1.   Introduction

The oral administration of a drug is the most frequent and convenient route of administration, and formulation researchers normally strive to formulate drugs so that they can be administered orally. Exceptions to this general rule occur when the site of action is accessible (e.g. creams and ointments for local treatment of skin conditions) or when the drug cannot be absorbed from the gastrointestinal tract (e.g. proteins like insulin). In most cases, orally administered drugs are intended to act systemically, so they have to be absorbed during their passage through the gastrointestinal tract. There are only a few drugs which are administered orally and are intended to act locally, e.g. some antacids like calcium carbonate or sucralfate, drugs used for pancreatic enzyme replacement therapy, and anti-inflammatory drugs which are used for the therapy of Crohn's disease and ulcerative colitis [1].

For a drug to be absorbed from the gastrointestinal tract into the systemic circulation, it has to be released from its formulation, dissolve within a reasonable time span (corresponding to the drug's passage through the regions in which it can be absorbed, e.g. in the small intestine and/or the colon), cross the gut wall, and enter into systemic circulation. If an orally administered drug does not dissolve within a reasonable time span, it cannot be completely absorbed and thus may not reach its target in sufficient quantities to exert its action. After reaching the blood stream, the drug is distributed in the body, and a certain fraction – depending on the distribution pattern– reaches the site of action. Parallel to drug absorption and distribution, elimination of the drug from the body starts. In most cases, the drug is metabolized in the liver, but there is also the possibility that the drug is metabolized in other organs, e.g. in the small intestine or in the lungs. After being metabolized, the drug is excreted, and the most important route of drug excretion is via the urine (renally). Other routes of excretion include e.g. pulmonary or biliary excretion or excretion via the sweat [1].

Scientifically speaking, the aforementioned processes can be described with the term "*pharmacokinetics*" (from the Greek φάρμακον [*pharmakon*; drug] and κινητικός [*kinetikos*; in motion]), and this term thus describes the process of absorption, distribution, metabolism, and elimination of all kinds of drugs (small or large molecules) and nutrients which are administered to living organisms. These processes are commonly referred to as "ADME" characteristics. By contrast, the term *"pharmacodynamics"* describes the (pharmacological) effect of the drug on the organism. It is thus reasonable that pharmacokinetics and pharmacodynamics must be linked together in order to describe the mutual relationship between drug and organism.

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In earlier times, active compounds were often discovered either by coincidence (e.g. the discovery of penicillin by Alexander Fleming) or empirically developed from "natural" structures, e.g. acetylsalicylic acid (which is derived from salicin, an ingredient in willow bark), antihypertensives like β-blocking agents (which are structurally related to adrenaline), angiotensin-converting enzyme inhibitors (which are derived from the poison of *Bothrops jararaca*, a South-American lance-head viper), and some anti-cancer drugs such as paclitaxel (which is derived from taxol, an alkaloid from the Pacific yew) [1, 2].

More recently, combinatorial chemistry has gained importance in drug discovery. In this approach, a large number of potential drug candidates are synthesized and subsequently screened for their affinity to a potential target, e.g. a receptor, within a short time-frame (so-called high-throughput screening). However, selection of potential drug candidates using this approach is based on *in vitro* pharmacology (e.g. receptor affinity). In many cases, lipophilicity is important to the interaction, and where this is the case, the selected candidates will often tend to have poor aqueous solubility [2-4]. The prediction of liberation, absorption, distribution, metabolism, and excretion (LADME) properties for these poorly soluble drug compounds is often more challenging than for highly soluble compounds, since various physiological factors such as (variations in) gastric emptying rates, small intestinal residence times, first pass metabolism, gastrointestinal fluid volumes, concentrations of natural surfactants, and/or the effective surface area at the site of absorption can all impact the pharmacokinetic profile after oral ingestion. As a consequence of this paradigm shift in oral drug development, the prediction of the intralumenal solubility and dissolution behavior of poorly soluble drug compounds and their consequences for oral drug absorption has gained importance in recent years.

