1

TRANSLATIONAL CONCEPT AND DETERMINATION OF DRUG ABSORPTION

1.1 DRUG ABSORPTION, MECHANISM, AND ITS IMPACT ON DRUG BIOAVAILABILITY, DRUG DISPOSITION, AND DRUG SAFETY

Discovery, development, and approval of a new drug is a long process that takes on average 12–14 years and costs an average of about $1.8 billion [1]. The financial burden and time for bringing to the market a new medicine are considered as major challenges in the pharmaceutical industry. In addition, the decrease in the number of truly innovative therapeutic areas that have been approved by the regulatory authorities around the globe was a reflection of higher attrition in late-stage drug development (Phase 2 and 3), despite the advancement of new technologies. However, the high-throughput screening; structure activity relationship (SAR) using absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties; and target efficacy-based molecular and cell biology in collaboration with advanced medicinal and combinatory chemistry have increased the number of drug candidates successfully reaching Phase 1 due to better preclinical characterization and improved ADMET properties. For example, the Phase 2 success rates for drug candidates have fallen from 28% in 2006 to 18% in 2009, with ~50% of success to progressing through Phase 2. The decrease in Phase 2 is mainly due to insufficient efficacy, undesired side effects, and/or poor pharmacokinetics (PK) of the newly developed drug, which account for 51% of the drug failures [2–4]. Therefore, correct prediction of the efficacy of novel drug candidates especially in the early stage preclinical phases is crucial.
The accurate assessment of absorbed drug dose, exposure, and disposition in in vitro and in preclinical animal models that translate to human clinical data may improve the success rate of bringing a needed medicine to the stage of reaching human patients.

For the drug to be absorbed in the intestine, several processes are involved. First, the physicochemical properties of drugs such as solubility, dissolution rate, lipophilicity, and molecular weight (MW) are major driving parameters for the drug absorption in the gastrointestinal (GI) tract, as a molecule should be in a solution to permeate the intestinal membranes, and the rate at which the molecule gets into the solution impacts its ability to get absorbed. Second, the drug has to cross several physiological parameters before it reaches the bloodstream, such as effect of pH, stomach emptying, intestinal transient time, disease state, age, diet, various GI fluids, and so forth. The sum of physicochemical and physiological parameters can either hinder or facilitate the permeability of a drug in the intestinal sections.

It is important to emphasize here, as the drug absorption will be discussed in detail, that the drug’s permeability is indeed the major determinant of its ability to be absorbed in the intestine. The permeability of drugs can be either by a passive diffusion mechanism, following the “rule of 5,” or by an active process driven by the intestinal transporters. Drug transporters can either promote the absorption (by uptake transporters such as OCT, OAT, PepT1, OATP) or hinder the absorption (efflux transporters such as P-gp, MRP2, BCRP).

Last, the drug absorption can be influenced by other significant factors, such as the metabolism by drug-metabolizing enzymes that are expressed mostly in the duodenal section of the small intestine. Thus, the intestinal metabolism, which may also cause drug–drug interactions (DDIs), changes the extent of oral drug absorption. As will be discussed later, effective orally absorbed drug will ensure systemic exposure. Absorption through membranes of the GI tract and metabolism by gut and hepatic metabolism are key players for drug exposure in systemic circulation—that is, oral bioavailability—before it reaches the other body organs.

### 1.1.1 Drug Absorption and Oral Bioavailability

In ADME processes that exert the pharmacokinetic properties of a new drug, absorption is a process by which a given extravascular dose (EV), that is, an oral dose (PO), reaches the systemic circulation. The absorption of a drug can be described by any drug dose that is administered orally, subcutaneously, intramuscularly, or any other way different from a direct injection into the vascular system. The term oral “bioavailability” (F) is a parameter that is used in pharmacokinetics to quantify the ability of a compound dosed orally to reach the systemic circulation, after surviving any first-pass extraction in the gut and liver. The systemic F can be determined from Equation (1.1):

\[
F = \frac{\text{AUC}_{\text{po}} \times \text{Dose}_{\text{iv}}}{\text{AUC}_{\text{iv}} \times \text{Dose}_{\text{po}}},
\]

where \( \text{AUC}_{\text{po}} \) refers to the area under the curve (AUC) from an oral administration and \( \text{AUC}_{\text{iv}} \) refers to the AUC from intravenous (iv) administration. Accordingly, the
drug becomes bioavailable when it overcomes the potential barriers to reach the systemic circulation. A compound with $F = 1$ (or 100%) indicates that a given oral dose produces an identical systemic exposure to that observed in the corresponding iv dose, indicating that it is fully absorbed and fully escaped any potential of metabolism in both the gut and liver. $F = 0.5$ (50%) indicates that in transit from the oral administration site to the systemic circulation, half of the compound is lost; in this case, the oral dose to systemic concentration relationship indicates that the oral dose must be twice that of an equivalent iv dose to achieve a similar systemic exposure.

Although there are several approaches for a drug to become bioavailable, the oral dosing route is the most convenient, well-tolerated, patient-compliant, and cost-effective route of drug administration; however, it is still a complex route of administration, as the absorption from the gut into the systemic circulation may require consideration to avoid inter- and intrapatient variability in a compound’s pharmacokinetic profile [5].

Oral administration, $F_{\text{oral}}$, can be described as shown in Equation (1.2):

$$F_{\text{oral}} = (f_a \cdot F_G) \times (F_H \cdot F_L),$$

where $f_a$ is the fraction of the dose absorbed from the gut, and $F_G$, $F_H$, and $F_L$ are the bioavailability of the compound in the intestine, liver, and lung (typically $F_L = 1$ and ignored), respectively. From Equation (1.2), it is clear that a lack of $f_a$ or bioavailability in any one of the organs will yield $F_{\text{oral}} = 0$, and fully no systemic exposure.

As mentioned above, oral bioavailability is determined by the absorption through membranes of the GI tract and by the extent to which gut and liver are able to extract the orally administered drug (see Figure 1.1). Therefore, gut and hepatic metabolism are also key players for drug oral bioavailability. In a study with a set of 309 drugs where bioavailability, fraction absorbed ($f_a$), fraction escaping intestinal extraction ($F_G$), and fraction escaping hepatic extraction ($F_H$) were known, the analysis was conducted to determine which physicochemical property influences these parameters to enhance the bioavailability of a new drug candidate [6]. It was shown that $f_a$ decreases with increasing MW ($> 500$), polarity (c log D $>-2$), polar surface area ($> 125 \text{ Å}^2$), total H-bond donors and acceptors ($> 9$), and rotatable bonds ($> 12$). Indeed, such properties limit the capability of small organic molecules to traverse lipid membranes. Molecules with a log P ranging from 1 to 3 are considered to be highly permeable. Lipinski et al. (2001) [7] showed that particular physicochemical properties are associated with high or low oral bioavailability. They established the famous “rule of 5” that predicts that poor absorption or permeation is more likely when there are more than 5 H – bond donors, 10 H – bond acceptors, the MW is $> 500$ g/mole, and the calculated log P $> 5$ [7].

However, it was noted in the above study [6] that high lipophilicity does not necessarily have a detrimental effect on $f_a$ and the analysis showed that the numbers of free rotatable bonds are negatively related, with all three processes leading to a dramatic effect on bioavailability. Also it has been noted that physicochemical properties that lead to high $f_a$ tend to be also associated with high rates of metabolism. That means
enough lipophilicity is needed to ensure good membrane penetrability but too much will lead to high extraction due to metabolism in gut and liver.

In other analysis by comparing basic, acidic, and neutral drugs, the data indicated that the higher first-pass effect due to higher metabolism and relatively lower protein binding of basic drugs leads to lower bioavailability than acids or neutral drugs, although basic drugs exhibits higher $f_a$ than the acidic and neutral drugs [6]. Indeed, the higher first-pass effect of basic molecules can be attributed to their affinity for metabolic enzymes.

1.1.2 Contribution of Intestinal Drug Transporters and Drug-Metabolizing Enzymes on Extent of Absorption and Mechanism

1.1.2.1 Intestinal Transporters  As the translational investigations of drug disposition grow, along with data generated from the bench and data generated from clinical pharmacology investigations, it becomes clear that drug transporters are widely considered as a critical determinant in PK, pharmacodynamics (PD), and, most importantly, drug safety (DDI). Specifically, the intestinal transporters, as mentioned earlier, are viewed as an important determinant of oral drug absorption, bioavailability, and DDI.

