1 Introduction

In earlier times active pharmaceutical ingredients (APIs) were discovered incidentally and derived from nature. Typical examples of such compounds are salicylic acid and penicillin, which were initially extracted from plants (bark of white willow) and fungi (*penicillum rubens*) [1, 2]. In the early 20th century drug research and development was based on empirical knowledge and little was known about drug pharmacokinetics (PK) or pharmacodynamics (PD). One of the first researchers to use a rational approach to drug development and therapy was Paul Ehrlich, who synthesized one of the first antibacterial chemotherapeutics in 1907, arsphenamine, which was later marketed by Hoechst AG under the trade name Salvarsan. Ehrlich hypothesized that by screening a number of compounds a drug with antibacterial activity could be discovered and selected for therapy without risking patients' lives.

After the Second World War new ways of conducting drug discovery emerged. Rational drug design was guided with the help of structure-activity relationship (SAR) in the second half of the 20th century. SAR enabled the identification of structural molecular components inducing an effect on a biological target [3]. Paul Ehrlich's approach of screening drugs before investigating them in humans was fully implemented through introduction of combinatorial chemistry as an industrial standard in late 1980s [4, 5]. This method made it possible to develop and use large compound libraries. High throughput screening (HTS) methods facilitate investigation of large numbers of potential candidates on target receptors or enzymes within a short amount of time. Molecules identified to interact with a target are called "hits". After being characterized and optimized, the most promising hits are selected as lead candidates for preclinical development. Based on the preclinical results certain lead candidates are then chosen for clinical development. In successful clinical programs, one or more of the clinical candidates are often able to reach the market.

Oral medication is the most convenient route of drug administration. Most compounds administered orally are designed to have systemic mode of action. Thus, if a compound is taken over the oral route it has to be liberated (from a formulation) and dissolved before being absorbed, distributed in the human body, metabolized

and excreted. The “Liberation” step in this so-called LADME scheme implies that poorly soluble compounds can have absorption issues: drug dissolution in the gastrointestinal (GI) tract has shown to be a key requirement for oral drug absorption [6]. Selection of drug candidates using HTS predestines many compounds for absorption issues related to solubility/dissolution, since it is an *in vitro* tool where compounds are pre-dissolved in organic solvents such as DMSO and then diluted in aqueous buffers to perform the screen [7]. For this reason most of the hit and lead molecules tend to exhibit high lipophilicity and thus poor aqueous solubility [8, 9].

Prediction of oral drug absorption has become one of the fundamental elements in clinical development. In the last few decades many methods have emerged for the investigation of factors involved in *in vivo* drug dissolution and absorption [10-12]. It is a topic which continues to be of major interest in the pharmaceutical sciences as well as in industrial research and development.

1.1 Drug transit through the upper GI tract

When a dosage form is taken orally it first enters the stomach and may subsequently be transferred through all segments of the GI tract. For immediate release dosage forms, disintegration and drug dissolution are intended to take place in the stomach and/or the upper small intestine. After being liberated, the API has to stay in solution in order to be absorbed through the intestinal epithelia. Figure 1.1 illustrates the passage of a dosage form through the proximal GI tract. Immediate release dosage forms are generally designed to disintegrate and liberate the API in the gastric fluid upon entering the stomach. The API (dissolved and non-dissolved) will then be emptied into the small intestine, where dissolution should be completed to make the entire dose available for absorption. By contrast, delayed release dosage forms have to remain intact until they reach the GI segment in which they are supposed to disintegrate and liberate the API, while a third category, extended release dosage forms, are intended to assure prolonged drug liberation over a large part of the GI tract.