1.1. Classification of Poorly Soluble Drugs

1.1.1. The Biopharmaceutics Classification Scheme (BCS)

The first approach to systematically classify drugs in terms of their biopharmaceutical characteristics was introduced by Gordon Amidon and colleagues in 1995, and the BCS addresses the interplay between the drug's solubility and permeability characteristics [5]. According to their solubility and permeability characteristics, drugs can be classified into four groups (Fig. 1-1.).
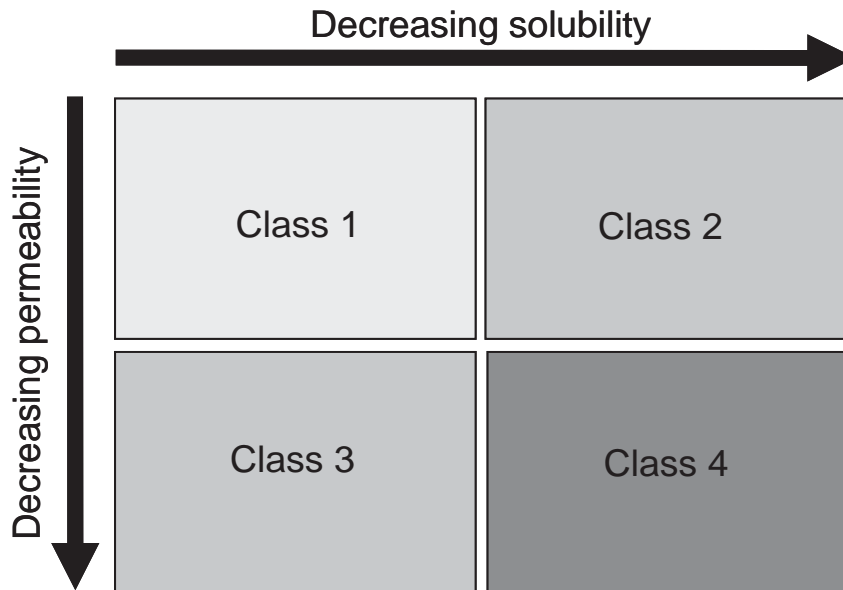
Fig. 1-1. Biopharmaceutics Classification Scheme, as proposed by Amidon et al. [5].

"Highly soluble" drugs show a dose/solubility ratio ≤ 250 mL in the *p*H range between *p*H 1.0 and 7.5, indicating that the whole dose can dissolve in a volume of 250 mL or less over this *p*H range. According to Amidon's definition [5], "high permeability" is defined as an extent of absorption ≥ 90%. However, the definition of "high permeability" has been modified somewhat by various regulatory agencies in the ensuing years [6-9]. It should additionally be emphasized that in all jurisdictions, the definition of "highly permeable" refers to the fraction of drug absorbed including metabolites formed after uptake in the intestinal membrane and not to the absolute bioavailability of the drug, which reflects only the fraction of drug reaching the systemic circulation intact.

Approximately 70% of the drugs currently available on the market can be classified as "poorly soluble" (BCS classes 2 and 4) [3]. Additionally, approximately 90% of the drugs under pharmaceutical development can be considered "poorly soluble", which makes the prediction of their pharmacokinetics more challenging [3].

1.1.2. The Biopharmaceutics Drug Disposition Classification Scheme (BDDCS)

The BDDCS provides an alternative approach to the BCS. In contrast to the BCS, the BDDCS not only considers the influence of a drug's solubility and permeability on its overall absorption, but also other factors, such as active transport (influx and efflux transporters), food effects, and the route(s) of metabolism and elimination. Consideration of this expanded group of factors is expected to improve the predictability of the drug's absorption and its *in vivo* disposition [3].

According to Wu and Benet, the drug's extent of absorption should no longer serve as criterion for classifying the drug in one of the four categories. As an alternative, elimination

should be used. For example, drugs with "extensive metabolism" (defined as ≥ 50% of a given dose metabolized) can be classified as class 1 (high solubility and permeability) or class 2 (low solubility, high permeability), whereas drugs with poor metabolism can be classified as either class 3 (high solubility, poor permeability) or class 4 (low solubility and permeability) compounds (Fig. 1-2.). The solubility characteristics are then used to assign the drug to a specific class.
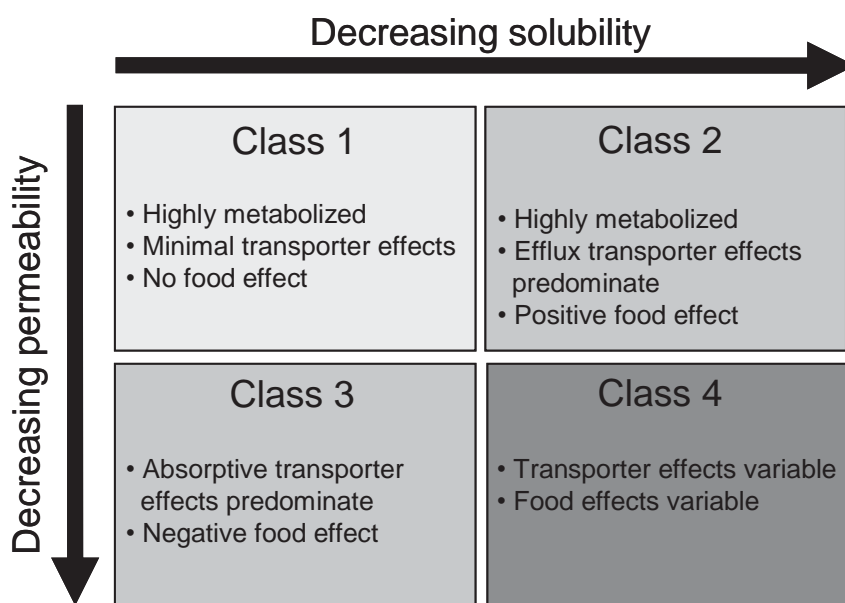

Fig. 1-2.   Biopharmaceutics Drug Disposition Classification Scheme, proposed by Wu and Benet [3].

The BDDCS suggests that there are strong links between the drug's solubility, permeability, and metabolic behavior, leading to a better understanding of the interactive role the LADME parameters play in both the pharmacokinetic profile and the clinical effect of the drug [3, 10-15].

1.2.   Intralumenal Events in the Context of Oral Absorption and Factors that Limit the Bioavailability of Orally Administered Drugs

Theoretically, a drug can be absorbed over the whole length of the digestive tract, i.e. from the oral cavity through the esophagus, the stomach, the small and large intestine all the way to the rectum. However, some of these regions are not associated with efficient drug absorption, either due to a short passage time, little amplification of the anatomical surface area compared to the geometrically-derived value, lack of active transport mechanisms, or a combination of these factors. Even though sublingual, buccal, and rectal absorption can be important routes for certain drugs [1], oral administration with intestinal absorption of the drug

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remains the most usual means of drug entry into the systemic circulation. For this route of administration, it is crucial for the formulation scientist to get a precise overview of how the drug and formulation behave in the stomach, small, and large intestine in order to optimize formulation performance. Bioavailability is key to the therapeutic success and is usually defined in terms of rate and extent of drug absorption. The maximum concentration reached in the blood plasma (c_{max}) and the time to reach this concentration (t_{max}) usually characterize the rate of absorption, whereas the area under the concentration-time curve (AUC) reflects the extent of drug absorption [16].

About half a century ago, it was recognized that poor solubility and/or low rate of dissolution of a drug at the site of absorption can be important factors to the *in vivo* performance of a drug [17-20]. But, even though these days most new drugs possess suboptimal properties in terms of aqueous solubility and dissolution rate, there are also various other factors that can lead to low bioavailability of a drug (paper 2, [21-31]). So in practice, a combination of factors often limits the bioavailability of an orally administered drug:

1) Poor solubility and/or dissolution rate, drug precipitation upon entering the (fasted) small intestine,

2) Removal of the drug from access to absorption pathways, e.g. by complexation with food components or via enzymatic and/or acid-induced degradation,

3) Site-specific absorption,

4) Poor permeability characteristics (e.g. because of ionization or a hydrophilic molecule structure),

5) Intestinal and/or hepatic first pass metabolism,

6) Exotransport, e.g. P glycoprotein (P-gp)-mediated efflux from enterocytes.

