1 Introduction

1.1 Oral bioavailability

The term "bioavailability" was coined in the late sixties. It describes the rate and the extent to which the fraction absorbed reaches the general circulation [1]. The primary parameter used to describe the bioavailability is the Area Under the Curve (AUC), reflecting the extent of absorption by an integration of the area underneath a plasma profile of a drug. The AUC after an intravenous (i.v.) injection corresponds to the maximum extent of drug since no absorption step is involved (100%). c_{max} is a further important parameter that provides information about the rate of absorption. In the i.v. case, the maximum plasma concentration is reached directly after injection at t_0.

The absolute bioavailability describes the rate and the extent of absorption from a formulation, e.g. a tablet, in comparison to i.v. data.

The relative bioavailability compares two formulations with each other, using one of them as reference. This is often evaluated in formulation development with an eye to improving the initial formulation e.g. to reach a different AUC and/or c_{max} profile. The development of generic drugs is also guided by the relative bioavailability, with the 90% confidence intervals around the point estimates of AUC and c_{max} in the plasma profiles needing to fall within the acceptance criteria based on the innovator product.

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1.2 Factors limiting oral bioavailability

In 1961, it was realized that low solubility correlates with low bioavailability [2]. In 1971, up to sevenfold differences in plasma levels from different digoxin-formulations were observed [3]. This prompted the question as to whether dissolution rates are responsible for these differences [4, 5]. In 1975, Jounela et al. [6] were able to correlate the particle size and subsequently the dissolution rates of digoxin with its bioavailability. So the aforementioned question could be answered with an unequivocal "yes".

These findings within the period from 1961 to 1975 can be considered as the origin of pharmacokinetically driven formulation development.

Incomplete drug absorption from an orally administered dosage form is usually due to one or more of four mechanisms [7]:

1.) The API is not delivered from its formulation in an appropriate time frame, to facilitate complete dissolution, resulting in elimination from the GI-tract before absorption is complete (dissolution-limited absorption).

2.) The drug degrades in the physiological GI fluids or non-absorbable complexes are formed.

3.) The drug is not transported efficiently across the GI-membrane (permeation-limited absorption).

4.) The drug is metabolized and/or eliminated before it enters the systemic circulation.

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The mechanisms 2.), 3.) and 4.) are difficult to address in formulation development. While degradation e.g. by gastric fluids can easily be resolved by coating processes using gastric fluid resistant polymers, permeation across the mucosa is still an issue that formulations scientists have to face. Despite some reports in literature enhancing permeation by the use of chitosan [8, 9], permeation issues are still not successfully worked out or fail due to lack of safety data about the excipient used to improve permeability [10]. Metabolism can sometimes be used for improvement of drug performance, but mostly these effects are caused by pharmacokinetic instead of galenical issues. An example for such effects is the boosting of anti-HIV-drug pharmacokinetics using subtherapeutic doses of Ritonavir. This drug inhibits CYP-enzymes. By a concomitant dosing of Lopinavir and Ritonavir, the degradation of Lopinavir by the CYP-enzymes is hindered and the duration of therapeutic plasma concentration of Lopinavir is prolonged [11].

But the major hurdle that can be addressed in formulation development is low aqueous solubility and subsequent slow dissolution, leading to insufficient bioavailability, as mentioned in point 1.) above.

1.3 Solubility and drug dissolution

As mentioned above it is obvious that an increase of dissolution rate can lead to an improved bioavailability. Several options are available to do so:

One of the classical approaches to increase the rate of dissolution is through decreasing particle size. Arthur Noyes and Willis Whitney identified the underlying

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basis for the correlation between particle size and dissolution rate in 1897. In the original publication its mathematical expression is:

$$\frac{dx}{dt} = C(S - x)$$

where *S* represents the solubility of the substance, or the concentration of its saturated solution; *x* is the amount of dissolved drug at time *t,* and C is a constant [12]. In 1904, the equation was modified by Nernst-Brunner and became what is nowadays one of the most important equations in pharmaceutical science.

$$DR = \frac{A_{Drug} \cdot D_{Drug}}{\delta} \cdot (C_S - C_t)$$

where *DR* is the dissolution rate, A_{Drug} is the drug surface area, D_{Drug} is the diffusion coefficient of the drug, *δ* is the diffusion layer thickness, C_S is the saturation solubility of the drug and C_t is the concentration of the dissolved drug at time *t.*