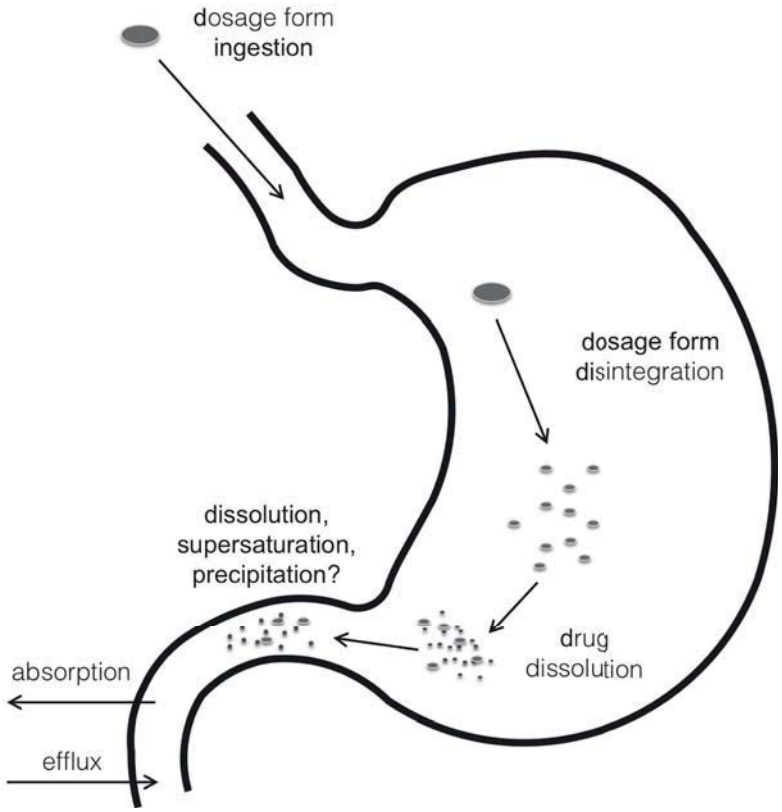

Fig. 1.1: Dosage form and drug transit in the upper GI tract

During the passage through the GI tract drug dissolution and absorption may be affected by a number of factors. After ingestion, dosage forms/APIs are exposed to drastic changes in environment. Shifts in pH and bile salt concentrations during the passage through the GI tract may significantly influence drug release, solubility and dissolution. If drugs are administered with food, they may additionally be subject to complexation with food components. Furthermore, food intake may significantly change GI motility and pH, and thus solubility and dissolution of certain compounds. Some drugs may even be subject to degradation. Moreover, poor permeability, site-specific absorption or drug efflux may restrict the extent of drug absorption. The next sections describe the interplay of factors which are crucial for prediction of drug absorption and the systems which attempt to describe them in more detail.

1.2 Determinants of drug absorption

1.2.1 The biopharmaceutics classification system

The biopharmaceutics classification system (BCS) was introduced in 1995 by Amidon and colleagues to describe factors involved in drug absorption [6]. The authors postulated that oral drug absorption is driven by two predominant parameters, drug solubility in the GI tract and *in vivo* drug permeability. According to the BCS framework, APIs are distinguished in four classes based on their solubility and permeability (Figure 1.2).

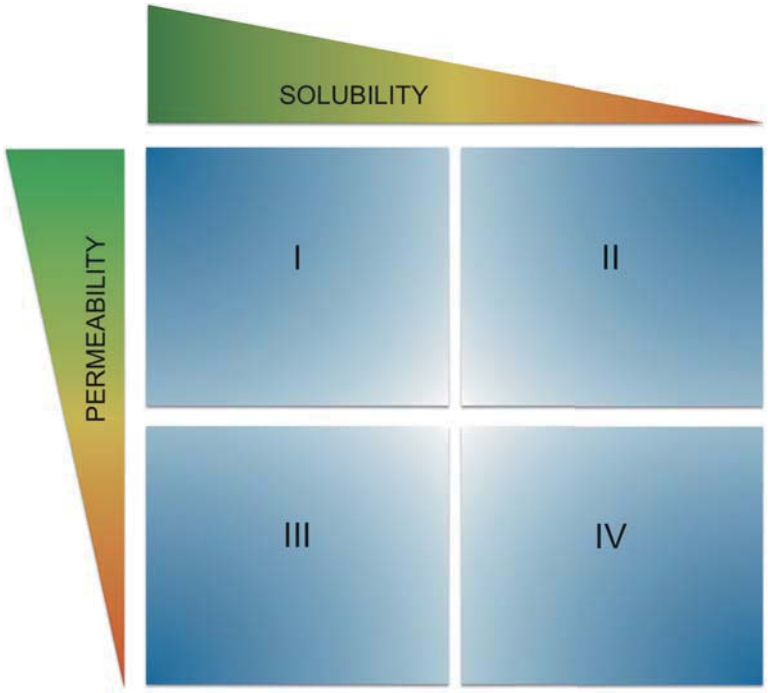

Fig. 1.2: Categorization of APIs based on the biopharmaceutics classification system

Poorly soluble weak bases, which are the focus of this work, are classified as BCS class II (highly permeable) and IV (poorly permeable) drugs. The BCS guidance established by the US-FDA suggests investigating drug solubility in aqueous media with pH values between 1 and 6.8. Drugs are regarded as highly soluble when the dose/solubility ratio is equal to or below 250 ml. Compounds are regarded as highly permeable when at least 85% of the administered dose is absorbed. Practically, permeability may be assessed through human bioavailability, mass balance studies and intestinal perfusion experiments [13, 14]. If human intestinal perfusion data are not available, drug permeability may be estimated through e.g. experiments in *in vitro* cell lines using a validated procedure [14-16].