Indeed, the efflux pump $ABCB1$ (P-glycoprotein, P-gp, or multidrug resistance 1, MDR1) is now one of the most evaluated transporters due to the many roles it plays, for example, differential bioavailability and DDI among human populations. Because
P-gp can play a role in limiting oral absorption of particular drugs [8–11], it has emerged as a potential determinant of oral bioavailability of those drugs.

As will be discussed in the following chapters, efflux transporters are expressed in many biological membranes of body organs, including the villus tip of the apical brush border membrane of gut enterocytes. They actively cause efflux of drugs from gut epithelial cells back into the intestinal lumen (see Figure 1.2). When a drug is orally administered, intestinal efflux transports limit the amount of drug absorbed into the intestine epithelia by pump drug to gut lumina, and this process presents a significant barrier toward drug absorption. Efflux transporter is one of the adenosine triphosphate (ATP)-binding cassette (ABC) family, as well as breast cancer resistance protein (BCRP; ABCG2) and multidrug transporter proteins (MRP; ABCC), but MDR1 is the most studied transporter [11]. Although P-gp activity limits oral drug absorption for specific drugs, these efflux transporters have a detoxification protecting function against the entry of exogenous toxins to the small intestine, colon, and other nondigestive organs like CNS and testis [12–14], and its role in blocking drug absorption makes the intestinal secretion a potential mechanism for drug elimination [15]. Although it is difficult to establish SAR for MDR1 substrates (and inhibitors), some features that are shared by many MDR1 substrates include the presence of a nitrogen group, aromatic moieties, planar domains, molecular size $\geq 300$ Da, presence of a positive charge at physiological pH, amphipathicity, and lipophilicity [16,17]. In the interaction between two modulators of P-gp, caution must be exercised when trying to extrapolate how the substrate/inhibitor may interact with an untested new drug, as MDR1 possesses multiple drug-binding sites and these sites are located in the middle of the lipid bilayer [18,19].

**Figure 1.2** The possible mechanisms of drug absorption across the intestinal epithelia monolayers, such as transepithelial passive diffusion (TC-PD), paracellular passive diffusion (PC-PD), and active transport by uptake (PepT1) and efflux transporters (P-gp, BCRP, MRP). Furthermore, the figure indicates the interplay between drug transporters and DMEs such as CYP3A that influence the drug absorption and bioavailability.
Several studies have focused on evaluating the impact of MDR1-mediated efflux activity and its potential attenuation of the overall bioavailability of its substrates. The studies revealed that P-gp could reduce the oral bioavailability via a couple of possible mechanisms:

1. It can attenuate the rate of substrate’s permeation from gut across intestinal enterocytes on apical membrane into blood, thus potentially delaying absorption time ($T_{\text{max}}$), reducing $C_{\text{max}}$, and possibly reducing total exposure (AUC).
2. It may enhance intestinal metabolic elimination (low absorbed substrate concentration below the Km of binding to P450 enzymes), thus indirectly reducing the amount of compound able to reach the bloodstream.

In clinical studies with substrates for P-gp like talinolol, the mean absorption time, AUC, and $C_{\text{max}}$ following oral administration of MDR1 substrates are affected by efflux activity of MDR1 in the intestine [20]. Furthermore, duodenal MDR1 mRNA content was significantly correlated with the AUC and $C_{\text{max}}$ of oral talinolol [21], and oral bioavailability of substrates such as tacrolimus and cyclosporin is known to be incomplete and variable in the clinic, as these are regulated by intestinal MDR1 and modulated by coadministered drugs, genetic polymorphisms, and disease states. Interestingly, the mRNA levels of MDR1, but not CYP3A4, correlated well with the ratio of concentration/oral dose and the oral dosage of tacrolimus [22]. In other clinical investigation with St John’s wort, an inducer of intestinal MDR, and talinolol revealed that talinolol AUC decreased with a corresponding increase in intestinal MDR1 expression after long-term treatment [23]. The impact of MDR1-mediated efflux activity on drug absorption and intestinal DDI was observed in clinical studies with key prototype P-gp substrate digoxin, as the latter bioavailability is influenced by absorption mediated by intestinal P-gp only and not by first-pass metabolism. Studies of orally administered digoxin in the presence of quinidine or digoxin and rifampicin resulted in a dramatic enhancement in digoxin $C_{\text{max}}$ and AUC [24,25]; in contrast, the treatment with the MDR1 inducer rifampicin decreased digoxin $C_{\text{max}}$ and AUC in humans [26], as inverse correlation between intestinal MDR1 and AUC of digoxin was seen.

To conclude, the impact of efflux-mediated drug absorption of P-gp substrates can vary depending on the permeability and solubility of these drugs, for example:

1. Unlike the low-solubility low-permeability drugs, the in vivo intestinal absorption of highly soluble and highly permeable MDR1 substrates is not limited by P-gp efflux pump by the in vivo absorption dominated by their high permeability. In this case MDR1 plays a minimal role in the intestinal absorption as reported for verapamile by Cao et al. (2005) [27]. These drugs possess a relatively high-absorbed fraction and the dissolution in GI tract is not the rate-limiting step.
2. For high-solubility but low-permeability MDR1 substrates, MDR1 limits the intestinal absorption in the distal segments of the small intestine but plays a minimal role in the proximal intestinal segments because of significant lower MDR1 expression levels in this region [28].
It is important to note that MDR1 efflux activity does not always predict a compound’s absorption profile. The magnitude of the effect of MDR1 efflux activity on a compound’s absorption profile ultimately depends on the MDR1 activity/expression profile in combination with solubility, permeability, and metabolism.

Unlike efflux transporters, uptake drug transporters, known as solute carrier transporters (SLC), do not require ATP and transport the drugs according to their concentration gradient, thereby improving the intestinal absorption of a wide range of drugs. They are localized in the intestine at the apical surface of epithelial cells, and most major SLC transporters are organic anion transporter families (OATP subfamilies; gene SLCO), SLC peptide transporter family (PepT1; gene SLC15A1), and organic zwitterion/cation transporters (OCTNs; gene SLC22) [29]. The clinical significance of intestinal SLCOs and OCTNs in drug absorption is still under investigation. In contrast, the impact of PepT1 transporter on drug absorption is well defined and investigated. PepT1 is expressed primarily in the small intestine, particularly in the duodenum [30], and the substrates for proton-coupled peptide transporters are mainly cationic, anionic, or zwitterionic di- and tripeptides; the free amino acids and larger peptides are excluded and peptide bond is not required for a substrate [31]. However, the transport function of PepT1 requires a proton gradient at the apical surface brush border membrane by the Na\(^+\)/H\(^+\) exchanger of epithelial cells; the system is known as a proton-dependent cotransport system, and then the influx of protons back into the epithelial cells is coupled by PepT1 to transport its substrates [32,33]. Drugs transported by PepT1 are prodrugs (e.g., acyclovir and l-dopa [34,35]), β-lactam antibiotics (e.g., penicillins and cephalosporins [36,37]), angiotensin-converting enzyme (ACE) inhibitors (e.g., captopril) [38], and anticancer agents (e.g., bestatin [39]). In general, PepT1 has generally been characterized as a low affinity/high capacity transporter with a wide variety of compounds as substrates. The impact of PepT1 on oral drug absorption has been well established in recent years, especially with the intestinal absorption of β-lactam antibiotics [37]. The affinity of PepT1 to β-lactam antibiotics as substrates is good due to resemblance to the backbone of its physiologically occurring tripeptides.

