing certain infections including symptomatic shigellosis. Compromised GI defense mechanisms may predispose patients to particular infections. Normal bowel microflora can inhibit pathogens; alteration of this flora with antibiotics allows for overgrowth of inherently pathogenic microorganisms or super infection with ordinarily commensal organisms such as Candida albicans.

1.2.4 Absorption Models

The barrier properties of the intestinal epithelium are generally investigated by assessing the permeability to various probe/marker molecules in vivo or in vitro with intestinal segments mounted in Ussing-type chambers. Although in vivo studies are more physiological, the in vitro approach makes it feasible to study epithelial permeability to a greater range of probes including proteins, and to determine the mechanisms and routes of passage involved. In vitro cell culture models, such as Caco-2, MDCK, or HT-29 cells, are used to measure drug permeability. The in vivo quantitative assessment models currently available are human jejunal perfusion technique, intravital microscopy of fluorescent bacteria, and in vivo fluorescence microscopic imaging for intestinal mucosa permeability [37, 38].

There are numerous in vivo, in situ, in vitro, and in silico models for assessment of absorption/transport-related mechanisms in addition to examining barrier properties [39, 40]. The absorption models and tools for in silico, in vitro, and in vivo experiments/methods used to get various parameters influencing human dosage regimen are shown in Figure 1.4. For example, an intestinal perfusion model allows for estimation of effective permeability (Peff), which enables estimation of bioavailability (F). Similarly, the Ussing chamber or Caco-2 model can provide apparent permeability [40], whereas the parallel artificial membrane permeability assay (PAMPA) enables estimation of passive permeability in a high throughput mode. Other in vitro models, such as everted gut sacs, brush border membrane vesicles (BBMVs), or intestinal rings, can serve as a means to investigate apparent absorption rates of drugs [40].

**FIGURE 1.4** Absorption models and tools for in silico, in vitro, and in vivo experiments/methods to get various parameters influencing human dosage regimen.
It is also feasible to calculate various descriptors, such as Lipinski’s Rule of Five, molecular weight (MW), polar surface area (PSA), clogP, solubility, and permeability, to a certain extent directly from the structure of a drug [40]. By taking the fundamental complexity of GI physiology and formulation characteristics into further consideration, the advanced absorption model could accurately estimate oral bioavailability and successfully predict an individual dosage regimen, which are crucial in the new drug development process.

Pharmacokinetics and biopharmaceutics are the fundamental areas for development of new drug formulations and evaluation of their clinical efficacy. Pharmacokinetics is divided into drug disposition features including the extent and rate of absorption, distribution, metabolism, and excretion. This is commonly referred to as the ADME scheme [41]. The kinetic approach using a compartment model has been frequently used, and the formation and ADME profiles of each metabolite have been broadly defined [42, 43]. Advanced evaluation and prediction tools for bioavailability/bioequivalence of drugs are also required in every stage of the drug assessment process. The drawbacks of biopharmaceutical estimation have mainly resulted from the physicochemical properties of drugs or the physiological factors of patients. Scientists have been enthusiastic to develop a theoretical model capable of predicting oral drug absorption in humans based on various mechanistic approaches, which can also address intrapatients’ variances.

The mechanistic approaches are classified into three categories based on their dependence on spatial and temporal variables [44]: quasi-equilibrium models, steady-state models, and dynamic models. The quasi-equilibrium models are independent of spatial and temporal variables, and they include the pH-partition hypothesis and absorption potential concept. The steady-state models are independent of temporal variables, but they are dependent on spatial variables and include the film model, macroscopic mass balance approach, and microscopic balance approach [45]. The steady-state models are restricted to prediction of the extent rather than the rate of oral drug absorption [44]. The dynamic models deals with the relationship between spatial and temporal variables and include dispersion models and compartmental models [3]. The dynamic models can predict both the rate and extent of oral drug absorption, and thus, they are considered an improvement over the steady-state models.

1.3 PHYSIOLOGICAL FACTORS INFLUENCING DRUG ABSORPTION

Gastrointestinal absorption of orally administered drugs is influenced by various factors including physiological, physicochemical, and formulation factors [2, 7]. Physiological factors are age, blood flow to the GI tract, gastric emptying and intestinal transit, disease state, first-pass effect, pH of luminal contents, a surface area of absorptive site, digestive enzymes, and microbial flora. Solubility, stability, buffers, complexation, particle size, crystal properties, pKa, and diffusion coefficient are classified as physicochemical factors that can affect drug absorption.