After the "digoxin-incident" [3, 5], the focus in dissolution testing for drug dissolution for poorly soluble substances shifted to establishing *in vitro − in vivo −* correlations (IVIVC). Since the gastrointestinal (GI) environment is a varying milieu with a pH-range from 1 - 7.4. and varying amount of electrolytes and bile salts, it is logical to adjust the composition of the dissolution media and the hydrodynamics of the test conditions to the human physiology [7]. In the ideal case, the *in vivo* performance is predicted by the

dissolution test using IVIVC. Oversimplification of the test conditions, for example by using compendial media which take only the pH-value of the GI tract into account, often leads to implausible predictions of *in vivo* behaviour [13]. Biorelevant media including FaSSIF and FeSSIF were introduced in 1998 [14]. Since these media better correspond to the *in vivo* situation [7], a better prediction of *in vivo* behavior of the drug formulation could be established, including the prediction of food effects. The media have been recently revised by Jantratid et al. [15], altering the media composition of FaSSIF and FeSSIF and introducing media for the gastric dissolution (FaSSGF, FeSSGF, "snapshot media"). The use of these media enables the prediction of food effects in combination with physiologically based pharmacokinetic modeling to generate *in silico* plasma profiles [16, 17].

1.4 The biopharmaceutics classification system

Dissolution testing can also be employed to facilitate drug product approval by allowing waiver of *in vivo* bioequivalence studies [18-20].

The interplay of solubility and absorption was acknowledged in 1995 in the Biopharmaceutics Classification System (BCS): Amidon et al. proposed a scheme, based on solubility and permeability, according to which a biowaiver can be granted [21]. Applicability of IVIVC to formulation development can also be determined according to this scheme:

Table 1.1: Probabilities for IVIVC as suggested by Amidon [13, 21]

Class	Solubility	Permeability	IVIVC Expectation
I	High	High	IVIVC if dissolution rate is slower than gastric emptying rate (e.g. controlled release dosage forms)
II	Low	High	IVIVC if dissolution rate is similar to the in vivo dissolution rate, unless dose is very high
III	High	Low	Permeability is rate determining, limited or no IVIVC
IV	Low	Low	Limited or no IVIVC expected

Active pharmaceutical ingredients (API) belonging to the BCS classes I and III are not likely to cause problems in terms of dissolution-limited bioavailability. The drugs of these classes are highly soluble, so dissolution is not an issue for the formulation. In case of BCS class III, poor bioavailability cannot be corrected easily by formulation since it is usually due to poor uptake at the gut wall. Taking a look at the current market share of drugs, more than 60% of the drugs belong to BCS class I or III (compare Figure 1.1 derived from [22]).

The formulation of poorly soluble substances has become more and more an issue in pharmaceutical industry over the past decades. The oral route is generally preferred. But since the early 90´s, APIs are often identified by high throughput screening and combinatorial chemistry. These approaches often lead to substances that possess physicochemical characteristics unfavorable for oral absorption. A major hurdle is their

low aqueous solubility and subsequent slow dissolution, resulting in insufficient bioavailability according to the Nernst-Brunner equation (compare Eq. 1.2). For the formulation scientist it is essential to resolve dissolution-limited absorption by the right choice of dissolution rate enhancing techniques, which will be described in detail in later sections.
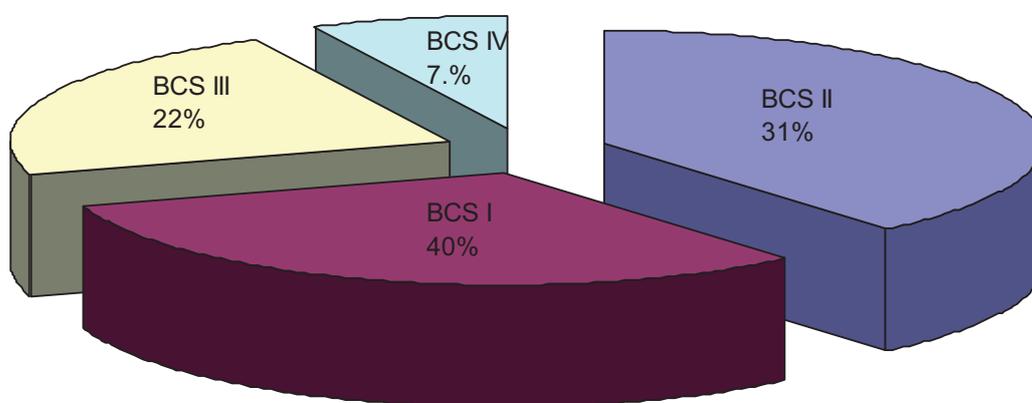

Figure 1.1: Current market share of drugs according to the Biopharmaceutics Classification System (derived from [23] and [22])