1.2.2 The biopharmaceutics drug disposition classification system

The biopharmaceutics drug disposition classification system (BDDCS), which was published by Wu and Benet in 2005, modified the BCS scheme to describe drug disposition kinetics based on two factors, metabolism and solubility [17]. Based on the BCS the investigators were able to distinguish predominant routes of drug elimination. They found correlations of drug permeability with metabolism, whereby BCS class I and II drugs typically exhibit high metabolism, while BCS class III and IV compounds are mostly excreted in bile and urine as unchanged drug.

Moreover, the investigators came to a conclusion that the BCS classes can be linked with the possibility of transporter mechanisms in intestinal epithelia. Drug uptake mechanisms can be generally distinguished into two types, passive and active drug transport. The latter mechanism is mediated through carrier molecules, which may actively bind and transport APIs into cells by energy-requiring interactions. A typical group of such uptake transport molecules is the OATP (organic anion transporting polypeptides) family, which is responsible for active transport of a variety of compounds, such as antibiotics, chemotherapeutic agents, antihistaminic drugs, and diuretics [18]. By contrast, efflux carrier molecules are able to eliminate certain compounds from the enterocyte. The most studied of the efflux proteins is P-glycoprotein (P-gp). It belongs to the MDR (multidrug residence protein) family and has a very wide substrate spectrum [18]. Class I drugs typically show minimal transporter effects. Because of their good solubility and permeability, saturation of both efflux and uptake transporters is probable. Due to the poor solubility of class II drugs, saturation of efflux transporters is considered improbable. Thus, efflux transporters are more likely to affect the net absorption of class II compounds. Class III drugs are highly soluble, however they often have to rely on uptake transporters, explaining their poor permeability. Alternatively, they may be absorbed by paracellular passive mechanisms. For class IV compounds both efflux and uptake mechanisms may be relevant. Based on their investigation Wu and Benet summarized the disposition kinetics of BCS drugs by introducing the BDDCS (Figure 1.3). This system facilitates the understanding drug characteristics and their absorption mechanisms on the basis of the BCS.

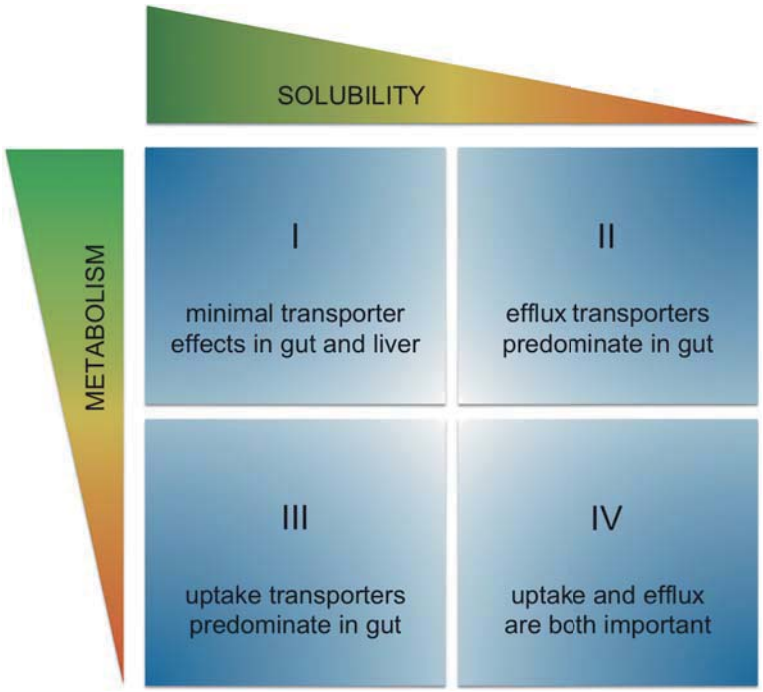

Fig. 1.3: Categorization of APIs based on biopharmaceutics drug disposition classification system

