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Development and evaluation of solvent-free processing techniques for poorly water soluble drugs.

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"Fomos todos assim, naquela encantada e quase fantástica Coimbra de há dez anos. Um sopro romântico, cálido mas balsâmico, fazia rebentar tumultuariamente as nossas primaveras em borbotões de flores; flores exóticas, estranhas, que a ciência impassível bania inexoravelmente das suas correctas classificações, mas a que dava um indizível encanto, um atrativo particular uma coisa: a mocidade"

ANTERO DE QUENTAL, Prefácio a
Primaveras Românticas, 1872

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AIMS

Poor water-solubility is becoming the leading hurdle for pharmaceutical formulation scientists working on oral drug delivery leading to the development of novel formulation techniques. **Chapter 1** gives a brief overview on the available literature on this topic. These new techniques often use organic solvents to transform the drug into a high-energy amorphous state or to produce crystalline micro- and nanoparticles. However, a major drawback of using volatile organic solvents is residual traces in the final product that are hard to remove. These not only involve toxicity issues, but might also induce phase transformation over prolonged periods of storage, impairing stability of the drug formulation [1]. In this regard, special guidelines have been issued by the European Pharmacopeia limiting the maximum allowed amount of residual solvents in pharmaceutical formulations [2-5].

Taking into account these considerations, the aim of this PhD thesis is to develop and evaluate processing techniques for poorly water-soluble drugs without using organic solvents. Therefore, several approaches were evaluated. In **Chapter 2**, the self-emulsifying excipient Gelucire®44/14 and its effect on the solubility of the poorly water-soluble drug carbamazepine is evaluated using melt granulation and spay drying followed by compression into tablets. In **Chapter 3**, Gelucire®44/14 was evaluated as stabilizing excipient during the production of drug nano- and microsuspensions via mechanical impact (ball milling and ultrasonication) and as solubilizing matrix for melt crystallization. In **Chapter 4**, an aqueous coacervation technique comprising a molten surfactant/drug mixture was developed in view of large scale drink water medication for veterinary purposes.

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CHAPTER 1
GENERAL INTRODUCTION

1.1. Poorly Water-Soluble Drugs and the BCS Classification

Drug development encompasses the entire process of bringing a new drug to the market and involves four stages: (1) drug discovery, (2) product development, (3) pre-clinical research and (4) clinical trials. Whereas for many decades, new active compounds were often identified via serendipity, nowadays high-throughput screening and combinatorial methods are applied. However, many of the found candidate drugs fail in clinical trial due to: adverse effects (10 %), toxicity (11 %), lack of efficiency (30 %) and poor bioavailability (30 %). Indeed, pharmaceutical industry witnesses a steady increase in poorly water-soluble drugs as lead compounds, i.e. 40 % of the drug molecules in development pipelines and 60 % of the drug molecules coming from chemical synthesis. This is of special concern for drug formulations intended for oral intake as good drug dissolution is a prerequisite for drug absorption and subsequently clinical response.

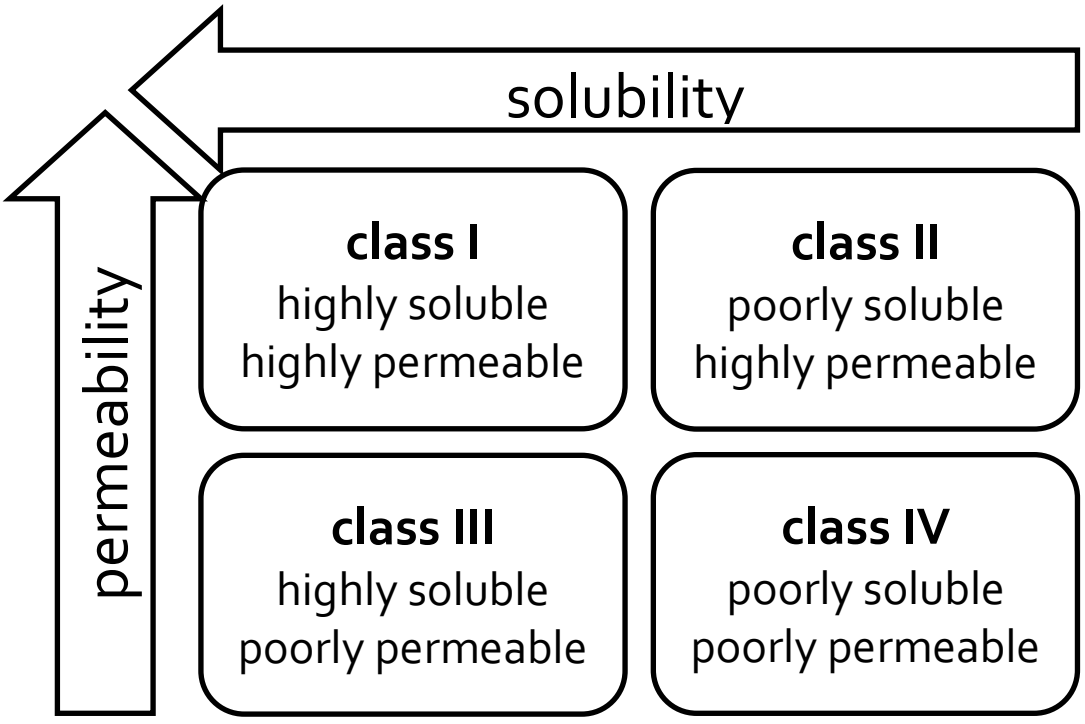

Figure 1. Schematic representation of the Biopharmaceutical Classification System (BCS).

Until now, comprehensive methods to predict oral absorption based on *in vitro* drug dissolution are limited, mainly due to the complexity of the processes in the gastro-intestinal tract and to complex pharmacokinetics. It became clear that to correlate *in vitro* drug dissolution and *in vivo* drug availability, one should consider drug dissolution and gastrointestinal permeability as

fundamental parameters controlling the rate and extent of drug absorption. For this purpose, Amidon and co-workers proposed the Biopharmaceutical Classification System (BCS) scheme that sets the basis for *in vitro-in vivo* correlations between drug dissolution and drug absorption. The BCS defines four classes of drug, as shown in **Figure 1**.

Recent advances in drug formulation have introduced a variety of strategies to enhance the dissolution rate of poorly water-soluble drugs, and are therefore promising for the formulation of BCS class II drug molecules. However, before attempting any physicochemical approach to enhance the solubility of such drug molecules, a profound understanding of drug solubilization phenomena is crucial.

1.2. Solubilization Phenomena

The solubility of a compound (termed the 'solute') is the maximum of the solute that can be dissolved in a certain volume of solvent at a specific temperature, and it's directly related to the solute's hydrophilicity. The process of solubilization involves the destruction of inter-ionic or inter-molecular bonds in the solute trough interaction with solvent molecules. Water is a electric dipole and as such a good solvent for molecules that can interact via ion-dipole interaction (e.g. ionized molecules), Van der Waals forces, dipole-dipole interaction (e.g. molecules containing highly electronegative elements such as O, S, N or halogens), dipole-induced dipole interaction (e.g. non-polar molecules where a dipole can be induced by presence of a polar molecule) and hydrogen bonding (e.g. molecules containing –OH or –NH groups). Hydrogen bonding contributes significantly to aqueous solubilization of drugs since water can act both electron donor and acceptor. Solubility can be expressed in many different ways and **Table 1** tries to give a general classification.

For drugs that ionize in water, solubility depends in most cases not only on the intrinsic solubility of the non-ionized drug but also on the extent of ionization. In general, aqueous solubility of the ionized from is much higher than the corresponding non-ionized one. When a weak acid/base drug is added to an aqueous medium, it will dissolve to the extent of the intrinsic solubility of the non-ionized form and then ionize. According to the Hendersson-Hasselbalch equation, as drug ionizes more drug will dissolve to maintain a saturated solution of the non-ionized form of the drug.

Table 1. Solubility defined in parts of solvent per one part of solute

Definition	parts of solvent required for one part of solute
very soluble	<1
freely soluble	1-10
Soluble	10-30
sparingly soluble	30-100
slightly soluble	100-1000
very slightly soluble	1000-10,000
Insoluble	>10,000

This process will persist until both the solubility and ionization equilibriums are achieved. These dynamic equilibrium processes are shown in the following equations:

weak acid drug $\quad HA_{solid} \leftrightharpoons HA_{solution} \leftrightharpoons H^+ + A^-$

weak base drug $\quad B_{solid} \leftrightharpoons B_{solution} + H^+ \leftrightharpoons BH^+$

Saturated solutions of weak acid/weak base will contain both the ionized and the non-ionized forms of the drug. The prevalence of either one depends on the pH of the aqueous medium, which this strongly influences the solubility of the drug. When the pH of the aqueous medium allows ionization of the weak acid/weak base, the solubility increases. Therefore, a weak base drug will show higher solubility at a low pH and a lower solubility at a high pH, while a weak acid drug will show the opposite behavior.

Another factor influencing drug solubility is temperature. The changes in temperature affect solubility in two different ways. If the solubilization process requires energy then solubility increases at increasing temperature (endothermic dissolution process). The opposite occurs for exothermic dissolution processes where increasing the temperature decreases solubility.

Other factors such as drug crystal size and the molecular weight of the drug limits its dissolution. The larger the molecule the more difficult is to solubilize [11]. The basis of solubility and solvent solubilization capacity depends on the number of interactions created between solute and solvent. The smaller the drug particle, the larger will be the surface available for interactions to take place and the higher will be its dissolution rate. Some compounds have the ability to

crystallize in more than one crystalline form (polymorphism), this results in different melting points for each polymorph and consequently different solubility [9].

1.3. Solubility enhancement techniques

Based on extensive knowledge of solubility phenomena, drug formulation scientists have developed different formulation techniques that enhance drug solubility to increase the bioavailability of poorly soluble drugs. **Figure 2** gives a schematic overview and below we go further review the different strategies used to enhance bioavailability of poorly water soluble drugs.

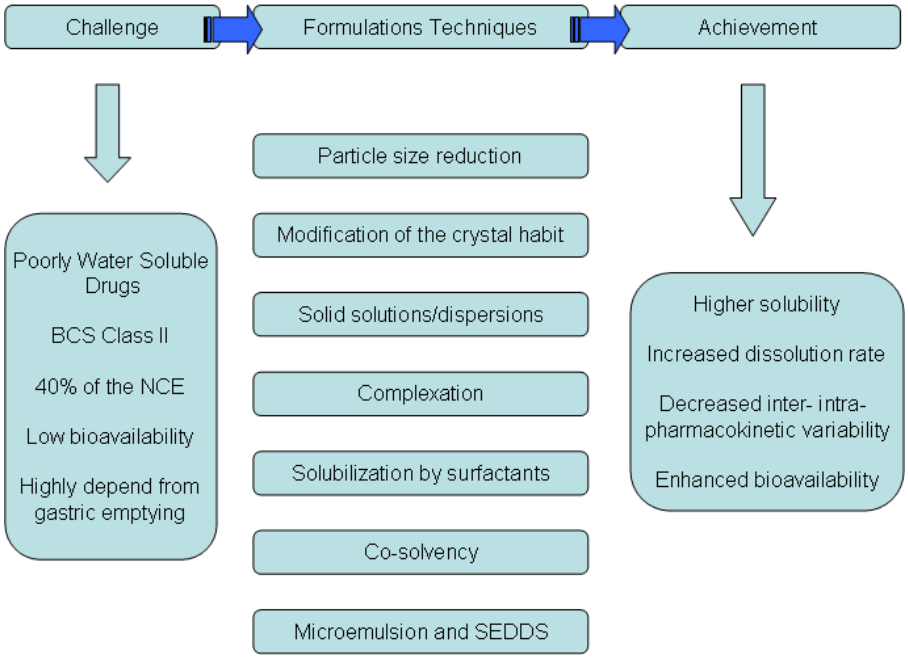

Figure 2. Formulation techniques to improve solubility of BCS Class II Drugs

1.3.1. Particle size reduction

Increasing the total drug surface, by particle size reduction, enhances drug saturation solubility and dissolution rate. Particle size reduction can be achieved through micronization and nanonisation.

1.3.1.1. Micronization

Table 2 gives an overview of the conventional methods based on mechanical force to disintegrate drug compounds. The principle of comminution is based in the fact that all crystalline materials have imperfections, and that mechanical stress will cause crack propagation. When mechanical stress is applied on a powder, the particles get strained. This stress-strain relationship is shown in **Figure 3**.

Table 2. Type of equipment and particle size outcome to reduce drug particle size

Method	Type of equipment	Approximate particle size (μm)
Cutting	Scissors, Shears, Cutter Mill	100-80,000
Compression	Roller Mill, Pestle-Mortar	50-10,000
Impact	Hammer Mill, Disintegrator	50-8000
Attrition	Colloidal Mill, Roller Mill, Pearl/Ball Mill	1-50
Impact and attrition	Ball Mill, Fluid Energy Mill	1-2000

The stress-strain curve can be divided into two distinct deformation regions, being elastic deformation and plastic deformation. Elastic deformation is temporary and fully recovers when the load is removed. This linear region is defined by Hooke's Law, which states that stress is proportional to strain. Plastic deformation is permanent and is not recovered when the load is removed.

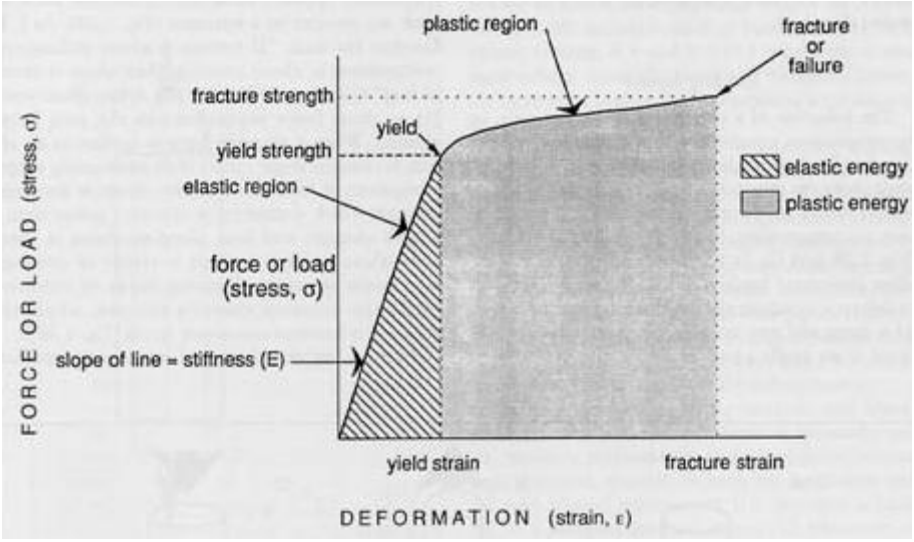

Figure 3. Stress-strain diagram for deformation of drug particles. (diagram adapted from www.brown.edu/.../EEB/EML/images/deformation.jpg)

The yield strength represents the stress required to generate permanent deformation and indicates the degree of easiness to deform the materials. The slope of the stress-strain curve in the elastic deformation region is the modulus of elasticity, which is known as Young's modulus. It represents the stiffness of the material-resistance to elastic strain. Beyond the yield point, the region represents irreversible plastic deformation. The increasing dislocation density makes plastic deformation more difficult. This phenomenon is the strain hardening. The strain at failure occurs after the particle fractures. It is the interaction point of the plastic recovery region in strain axis also known as ductility. The ductility is the percentage of elongation at failure and indicates that the general ability of the material to be plastically deformed. The toughness is used to describe the combination of strength and ductility. The area under the curve represents the fracture toughness; this is a proximal measure of the impact strength of the material. The fracture of a particle can be obtained when the mechanical force exceeds the elastic limit [12-19].

Particle size reduction to micrometric range influences greatly the dissolution rate and is described by the Noyes-Whitney equation. The ratio dm/dt is thereby defined as dissolution rate. From equation it is clear that increasing the surface area (S) of a drug particle enhances its dissolution rate.

$$\frac{dm}{dt} = \frac{D.S}{V.h}(C_s - C_t)$$

dm = amount of drug(Kg) **h** = thickness of the diffusion film

$\mathbf{d_t}$ = time interval (s) adjacent to the dissolution surface(m)

S = surface (m^2) **D** = diffusion coefficient(m^2/s)

$\mathbf{C_t}$ = concentration of $\mathbf{C_s}$ = saturation solubility of the drug molecule (mol/Kg H_20)

the dissolved drug (mol/L) **V** = volume of dissolution medium (L)

Vogt et al. [20] made a comparative study between different micronisation techniques (bead milling, jet milling) and their effect on the solubility of fenofibrate, a poorly soluble drug. Micronization of the fenofibrate was shown to enhance its dissolution (8.2% in 30 min) compared to the crude material (1.3% in 30 min).

1.3.1.2. Nanonization

Currently, most of the newly discovered drugs are so poorly water soluble that micronization is no longer sufficient to achieve the required bioavailability. The next step was to move from micronization to nanonization. By moving one dimension further to smaller particles, surface is further enlarged and the dissolution rate further enhanced. Saturation solubility is assumed as a constant depending on the compound, temperature and dissolution medium. However, bellow a critical size of 1 μm, saturation solubility (C_s) becomes also function of the particle size, increasing with the decrease of particle size. This effect on solubility is described by the Ostwald-Freundlich equation:

$$\frac{S(d)}{S_0} = \exp \frac{\gamma . V_m}{RTd}$$

S(d) = solubility (mol/Kg H_20)

d(m)= particle diameter) (m)

T= temperature (K)

R= gas constant (J/mol.Kg)

$\mathbf{V_m}$= molar volume (m^3/mol)

γ= surface free energy (surface tension) (mJ/m^2)

$\mathbf{S_0}$= solubility of the bulk material ($d \to \infty$) (mol/Kg H_20)

Nevertheless, for the solubility S(d) to differ significantly from the solubility S_0 of the bulk material, the exponential term needs to be much smaller than 1 as is the case for particles in the nanorange. Dissolution pressure increases with the increase of the curvature of the surface, i.e. by the decreasing particle size. The transfer of molecules from liquid phase to a gas phase (vapour pressure) is theoretically similar to the transfer of molecules from solid phase (nanocrystal) to liquid phase (dissolution pressure). At saturation solubility the equilibrium between molecules dissolving and molecules re-crystallizing is reached. This equilibrium can be altered in case the dissolution pressure increases. Taking into account the Kelvin equation allows a better understanding of the enhancement of saturation solubility by particle size reduction:

$$\ln\left(\frac{P}{P_0}\right) = \frac{-\gamma * V_L * \cos\theta}{r_k * RT}$$

P = vapor pressure(Pa)

$\mathbf{P_0}$ = equilibrium pressure(Pa)

Cos θ = contact angle

$\mathbf{r_k}$ = radius of droplet(m)

γ = surface tension (mJ/m^2)

$\mathbf{V_L}$ = molar volume (m^3/mol)

R = universal gas constant (J/mol.Kg)

T = absolute temperature (K)

Applying Kelvin equation to compounds with different vapor pressures, demonstrated that the increase in vapor pressure with the decrease in droplet size is more significant in compounds with initial lower vapor pressure [21]. In analogy, the increase of dissolution pressure

in solid particles is more pronounced in those with an initial lower dissolution pressure, i.e. the relative increase is higher in poorly soluble drugs. Such effect is exponential for particles with sizes bellow 100 nm [22].

Several techniques are used to obtain drug particles in the nanorange [27], and can be divided in either top down or bottom up processes as listed in **Table 3**. The most common way is the size reduction of previously formed particle (top down process), using mechanical energy to induce **impact**, **shear**, **attrition and cavitation.** In a different approach bottom up techniques aim to produce small particles from solution by stimulating recrystallization into particles of a desired size, e.g. via spray drying or solvent displacement.

Table 3. Processes and techniques for drug particle nanonization

Nanonization methods	Nanonization techniques
Top down	Pearl/Ball milling
	High pressure homogenization
Bottom up	Spray Freezing into liquid
	Liquid solvent exchange
	Supercritical fluids
	Controlled crystallization

Milling can be carried out both in dry and wet state. This methodology is typically applied in the production of micro- or nano-suspensions for pulmonary administration [23]. For stabilization of the produced suspensions, stabilizing agents are used, including polyvinylpyrrolidone and poloxamers [24, 25]. When milling is performed in the presence of a surfactant a significant enhancement of drug dissolution rate and bioavailability can be achieved [26]. Liversidge et al. [28] investigated the effect on bioavailability of increasing the particle surface area by decreasing drug crystal particle size below 200 nm and stabilizing the particles to prevent agglomeration in the GI tract. A randomized three-way crossover study was conducted to compare absolute oral bioavailability of danazol from three formulations: (a) an aqueous

dispersion of nanoparticulate danazol (mean particle size 169 nm); (b) danazol-hydroxypropyl-beta-cyclodextrin (HPB) complex and (c) an aqueous suspension of conventional danazol particles. The bioavailability of nanoparticle suspension demonstrated that the nanoparticle dispersion had overcome the limited bioavailability observed with conventional suspensions of danazol. Alternatively, similar results were achieved by Muller et al. [30] by means of high-pressure homogenization and by Pace et al. [29] using microfuidation. Milling is used by Elan for its NanoCrystal technology, which is currently licensed to Wyeth to produce Rapamune and to Merck to produce Emend.

Contrary to nanocrystalline drug formulation via milling, a bottom up approaches allow the production of drug particles directly in the required small particle size range. These methodologies are based on controlled crystallization phenomena. From dissolved state, assembly of drug molecules into agglomerates is induced under controlled conditions. The challenge in creating stable drug nanosuspensions is twofold, as besides preparing nanoparticles, they also have to be kept in the nanorange and thus prevent them from growing via Ostwald ripening or re-agglomeration. A common route to avoid particle growth is via the use of surfactants that coat the surface of the drug particles, preventing them from growing and provide steric or electrostatic repulsion to avoid particle aggregation.

Furthermore, due to the Ostwald-Meyers rule, when engineering drug particles via a bottom up approach, the speed of the precipitation process is often crucial as super saturated conditions, smaller particles are obtained when nucleation occurs faster [31]. For this purpose, several techniques have been developed, including spray freezing into liquid, liquid solvent exchange and the use of supercritical fluids.

Spray freezing into liquid (SFL) is a cryogenic particle engineering technique involving atomization of a drug solution into a cryogenic liquid (e.g. nitrogen) to produce small particles. The frozen particles are then lyophilized to obtain a free flowing powder. This technique has been extensively studied by Rogers et al. and Hu et al. to produce danazol nanoparticles. The obtained SFL powders significantly enhanced drug dissolution rate compared with micronized danazol [32, 33]. Similar results were obtained by the same research group using the EPAS (Evaporative Precipitation into Aqueous Solution) process. In this process, drug precipitation is induced by evaporation of an organic solvent from the feed solution near or above the boiling point upon contact with a heated aqueous solution [34]. Both techniques are presently commercialized by Dow Chemical Company. Based on the solubilization power of supercritical fluids (e.g. supercritical CO_2) Li et al. developed the RESS (Rapid Expansion from a liquefied-gas

solution) technique to improve the dissolution of griseofulvin. After loading the supercritical fluid with drug, an extremely fast reversal from the supercritical phase to the gas phase occurs during expansion in a supersonic jet. This phase reversal induced super-saturation and subsequent particle precipitation. The obtained griseofulvin particles have sizes below 50 nm and exhibited an enhanced dissolution rate [35]. Young et al. achieved equivalent results upon preparing cyclosporine nanoparticles by RESS in a size range of 500-700 nm. In this case, Tween 80 was used as surfactant to prevent flocculation and aggregation of the nanoparticles [36].

These approaches have been further explored by Pace et al. who combined the RESS technique with high-pressure homogenization to prepare a stable nanosuspension. In this process, a poorly water-soluble drug was first dissolved in a supercritical fluid, which was subsequently expanded into an aqueous solution containing surfactant. The thus formed suspension was further subjected to high-pressure homogenization, producing a stable nanosuspension [37]. Baxter has developed a similar process by combination of precipitation and shear forces, termed Nanoedge.

1.3.2. Modifications of crystalline structure

1.3.2.1. Polymorphs

Although chemically identical, polymorphs exhibit different physicochemical properties such as solubility, melting point, density, texture and stability. Polymorphs can be classified as enantiotropes and monotropes, according to their thermodynamic properties. In enantiotropic systems, one polymorph form can change reversibly into another at a certain defined transition temperature below the melting point, while in monotropic systems such transition is irreversible. Drug molecules that fit into one of these categories undergo a 3-stage transition:

Amorphous > Metastable polymorph > Stable polymorph.

The metastable forms are associated with higher energy with increased surface area and consequently with increased solubility. The amorphous (i.e. no internal crystalline structure) form is more suitable to enhance drug solubility due to its higher energy state. Some drugs can already exist in an amorphous form and are commonly recognized as super cooled liquids. These have greater solubility than the crystalline forms because less energy is needed to transfer the molecule into solvent.

Similarly, an anhydrous form has better solubility than a hydrated form, since the latter one is already associated to water and have less energy for crystal breakup than anhydrates. Therefore in regard to bioavailability enhancement, it is better to use anhydrous forms or develop formulations of metastable and amorphous forms to enhance drug solubility. Ma et al. studied the two different polymorphic forms of 5-chloro-8-hydroxyquinolone, showing a much higher solubility and dissolution rate of polymorph form II [38]. Nevertheless, the possibility of a conversion of the high-energy form into a low-energy crystal with low solubility cannot be ruled out. During manufacture and storage, recrystallization and water incorporation may occur, endangering drug solubility performance and bioavailability. Greco et al. evaluated the crystallization of amorphous indomethacin during dissolution. The amorphous indomethacin was prepared either via quench cooling the melted solid or via cryogrinding. Both techniques apparently yielded amorphous drug. Nevertheless, only drug processed via quench cooling showed enhanced dissolution. Deeper investigation into the solid state characteristics of the drug formulations indicated the presence of small crystalline nuclei in the product obtained via cryogrinding that impaired the drug dissolution rate [39].

1.3.2.2. Co-crystals

Co-crystals have emerged as a promising means to enhance drug solubility, dissolution and bioavailability [7, 8]. A co-crystal may be defined as a crystalline material that consists of two or more molecular (electrically neutral) species held together by non-covalent forces [40]. The formation of co-crystals requires the development of a supramolecular library of co-crystallizing agents derived from examining structure-properties relationships present in classes of known crystal structures [42]. Co-crystals are more stable, particularly as the co-crystallizing agents are solids at room temperature. Only three co-crystallizing agents are classified as generally recognized as safe (GRAS), including saccharin, nicotinamide and acetic acid, thereby limiting the pharmaceutical applications [41]. Co-crystallization between two active pharmaceutical ingredients has also been reported. At least 20 have been reported to date, including caffeine and glutaric acid polymorphic co-crystals [43]. Co-crystals can be prepared by different techniques including solvent evaporation, simultaneous grinding of different components, sublimation, melt crystallization, and slurry processing [44]. The formation of molecular complexes and co-crystals is becoming increasingly important as an alternative to salt formation, particularly for neutral compounds or those having weakly ionisable groups. There is nevertheless lack of precedence and emergent concerns about the safety and toxicity of co-crystal forming agents.

1.4. Solid solutions and solid dispersions

A solid dispersion is a solid formulation consisting of at least two different components, generally a hydrophilic matrix (crystalline or amorphous) and an, often hydrophobic, drug. The drug compound in a solid dispersion does not necessary exist in a micronized state. Often a fraction of the drug is dispersed solubilized in the matrix or can be in amorphous state [45-48]. Upon exposure to aqueous medium, the carrier dissolves and releases the drug as fine colloidal particles. This will result in enhanced surface area and higher drug dissolution rate. During such process a portion of the drug goes immediately into solution (until saturation point is reached) while the remaining excess precipitates as fine colloidal particles. Based on the molecular arrangement of the drug within the matrix, different types of solid dispersions can be distinguished as listed in **Table 4**.

Table 4 - Types of solid dispersions, adapted from Dhiendra K. et al [53].

	Solid dispersion type	Matrix *	Drug**	Remarks	No. Phases
I	**Eutectics**	C	C	The first type of solid dispersion prepared	2
II	**Amorphous precipitations in crystalline matrix**	C	A	Rarely encountered	1
III	**Solid solutions**				
	Continuous solid solutions	C	M	Miscible at all composition, never prepared	1
	Discontinuous solid solutions	C	M	Partially miscible, 2 phases even though drug is molecularly dispersed.	2

Table 4 (CONT.) - Types of solid dispersions, adapted from Dhiendra K. et al [53](Cont.)

	Substitutional solid solutions	C	M	Molecular diameter of drug (solute) differs less than 15% from the matrix (solvent) diameter. In that case the drug and matrix are interchangeable. Can be continuous or discontinuous. When discontinuous: 2 phases even though drug is molecularly dispersed.	1 or 2
	Interstitial solid solutions	A	M	Drug (solute) molecular diameter less than 59% of matrix (solvent) diameter. Usually limited miscibility, discontinuous. Example: Drug in helical interstitial spaces of PEG.	2
IV	**Glass suspension**	A	C	Particle size of dispersed phase dependent on cooling/evaporation rate. Obtained after crystallization of drug in amorphous matrix	2
V	**Glass suspension**	A	A	Particle size of dispersed phase dependent on cooling/evaporation rate many solid dispersions are of this type	2
VI	**Glass solution**	A	M	Requires miscibility OR solid solubility, complex formation or upon fast cooling or evaporation during preparation, many (recent) examples especially with PVP	1

*A:matrix in the amorphous state, C: matrix in the crystalline state
** A: drug dispersed as amorphous clusters in the matrix, C: drug dispersed as crystalline particles in the matrix, M: drug molecularly dispersed throughout the matrix

1.4.1. Simple eutectic mixtures

These solid dispersions are characterized by a molten mixture of a component A and B, in which, upon cooling both A and B crystallize simultaneously (**Figure 4**). Solid eutectic mixtures are commonly prepared by rapid cooling of a molten mixture, resulting in a mixture of very fine crystals of both components. When these mixtures incorporate a poorly soluble drug, its dissolution rate is strongly enhanced by increasing the crystal contact surface. Liu et al.

reported on the creation of a eutectic mixture of itraconazole and poloxamer 188, leading to at least a 33-fold increase in itraconazole solubility in water. [49].