Figure 1.1 indicates that nearly 50% of marketed drugs exhibit low aqueous solubility. However, if only APIs approved in the last decade are taken into account, the market share shows a tendency towards more BCS Class II and IV compounds:
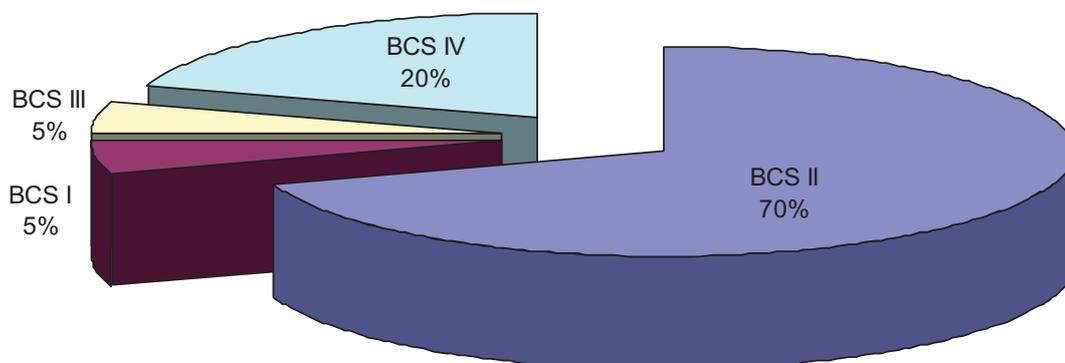
Figure 1.2: Market share of recently developed drugs according to the BCS [22, 23]

Figure 1.2 shows that solubility improvement is necessary for up to 90% of recently developed drugs and clearly illustrates the need for new formulation approaches to increase bioavailability.

The possibilities to formulate BCS class II and IV substances for oral administration will be described in the next section.

1.5 Formulation of poorly soluble substances

The flow process chart in Figure 1.3 gives an overview of how to find a suitable formulation for a poorly soluble API [24].
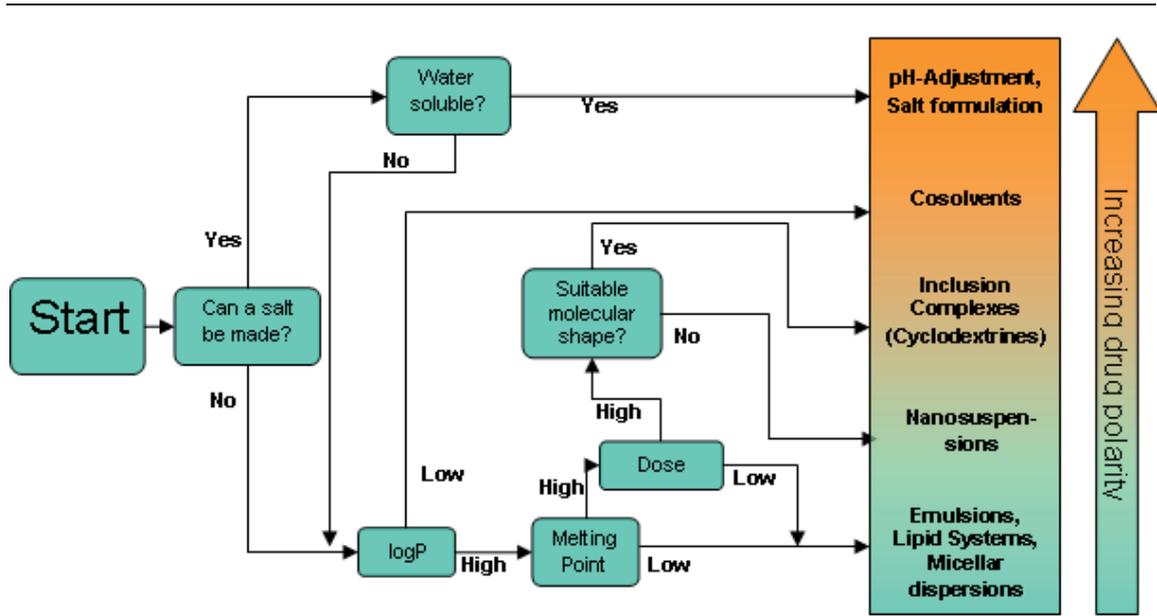
Figure 1.3: Decision tree in formulation finding for poorly soluble compounds (derived from [24])

Formation of a water-soluble salt from a water-insoluble compound is the method of choice to increase bioavailability, since salt formation is an easy and well-described technique [25-27]. There is a broad variety of counterions for anions as well as for cations, although most frequent used counterions are sodium and hydrochloride [28]. In addition to solubility improvement and hence dissolution rate enhancement, salt formation can positively influence hygroscopicity, chemical stability, crystal form and mechanical properties [29].