1.2.3 The developability classification system

Even though the BCS has shown to be a useful tool to assist drug development in the pharmaceutical industry, the boundaries for solubility and permeability are too strict to apply it as a screening tool for selection of lead compounds. In 2010 Butler and Dressman modified the BCS to better address candidate selection for drug development [19]. The developability classification system (DCS) utilizes a modified approach of classifying drugs (Figure 1.4). According to the DCS, *in vivo* drug solubility would optimally be assessed with the help of aspirated fasted state human intestinal fluids (FaHIF). If FaHIF is inaccessible, biorelevant media, which mimic the fluids of the GI tract, may be used for the estimation of *in vivo* drug solubility [20-22]. The comparator intestinal volume of liquids is set at 500 ml, indicating that solubility is not expected to constitute a major limitation to drug absorption at dose/solubility ratios ≤500 ml. The authors assumed compensatory effects of solubility and permeability for some class II compounds, distinguishing them into two groups. Class IIa compounds exhibit dissolution rate limited extent of absorption. For these APIs, the high permeability facilitates drug dissolution, which in turn enables absorption of the entire drug dose as long as the dissolution rate can be adequately improved via formulation. Class IIb drugs are solubility limited. For these, the compensatory

permeability effect is not sufficient enough to ensure dissolution and absorption of the entire drug dose and the formulation effect should be directed towards improving drug solubility.

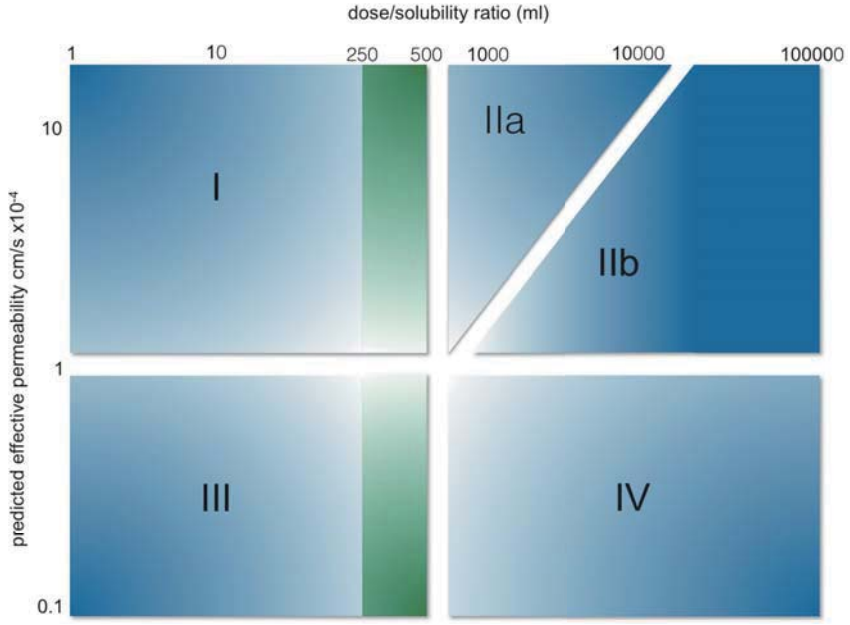

Fig. 1.4: Characterization of APIs based on developability classification system. Green bars describe the extension of the dose solubility ratio from 250 ml to 500 ml.

Interestingly, poorly soluble weakly basic drugs have a unique status according to the DCS, since they may undergo complex dissolution processes, which cannot easily be predicted by simple *in vitro* methods. The dissolution behavior of poorly soluble weak bases in the GI tract will be discussed in more detail in the following sections.

1.3 Weak bases and the GI tract

1.3.1 pH in the GI tract

Weakly basic and acidic drugs usually have pH dependent solubility and react sensitively to the pH changes that occur during GI transit. Figure 1.5 illustrates pH dependent solubility profiles of weak bases and acids, together with the pH values that predominate in the upper GI tract in the pre- and postprandial state.

The pH in the fasted stomach is estimated to generally lie between 1.4 and 2.1. When entering the upper small intestine compounds are exposed to significantly higher pH values of 4.4 to 6.6. After food ingestion the stomach pH increases to

values between 4.3 and 5.4, whereas a slight decrease in pH is observed in the upper small intestine (pH 4.9 to 6.0) [23-25].