Fig 1-3. gives an overview over the processes that may limit the bioavailability of an orally administered drug.
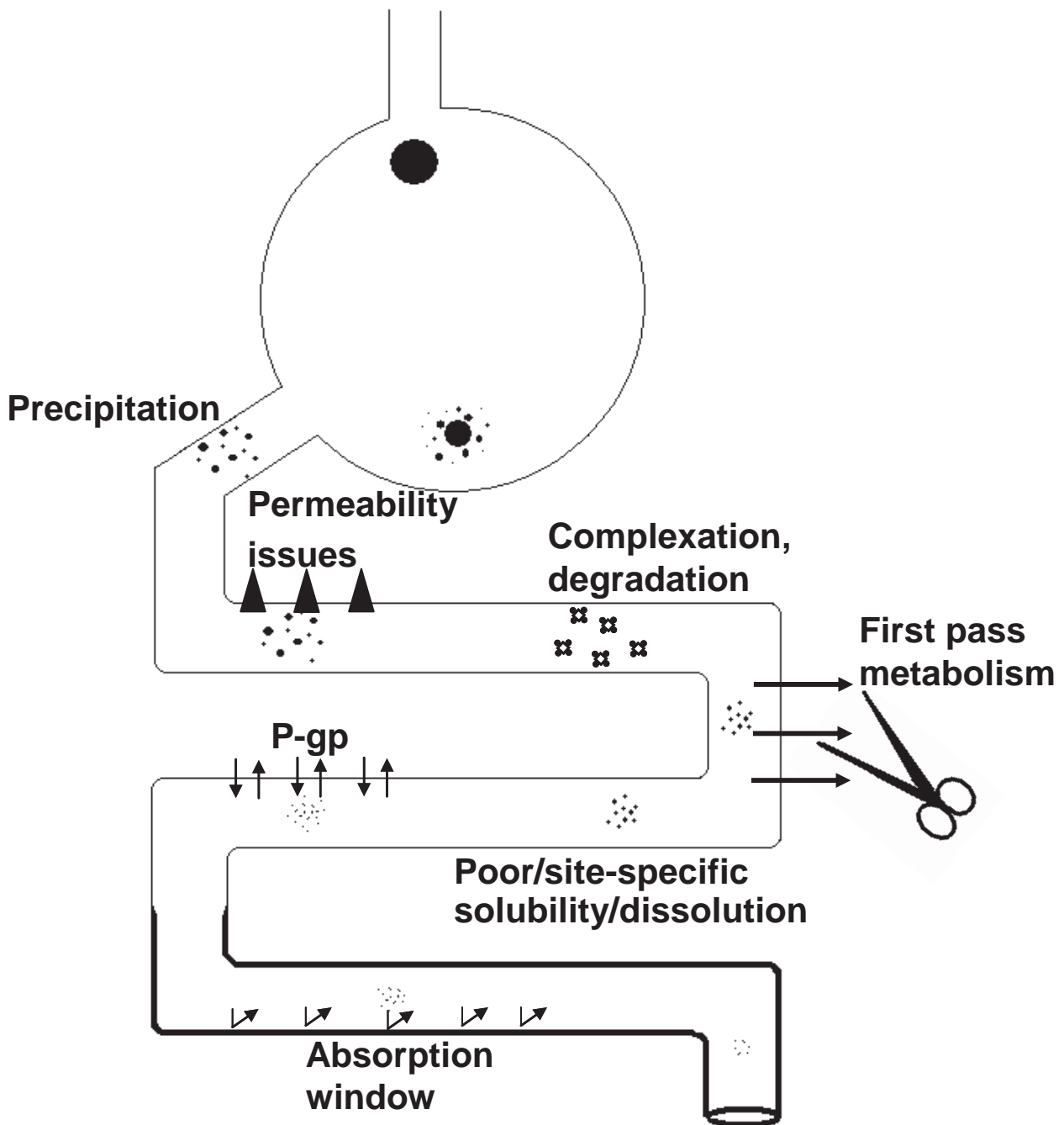
Fig. 1-3.   Overview of the most important factors that may limit the bioavailability of orally administered drugs.

1.2.1. Solubility, Dissolution, and Precipitation

It is widely accepted that undissolved drug cannot be absorbed quantitatively into the systemic circulation from the gastrointestinal tract, and that drug release from the formulation and subsequent dissolution are therefore crucial steps for the bioperformance of the drug. Poor solubility of the drug may result in non-linear drug absorption and bioavailability with

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increasing dose. As many of the drugs which are currently on the market can be classified as "poorly soluble", the bioavailability of these drugs may thus be limited by the drug's solubility and/or rate of dissolution [3].

There are several properties that may lead to poor aqueous solubility of a drug [32, 33]:

1) Lipophilicity: As a result of rational drug design (section 1), many new compounds tend to be rather lipophilic, and highly lipophilic drugs in general show a lower aqueous solubility compared to more hydrophilic drugs.

2) Ionization: For ionizable drugs, the percentage of the ionized form, which is more soluble than the non-ionized form, depends on both the pH of the medium and the pKa of the drug. Weak bases show a higher solubility at low pH values, and weak acids at high pH values. By contrast, for strong bases and acids, the fraction of the ionized species is usually large over the gastrointestinal pH range.

3) Crystal structure: Before a crystalline drug goes into solution, the crystal lattice has to be broken. As the melting point of a drug depends on the crystal lattice energy, drugs with higher melting points tend to be less soluble than drugs with lower melting points. Additionally, one should bear in mind that drugs may show polymorphs which differ in their melting point and thus ability to dissolve under physiological conditions.

In addition to these drug-related factors, there are also various physiology-related factors which influence the solubility of a drug (section 1.6.).

Due to the presence of components which enhance the drug's solubility, such as bile salts and lecithin which are secreted via the gall bladder, the solubility for most poorly soluble drugs in simple aqueous buffer systems (which do not contain any surfactants) is lower than in human intestinal fluid (HIF). In the fed state, food components, principally the fats and their digestive products including fatty acids and mono- and diglycerides, may also lead to an increase in drug solubility. To better reflect these physiological conditions and hence better estimate *in vivo* solubility of the drug, biorelevant media have been developed over the past 15 years (section 1.4.2.).

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A crucial step in making the drug available for absorption is to ensure its dissolution in the gastrointestinal tract. There are several commonly accepted approaches to enhance the drug's dissolution rate and/or solubility:

1) Formation of more soluble salts, esters, polymorphs, complexes, and prodrugs [34-39],

2) Decreasing the drug's particle size by micronization or nanosizing [40-43],

3) Formation of solid solutions or at least stabilizing the drug in its amorphous (often more soluble) form [44-48], and

4) Formulations in which the (lipophilic) drug is dissolved in a lipid-based vehicle (often with the help of surfactants and/or co-solvents) [49-51], or in a solvent system with similar polarity to the drug (e.g. hydroalcoholic solutions) [52].

For drugs with solubility- and/or dissolution rate limited bioavailability, these "enhanced formulations" often provide a significant step forward in improving the bioavailability of a poorly soluble drug. However, one should bear in mind that "enhanced formulations" may lead to a thermodynamically unstable, supersaturated solution, i.e. a solution in which the actual concentration of the drug is higher than one would expect from its equilibrium solubility. Depending on the degree of supersaturation, the drug may precipitate which, in turn, can result in a decreased rate and possibly also extent of drug absorption. Especially for lipid and alcoholic formulations, drug precipitation is likely to occur due to the dilution and digestion process of the lipid contents of the formulation [53, 54]. For instance, non-linear pharmacokinetics have been observed for Adalat®, a nifedipine immediate release (IR) formulation in which the drug is dissolved in a vehicle of polyethylene glycol 200 and peppermint oil and encapsulated in a soft gelatine capsule. In comparison to a 5 mg and a 10 mg dose, administration of a 20 mg dose of Adalat® to healthy volunteers led to a non-linear increase in c_{max}, although the increase in AUC remained linear [55]. This result was explained by gastric precipitation after release of the drug solution from the 20 mg capsule in *in vitro* experiments, suggesting that at a 20 mg dose, the rate of absorption was limited by re-dissolution of the precipitated nifedipine [56]. No precipitation was observed for the 5 mg and the 10 mg dose.

Indeed, precipitation may be an issue not only for "enhanced" formulations, but also for weakly basic drugs: under normal circumstances, the drug exhibits high solubility at low pH values, i.e. in the fasted stomach. Due to the "pH gap" between the stomach and the small

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intestine, the drug may precipitate as soon as it encounters the more neutral duodenal environment. Depending on the precipitation conditions, the drug may precipitate in a crystalline or in an amorphous form, usually as the free base. Re-dissolution of the precipitate might thus be substantially different compared to the dissolution kinetics of the "parent" drug, and this may in turn affect the *in vivo* pharmacokinetics of the drug [29, 31, 57-62].

In order to get an estimate of the solubility, dissolution, and possible precipitation behavior of the drug in the gastrointestinal tract, it is hence of great importance to mimic gastrointestinal conditions as closely as possible (section 1.4.2.).