One of major areas that the human intestinal peptide transporter appear to target for increasing intestinal absorption of some small molecular weight drugs is the prodrug delivery. Because of its high capacity, broad substrate specificity, high expression in the intestinal epithelium, and low occurrence of functional polymorphisms [40,41], the intestinal peptide transporters have a significant impact on delivery of prodrugs. By using bonds that hydrolyze enzymatically in the preparation of PepT1-targeted prodrugs, it is possible to dramatically improve the systemic availability of poorly absorbed drugs, with limited systemic exposure to the intact prodrug. This general strategy of peptide transport associated with prodrug therapy [42] with valacyclovir is the most widely studied [43]. It is also used to deliver the prodrug LY544344, demonstrating the utility of PepT1-targeted non-ester prodrugs to overcome poor permeability and low bioavailability [44]. This compound exhibits near-ideal prodrug properties, with good solubility and chemical stability, extensive and reproducible absorption across species, low concentrations of circulating nontoxic prodrug, and pharmacokinetic linearity across a wide dose range [44].
1.1.2.2 The Impact of Intestinal Metabolism on Drug Absorption

When a drug has been ingested, the first site capable of metabolism is the small intestine. Because both phase I metabolic enzymes (e.g., oxidative metabolic pathways) and phase II metabolic enzymes (conjugating metabolism pathways) are expressed in the intestine, metabolism in the small intestine can play an important role in the first-pass metabolism of drugs [45]. The intestinal metabolism, in animals or humans, has been extensively studied. Many difficulties have been encountered, leading sometimes to discordant results. These include (1) the low expression levels of intestinal metabolic enzymes relative to the liver; (2) intra- or interspecies variability of expression of biotransformation enzymes; (3) ethical and technical limitations for obtaining biological samples for translational studies in humans; (4) variability of sample preparation techniques; and (5) structural and functional heterogeneity of the intestine.

Several drugs such as cyclosporine [46], verapamil [47], and midazolam [48] undergo extensive intestinal first-pass metabolism, and in turn affect the intestinal bioavailability. The human intestine is divided into two parts: the small intestine, subdivided into duodenum, jejunum, and ileum; and the colon. These two parts differ in their histological structure and by their metabolism. The highest metabolic activity of the intestine is in the upper part of the small intestine, with a maximum observed at the proximal jejunum [49]. The total P450 content increases slightly between the duodenum and the jejunum, then decreases markedly at the ileum. If this heterogeneous distribution concerns phase I enzymes (i.e., CYP3A4, 2C9, or 2C19), phase II enzyme (i.e., GST, UDPGT) distribution is relatively homogeneous in small intestine but with a lower level of expression in the colon. P450 enzymes such as CYP3A and CYP2C9 were found to be the most intestinal P450 enzymes, accounting for 80% and 15% of the total immunoquantified P450s, respectively [50], but the expression of CYP3A4 in human donors varied along the length of the small intestine, decreasing from the duodenum to the distal ileum, and content is estimated to be <1% of that in the liver [50]. The variability in expression of phase I and II metabolic enzymes in small intestine among human donors has been observed more frequently compared to the liver [51], possibly due differences in diet and environment [52].

Many reports have been published to demonstrate the large degree of overlap between the broad substrate specificities of MDR1 and CYP3A4 [53], and their combined actions could contribute to the low oral bioavailability determined for many of their dual substrates. Transport studies of cyclosporin A across in vitro intestinal absorption model Caco-2 cell monolayers have shown that the actions of MDR1 and CYP3A4 may act coordinately to enhance the attenuation of apical to basolateral transport of this drug. It was observed that cyclosporin A metabolism was much greater when the compound was transported in the apical to basolateral (absorptive) direction than in the basolateral to apical (secretory) direction [54]. The rationale was that the reduction in the apical to basolateral flux of cyclosporin A, due to the P-gp efflux pump, increased the metabolism by CYP3A4 [54]. These findings that CYP3A metabolism increases with P-gp efflux have failed to be produced using PK models [55,56]. A physiologically based model showed that intestinal drug metabolism is influenced directly by drug concentrations within the enterocyte, which is governed
by the degree of MDR1-mediated drug efflux and drug permeability through the entereocytes [57]. For drugs possessing high apparent permeability, increasing the efflux ratio (indicative of MDR1 substrate) predicts an increase in CYP3A drug clearance by up to 12-fold in the distal intestine.

For DDIs in intestine, as in liver, selective inhibition or induction of intestinal metabolizing enzymes either by dietary or environmental xenobiotics or by co-administered drugs has been identified as an effective method of drug interaction and a contributor to variability in oral drug bioavailability [58].

1.2 EFFECT OF PHYSIOCHEMICAL PROPERTY–RELATED FACTORS ON DRUG ABSORPTION

1.2.1 Lipophilicity, Solubility and Dissolution, and Permeability

While the physiochemical properties of drugs that are favorable and provide optimal biological activity with minimal safety liabilities have been evaluated and summarized by many medicinal chemists, the most popular is Lipinski’s “rule of five.” This rule is considered as the guide for drug discovery by almost all of the pharmaceutical industry. It supports the synthesis of new chemical entities [7]. The compounds with MW < 500, lipophilicity < 5 (calculated log P), total hydrogen bond acceptors < 10 (acceptors being O and N), and total hydrogen bond donors < 5 (donors being O-H and N-H groups) should have good oral absorption. It was proposed that when a compound violates two or more of these five rules, it is likely have poor intestinal absorption. Molecules within the “rule of 5” chemical space are usually absorbed through a passive process. When a drug is delivered orally, it transports from the GI fluid, primarily across the jejunum and the ileum segments of the small intestine into the portal blood system (Figures 1.1 and 1.2). This process involves transporting the drug from the GI fluid across the segments of the small intestine into the portal blood system. Before it reaches systemic circulation, it must pass through to one or more layers of membranes, either by passive/facilitated diffusion, transcellular or paracellular mechanism, active uptake or efflux, or endocytosis. Drugs cross the epithelial or mucosal cell walls, endothelial and capillary cell walls (bloodstream), and cellular plasma membranes to exert effect. These mechanisms will be discussed in more detail later in this chapter.

Ritchie et al. (2011) [59] have shown that increasing aromatic ring count influences several developability parameters such as lipophilicity and molecular weight and contributes to poor oral bioavailability. They also suggested that fused aromatic systems might have a beneficial effect relative to their nonfused counterparts. Their analysis revealed that newer drugs have increased heteroaromatic rings more than nonfused aromatic rings.

1.2.1.1 Lipophilicity Lipophilicity is the tendency of a compound to partition into a nonpolar lipid matrix versus an aqueous matrix. It is commonly determined by using log P from octanol/water partitioning. As has been mentioned earlier in this chapter,
in order for compounds to permeate across biological membranes to be absorbed and become bioavailable, a certain balance of lipophilicity to hydrophilicity is required. The drug lipophilicity is inversely related to solubility; higher lipophilicity leads to lower aqueous solubility. However, the balance between these two properties is very critical for compounds to be absorbed from the GI tract, and the relationship between log D/log P and permeability across biological membranes is nonlinear. The permeation decreases in both the low and high end of log D values, and often a log D value of 5 is considered as an upper limit of desired lipophilicity, as indicated in Table 1.1. It has been recognized that the higher the log P, the lower the solubility [60], while neutral molecules have been shown to be more poorly soluble compared to ionizable molecules. When clog P < 3, the average solubility of neutral molecules approaches the average solubility of ionizable molecules. The same trend has been shown with molecular weight: as it increases, solubility decreases [60]. To generalize the relationship between log P value (lipophilicity) and solubility/permeability:

1. A drug with log P value of 0–3 is considered optimal for passive diffusion [61].
2. A log P value of < 1 suggests that a compound will have good solubility by being hydrophilic but will have poor permeability.
3. A drug with log P value > 3 indicates that a compound is highly lipophilic, may possess low solubility, and is subject to metabolism and/or biliary excretion.

Determination of the extent of ionization in discovery of new chemical entities (NCEs) can be valuable in the selection process for potential drug candidates. The ionization is determined by measuring the dissociation constant pKₐ, which is indicative of compound capability to be ionized at various pH ranges, hence influences its solubility and absorption across the GI tract [62,63]. In humans, the pH of stomach is close to 2 and in the small intestine around 6, which can be affected by food.