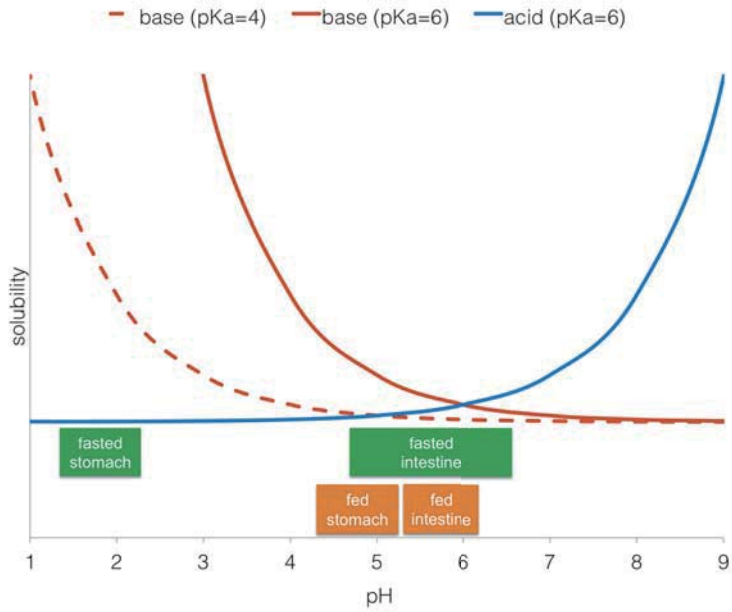

Fig. 1.5: pH solubility behavior of weak bases and acids in relation to the upper GI pH

Based on the pH/solubility profile, a large difference in solubility is expected for poorly soluble weak bases between the fasted stomach and intestine. A substantial drug amount is expected to dissolve in the fasted stomach. Upon entering the fasted intestine three general scenarios may be considered for poorly soluble weakly basic drugs (Figure 1.6):

1. Direct drug precipitation to the intestinal solubility

2. Drug supersaturation for extended period of time

3. Drug supersaturation and with subsequent precipitation towards the intestinal solubility.

Thus, supersaturation and precipitation may be important forces of the absorption of poorly soluble weakly basic drugs. It is therefore advisable to investigate factors which may influence drug supersaturation and precipitation following gastric emptying.

Additionally, this description evokes the assumption that bioavailability of poorly soluble weak bases may be vulnerable to gastric pH [26]. Indeed, absorption of weakly basic drugs is often negatively affected when H_2 receptor antagonists and proton pump inhibitors are co-administered, since these elevate the gastric pH [27, 28].

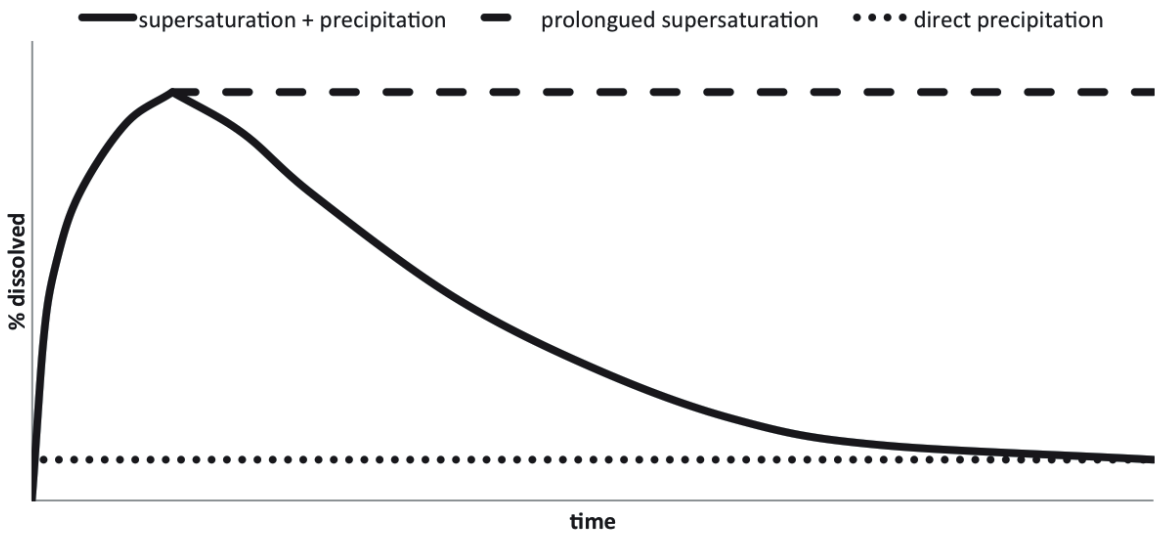

Fig. 1.6: Possible dissolution scenarios for weak bases upon entering the upper small intestine

1.3.2 Bile salts and food in the GI tract

Bile salts are secreted in the upper small intestine. Due to their formation of micellar structures they may enhance the solubility of lipophilic APIs in the GI tract via solubilization [29-31]. Hence, their presence may mitigate the pH mediated decrease in solubility of weakly basic compounds during gastric emptying and partly influence drug supersaturation and precipitation. Bile salt secretion depends on the prandial state. After food intake, luminal bile salt concentrations in humans are higher than in the fasted state [32]. Human intestinal aspirate data reveals bile salt concentrations of approximately 3 mM in the fasted state and of 10 mM in the fed state [33-35].