1.2.2. Complexation, Degradation

Complexation and degradation of the drug are further issues that may limit the oral bioavailability of a drug. Depending on the mechanism, the drug can theoretically be subject to gastric or small intestinal, acid- and/or enzyme-induced degradation. Additionally, potential interactions between drugs like antibiotics or thyroid hormones and polyvalent cations can reduce the drug's solubility, leading to a decrease rate and extent of absorption [22, 23, 28, 30, 63].

1.2.3. Permeability Considerations

The absorption process is very complex and involves passive (no energy consumption) para- and transcellular transport mechanisms as well as active, carrier-mediated (energy-consuming) transport mechanisms. Besides transporter proteins which actively uptake dissolved drug molecules possessing certain structural features, e.g. peptide transporters and organic anion transporters (OATP), there are also transporters which eliminate the drug from the enterocytes back into the intestinal lumen, e.g. P-gp. Additionally, the blood flow in the gut wall, the thickness of the mucus layer, and lymphatic transport can influence the rate and extent of drug absorption [1, 64-67].

To be absorbed transcellularly in the intestinal tract, the drug has to cross the lipid membrane of the enterocytes. Thus, hydrophilic, large molecules with a high polar surface area (PSA) tend to have lower absorption rates than small, lipophilic drug molecules. Additionally, ionized drugs tend to be less permeable than their neutral counterparts.

According to the BCS, a drug is "highly permeable" when the fraction of drug absorbed is higher than 90% [5]. If no clinical data about a drug's fraction absorbed is available, the apparent Caco 2 (human epithelial colorectal adenocarcinoma cells) permeability ($P_{app\ Caco\ 2}$) can serve as an estimate for the fraction absorbed, and the cut-off point to ensure (almost) complete absorption is approximately 10^{-5} cm/sec [68-70]. For drugs with lower apparent permeability values, complete absorption cannot be assured. However, lower P_{app} values do

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not necessarily mean that the drug is not absorbed completely, as the drug may be taken up by an active transport mechanism. It should be noted that these permeability values must be established on a relative basis due to laboratory-to-laboratory variability on the one hand and variability between the cell clones on the other hand (section 1.3.2.4.).

Some examples of drugs with less than optimal permeability characteristics are aciclovir (BCS class 3), atenolol (3), cimetidine (3), furosemide (4), levothyroxine (3), nelfinavir (4), and ritonavir (4) [71].

1.2.4. Site-Specific Absorption

The presence of site-specific drug absorption (so-called "absorption window"), which can be due to pH-dependent drug solubility, insufficient effective surface in some regions (e.g. in the colon), paracellular absorption via tight junctions, or special uptake- or efflux-transporter characteristics, might also be a limitation for the drug's oral bioavailability. In this case, the passage time of the drug through the site/-s of absorption may be inadequate to effect complete absorption, and the fraction of drug which is not absorbed will be eliminated with the faeces. In other cases, drugs like *L*-dopa, angiotensin-converting enzyme (ACE) inhibitors and some antibiotic drugs are substrates of aminoacid or peptide transporters. As these transporters are not distributed equally over the whole length of the gastrointestinal tract, these drugs also exhibit site-specific absorption [72-76].

1.2.5. Exotransport

P-gp, also known as multi-drug resistance protein (MDR1) or adenosine tripohosphate (ATP)-binding cassette sub-family B member (ABCB1), is a transmembrane protein that is located in the gut wall, hepatocytes, and in the cerebral capillary endothelium, where it forms part of the blood-brain barrier [1, 77, 78]. For some orally administered drugs, e.g. carvedilol, diltiazem, furosemide, loperamide, paclitaxel, and phenytoin [79, 80], this active, energy-consuming transport protein is an important limitation to bioavailability. Theoretically, the concomitant administration of efflux pump inhibitors, such as cyclosporine, erythromycin, nelfinavir, ritonavir, and verapamil, can increase the bioavailability of other P-gp substrates, but this strategy is seldem applied in the clinic as this may result in an increase in side effects [77].

1.2.6. First Pass Metabolism

Intestinal and/or hepatic first pass extraction is one of the most important factors that may limit the oral bioavailability of orally administered drugs. Intestinal first pass metabolism, for example, is mediated mainly via enzymes that belong to the cytochrome P 450 (CYP) 3A subfamily, and these enzymes account for up to 80% of the small intestinal CYP enzymes