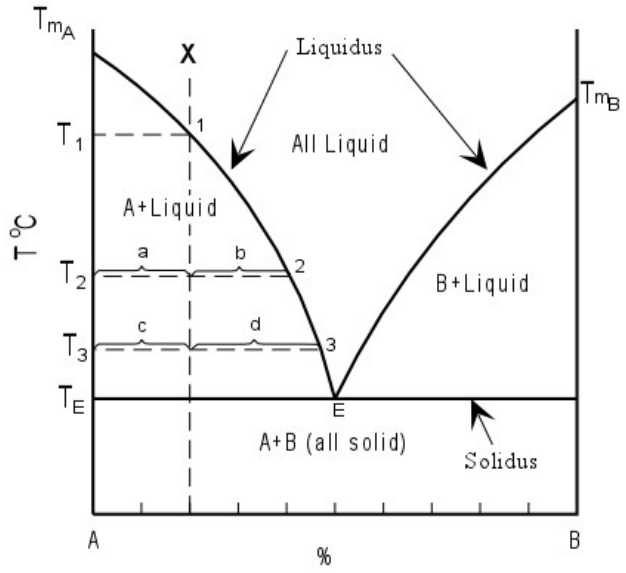

Figure 4. Eutectic system phase diagram

1.4.2. Solid solutions

Solid solutions can be divided in several subdivisions. In **continuous solid solutions**, all components are miscible in all proportions. In theoretical terms it means that the bonding strength between components is stronger that the bonding strength between the molecules within each individual component. This is an ideal situation, but yet to be reported for pharmaceutical applications.

In **discontinuous solid solutions**, the solubility of each component (α, β) in the other is limited. A typical diagram of a discontinuous solid solution is shown in **Figure 5**. The region labeled "α" is a solid solution, with β acting as the solute in a matrix of α. On the other end of the concentration range, the region labeled "β" is also a solid solution, with α acting as the solute in a matrix of β. The large solid region in between the α and β solid solutions, labeled "solid α and β", is *not* a solid solution. Instead, examination of the microstructure of a mixture in this range would reveal two phases — a solid solution α-in-β and a solid solution β-in-α would, forming two separate phases.

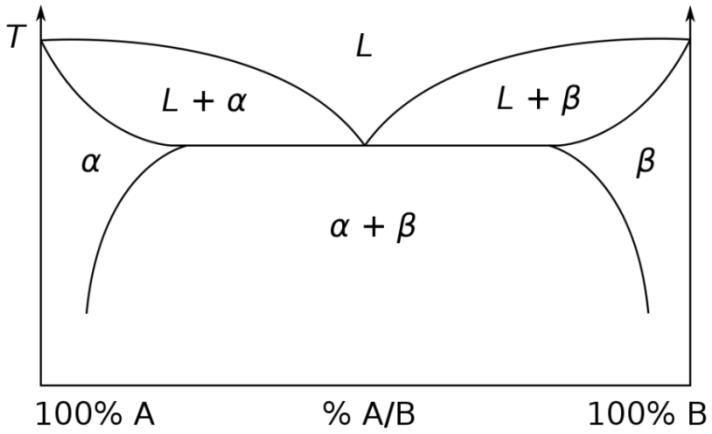

Figure 5. Diagram of a discontinuous solid solution

In a classic solid solution within its crystalline structure the solute molecules can either substitute the matrix molecules if the size of the solute molecules differs less than 15% from the solvent molecule [50] forming a **crystalline substitutional solid solution** (**Figure 6**), or simply occupy the interstitial spaces between the matrix molecules in the crystal lattice and forming a **interstitial solid solution**. In an interstitial solid solution, the molecules of solute should have a diameter no greater than 0.59 of the matrix molecule [51] and a volume of less than 20% of the matrix.

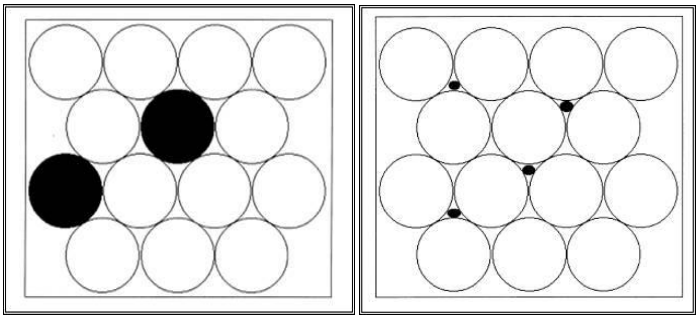

Figure 6. Substitutional solid solution (left) interstitial solid solution (right)

In **amorphous solid solutions**, the solute molecules are molecularly solubilized in an amorphous matrix (**Figure 7**). Chiou and Riegelman [52] were the first to report an amorphous solid solution by using griesofulvin in citric acid, achieving an enhanced dissolution of the drug. Many other carriers have been used like urea, sugars (e.g. sucrose, dextrose, galactose) and more recently organic polymers such as polyvinylpyrrolidone (PVP), polyethylene glycol (PEG) and several cellulose derivates. These polymeric carriers are particularly interesting since they are likely to form amorphous solid solutions due to their inherent amorphous nature. The drug molecules

incorporated within these polymeric matrixes could additionally act as a plasticizer, reducing the glass transition temperature of the polymeric matrix.

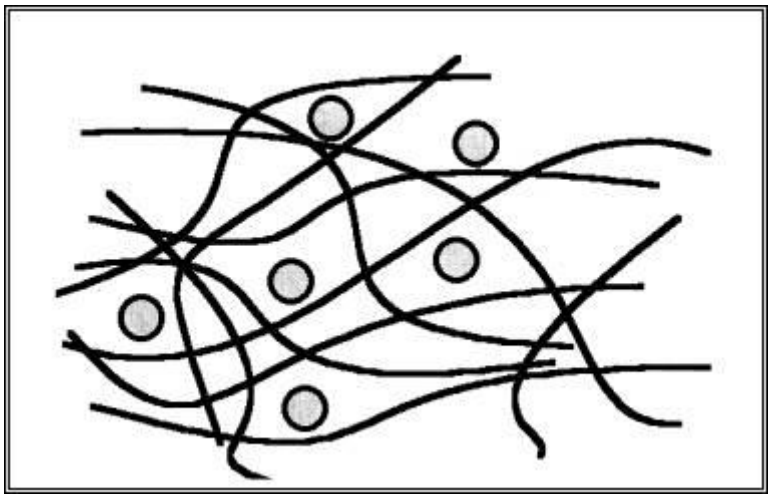

Figure 7. Amorphous solid solutions

1.4.3. Glass solutions and suspensions

A glass solution is a homogeneous glassy system where the solute is dissolved in a glassy matrix that acts as solvent. Such glassy or vitreous state is usually obtained by an abrupt cooling of a melt. This system is characterized by its transparency and brittleness below its glass transition temperature. Upon heating it progressively softens without a sharp melting point [51].

1.4.4. Methods for preparation of solid dispersions

The **fusion method**, sometimes referred as the **melt method** (when the starting material is crystalline), was first proposed by Sekiguchi and Obi [53]. This process involves the preparation of a physical mixture of a drug a water-soluble carrier subjected to direct heat until a molten phase is obtained. The molten mixture is then rapidly solidified. The final mass is pulverized and sieved. Although frequently applied, the fusion method has some limitations: incompatibility between components results in a inhomogeneous solid dispersion [54], under slow cooling conditions, crystalline drug precipitates [55, 56], degradation of drug and or matrix during the heating to temperatures necessary to fuse matrix and drug and it can only be applied when matrix and drug are compatible and mixable at the melting temperature.

Hot melt extrusion is essentially similar to the fusion method with the exception that intense mixing of the components is induced by an extrusion process. Compared with the

traditional fusion method, this technique allows obtaining a similar stability and dissolution, but offers the potential to shape the heated drug-matrix system into a desired form. Furthermore, it allows continuous production, making it suitable for large scale production. Den et al. [57] have investigated the solubility enhancement of fenofibrate by producing polymeric matrix pellets of low molecular weight (LMW) hydroxypropylcellulose polymers (Klucel EF, Klucel ELF and Klucel ELXF by Ashland Specialty Ingredients, USA) by hot melt extrusion (HME). Drug dissolution from the HME pellets was much faster rate than the pure drug.

The **solvent method** involves a first step where matrix material and drug are solubilized in a common solvent. In a second step, the solvent is removed, forming a solid dispersion. The main advantage of this method is that thermal decomposition of the matrix material and/or drug can be prevented. Nevertheless some disadvantages are inherent to this method as super-saturation of the drug in the matrix material can only be reached in systems with high viscosity [58]. Furthermore it might be difficult to find a common solvent for both matrix material and drug, as well as the elevated cost of preparation due to the use of organic solvent and posterior removal.

Supercritical fluid techniques are mostly applied using carbon dioxide (CO_2). The supercritical (Sc) CO_2 is used either as a solvent for drug and matrix or as an non-solvent [59, 60]. When used as a solvent, both drug and matrix are solubilized in $ScCO_2$ and the resulting solution is sprayed through a nozzle into an expansion chamber where upon adiabatic expansion the $ScCO_2$ evaporates and particles are instantly formed. This technique is known as Rapid Expansion of Supercritical Solution (RESS), and considered a "solvent free" process since the CO_2 is environmentally friendly.

Other supercritical techniques are precipitation methods that take advantage of $ScCO_2$ as an non-solvent. In a first stage the drug and matrix material are solubilized in an organic solvent, followed by precipitation induced by $ScCO_2$. Some of those techniques are: Gas-Anti-Solvent (GAS) or Precipitation from Gas Saturated Solutions (PGSS), where a solution of matrix material and drug is brought in contact with $ScCO_2$, and then after expansion and decrease of solvent strength, precipitation of particles occurs. In Precipitation with Compressed Anti-Solvent (PCA), a solution of drug and matrix is sprayed into a vessel containing a supercritical non-solvent, which rapidly penetrates into the droplets inducing particle precipitation [61].

In a somehow different approach but still considered a supercritical technique, Super Critical Fluid Impregnation is based on the solubilization of only the drug in the Sc fluid and

exposed to a solid matrix material that swells and absorbs the supercritical solution. By inducing a change in pressure and time of exposure, the diffusion process of the Sc fluid can be controlled [62]. The use of $ScCO_2$ is advantageous as it is much easier to remove it from the polymeric materials in the end of the process, even the reminiscent amount will pose no danger to the patient.

1.5. Complexation

Complexation can be described as association between two or more molecules, forming a non-covalently bound entity with a well-defined stoichiometry. This association relies on relatively weak forces such as Van der Waals forces, hydrogen bonding and hydrophobic interactions. Several types of complexes exist depending on the complexing agent that is used. Some compounds are known to form stacking complexes (e.g. nicotinamid, salicylic acid, caffeine), due to large planar non-polar regions in the molecule. Stacked complexes can be homogeneous (association) or mixed (complexation). This causes some molecules to minimize the contact with water by aggregation of their hydrocarbon moieties.

The exclusion of water from contact with non-polar molecules or molecular regions, can be obtained through inclusion into a host molecule. Complexation by inclusion takes place when non-polar molecules or molecular group (guest) are inserted in the cavity of another molecule (host). The cavity of the host should be sufficiently large to accommodate the guest and small enough to exclude water of contacting the guest. In pharmaceutical formulations, the most used host molecules are cyclodextrins. These compounds are obtained by degradation of starch by cyclodextrin-glycosyltransferase (CGT). Cyclodextrins are cyclic oligomers, crystalline and water-soluble. There are three major groups of these cyclic oligosaccharides, α-, β- ,γ-cyclodextrins composed of six, seven and eight D-(+)-glucopyranose units. These compounds have a toroidal structure with primary and secondary hydroxyl groups oriented outwards, creating a hydrophilic exterior and hydrophobic internal cavity. Molecules or functional groups less hydrophilic can be included into the hydrophobic cyclodextrine cavity in the presence of water. The cavity size and possible chemical modification determines the affinity of cyclodextrins to various molecules. The solubility of a compound can be enhanced by inclusion within the cycodextrin cavity [63]. As an example it was shown that cyclodextrin complexation increases the solubility of paclitaxel with a 950 fold [64] and similar were obtained for rofecoxib, celecoxib, clofibrate, melarsopol and cyclosporine A [65-69].

1.6. Solubilization by surfactants

Surfactants are characterized by a tendency to absorb at surfaces and interfaces lowering the free energy of the phase boundary. The interface defines a boundary between to immiscible phases. When such boundary is covered with a surfactant compound, the energy necessary to expand the interface (surface tension) is greatly reduced. Surfactant molecules have distinct polar (or hydrophilic) and non-polar (or hydrophobic) regions, and are commonly designated as amphiphilic (**Figure 8**). Most surfactants are composed of a hydrocarbon chain (tale) connected to a polar group (head). The polar group can be anionic, cationic, zwitterionic or non-ionic [70]. **Table 5** gives an overview of the different types of surfactants that are used for pharmaceutical applications. Besides the ability to decrease surface tension, surfactant molecules are also know by the capability to create association structures above their critical micelle concentration (**Figure 9**). These two characteristics are of great importance in enhancing the solubility of poorly water-soluble compounds. This occurs either by covering the interface of the poorly water-soluble drug particle will decrease the surface tension, allowing better wettability or by forming micelles that integrate the drug within their hydrophobic core.

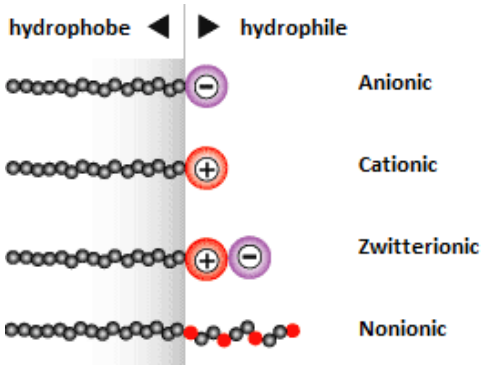

Figure 8. The primary classification of surfactants is made on the basis of the charge of the polar head group.

Park et al. have extensively documented the effect of different types of surfactants (cetyltrimethylammonium bromide (CTAB) as cationic surfactant, sodium lauryl sulfate (SLS) as anionic surfactant, and polysorbate 80 as a non-ionic surfactant) on the dissolution behavior of poorly water-soluble acid drugs, including mefenamic acid, nimesulide and ibuprofen. The dissolution of these acidic drugs was substantially enhanced in aqueous media containing CTAB. For acidic drugs, the ability of aqueous media containing a cationic surfactant to enhance dissolution of acidic drugs appeared greater than that of media containing other surfactant types [71].

Table 5 - Surfactants classification versus structure

Surfactant type	Hydrophilic group	Hydrophobic group	Comments
Anionic	Negatively charged group **e.g.** carboxyl, sulfonate, phosphate	Long, straight alkyl groups Branched-chain alkyl groups Unsaturated alkenyl chains Alkylbenzenes Alkylnaphatalenes Fluoroalkyl groups Polydimethylsiloxanes Polyoxypropylene glycol derivatives Others (e.g. biosurfactants, derivates of natural and synthetic polymers)	Generally non compatible with cationic surfactants, sensitive to water hardness.
Cationic	Positively charged group **e.g.** quaternary ammonium halides		Generally non compatible with anionic surfactants. They adsorb strongly to surfaces and show high aquatic toxicity
Zwitterionic	Contains both positive and negative charge **e.g.** betaines, sulfobetaines		Compatible with all other types of surfactants, not sensitive to hard water. Generally stable in acids and bases. Highly tolerated by the skin
Nonionic	No charge, highly polar group **e.g.** polyoxyetheylene, polyhydroxyl		Compatible with all other types of surfactants, not sensitive to hard water. Contrary to ionic surfactants, their physicochemical properties are not affected by electrolytes.

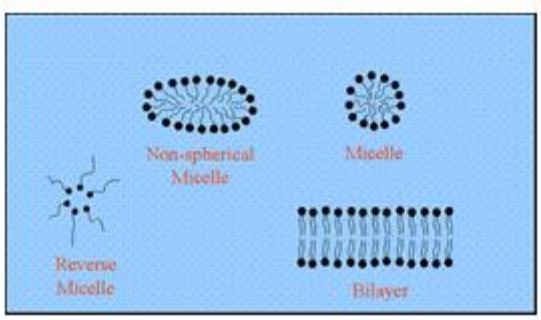

Figure 9. Different types of surfactant associations

1.7. Use of co-solvents

A common approach to solubilize lipophilic drugs is the use of co-solvents [72]. A co-solvent must have solvent capability towards a solute and be mixable with the solvent in which the solute is insoluble. [73]. The co-solvent decreases the surface tension between the aqueous solution and the hydrophobic solute [74]. Most co-solvents have hydrogen bond donors and/or receptors as well as a hydrocarbon chain. Through a mutual bonding to water and to lipophilic substances, a co-solvent is able to prevent precipitation of the insoluble compounds in water. In pharmaceutical formulation, currently the most used organic compounds with co-solvent activity are polyethylene glycol 400 (PEG 400), ethanol, propylene glycol and glycerine. An overview of solubilizing excipients in commercial oral and injectable formulations is given in **Table 6**.

Badu et al. investigated the effect co-solvency with biocompatible solvents such as ethanol, propylene glycol, glycerin, and PEG 400, on the solubility enhancement of Cox-II inhibitors (meloxicam and rofecoxib). They found that less polar solvents were more potent enhancers of drug dissolution, with the solvent blend water-PEG 400 yielding the best results [75].

Table 6 – Solubilizing excipients in commercial oral and injectable formulations

Water Soluble	Water Insoluble
Dimethylacetamide (DMA)	Oleic acid
Ethanol	Soy fatty acids
Glycerin	d-α-tocopherol (Vit. E)
N-methyl-2-pyrrolidone (NMP)	Corn oil mono-di-triglyecerides
PEG 300	Medium chain triglycerides
PEG 400	Long-chain triglycerides
Poloxamer 407	Castor oil
Propylene Glycol	Corn oil
Hydrogenated Soy	Peanut oil
	Caprylic/capric triglycerides

1.8. Microemulsions and Self-emulsifying drug delivery systems (SEDDS)

A microemulsion is a thermodynamically stable isotropical four-component system composed of an external phase, internal phase, surfactant and co-surfactant. The addition of the surfactant, soluble in the internal phase, results in the spontaneous formation of a microemulsion (internal dispersed phase is <0.1μm in droplet diameter) without the input of external energy as is the case in common emulsification processes. The formation of a mixed film composed of surfactant and co-surfactant at the interface between internal and external phase, contributes to the stability of the microemulsion [76]. Non-ionic surfactants (e.g. polysorbates and

polyoxyethylated oleic acids) with a high hydrophilic-lipophilic balance (HLB) are frequently used to ensure an immediate formation of the microemulsion mixing of both phases [77].

Microemulsions have potential as drug delivery systems for poorly soluble drugs due to their capability to solubilize the lipophilic molecules in the internal phase. These formulations present several advantages over conventional emulsification, including thermodynamic stability, increased drug loading, decreased inter- and intra variability in drug pharmacokinetics and enhanced bioavailability [78, 79].

In the absence of an external phase, the mixture of the remaining components can form a so-called self-emulsifying drug delivery system (SEDDS). These systems are able, upon dilution in an aqueous phase, to spontaneously form fine O/W emulsion or microemulsions, improving water solubility of lipophilic drug. However, potential drawbacks of these systems include possible drug instabilities and high concentration of surfactants, leading to irritation in the gastro intestinal tract. Kadu et al. have studied the impact of self-emulsifying drug delivery systems in the oral bioavailability of atorvastatin calcium. The solubility of atorvastatin calcium was determined using various self-emulsifying components, including, Glyceryl Tricaprylate/Tricaprate (Captex®355 EP/NF, Abitec, USA), Ethyl oleate, Medium Chain Mono- and Diglycerides (Capmul®MCM, Abitec, USA), Propylene Glycol Monocaprylate (Capmul®PG-8, Abitec, USA), Lauroyl macrogol-32 glycerides EP (Gelucire®44/14, Gattefosse, France), Polyoxyethylene (20) sorbitan monooleate (Tween 80, Tween 20 by ICI Americas Inc, USA) and Polyetheylene glycol 400. *In vivo* these formulations significantly reduced serum lipid levels compared to atorvastatin calcium [80].

1.9. Research work considerations

This overview of both the solubility phenomena and the different formulation approaches used by formulation scientists towards the enhancement of drug solubility, allowed to define this research work. Two distinct approaches were used: particle size reduction and the use of surface active substances, in order to enhance the solubility of three poorly soluble model drugs (carbamazepine, itraconazole and febantel) non using organic solvents during the process.

These compounds qualify as BCS Class II drugs, demonstrating low aqueous solubility and variable bioavailability.

In this work carbamazepine solubility is enhanced with the use of PEG-32 glyceryl laureate (Gelucire®44/14, Gattefossé, France). Perissuti et al, have described similar results for both Gelucire®44/14 and Gelucire®50/13 when used in the preparation of solid dispersion of carbamazepine with enhanced dissolution rate [81]. Furthermore, the use of Gelucire®44/14 and its capability to enhance carbamazepine dissolution rate has been further explored by Sethia et al, using solid dispersions of carbamazepine formulated by supercritical fluid processing (SCP) and conventional solvent evaporation in polyethylene glycol (PEG) 8000 with either Gelucire 44/14 or vitamin E TPGS NF (d-alpha-tocopheryl PEG 1000 succinate), which contributed to an enhanced drug solubility [82].

Gelucire®44/14 was also used for the production of nano- and micro suspensions of itraconazole and febantel. Sun et al investigated the effect of particle size on the in vitro dissolution and oral absorption of itraconazol. The nanosuspensions were prepared by high pressure homogenization and stabilized by Lutrol F127 and sodium lauryl sulfate. These studies revealed faster drug dissolution rate with significant increase in both maximal plasma concentration of drug and area under the drug concentration-time curve [83]. The presence of Gelucire®44/14 and its effect on itraconazole solubility behavior has been described by Robini and collaborators, they enhanced dissolution of itraconazole by developing solid dispersions using water-soluble carriers polyglycolized fatty acid esters (Gelucire®44/14 and Gelucire®50/13), Vitamin E TPGS (d-alpha tocopheryl polyethylene glycol-1000 succinate), polyethylene glycol 6000 (PEG 6000) and urea. These solid dispersions of itraconazole and water-soluble carriers exhibited the highest dissolution rate for the observed period of 2 hours, which was about two-fold as compared to that of control itraconazole formulation [84].

In parallel, similar tests were conducted with febantel as model drug. Up until today no data on solubility enhancement of this probenzimidazole are available. This drug is widely use in animal

large scale medication and its primary way of administration is through drinking water. Its low solubility together with everyday application in veterinary practice made it the ideal candidate for the development of a new solubilization technique based on the preparation of a highly loaded drug coacervate phase by using polyethoxylated castor oil (Cremophor®EL, BASF).

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CHAPTER 2
GELUCIRE®44/14 -BASED IMMEDIATE RELEASE FORMULATIONS FOR POORLY WATER- SOLUBLE DRUGS

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2.1. INTRODUCTION

Rapid and complete drug absorption is one of the key objectives in designing conventional oral solid dosage forms. Most orally administered drugs are absorbed via passive diffusion through the small intestine, where the extent of absorption is largely influenced by drug solubility in the gastrointestinal fluids and drug permeability through the gastrointestinal membrane [1]. Many of the currently developed new chemical entities are poorly water-soluble. To overcome this challenge, polymeric non-ionic surfactants are attractive excipients to enhance drug solubility. Amongst these, Gelucires® are polyethylene glycol (PEG) glycerides composed of mono-, di- and triglycerides and mono- and diesters of PEG [2]. They are inert semi-solid waxy amphiphilic excipients with surface-active properties that spontaneously form a fine dispersion or emulsion upon contact with water [3]. The different varieties of Gelucires® are characterized by a range of melting points and hydrophilic-lipophilic balance (HLB) values, with high HLB Gelucires® allowing faster drug release due to their increased hydrophilicity [4, 5]. Mehuys and co-workers demonstrated that hot-melt extruded core-shell rods comprising a core of Gelucire® 44/14 could release the poorly water-soluble drug hydrochlorothiazide in a controlled fashion [6]. Capsules filled with a Gelucire®44/14 matrix were reported by Massik to enhance the dissolution rate of phenytoin [7]. Spray-drying from organic medium was demonstrated by Chauhan to yield solid dispersions of etoricoxib with enhanced dissolution kinetics [8].

However, the use of Gelucire®44/14 in the above mentioned examples has several limitations regarding the development of immediate release formulations of poorly water-soluble drugs. The long erosion time of monolithic Gelucire®44/14 structures is often the rate-limiting step in these formulations while the need for organic solvents involves the burden of toxic residual solvents.

In this paper we aim to develop a formulation strategy based on Gelucire® for immediate release of poorly water-soluble drugs without using organic solvents. As a model drug carbamazepine is used while melt granulation and spray-drying from aqueous medium are used to produce granules and respectively powder that is then further compacted into tablets.

2.2. MATERIALS AND METHODS

2.1.1. Carbamazepine

Carbamazepine (**Figure 1** shows scanning electron microscopy images of the crude drug and **Figure 2** shows its molecular structure) is a poorly water-soluble (water solubility of 17 mg/L) Biopharmaceutical Classification System (BCS) Class II drug. It is a weak base with a pKa of 13.9. Carbamazepine is used as an anticonvulsant and mood-stabilizing drug for treatment of epilepsy and bipolar disorder. It is also used off-label for a variety of indications including attention-deficit hyperactivity disorder (ADHD), schizophrenia, phantom limb syndrome, paroxysmal extreme pain disorder and post-traumatic stress disorder. The mechanism of action of carbamazepine occurs through interaction with the neuronal sodium channels as well as the gamma-aminobutyric acid (GABA) receptors. The carbamazepine used in the present work was purchased from Fagron.

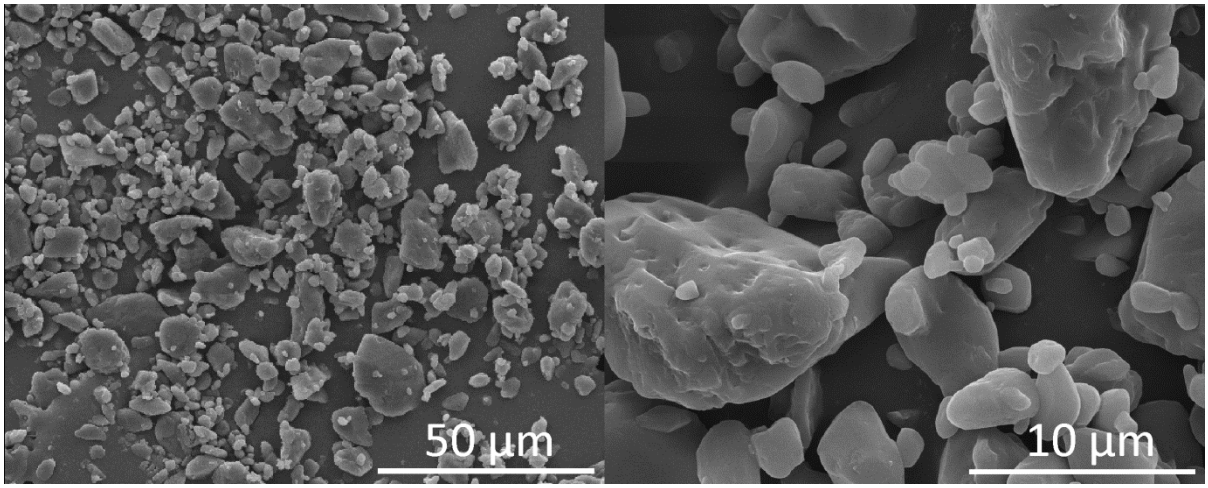

Figure 1. Scanning electron microscopy (SEM) images at different magnification of crude carbamazepine.

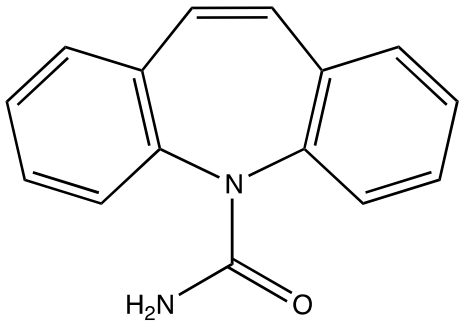

Figure 2. Molecular structure of carbamazepine

2.2.2. Gelucire ®44/14

Gelucire®44/14 is the trade name under which PEG-32 glyceryl laureate is sold by Gattefossé. It is an inert semi-solid waxy amphiphilic material with a melting point of 44°C and an HLB of 14. Gelucire®44/14 is GRAS (generally regarded as safe) and obtained by polyglycolysis of hydrogenated palm kernel oil with poly(ethylene glycol) 1500. Due to its composition of hydrophilic and hydrophobic parts, it has self-emulsifying properties, spontaneously forming a fine emulsion upon contact with water.

2.2.3. Other excipients

Citric acid and sodium bicarbonate were purchased from Fagron. Hard gelatin capsules size 000 were purchased from ACA Pharma. Microcrystalline cellulose (Avicel® PH 102) and cross-linked sodium carboxymethyl cellulose (Ac-di-sol®) were purchased from FMC Biopolymer. Pre-gelatinzied starch (C*Gel-instant 12410) was obtained from Cerestar. Polyplasdone® XL was purchased from ISP, Explotab® from Penwest.