Classically, ionization constant (pKₐ) is expressed using Henderson–Hasselbalch equation, which can provide the extent of ionized versus unionized compound at a particular pH:

For acidic compounds: $\text{pH} = \text{pK}_a + \log \left( \frac{[\text{ionized compound}]}{[\text{unionized compound}]} \right)$

For basic compounds: $\text{pH} = \text{pK}_a + \log \left( \frac{[\text{unionized compound}]}{[\text{ionized compound}]} \right)$

### Table 1.1 Log D Values and Impact in Drug Permeability and Oral Absorption.

<table>
<thead>
<tr>
<th>Log D at pH 7.4</th>
<th>Impact on Drug Disposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0</td>
<td>Poor absorption across GI tract and BBB. Potential rapid renal clearance</td>
</tr>
<tr>
<td>0–1</td>
<td>Balance between solubility and permeability, good oral absorption, but poor CNS permeability</td>
</tr>
<tr>
<td>1–3</td>
<td>Optimum oral absorption and CNS permeability. Low metabolism</td>
</tr>
<tr>
<td>3–5</td>
<td>Lower solubility, higher metabolic rate</td>
</tr>
<tr>
<td>&gt; 5</td>
<td>Poor solubility and absorption across the GI tract, high metabolism</td>
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</table>
To measure the $pK_a$, two methods have been used that employ cosolvents or surfactants. The cosolvent approach has been used successfully to solubilize unionized compounds. By mixing organic polar solvents like methanol, dioxin, or acetonitrile with water the solubility can be enhanced (MDM), though it was found that not all compounds would dissolve in cosolvent–water mixture. It is effective to dissolve the lipophilic compounds [62], and it can be used for compounds that are not soluble in methanol–water or other single organic cosolvent mixtures (e.g., 2-propanol, DMF, DMSO, and acetone). However, MDM also dissolves polar compounds, so it can be considered as an efficient cosolvent for $pK_a$ determination in drug research. In an investigation by Ravichandran et al. (2011) [63], they argued that the cosolvent approach might result in erroneous $pK_a$ determinations due to existence of two liquid boundaries, which may lead to variation in the ionization behavior of the NCE. These investigators used nonionic surfactants such as Tween 80, Cremophore EL, or Labrasol to determine $pK_a$ values of poorly soluble compounds where the solubilization occurs by changing the ionic, hydrophobic, and amphiphilic molecule to micellar structure formed by the surfactant.

1.2.1.2 Solubility Solubility is a critical parameter for absorption because drugs have to be in solution to permeate the GI membrane, and solubility has long been recognized as a limiting factor in the absorption process. By definition, solubility is the extent to which molecules from a solid are removed from its surface by a solvent. Solubility of solid drugs in a solvent matrix reaches maximum concentration at equilibrium, and that can be optimized by modifying the structures, hence the physicochemical properties, dissolution rate, and the solvent matrix used. Aqueous solubility can be estimated by determining the ability of a drug to partition from lipid to aqueous environments, which is dependent on the ionization of the drug tested. Most drugs are weakly acidic or weakly basic compounds that cannot ionize completely in aqueous media and therefore only partly ionize. Since drug ionization is greatly dependent on the solvent pH, the above partition behavior is often considered as a function of solvent pH, and $pK_a$ is often used as a parameter describing a compound’s dissolution characteristic. In general, ionized drugs tend to exhibit far greater aqueous solubility than the unionized counterpart. As a result, the rate of solute dissolution in aqueous media can be markedly affected by the pH of the solvent. Introducing ionizable groups, reducing lipophilicity, introducing hydrogen bonding and polar groups, reducing MW, and introducing out-of-plane substitutions can improve the solubility of NCE, though the introduction of ionizable groups may impair the permeability. Interestingly, while neutral molecules and larger molecular weight drugs have been shown to be more poorly soluble compared to ionizable and small molecules, at clog $P < 3$, the average solubility of neutral and ionizable molecules, smaller or larger MW is similar [60].

As pH decreases, there is a higher solubility and in turn greater concentration of neutral molecules and lower concentration of anionic acid molecules. At basic pH it is the opposite. When drug is delivered in the GI tract and as the luminal pH changes along different sections from acidic to basic, and in the presence of food, solubility of acids and bases will vary in an opposite way, if ionization drives the solubility.
The contributions of medicinal chemistry to improve solubility, via introduction of ionizable, N-containing basic groups [64,65], or disruption of planar crystal structure, have been highlighted in various examples within drug discovery programs [66,67].

It has been estimated that up to 90% of current NCEs suffer from low solubility according to the Biopharmaceutics Classification System (BCS) [68]. Because limited solubility may compromise absorption and thus drug likeness, it is important to assess the solubility and any potential issues as early as possible to avoid the risk of advancing of drug candidates in the development stage. Methods for measurement of solubility in the early discovery phase include kinetic and thermodynamic solubility. Kinetic solubility determination is carried out by spectrophotometry, turbidimetry, or nephelometry. Solubilities in simulated gastric fluid, simulated intestinal fluid (SIF), and fasting state SIF can be determined for selected compounds from the early discovery phase to assess its solubility in biological fluids ex vivo.

When assessing the solubility by determining the dissolution rate, which is defined as the rate at which the molecule dissolves into a solvent from a solid form, a molecule with a high dissolution rate will dissolve into solution quickly, leading to a quick absorption phase and increasing its chance to be absorbed within the GI transit time while its solubility remains constant. The dissolution rate depends on the particle size and compound physical and salt form. Reducing the particle size increases the surface area of the solid in contact with the solvent, which increases the dissolution rate.

The most frequent physical form in drug discovery is amorphous, the solid with no specific organization of molecules, unlike a crystal, which is a highly organized set of molecules. The amorphous form is often more soluble and less stable than the crystalline form. When an oral dose of poorly soluble amorphous compounds is delivered, there is potential that these compounds may precipitate in the GI tract to more stable and less soluble crystalline solid form, thus leading to lesser absorption. To increase the dissolution rate, a salt form can be developed. Salts can stay in solution in a supersaturated state and delay the compound’s precipitation. However, salts of weak acid or base can precipitate because they will convert to the free acid or base, leading to reduced intestinal absorption. By introducing the use of formulations or excipients, improvement in the molecule’s dissolution rate might be achieved.

1.2.1.3 Permeability As mentioned earlier, when a drug is delivered orally, it transports from the GI fluid across primarily the jejunum and the ileum segments of the small intestine into the portal blood system (Figure 1.1). This process involves transporting the drug across layers of lipid biolayer membranes, either by passive/facilitated diffusion, transcellular or paracellular mechanism, or active uptake or efflux, as shown in Figure 1.2, or by endocytosis. A drug has to cross the epithelial or mucosal cell walls, endothelial or capillary cell walls (bloodstream), and cellular plasma membranes to be absorbed, become bioavailable, and to be efficacious. Membrane permeability is not just critical for absorption of drugs across the biomembrane of the GI tract but also plays a very significant role in their distribution to all other tissues of the body (discussed in a later chapter). Most of the neutral lipophilic drugs enter a cell’s lipid membrane by the transcellular passive diffusion route, but hydrophilic or charged drugs cross the intestinal epithelial cell
membrane by paracellular passive diffusion through the tight junctions (TJs). Unlike the passive transcellular route, which takes place across most of the surface area of apical membrane of microvilli in the enterocytes, the paracellular route of absorption is limited, since the surface area of the TJ is about 0.01% of the small intestine surface area. However, the absorption route associated with the uptake and efflux functions of intestinal transporters is now considered as a very important mechanism of drug delivery. Those mentioned mechanisms of drug absorption are depicted in Figure 1.2 and will be discussed in more detail below.

The predominant mechanism of absorption for marketable drugs is passive diffusion [7], though other mechanisms can be involved when the physicochemical properties of drugs are beyond the “rule of 5.” It has been established that transport of drugs by passive diffusion requires no energy but is driven by concentration gradient and follows Fick’s law of diffusion:

\[
\frac{dQ}{dt} = DAK \frac{h}{h} (C_{GI} - C_p),
\]  

(1.3)

where \(\frac{dQ}{dt}\) = rate of diffusion, \(D\) = diffusion coefficient, \(K\) = lipid – water partition coefficient of drug in the biologic membrane, \(A\) = surface area of the membrane, \(h\) = membrane thickness, \(C_{GI}\) = drug concentration in the GI tract, and \(C_p\) = drug concentration in the plasma.

In explaining the law and its use in assessing drug permeability, these parameters are explained in more detail:

1. The term \((C_{GI} - C_p)\) represents the difference between free drug concentrations in the lumen and free drug concentrations in the plasma. There is a high degree of drug dilution due to high GI blood flow rate after permeation of drug into the GI membranes and the relatively high drug dose given orally (in the milligram range), which thus creates a large concentration gradient between the intestinal lumen and the bloodstream.

2. \(K\) which represents the lipid–water partitioning coefficient of a drug across the theoretical GI membrane; lipophilicity (log P) drug property can significantly impact this parameter.

3. \(A\) represents the surface area of the GI membrane accessible to the drug; the larger the surface area of the GI tract, the faster the drug can permeate. The duodenum is involved most in drug permeation because it has the largest surface area in the GI tract.

4. \(h\) represents the thickness of the theoretical GI membrane and the assumption it is constant across the GI tract.

5. \(D\) represents the amount of a drug that diffuses across a membrane for a given unit area when the concentration gradient is unity.