Another factor which may influence solubility of compounds is food. Food ingestion usually induces an increase in gastric pH and bile salt secretion, both of which can influence the solubility of weak bases. Food components or products of digestion may also influence solubilization of poorly soluble compounds and have to be

considered when investigating drug dissolution [36]. Further, some food components can interact with gut epithelia and affect drug permeability. A typical representative of such food constituents is grapefruit juice, which exhibits inhibition of P-gp and can be therefore alter drug bioavailability [37].

1.3.3 Gastric emptying

In the fasted state, gastric motility is best characterized by cyclical fluctuations, which constitute the migrating motor complex (MMC). The MMC is divided into three phases: phase I is a quiescent period with almost no movement, phase II consists of intermittent and irregular contractions, which progressively increase in strength culminating in maximum frequency and strength of contractions in phase III. The mean cycle duration is estimated to be approximately 90-120 minutes, although it may range between 15 minutes to 3 hours [38-41]. Gastric emptying of liquids depends on the phase of the MMC and on the amount of liquids ingested [42, 43]. Whereas phase III includes an almost bolus gastric emptying, phases I and II motility patterns generally show first order emptying kinetics, with a high variability of 1 to 14 h^{-1} [41-47]. Thus, dissolution, supersaturation, precipitation and absorption of weakly basic compounds could all depend on the MMC phase and on the volume of liquid taken with the drug. By contrast, the fed state gastric emptying follows linear kinetics (zero order) and depends mainly on the number of calories contained in the meal [48, 49]. With values between 1 and 4 kcal/min the gastric emptying rate is slower and less variable in the fed state than in fasted state [50, 51].

1.4 Simulation of *in vivo* drug dissolution

1.4.1 Dissolution media simulating GI fluids

Dissolution media have been developed and refined over the last two decades to mimic the *in vivo* gastric and intestinal conditions. Compendial dissolution media consist of simple aqueous solutions and buffers, and mimic solely the pH conditions of the GI environment. The US pharmacopeia (USP) introduced simulated gastric fluid (SGF) with a pH of 1.2 and simulated intestinal fluid (SIF) with a pH of 6.8 as standard dissolution media for oral dosage forms. SGF may contain pepsin to represent gastric conditions more physiologically. Sodium dodecyl sulfate (SDS) or

other surfactants may be added to SIF to increase solubility when investigating poorly soluble compounds [52]. The compendial media are especially useful for quality control assessment of solid oral dosage forms, however they have relatively little predictive power when it comes to *in vivo* dissolution behavior of poorly soluble compounds. In addition, use of synthetic detergents, such as SDS for the imitation of bile salts of human intestinal fluids is not necessarily satisfactory. In 1998 the simplistic pharmacopeial simulated gastric and intestinal fluids (SGF and SIF) were transformed into more biorelevant dissolution media FaSSGF (Fasted State Simulated Gastric Fluid), FaSSIF (Fasted State Simulated Intestinal Fluid) and FeSSIF (Fed State Simulated Intestinal Fluid) by adapting buffers and bile salt contents to human aspirate data [20, 21]. In 2008 Jantratid *et al.* further refined FaSSIF and FeSSIF to better represent the *in vivo* intestinal conditions by introducing new versions (version 2 – V2) of the two media, FaSSIF-V2 and FeSSIF-V2. To represent the higher bile salt secretion in the fed state, bile salt and lecithin concentrations are greater in FeSSIF-V2 than in FaSSIF-V2. Moreover, glycerol monooleate and sodium oleate were incorporated into FeSSIF-V2 to represent products of lipolysis of dietary fats of the fed intestine [22]. In addition, Jantratid and co-investigators introduced the fed state simulating gastric fluid (FeSSGF) to imitate the postprandial stomach. The introduction of biorelevant media of the ascending colon (FaSSCoF and FeSSCoF) in 2010 made it possible to simulate the dissolution behavior of extended release dosage forms or formulations with colon targeting [53]. Biorelevant media enable qualitative predictions of the *in vivo* dissolution performance of poorly soluble compounds. Today they are widely utilized for quantitative predictions of *in vivo* drug performance using *in vitro-in vitro* correlation (*IVIVC*) methods [54-57].