2.2.4. Formulation of carbamazepine in gelatin capsules

Carbamazepine was dispersed in molten Gelucire®44/14 at 60°C under stirring during 10 min. **Table 1** list the different ratios used for this formulation. The molten mass was immediately injected into hard gelatin capsules and allowed to cool to room temperature for 24h.

Table 1: Carbamazepine/Gelucire®44/14 ratios used to formulate hard gelatin capsules

Drug/excipient ratio	Carbamazepine (mg)	Gelucire®44/14 (mg)	Total (mg)
1 to 1	200	200	400
1 to 2	200	400	600
1 to 3	200	600	800
1 to 4	200	800	1000
1 to 5	200	1000	1200

2.2.5. Formulation of carbamazepine via melt granulation

A mortar was heated in a water bath set at 60 °C. Carbamazepine was first mixed with microcrystalline cellulose or pre-gelatinized starch and then granulated in the heated mortar with molten Gelucire®44/14. Finally the granules were passed through a 1.25 mm sieve. To enhance the dissolution rate of the carbamazepine a series of granules were fabricated with the addition of excipients that should enhance the disintegration of the granules. **Table 2** gives an overview of the respective amounts of drug and excipient to produce the formulations.

Table 2: Carbamazepine/excipient ratios used for melt granulation

Granules	Carbamazepine (g)	Excipients	Amount (g)	Total (g)
Formulation 1 (G1)	20	Gelucire®44/14	20	60
		pre-gelatinized Starch	20	
Formulation 2 (G2)	20	Gelucire® 44/14	20	60
		microcrystalline cellulose	20	
G2+ Ep*	20	Explotab®	3	63
G2+ Ac*	20	Ac-di-sol®	3	63
G2+ Py*	20	Polyplasdone® XL	3	63
G2+ EF mix	20	mixture citric acid with sodium bicarbonate	3	63

*Excipients added to the dry powder mixture before granulation

2.2.6. Formulation of carbamazepine via spray-drying

An aqueous suspension was prepared by first dissolving 100 g of Gelucire®44/14 in 1 L demineralized water at 50 °C. Subsequently, carbamazepine and additional excipients were added and the suspension was homogenized using a Silverson L4R high shear mixer at 6000 rpm. The

ratios of the different components are listed in **Table 3.** The mixture was spray-dried in a Mobile Minor (GEA Niro) spray-dryer equipped with a two-fluid nozzle (1 mm diameter) and a Watson Marlow 520U peristaltic pump set at 20 mL/min. The spray-dryer was operated in a co-current flow with a constant inlet temperature of 140 °C and an outlet temperature of 55 °C. After spray-drying a free-flowing powder was collected.

Table 3: Carbamazepine/excipient ratios used for spray drying

Spray dried powder	Carbamazepine (g)	Excipients	Amount (g)	Total (g)
Formulation 1 (SD1)	100	Gelucire®44/14	100	600
		Pre-gelatinized Starch	45	
		Mannitol	45	
		Silicon dioxide	20	
		Demineralized water	1000	
Formulation 2 (SD2)	100	Gelucire®44/14	100	600
		Microcrystalline cellulose	45	
		Mannitol	45	
		Silicon dioxide	20	
		Demineralized water	1000	
SD2+ Ep*	100	Explotab®	30	630
SD2+ Ac*	100	Ac-di-sol®	30	630
SD2+ Py*	100	Polyplasdone®XL	30	630

*Excipients that were added to the spray dried powder

2.2.7. Production of carbamazepine tablets

The spray-dried powder and the granulates were compacted (10 kN) using an eccentric Korsch EKO tablet press equipped with a 13 mm circular flat edge punch. The tablets were tested for their disintegration time on a Pharma Test PTZ disintegrator in 900 mL of a 0.1 M HCl solution at 37 °C.

2.2.8. X-ray diffracion

X-ray diffractograms of the spray-dried powder and granules were recorded on a D5000 Cu Kα diffractor (λ=0.154 nm) (Siemens, Karlsruhe, Germany) with a current of 40 mA in the angular range of $10° < 2\theta < 60°$ using a step scan mode (step width 0.02°, counting time 1 s/step).

2.2.9. Optical microscopy

Optical microscopy images were recorded on Leica DM2500P microscope equipped with a 40x objective and a DFC360FX CCD camera. Hot stage microscopy was performed on the same microscope, but then equipped with a THMS600 heating stage.

2.2.10. Scanning electron microscopy (SEM)

SEM images were recorded on gold-sputtered samples using a Quanta 200 FEG FEI scanning electron microscope, operating at an acceleration voltage of 5 kV.

2.2.11. In vitro drug release

Carbamazepine release from the respective formulations was evaluated (N=6) under sink conditions in a VanKel VK7010 dissolution tester combined with a VK8000 automatic sampling station. Per dissolution vessel, formulations containing 200 mg of carbamazepine were used. The dissolution medium was 900 ml 0.1 M HCl at 37 °C. 5 mL samples were withdrawn from the dissolutions vessels at 5, 10, 15, 30, 30, 45 and 60 min (for the granules, spray-dried powder and tablets) and at 5, 10, 15, 20, 30, 45, 60, 75, 120 and 180 (for the capsules).

2.2.12. Quantification of carbamazepine release via HPLC

Carbamazepine concentrations in filtered (to remove non-dissolved particulates) dissolution samples were determined via a high performance liquid chromatography (HPLC) method according to the European Pharmacopoeia. Prior to analysis, samples were diluted 1:4 with methanol. The HPLC set-up consisted of a solvent pump (L-7100 pump, Merck Hitachi) set at a constant flow rate of 1.5 mL per minute, an automatic injection system (L-7200, Hitachi,), a variable wavelength UV-detector (L-7400, Merck Hitachi,) set at 230 nm, a reverse phase column (LiChrospher®100 CN -10 µm, Merck) and an automatic integration system (D-7000, Hitachi). The mobile phase consisted of 3% tetrahydrofuran, 12 % methanol, 85 % water, 0,02 % anhydrous formic acid and 0.05 % triethylamine.

2.3. RESULTS AND DISCUSSION

In this work several formulation strategies based on Gelucire®44/14 were evaluated to enhance the dissolution kinetics of the poorly water-soluble drug carbamazepine. To set a reference formulation, we first dispersed carbamazepine in molten Gelucire®44/14, followed by filling this dispersion into hard gelatin capsules. To assess whether melting of Gelucire®44/14 to 60 °C followed by mixing with carbamazepine leads to either a solid dispersion or a solid solution, X-ray diffraction was performed. As shown in the diffractograms depicted in **Figure 3A**, no substantial influence of the heating and mixing process on the crystallinity of the carbamazepine is observed. Furthermore, optical microscopy images recorded on a heating-stage set up (**Figure 3B**) showed that at 60 °C, the carbamazepine is dispersed as solid crystalline material in a molten Gelucire®44/14 phase. This eliminates the possibility that the crystalline peaks observed in the X-ray diffractogram of the heated carbamazepine: Gelucire®44/14 mixture result from carbamazepine that was first dissolved in the molten Gelucire®44/14 phase and then recrystallized upon cooling to room temperature.

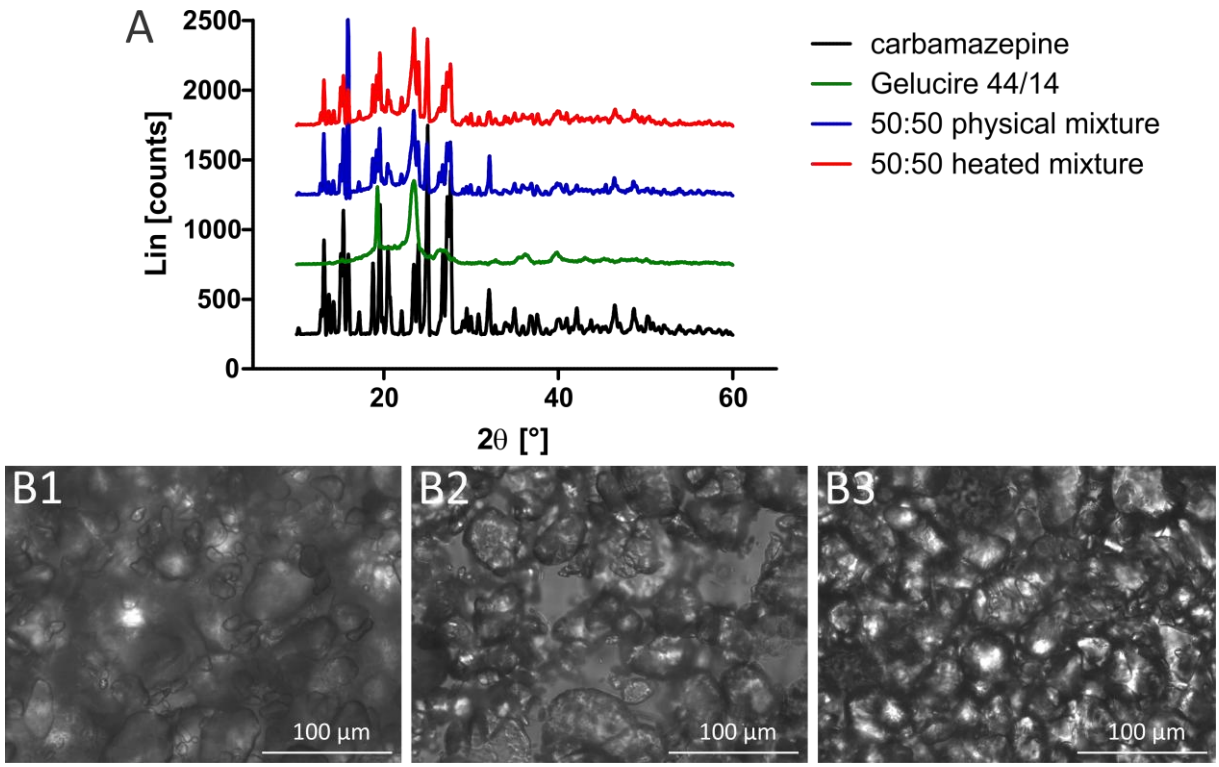

Figure 3. (A) X-ray diffractograms of carbamazepine, Gelucire®44/14, a physical mixture of both components and a mixture that was prepared under heating at 60 °C. (B) Hot stage microscopy images recorded from a 50:50 carbamazepine/Gelucire®44/14 mixture (B1) at room temperature, (B2) at 60 °C and (B3) after cooling back to room temperature.

The influence of different carbamazepine/ Gelucire®44/14 ratios on the drug dissolution kinetics were evaluated *in vitro* and compared to crude carbamazepine. 0.1 M HCl was used as dissolution medium, set at a constant temperature of 37°C. As shown in **Figure 4**, crude carbamazepine resulted in only 10 % dissolution under the given conditions. By contrast, the hard gelatin capsules filled with the solid carbamazepine/Gelucire®44/14 dispersion yield a nearly complete drug release within 2 hours. Furthermore, no influence of the carbamazepine/Gelucire®44/14 ratio was observed, indicating that the rate-determining step in drug release from these formulations was the dissolution of the Gelucire®44/14 itself, which occurs through surface erosion. Since nearly-complete drug release was observed for a 1:1 drug: excipient ratio, this ratio was used for further experiments.

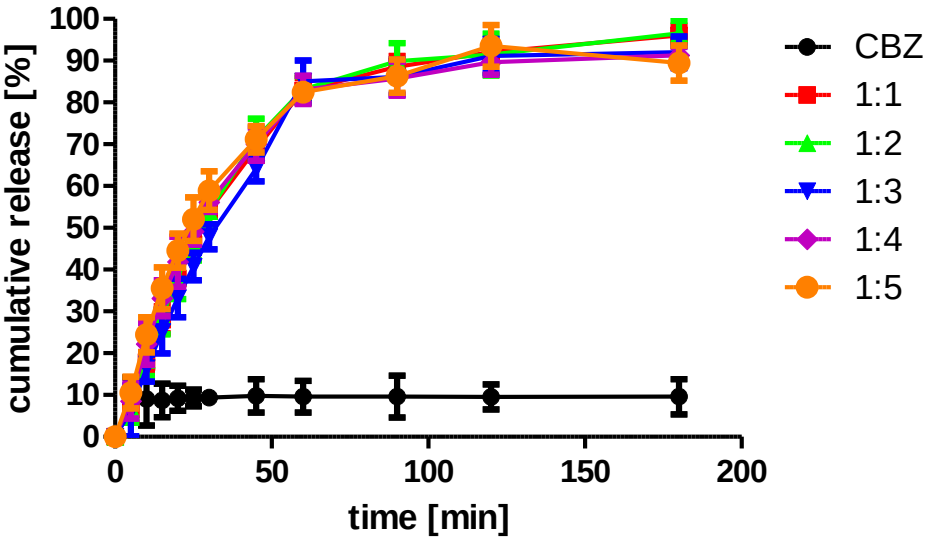

Figure 4. Cumulative *in vitro* release of carbamazepine formulated in hard gelatin capsules filled with a solid carbamazepine/Gelucire®44/14 dispersion in different drug: excipient ratios. Crude carbamazepine (CBZ) was used as reference.

In a next series of experiments we aimed to enhance the drug dissolution rate of carbamazepine, envisioning an immediate release formulation. Therefore, we sought for production methods that enhance the surface to volume ratio of the obtained formulations. This is especially the case for particulate formulations, which are expected to exhibit accelerated drug release relative to a monolithic structure of the same material. First, we attempted to perform melt granulation using Gelucire®44/14 both as liquid and as binder. As carrier excipient for granulation, microcrystalline cellulose (referred to as G1) and pre-gelatinized starch (referred to as G2) were evaluated. This would allow us to investigate whether the addition of a hydrophilic excipient could further enhance the dissolution kinetics of the obtained formulations. In both cases, the total drug load was 30 %. **Figure 5** shows SEM images of the obtained granules. Secondly, we evaluated spray-drying to produce immediate release solid dosage forms. Therefore, Gelucire®44/14 was first dispersed in demineralized water followed by the addition of carbamazepine, and either microcrystalline cellulose (referred to as SD1) or pre-gelatinized starch (referred to as SD2). Furthermore, to increase the yield of the spray-drying process, silicon dioxide as glidant and mannitol as bulking agent were added prior to spray-drying. **Figure 5** shows SEM images of the obtained microparticles.

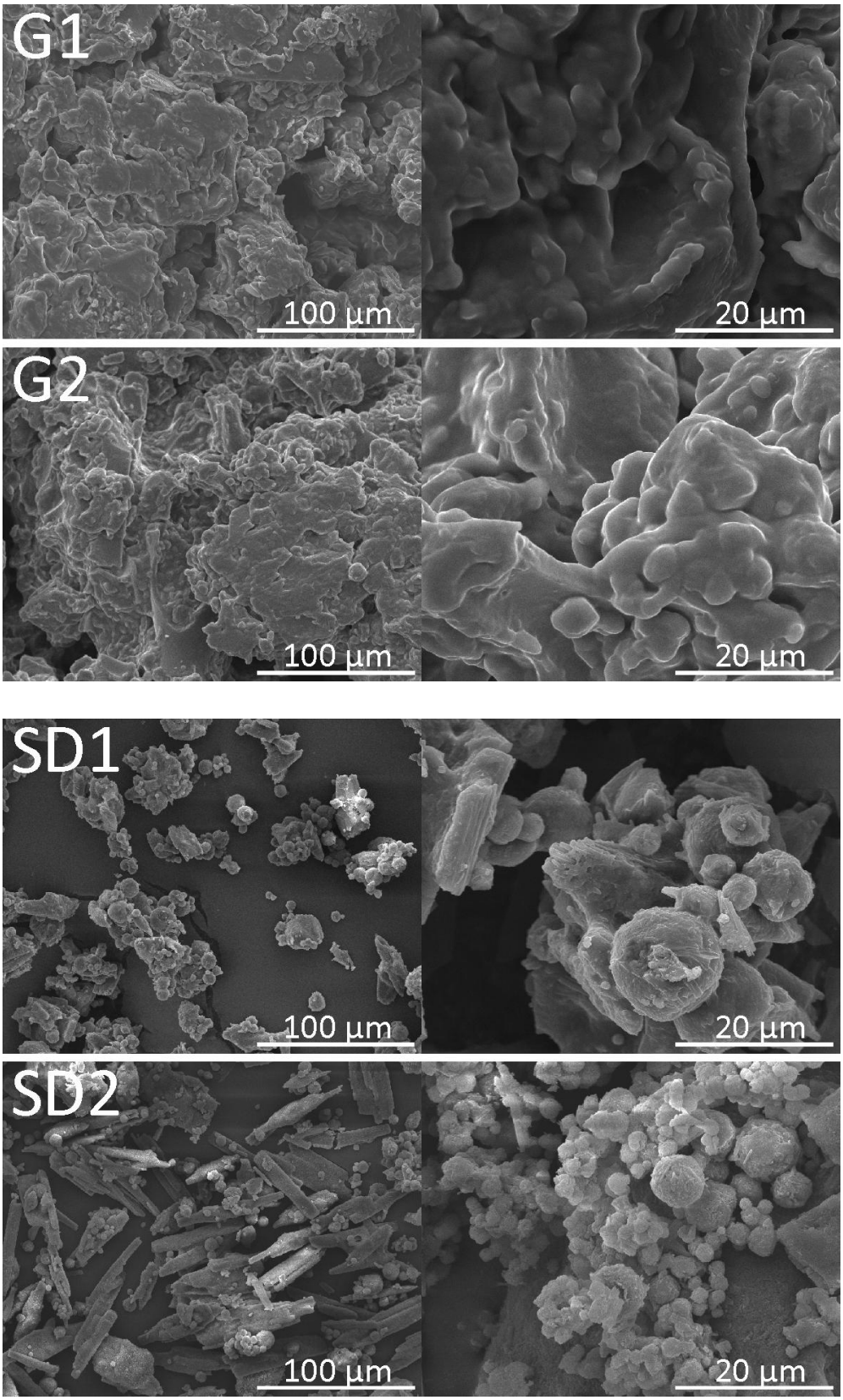

Figure 5. SEM images at different magnifications of the respective granules and spray dried microparticles. G1, G2, SD1 and SD2 are defined in Tables 2 and 3.

Figure 6 shows the effect on drug release of these different formulation strategies, with a nearly complete release being achieved within 30 min. Clearly, both the granulation and the spray-drying process strongly increase the rate of drug dissolution compared to the hard gelatin capsules filled with a solid dispersion of carbamazepine in Gelucire®44/14. This demonstrates that formulating carbamazepine and Gelucire®44/14 in a particulate rather than a monolithic form is beneficial to obtain an immediate release formulation. The use of either microcrystalline cellulose or pre-gelatinized starch was not found to influence drug release profile, neither in the granulated nor in the spray-dried formulation. Between the latter two groups a slightly faster release was observed for the spray-dried formulation. This is likely due to the smaller particle size of the spray-dried powder relative to the granules as evident from the SEM images in **Figure 5**, showing large (> 100 μm) chunky granules and spray dried microparticles which all have a size below 20 μm.

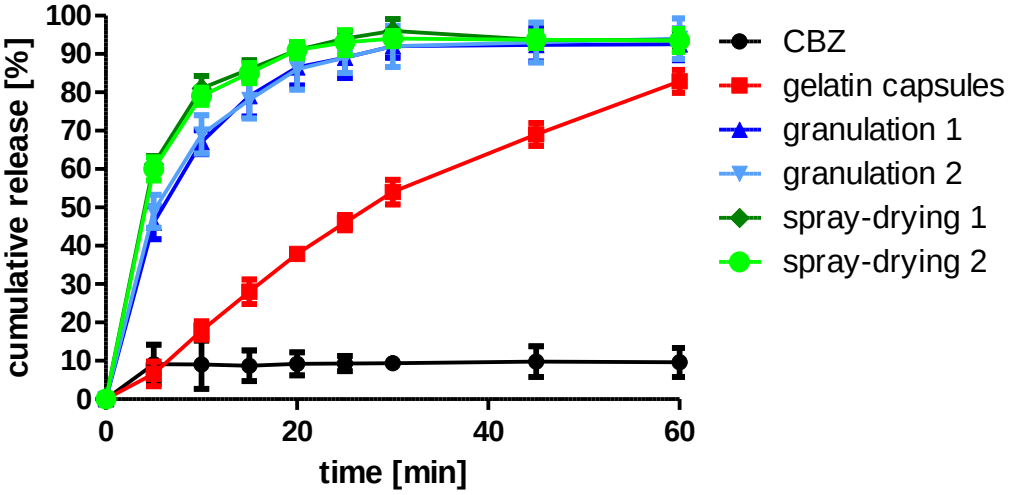

Figure 6. Cumulative *in vitro* release of carbamazepine formulated with Gelucire®44/14 via granulation or spray-drying. '1' refers to formulations containing pre-gelatinized starch as co-excipient, while '2' refers to microcrystalline cellulose as co-excipient. Crude carbamazepine (CBZ) and hard gelatin capsules filled with a solid carbamazepine/Gelucire 44/14 solution in a 1:1 drug: excipient ratios were used as reference.

In a next step, we aimed to transform the granulated and spray-dried formulations into a form that is suitable for oral intake. Therefore, these particulate forms were compacted into tablets under a compaction force of 10 kN using 13 mm flat end punches. **Figure 7** shows SEM images of the tablets and their interior, obtained by splitting the tablets in half. **Figure 8** shows the cumulative drug release profiles of the respective tablet formulations. These profiles indicate a significant decrease of the drug release rate upon compaction into tablets, being quasi equal to

the hard gelatin capsules filed with a solid dispersion of carbamazepine/Gelucire®44/14. The major reason for this is likely the long time it takes for the tablets to disintegrate, while this step is crucial to allow further wetting of the bulk of the tablets prior to drug dissolution. **Table 4** lists the disintegration time of the respective tablet formulations. Gelucire®44/14 has a melting point of 44°C and behaves at room temperature as a waxy plastic solid. Therefore, under compression, Gelucire will redistribute and bridge multiple granules or spray-dried particles, forming a monolithic structure as shown by SEM imaging of the tablet surface and tablet interior (**Figure 7**). Apparently, the presence of the water-soluble pre-gelatinized starch in both the tablets produced from granules and spray-dried powder, does not play a role in promoting tablet disintegration.

Table 4: average disintegration time of CBZ tablets

Tablet	**Average(±SD) disintegration time (min) in 0.1N HCl at 37°C (±0.5)**
TG1	27±0.69
TG2	25±0.82
TSD1	22±0.69
TSD2	21±0.9

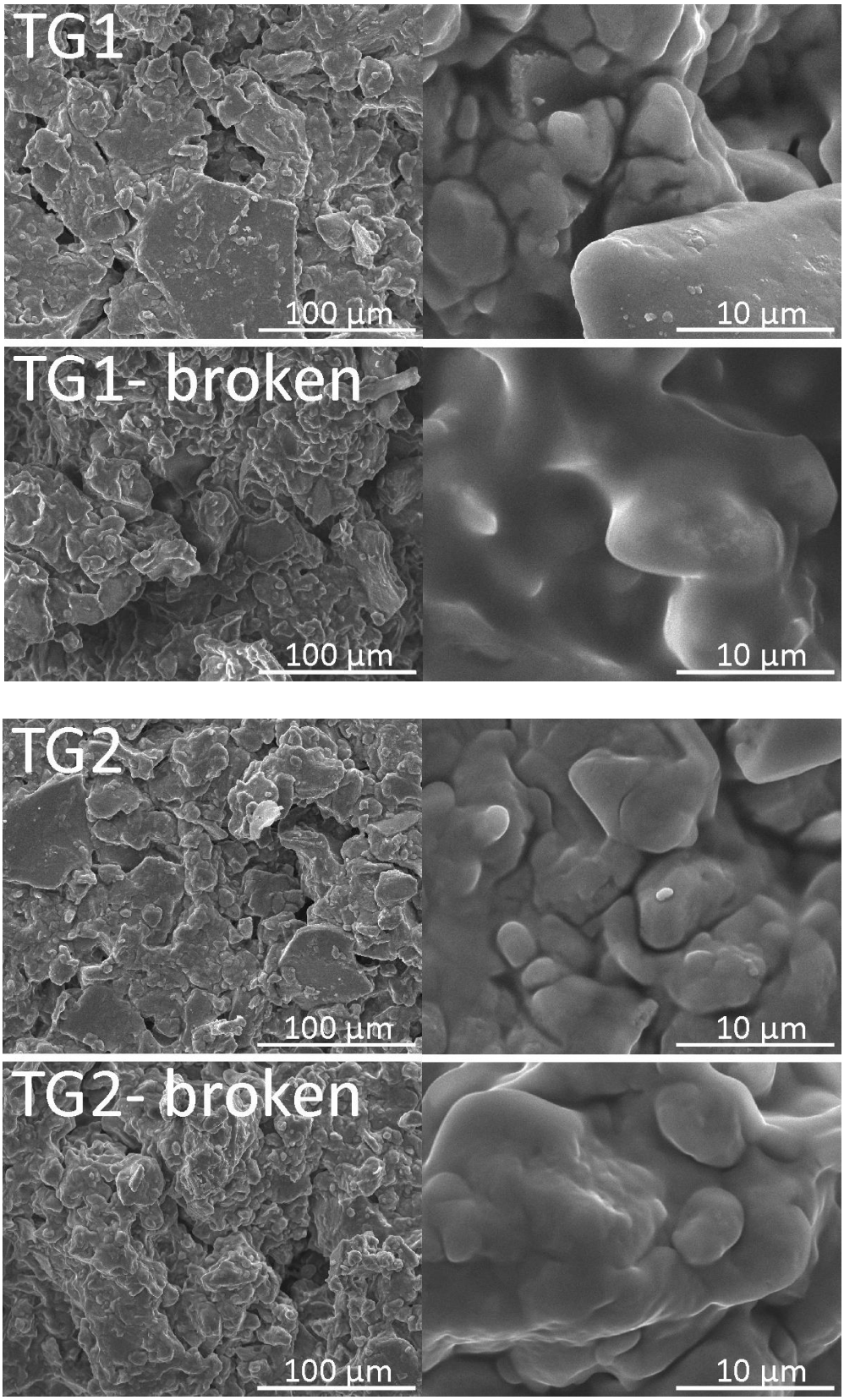

Figure 7. SEM images at different magnification of the respective tablet formulations. To image the interior of the tablets, the tablets were manually split in half and imaged perpendicular on the fraction plane. TG1, TG2, TSD1 and TSD2 are defined in Tables 4.

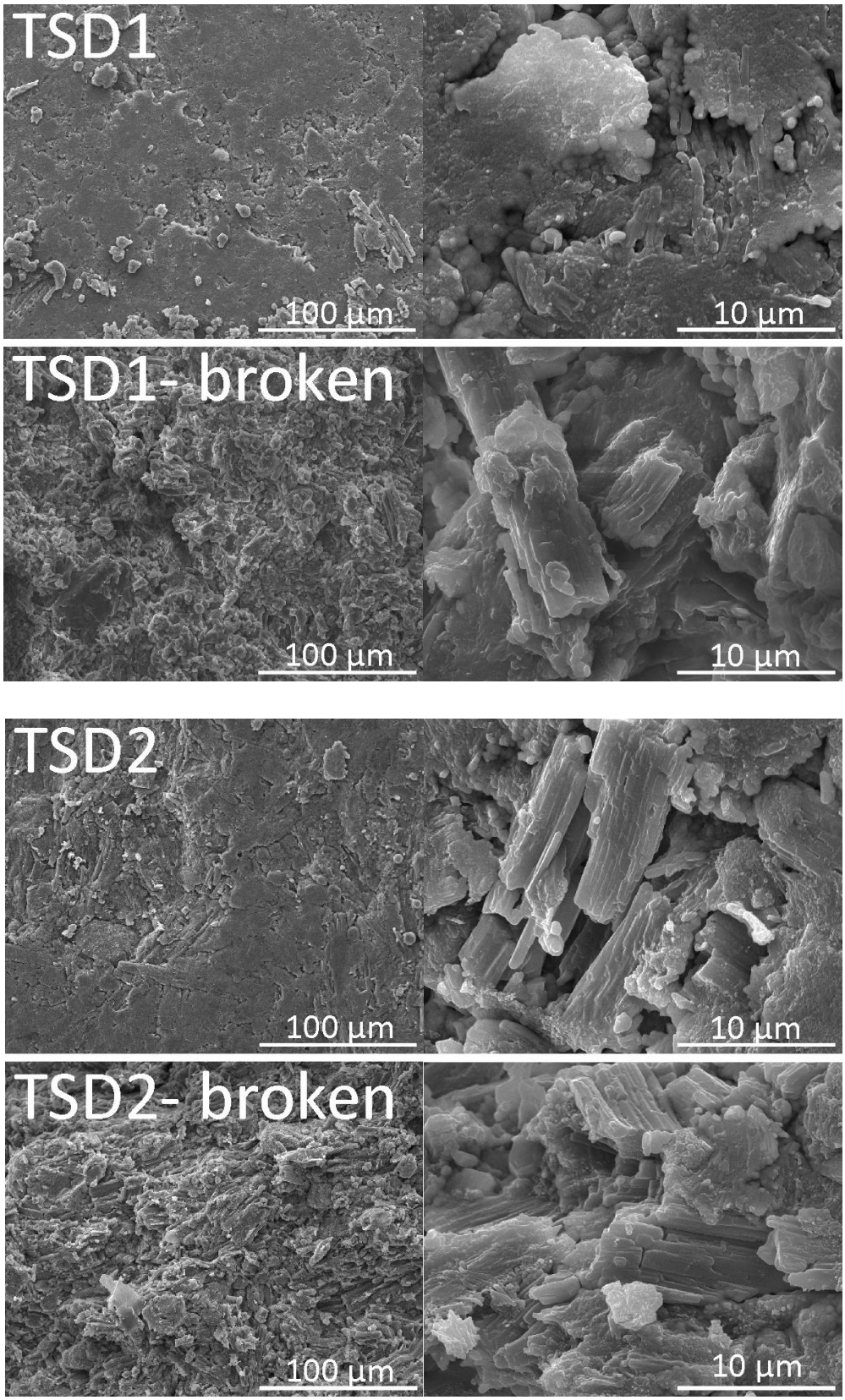

Figure 7. SEM images at different magnification of the respective tablet formulations. To image the interior of the tablets, the tablets were manually split in half and imaged perpendicular on the fraction plane. TG1, TG2, TSD1 and TSD2 are defined in Tables 4. (Cont.)