It is worth mentioning that \(K\), \(A\), \(h\), and \(D\) are constant for a given molecule with a given oral formulation and define the permeability \(p\) of a drug in Equation (1.4):

\[
p = \frac{DAK}{h}.
\]  

(1.4)
In addition, since the free plasma concentration is extremely low, $C_p$ is considered negligible. Therefore, Equation (1.5) can be a simple calculation of Fick’s law and used to determine the passive drug permeation through the GI membrane, which is a first-order process:

$$\frac{dQ}{dt} = P(C_{GI}).$$  \hspace{1cm} (1.5)

Since a majority of molecules permeating through the membrane are in a neutral form, accordingly, pH of the lumen and pK$_a$ of the molecules impact the degree of gradient concentration. As an example, without taking blood flow into account, the amount of neutral form of an acidic compound in the duodenum lumen is much higher than that in the plasma, and that further drives the gradient concentration in the direction toward greater intestinal drug permeation. However, a formal electrical charge can be highly delocalized and therefore be less of a barrier than believed, especially when lipophilicity is sufficiently high.

For the other passive diffusion mechanism through the TJs, paracellular permeation is a less frequent mechanism of intestinal absorption. TJs or zonula occludens constitute the major rate-limiting barrier toward hydrophilic drugs that are transported by paracellular mechanism. The dimensions of the paracellular space are between 10 and 50 Å, indicating the exclusion of any particles with a molecular radius exceeding 15 Å ($\approx 3.5\text{ kDa}$).

For the paracellular passive diffusion mechanism, transepithelial electrical resistance (TEER) tightens the intercellular junctional complex. There are gradients in TEER values across the GI regions, less tight in duodenal than colon. When TEER data are corrected for differences in mucosal surface area (see Figure 1.3), the permeability of small intestinal and colonic epithelium is determined to be virtually identical [69,70]. However, paracellular absorption is more likely to occur in the small intestine, not due to the more leaky TJs but because of a larger mucosal surface area. Endocytosis is a constitutive process observed in most mammalian cells for the uptake of macromolecules. It requires metabolic energy and it is a slow uptake mechanism resulting in a fusion of endocytic vesicles with lysosomes containing high levels of enzymatic activity. Endocytosis may involve specific receptors, for example, vitamin B12 receptor [71]. Endocytosis of compounds, like leptin, is believed to be limited in the small intestine, and in general is not a significant mechanism for drug absorption in the intestine.

### 1.3 EFFECT OF GI-PHYSIOLOGICAL FACTORS AND PATIENT CONDITION ON DRUG ABSORPTION

#### 1.3.1 Effect of pH, Intestinal Surface Area, Gastric Emptying, Transient Time, and Bile Acid

##### 1.3.1.1 Effect of pH and Surface Area

After oral dosing, the compound first encounters the buccal mucosa, where it can be absorbed, though the absorption at the buccal mucosa is negligible. The most important nonintestinal absorption site is
the stomach, which can take up nonionized, lipophilic molecules of moderate size. Compared to that of the intestines, gastric absorption is limited by the comparatively small epithelial surface area, relatively large volume, and brief amount of time that substances are in contact with the stomach epithelium. After ingestion via the esophagus, the drug arrives at the first region of the GI tract, the stomach. In the stomach, the drug is mixed with gastric acids, pepsinogen, and mucus secretions. The absorption in stomach is limited for most drugs due to the relatively small surface area ($< 0.1 \text{ m}^2$), the low blood flow perfusion rate (150 mL/min), and the rapid gastric emptying time (0.5–1 h). Although the acidic pH in the stomach can facilitate the absorption of acidic compounds in the stomach, the absorption of acidic compounds is faster in the small intestine.

The small intestine consists of three consecutive sections: duodenum, jejunum, and ileum. The pH of each section as shown in Table 1.2 increases gradually, creating a gradient from the stomach to the ileum. As has been mentioned before, the absorption in the small intestine is greater because of the larger surface area ($\sim 200 \text{ m}^2$), high blood flow perfusion rate (1 L/min), and lengthy transit time (2–4 h). To manage the intestinal drug absorption, the gastric emptying time can be the step to control the speed of drug absorption.

In the second part of the duodenum, the bile that is secreted from the gallbladder into the GI tract gets mixed with the digested drug. Bile, with its detergent-like properties, facilitates the solubilization and chemical breakdown of lipids, which explains why bile is secreted in the presence of lipids in the duodenum. The presence
TABLE 1.2 Physiological Parameters of GI Tract Regions.

<table>
<thead>
<tr>
<th>Region of GI Tract</th>
<th>Length (cm)</th>
<th>Range of pH Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>25</td>
<td>1.5–5.0</td>
</tr>
<tr>
<td>Small intestine:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>25</td>
<td>5.0–7.0</td>
</tr>
<tr>
<td>Jejunum</td>
<td>260</td>
<td>6.0–7.0</td>
</tr>
<tr>
<td>Ileum</td>
<td>395</td>
<td>7.0–7.4</td>
</tr>
<tr>
<td>Large intestine:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cecum</td>
<td>7</td>
<td>5.7–5.9</td>
</tr>
<tr>
<td>Colon</td>
<td>93</td>
<td>5.5–7.5</td>
</tr>
<tr>
<td>Rectum</td>
<td>55</td>
<td>6.5–7.0</td>
</tr>
</tbody>
</table>

of peptides and amino acids in the duodenum activates the secretion of pancreatic enzymes: amylases, lipases, and proteases. The pancreatic enzymes can hydrolyze some molecules that contain hydrolyzable functional groups, resulting in the deactivation or activation of the drug in the GI tract. In addition, the pancreas secretes bicarbonates ion, which neutralizes acid stomach secretions. At the ileocecal junction, the small intestine connects with the large intestine. Because of the modest surface area of the human large intestine (0.35 m², which is less than 0.15% that of the small intestine), the large intestine tends to play a minor role in absorption compared with the small intestine. Although the large intestine transit time is long (7–20 h), drug absorption is still limited mainly because of its small surface area, as mentioned, and its lack of villi.

The pH of the luminal contents (as seen in Table 1.2) is the lowest in the stomach and increases as the chyme progresses distally through the GI tract, approaching neutrality. This modification in pH as the chyme travels through the GI tract is accomplished through the secretion of various acidic and alkaline fluids. In most regions of the digestive tract, the secretions are slightly alkaline, and the luminal contents exhibit a pH of 7–8 as chyme approaches the distal small intestine. The stomach is the lone exception to this general statement. The secretion of acid by the gastric mucosa results in acidification of chyme. The pH of chyme affects the ionization state of certain molecules and, therefore, can affect absorption. The fasting versus fed, as will be explained later, can alter the intestinal pH and thus the rate of drug absorption. In addition to the pH gradient that exists along the linear axis of the GI tract, a pH gradient also exists from the center of the lumen moving radially toward the epithelial surface. The pH of the lumen is more acidic than the pH of contents at the epithelial surface, a function of the unstirred layer of water with the brush border [72] and the alkaline secretions of the intestinal epithelia [73]. This gradient may also affect the rate of uptake of endogenous and exogenous chemicals.

Many factors are involved in oral drug delivery, and the oral bioavailability of a particular drug can be a reflection of several components related to its delivery to the intestine (e.g. relative surface area, gastric emptying, pH, food). Because the surface
area is a dominant factor in the passive diffusion permeability mechanism, the rate and extent of absorption is increased by expansion of the absorptive surface area.

The luminal content is also changed through the progressive absorption of fluids, electrolytes, nutrients, and xenobiotics as chyme moves through the GI tract. It should be noted that of the 8–9 L of fluid that enters the human upper digestive tract each day (~1.5 L of ingested fluid plus ~7 L of secreted digestive juice), only ~1 L enters the large intestine and only about 100 mL of water is found in the daily output of feces [72]. Surface area can be altered by physiological changes that occur in response to nutritional challenges, for example, during times of starvation; in disease states, as in diabetes (discussed later); during normal physiologic stress in pregnancy; and during human growth, though the villi of the small intestine decrease in height during old age, and that will reduce the extent of absorption.

1.3.1.2 Effect of Gastric Emptying and Intestinal Transit Time In addition to the influence of pH and surface area, there are other important parameters in initiating and enhancing the extent of drug absorption. Gastric emptying rate is an important factor because it affects the plasma concentration profile (AUC, $T_{\text{max}}$ and $C_{\text{max}}$) of orally administered drugs. The significant effect of gastric emptying has been reported, using celiprolol as an example [74]. The rate and the extent of celiprolol absorption and the influence of gastric emptying on the occurrence of double peaks were demonstrated. It has been revealed that variability in gastric emptying rates due to the motility cycle can account for plasma level double peaks [75]. Furthermore, variable gastric emptying rates combined with the short plasma elimination half-life and poor gastric absorption of cimetidine cause the observed plasma level double peaks. The effect of altered gastric emptying and GI motility on the absorption of metformin in healthy subjects has been reported [76]. The results indicated that $AUC_{(0,\infty)}$ and percent dose excreted unchanged in urine increase with the increase in gastric emptying time and small intestinal transit times; that is, the extent of metformin absorption is improved when the GI motility is slowed. By using previously developed GI-Transit-Absorption Model, the prediction method for the plasma concentration-time profile of N-methyltyramine (NMT) was achieved [77]. By estimating the permeability of NMT at each GI segment, it is revealed that NMT is absorbed mainly from the small intestine and that permeability is at the highest level in the duodenum and jejunum. However, the contribution of these regions to the total absorption in vivo is found to be small. The substantial absorption sites in vivo were suggested to be the regions from lower jejunum to lower ileum, which have a longer residence time than duodenum and upper jejunum, thus the substantial absorption is a function of longer residence time.

Furthermore, the intestinal transit rate significantly influences the drug absorption and can make a difference in absorbability for some drugs because it determines the residence time of the drug in the absorption site.

1.3.1.3 Effect of Bile and Bile Salts Humans secrete from the gallbladder 2–22 mL of bile per kilogram body weight each day [78], at a rate of 3–45 mmol/L. Bile acids are an extensive group of molecules that share a structural similarity to
cholesterol but exhibit differences with regard to substituent side groups. Bile acid in humans is moderately lipophilic and is a complex mixture of organic and inorganic materials, of which the various bile acids are the major component, composed of cholic acid, deoxycholic acid, and chenodeoxycholic acid.

Bile secreted into the GI tract may improve the bioavailability of poorly water-soluble drugs by enhancing their rate of dissolution and/or solubility. Bile salts can increase drug solubility via micellar solubilization. The increase in the rate of dissolution also may occur via a decrease in the interfacial energy barrier between solid drug and the dissolution media (via enhanced wetting), leading to an effective increase in surface area [79]. A study to examine the dissolution of low solubility–high permeability compared to high solubility–low permeability class of drugs provided results that in general low solubility–high permeability drugs depend much more on the medium, including the presence of bile salt, than high solubility–low permeability drugs [80]. The ability of sodium taurocholate (one of the bile salts secreted in the GI tract) to increase the initial dissolution rate of five steroids (hydrocortisone, triamcinolone, betamethasone, dexamethasone, and danazol) was tested [81]. The result showed that at bile salt concentrations representative of the fasted state, the wetting effects predominated over solubilization effects for all compounds [81]. At the higher bile salt concentrations, typical of the fed state, the increase in solubility was the predominant factor for the more lipophilic danazol. Furthermore, the extent to which bile salts can enhance the solubility of a drug can be predicted based on the physicochemical properties of the compound. The increase in solubility as a function of bile salt concentration can be estimated based on the partition coefficient and aqueous solubility of the compound [82].

1.3.2 Impact of Age and Disease State on Drug Absorption

1.3.2.1 Drug Absorption in Pediatric Populations Immaturity of the sphincter may lead to increased reflux of the stomach contents, which may contain drugs; drug absorption may thus be reduced [83]. Maturation of an effective antireflux barrier is not achieved until ~3 months postnatal [84]. It has been mentioned that the limited absorption from the stomach can be affected by gastric pH, which is neutral at birth but falls to ~1 – 3 within the 24 h after birth, followed by a gradual return to neutrality by day 8 [85] and slow decline again thereafter (e.g., pH = 2 – 3 by the age of 2–3 years) to reach adult values. At age 3 years, the amount of gastric acid excreted per kilogram body weight is similar to that excreted in adults [86]. Consequently, younger children, because of the reduced gastric acid production and secretion, have low absorption of drugs that need gastric acid for dissolution/absorption. Acid-labile drugs, such as penicillin G, ampicillin, amoxicillin, flucloxacillin, and erythromycin, are more efficiently absorbed when orally administered in the neonate and infant than in the adult [87–89]. However, such changes are unlikely to affect the absorption of non-acid-labile drugs, for which absorption will continue efficiently in the small intestine.

Gastric emptying (rate of removal of a drug from the stomach) is delayed in the neonate and infant [85,90], and emptying times of 6–8 h have been reported in
neonates. This may result in delayed absorption of orally administered drugs. The $T_{\text{max}}$ is delayed and the $C_{\text{max}}$ is also likely to be lower, although in most cases the AUC will not be affected. The exact age at which gastric emptying time approaches adult levels is unclear.

Intestinal transit time appears to be shorter in young children, thus suggesting that sustained-release products may demonstrate incomplete absorption [91,92]. The unreliability of the sustained-release mechanisms seems to be more marked in children than in adults [93]. Therefore, extrapolation of adult data to children is particularly delicate for sustained-release formulations, as has been extensively demonstrated for theophylline [94–97].

Immaturity of secretion and activity of bile and pancreatic fluid leads to impaired fat digestion in neonates and infants in the first few months. The absorption of fat-soluble vitamins (vitamins D and E) is reduced in neonates probably because of the inadequate bile salt pool in the ileum [98]. Impaired fat digestion could be of toxicological importance when lipophilic compounds such as dichlorodiphenyl-trichloroethane and structurally similar compounds are ingested, leading to a reduced uptake of these compounds in neonates and young infants. After a few months, the infant is capable of efficiently absorbing fat-soluble compounds due to a postnatal maturation of bile salt metabolism [99,100]. Ethnic/racial dietary differences can be a source of confounding for adult–pediatric comparisons pertaining to absorption. Moreover, many pediatric formulations are liquid or suspensions, as opposed to their adult-equivalent solid dosage forms, which also constitutes a source of confounding for adult–pediatric comparisons pertaining to absorption and bioavailability.

Intestinal expression of drug transporter-mediated absorption of substrates that are administrated in children was recently reported [101]. MDR1, MRP2, and OATP2B1 was determined in surgical removed small bowel samples (neonates $n = 15$, infants $n = 3$, adults $n = 14$). In adults, drug transporters are recognized as key determinants of variation in the pharmacokinetics of many drugs, as shown in studies using primary cell and ex vivo organ cultures, as well as clinical studies. In contrast, such data in children are scarce, and clinical studies are absent (Yanni et al. (2011) [102]). Neonatal intestinal expressions of MDR1 and MRP2 were comparable to those in adults, while intestinal OATP2B1 expression in neonates was significantly higher than in adults. This suggests that drug absorption mediated by these transporters may be subject to age-related variation in a transporter-dependent pattern.

1.3.2.2 Drug Absorption in Disease State

Diabetes and its effect on altering the absorption of drugs in patients is discussed in this section. One factor that has an impact on drug absorption is drug transporters, specifically, as mentioned in this chapter, intestinal P-gp, which acts as the first barrier for drugs administered via the oral route [103]. Indeed, intestinal P-gp is likely to play a critical role in the extent of absorption of its orally delivered substrate drugs.

As has been reported, the expression and/or drug efflux activity of P-gp can be modulated not only by inherited factors, such as genetic polymorphisms [104], but also by environmental factors, such as diet [105], drugs [106], or diseases [107] like epilepsy, seizure, and diabetes.
Among these diseases, the prevalence of diabetes—classified as either type 1, characterized by dysfunction of pancreatic β cells, leading to insulin depletion, or type 2, characterized by dysfunction of the insulin receptor (insulin resistance) or by impairment in insulin secretion—is estimated as 9.2–9.8% of the adult population worldwide [108]. In addition to the modulation of P-gp in intestine, the expression and functional activity of P-gp was found to be altered in the brain, liver, and kidney under diabetic conditions [109].

Several antidiabetic drugs, such as glibenclamide [110], rosiglitazone, metformin [111], repaglinide [112], and linagliptin [113], have been found to be substrates for P-gp. Changes in the expression of intestinal P-gp due to the disease may have a critical role in the absorption process of these drugs, though few studies have been conducted to address the changes in intestinal P-gp on the PK/PD of antidiabetic drugs under diabetic conditions.