1.4.2 Simulation of GI dissolution conditions

The USP provides different apparatus for the investigation of dissolution behavior of solid oral dosage forms:

1. USP apparatus 1 (Basket apparatus) consists of a vessel filled with a defined volume of dissolution medium (usually 500 or 900 ml) and a perforated basket, which holds the dosage form. The rotary movement of the basket in the vessel

is used to disperse the dosage form and release the API. The rotation speed is routinely set at 100 rotations per minute (rpm).

2. USP apparatus 2 (Paddle apparatus) is similar to the apparatus 1. The basket is exchanged for a paddle, which rotates inside the vessel. The rotation speed is usually set at 50 or 75 rpm. The dosage form initially resides at the bottom at the vessel in most cases. To prevent dosage forms like capsules from floating a sinker can be introduced.

3. USP apparatus 3 (reciprocating cylinder) is a tester consisting of six dissolution compartments in a row. Up to six rows, each with six vessels can be utilized in the apparatus. The vessels are smaller than in apparatus 1 and 2 and have maximal volume capacity of 250 ml. The dosage form is introduced into a holder, which is dipped into the medium at a predefined rate. This method enables the simulation of gastrointestinal transit of enteric coated or extended release dosage forms by sequentially transferring the dosage form holder from one vessel to the next. The core advantage of apparatus 3 is that dipping rates can be adjusted for each of the individual rows without stopping the apparatus.

4. USP apparatus 4 (flow-through tester) is commonly used for the analysis of extended release dosage forms. A glass cell is used to hold the formulation. The dissolution medium is pumped through the cell at a preset pumping rate.

The USP apparatus 1 to 4 have been widely used not only for quality control of oral dosage forms, but also for biorelevant dissolution testing. The apparatus 2 is the most typically utilized approach for the dissolution analysis of poorly soluble compounds in fasted and fed state simulated gastric and intestinal fluids. Additionally, a scaled down apparatus 2 (Mini Paddle apparatus) comprising a maximum volume of 500 ml was implemented to better represent volumes available for dissolution in the fasting stomach [58].

Individual dissolution testing has proven to be a useful tool to assess the *in vivo* behavior of poorly soluble drugs. However, it may have limited predictive power in the case of poorly soluble weak bases. In order to evaluate the behavior of weak bases during gastric emptying, Kostewicz *et al*. introduced the transfer model in 2004 (Figure 1.7) [59]. Unlike individual dissolution tests, the transfer model simulates changes in drug concentration induced by the sudden change in environment when the drug leaves the stomach and enters the small intestine.