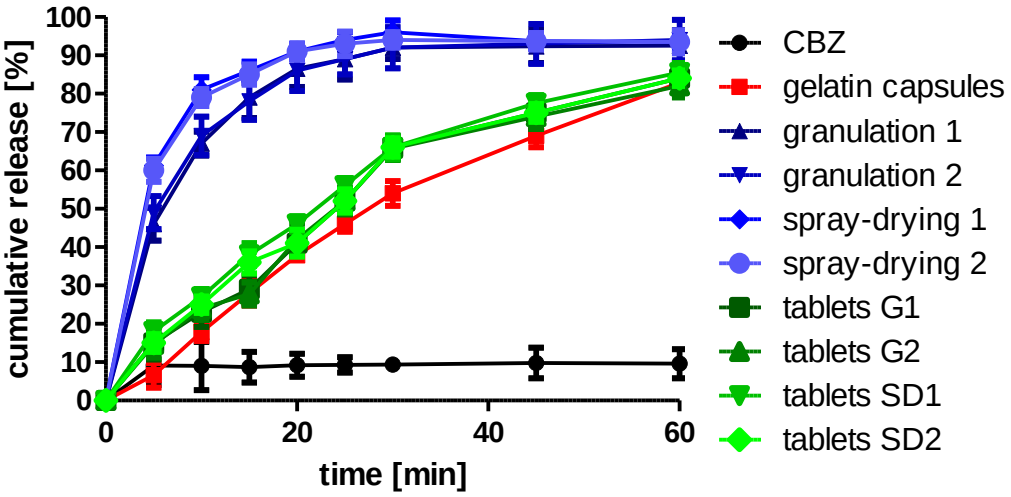

Figure 8. Cumulative *in vitro* release of carbamazepine formulated with Gelucire®44/14 via either granulation or spray-drying, followed by compaction into tablets. 'G' refers to tablets produced from granules while 'SD' refers to tablets produced from spray-dried powder. Crude carbamazepine (CBZ) and hard gelatin capsules filled with a solid carbamazepine/Gelucire® 44/14 solution in different drug: excipient ratios were used as reference.

To cope with these issues and thus to obtain an immediate release tablet formulation, we evaluated several types of super-disintegrants [9 -11] to be added during the formulation steps. For this purpose, 10 % of either Explotab®, Ac-di-sol® or Polyplasdone®XL were added to the spray-dried powder (after spray-drying, but prior to compaction) (**Table 3**) or to the powder mixture prior to melt granulation (**Table 2**). For these experiments only microcrystalline cellulose was used as co-excipient while pre-gelatinized starch was discarded. The reason for this is that the previous experiments showed no beneficial effect of pre-gelatinized starch on the dissolution kinetics. From the obtained granules and spray-dried powder, tablets were produced using identical settings regarding compaction force and punch size as described earlier in this work. These tablets were evaluated for their degradation time and drug release and as shown on **Table 5** and **Figure 9**, the addition of super-disintegrants only leads to a minor improvement in the drug dissolution kinetics. In the presence of super-disintegrants slightly more drug is released during the first 30 min, for all three types of super-disintegrants and for both the tablets produced from granules as well as those produced from spray-dried powder. Although differing in chemical structure, these super-disintegrants exert their effect in a similar way, i.e. by fast water uptake leading to swelling. This swelling is intended to disrupt the binding forces that hold the tablet. However, the limited effect of super-disintegrants on the disintegration time (**Table 5**) of the tablets produced in the present work is likely due to the presence of Gelucire®44/14.

Table 5: average disintegration time of CBZ tablets containing Explotab®; Ac-di-sol® and Polyplasdone® XL.

Tablet	Average(±SD) disintegration time (min) in 0.1N HCl at 37°C (±0.5)
TG2 EP	16±1.21
TG2 AC	16±0.94
TG2 PY	17±0.75
TSD2 EP	15±0.76
TSD2 AC	16±0.96
TSD2 PY	16±0.69
TG2 EF mix	2±0.29

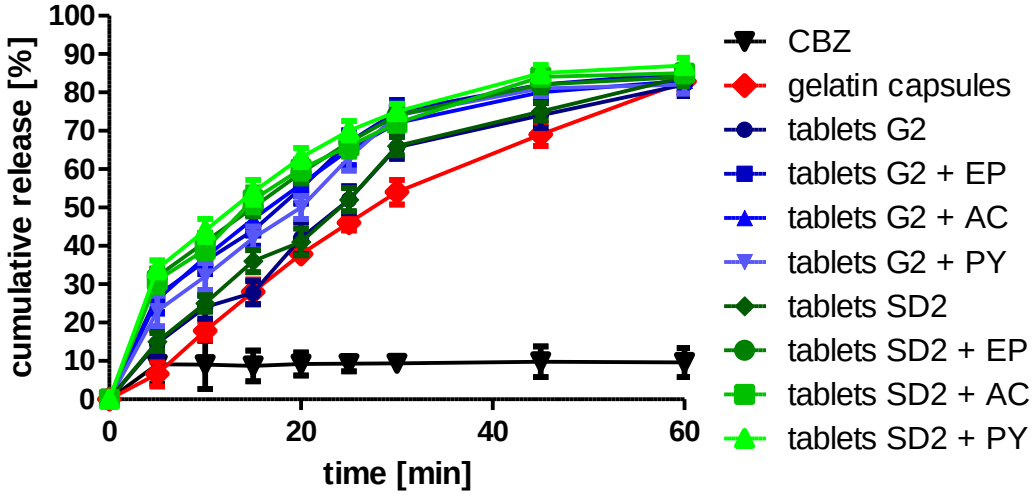

Figure 9. dissolution profiles of CBZ tablets obtained by compression of spray dried powder mixture and granules containing Explotab® (EP); Ac-di-sol® (AC) and Polyplasdone® XL (PY).

In a final series of experiments we replace the super-disintegrants by an effervescent mixture composed of citric acid and sodium bicarbonate in a 1:1 ratio. An effervescent powder mixture is generally composed of an acid and a bicarbonate, which readily react upon contact with water, releasing carbon dioxide. This effervescent mixture was added to the carbamazepine/microcrystalline cellulose mixture prior to melt granulation with Gelucire®44/14, in the same ratios as used for the earlier super-disintegrants. From the obtained granules, tablets

were produced using the same compaction force and punch size as described above. As shown in **Figure 10**, addition of the effervescent mixture to the formulation dramatically increased the drug dissolution rate, reaching more than 90 % of drug release in less than 10 min. Measuring the disintegration time of these tablets also elucidated that complete tablet decomposition took place in less than 2 min. These data indicate that, unlike swelling exerted by common super-disintegrants, the mechanical force exerted by the formation of CO_2 gas bubbles is capable of disrupting the bonds within the tablet.

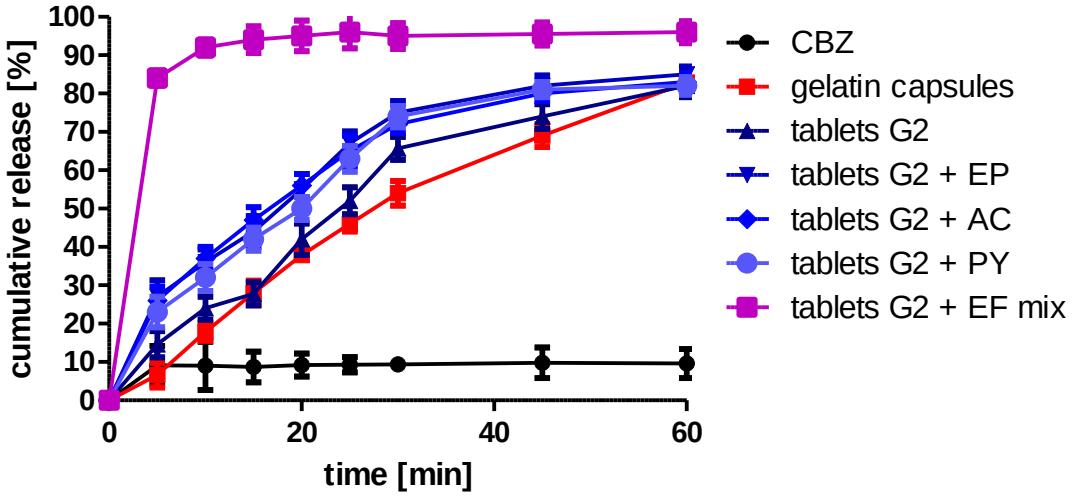

Figure 10. Dissolution profiles of CBZ tablets obtained by compression of spray dried powder mixture and granules containing Explotab® (EP); Ac-di-sol® (AC) and Polyplasdone® XL (PY) and the effervescent mixture (EF mix).

2.4. CONCLUSIONS

In this chapter we have demonstrated that the dissolution rate of a poorly water-soluble model drug, carbamazepine, can be greatly enhanced through formulation with Gelucire®44/14. However, to achieve immediate drug release it was found that a particular rather than a monolithic structure was found to be crucial. However, when granules or spray-dried powder were further processed into tablets this was found to reduce the drug dissolution rate to the level of a monolith. Addition of super-disintegrants to the tablet formulation did not result in similar dissolution properties as those of the granules of spray-dried powder prior to tabletting. However, replacing the super-disintegrants by an effervescent mixture of citric acid and sodium bicarbonate was found to overcome the tablet integrity and lead to immediate drug release.

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CHAPTER 3
SOLVENT-FREE DRUG CRYSTAL ENGINEERING FOR DRUG NANO- & MICROSUSPENSIONS.

Parts of this chapter were published in:

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3.1. INTRODUCTION

One of the major causes of new drug molecules failing to reach the market is poor bioavailability. Indeed, many of the newly discovered drug candidates suffer from low to extremely low water solubility and hence are prone to limited bioavailability. To cope with these issues, drug formulation scientists are developing formulation strategies that enhance the dissolution, and thus bioavailability of these poorly water-soluble drugs. These strategies include, formation of inclusion complexes with cyclodextrins, salt formation, transformation of crystalline to amorphous, particle size reduction, etc...

Amongst these techniques, particle size reduction is highly promising. Micronization of drug crystals to the lower micron range increases the surface area, thereby enhancing the drug dissolution rate in the gastrointestinal tract. For the simplified case of spherical particles, size reduction with a factor 10 (e.g. from 100 μm to 10 μm), a 100-fold increase in surface area is obtained. However, for extremely hydrophobic drug molecules, micronization is not sufficient to achieve high enough bioavailability [1, 2]. Therefore, reducing particle size to the nanorange (increasing the surface area of the drug crystals with multiple orders of magnitude) has been investigated through several approaches. Basically, these can be divided into two main groups: top-down and bottom-up techniques. In a top-down approach, nanoparticles are produced by exposing drug particles to mechanical energy, either through impact, shear or ultrasound induced cavitation. Drawbacks of these approaches are the limited control over the final particle size and morphology and the risk for potential contamination originating from the grinding media or ultrasonication set-up. Bottom-up approaches involve controlled recrystallization of dissolved (typically in organic solvents) drug molecules into nanoparticles via e.g. spray-drying or solvent-displacement. These approaches allow, by optimizing solvent and stabilizer (e.g. surfactants) a better control over particle size and morphology. However, a major drawback is the use of organic solvents that holds the risk of residual traces of organic solvents that can hardly be removed.

When producing crystalline drug nanosuspensions, several considerations should be taken into account. First of all, colloidal stability of the nanosuspension is an important issue as reaggregation of hydrophobic nanocrystals readily takes place when these are not stabilized by means of surface active molecules. Reaggregation decreases the total drug surface area, thereby decreasing drug dissolution kinetics and should thus be avoided. Secondly, drug concentration plays an important role as for practical reasons excessive dilution of the nanosuspensions should be limited. Related to the latter is the further processing of the nanosuspensions into a solid

form, while still allowing a nanosuspension to reform in aqueous physiological medium (e.g. in the gastrointestinal tract).

This study aims to evaluate different solvent-free approaches to produce nanosuspensions of poorly water-soluble drugs in order to enhance their dissolution behavior. As model drugs, itraconazole (**Figures 1A and 2A)**; and febantel (**Figures 1B and 2B**) were chosen due to their extremely low water solubility.

In order to stabilize the drug nanosuspensions, we will co-formulate the drug nanocrystals with non-ionic surfactants. Whereas a first top-down approach is based on simple grinding of crystalline drug suspended in liquid surfactant, we focus in the other approaches on using cavitation induced by ultrasonic waves to control the drug particle size. Cavitation is the formation of gas bubbles in a liquid phase in regions where the pressure of the liquid falls below its vapor pressure. Due to acoustic waves, the gas bubbles oscillate in size and upon collapse of the bubbles, a shock wave is produced. These shock waves have sufficient power to erode crystalline material into smaller particles in a top-down process as well as avoiding nanoparticle aggregation in a bottom-up process [3].

3.2. MATERIALS AND METHODS

3.2.1. Materials

Febantel (melting point of 166 °C and water solubility of 1.9 μg/ml) was obtained from Fagron (Belgium) and itraconazole (melting point of 130 °C and water solubility of 1 ng/ml) was obtained from Johnson & Johnson (Belgium). **Figure 1** shows optical microscopy images of both drugs in crude form and their molecular structure is shown in **Figure 2**. Febantel is a broad-spectrum probenzimidazole that is widely used against gastrointestinal nematodes and lung worms in live stock [3-8]. It is a prodrug that is metabolized *in vivo* to fenbendazole and further to oxfendazole, 4-hydroxyfembendazole, 4-hydroxyfendazole, and an inactive metabolite febendazolesulfone [9-14]. Itraconazole is a triazole antifungal drug, used in the treatment of fungal infections.

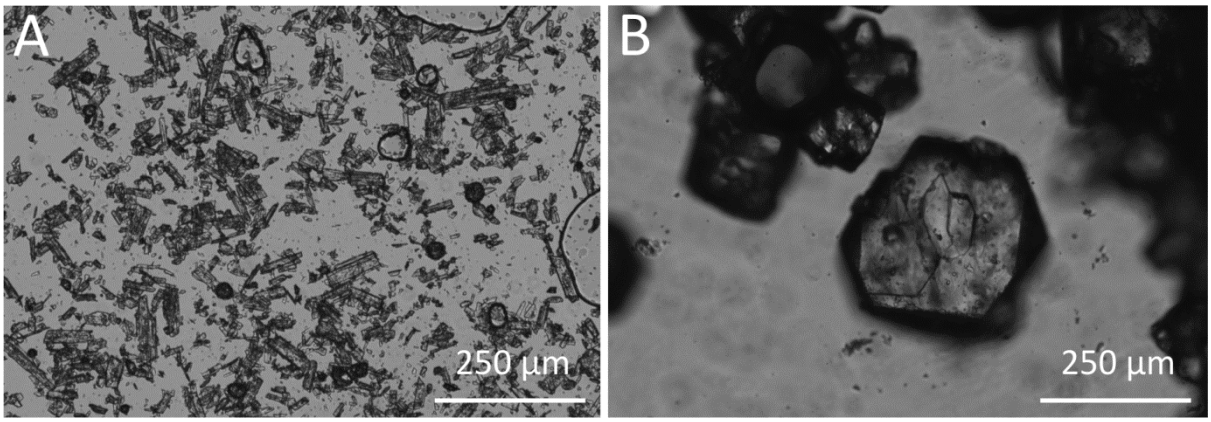

Figure 1. Optical microscopy images of (A) itraconazole and (B) febantel.

Gelucire®44/14 (i.e. PEG-32 glyceryllaurate) was obtained from Gattefossé (France). Gelucire®44/14 is an amphiphilic semisolid with a melting point of 44°C and an HLB of 14. Gelucire®44/14 is GRAS (generally recognized as safe) and is obtained by polyglycolysis of hydrogenated palm kernel oil with polyethylene glycol 1500. Due to its unique composition, comprising mono- and diesters of PEG, mono-, di- and triglycerides, it spontaneously forms a microemulsion upon contact with water [15]. These properties allow Gelucire®44/14 to be used in the solubilization enhancement of poorly soluble drugs and increase of their solubility rate [16, 17].

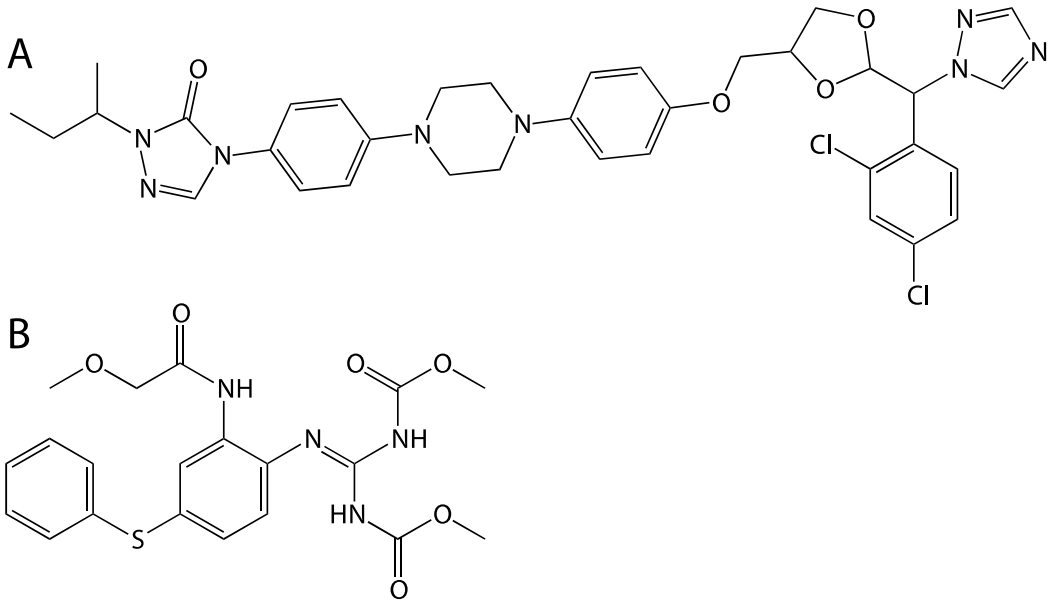

Figure 2. Molecular structure of (A) itraconazole and (B) febantel.

3.2.2. Preparation of the nanosuspensions

The composition of the nanosuspensions containing itraconazole (water solubility 1 ng/mL) and febantel (water solubility 1.9 μg/mL) is displayed in **Table 1**.

Table1. Composition of the crude febantel and itraconazole suspensions

Formulation components	(wt %)	(g)
Gelucire®44/14	10,1	20
water	75,7	150
febantel	14,2	28,14
Gelucire®44/14	11,5	20
water	85,9	150
itraconazole	2,6	4,54

3.2.2.1. Wet milling

Drug, Gelucire®44/14 and water were added to a 500 mL zirconium dioxide grinding bowl loaded with 25 zirconium dioxide grinding beads of 20 mm in diameter. The bowl was closed with a zirconium dioxide lid, placed in a ball mill type Pulverisette 6 (Fritsch, Idar-Oberstein, Germany) and allowed to rotate at 350 rpm for a fixed time interval.

3.2.2.2. Indirect sonication

First, Gelucire®44/14 was dispersed in water followed by the addition of the crude crystalline drug powder. The experimental set-up is schematically shown in **Figure 3.** The ultrasonic mini flow cell was obtained from Hielscher Ultrasonics (Teldow, Germany) and attached to a titanium sonotrode type BS2d22 (HielscherUltrasonics) and an ultrasound generator type UIP1000 (Hielscher Ultrasonics), operating at 20 kHz (± 1kHz) and an intensity of 20 W/cm². The ultrasonic mini flow cell consists of a glass tube connected to a closed tubing circuit in which the aqueous drug/Gelucire®44/14 suspension is injected through a syringe connected to

a valve. The total volume of the system is approximately 30 ml. The tubing is inserted in a peristaltic pump to allow circulation of the suspension. The residence time of the suspension in the flow cell can be controlled via the peristaltic pump. Additionally, the ultrasonic mini flow cell contains a water jacket (room temperature), surrounding the glass tube (4 mm in diameter), connected with a pressurized water reservoir. This water jacket allows propagation of the generated ultrasonic waves, transmitting them to the suspension within the glass tube. Furthermore, the water jacket allows controlling the temperature of the glass tube limiting the heating caused by the ultrasonication.

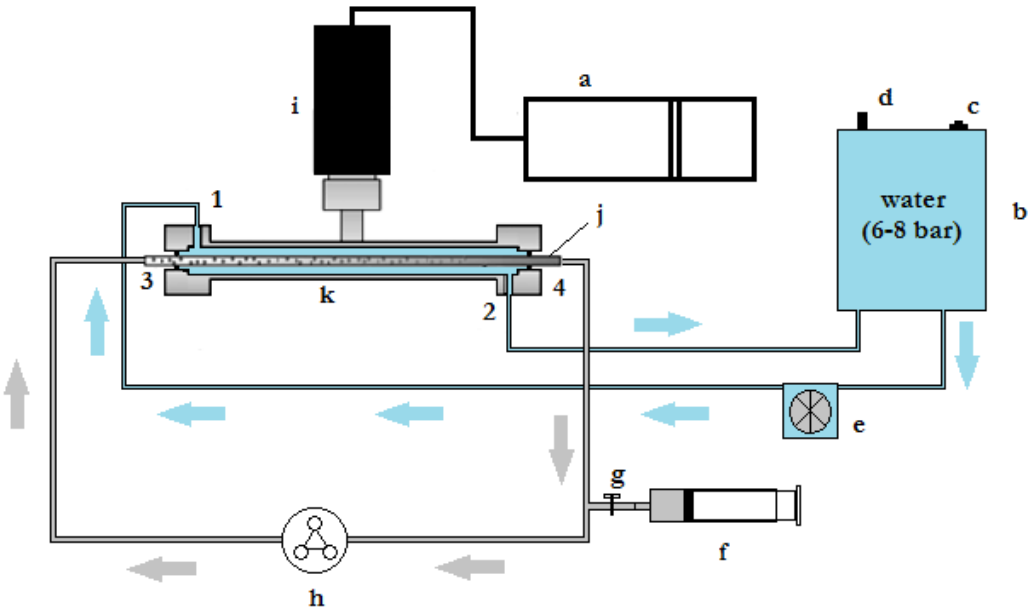

Figure 3. Schematic representation of the experimental set-up for indirect sonication of drug suspensions. (a) ultrasonic processor, (b) pressurized water tank, (c) security pressure valve, (d) compressed air input, (e) water pump, (f) syringe, (g) close cycle valve, (h) peristaltic pump, (i) sonotrode, (j) glass tube, (k) ultrasonic mini flow cell, (1:2) pressurized water input/output, (3;4) suspension input/output.

3.2.2.3. Ultrasonic melt crystallization

Figure 4 shows a schematic representation of the experimental set-up to produce drug nanosuspensions via ultrasonic melt crystallization. A photograph of the set-up is shown in **Figure 5**. Itraconazole and febantel were first dissolved in Gelucire®44/14 at respectively 150°C and 140°C, which is above their respective melting point. Subsequently, the liquid phase was atomized through a 2 fluid nozzle into a dual wall stainless steel flow cell containing 150 mL water. The water was thermostatized at 10°C to allow prompt cooling of the atomized liquid. The temperature was monitored by an in line temperature probe. During the atomization process, cavitation was induced to the system by means of an ultrasonic probe (HielscherUltrasonics, Teldow, Germany) composed of a titanium sonotrode type BS2d22 (HielscherUltrasonics), a booster type B2-1.4 (HielscherUltrasonics) and an ultrasound generator type UIP1000 (HielscherUltrasonics) operating at 20 kHz ± 1 kHz, and an intensity of 100W/cm^2. After 5 minutes of sonication, milky suspensions of both drugs were obtained

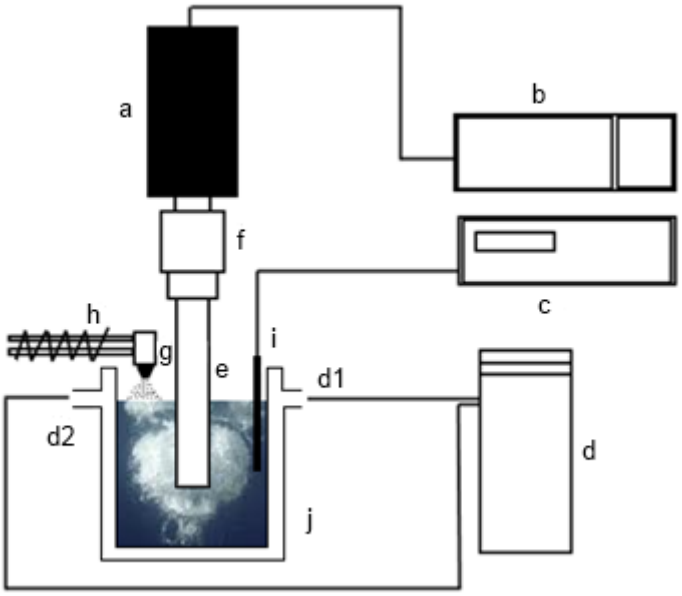

Figure 4. Schematic representation of the ultrasonic melt crystallization set-up. (a) sonotrode, (b) ultrasonic processor, (c) digital thermometer, (d) cooling system, (e) titanium tip, (f) booster, (g) two fluid nozzle, (h) heating cord, (i) temperature probe, (j) dual wall flow cell, (d1, d2) inputs for the cooling circuit.

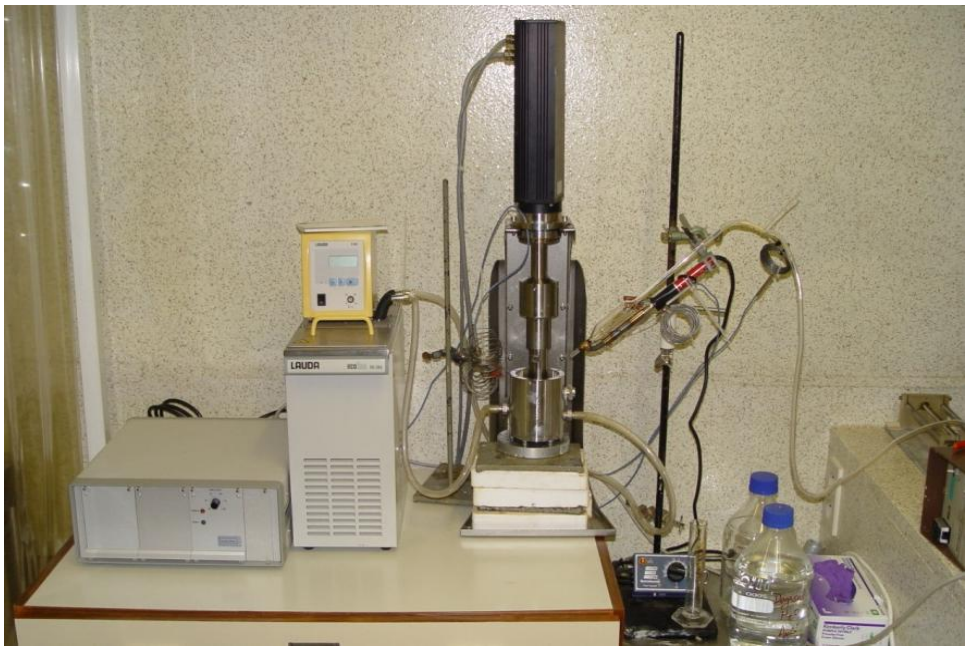

Figure 5. Photograph of the experimental ultrasonic melt crystallization set-up.

3.2.3. Particle size distribution

The particle size distribution of the drug crystals in the nanosuspensions was determined by laser diffraction using a Malvern Mastersizer S (Malvern Instruments, Spring Lane South, UK) equipped with a small volume dispersion unit and a 300 RF lens. Water was used as dispersion medium for all measurements.

3.2.4. Optical microscopy

Optical microscopy images were recorded under bright field and polarized light using a Leica 2500P microscope equipped with 10x and 63x oil-immersion objectives, a DFC360FX CCD camera and a Linkham THMS600 heating stage. To allow proper visualization, drug crystals and nanosuspensions were squeezed between two glass cover slides.

3.2.5. Experimental design

An experimental design was set up to evaluate the influence of the drug to Gelucire® 44/14 ratio and ultrasonication time on the particle size of the nanosuspensions produced via ultrasonic melt crystallization. As model drug only febantel was evaluated and a central composite experimental design was chosen. The febantel to Gelucire®44/14 ratio (ranging from 0.1 to 0.5) and the ultrasonication time (ranging from 0 to 60 minutes) were used as variables and the

D(v,0.9) particle size was the response factor. In total 13 experiments were performed in randomized order as listed in **Table 2**. The experimental runs and the output results were processed via Design Expert-software (version 6.0.10, Stat-Ease, USA).

Validation of the prediction model was performed by comparing the predicted particle size to the observed value for a random point of the design (sonication time: 20 min, febantel to Gelucire ratio: 0.3). **Table 3** shows that the 95% confidence interval of the observed value was within the 95% prediction interval.