In the intestine, there is insufficient information about the features of PK/PD after oral administration of substrate drugs for P-gp. However, to design appropriate or individually targeted pharmacotherapy, it has been recommended [114] to consider the influence of changes in the expression and function of P-gp in the intestine under diabetic conditions.

Other conditions that will have an impact on drug absorption are starvation, physiologic stress in pregnancy, and during human growth decrease in absorptive surface area and, consequently, reduced efficiency in the absorption of nutrients and other materials from chyme.

### 1.4 EFFECT OF FOOD AND FORMULATION ON DRUG ABSORPTION

#### 1.4.1 Effect of Food

The impact of food on intestinal absorption through bioavailability is extremely complex. Food delays gastric emptying, stimulates bile flow, changes pH of GI segments, alters the luminal metabolism, or interacts with prescribed drugs [115]. Clearly, the extent of the food’s effect is variable and can be dependent on the meal content of protein, carbohydrate, or fat; volume; and fluid ingestion. Also, the effect of food can be based on the physicochemical properties of the compounds.

Food also increases blood flow to the liver, thus may change the first-pass effect and bioavailability between the fed and fasted state. The pH differences in the contents of the upper GI tract between fed and fasted states, as seen in Table 1.3, can influence the dissolution and absorption of weakly acidic and basic drugs. Elevation of gastric pH following a meal may enhance the dissolution of a weak acid in the stomach but inhibit that of a weak base. Furthermore, food inhibits the rate of gastric emptying, and prolonged retention in the stomach may increase the proportion of drug that dissolves prior to passage into the small intestine, which is the primary site of drug absorption [116]. Elevated gastric pH may afford enhanced bioavailability of acid-labile drugs such as penicillin, erythromycin, and digoxin. For ionic drugs, changing pH values can alter the fraction absorbed and thus the
permeability. Changing the pH can affect the dissolution of some formulations and can vary excipients–drug release.

Food has a significant effect, as it can cause drug–food interactions. As an example, if a drug chelates with ions present in the ingested meal, drug dissolution and/or absorption may be reduced. Furthermore, the meal itself may pose a physical barrier that prevents drug diffusion to the site of absorption, resulting in decreased bioavailability. Also, drug instability, as a result of acid degradation, may be exacerbated by prolonged gastric residence after food ingestion. For highly lipophylic drugs or large MW macromolecules, lymphatic uptake can be increased by the presence of a high-fat meal, thereby lowering plasma drug levels.

In general, food effect is not always predictable; however, its effect on bioavailability is the greatest when the drug is administered shortly after a meal is ingested and when meals are high in total calorie and fat content. Accordingly, the Food and Drug Administration (FDA) recommends the use of high-calorie and high-fat meals to study the effect of food on the bioavailability and bioequivalence of drugs (hamburger meal). Furthermore, qualitative prediction of food effect is often possible based solely on the BCS class of the drug, but food effect whether negative, positive, or no effect was classified for 80% of a set of 92 drugs, based simply on their dose, solubility, and permeability [117].

1.4.2 Formulation Effect

Formulation is an additive component of the dosed drug that facilitates or enhances the absorption and hence the bioavailability of the drug by ensuring drug dissolution/solubility. There are several variables that affect the dissolution rate as described by the Noyes–Whitney equation [118], as it shows the dissolution rate as a function of diffusion coefficient, surface area of particle, the thickness of the dissolution film adjacent to the dissolving surface, the saturation solubility of the drug molecule, the concentration of the dissolved solute, and the volume of the dissolution medium. Drug formulation can control the surface area and solubility but not the other variables. Clearly the most important factor achieved by formulation is the change in surface area. Increasing the surface area of a drug particle can enhance the dissolution rate of the drug. Using wetting agents that lower the surface tension of the dissolution medium and that reduce drug particle size thus increase the effective surface area for dissolution. However, due to the amount of the above surface-active

### Table 1.3 pH of the Gastrointestinal tract under Fed vs. Fasted State.

<table>
<thead>
<tr>
<th>GI Tract Section</th>
<th>pH, Fasted</th>
<th>pH, Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>1.4–2.1</td>
<td>3–7</td>
</tr>
<tr>
<td>Duodenum</td>
<td>4.4–6.6</td>
<td>5.2–6.2</td>
</tr>
<tr>
<td>Jejunum</td>
<td>4.4–6.6</td>
<td>5.2–6.2</td>
</tr>
<tr>
<td>Ileum</td>
<td>6.8–8</td>
<td>6.8–8</td>
</tr>
<tr>
<td>Colon</td>
<td>5.5–7</td>
<td>5.5–7</td>
</tr>
</tbody>
</table>
agents needed to enhance in vivo drug dissolution rate, these agents are not generally used in product formulations [118]. The solubility of weak acid and weak base can be modified by using a buffer agent that slightly changes the surrounding pH. Besides buffering agents, some excipients are known to have effects on physiological conditions, such as decreasing GI transit time [119], affecting membrane permeability [120], and inhibiting efflux pumps [121].

1.4.3 The BCS in Relation to Intestinal Absorption

The BCS has been one of the most significant prognostic tools created to promote product development in recent years [122]. It is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability characteristics, which will substantially facilitate the drug product selection and approval process for a large group of drug candidates. The goal of the BCS is to function as a tool for developing in vitro dissolution specifications for drug products that are predictive of their in vivo performance [123]. According to the BCS, drug substances are classified as follows:

Class 1: high solubility–high permeability: generally very well-absorbed compounds. Effect of drug transporters is minimum
Class 2: low solubility–high permeability: exhibit dissolution rate-limited absorption. Effect of efflux transporters is predominant
Class 3: high solubility–low permeability: exhibit permeability rate-limited absorption. Effect of absorptive transporters is predominant
Class 4: low solubility–low permeability: very poor oral bioavailability. Effect of absorptive and efflux transporters could be both important

There some limitations in these classified system, such as:

1. A drug substance is considered highly soluble when the highest dose strength is soluble in \( \leq 250 \) ml water over a pH range of 1–7.5.
2. A drug substance is considered highly permeable when the extent of absorption in humans is determined to be \( \geq 90\% \) of an administered dose, based on mass balance or in comparison to an intravenous reference dose.
3. A drug product is considered to be rapidly dissolving when \( \geq 85\% \) of the labeled amount of drug substance dissolves within 30 minutes using USP Apparatus 1 or 2 in a volume of \( \leq 900 \) ml buffer solution.

The pH-solubility profile of the test drug substance should be determined in aqueous media with a pH in the range of 1–7.5 using the traditional shake-flask method as well as acid or base titration methods. A sufficient number of pH conditions should be evaluated to accurately define the pH-solubility profile. Concentration of the drug
substance in selected buffers (or pH conditions) should be determined using a validated stability-indicating assay that can distinguish the drug substance from its degradation products.

The permeability class of a drug substance can be determined in human subjects using mass balance, absolute bioavailability, or intestinal perfusion approaches:

1. Pharmacokinetic studies in humans: mass balance studies or absolute bioavailability studies.
2. Intestinal permeability methods: the following are the methods that can be used to determine the permeability of a drug substance from the GI tract: (a) in vivo intestinal perfusion studies in humans; (b) in vivo or in situ intestinal perfusion studies using suitable animal models; (c) in vitro permeation studies using excised human or animal intestinal tissues; or (d) in vitro permeation studies across a monolayer of cultured epithelial cells.
3. Instability in the GI tract: determining the extent of absorption in humans based on mass balance studies using total radioactivity in urine does not take into consideration the extent of degradation of a drug in the GI fluid prior to intestinal membrane permeation.

For immediate-release formulations of Class I drugs, during the dissolution tests, verify that the drug is rapidly released from the dosage form under mild aqueous conditions.

For Class II drugs, to establish a strong correlation between the results of dissolution tests and the in vivo absorption rate, it is essential to reproduce the conditions existing in the GI tract following administration of the dosage form. Adequate comparison of formulations for Class II drugs requires dissolution tests with multiple sampling times in order to characterize the release profile. Use of more than one dissolution medium might be applied.

Class III drugs are defined as being rapidly dissolved as Class I drugs, then the formulation can be used to release the drug under mild aqueous conditions within a predetermined time. The duration of the dissolution test should be at least as stringent for Class III drugs. For Class I drugs, the contact time between the dissolved drug and intestinal epithelia needs to be maximized, thus increasing the bioavailability.