The use of cosolvents can be applied to poorly soluble compounds for injection, but for orally administered drugs as solid dosage forms, this is obviously not the method of choice.

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Cyclodextrins are able to increase the saturation solubility of a compound by its complexation. In this way, supersaturated systems are created. According to Eq. 1.2 the dissolution rate, and subsequently the bioavailability, for drugs of BCS class II and IV is increased, especially when cyclodextrins are also able to function as a permeability enhancer [30, 31]. Nevertheless, cyclodextrins are not the answer to every challenge in oral drug formulation. For a variety of reasons, including toxicological considerations, formulation bulk and production cost, the use of as small amounts of cyclodextrins as possible in pharmaceutical formulations is preferred [32]. Since cyclodextrins often bind to the drug in a certain molecular ratio (usually 1:1 or 2:1), they are less suitable for highly dosed drugs. Nevertheless, cyclodextrins and their derivatives are used for orally administered pharmaceutical products, and there is even a sublingual tablet Prostarmon E® (Ono Pharmacaceutical Co, Japan), which contains β-cyclodextrin to solubilise prostaglandine E [33]. Especially for low dosed APIs, derivatives of cyclodextrins with improved physicochemical characteristics appear to be suitable for enhancement of drug solubility [34].

The enhancement of bioavailability by the use of lipid-based systems has been recognized for many years [35]. These formulations are able to keep the poorly soluble compound in solution and thus maintain a concentration of dissolved drug as the driving force for absorption [36]. Pouton has proposed the lipid formulation classification system (LFCS) [37]:

Table 1.2: The LFCS as proposed by Pouton [37]

LFCS type	Characteristics	Advantages	Disadvantages
Type I	Non-dispersing, requires digestion	GRAS status; simple; excellent capsule Compatibility	Formulation has poor solvent capacity unless drug is highly lipophilic
Type II	SEDDS without water-soluble Components	Unlikely to lose solvent capacity on Dispersion	Turbid o/w dispersion (particle size 0.25–2 µm)
Type IIIa	SEDDS/SMEDDS with water-soluble Components	Clear or almost clear dispersion; drug absorption without digestion	Possible loss of solvent capacity on dispersion; less easily digested
Type IIIb	SMEDDS with water-soluble components and low oil content	Clear dispersion; drug absorption without digestion	Likely loss of solvent capacity on Dispersion
Type IV	Oil-free formulation based on surfactants and cosolvents	Good solvent capacity for many drugs; disperses to micellar solution	Loss of solvent capacity on dispersion; may not be digestible

Nowadays lipophilic drug delivery systems are used for about 2% of oral formulations. An example for such patented systems is the Lidose® platform technology by S.M.B. Laboratoires [38]. This technology utilizes a mixture of API and lipophilic excipients in a hard gelatine capsule, either semi-solid or solid. The mixture melts at body temperature, forming a stable suspension or solution, which in turn leads to increased absorption of drug and subsequently more reliable blood plasma profiles. This patent was commercialized using fenofibrate (Fenogal©). The dosage form shows bioequivalence to the micronized, suprabioavailable formulation Tricor® or Lipidil Ter®,

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products marketed by Abbott and Solvay, respectively (compare section 3.2.1). The challenge in using lipid formulation approaches is the potentially metastable state of the formulation in the GI tract: with a change of environment, e. g. by dilution in the stomach depending on its content, the system might not be able to hold the API in solution and precipitation can occur before the drug enters the small intestine, the main site of absorption. But even when the formulation reaches the intestine intact, the digestion of lipid components of the formulation might cause a rapid disassembly of the formulation, resulting in precipitation of the API (if in solution) or a change of crystalline state (if in suspension).

The crystalline state of the drug can influence solubility and dissolution behaviour significantly. Depending on thermodynamics, form A of a drug might be more stable than form B, resulting in a longer shelf life of the dosage form. But often the more stable form possesses disadvantageous characteristics regarding drug solubility and dissolution. An amorphous state is more favorable in this respect, since no crystal lattice energy has to be overcome to dissolve the drug. But amorphous states are metastable by definition, and a transition of the desired amorphous form into an unfavorable crystalline form can occur [37, 39, 40].

The possibility of an unwanted phase transition remains a challenge for formulations containing amorphous API. An example is the conversion in a liquid formulation of ritonavir from form I to the less soluble form II in the soft capsule. This results in supersaturation and subsequent precipitation of drug within the capsules [41].