Table 2: Experimental design variables and response factor, for a total of 13 experiments

	Variables		Response factor
Experiment Number	Ratio Drug/Gelucire®44/14 (w/w)	Sonication Time [min]	Particle size [µm]
1	0.5	30.0	4.58
2	0.3	60.0	4.97
3	0.4	8.8	9.45
4	0.16	8.8	0.28
5	0.3	30.0	4.74
6	0.3	30.0	4.36
7	0.3	30.0	3.18
8	0.44	51.2	15.17
9	0.3	0.0	2.38
10	0.1	30.0	0.26
11	0.3	30.0	9.39
12	0.3	30.0	7.11
13	0.16	51.2	0.32

$$Dv(0,9) = -1.170 + 10.144 * ratio + 0.011 * time$$

Table 3: Validation of the prediction model.

Response factor	Predicted value [μm]	95% Prediction interval [μm]	Observed value [μm]	95% Confidence interval [μm]
D(v,0.9)	4.42	[1.07; 10.05]	5.62	[3.010; 5.97]

3.2.6. In vitro drug dissolution

Drug release from the produced nanosuspensions was evaluated in a VK7010 dissolution bath (VanKel Industries, NJ, USA) equipped with a VK8000 automatic sampling station (VanKel Industries, NJ, USA). All dissolution tests were run in triplicate. Note that since this study focused on the development of formulations that enhanced the dissolution rate of extremely poorly water-soluble drugs, sink conditions were not met. As dissolution medium, 900 ml 0.1 M HCl in demineralized water was used at a temperature of 37°C. At 5, 10, 15, 20, 30, 45 and 60 minutes, 5 ml samples were withdrawn from the dissolution vessels without medium replacement, filtered through a 200 nm filter to remove particulate matter and analyzed by spectrophotometry for their drug content.

3.2.7. Analytical method validation

The febantel concentration was measured at 280 nm and itraconazole concentration was measured at 260 nm using a double beam spectrophotometer (Shimadzu UV-1650 PC, Shimadzu CO., Kyoto, Japan).

The UV-spectroscopy method was validated based on the guidelines stated by the International Conference on Harmonization (ICH) for validation of analytical procedures (1994). The following characteristics were considered: specificity, linearity, accuracy, precision, detection and quantification limit. The parameter of recovery was not evaluated since no extraction step is involved.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of other compounds. Such can be assessed by comparing the UV spectrum of the different components of the formulation on **Figure 6** and verifying that no interference is observe at the considered wavelengths of 260nm (itraconazole) and 280nm (febantel). It is clear that both methods are selective to determine the amount of each drug in the dissolution medium.

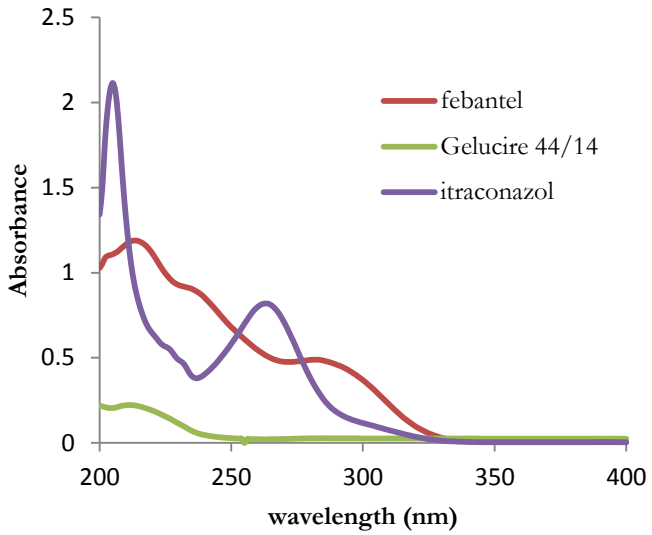

Figure 6. Specificity of the UV method.

Linearity

The linearity of the analytical procedure is its ability – within a given range – to produce results which are directly proportional to the concentration of drug in the dissolution medium sample. For the validation of the present UV-spectroscopy methodologies several calibration curves were performed for both drugs. The linearity of the calibration curves was evaluated by the determination of the coefficient R^2. The mean determination of the calibration curves (n=6) was 0.999 for itraconazole and 0.999 for febantel, indicating a linear relationship between absorption and concentration. Equation of the mean calibration curve was:

Y=35.77±0.35x + 0.0009±0.00003 for febantel (concentration range 0.003 to 0.031mg/mL)

Y=40.02±0.8x + 0.0061±0.0007 for itraconazole (concentration range of 0.001 to 0.021mg/mL)

With **x**= drug concentration (mg/mL) and **y**=absorption.

Accuracy

The accuracy of analytical procedure expresses the closeness of agreement between the true value and the determined value; it's expressed as the percentage agreement between the mean determined and the true concentration.

The accuracy was investigated in three concentration levels. Each concentration was determined six times. The mean accuracies (±SD) are listed in **Table 4** and **Table 5**.

Table 4: Accuracy (±SD) of UV-method for febantel (n=6)

Concentration (mg/mL)	**Accuracy %**	
	Within-day	Between-day
0.003	106.9 ± 3.4	109.9 ± 6.6
0.015	101.2 ± 2.3	98.9 ± 2.9
0.031	99.7 ± 1.8	101.3 ± 1.2

Table 5: Accuracy (±SD) of UV-method for itraconazole (n=6)

Concentration (mg/mL)	**Accuracy %**	
	Within-day	Between-day
0.001	97.7 ± 8.6	101.6 ± 10.5
0.011	91.0 ± 2.5	91.8 ± 2.9
0.021	101.1 ± 0.8	100.7 ± 1.0

All mean values are within ±15% of the actual concentration.

Precision

Precision expresses the closeness of agreement between repeated determinations. It may be considered at three levels: repeatability (intra-intra assay precision), intermediate precision (within laboratory precision) and reproducibility (between laboratory precision). Precision is expressed as the coefficient of variation (%) of a series of measurements of the calibration standards. Since the measurements were performed in the same laboratory, by the same person using the same equipment only the repeatability (within-day and between day) of the measurements was evaluated. The precision was assessed by assaying three standards in six replicate in one day for within-day precision and once daily for six days for between-day precision (**Table 6** and **7**), the acceptance criteria are coefficients of variation less than 15%.

Table 6: Precision (expressed as coefficient of variation) of the UV-method for febantel (n=6)

Concentration (mg/mL)	Within-day			Between-day		
	$\bar{x}$	SD	**Precision (%CV)**	$\bar{x}$	SD	**Precision (%CV)**
0.003	0.14	0.0015	1.1	0.15	0.005	3.5
0.015	0.55	0.0045	0.8	0.54	0.004	0.75
0.031	1.12	0.0047	0.4	1.11	0.004	0.39

Table 7: Precision (expressed as coefficient of variation) of the UV-method for itraconazole (n=6)

Concentration (mg/mL)	Within-day			Between-day		
	$\bar{x}$	SD	**Precision (%CV)**	$\bar{x}$	SD	**Precision (%CV)**
0.001	0.043	0.0053	12.3	0.15	0.0044	9.7
0.011	0.412	0.0223	5.4	0.54	0.0217	5.2
0.021	0.861	0.059	6.8	1.11	0.0148	1.7

Detection and quantification limit

The detection limit of an analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value. The quantification limit is the lowest amount of analyte in a sample that can be adequately determined with suitable precision and accuracy. Both values are estimated from the mean calibration curve (ICH, 1995).

$$\boldsymbol{DL = \frac{3.3 \times \sigma}{S}}$$

DL= detection limit
σ= standard deviation of the Y-intercept of the mean calibration curve
S= slope of the mean calibration curve

$$\boldsymbol{QL = \frac{10 \times \sigma}{S}}$$

QL= quantification limit
σ= standard deviation of the Y-intercept of the mean calibration curve
S= slope of the mean calibration curve

The detection and quantification limits of febantel and itraconazole in the dissolution samples were DL=8.3×10^{-5} mg/mL ,QL=2.5×10^{-4} mg/mL and DL=5×10^{-4} mg/mL, QL=1.5×10^{-3} mg/mL respectively.

3.2.7. *X-ray diffraction (XRD)*

X-ray diffractograms were recorded on a PANalytical X'Pert PRO X-ray diffractometer (Siemens). XRD patterns were obtained with Cu KR radiation (45 kV x 40 mA; λ = 1.5406 A) at a scanning speed of 25° (2θ)/min and step size of 0.03° (2θ). Measurements were done in the reflection mode in the 2θ range of 5-40°.

3.3. RESULTS AND DISCUSSION

3.3.1. Preparation of nanosuspensions

As mentioned above, itraconazole and febantel are used in this study as model poorly water-soluble drugs. Both drugs are highly crystalline with a mean diameter D (v, 0.5) of respectively 26 µm (itraconazole) and 232 µm (febantel) as measured by laser diffraction. **Figure 1** shows optical microscopy images of both drugs in crude, unprocessed form. A first step in our formulation strategy was suspending the crystalline drug in Gelucire®44/14 and water. A first series of preliminary screening experiments served to determine the Gelucire®44/14 to drug ratio that allows processing via all 3 formulation processes. The respective formulation parameters are shown in **Table 1**.

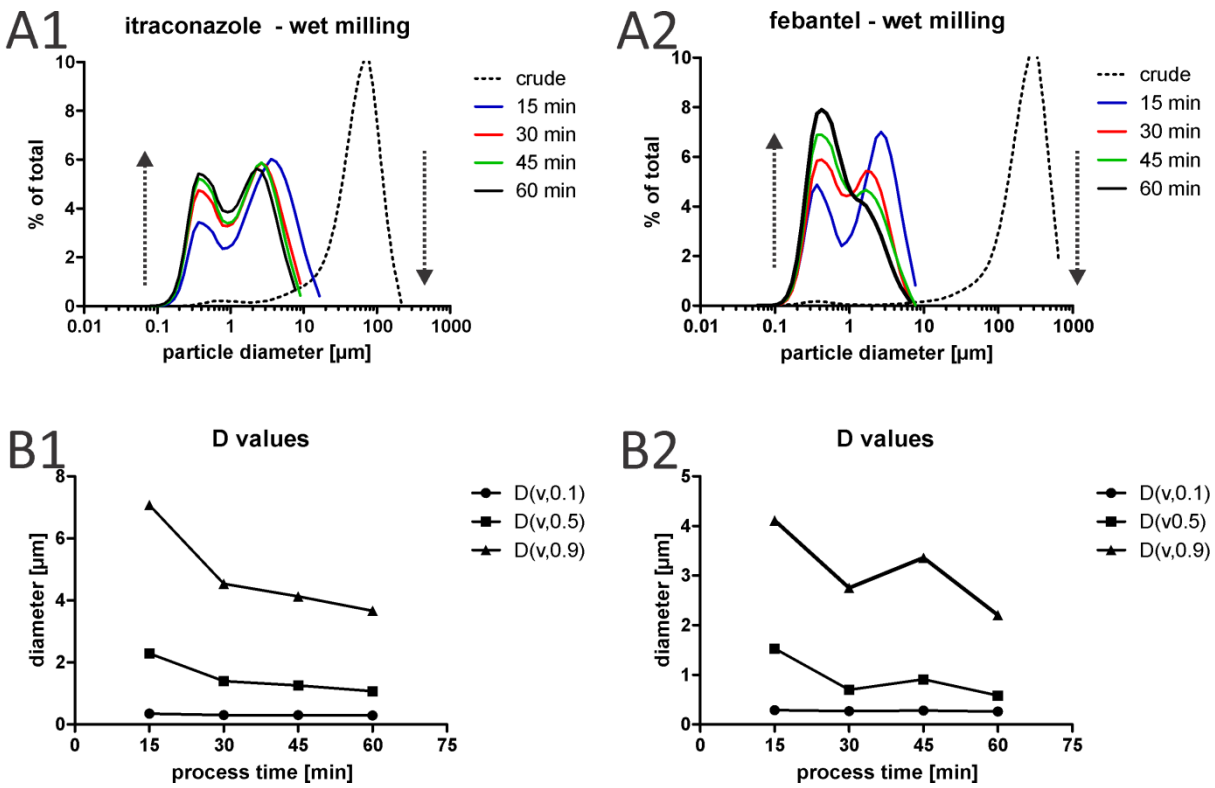

Figure 7.(A) Size distribution, measured by laser diffraction, at different time points during wet milling of respectively itraconazole and febantel suspensions in Gelucire®44/14 . The dotted arrows indicate the 'micro' drug crystal population to diminish as function of process time in favour of the 'nano' population. (B) Corresponding evolution of the D values as a function of process time.

3.3.1.1. Wet milling

Wet milling is frequently used to produce drug nanosuspensions, and was used in this work as reference process. The crude aqueous drug/Gelucire®44/14 suspensions were added to a bowl containing zirconium dioxide as grinding medium, and were subsequently placed during 1 h in a ball mill at 350 rpm. **Figure 7A**, shows the evolution of the particle size distribution and the mean particle diameter, measured by laser diffraction, during the wet milling process. These data demonstrate that for both febantel and itraconazole the initial particle population decreases as a function of process time, while a new sub-micron population emerges. Although efficient to produce drug nanosuspensions, the wet milling process bears several disadvantages. First of all, relatively long milling times are required. Secondly, there is a potential risk of contaminating the drug formulation with particulate matter resulting from erosion of the milling components. A third limitation is the possibility of only batch wise production.

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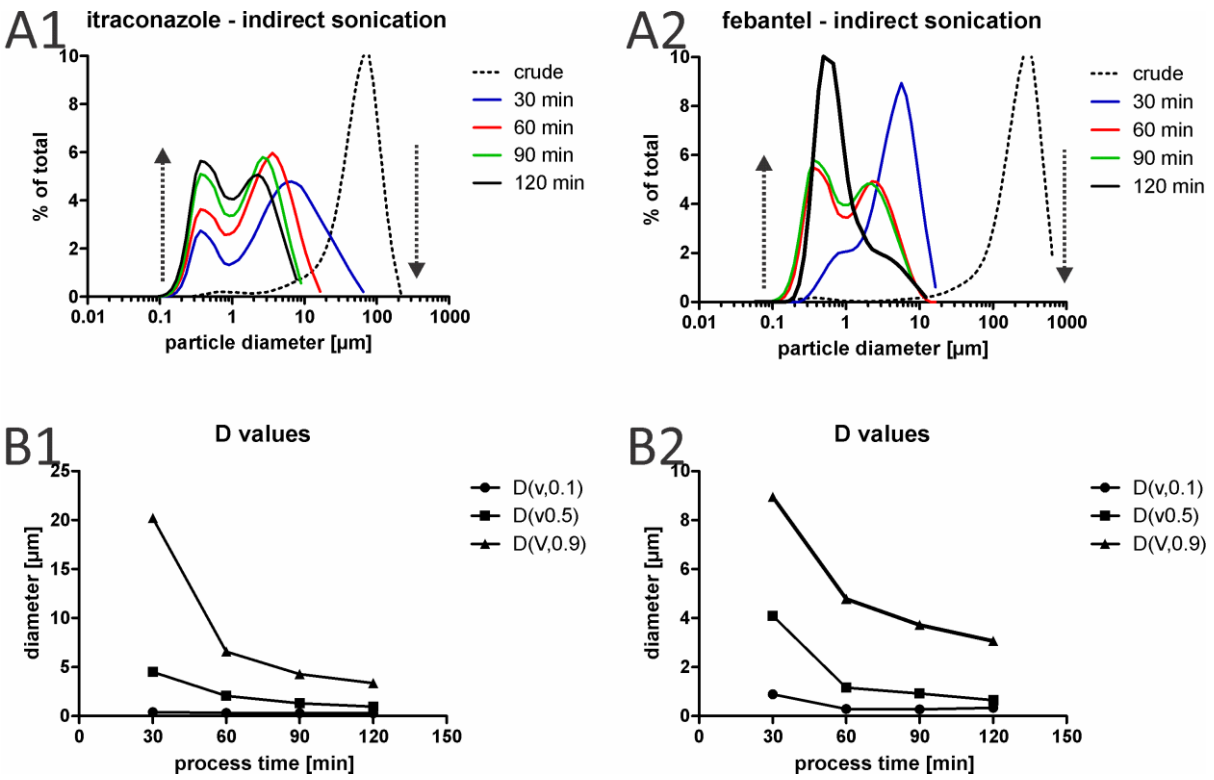

Figure 8. (A) Size distribution, measured by laser diffraction, at different time points during indirect sonication of respectively itraconazole and febantel suspensions in Gelucire®44/14 . The dotted arrows indicate the 'micro' drug crystal population to diminish as function of process time in favor of the 'nano' population. (B) Corresponding evolution of the D values as a function of process time.

3.3.1.2. Indirect sonication

To cope with the above mentioned issues related to wet milling, we aimed to design a process that allows to generate energy input without exposing the drug molecules to potential sources of contamination. Therefore, we constructed in-house an 'indirect sonication' set-up.

Table 8. Overview of the D values, measured by laser diffraction, of itraconazole and febantel drug suspension after different process times during respectively wet milling and indirect sonication.

process time [min]		**D(v,0.1)** [μm]		**D(v,0.5)** [μm]		**D(v,0.9)** [μm]	
				itraconazole			
milling	*sonication*	*milling*	*sonication*	*milling*	*sonication*	*milling*	*sonication*
15	30	0.35	0.4	2.29	4.51	7.08	20.21
30	60	0.3	0.33	1.4	2.08	4.54	6.57
45	90	0.3	0.3	1.26	1.3	4.13	4.29
60	120	0.29	0.28	1.07	0.96	3.66	3.36
				febantel			
milling	*sonication*	*milling*	*sonication*	*milling*	*sonication*	*milling*	*sonication*
15	30	0.29	0.89	1.53	4.1	4.11	8.95
30	60	0.27	0.29	0.7	1.16	2.75	4.78
45	90	0.28	0.27	0.91	0.92	3.36	3.72
60	120	0.26	0.34	0.58	0.65	2.2	3.06

Figure 8 displays the evolution of the particle size, measured by laser diffraction. Similar to the wet milling process, the particle size decreased as function of the process time. Again, febantel showed a more profound down-sizing of the particle diameter than itraconazole. We also observed that the efficiency of down-sizing can be optimized by extending the residence time of the liquid in the flow cell by controlling the pump rate. Lower pump rates lead to longer residence times in the flow cell and allowed to shorten the total process time. The indirect sonication process clearly has the advantage that the drug is not in contact with the physical

energy source, thus avoiding potential contamination. Furthermore, this process is expected to be more suitable for up-scaling, either by enlarging the flow-through cell or by putting several cells in series. Additionally, this process should allow working in a continuous way with constant in-and-out flow of fresh drug suspension, while the earlier described wet milling process is a batch process. Nevertheless, the indirect sonication process still suffers from relatively long, i.e. 2h, process times that are required to significantly reduce the drug crystal size. To summarize, **Table 8** gives an overview of the volume distributions (D values) of the drugs particles during processing via wet milling and indirect sonication.

3.3.1.3. Ultrasonic melt crystallization

To cope with the issue of prolonged process times associated with both wet milling and indirect sonication, we developed a novel bottom up approach that we termed 'ultrasonic melt crystallization'. In this process, drug nanosuspensions are generated by dissolving the drug in a molten phase of Gelucire®44/14 at respectively 140 °C (itraconazole) and 150°C (febantel). The process of drug dissolution in a molten Gelucire®44/14 phase was deeper analyzed by hot stage microscopy and presented in **Figure 9**. At 60 °C a liquid Gelucire®44/14 phase exists in which the crystalline drug is suspended. As soon as the melting point of the respective drugs is reached, they dissolve in the liquid Gelucire®44/14 phase. Upon cooling to room temperature, the drug (or a part of the drug) recrystallizes from the solution into very fine particles in case of febantel and larger precipitates in case of itraconazole, as shown in **Figure 9**.

During the ultrasonication process, the molten drug/Gelucire®44/14 mixture was atomized through a two-fluid nozzle into a flow cell containing water thermostatized at 10°C. Furthermore, during atomization of the molten drug/Gelucire®44/14 phase, cavitation is induced by immersion of an ultrasonic probe. After 5 min of sonication, milky suspensions of both drugs were obtained. Figure 4 gives a schematic representation of the experimental set-up and Figure 5 shows a photograph.

As shown in **Figure 10** on the size distribution graphs, the ultrasonic melt crystallization approach allowed to dramatically reduce the drug particle size. Importantly, whereas wet milling and indirect sonication yielded a bi-modal size distribution, the ultrasonic melt crystallization approach resulted in a mono-modal drug nanosuspension. In case of itraconazole, the D(v,0.5) reduced from 20.6 μm (i.e. the crude crystalline material before melting in Gelucire®44/14) to 0.51 μm, while in case of febantel the D(v,0.5) was reduced from 153.7 μm to 0.38 μm (**Table 9**).

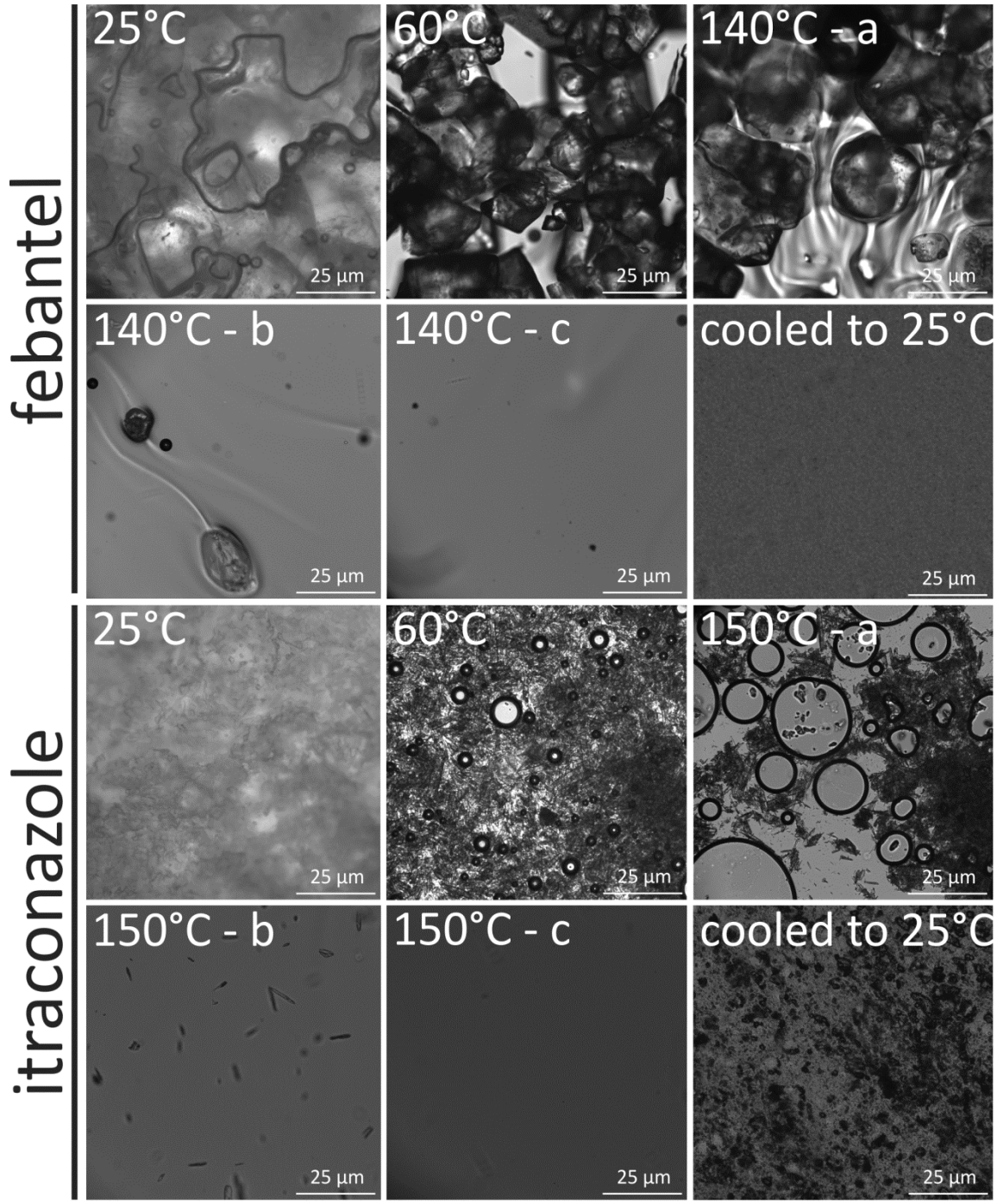

Figure 9. Optical microscopy images recorded using a hot stage microscopy set-up, demonstrating the dissolution of febantel, respectively itraconazole in a liquid Gelucire®44/14 phase as soon as the melting point of the drug is reached. The panels a, b and c show images at 10 s time intervals. Upon cooling to room temperature, (partial) re-crystallization occurs.

This is due to a series of events that occur during the process. First, the drug is dissolved in a molten Gelucire®44/14 phase through heating. Secondly, the atomization of liquid Gelucire®44/14 droplets into cold water under intense cavitation induces flash precipitation of the drug into small crystals. Cavitation plays a crucial role in this process, preventing

agglomeration of the newly formed crystals [18]. Indeed, when we conducted control experiments in absence of ultrasonication, the atomized liquid immediately gelified upon contact with the cold water, followed by nucleation of drug crystals and further on the formation of large crystalline precipitates. By contrast, performing the process under ultrasonication readily allowed the Gelucire®44/14 to emulsify upon contact with the water and to control the nucleation of drug crystals, stabilizing these within the nano-range.

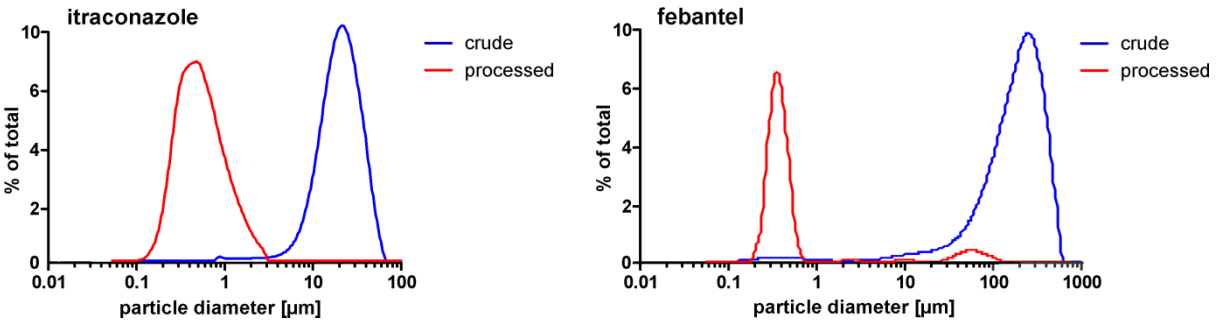

Figure 10. Size distribution, measured by laser diffraction, of the drug (itraconazole, respectively febantel) crystals in crude state (red curves) and after ultrasonic melt crystallization.

Table 9. Overview of the D values, measured by laser diffraction, of itraconazole and febantel before and after ultrasonic melt crystallization.

	D(v,0.1) [µm]	**D(v,0.5)** [µm]	**D(v,0.9)** [µm]
		itraconazole	
crude	9.1	26	71
processed	0.26	0.51	1.25
		Febantel	
crude	36	232	480
processed	0.26	0.38	0.56

To investigate the influence of sonication time and drug to Gelucire®44/14 ratio on the final particle size, an experimental design was constructed. For this purpose we took only

febantel as model drug and in total 13 series of experiments were performed as listed in **Table 2**. As visualized by the 3D surface plot in **Figure 11**, particle size reduction is more efficient at lower drug to Gelucire®44/11 ratios and at shorter sonication times. Reducing the drug to Gelucire®44/14 ratio evidently decreases the total amount of drug in the system thus requiring more time for nucleating drug crystals and drug molecules to reach each other through diffusion. Thereby, the increased amount of Gelucire®44/14 allows a better stabilization of the newly created drug crystal surface, preventing agglomeration of the drug crystals. The influence of sonication time on the resulting particle size is less pronounced. However, the relative short required sonication times compared to the indirect sonication process described above is certainly beneficial to limit potential contamination of the drug formulation with metal nanoparticles that are eroded from the metal ultrasonication tips over prolonged sonication periods.

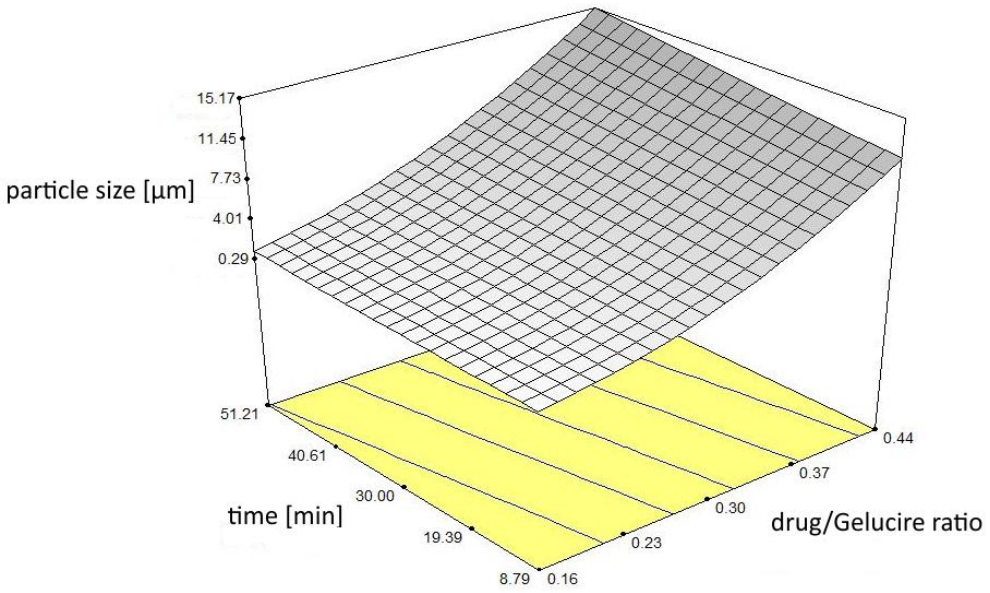

Figure 11. 3D surface plot demonstrating the influence of drug to Gelucire®44/14 ratio and sonication time on the particle size of febantel crystals produced via the ultrasonic melt crystallization process.

3.3.2. *In vitro* drug dissolution of drug nanosuspensions

Finally, the itraconazole and febantel nanosuspensions were evaluated for their *in vitro* dissolution behavior. The cumulative release curves of the respective formulations and their controls are shown in **Figure 12**. **Table 10** summarizes the maximum concentration of drug that was dissolved. Crude drug, as well as a physical mixture of drug and Gelucire®44/14 give very

low drug concentrations, for both febantel and itraconazole. By contrast, the cumulative drug release curves depicted in **Figure 12A**, show a pronounced enhancement in total drug release for febantel processed via all three formulation strategies. Febantel formulated via ultrasonic melt crystallization achieves the highest total drug dissolved followed by indirect sonication and wet milling in a third place. Importantly, maximum drug concentration is reached within 10 min after addition of the nanosuspensions to the dissolution medium. In case of itraconazole only ultrasonic melt crystallization (**Figure 12B**) was able to increase drug dissolution.

For febantel, on the first sight, merely reducing the particle size (**Figure 10**) is effective to enhance drug dissolution; however, this is not the case for itraconazole. Probably this is due to the extremely poor water solubility of itraconazole (i.e. 1 ng/ml), which is even several orders of magnitude lower than febantel that has a water solubility of 1.9 µg/ml. To further investigate why the ultrasonic melt crystallization is the only process that effectively enhances the dissolution of itraconazole we assessed the crystallographic state of the formulated drug by X-ray diffraction (XRD). **Figure 13** shows the corresponding X-ray diffractograms for all febantel and itraconazole formulations and the respective controls. These diffractograms show, relative to a physical mixture of drug/Gelucire®44/14 in the same ratio as used for the respective formulations, a significant reduction in crystallinity only for the formulations (of both drugs) that were processed via ultrasonic melt crystallization. The other formulations strategies had no detectable influence on the crystalline state of the drug. Hence, it is likely that ultrasonic melt crystallization produces a certain fraction of amorphous drug that contributes to the observed enhanced drug dissolution.

Table 10. Maximum drug solubility in 0.1 M HCl at 37°C obtained via the respective formulation strategies.

Formulation	Febantel (µg/mL)	Itraconazol (µg/mL)
crude drug	0.55 ± 0.05	1.5 ± 0.1
physical mixture drug/Gelucire 44/14	0.8 ± 0.05	2.8 ± 0. 2
wet milling	8.8 ± 0.5	6.3 ± 0. 1
indirect sonication	17.45 ± 1.5	3.0 ± 0. 2
ultrasonic melt crystallization	21.3 ± 0.5	4.5 ± 0. 5

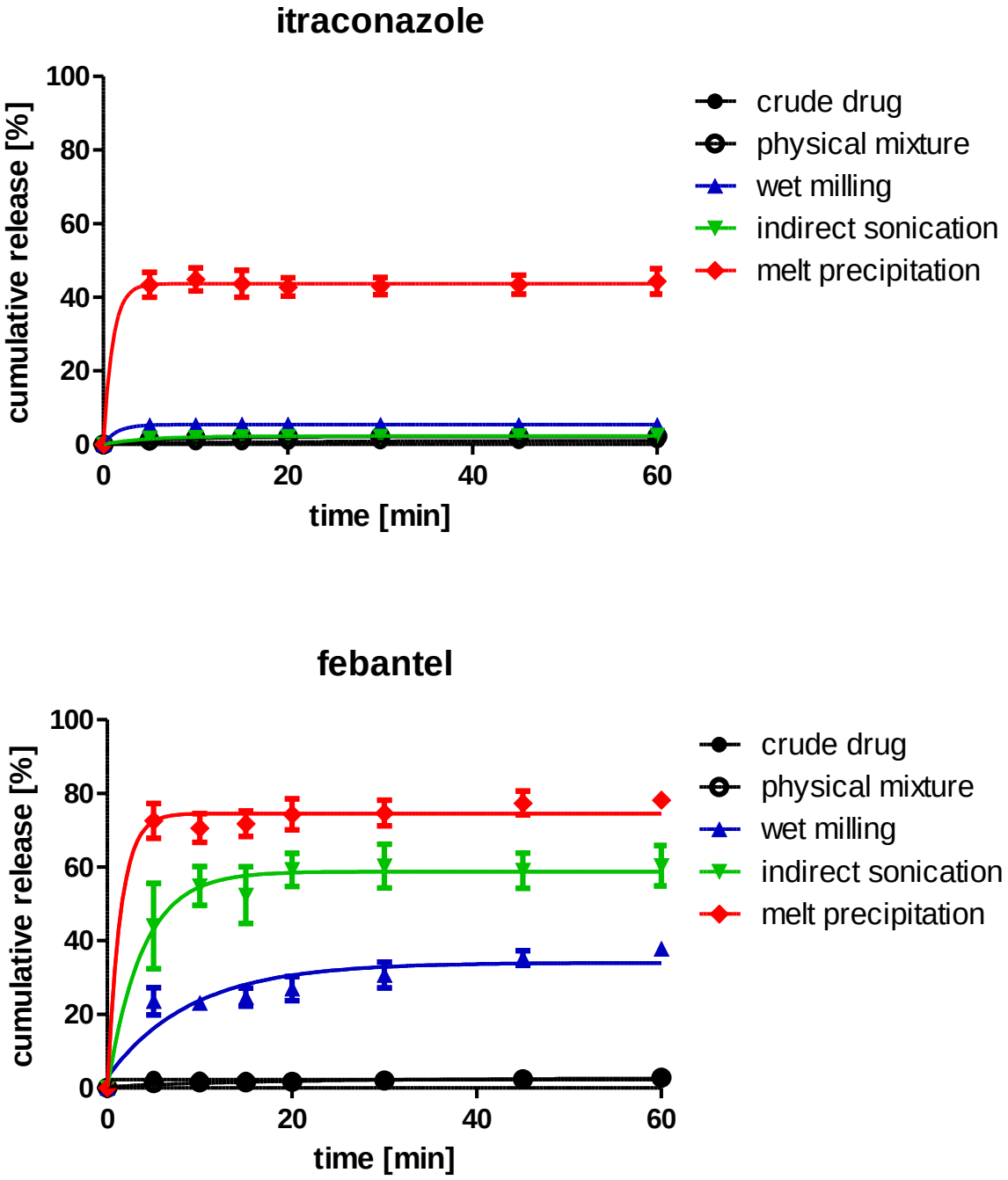

Figure 12. Cumulative *in vitro* in 0.1 M HCl at 37 °C release of (A) febantel and (B) itraconazole formulated via either wet milling, indirect sonication or melt crystallization. As controls, crude unprocessed drug and a physical mixture of drug and Gelucire®44/14 were taken.

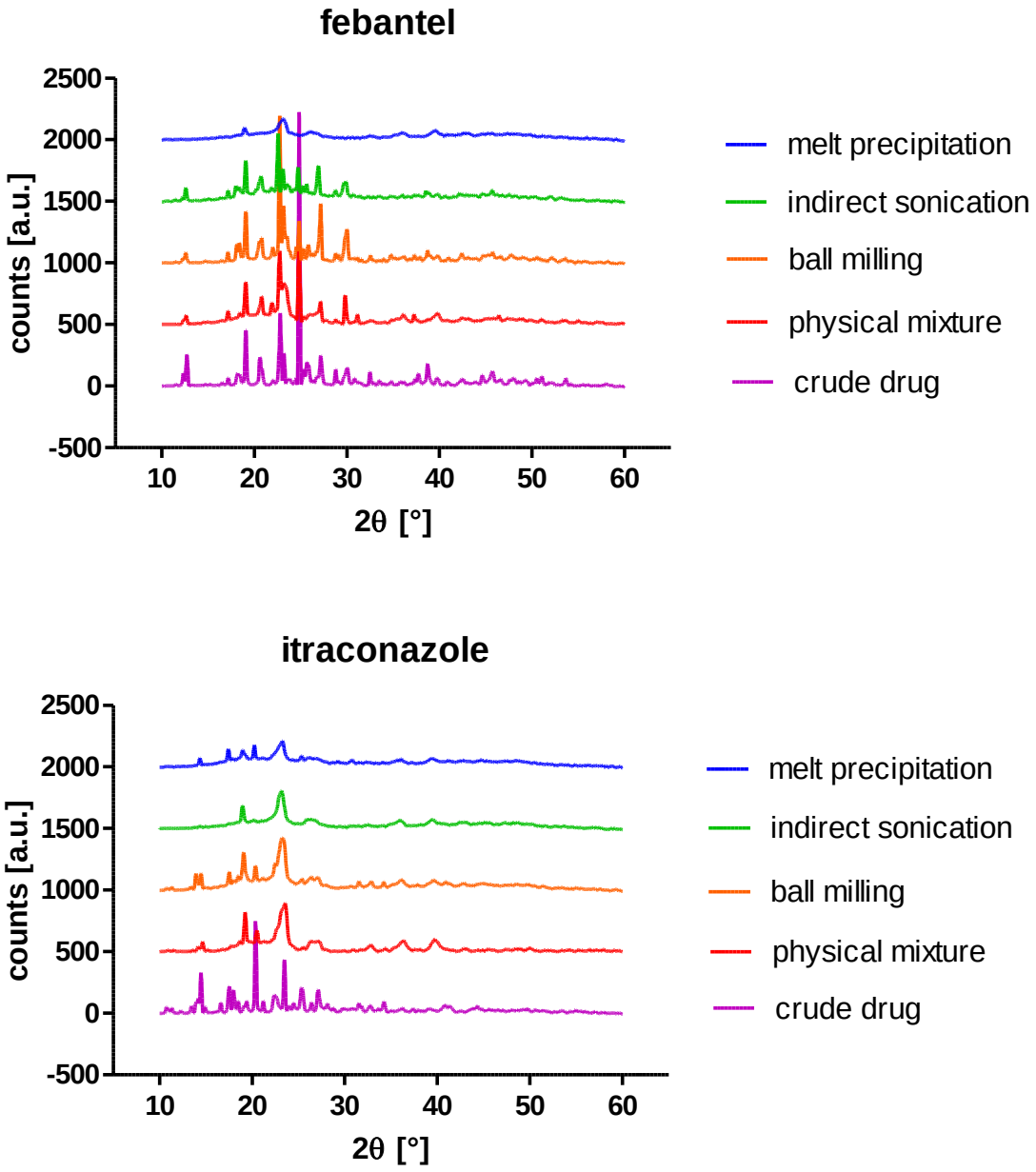

Figure 13. X-Ray diffractograms of the respective (A) febantel and (B) itraconazole formulations.

The relevance of both itraconazole nanoparticles and amorphous state in enhancing drug solubility has been acknowledged by several research works. Van Eerdenbrugh et al, evaluated the effect of different stabilizing systems (povidone-K25, poloxamer-188 and TPGS-1000) and concentrations on the media milling efficiency of itraconazole and their impact on the dissolution behavior of the freeze-dried nanosuspensions, observing a faster dissolution rate compared with pure drug [19].

Miller et al, produced by means of rapid flocculation amorphous itraconazole nanodispersions stabilized with hydroxypropylmethylcellulose (HPMC) or the pH-sensitive Eudragit® L100-55 with enhanced drug solubility and higher *in vitro* supersaturation (AUC) of the flocculated nanoparticle dispersions, relative to the AUC for a commercially available itraconazole formulation, Sporanox [20]. Similar results have been described by Choi and Collaborators, when developing amorphous solid emulsions of itraconazole by the combined use of membrane emulsification and spray drying solidification technique using Transcutol HP and Span 20 as a stabilizer, and dextran as solid carrier. The solid emulsion containing amorphous itraconazole displayed a significant increase in the dissolution rate than that of pure itraconazole. Furthermore, the solid emulsion after oral administration gave about eight-fold higher AUC and about ten-fold higher C(max) in rats than pure itraconazole powder [21]. The use of drug amorphous state as solubility enhancement alternative has been further explored by DiNunzio et al in the production of itraconazole solid dispersions by a novel high energy fusion technique KinetiSol® Dispersing (KSD). The obtained amorphous solid dispersions of itraconazole (ITZ) and hypromellose exhibited near complete drug supersaturation in dissolution and enhanced AUC upon oral bioavailability compared to crystalline itraconazole [22].

3.4. CONCLUSIONS

In this chapter, we have developed several solvent-free strategies to produce micro- and nanosuspensions of poorly water-soluble drugs. To enhance the water-solubility of these drugs, Gelucire®44/14, a self-emulsifying biocompatible excipient, was used. Wet milling and indirect sonication yielded drug suspensions with a bimodal size distribution, comprising submicron particles and particles in the lower micron range. Ultrasonic melt crystallization was able to yield a unimodal drug suspension with submicron drug particles. All three strategies to reduce the size of the drug crystals in the presence of Gelucire®44/14 strongly enhanced the dissolution rate and total amount of dissolved drug for febantel. In case of itraconazole, only ultrasonic melt crystallization was able to enhance the dissolution rate and total amount of dissolved drug. This was attributed to the lower water solubility of itraconazole, relative to febantel, and the fact that ultrasonic melt crystallization produces a fraction of amorphous drug that is stabilized by Gelucire®44/14.

Although enhancing dissolution rate and solubility of itraconazole using melt precipitation this technique remains inferior to other techniques described in literature for amorphisation.

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CHAPTER 4
FORMULATION OF POORLY WATER- SOLUBLE DRUGS VIA MELT- COACERVATION

Parts of this chapter will be submitted for publication

4.1. INTRODUCTION

For the treatment of veterinary diseases of livestock, mass medication is often preferred over individual treatment of animals. For this purpose, drug administration via the drinking water is highly convenient. This strategy is not only cost- and labor-saving but also targets sub-clinically affected animals as well as animals in the incubation stage of the disease. Compared to feed medication, drinking water medication allows a faster initiation of the treatment and faster dose-adjustment while the risk of disease carry-over between consecutive food containers is less [1,2]. However, the administration of medication via drinking water offers specific challenges for poorly water-soluble drugs. Whereas drug resorption of solid oral dosage forms of poorly water-soluble drugs is enhanced by surface-active properties of bile salts, this is not the case when poorly water-soluble drugs are added to a drinking water reservoir. By consequence, drug precipitation is likely to occur, leading to inhomogeneous drug distribution and thus large variation in medication levels in the drinking nipples [3, 4].

The above-mentioned issues are of particular relevance for antiparasitic and antibacterial drugs such as chloramphenicol, febendazol and febantel, which are extremely poor water-soluble. To enhance the dissolution of poorly water-soluble drugs, several strategies have been developed, including size reduction of the drug crystals to the nano-scale, the formation of solid solutions, cocrystals, complexation with cyclodextrins, formation of microemulsions etc… However, for veterinary purposes as drinking water medication, the above-mentioned formulation strategies suffer from elevated cost and also retain the drug molecules in a crystalline state. Aggregation of drug crystals is likely to occur win the drinking water reservoir and will not only limit drug dissolution kinetics, but will also lead to inhomogeneous drug distribution in the drinking water reservoir and thus large variation in drug concentration that is administered to livestock animals.

Therefore, we aimed to develop a formulation strategy for poorly water-soluble veterinary drugs that allows prompt drug dissolution in drinking water reservoirs. An attractive strategy to enhance drug solubility is the use of biocompatible non-ionic surfactants. Commonly, these are used to enhance the drug dissolution kinetics by lowering the surface tension and to increase the saturation concentration. In the present work, we modified this approach by directly dissolving the drug in a liquid surfactant phase. For this purpose, we used febantel, an extremely poor water-soluble broad-spectrum probenzimidazole that is widely used against gastrointestinal nematodes and lungworms in livestock. It is a prodrug that is metabolized *in vivo* to fenbendazole and further to oxfendazole, 4-hydroxyfembendazole, 4-hydroxyfendazole, and an inactive metabolite febendazolesulfone [5-7]. Following solubilisation in a liquid surfactant phase, we

sought to transform the formulation into a solid dosage form allowing easy handling, transportation and use.

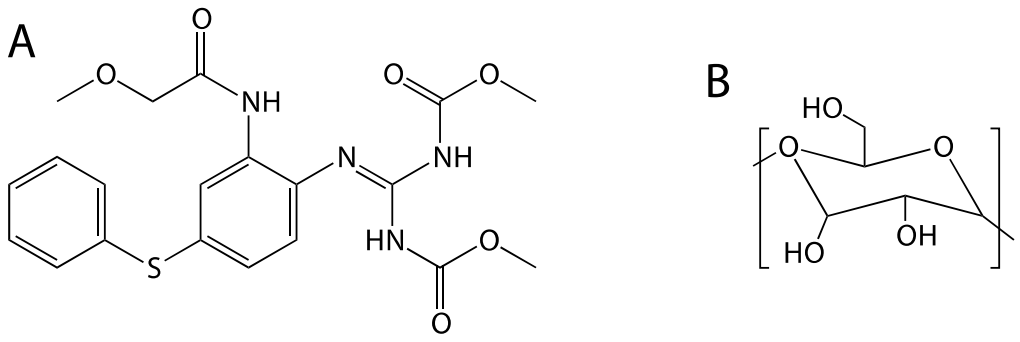

Figure 1. Molecular structure of (A) febantel and (B) maltodextrin.

4.2. MATERIALS AND METHODS

4.2.1. Materials

Febantel was purchased from Fagron. Maltodextrin with a dextrose equivalent of 8 (Pharmadry C*01983) was obtained from Cargill. Tapioca dextrin (k-4484) was purchased from National Starch. Cremophor®EL was obtained from BASF. FITC-dextran (Mw~500 kDa) was obtained from Invitrogen. Rhodamine B Octadecyl ester was obtained from Fluka. The deuterated solvents D_2O and MeOH-d_4 were obtained from Acros.

4.2.2. Production of the liquid coacervate

150 g of Cremophor®EL was heated to 130°C, which is just above the melting point (MP) of febantel (i.e. MP of 129°C). Subsequently, 60 g febantel was added and allowed to dissolve under stirring. Dissolution took about 2.5 min and the solution was left to stir for another 2 min to assure homogenization. The liquid was then immediately poured in 1L of a 30 % (w/w) aqueous maltodextrin solution at room temperature and homogenized for 10 min with a high-shear mixer (Silverson L4R) set at 8000 rpm. Finally, the liquid was allowed to stand under static conditions for 8h to allow coacervate formation and phase separation.

4.2.3. Experimental design

Several experiments were carried out to evaluate the influence of different ratios of water (g), maltodextrin (g) and Cremophor EL (g) in the amount of coacervate formed. A classical central composite design was applied. The design runs are listed in **Table 1**. Because interactions between the variables were expected, the following quadratic model was proposed in Eq. (1):

$$\textit{Eq. (1)} \quad Y = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{1} \sum_{j=i+1}^{2} \beta_{ij} X_i X_j + \sum_{i=1}^{2} \beta_{ii} X_i^2$$

where Y is the response, X_iX_j are the set points of the factors 'i' and 'j', respectively, in the mixture and β_0, β_i, β_{ij} and β_{ii} are the coefficients.

The design points were chosen by the software (Design-Expert version 6.0.10, Stat-Ease Inc., Minneapolis, USA). Manual regression was performed. The significant highest order polynomial was selected without destroying the model hierarchy. Outlier-t limit was set at 3.5. The significant model was used for fitting the response. The lack-of-fit test and a normal probability plot of the residuals were performed in order to evaluate the model and to detect outliers. The models provide several comparative measures for model selection. R^2 statistics, which give a correlation between the experimental response and the predicted response, should be high for a particular model to be significant. Adjusted R^2, which gives a similar correlation after ignoring the insignificant model terms, should have good agreement with predicted R^2 for the model to be fit. Predicted and adjusted R-squares should be within 0.20 of each other. Contour plots for the response were drawn for determination of the optimal variable settings. The response was the amount of coacervate formed. Equations in terms of actual factors:

*Coacervate (g) = -1.45544 + 0.00524 * A - 0.04747 * B + 0.04096 * C + 0.00024 * B² - 0.000016 * B * C*

Table 1: Design runs of the central composite design experiments and response results

Run	Factors			Response
	A:X_1 Water (g)	B:X_2 Maltodextrin (g)	C:X_3 Cremophor®EL (g)	Coacervate (g)
1	125	95	67.5	1.5
2	150	70	35	1.2
3	125	95	35	0.8
4	150	95	67.5	1.6
5	100	70	100	2.8
6	100	120	100	2.0
7	125	95	67.5	1.6
8	125	95	67.5	1.6
9	125	95	67.5	1.5
10	150	120	35	0.8
11	125	70	67.5	2.1
12	150	70	100	2.2*
13	100	95	67.5	1.5
14	125	95	100	2.5
15	125	95	67.5	1.5
16	100	70	35	0.8
17	125	95	67.5	1.4
18	150	120	100	2.2
19	125	120	67.5	1.5
20	100	120	35	0.5

Analysis of variance of the response (**Table 2**) indicated that the response surface model developed for amount coacervate was significant, without significant lack of fit. In addition, the model summary statistics for the selected significant models were detailed (**Table 2**). It can be observed that for coacervate, R^2, predicted R^2 and adjusted R^2 were in good agreement, resulting in reliable models.

Table 2: ANOVA – Influence of concentration the amount of water (g), Maltodextrin c*01983 (g) and Cremophor®EL (g) on the response factors

Response factor	Model F-value	Prob>F	Lack of Fit F-value	Prob>F
Coacervate	153.17	<0.0001	1.71	0.2870
	St.dev.	R^2	Adjusted R^2	Predicted R^2
Coacervate	0.09	0.9833	0.9769	0.9611

4.2.4. Production of a solid dosage form via spray-drying

The two-phase liquid was again homogenized under high shear and spray-dried in a Mobile Minor spray-dryer (GEA Niro) using a two-fluid nozzle. The spray-dryer was operated in co-current flow at a constant inlet temperature of 130°C and an outlet temperature of 60°C. The liquid feed rate was 25 ml/min, controlled by a peristaltic pump. After spray-drying a free-flowing powder was obtained.

4.2.5. Production of a solid dosage form via granulation

The two-phase liquid was separated in an upper and lower phase. The upper phase was mixed in a 1:3.5 (w/w) ratio with tapioca dextrin in a mortar and allowed to dry at 40°C (to allow the residual water to evaporate). Finally, dry granules were obtained, indicating that the tapioca dextrin was capable of absorbing the liquid Cremophor®EL.

4.2.6. Characterization

4.2.6.1. Fluorescence microscopy

Fluorescence microscopy was used to determine the distribution of the respective components in the respective phases during coacervate formation. For this purpose, the amount of material was down-scaled with a factor 10. Fluorescent labeling of the febantel/Cremophor® EL phase was performed by addition of 1 μL of a 10 mg/mL rhodamine B octadecyl ester solution in ethanol, after complete dissolution of the febantel in the Cremophor®EL. The liquid was thoroughly vortexed to ensure homogeneous distribution of the fluorescent dye. The aqueous maltodextrin phase was fluorescently labeled by addition of 10 μL of a 1 mg/mL FITC-dextran solution in water. The liquid was thoroughly vortexed to ensure homogeneous distribution of the fluorescent dye. The formation of the coacervate was performed as described in section 4.2.2. Fluorescence microscopy images were recorded on Leica DM2500P microscope equipped with 10 x and 63x oil immersion objectives and a DFC360FX CCD camera. Hot stage microscopy was performed on the same microscope, but then equipped with a THMS600 heating stage.

4.2.6.2. ^{1}H-NMR spectroscopy

^{1}H-NMR spectra were recorded separately in D_2O and/or MeOH-d_4, depending on the solubility in the respective solvent of the components of interest. The ^{1}H NMR spectra were recorded on a 300 MHz Varian Inova spectrometer. Spectra were acquired with a 90° pulse of 5 μs, a spectral width of 4500 Hz, an acquisition time of 5s, a preparation delay of 7s and 100 accumulations. The chemical shift axis was calibrated by means of the D_2O resonance at 4.72 ppm from tetramethylsilane.

4.2.6.3. X-ray diffractometry

X-ray diffractograms of the spray-dried powder and granules were recorded on a D5000 Cu Kαdiffractor (λ=0.154 nm) (Siemens, Karlsruhe, Germany) with a current of 40 mA in the angular range of $10° < 2\theta < 60°$ using a step scan mode (step width 0.02°, counting time 1 s/step).

4.2.7. *In vitro* drug release and stability

In vitro release of febantel from the respective formulation was evaluated using a VanKel VK7010 dissolution bath connected to a VK8000 automatic sampling station. Demineralized water (900ml) at room temperature (20±2°C) was used as dissolution medium. 5 mL samples were withdrawn after 5, 10, 15, 20, 30, 45 and 60 minutes and the febantel concentration was determined by measuring the UV-VIS absorbance at 280 nm using a Shimadzu UV-1650 PC spectrophotometer.

To evaluate the effect of storage on the dissolution rate and solid state of the spray dried powder, the powder was stored for 6 months in sealed aluminum bags at different environmental conditions (25°C/ 60% RH, and 40°C/75% RH). Afterwards effect of the storage on the *in vitro* release kinetics of febantel was assessed as described above.

Evaluation in a drinking water system for live stock (**Figure 2**) was performed to compare the spray-dried powder with the commercial febantel formulation Rintal (granules containing 10% febantel, Bayer), regarding their ability to provide a constant febantel level in the drinking water. Therefore, 8.12 g of spray dried powder, containing 950 mg of febantel, and 9.5 g of Rintal granules, also containing 950 mg of febantel, were added to a 250 L drinking water reservoir and allowed to dissolve for 10 min. Subsequently, the valve between the reservoir and the piping system, that connect the drinking nipples, was opened and samples were taken from the drinking nipples every 15 min for a period of 6h and analyzed for their febantel content by UV-VIS spectrophotometry.

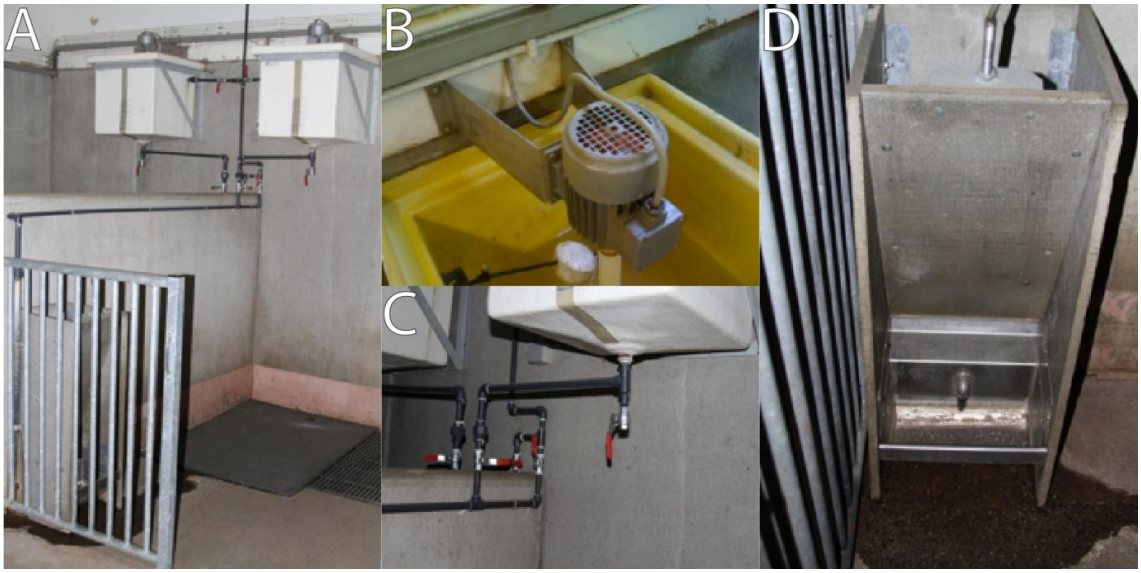

Figure 2: (**A**) drinking water system used in drinking water medication, (**B**) detail of the homogenization system. (**C**) PVC tubing, (**D**) drinking nipple.

4.2.8. In vivo evaluation

The bioavailability of febantel in the spray dried formulation and in Rintal as reference formulation was evaluated in pigs (n=6, body weight 20-30 kg, age ±9 weeks). The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine (Ghent University). The animals were individually housed in the live stock facilities of the Faculty of Veterinary Medicine at Ghent University. After one week acclimatization the trial was conducted as a cross-over design with a 1 week wash-out period. All animals were weighed individually one day before administration of the formulation. A dose of 5 mg febantel per kg bodyweight was orally administered to the pigs. Therefore, the required amount of spray-dried powder (or Rintal granules) was added to 50ml water and then administered via a canule into the pig's stomach. A volume of 50ml of water was used to rinse the canule. During drug administration the animals were immobilized.

Blood samples were taken via the vena jugularis using a Venoject® system. A blood sample (3-5ml) was taken prior to each treatment and 0.5, 1, 2, 4, 8, 12, 24, 32 hours after administration of the febantel formulations. After sampling, the blood was centrifuged (4°C, 800g_n, 10 min) to separate the plasma. After centrifugation, the supernatant was transferred into another test tube and frozen at -18 °C.