As for Class IV drugs, which are generally considered poorly absorbed, special attention should be given to the formulation to avoid further deterioration in the rate and extent of drug absorption caused by poor formulation.

The BCS is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability and it takes into account three major factors that govern the rate and extent of drug absorption from immediate-release solid oral dosage forms: dissolution, solubility, and intestinal permeability. The BCS classification can be used to request a waiver from the FDA for in vivo bioavailability and/or bioequivalence studies for immediate-release solid oral dosage forms.
1.5 TRANSLATIONAL APPROACHES TO DETERMINE DRUG ABSORPTION IN CLINICAL STUDIES

1.5.1 Cellular Intestinal Model

The use of epithelial cell cultures for studies that predict the extent of oral drug absorption and mechanism of drug transport has been widely introduced in drug discovery and development programs within the pharmaceutical industry. Cell lines originated from human tumor, which possess properties and most of the morphological and functional characteristics of enterocytes, were used since the use of primary intestinal epithelial cells is limited due to the rapid loss of their differentiated characteristics during culture. Most commonly used cell lines are Caco-2, HT-29, and T84 cells. Other cell lines, such as HCT8, HRT18, or SW1116, express only a partially intestinal differentiated phenotype [124]. Although these cell lines have some recognized limitations, the simplicity of their use as in vitro models provides a clear advantage over their disadvantages. Caco-2 cells, which derived from a human colorectal adenocarcinoma, express the majority of the morphological and functional characteristics of small intestinal absorptive cells, including the spontaneous formation of polarized membranes, formation of TJs, expression of drug transporters, and phase I and phase II drug-metabolizing enzymes. Upon cultivation as monolayers on semipermeable membrane of Transwell format for 21 days, the differentiated Caco-2 cells form the apical compartment and basolateral compartment, which correspond to the intestinal lumen side and the serosal side, respectively. However, because Caco-2 cells are originated from colon and tumors that do not resemble the in vivo physiological environment of the small intestine, extrapolation of the data to the in vivo situation may be difficult.

The typical experimental setup for a Caco-2 permeability experiment is to measure the apparent rate of permeability (P_app) of the compound in both an apical to basolateral (A to B) and a basolateral to apical (B to A) direction after incubation at 37°C for a given period of time. The relative rates of permeability from A to B (influx) and B to A (efflux) directions and ratio (B to A/A to B) can be indicative of the mechanism of permeability. This is a well-established technique in academia and industry for high-quality permeability measurements, but the culture of the cell line is expensive and manually intensive when used as a screen for permeability [125,126].

On the other hand, MDCK (Madin–Darby canine kidney) is a dog kidney cell line that has also been applied to permeability measurements [127–129]. Although this cell line requires 3 days of culture compared to 15–21 days for most Caco-2 models, it is still relatively expensive and requires cell culture expertise. It is also less physiologically relevant, as this is a canine kidney cell line and generally the permeability measured is used to estimate intestinal absorption in humans.

1.5.2 In Vitro Artificial Membrane

In order to meet the need for higher throughput and less cost to measure the permeability, the parallel artificial membrane permeability assay (PAMPA) is utilized.
The artificial membranes are designed to mimic the GI membranes [130–133]. The amount of compound that moves from the donor chamber to the acceptor chamber after a period of incubation is measured, along with the amount left in the donor chamber. These measurements and other parameters characteristic of the experimental setup are used to determine the passive diffusion permeability, \( P_e \), and the retention factor, \( R_f \) [132]. PAMPA is performed in a 96-well plate and suitable for automation setting; accordingly PAMPA has been used as a primary screen for measuring the intrinsic permeability for thousands of NCEs per week prior to selecting leads to be tested in the cell model assay [130,133]. Because PAMPA does not provide information about whether a compound is a substrate for active influx or efflux transporters, further mechanistic information could be determined using the Caco-2 cell line. Also, PAMPA does aid the chemist, as the output can be a simple measure of permeability that allows for clearer SARs and easier interpretation when attempting to design a more permeable series.

As the FDA guideline recommended, the process to assess drug absorption using in vitro tools is as follows:

1. Examine bidirectional transport of the test compounds in Caco-2 or MDR1-MDCK cell monolayers.
2. Select likely substrates based on an efflux ratio > 2.
3. Confirm P-gp substrate activity using specific inhibitors, determining \( K_i \) or \( [I]/IC_{50} \) of the test compounds.
4. Select compounds for in vivo interaction studies with a P-gp substrate such as digoxin if efflux ratio > 2 and in step 3, compounds possesses \( K_i \) or \( [I]/IC_{50} > 0.1 \).

1.5.3 Non–In Vitro Models: In Situ and In Vivo

To predict the human absorption in more accurate fashion before first injection in humans (FIH), studies using in situ models and in vivo models were applied. Although in situ models are seldom used in a drug discovery setting, in situ single-pass perfusion of the rat intestine (sometimes mice model) can provide useful mechanistic information. The drug concentration, usually radiolabeled, in the intestine is known and controlled and the subsequent barriers that a compound has to cross to reach the portal blood circulation are identical in the in situ and in vivo situation. The model dimension certainly offers reliable data and justifies the use of an in situ model despite the technical challenge in its setting, labor intensity, and low throughput.

The in vivo pharmacokinetic studies to give a sense of the absorption profile of their molecules are always the most applicable approach. These studies depend on using radiolabeled drugs and including as one element the preclinical mass balance study. Rat is normally used to determine the fraction absorbed and the bioavailability, and hence to understand any obstacles that may hinder these parameters in humans. By comparing rat absorption with human absorption for approximately a hundred
Drugs, a similarity of percentage of dose absorbed was presented [134]. Consequently, the authors recommended that the in vivo absorption information in rats could be used as a translational method to predict the extent of intestinal absorption in humans. In addition, by finding a moderate correlation in the expression of all known transporters in both human and rat duodenum, the mechanism of human absorption can be determined from rat data. However, there was no correlation in the expressions of drug-metabolizing enzymes, indicating a disconnection in the bioavailability between rat and human [135]. It is worth mentioning that although dog was commonly used as a nonrodent preclinical species for drug discovery and development in humans, there was poor correlation between fraction absorbed based on a retrospective study in human and dog for approximately 40 drugs [136].

Drug development studies that determine drug absorption by using radiolabeled drug are generally conducted by introducing radiolabel of $^3$H or $^{14}$C. The drug is administered orally (PO) and intravenously (IV) to intact or bile duct–cannulated animals or intact humans. The urine, bile (in animals only), plasma, and feces are collected until a full recovery of radioactivity has occurred. The fraction absorbed ($f_a$) can then be assessed using several approaches.

1. Calculating the percentage of cumulative excretion of radioactive drug-related material in urine and bile from bile-duct animals following oral administration:

$$f_a = \frac{\% \text{ total dose excreted in urine and bile}}{100}$$

2. Measuring the amount of unchanged drug in feces after oral administration from intact animals:

$$f_a = \frac{\% \text{ dose of parent drug excreted in feces}}{100}$$

3. Comparing the amounts of total radioactivity excreted in the urine after PO and IV dose:

$$f_a = \frac{\% \text{ dose excreted in urine after PO dose}}{\% \text{ dose excreted in urine after IV dose}}$$

4. Comparing the exposure (AUC) of total radioactivity after PO dose and IV dose:

$$f_a = \frac{\text{AUC total radioactivity after PO dose}}{\text{AUC total radioactivity after IV dose}} \times \frac{\text{Dose IV}}{\text{Dose PO}}$$

By employing these approaches, the fraction absorbed and mechanisms of excretion for several drugs were assessed [137–141].

In a more recent investigation, Augustijns et al. (2014) [142] reported the use of simulated media as an attractive approach for the prediction of oral absorption, especially for drugs that have poor aqueous solubility. Solubility assessment in human intestinal fluid (HIF) can be considered as the most optimum approach, but unfortunately, HIF is not commercially available and its collection is impractical as
well as requires institutional review board approval because the use of biological samples in an industry setting is regulated by strict legality rules. The use of simulated media was found to be a practical approach and found to mimic the HIF. Augustijns [142] and his colleagues demonstrated a correlation between simulating intestinal fluid for fasting and fed state (FASSIF and FESSIF, respectively) with HIF for fasting and fed state (FaHIF and FeHIF, respectively) for hundreds of drugs as a simple but effective way of getting adequately accurate estimates of intestinal solubility.

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