Formulation factors include dosage forms and pharmaceutical excipients that are needed to secure stability, acceptability, bioavailability, or functionality of the drug product [39]. Oral dosage forms, such as solution, suspension, tablet, or capsules, are influenced by these factors. Multiple excipients in a dosage form may cause poor absorption and low bioavailability of drugs [39]. Excipients commonly used are binders, buffers, coatings, diluents, disintegrants, lubricants, suspending agents, sweeteners, colorants, and surfactants [39]. Since drug absorption is concomitantly affected by various factors, it is quite difficult to determine which factors are mainly responsible for the poor bioavailability of specific drugs.

1.3.1 Epithelial Membranes

All cells are bound by membranes [46], which consist of the phospholipids bilayer with embedded proteins as described in Figure 1.5. Cell membranes are involved with a variety of cellular processes, such as cell adhesion, ion conductivity, and cell signaling. Cell membranes serve as the attachment surface for the extracellular glycocalyx, cell wall, and intracellular cytoskeleton. The lipid bilayer of cell membranes is impermeable to most water-soluble molecules.

Membrane transport processes in most biological events occur during the formation process of electrochemical potentials and the uptake process of nutrients, such as sugars and amino acids, removal of wastes, endocytotic internalization of macromolecules, and oxygen transport in respiration [4, 47]. The movement of many ions, nutrients, and metabolites across cellular membranes is catalyzed by specific transport proteins, i.e., transporters that show saturation and substrate specificity [48]. Thus, cell membrane is selectively permeable to ions and organic molecules. The epithelium lies on top of connective tissue, from which it is separated by a basement membrane. It is composed of tightly clustered cells connected by tight junctions and desmosomes. The gastrointestinal barrier that separates the lumen of the stomach and intestines from the systemic circulation has the properties similar to a semipermeable membrane with the complex structure composed of lipids, proteins, lipoproteins, and polysaccharides. Lipid-soluble molecules penetrate the barrier directly through the lipophilic portion of the membrane.
1.3.2 Absorption Processes

Absorption processes include passive or facilitated diffusion, ion channels, primary and secondary active transporters, and macromolecular and bulk transporters [48]. The characteristics of the absorption processes were summarized in Table 1.3. The mechanisms involved with drug transport through epithelial membranes are shown in Figure 1.3.

1.3.2.1 Passive Diffusion

Passive diffusion is the movement of a solute across the membrane down the electrochemical gradient in the absence of the assistance of a transport protein. It does not require any biological energy but follows Fick’s law:

\[
V = P \cdot A \cdot \Delta C = \left( \frac{D \cdot K}{\delta} \right) \cdot A \cdot \Delta C
\]

(1.1)

![Cell membrane](cell_membrane.png)

**FIGURE 1.5** Illustration of a eukaryotic cell membrane. The cell membrane is a biological membrane that separates the interior of all cells from the outside environment [46].

<table>
<thead>
<tr>
<th>Type</th>
<th>Transport Protein</th>
<th>Saturation</th>
<th>Concentration Gradient</th>
<th>Energy Dependence</th>
<th>Examples</th>
<th>Energy Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple diffusion</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Oxygen, water</td>
<td>ATP, light, substrate oxidation</td>
</tr>
<tr>
<td>Ion channels</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Na(^+) channel</td>
<td></td>
</tr>
<tr>
<td>Facilitated diffusion</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Glucose transporter</td>
<td></td>
</tr>
<tr>
<td>Primary active transport</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>H(^+)-ATPase, Ca(^{2+})-ATPase, Na(^+), K(^+), Ca(^2+), Na(^+) ATPase, MDR1, BCRP, MRPs</td>
<td>ATP, light, substrate oxidation</td>
</tr>
<tr>
<td>Secondary active transport</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Na(^+)/Ca(^{2+}) antiporter, Na(^+)/amino acid symporters, SGLT, H(^+)/peptide transporter, OATP, MCT</td>
<td>Ion gradient</td>
</tr>
</tbody>
</table>

*Source: Adapted from Ref. 48.*
where \( V \) is a transport rate, \( A \) is the surface area, \( \Delta C \) is a concentration difference across the membrane, \( D \) is the diffusivity of a solute, \( K \) is the partition coefficient between membrane and water, and \( \delta \) is the membrane thickness. The general plot for a simple diffusion is shown in Figure 1.5. As expected from Equation (1.1), the transport rate is proportional to the substrate concentration, displaying a linear relationship (Pattern A). Numerous small lipid-soluble molecules, oxygen, \( \text{N}_2 \), \( \text{CO}_2 \), and \( \text{NH}_3 \), are transported through biological membranes via simple diffusion [4].