The plasma concentrations of both febantel metabolites (fenbendazol and oxfendazole) were determined using two separate HPLC methods developed [3]. The samples were prepared as follow: 40µL of the internal standard solution containing 10µl/mL of flubendazol (Janssen Pharmaceutica, Beerse, Belgium) in dimethylacetamide and 40µL of a 0.1M $ZnSO_4$ solution were added to 150µL of plasma sample (mixed for 2 minutes centrifuged 1500g_n for 3min). A 50µL aliquot of supernatant was injected (automatic injection system type L-7200, Hitachi, Tokyo, Japan) and eluted white a mobile phase consisting of an aqueous buffer (0.01M citric acid anhydrous and 0.004M disodium hydrogenium phosphate) and acetonitrile 60/40 (v/v) for the elution of fenbendazol and a mobile phase consisting of an aqueous buffer (0.01M citric acid anhydrous and 0.004M disodium hydrogenium phosphate) and acetonitrile 72.5/27.5 (v/v) for the elution of oxfendazole. A reversed phase column (C-18, 5µm, 12.5cm x 4mm, Lichrocar 125-4, Merck, Darmstadt, Germany) was used for both analyses. The pump (Type L-7100 Merck Hitachi, Tokyo, Japan) flow rate was 1.0ml/min at room temperature. The variable wavelength UV-detector (L-7400, Merck Hitachi, Tokyo, Japan) was set at 290nm. Calibration curves were performed using known concentrations of fenbendazol (Hoeschet, Brussels, Belgium),

oxfendazol (Syntex Research, Palo Alto, Ca, USA) and febantel in drug free plasma collected from pigs.

Pharmacokinetic parameters (T_{max}, C_{max} and AUC_{0-t}) were calculated using the Microsoft® Excel based program Pk Functions (Joel I. Usansky, PhD, Atul Desai, MS and Diane Tang-Liu, PhD, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA 92606, USA) [4].

4.3. RESULTS AND DISCUSSION

4.3.1. Formation of a liquid febantel/Cremophor®EL phase

A first key step in our formulation strategy was the dissolution of the poorly water-soluble drug in a liquid surfactant phase. Therefore, febantel (**Figure 1A**) was dissolved in the liquid surfactant Cremophor®EL. Cremophor®EL is a non-ionic surfactant obtained by reacting ethylene oxide with the hydroxyl groups of castor oil, leading to the formation of poly(ethylene glycol) chains onto a hydrophobic backbone. It has an HLB value between 12 and 14 and a critical micelle concentration (CMC) of 0.02% [8]. Cremophor®EL was heated to 130°C under stirring, which is slightly above the melting point of febantel (i.e. 129 °C), followed by the addition of crystalline febantel powder under stirring. Note that thermal degradation of febantel only starts at 175 °C and thermal degradation of Cremophor®EL at 300°C. Visual observation allowed to determine that around 2.5 min was required to reach complete solubilization of febantel in Cremophor®EL. An additional 2 min stirring at 130 °C was performed to ensure homogenization of the liquid. **Figure 3A1-3A2** depicts the dispersion of febantel in Cremophor® EL before and after heating. The experiment was repeated on a small scale and the behavior of the febantel was followed by hot stage microscopy under bright field and polarized light. As shown in **Figure 3B**, the crystalline febantel gradually dissolves in the Cremophor EL medium with no residual crystallinity remaining.

The liquid febantel/Cremophor®EL formulation was stable over prolonged periods of time (48h) without drug precipitation to occur. However, when this liquid was poured in aqueous medium a translucent gel was formed that readily became opaque (**Figure 3A3**). **Figure 3C** shows optical microscopy images taken at 1 min time intervals of this gelation process. Drug precipitates were formed that gradually grow over time. The optical microscopy image in **Figure 3C4**, recorded under polarized light, clearly demonstrated the crystalline nature of these precipitates. Most likely, upon contact with water the drug precipitation occurs faster than the formation of Cremophor®EL micelles that could stabilize the febantel in an amorphous state in

their hydrophobic cavities. As this will severely reduce the drug dissolution kinetics we sought to optimize our formulation further by transforming the Cremophor®EL into a micellar state before febantel precipitation occurs, thus allowing stabilization of the febantel upon contact of the febantel/Cremophor®EL solution with water.

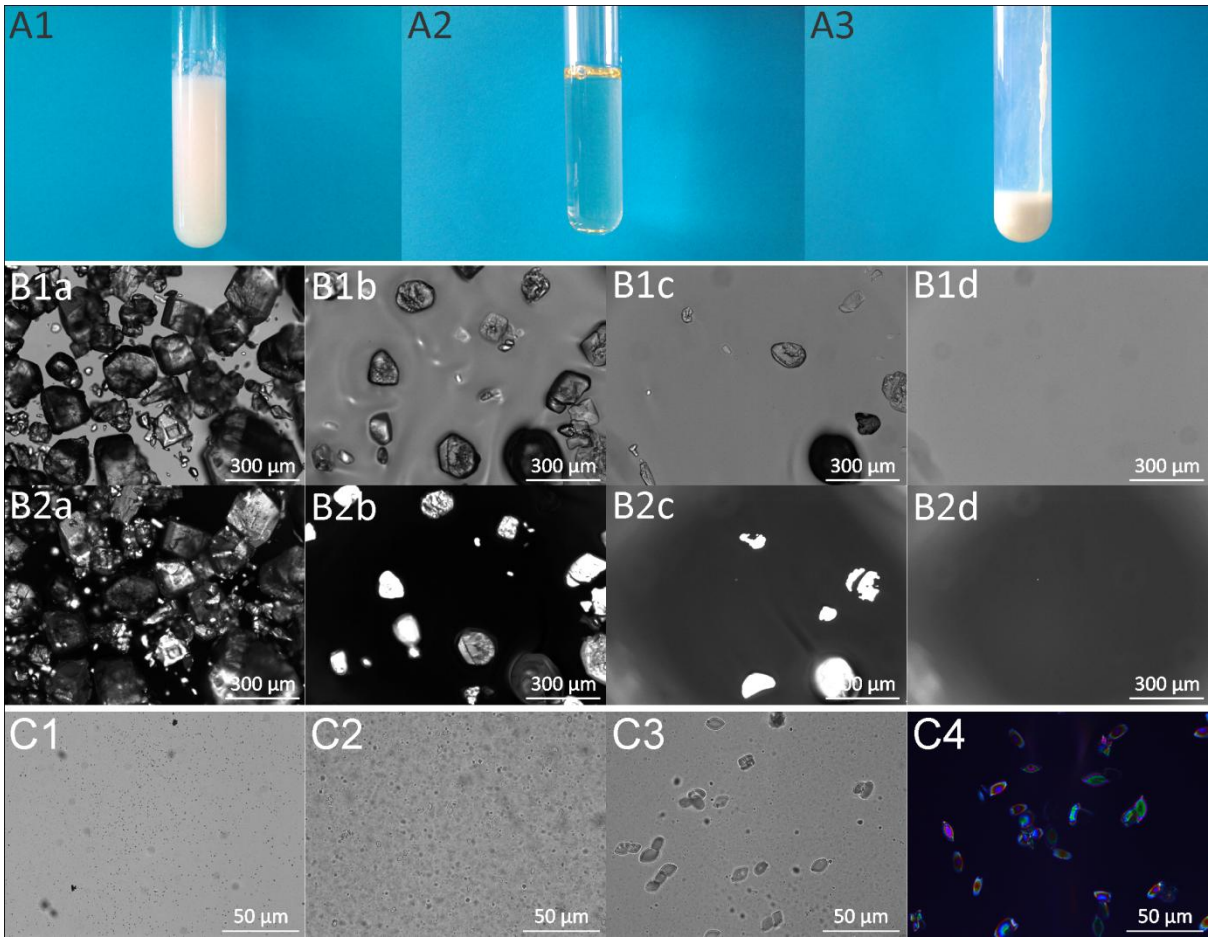

Figure 3.(**A**) Photographs of test tubes containing a (A1) febantel/Cremophor®EL suspension, (A2) febantel/Cremophor®EL solution obtained after heating at 130 °C during 2 min and (A3) gel obtained by addition of water to the febantel/Cremophor®EL solution. (**B**) Hot stage microscopy images taken at 1 min time intervals after reaching 130 °C under (B1) bright field and (B2) polarized light during the heating of the febantel/Cremophor® EL suspension. (**C**) Bright field microscopy images taken at 1 min intervals after the addition of water to the febantel/Cremophor®EL solution. Panel C4 was is identical to C3, but recorded under polarized light to evidence the presence of crystallinity in the formed precipitates.

4.3.2. Formation of a liquid coacervate

To resolve the issue of instantaneous drug precipitation upon contact of the febantel/Cremophor®EL solution with water, we sought a way to delay the hydration kinetics of the febantel/Cremophor®EL phase. We reasoned that this would lead to a slow transformation of the Cremophor®EL to a micellar state which would give sufficient time to the febantel to be distributed in the hydrophobic micellar cavities [9-11]. To achieve this goal, we poured the febantel/Cremophor®EL solution into a concentrated (i.e. 30 % (w/w)) aqueous maltodextrin solution. It is known that at elevated concentration, aqueous solutions of macromolecular species (as are Cremophor®EL and maltodextrin) are immiscible and phase separate [12]. Therefore, we hypothesized that addition of an aqueous macromolecular phase would therefore prevent immediate dissolution of the Cremophor®EL, which is also a macromolecule, thus preventing instantaneous precipitation of febantel, but would rather slowly draw water to the Cremophor®EL phase, allowing micelles to form, stabilizing the febantel in amorphous state [13-15].

As good water-soluble polymer maltodextrin (**Figure 1B**) was chosen because this component could in a subsequent formulation step serve as carrier material to obtain a solid dosage form after spray-drying by absorbing the Cremophor®EL. The febantel/Cremophor®EL solution was added to a 30 % (w/w) aqueous maltodextrin solution under vigorous stirring. Immediately an opaque liquid formed (**Figure 4A1**), although both maltodextrin and Cremophor®EL are both readily water-soluble. Optical microscopy revealed that the opacity of the liquid is due the formation of an emulsion and not due to precipitation of febantel. This clearly demonstrates that the presence of maltodextrin the aqueous phase prevents the Cremophor®EL to dissolve in the aqueous phase. When left to stand under static conditions, both phases demixed (**Figure 4A2-4A3**) and a translucent lower phase (+/- 35 % of the total volume) and a slightly opaque yellowish upper phase (+/- 65 % of the total volume) were formed. This two-layer system was stable over a period of several months without precipitation of febantel to occur. It is noteworthy that in absence of febantel a similar phase behavior was observed. Importantly, when the two-phase liquid is diluted in aqueous medium, a clear solution is readily obtained (**Figure 4B**) and no drug precipitation is observed.

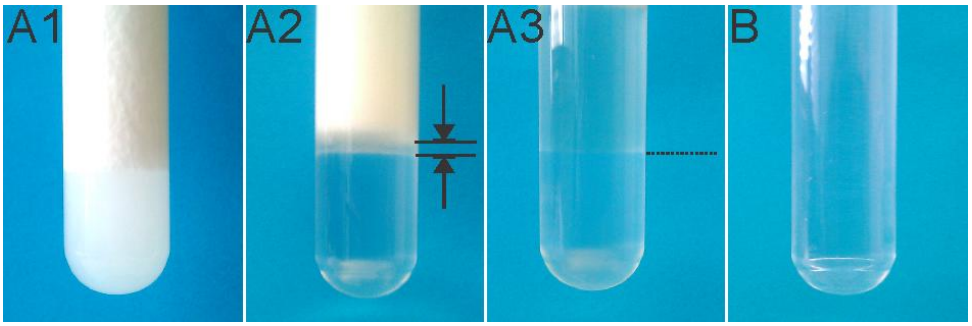

Figure 4. Photographs of test tubes containing the mixture of febantel/Cremophor®EL with maltodextrin (**A1**) directly after mixing, (**A2**) after 2h of demixing, (**A3**) after complete demixing.(**B**) Photograph of after addition of water to the coacervate phase.

In a next series of experiments, we aimed to investigate the constitution of both phases and the phenomena that play a role in their formation. Therefore, we spiked before mixing the febantel/Cremophor®EL and the aqueous maltodextrin phase, with a fluorescent dye. Rhodamine B octadecyl ester (red fluorophore) was used as hydrophobic dye and is expected to be distributed in the same phase as hydrophobic drug febantel while FITC-dextran (green fluorophore) was used as hydrophilic dye that is expected to be distributed in the same phase as the maltodextrin. Upon mixing of both phases, foam was formed (**Figure 5A**) which slowly phase-separated over time, similarly as described above for the non-fluorescent labeled sample. After 8h, two distinct phases were formed with the lower phase stained yellow and the upper phase stained red. This already gives a strong indication that the maltodextrin will be distributed in the lower phase while the febantel will be distributed on the upper phase.

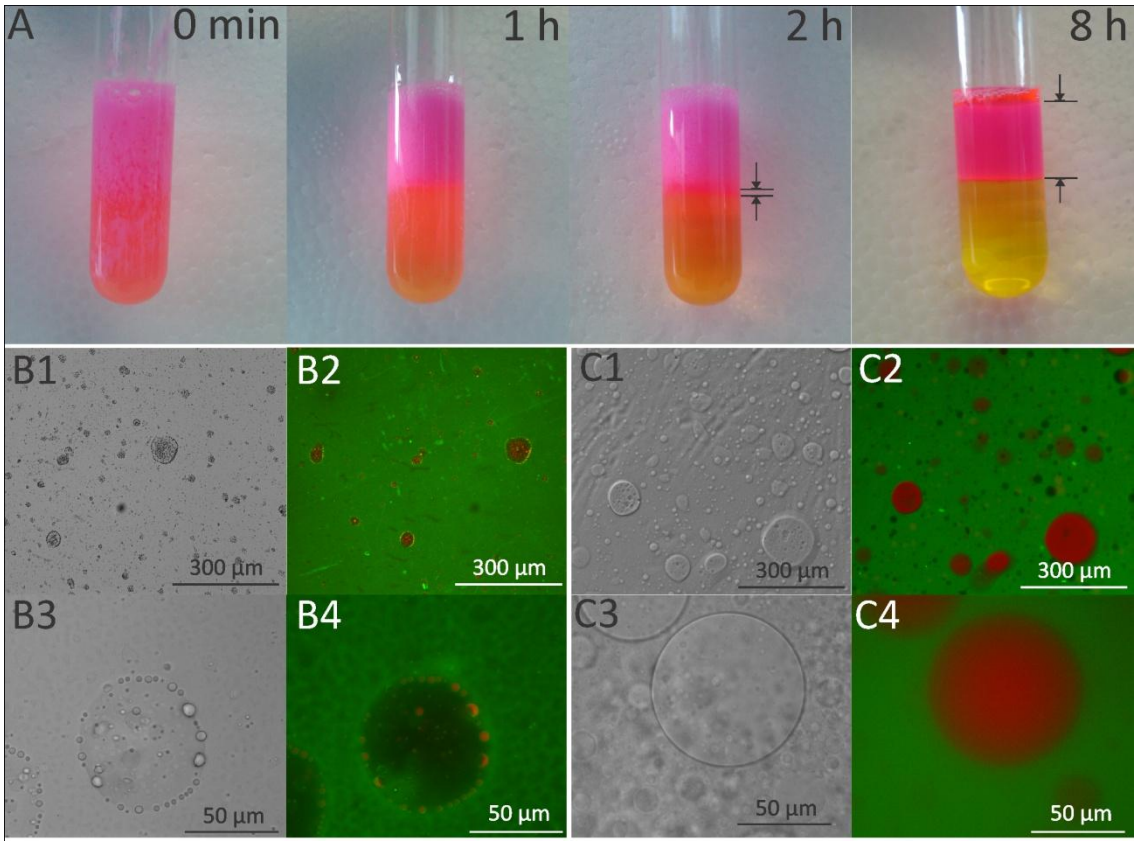

Figure 5.(A) Photographs showing the unmixing of the two-phase system. The red color is due to rhodamine B octadecyl ester (i.e. a hydrophobic dye), staining the Cremophor®EL phase while the yellow color is due to FITC-dextran (i.e a hydrophilic dye), staining the aqueous maltodextrin phase. The arrows on the images recorded after 2h and 8h represent the formation of the coacervate phase. **(B-C)**Optical microscopy images at low (10x; panels 1 and 2) and high (63x; panels 3 and 4) magnification recorded in DIC (panels 1 and 3) and fluorescence (panels 2 and 4) mode, **(B)** immediately upon mixing of the febantel/Cremophor EL and maltodextrin phases and **(C)** after 8 h and re-homogenization of the system.

To gain more insight into the formation of both phases, optical microscopy images were recorded immediately upon mixing of the febantel/Cremophor®EL and the maltodextrin phase, and after phase separation and re-homogenization. As shown in **Figure 5B**, immediately upon mixing of both phases an emulsion was formed with red fluorescent febantel/Cremophor®EL droplets being dispersed in a continuous green fluorescent maltodextrin phase. Within a short time, the red fluorescent febantel/Cremophor®EL droplets agglomerated, forming a new coacervate phase. This third phase likely originated from the hydration of the Cremophor®EL droplets by water that was transported from the aqueous maltodextrin phase to the febantel/Cremophor®EL phase. Subsequently, the larger coacervate droplets coagulated and formed the upper phase of a biphasic liquid (**Figure 5C**).

These observations indicated indeed that upon contact with the aqueous maltodextrin phase, the febantel/Cremophor®EL phase withdraws water from the aqueous maltodextrin phase. Subsequently, upon contact with the attracted water, the Cremophor®EL is expected to form a micellar phase, distributing the febantel in the hydrophobic domains of the micelles. To confirm this hypothesis we analysed the composition of the lower and upper phase by ^{1}H-NMR spectroscopy. Therefore, the two-phase system was prepared using deuterated water (D_2O) instead of H_2O. **Figure6** shows the NMR spectra of the upper (**Figure 6A**) and lower phase (**Figure 6B**) as well as the spectra of the individual components. As febantel is poorly water-soluble, but soluble in methanol, and maltodextrin is soluble in water but not in methanol we recorded the ^{1}H-NMR spectra of the upper and the lower phase both in deuterated water (D_2O) and in deuterated methanol (d_4-MeOH). This should allow us to detect e.g. febantel in the most hydrated phase and maltodextrin in the least hydrated phase. From the data in **Figure 6,** it is clear that the upper phase contained quasi all Cremophor®EL and febantel while no Cremophor® EL and febantel was detected in the lower phase. Furthermore, maltodextrin was abundantly present in the low phase but also to a limited extent in the upper phase. Also D_2O was observed in the spectrum of the upper phase that was recorded in d_4-MeOH, this attributed to the hypothesis that the Cremophor®EL was present in a micellar state with the febantel solubilized in the hydrophobic micellar domains.

Due to the detection limit of NMR spectroscopy, UV-VIS spectroscopy was used to determine the febantel concentration in the respective phases. We found that 59.4 g (i.e. 99 % of the initial amount of febantel) was present in the upper phase, supporting our NMR observations. Next, we aimed to assess whether Cremophor®EL is present in the upper phase as jammed micelles orienting their hydrophilic domains to a thin continuous water phase that surrounds the micelles or contrary, that the Cremophor®EL is present as inverted micelles with their hydrophilic domains oriented inwards. In the latter case, the febantel would be distributed in what could be considered as the continuous phase. We isolated the upper coacervate phase prepared with tap water and measured the conductivity of this phase, as commonly performed to determine the oil-in-water/water-in-oil nature of emulsions. From the value of 1.4 mS (vs. 0.3 mS for an water-in-oil emulsion of silicon oil in tap water, stabilized by Cremophor®EL) is clear that Cremophor®EL is present in micellar state with the hydrophilic domains oriented towards a continuous aqueous phase.

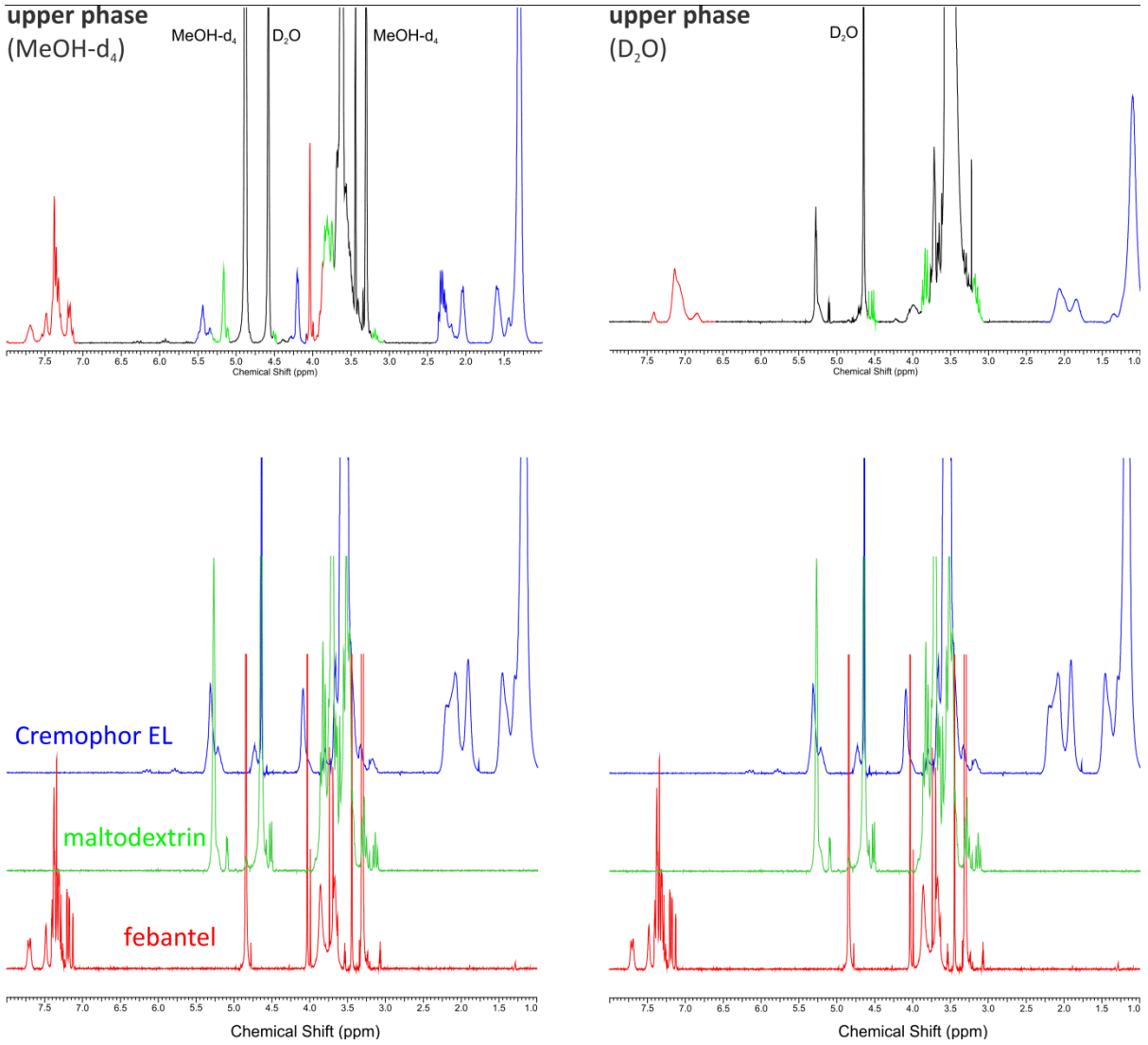

Figure 6. ^{1}H-NMR spectra of the upper and lower phase of the biphasic liquid recorded in respectively deuterated methanol (MeOH-d_4) and deuterated water (D_2O). To allow proper comparative analysis, the spectra of the single components are colored.

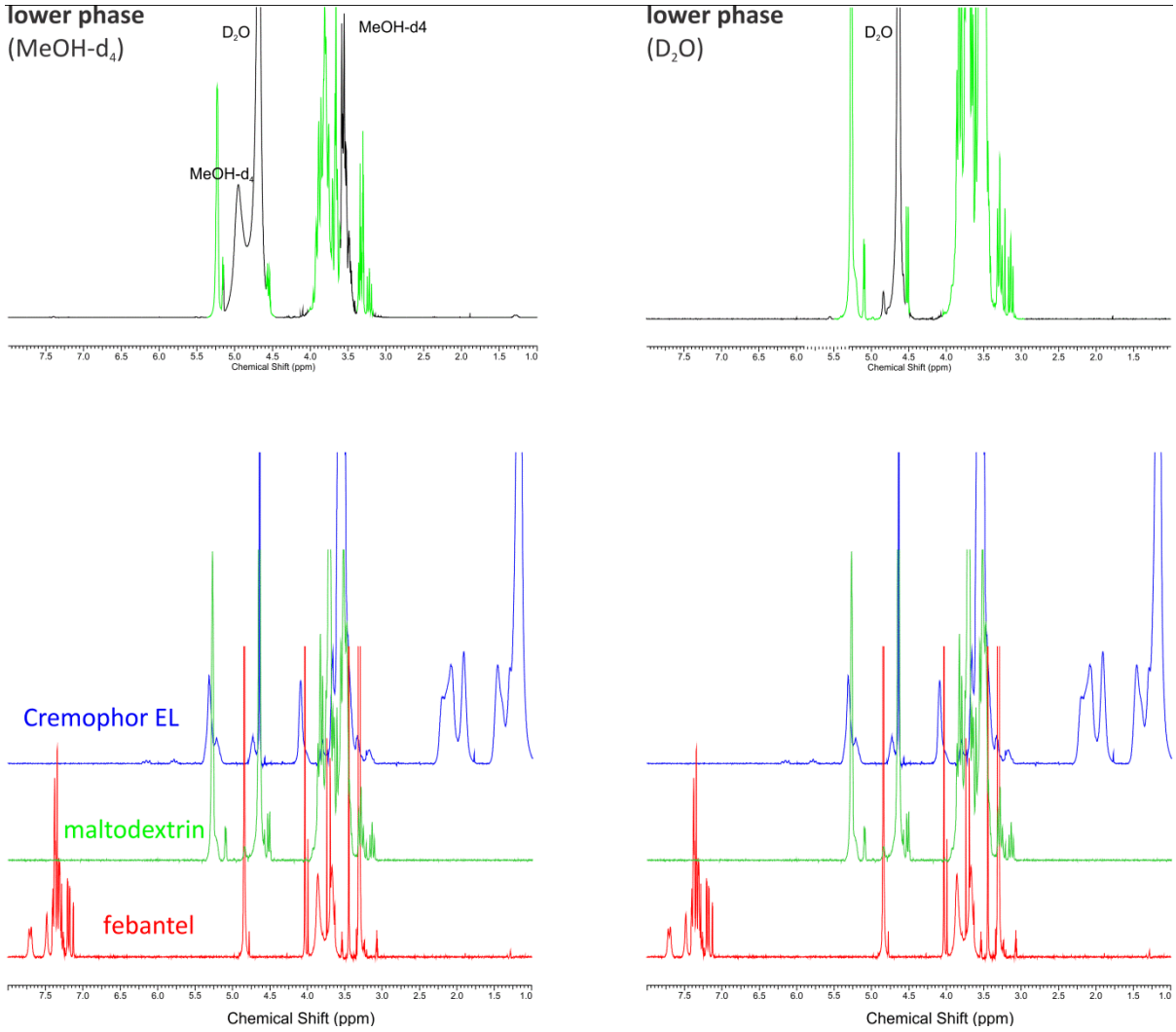

Figure 6 (Cont.). ^{1}H-NMR spectra of the upper and lower phase of the biphasic liquid recorded in respectively deuterated methanol (MeOH-d_4) and deuterated water (D_2O). To allow proper comparative analysis, the spectra of the single components are colored.

The above-mentioned observations allowed us to postulate a model for the formation of the coacervate as schematically shown in **Figure 7**. First, upon addition of the febantel/Cremophor®EL solution to the aqueous maltodextrin solution, an emulsion is formed with febantel/Cremophor®EL as discrete phase and maltodextrin/water as continuous phase. Subsequently, over time water is drawn through osmosis in the febantel/Cremophor®EL droplets that also coagulate and form an upper phase. Meanwhile, the Cremophor®EL molecules orient as micelles and keep the febantel dissolved in their hydrophobic cavity. These micelles are presumably in a jammed state, surrounded by a thin water layer that also contains a relatively small amount of maltodextrin.

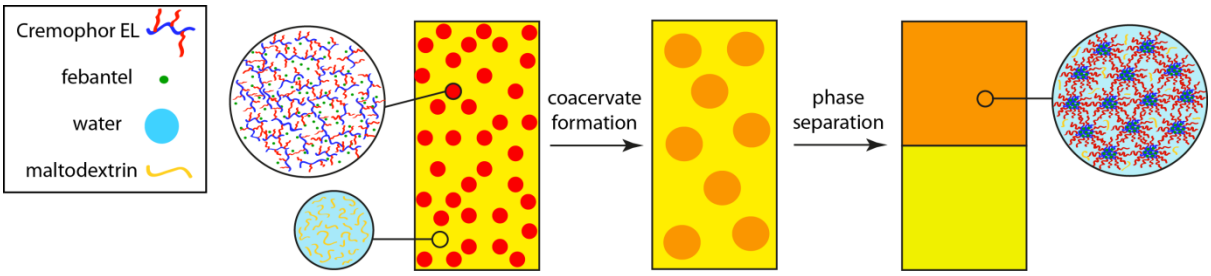

Figure 7. Schematic representation of the formation of the two-phase liquid with the upper liquid being a coacervate phase.

To assess the influence of the water, maltodextrin and Cremophor®EL concentration on the amount of coacervate formed, an experimental design was performed.