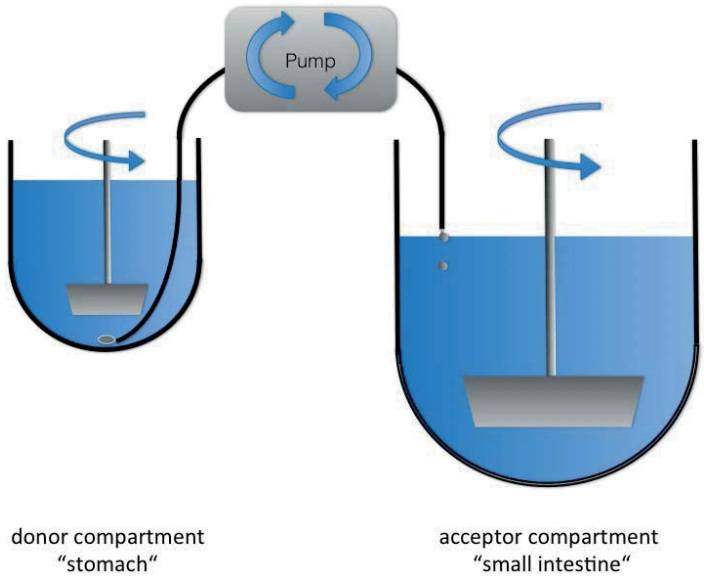

Fig. 1.7: Transfer model for the *in vitro* simulation of gastric emptying

The transfer model consists of a donor compartment (e.g. Mini Paddle apparatus) containing a medium simulating the stomach and an acceptor compartment (e.g. USP 2 apparatus), which represents the upper small intestine. Gastric emptying is mimicked with the help of a peristaltic pump. The pumping rate can be set up according to the desired simulation of the GI state. Contemporary pumps can be programmed to run nonlinear transfer kinetics, and thus enable the imitation of first order gastric emptying in the fasted state. Kostewicz and co-workers initially utilized the model by pre-dissolving weakly basic compounds (dipyridamol, BIBU 104 XX and BIMT 17 BS) in SGF and subsequently transferring the solutions into FaSSIF or FeSSIF [59]. Nowadays, sophisticated biorelevant media such as FaSSGF, FaSSIF-V2, FeSSGF and FeSSIF-V2 can be utilized in transfer experiments to simulate fasted and fed state gastric emptying conditions [60, 61]. The transfer model has proven to be an important tool not only for formulation development but also for the

assessment of possible *in vivo* performance of several poorly soluble weak bases [60-65].

1.5 Drug permeability assessment

Human intestinal perfusion experiments enable a direct permeability assessment of compounds. Since experiments in volunteers need to be conducted to assess the effective permeability, such data are unfortunately not available for most APIs. Several alternative theoretical and practical tools are available today for the prediction of human intestinal permeability. The theoretical permeability assessment uses the chemical structure or physicochemical properties of the compound. Lipinski was one of the first to describe permeability of compounds with the help of their structural characteristics [66]. He introduced the “rules of five”, which postulates that poor absorption of a compound is more likely when there are more than 10 H-bond acceptors (HBA), 5 H-bond donors (HBD), the molecular weight (MW) is greater than 500 and the logP is greater than 5. More recently, different statistical methods have emerged to predict the permeability of compounds based on their molecular structure or physicochemical properties, e.g. MW, HBD and logP. Polar surface area (PSA) also provides information about API lipophilicity and thus may be used for permeability assessment. On the basis of these properties, several correlations have been proposed for the prediction of jejunal permeability [67-69].

However, the most commonly used methods for the investigation of intestinal permeation are *in vitro* tools. *In vitro* permeability studies can be distinguished into cell-based and non cell-based analyses. Surface activity profiling (SAP) and parallel artificial membrane permeability assay (PAMPA) represent non cell-based techniques.

SAP is an *in vitro* investigation of concentration dependent surface pressure profiles of compounds in aqueous buffers. The maximum surface pressure has been correlated with absorption (fraction absorbed) for a range of compounds. Therefore, it may be utilized as the predictive parameter for permeability of compounds. The

method has proven to be a practical tool to evaluate both oral absorption, and with some modifications, blood brain barrier penetration of drugs in humans [70, 71].

PAMPA uses an artificial semipermeable membrane, which separates a donor and an acceptor compartment, both filled with aqueous buffer. The compound is introduced into the donor solution and allowed to permeate the membrane. Time-concentration profiles are then generated to assess permeability of the compound. Like SAP, this method only allows the investigation of passive perfusion of compounds [72].

Cell-based permeability assessment may be performed using different kinds of cell systems, such as Caco-2, HT-29, MDCK II and LLC-PK_1. Alternatively, animal intestinal membranes, such as rat jejunum or ileum, may be used for this kind of investigation.
Caco-2 (heterogeneous human epithelial colorectal adenocarcinoma cells) and HT-29 (mucus producing human colon cells) cell lines are based on human derived intestinal cell systems. Caco-2 is the most commonly used assay for the investigation of permeation. The cell line was derived from heterogeneous human epithelial colorectal adenocarcinoma cells [73]. Interestingly, Caco-2 cells are morphologically similar to cells in the human upper intestinal epithelia. They express P-gp, metabolizing enzymes and several active uptake transporter proteins. The expression of those proteins may be inconsistent from batch to batch. Therefore, the permeability is optimally assessed on a relative basis using high and low permeability reference compounds. HT-29 cultures pose an alternative to Caco-2 cell lines. A fraction of the cells differentiate into goblet cells, which are capable of producing mucus [74]. HT-29 and Caco-2 cell assays may be combined by differentiating the HT-29 cells (into HT 29-H or HT29-MTX cells) and by co-culturing both cell lines [75].
MDCK-II (Madin-Darby canine kidney) and LLC-PK_1 (Lewis lung carcinoma - porcine kidney) are animal derived cell systems and pose alternatives to Caco-2 cultures. The main advantage of these cell lines is the shorter culture time. Each of the cell strains can be transfected with P-gp to mimic efflux kinetics. Since neither system is human derived, permeability values are expected to be significantly different to Caco-2 cultures [76, 77].