1.3.2.2 Ion Channels As ion fluxes are involved with regulation of inorganic ions, such as \( \text{Na}^+ \), \( \text{K}^+ \), \( \text{Ca}^{2+} \), and \( \text{Cl}^- \), ion transport across the cell membranes plays a critical role in numerous cell processes, such as cell growth and proliferation [4]. As previously reported, the high concentration of \( \text{Na}^+ \) ion outside the cell is balanced mainly by extracellular chloride ions [4]. On the other hand, the high concentration of \( \text{K}^+ \) ion inside the cell is balanced by a variety of negatively charged intracellular ions, such as \( \text{Cl}^- \), \( \text{HCO}_3^- \), and \( \text{PO}_4^{3-} \), or negatively charged organic molecules [4].

Various enzymes and transport mechanisms regulate the pH and the concentrations of \( \text{Na}^+ \), \( \text{K}^+ \), \( \text{Ca}^{2+} \), and \( \text{Cl}^- \) and other anions in cell, mitochondria, or organelle compartments [49]. Ionic gradients, established at the expense of metabolic energy, e.g., ATP hydrolysis, are used to transfer solutes across the membranes (e.g., amino acids and sugars) into the cell by multitransporters and protons out of the cell by antiporthers [4]. The outward \( \text{K}^+ \) gradient generated across the plasma membrane is a major determinant of the inside negative transmembrane potential of cells [4]. In epithelial tissues, the polarized distribution of enzymes and ion carriers provides the driving force for movement of ions and molecules across the cell interior.

Ion channels have basically two conformations: open or closed. When they are open, ions flow through the channel and reproduce an electric current. For example, the rate of \( \text{Na}^+ \) movement through the acetylcholine receptor ion channel has a linear relationship with extracellular \( \text{Na}^+ \) concentration and the process is not saturable (Pattern A in Figure 1.6), which is similar to simple diffusion [48]. The ion channel of the acetylcholine receptor behaves as though it provides a hydrophilic pore within the lipid bilayer through which an ion of the right size, charge, and geometry can diffuse down its electrochemical gradient [48].

1.3.2.3 Facilitated Diffusion Molecules transport through cells according to their electrochemical potential gradient when an energy source is not required [4]. The facilitated diffusion process does not require energy and is accelerated by the specific binding process between a solute and membrane proteins [4].

Some essential features of the facilitated diffusion process can be summarized as follows [50]: 1) The facilitated diffusion process by a mobile transporter system operates such that solute flows from a higher to a lower electrochemical potential; 2) the solute flows at a rate greater than that predicted based on its size or hydrophilicity; 3) the penetration rate does not follow Fick’s law except at very low concentrations (at higher concentrations, saturation kinetics are observed as seen in Figure 1.6 (Pattern B)); 4) competitive inhibition occurs between chemically and sterically similar substrates; 5) inhibitory action may be exerted by other compounds, especially those reacting with or ligating reactive groups in proteins; and 6) it is often feasible for the flow of substrate to propel temporarily in the opposite direction against the electrochemical potential gradient of analogs (an overshoot phenomenon) for the accumulation of an analog (or isotope). A well-known example of facilitated diffusion is displayed with erythrocyte glucose transport, in which glucose enters the erythrocyte via a specific transporter that allows for glucose entry into the cell at a rate about 50,000 times greater than its simple diffusion through a lipid bilayer [4].

1.3.2.4 Active Transporters Active transport results in the accumulation of a solute on one side of the membrane and often against its electrochemical gradient. It occurs only when solute accumulation is coupled with the exergonic process directly or indirectly [4]. During the transport process, energy sources, such as adenosine triphosphate (ATP), electron transport, or an electrochemical gradient of another ion, are used to drive ions or molecules against their