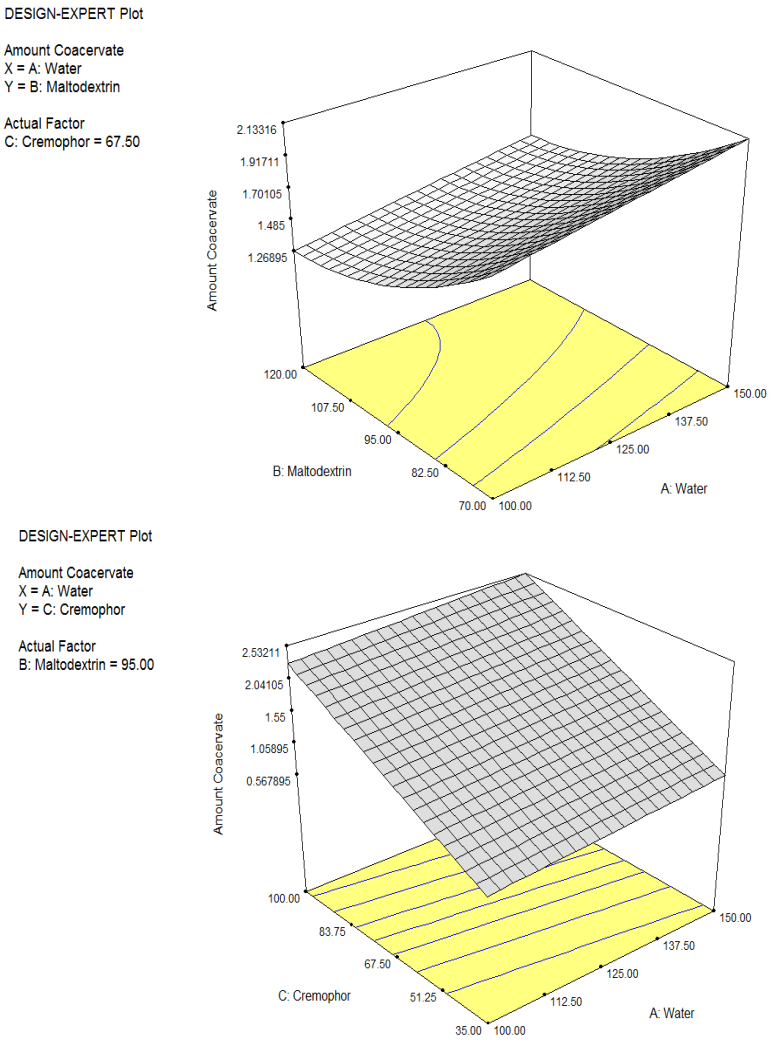

Figure 8. 3D surface plots showing the effect of the amount of water (g), maltodextrin (g) and Cremophor®EL (g) in the quantity of obtained coacervate phase (g).

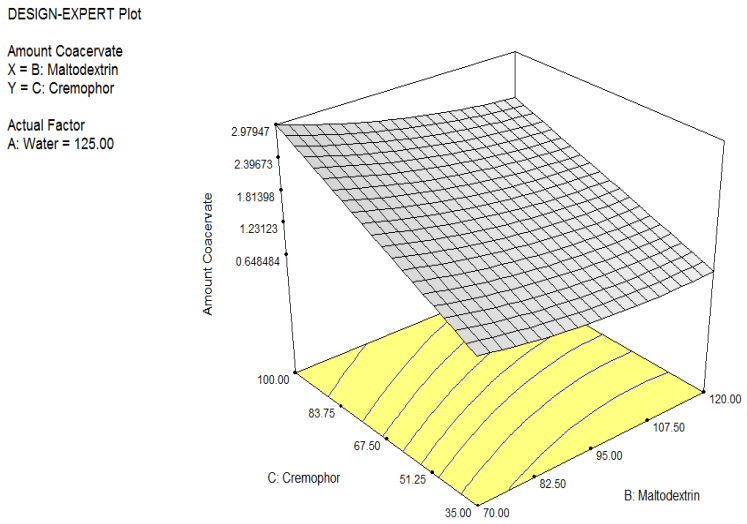

Figure 8 (Cont). 3D surface plots showing the effect of the amount of water (g), maltodextrin (g) and Cremophor ®EL (g) in the quantity of obtained coacervate phase (g).

The resulting density plots are shown in **Figure 8** and indicate that the concentration of Cremophor®EL is the main factor determining the amount of coacervate phase that is formed.

4.3.3. Transformation into a solid dosage form and in vitro release kinetics

A third and final step in our formulation strategy was the transformation of the liquid phase into a solid form. Therefore, two routes were pursued (1) spray-drying of the homogenized two-phase system and (2) granulation using the upper coacervate phase. In the first strategy, maltodextrin played a role as carrier, intended to absorb the liquid Cremophor®EL upon evaporation of the water. In the case of granulation, tapioca dextrin was used as absorbing carrier material to obtain solid granules. To analyze whether these approaches allowed retaining the febantel in an amorphous state, X-ray diffraction was performed on the spray-dried powder and the granules. **Figure 9** shows the X-ray diffractograms of the crude components. Whereas febantel is highly crystalline, the produced solid dosage forms, either via spray-drying or granulation, completely lacked the crystalline peaks, indicating that upon processing into solid form, febantel was retained in amorphous state.

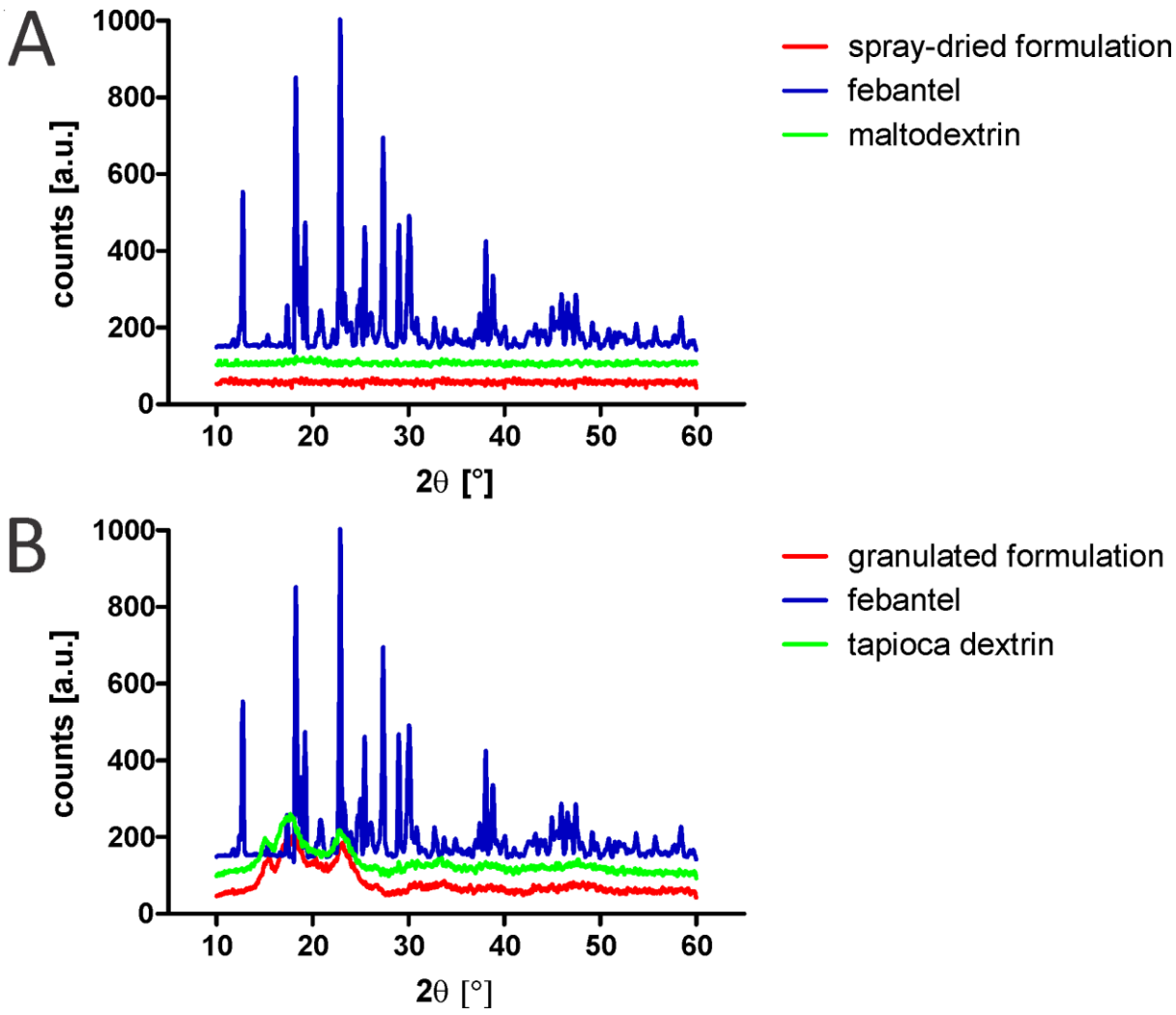

Figure 9: (**A**) X-Ray diffractograms of febantel, tapioca dextrin and febantel formulated by granulation of the upper coacervate phase with tapioca dextrin. (**B**) X-Ray diffractograms of febantel, maltodextrin and febantel formulated by the homogenized two-phase liquid.

Subsequently, *in vitro* drug release kinetics were investigated in dissolution vessels at room temperature and compared to Rintal and a physical mixture of the respective components. Rintal is a commercial febantel formulation consisting of granules that contain a drug load of 10% formulated with sodium docusate and saccharine. As shown by the cumulative release curves in **Figure 10A**, drug release from both the spray dried powder and the tapioca dextrin granules was fast and complete compared to the physical mixture and Rintal. The poor performance of Rintal in this experimental *in vitro* set-up is likely due to the crystalline state of the febantel within this formulation whereas the febantel in our coacervate-based formulation is in amorphous state.

Formulations containing amorphous drug are inherently prone to drug recrystallization. Therefore, we evaluated the long-term stability of the spray-dried powder. The powder was filled in sealed aluminium bags and stored at either 25°C (relative humidity (RH) of 60%) and 40°C (relative humidity (RH) of 75%). As shown in **Figure 10B**, drug release kinetics were not

influenced by this long period of storage. Furthermore, we recorded the X-ray diffractograms of the respective samples and no crystalline peaks could be detected. These data indicated that the formulation successfully retains febantel in amorphous state for prolonged periods of time.

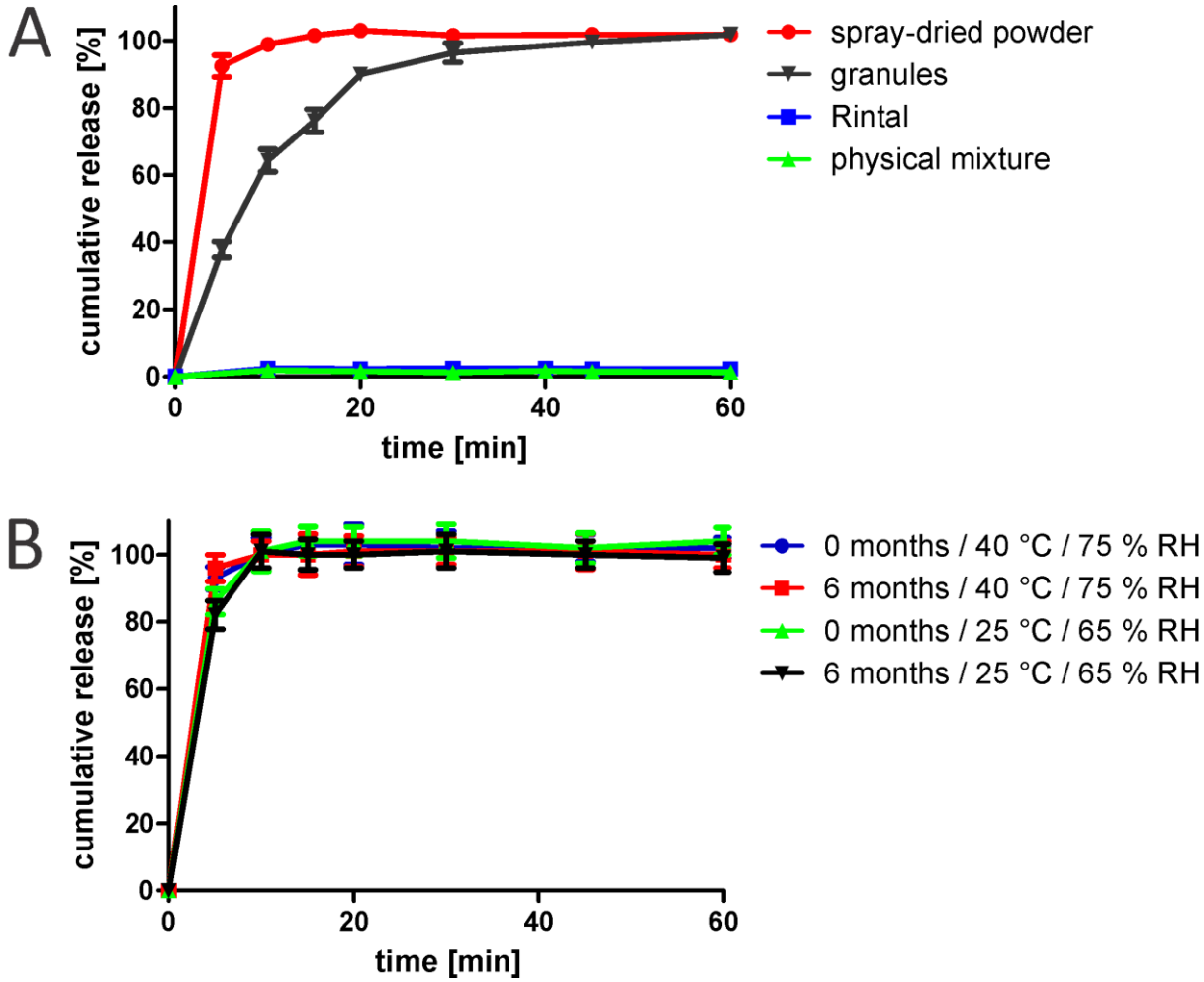

Figure 10. (A) Cumulative febantel release from the spray-dried and granulate formulations. As control formulations, we used a physical mixture of the components as well as a commercial febantel formulation (i.e. Rintal) **(B)**Cumulative febantel release curves before and after storage for 6 months at constant temperature and relative humidity.

4.3.4. Evaluation of the formulation for drinking water medication

For application in drinking water medication, it is essential that upon addition of the formulation to the drinking water reservoir it readily distributes over the entire reservoir, allowing constant febantel levels to reach the drinking nipples. Therefore, a series of experiments were performed to compare the spray-dried powder formulation with the commercial Rintal formulation. Both formulations were added to a drinking water reservoir and after 10 min (allowing the formulation to dissolve/disintegrate), non filtered samples were withdrawn from the drinking nipples at regular time intervals and analyzed for their febantel content. As shown in **Figure 11**, the spray-dried formulation provided a quasi-constant drug level over a period of 6 hours. Contrary, large alterations in drug levels were observed for the Rintal formulation. This is likely due to the presence of non-water soluble granules, leading to inhomogeneous drug distribution in the drinking water reservoir.

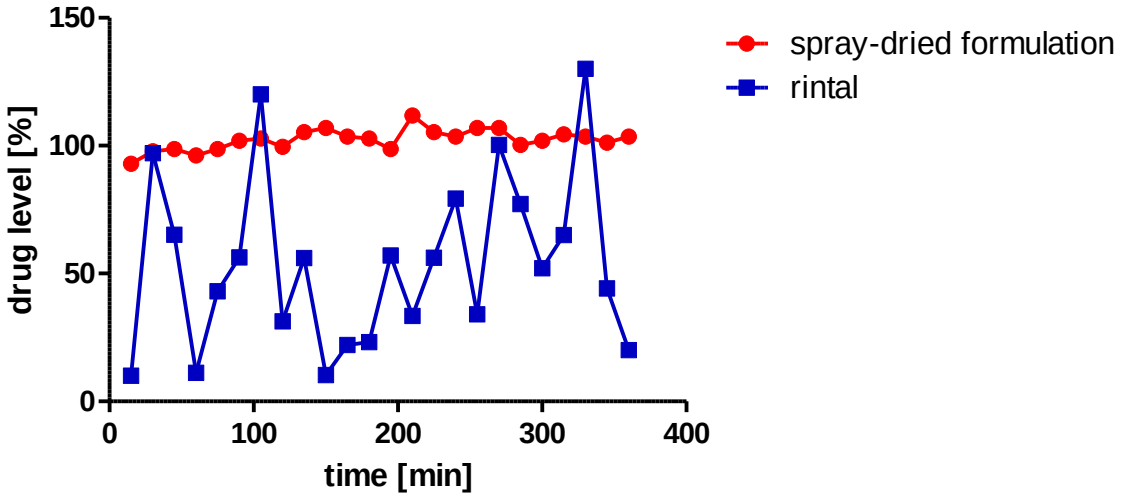

Figure 11: Febantel levels over time at the drinking water nipples 10 minutes after the addition of respectively the spray-dried formulation and Rintal to the drinking water reservoir. 100 % drug corresponds to the theoretical maximum of febantel that can be released in the drinking water reservoir upon complete dissolution of febantel.

4.3.5. *In vivo* evaluation

Pigs received either the spray dried powder formulation or the commercial Rintal reference formulation, in both cases at a dose of 5 mg of febantel per kg bodyweight. The formulations were first dispersed in water and administered via a canule directly into the stomach of the animals. Subsequently, blood samples were withdrawn at regular time intervals and analyzed for the respective metabolites of febantel, being febendazole and oxfedazole.

The individual maximal plasma concentrations (C_{max}) and the area under the curve (AUC)-values are shown in **Table 3** and the concentration of the metabolites over time in blood samples shown in **Figure 12**. Statistical analysis revealed no significant difference between the T_{max}, C_{max} and AUC_{0-t} levels of the spray-dried formulation and Rintal. For fenbendazole the mean difference between the formulations was 90 (95% CI -400 to 220) and for oxfendazole the mean difference between the spray-dried formulation and Rintal was 829 (95% CI -849 to 2507), while the median change in T_{max} values for oxfendazol and fenbendazol between the spray dried formulation and Rintal was not significantly different from 0 (i.e. respectively 0.141 and 0.236).

Table 3: Statistical evaluation of pharmacokinetic parameters (T_{max}, C_{max} and AUC_{0-t}) of the metabolites fenbendazole (Fen) and oxfendazole (Oxf) in blood plasma.

	Drug metabolite	**spray dried formulation (µg/L)**		**Rintal (µg/L)**		**95% CI**			
		Mean	SD	Mean	SD	Mean dif.	Lower	Upper	p-value
AUC_{0-}	Fen	645	229	735	204	-90	-400	220	0.4644
	Oxf	5655	1553	4826	837	829	-849	2507	0.2419
C_{max}	Fen	143	77	61	3	82	-28	191	0.1064
	Oxf	424	137	276	44	148	-20	316	0.0705

		Median	**Min.-Max.**	**Median**	**Min. - Max.**	**Nonparametric Wilcoxon signed rank test p-value**
T_{max}	Fen	4	2-8	6	4-12	0.236
	Oxf	0.5	0.5-4	2	2-8	0.141

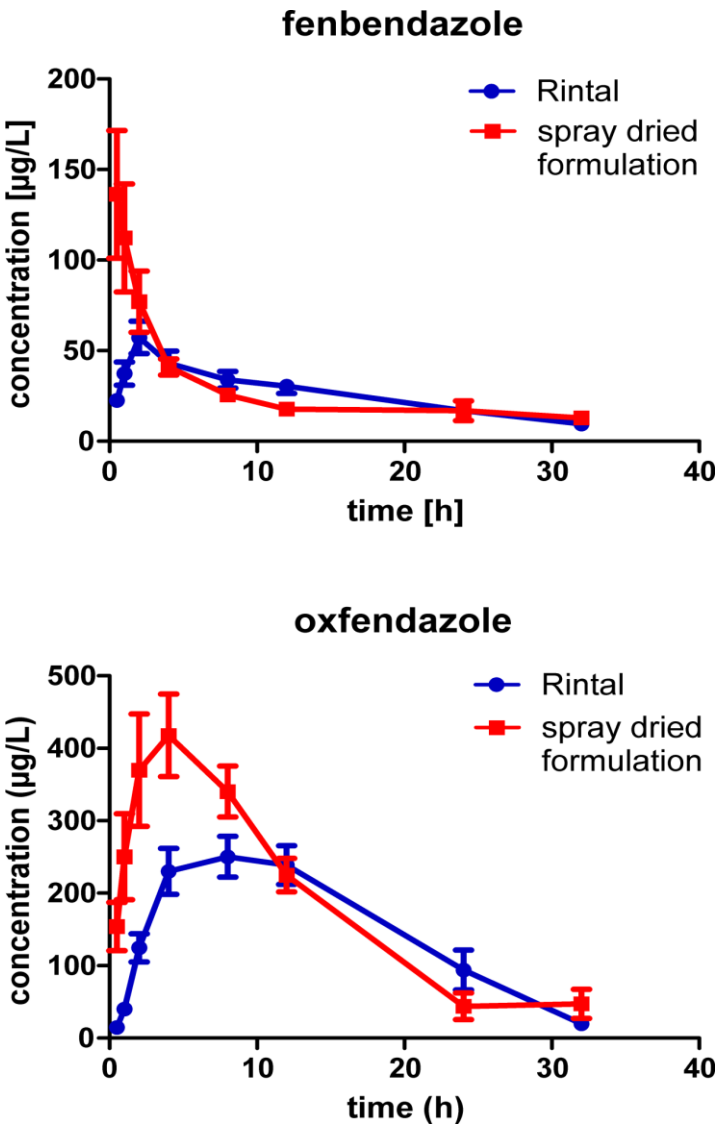

Figure 12: Plasma concentration profiles for both febantel metabolites (fenbendazole and oxfendazole) after administration of respectively the spray-dried formulation and Rintal.

4.4. Conclusions

In this work we have introduced drug melting in a liquid surfactant, followed by the formation of a coacervate, as an attractive route to dramatically enhance the dissolution kinetics of poorly water-soluble drugs. Febantel, a crystalline drug to treat helminthic infections in livestock was used as model drug. In a first step, febantel was dissolved in Cremophor®EL, a non-ionic liquid surfactant, and subsequently poured into a concentrated aqueous maltodextrin solution. The presence of maltodextrin avoided instantaneous precipitation of the febantel and induced the formation of a coacervate phase comprising highly concentrated Cremophor®EL micelles. These micelles stabilized the febantel, avoiding recrystallization. Subsequently, the obtained two-phase liquid was further processed into a solid form via either spray-drying or

granulation. Compared to crude febantel and Rintal (a commercial febantel formulation), the formulations produced via coacervation exhibited a dramatic increase *in vitro* dissolution kinetics. Finally, we demonstrated in a realistic veterinary setting, comprising a drinking water reservoir connected to drinking nipples, that the coacervate formulation yielded constant drug levels at the drinking nipples, which was found not to be the case for the commercial Rintal formulation. A first in vivo evaluation did not indicate significant differences in pharmacokinetic parameters between the spray dried formulation and the Rintal reference formulation. However, these experiments were performed by direct administration of the formulations into the stomach of pigs, while more extensive studies are required, involving administration to the formulations to the drinking water reservoirs, to obtain a clear and conclusive view on the potential of the developed formulation strategy.

In conclusion, these data showed that the coacervation formulation strategy shows promise for mass drinking water medication. Furthermore, as the formulation can easily be transformed into a solid state, it also holds potential for further development towards solid dosage forms for oral intake.

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SUMMARY AND GENERAL CONCLUSIONS

One of the major causes of new drug molecules failing to reach the market is poor bioavailability. The introduction of the Biopharmaceutical Classification System (BCS) has provided a basis to categorize drugs based on the two major parameters affecting absorption, being solubility and permeability. Several techniques have been developed to enhance the absorption and bioavailability of poorly soluble and poorly permeable drugs based on the BCS concept. Molecules with poor solubility frequently referred to as BCS Class II (poorly soluble) and Class IV (poor solubility and permeability), possess insufficient aqueous solubility to allow adequate and consistent gastrointestinal absorption ensuring pharmacological effect, and thereby pose additional challenges to drug formulation scientists.

Chapter I gives an overview of the current options available to the formulation scientist to improve the bioavailability of BCS Class II and IV drugs. The majority of the formulation techniques described in this chapter takes advantage of the higher drug solubility in organic solvents to transform the drug compounds into a high-energy amorphous state or into small size crystalline particles in order to significantly enhance drug solubility. However, the use of organic solvents in drug formulation to overcome poor aqueous solubility of drugs has an important drawback. It is unrealistic to assume that organic solvents can be completely eliminated from the final drug product. Usually some small amounts of solvents may remain in the final product, which raises concerns regarding their in vivo toxicity and effect on the physicochemical stability of the active compound. Therefore, this PhD thesis aimed to evaluate different approaches to formulate poorly water- soluble drugs without the use of organic solvents.

Chapter 2 describes a first approach to the drug-solubility issue by formulating the poorly water-soluble drug carbamazepine into tablets, intended for oral intake, with a self-emulsifying excipient to increase drug dissolution. For this purpose, Gelucire ®44/14 was chosen as previous work reporting on the use of Gelucire®44/14 as excipient in drug formulation highlighted its capability to enhance drug dissolution but also mentioned difficulties to formulate Gelucire 44/14 based formulations into immediate-release tablets for oral intake. Due to its low melting point and waxy behavior, Gelucire®44/14 acts as a binder, thereby strongly increasing the disintegration time of tablets produced via compression.

In the pursuit of an immediate release tablet formulation, carbamazepine was first processed with Gelucire®44/14 via either granulation or spray drying and subsequently compressed into tablets. Compression was observed to strongly decrease drug dissolution, relative to the non-tableted granules and spray-dried powder. This could be attributed to the relatively long disintegration time of the tablets. To address this issue, super disintegrating excipients were added to the formulations. Unfortunately, these did not accelerate drug

dissolution since no significant effect on tablet disintegration was observed. Likely this has to be attributed to the binding capacity of Gelucire®44/14. However, fast and complete tablet disintegration was obtained by incorporation of an effervescent mixture in the formulation. In this case tablet disintegration and full drug dissolution was reached within 15 min, thus presenting a suitable immediate release formulation for the poorly water-soluble drug carbamazepine.

Chapter 3 focused on the formulation of drugs (i.e. itraconazole and febantel) with an even lower water-solubility for which the formulation approach, developed in **Chapter 2**, is insufficient. To formulate these drugs, we aimed to produce micro- and nanocrystalline suspensions containing Gelucire®44/14 as additional solubility enhancement agent. For this purpose, two top down and one bottom up approach were developed. The top down approaches involved either ball milling or ultrasound treatment, both in the presence of Gelucire®44/14, to reduce the size of existing drug crystals. Both techniques allowed to significantly reduce the size of the drug crystals, with ultrasound treatment being the most powerful method. Both methods also allow to enhance the dissolution of febantel with the ultrasound treated formulation providing the best dissolution. In case of itraconazole, no influence of both processing techniques was observed, which is likely to be attributed to its even lower water solubility.

In the bottom up approach, itraconazole and febantel nanosuspensions were produced by dissolving the drug in molten Gelucire®44/14. This molten mixture was atomized into cold water. During the atomization, cavitation was induced by ultrasonication. This process yielded milky suspensions in the submicrometer range. Furthermore a fraction of the drug was found to be in amorphous state. The nanosuspensions of both drugs showed enhanced dissolution. In case of febantel drug dissolution was superior to the formulations produced via ball billing or sonication, while in case of itraconazole drug dissolution was obtained, which was not the case for any of the other formulations.

Chapter 4 describes a melt-coacervation technique for the formulation of febantel intended for mass drink water medication for veterinary purposes. In this regard it is advantageous to obtain a solid formulation, as this would not only avoid stability issues related to nanosuspensions, but also offers benefits regarding easy transport, storage and dosing. In a first step in the melt-coacervation approach, febantel was dissolved in liquid micelle-forming surfactant (i.e. Cremophor®EL) under heating. Next, the liquid was added to an aqueous maltodextrin solution under high shear homogenization. Spontaneously, a coacervate is formed, comprising a maltodextrin rich phase and a Cremophor®EL rich micellar phase, with the febantel being distributed within the hydrophobic core of the Cremophor®EL micelles. This phenomenon

is due to the incompatibility between concentrated aqueous solutions of maltodextrin and Cremophor®EL. Further processing via spray drying yielded a dry powder as the excess of maltodextrin was able absorb the liquid Cremophor®EL phase. Alternatively, a dry formulation could also be obtained by granulation of the Cremophor EL rich phase as granulation liquid and tapioca dextrin as solid carrier. Importantly, X-ray diffraction elucidated that both techniques allowed retaining febantel in amorphous state. Drug dissolution was found to be fast and complete, with the spray dried formulation performing the best. The spray-dried formulation showed long-term stability and could provide constant drug levels in a drinking water system, whereas large drug fluctuations were observed for the commercial Rintal formulation. Preliminary *in vivo* studies in pigs, further demonstrated the potential of the melt-coacervation technique.

Summarizing, this PhD thesis explores several solvent-free approaches to enhance the dissolution of poorly water-soluble drugs. For this purpose it was found essential to use bio-compatible surface-active agents and to reduce the particle size of the drug crystals. Especially the formation of drug crystals in the nano-range was found to be a potent strategy to enhance drug dissolution. These nanosuspensions, however, are prone to re-agglomeration of drugs nanocrystals and further research is required to allow transformation to solid formulation. In this regard it will be essential to assure that upon redispersion in water again stable nanoparticles are generated, to allow obtaining enhanced drug levels *in vivo*.

Furthermore, methods that yield drug in an amorphous are also potent enhancers of drug dissolution. In this regard, further research is required on long-term stability of the amorphous state. Also it is yet unknown whether results obtained via a specific formulation technique for one type of drug can be translated to other drug molecules as well.

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