Nowadays there are often approaches to utilize the benefits from amorphous formulations. By dispersing the API into polymers, forming solid dispersions, the amorphous state can be preserved at least temporarily. Of course, phase conversion can happen here as well, depending on the drug. A well described technique to form amorphous dispersions in solid states is the melt extrusion of polymers. The API, amorphous dispersed or dissolved in the polymer melt, is immobilized in a matrix by dropping the temperature below the glass transition temperature. Even if recrystallisation occurs within a hydrophilic polymer, often only very small crystals are formed due to the inability of the lipophilic drug to mix with the polymer [42]. Consequently, the performance of the dosage forms may not be drastically affected by such a transformation. Not only the bioavailability but also the applicability and subsequently the compliance of the patient can be improved by melt extrusion (e.g. there is no need for refrigeration of the melt extrusion product Kaletra$^©$ in contrast with the predecessor liquid formulation). Drawbacks include the high input of energy in form of temperature and shear forces, which may lead to a high degree of degradation for thermolabile drugs [37, 42].

1.6 Particle size reduction in pharmaceutical technology

By optimizing the particle size within a drug powder, the following aims can be achieved:

(i) The application can be made easier, e.g. pulverization of a drug can lead to shorter extraction times.

(ii) The accuracy of dosing can be improved. This is certainly not the case for very high dosages, but in cases of very potent drugs a narrow particle size distribution is a prerequisite for accurate dosing.

(iii) Flowability is often related to particle size. By matching the right particle size and its distribution, tabletting and dispergibility of a powder, e.g. in ointments can be improved

(iv) According to the Nernst-Brunner equation the reduction of particle size leads to an increase surface area and subsequently to an enhanced dissolution rate. In consequence, a larger amount of dissolved drug is provided for absorption and bioavailability can be increased. Several studies prove the success of this formulation approach [6, 43-45].

1.6.1 Particle size reduction

From the very first attempts to manufacture pharmaceutical preparations hundreds of years ago, pharmacists reduced the particle size of powders and drugs using a mortar and a pestle. By using this easy but effective technique, it was possible to grind plant components into powders with a subsequent release of essential oils that have a healing effect. The mortar and pestle are still in use in pharmacies, e.g. for the preparation of extemporaneous prescriptions. Nowadays, these simple tools are more common in the kitchen at home for grinding of spices and for preparation of food.

Here the mortar and pestle demonstrates superiority to many modern tools for particle size reduction: They can be used for grinding both dry substances like peppercorns as well for wet milling operations as the preparation of a delicious pesto [46].

Nevertheless, particle size reduction has become its own science that divides the mechanism of particle size reduction into (i) pressure and friction, as is the case for the mortar and pestle method, (ii) collision and (iii) hammer and (iv) shear forces.

The reduction ratio can be easily expressed as

$$Z = \frac{d_0}{d_1}$$

Eq. 1.3

Where Z is the reduction ratio, d_0 is the initial particle size and d_1 the particle size after the milling process. The reduction ratio is dependent on (i) the type of technical operation, (ii) the operating conditions and (iii) the physicochemical characteristics of the milled substance. Solid substances contain predetermined fission points in each particle, caused by imperfections of the crystal lattice. As particle size is decreased, these imperfections become fewer and the energy needed for further reduction increases. The maximum energy that can be applied is determined by the choice of equipment and the operating conditions [47].

1.6.2 Dry milling methods

An overview of tools used for dry milling is provided in Table 1.3 [48].

Table 1.3: Dry milling operations [48]

Tool	Working principle	Degree of fineness	Principle of particle size reduction
Roller crusher	Rollers running against each other, smooth or spiny	1 – 2 mm	Pressure, friction
Cutting mill	Rotating kniferoll, stationary cutting edge	1 – 5 mm	Chopping
Hammer mill	Rotating hammer (rotor), stationary jaw (stator)	0,3 – 2 mm	Collision, hammer
Ball mill	Cylindric, rotating vessel containing spherical grinding elements	ca. 20 µm	Collision, hammer, pressure
Pin disc mill	Rotating pair of discs with interleaved pins	20 – 200 µm	Collision, hammer
Mortar mill	Mortar with stationary, versatile pestle	n.a.	Pressure, friction
Jet mill	No moving parts, powder-loaded jet-stream	1-30 µm	Hammer, friction

Using a dry milling technique, climate control within the production rooms is very important. Hygroscopic substances may bind more water in a humid atmosphere, making them more resistant to milling. In addition, it has to be guaranteed that no explosive dusts are generated.

For poorly soluble substances the need for climate control of production rooms can be circumvented by applying a wet milling technique. These are described in the next section: