Dissolution rate of poorly soluble drugs

Potential influence on dissolution rate using calcium sulfate as carrier in drug formulations

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Abstract

Since the mid 1990s the number of hydrophobic drugs has increased. This is an ongoing challenge for the pharmaceutical industry since poorly soluble drugs in general exhibit low bioavailability after oral administration. Several methods that increase solubility and/or dissolution rate of poorly soluble drugs have been developed, however, there are still drawbacks with each method. Therefore additional methods or improvement of existing methods are desirable. The aim of this master thesis was to investigate the influence on dissolution rate when using the ceramic material calcium sulfate as a carrier material. Zolpidem was used as a model substance.

Zolpidem was mixed with alpha respectively beta calcium sulfate hemihydrates. Two mixing technique were investigated; dry mixing method and solvent evaporation method. Both techniques included addition of water and setting of calcium sulfate into the dihydrate form. Dissolution tests, of each powder mixture, were performed in pH-values equivalent to pH-value in the gastrointestinal tract (i.e. pH 1.0 and pH 6.8). Results were analyzed in UV-spectrophotometer and presented as percent drug substance dissolved versus time. The properties of included materials and mixtures were analyzed using external surface area- and density measurements, x-ray diffraction (XRD) and scanning electron microscopy (SEM).

The dissolution rate, in pH 1.0, was fast irrespective of mixing method and type of calcium sulfate used. Within five minutes at least 96 percent of zolpidem was dissolved from each powder mixtures. In pH 6.8 the dissolution was slower with a maximum release of 54 percent within five minutes. The powder mixture prepared by the solvent evaporation method showed the fastest dissolution. The porosity and size of the drug-carrier complex and the particle size of the drug were of insignificant importance in gastric pH but relevant to the pH corresponding to that of the small intestine (p=0.05).

It was concluded that calcium sulfate have potential to be used in drug formulations since it provides a fast dissolution of the drug-carrier complex in gastric pH. However, additional tests on drug substances with poorer solubility than that of the model substance have to be evaluated to confirm this.

Keywords: dissolution rate, solubility, poorly soluble drugs, ceramic materials, drug delivery system
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1. Introduction

The introduction of combinatorial chemistry and high throughput screening in the mid 1990s resulted in a change in chemical properties, towards higher molecular weight and increased lipophilicity, of new potential drug substances. In fact 35-40 percent of the lead substances exhibit an aqueous solubility less than 5 mg/ml at physiological pH [1]. This is an ongoing challenge for the pharmaceutical industry since poorly soluble drugs in general exhibit low bioavailability after oral administration [2, 3].

Bioavailability of a drug refers to the fraction of a drug that enters the systemic circulation after administration. After intravenous administration this fraction is considered to be 100 percent [3]. However, when administrated via the oral route several factors influence the bioavailability, such as; poor dissolution or solubility, inability to permeate the gastrointestinal membrane or metabolic transformation during absorption [4].

Also introduced in the mid 1990s was the Biopharmaceutics Classification System (BCS) which classifies drugs into four different classes depending on their aqueous solubility and membrane permeability, table 1 [1, 5].

<table>
<thead>
<tr>
<th></th>
<th>High permeability</th>
<th>Low permeability</th>
</tr>
</thead>
<tbody>
<tr>
<td>High solubility</td>
<td>Class I</td>
<td>Class III</td>
</tr>
<tr>
<td>Low solubility</td>
<td>Class II</td>
<td>Class IV</td>
</tr>
</tbody>
</table>

The BCS predicts the in vivo bioavailability of a drug in correlation with the in vitro dissolution tests. Class I drugs exhibit optimal properties regarding permeability and solubility. These drugs have potential to be sufficiently absorbed due to their high solubility and high permeability properties. Class II drugs also exhibit high permeability properties but they have low aqueous solubility, hence, the absorption will be limited by the dissolution rate of the drug. These drugs require improved solubility or dissolution rate in order to be sufficiently absorbed. Class III drugs exhibit high solubility but low permeability and therefore solubility enhancement or improved dissolution rate will not have any effect on the bioavailability of the drugs. Class IV drugs exhibit both poor solubility and low permeability, which requires both improved solubility and permeability properties in order to be pharmacologically active [1, 3, 5]. Between 1995 and 2002 most drug candidates were classified as class III and class IV drugs, 28 and 48 percent, respectively [1].

1.1 Drug absorption

In order for a drug to be pharmacological active, after oral administration, it must pass through the gastrointestinal mucosa. This requires at least some degree of aqueous solubility. In a dissolved state the drug exists in its molecular form and is available for absorption. If a drug exhibit poor water solubility it might not by totally dissolved in the gastrointestinal fluid, hence, the whole administrated dose will not be available for absorption [3, 4]. Potentially, this might prevent a considerable amount of the active pharmaceutical substance (API) from being absorbed through the gastrointestinal mucosa.
During the last two decades several methods have been developed in order to increase water solubility of hydrophobic drugs, and hence, increase bioavailability. This includes preparation of soluble salts and pro-drugs. Since solubility is an intrinsic material property, it can only be altered throughout chemical modifications. As a result, the effect of the drug might be affected [1].

Another method to increase the bioavailability of a drug is to increase the dissolution rate of the drug. This approach requires no alteration in chemical properties of the drug, since dissolution is an extrinsic material property [1].

1.2 Dissolution rate

After oral administration of a solid dosage form, two consecutive steps take place. First, the drug molecules on the surface of the solid dissolve. This results in a saturated solution in immediate vicinity of the solid, also called the boundary layer. Secondly, the dissolved drug molecules diffuse through the boundary layer into the bulk (gastrointestinal fluid). When the dissolved molecules interact with the gastrointestinal mucosa they are absorbed. This mechanism is regulated throughout equilibrium and as long as the drug molecules are absorbed, additional drug molecules will diffuse into the bulk from the boundary layer. This results in more solid particles being dissolved. If the dissolution rate of a drug is fast, the absorption will primarily be dependent on the drugs ability to permeate the gastrointestinal membrane. When the drug dissolves slowly, then the dissolution rate of the drug will be the rate-limiting step in absorption, and hence, influence the bioavailability of the drug [3].

The fundamental theory of dissolution rate and solubility of a drug substance can be explained by the Noyes-Whitney equation (Eq. 1) [3, 6].

\[
\frac{dm}{dt} = \frac{DA(C_s - C_t)}{h} \tag{1}
\]

In figure 2, a schematic description of the dissolution of a spherical particle is shown. The dissolution rate (dm/dt) is influenced by the diffusion coefficient (D), the surface area of the solid available for dissolution (A), the saturation solubility of the drug (C_s), the concentration of the drug in the dissolving media at a specific time (C_t) and the thickness of the boundary layer (h) [3]. Two easily manipulated parameters, in order to increase the dissolution rate, are the saturation solubility and the surface area of the solid [1]. A large surface area and/or high saturation solubility will result in an increased dissolution rate according to the Noyes-Whitney equation (Eq. 1) [1, 3].
1.3 Drug delivery systems

In order to increase the dissolution rate, several drug delivery systems (DDS) have been developed during the last two decades. One common strategy is particle size reduction which increases the total surface area of the drug particle, and hence, increases the dissolution rate [1, 6]. Griseofulvin, a poorly soluble drug showed a twofold increase in bioavailability when the particle size was reduced from 10 µm to 2.7 µm [3]. This is why many poorly soluble drugs are present in micronized form, for example; digoxin, aspirin and ibuprofen. However, dry particle size reduction (i.e. micronization) can result in aggregation of particles and a decreased dissolution rate. To prevent this, wetting agent or hydrophilic carriers can be used during micronization or milling procedures [3, 7].

Solid dispersion (SD) is another method to increase the dissolution rate of poorly soluble drugs. The basic concept is to disperse the drug, either as fine particles or in molecular form, in an inert/biocompatible amorphous or crystalline carrier. Several types of solid dispersion have been described in the literature; eutectic mixtures, solid solutions, amorphous precipitates and glass solutions, however, the main purpose are the same, to increase the dissolution rate [8]. This is achieved by presenting the drug to the fluid as small–sized particles, thereby increasing surface area, improving wetting characteristics and reducing agglomeration [6].

SD can be prepared with either fusion or solvent methods. It is crucial to choose an appropriate method, since the physiochemical properties might be altered during processing. A simple fusion method includes heating of the carrier into a molten/liquid state before adding the drug. The mixture is then cooled whilst stirring whereupon the solid mass is milled and sieved to obtain a powder. The method is fast and simple, but requires the drug and carrier to be miscible in liquid state and that the drug is thermo stable. Disadvantages of the procedure are related to potential phase separation and formation of large drug particles [4, 6]. Also, the physiochemical properties might be altered during the process, which might result in undesirable storage problems. This is correlated to the formation of amorphous drug particles during milling. Since amorphous materials are able to absorb water from the surrounding and thereby facilitate mobility of molecules within the material, molecules are able to align and crystallize [3].

Hot melt extrusion is another fusion method, which provides better temperature control during mixing of the drug and carrier, compared to the simple fusion method. Direct extrusion of tablet is also an advantage of this technique, since no compaction
of tablets will be necessary. However, the method still requires thermo stable drugs and carriers [4].

The solvent method includes dissolution of the drug in an organic solvent. This facilitates incorporation of the drug, either as a solution or suspension, into the carrier without heating. As a result, no thermal degradation of the drug occurs. However, this requires an extra solvent-removal step, which is of economic and environmental concerns [4, 6].

Cyclodextrins (CD) are cyclic sugar compounds with a hydrophobic cavity and a hydrophilic external surface. Natural CDs used in pharmaceutical industry are; α-CD, β-CD and γ-CD. They differ in water solubility and interior cavity size. A poorly soluble drug forms non-covalent bonds within the CD-molecule. As the CD has a higher solubility than the drug itself the complex will dissolve in the gastrointestinal fluid and the drug will be quickly absorbed. Since the free drug and the drug-CD complex are in equilibrium, new drug-CD complex will dissolve as the free drug permeates the gastrointestinal membrane. Disadvantages using CDs are related to toxicology which particularly refers to β-CD with the lowest water solubility, with nephrotoxicity as a result. Also randomized methylation and increased lipophilicity after oral administration is of concern [1, 6].

The vast majority of pharmaceutical products e.g. tablets contain more than one component (e.g. binder, diluent, disintegrant, lubricant and carrier). In order to achieve an even distribution of the active substance within the powder mixture, a mixing step is required during manufacturing. This also ensures content uniformity of the API within the mixture. In practice powder mixtures tend to segregate, which might result in a failure in a content uniformity test. This is related to different particle size, shape and density of the components within the mixture. In order to prevent segregation, several approaches are available. For example; use components with approximately the same size and density, use granulation techniques or reduce the extent of vibration of the powder after mixing. Another available method is production of an ordered mixture. This technique provides an even distribution of the API within the mixture [9]. The method requires an API in micronized form and a carrier with considerably larger particle size. This facilitates adsorption of the API onto the carrier particles during mixing because the attraction forces will be greater than the gravitational forces trying to separate them [3, 9]. Ordered mixtures reduce potential agglomeration of the micronized drug and thereby a larger surface area of the drug is maintained [10]. According to Noyes-Whitney equation (Eq. 1), this increases the dissolution rate of a drug [3].

1.4 Ceramic materials

Calcium sulfate (CaSO₄), a ceramic material, will be used as a carrier in this master thesis. The raw material is also known as “Gypsum” and contains calcium sulfate dihydrate (CaSO₄ x 2H₂O) and impurities, which must be removed before medical applications. Gypsum is heated to 110 °C and during the procedure loss of water occurs. The product is calcium sulfate hemihydrate (CaSO₄ x ½H₂O). The hemihydrate exists in two different forms, α and β. Despite that they are chemically identical they differ in physical properties, which are due to differences in lattice structure, crystal size and surface area. The α-form contains rod- and prism-shaped crystals and cleavage fragments, whereas the β-form contains aggregate of irregular crystals and capillary pores. This makes the α-form less soluble compared to the β-form [11]. Addition of water to the hemihydrates reverses the reaction and the dihydrate is reformed. During the reaction a coherent mass of rod-like crystals are
formed and the material sets. In general only 18.6 wt % of water is required to reform the dihydrate, but in practice more water is added to increase the fluidity needed for casting. When excess water evaporates it causes an increased porosity within the dihydrate [12]. Because the hemihydrates differ in particle structure, they require different amount of water to rebuild the dihydrate form. The α-form requires 0.3 g water/gram hemihydrate and the β-form requires 0.6 g water/gram hemihydrate. This gives a denser α-form, which is stronger and less solubility [11].

The pharmaceutical industries have developed several formulation methods that improve the dissolution rate of poorly soluble drugs. These methods includes formulations in the micro- or nano-size region, formulations of solid dispersions, formulations of cyclodextrins and strategies that stabilize the drug in amorphous form [3]. However, there are still drawbacks with each method. Further improvements of these methods and/or development of new methods in drug formulation are therefore desirable. The use of ceramic materials, in drug formulation, can be a successful approach. Since these materials are highly biocompatible, chemically inert, inexpensive and can be made porous they have potential to be successfully used in drug delivery systems [11]. So far, ceramic materials have mainly been used in dental application, as bone regeneration material [13] or in sustained release system [14, 15].

As a model substance, zolpidem will be used (figure 2). The drug belongs to the imidazopyridine class, which is structurally different from other hypnotic agents. Zolpidem is a fast-acting hypnotic, with selective affinity to omega-1-receptors, which composes a subtype of the α-unit of the GABA_A receptor system. The drug has a fast onset and should be administrated immediately before bed time [16]. The solubility of zolpidem is 23 mg/ml [17], and according to USP definition the drug is sparingly soluble [1]. Zolpidem is classified as a very weak base with a pK_a-value of 6.2 [17]. The degree of ionization will therefore increase when the pH-value decreases. Therefore the solubility of zolpidem will be higher at pH 1.0 compared to pH 6.8 [3].

![Figure 2: Chemical structure of zolpidem][18]

**1.5 Aim of the master thesis**

The aim of this project was to investigate if calcium sulfate could be used as a carrier, in drug delivery systems, in order to increase the dissolution rate of poorly soluble drugs.
2. Materials and Methods

2.1 Materials

α-calcium sulfate hemihydrate (Ernst Hinrich GmbH, Germany) and β-calcium sulfate hemihydrate (Sigma Aldrich, Germany) were supplied by the Ångström laboratory, Uppsala University, Uppsala, Sweden. Micronized (<10 µm) zolpidem tartrate (Cambrex, USA) was provided by Orexo AB, Uppsala, Sweden. Methanol 99.9% (Sigma Aldrich, Germany), ethanol 99.5% (Kemetyl AB, Sweden), hydrochloric acid 37% (Sigma Aldrich, Germany), sodium hydroxide (Merck, Germany) and potassium phosphate monobasic (Sigma Aldrich, Reagent ≥ 99%, Germany) were supplied by the Ångström laboratory, Uppsala University, Uppsala, Sweden.

2.2 Methods

2.2.1 Preparation of solutions

**Phosphate buffer 0.05 M, pH 6.8**
The buffer was prepared by adding 22.4 g NaOH and 170.1 g KH₂PO₄ into 300 ml deionized water, thereafter the volume was adjusted to 500 ml and the solution was stirred, using a magnet bar, until completely dissolved. The phosphate buffer was then diluted 50 times and the pH was adjusted, by using 1.0 M H₃PO₄ or 1.0 M NaOH, when needed.

**Hydrochloric acid 0.1 M, pH 1.0**
Concentrated hydrochloric acid, 49.27 ml, was added into 300 ml deionized water. The volume was then adjusted to 500 ml and agitated, followed by a ten times dilution and pH adjustment, using deionized water or concentrated hydrochloric acid, when needed.

2.2.2 Solubility determination

The solubility of zolpidem was determined in 0.1 M hydrochloric acid (pH 1.0), 0.05 M phosphate buffer (pH 6.8) and 99.5% ethanol, by adding an excess amount (~150 mg) of the drug into 5 ml of each medium. The solubility determinations were performed in duplicates, and each sample was stored in 15 ml plastic tubes (BD Falcon), covered with parafilm and aluminum foil, on a swag board at 37°C for 24 hours. Before determination of the concentration, the sample tubes containing hydrochloric acid and phosphate buffer were pH adjusted and stored for an additional two hours. Tubes containing 99.5% ethanol were not pH adjusted. The sample tubes were centrifuged (Heraeus Biofuge Primo, Thermo Electron LED GmbH, Germany) at 3000 rpm for five minutes. Finally the supernatant was filtered through a 0.2 µm PTFE membrane filter and analyzed using UV spectroscopy (Shimadzu 1800, Shimadzu Corp., Japan) at wave length 300 nm. Results were presented as mean values.

2.2.3 Preparation of powder mixtures

**Dry mixing method**
Zolpidem and α-CaSO₄ (batch 1) and zolpidem and β-CaSO₄ (batch 2) were mixed in weight ratios of 1:9. The total size of each batch was approximately 20 g. The mixing
procedure was performed in a tumbling mixer (Turbula mixer T2F, W.A. Bachofen AG, Switzerland) for 30 minutes. After mixing, deionized water was added in weight ratios of 0.3:1 (water:α-CaSO₄) and 1.1:1 (water:β-CaSO₄) and blended into a homogenous paste. Batch 1 was blended for approximately three minutes using a glass mortar. Batch 2 was blended in a vacuum mixing machine (Twister, Renfert GmbH, Germany) for three minutes at 400 rpm. The paste was then wiped out on a petri-dish to a thickness of 2 mm. The plates were weighed and then stored for 48 hours at room temperature (batch 1) or 144 hours at 37°C (batch 2). Evaporation of excess water was calculated by noting the weight of each batch after storage. The percentage of drug within each powder mixture was also calculated.

After curing, the cake was milled using a glass mortar and finally sieved and collected into three different size fractions; <50 μm, 50-100 μm and 200-400 μm, using test sieves (50-400 μm, Retsch GmbH, Germany) and a vibration machine (Vibro, Retsch GmbH & Co, Germany). The dissolution rate of size fraction 50-100 μm was first determined for each powder mixture. The dissolution rates of the two remaining size fractions were only tested for the powder mixture showing the best dissolution profile and/or the powder mixture that was easiest to manufacture.

**Solvent evaporation method**

Methanol and zolpidem were mixed under heating and stirring conditions, using a water bath at a temperature of 50°C, magnet stirring equipment (Heidolph, Germany) and a magnet bar. The solubility was estimated by testing a small quantity of zolpidem and methanol during specified conditions. An eppendorf tube of 2 ml was filled with 50 mg zolpidem and 0.25 ml methanol and after ten minutes an additional 0.25 ml methanol was added. The procedure was repeated until a clear solution was obtained. The solubility was estimated to 70-100 mg/ml.

The total amount of powder mixture (batch 3) needed for further tests were estimated to 10 g. The same ratio between zolpidem, α-CaSO₄ and deionized water was used as in the previous mixing method. 1.0 g zolpidem was placed in a 50 ml plastic tube (BD, Falcon) and mixed with 15 ml methanol. When the powder was totally dissolved the solution was mixed with 9.0 g α-CaSO₄ in a vacuum mixing machine (Twister, Renfert GmbH, Germany) for one minute at 150 rpm. The mixture was covered with aluminum foil and stored for 48 hours at 37°C. After evaporation of methanol a dry powder was obtained. The setting of α-CaSO₄ was achieved by adding 2.7 ml deionized water. The mixing was performed in a vacuum mixing machine (Twister, Renfert GmbH, Germany) for 30 seconds at 400 rpm. The homogenous paste was then wiped out on a petri-dish to a thickness of 2 mm.

The plate was weighed and stored at 37°C for 48 h. After storage the plate was weighed again and the drug content within the powder mixture was calculated. After curing the cake was milled using a glass mortar and finally sieved and collected into the size fraction 50-100 μm using test sieves (50-100 μm, Retsch GmbH, Germany) and a vibration machine (Vibro, Retsch GmbH & Co, Germany).

**2.2.4 Characterization of the materials and the drug delivery system**

**Materials**

The structure of the powder (calcium sulfate and zolpidem) was analyzed with X-Ray diffraction (XRD) using D5000 diffractometer (Siemens/Bruker, Germany). Bragg Brentano Focusing Geometry analysis method was used throughout the sample analysis. The samples were analyzed between 10° - 90° at a power supply of 45 kV and
40 mA. The results were used as a reference to the x-ray diffraction analysis of the powder mixtures (batch 1, 2 and 3).

The surface area of the powders was investigated with gas adsorption by using BET (ASAP 2020, Micromeritics Corp., USA). Samples were prepared by storing them in an oven at 80°C for five days. This procedure was used in order to reduce time required for degassing of each sample. Before analysis, the samples were degassed at 95°C for five hours. Each analysis was performed on approximately 0.5 g degassed powder and a 5-point measurement was used during analysis.

Density measurements were performed using a helium pycnometer (AccuPyc 1340, Micromeritics Corp., USA). The parameters in setup mode were adjusted to: standard, number of purges: 20, purge fill pressure: 19.500 psig, number of cycles: 10, cycle fill pressure: 19.500 psig, equilibration rate: 0.0500 psig/min, use run precision: yes and percent full-scale: 0.05%. The empty metal cylinder of 1.0 cm³ was calibrated: thereafter the attached metal ball was added to the cylinder and calibrated. Prior to analysis 2/3 of the cylinder was filled with sample and the weight was recorded and specified in analysis mode before measurements.

Drug delivery systems
The surface area of the drug delivery system was investigated with gas adsorption by using BET (ASAP 2020, Micromeritics Corp., USA). Measurements were performed on each size fraction used in the dissolution measurements. Sample preparation and analysis condition were the same as in analysis of materials.

Density measurements were performed by using helium pycnometer (AccuPyc 1340, Micromeritics Corp., USA). Calibration procedure and settings were the same as in analysis of materials.

The drug delivery systems were also analyzed with X-ray diffraction using D5000 diffractometer (Siemens/Brucker, Germany) in order to investigate the structure of the final product. The analysis was performed under the same conditions as in analysis of materials. The results were compared with the results prior to setting.

Scanning electron microscopy (SEM) analysis was performed using a Leo 1550 FEG microscope (Zeiss, UK). Analysis was performed on batch 1 and no coating was applied onto the sample during analysis. The image was compared to a reference image of pure alpha calcium sulfate dihydrate.

2.2.5 Dissolution test

Analysis of powder mixtures
The dissolution tests were performed in an USP-2 dissolution bath (Sotax AT7 smart, Switzerland) at 37°C and 50 rpm. Each sample was run in triplicates and the results were presented as mean values with corresponding standard deviation. Measurements were performed in both 0.05 M phosphate buffer (same conditions as the small intestine, pH 6.8) and 0.1 M hydrochloric acid (same condition as the stomach, pH 1.0). Sink conditions (i.e. Cₜ <10 % of Cₛ) were maintained during each dissolution test [3]. Therefore, the amount of powder added to each vessel was determined by the saturation solubility of zolpidem in each medium. The amount of powder added to 0.1 M hydrochloric acid was equivalent to 10 mg of zolpidem and the amount of powder added to 0.05 M phosphate buffer was equivalent to 5 mg of zolpidem. Batches 1, 2 and 3 were compared by measuring the dissolution rate of the
size fraction 50-100 μm. The powder mixture produced by the dry mixing method that showed the best dissolution profile (batch 1 or 2) was further investigated by measuring the size fractions; <50 μm and 200-400 μm.

**Analysis of controls**

Pure zolpidem powder and suspension of zolpidem (5 mg/ml) were used as controls and the dissolution tests were performed under the same condition as with the powder mixtures. The suspension was produced by adding 1 ml of 0.05 M phosphate buffer to approximately 5 mg of zolpidem.

In all dissolution tests, aliquots of 1.0 ml were collected at specific time intervals: 3, 5, 10, 15, 30, 60 and 120 minutes. Each sample was filtrated through a 0.2 μm PTFE membrane filter and placed in an optical glass cuvette of 1 ml. Absorbance measurements were performed at wave length 300 nm using a UV-spectrophotometer (Shimadzu UV-1800, Shimadzu Corp., Japan). The dissolution rate of zolpidem was analyzed by plotting dissolved zolpidem in percent versus time.

The results were presented as mean values with corresponding standard deviation. The statistic program SASW_statistics_18(SPSS) was used to evaluate potential differences in dissolution rate between each powder mixture. The method used was a Mann Whitney U-test. Results with p≤0.05 were considered as a significant difference.

### 3. Results

#### 3.1 Solubility measurement

The saturation solubility (C_s) of zolpidem in 0.1 M hydrochloric acid (pH 1.0) and 0.05 M phosphate buffer (pH 6.8) was determined to 28 mg ml⁻¹ and 0.15 mg ml⁻¹, respectively. The saturation solubility in 99.5% ethanol was determined to 11 mg ml⁻¹.

#### 3.2 Preparation of powder mixtures

At curing time (t₀) the total weight of the powder mixtures (batch 1 and 2), including the weight of the petri-dish was 37.97 g and 82.41 g. After 48 h the weight of batch 1 was 37.34 g. Batch 2 required additional curing time and after 144 h the weight was 68.50 g. This contributed to a water loss of 11.8% and 69.5% respectively and resulted in a theoretical drug content of 8.1% and 7.7% within each batch. Therefore 123.8 mg (batch 1) respectively 130.5 mg (batch 2) of the powder mixtures were equivalent to 10 mg of zolpidem (Eq. 2).

\[
m \text{ (powder)} = \frac{\text{Amount of zolpidem (mg)}}{\text{zolpidem content within the mixture (%)}} = \text{powder (mg)} \quad (2)
\]

The amount of powder mixture equivalent to 5 mg of zolpidem was calculated to 61.9 mg (batch 1) and 65.2 mg (batch 2) using equation 2.

The same method was used when calculating the drug content within the powder mixture produced by the solvent evaporation method (batch 3.) and the drug content was determined to 8.5%.
3.3 Characterization of the materials and the drug delivery systems

The results from density and external surface area measurements of the included materials are specified in table 2. When comparing properties of the pure substances (α-CaSO₄, β-CaSO₄ and zolpidem), the latter showed the lowest density and the highest external surface area. Density of the alpha and beta form was comparable. However, the beta form showed a 14 percent lower external surface area compared to the alpha form (3.56 vs. 4.15 m² g⁻¹).

<table>
<thead>
<tr>
<th>Material</th>
<th>Density (g cm⁻³)</th>
<th>External surface area (m² g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-CaSO₄</td>
<td>2.77 ± 0.003</td>
<td>4.15</td>
</tr>
<tr>
<td>β-CaSO₄</td>
<td>2.75 ± 0.002</td>
<td>3.56</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>1.29 ± 0.002</td>
<td>4.18</td>
</tr>
</tbody>
</table>

Density measurement: n=10 (run precision mode). External surface area was analyzed using a 5-point measurement, using nitrogen.

After processing (i.e. setting and milling), the powder mixtures produced by the dry mixing method (batch 1 and 2) showed a two-fold increase in the external surface area (table 3). Additional tests of batch 1 showed an increased external surface area when the particle size was reduced. The surface area of the powder mixture produced by the solvent evaporation method (batch 3) showed a 35 percent increased of the external surface area after processing (4.15 m²g⁻¹ versus 6.39 m²g⁻¹).

<table>
<thead>
<tr>
<th>Powder mixture</th>
<th>Density (g cm⁻³)</th>
<th>External surface area (m² g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 1ᵃ, &lt;50 µm</td>
<td>2.24 ± 0.0029</td>
<td>8.39</td>
</tr>
<tr>
<td>Batch 1ᵇ, 50-100 µm</td>
<td>2.24 ± 0.0029</td>
<td>8.34</td>
</tr>
<tr>
<td>Batch 1ᶜ, 200-400 µm</td>
<td>2.24 ± 0.0029</td>
<td>5.78</td>
</tr>
<tr>
<td>Batch 2ᵇ, 50-100 µm</td>
<td>2.15 ± 0.0061</td>
<td>7.07</td>
</tr>
<tr>
<td>Batch 3ᶜ, 50-100 µm</td>
<td>2.27 ± 0.0011</td>
<td>6.39</td>
</tr>
</tbody>
</table>

ᵃ DDS produced by dry mixing method, weight ratio of zolpidem and α-CaSO₄, 1:9.
ᶜ DDS produced by solvent evaporation method, weight ratio of zolpidem and α-CaSO₄, 1:9.

Density measurement: n=10 (run precision mode). External surface area was analyzed using a 5-point measurement, using nitrogen.

The X-ray diffraction analysis is shown in figure 3. The alpha and beta form was difficult to differentiate using x-ray diffraction as analysis method, since the diffractograms were almost identical. The powder mixtures (batch 1-3) were also similar in their diffractograms. All three powder mixtures showed the characteristic peaks for calcium sulfate dihydrate [19]. The intensity of the peaks was lower for batch 3 compared to batch 1 and 2.
Figure 3: X-ray diffractogram of alpha and beta calcium sulfate hemihydrates. Batch 1, 2 and 3 (drug substance and calcium sulfate dihydrate). The characteristics peaks of calcium sulfate dihydrate are marked in the figure (x). The peaks were found for all tested powder mixtures.

Scanning electron microscope (SEM) of alpha calcium sulfate dihydrate and alpha calcium sulfate dihydrate mixed with zolpidem (batch 1) are shown in figure 4. The rod-shaped crystals are present in both figures. The transformation of the hemihydrate form into the dihydrate form was performed under the same conditions using a L:P\(^1\) ratio of 0.3:1.

\(\text{Liquid to powder ratio, volume deionized water (ml)} : \text{amount of powder (g)}\)
Figure 4: SEM images of the surface structure of alpha calcium sulfate dihydrate\textsuperscript{2} in magnification 1000 x (at the top) and alpha calcium sulfate dihydrate mixed with zolpidem in magnification 50000 x (at the bottom).

3.4 Dissolution test

The dissolution rates of zolpidem, in pH 1.0, are shown in figure 5. All tested powder mixtures showed a fast and complete dissolution of zolpidem. Within five minutes 98, 96 and 98 percent of zolpidem were released from batch 1, 2 and 3 respectively. The amount of drug released from the controls (powder and suspension) was determined to 99 and 107 percent respectively (figure 5). The variability of drug

\textsuperscript{2} Reference picture from previous work, alpha calcium sulfate dihydrate, L:P 0.3:1. Jonas Åberg.
dissolved (expressed as standard deviation) was determined to <0.11, <0.48 and <0.04 percent for batch 1, 2 and 3 respectively.

The mean values of triplicates with related standard deviation are shown in the figure. After five minutes there was a significant difference in dissolution rate between the suspension and the batches 1-3 (p=0.05). There was no significant difference in dissolution rate between the batches.

Results from the dissolution tests of zolpidem in pH 6.8 are shown in figure 6. Batch 3 showed a slightly faster dissolution rate compared to batch 1 and 2. Overall, a slower dissolution rate was obtained in pH 6.8 compared to pH 1.0. Within five minutes 47, 44 and 54 percent of zolpidem were dissolved from batch 1, 2 and 3 respectively. There was no complete dissolution of the powder mixtures during the dissolution tests. After two hours the percentage dissolved was below 74 percent for all powder mixtures tested. The standard deviation showed a larger variability in drug release when the dissolution test was performed in pH 6.8 (~four percent) compared to when pH 1.0 was used. The percent of drug released from the controls (powder and suspension) within five minutes, was determined to 76 and 55 percent, respectively. After two hours the amount of drug released from the controls (powder and suspension) were determined to 79 and 61 percent respectively.
Figure 6: Dissolution rate of zolpidem in pH 6.8. Mixing ratios of zolpidem and calcium sulfate hemihydrate 1:9, size fraction 50-100 µm. The mean values of triplicates with related standard deviation are shown in the figure. After five minutes the dissolution rate of each batch (1, 2 and 3) were significantly slower compared to the zolpidem powder (p=0.05). Batch 3 also showed a significantly faster dissolution rate compared to batch 2 (p=0.05).

The dissolution rates of the three different size fractions (batch 1) are shown in figure 7. In pH 1.0, size fraction 200-400 µm showed the slowest dissolution rate followed by size fraction 50-100 µm and <50 µm. However there was no significant difference between the two smallest size fractions. Within five minutes, 91, 97 and 98 percent of zolpidem were dissolved from each size fraction (decreasing particle size). In pH 6.8 the pattern was the same as in pH 1.0. The largest size fraction also showed the slowest dissolution rate followed by the other two size fractions with similar dissolution. Within five minutes, 24, 47 and 43 percent of zolpidem were dissolved from each size fraction (decreasing particle size).
Figure 7: Dissolution rates of batch 1 in pH-value 1.0 (at the top) and 6.8 (on the bottom). The mean values of triplicates with related standard deviation are shown in the figure. No significant difference could be detected in pH 1.0 between the size fractions. In pH 6.8, the largest size fraction (200-400 µm) showed a significantly slower dissolution rate compared to the other size fractions ($p=0.05$).

4. Discussion

To my knowledge there are no previous studies on the use of calcium sulfate as a drug dissolution rate enhancer. Previous studies using ceramic materials have mainly focused on dental applications, bone regeneration methods or sustained drug release systems [13-15]. Since ceramic materials can be made porous during the setting procedure are highly biocompatible and relatively inexpensive they have potential to be successful in drug formulations [11].

4.1 Evaluation of mixing methods

The dissolution tests were performed in triplicates and the standard deviation within each test revealed a low variability of drug content within batch 1 and batch 3 (i.e. <0.11 and <0.04 percent, respectively). The results indicated that the used mixing method generated an even distribution of zolpidem within the powder mixtures. The results also revealed a small advantage of using the solvent evaporation method as a mixing method since it created less variability of drug content within the mixture (<0.04 percent). Since zolpidem exists in its molecular form, when using the solvent
evaporation method, it theoretically enables a more even distribution of the drug within the powder mixture.

During preparation of the powder mixtures, the beta form of calcium sulfate was more difficult to handle. More water and a prolonged mixing time were needed to achieve a homogenous paste. Therefore, a vacuum mixing machine was used during preparation of batch 2. Since this mixing technique provides a better repeatability it was also used when batch 3 was mixed. The mixing of batch 1 was performed manually using a glass mortar. Since a homogenous paste were achieved during mixing of batch 1, 2 and 3, the choice of mixing method was not expected to influence the mixtures.

Overall, the dissolution rates from each triplicate showed a larger variability in pH 6.8 compared to pH 1.0. Since the dissolution tests were stopped after two hours, a large amount of the powder mixture was still undissolved.

The dry mixing method used during this experimental has advantages compared with methods used to prepare solid dispersions. Since no heating is needed during the mixing procedure it enables heat sensitive drugs to be mixed with the carrier. When preparing a solid dispersion using the fusion method the carrier needs to be in a liquid/molten state before adding the drug. This requires heating of the carrier and that the carrier and drug are miscible in liquid state [4].

4.2 Microstructure analysis

The x-ray diffractograms showed almost identical pattern of the alpha and beta form of calcium sulfate hemihydrates. This was expected since previous studies have failed to differentiate between the alpha and beta form using the x-ray diffraction analysis method [20]. When comparing the powder mixtures, batch 1, 2 and 3, they also showed almost identical diffractograms. The characteristic peaks for the dihydrate form was shown in all tested powder mixtures, which confirms a successful handling of the used method and that transformation of calcium sulfate hemihydrate into calcium sulfate dihydrate occurred during the preparation process [19]. It also indicated that the addition of drug substance prior to setting had none or small effect on the crystal shape.

The SEM images of calcium sulfate dihydrate and calcium sulfate dihydrate mixed with drug substance shown in figure 4, confirmed that the drug had little or no effect on the crystal shape during setting. Both pictures showed rod-shaped crystals which are characteristics for calcium sulfate after setting.

The external surface area is a measurement of the particle size including the pores on its surface [3]. It was shown in this report that alpha calcium sulfate dihydrate had a higher external surface area compared to the hemihydrate form (8.39 m² g⁻¹ versus 4.15 m² g⁻¹). This was expected since the crystals rearrange during evaporation of excess water. This contributes to an increased porosity and a decreased density within the material [12]. However, the values before and after setting are not directly comparable since the size fraction is different and the dihydrate form contains ten percent zolpidem. The differences in density (before and after setting) were also confirmed in this report (2.77 g cm⁻³ versus 2.24 g cm⁻³). These results are in agreement with the literature (2.75 g cm⁻³ versus 2.32 g cm⁻³) before and after setting [12]. The density values after setting were not comparable with values from the literature, since the powder mixture in this report also contained ten percent zolpidem. Therefore a slightly lower density was achieved. An increased porosity
within the material increases the wetting characteristics of the material. As a result, the fluid surrounding the particle will easier penetrate into the material and hence increase the dissolution rate [3]. This was confirmed in this report since the size fraction with the lowest surface area also exhibited the slowest dissolution rate (figure 7).

The solvent evaporation method (batch 3) generated a powder mixture with a smaller external surface area compared to the other batches (table 3). In theory this should have decreased the dissolution rate since the material becomes less porous. Figure 6 shows that this was not true since batch 3 showed the fastest dissolution rate. This confirms that the solvent evaporation method was the most successful mixing method and that the drug particle size had influence on the dissolution rate. It is likely that mixing, using the solvent evaporation method, would increase the dissolution rate further if a smaller size fraction were tested (i.e. <50-100 μm).

4.3 Dissolution rate, pH 1.0

The dissolution rate in pH 1.0 was fast for all included powder mixtures (size fraction 50-100 μm) with a minimum of 96 percent drug released within five minutes. However a fast dissolution also occurred from the controls (powder and suspension). The dissolution within five minutes was determined to 99 and 107 percent, respectively. The percent released from the suspension was higher than 100 percent, which partly could be explained by accuracy of used analysis methods.

During the dissolution tests, the drug was presented as particles in the micro-size region (batch 1 and 2). Since batch 3 was prepared by the solvent evaporation method the drug particle size is expected to be reduced. This is because the drug is presented in its molecular form during mixing. When the solvent evaporates the drug precipitates as smaller drug particles, thus resulting in an increased surface area and an increase in dissolution rate. The lack of differences between these batches (i.e. batch 1, 2 and 3) indicated that the dissolution rate was not affected by differences in drug particle sizes. This is probably attributed to the solubility of zolpidem (28 mg/ml) which is defined as sparingly soluble [1]. The dissolution tests also confirmed that no reduction in drug release occurred using calcium sulfate as a carrier. This verifies that calcium sulfate undergo a fast dissolution, and therefore a fast release of the drug is achieved. This reinforce the theory that calcium sulfate can be used in drug formulations and potentially increase the dissolution rate of poorly soluble drugs.

There was no significant difference in dissolution rate between alpha and beta calcium sulfate and the alpha form was easier to handle during production. This was attributed to an extended mixing time and a larger amount of water needed for setting. Therefore the alpha form was used in the further evaluation.

4.4 Dissolution rate, pH 6.8

The dissolution rate in pH 6.8 was slow for all tested powder mixtures (size fraction 50-100 μm) with a maximum of 54 percent drug released within five minutes. The controls also showed a slow dissolution rate compared to the dissolution rate in pH 1.0. The percent released within five minutes was determined to 76 percent (powder) and 61 percent (suspension) respectively. Since the solubility of zolpidem was considerably lower in pH 6.8 compared to pH 1.0 (0.15 mg/ml versus 28 mg/ml), it was also expected that the dissolution of zolpidem would be slower in pH 6.8.
There was no complete dissolution during the test time neither of the powder mixtures or the controls. The low dissolution of the suspension can be a result of an unsuccessful pouring of the suspension into the dissolution vessel during the experimental procedure, leaving a residue of drug inside the suspension container. The dissolution of the zolpidem powder reached a plateau value after approximately one hour (79 percent). This was unexpected since the whole amount of powder should have been dissolved within the test time of two hours. One potential explanation is that a small amount of the powder never reached the bottom of the dissolution vessel. Therefore, it is possible that undissolved drug particles were removed from the dissolution vessel during sample collection. Consequently, a lower concentration than expected will be obtained during measurements. This theory can also be applied to the dissolution of the powder mixtures (batch 1, 2 and 3). Therefore, it is likely that the dissolution rates during these experimental trials actually should be higher than declared. An extended sample collection time could have been used, to verify that zolpidem reached its maximum concentration during the dissolution tests.

When comparing the dry mixing method (batch 1 and 2) with the solvent evaporation method (batch 3) the latter showed a slightly faster dissolution rate (47, 44 and 54 percent, respectively). The difference between batch 2 and 3 was significant (p=0.05). However, no significant difference could be detected between batch 1 and 3. Since the solvent evaporation method provides a dispersion of smaller drug particles the surface area of the drug is increased. According to Noyes-Whitney equation the dissolution rate of a spherical particle increases when the surface area increases [3, 6]. The solubility of zolpidem was determined to 0.15 mg/ml in pH 6.8. The results from this work indicated that the size of the drug particles and the size of the drug-carrier complex were of greater importance when the solubility of the drug was low. The disadvantage of using an organic solvent in drug formulations is that it requires an additional solvent removal step which is of environmental and economic concerns [4, 6].

4.5 Particle size fraction

Additional dissolution tests were performed on the powder mixture containing alpha calcium sulfate produced by the dry mixing method (batch 1). When comparing the dissolution rate of the drug from each size fraction, the largest size fraction showed the slowest dissolution of zolpidem irrespective of dissolution medium. However, the difference in dissolution rate was only significant in pH 6.8 (p=0.05). Since a larger particle size decreases the external surface area of the drug-carrier complex, the dissolution rate of the drug-carrier complex consequently decreases. Analysis of the external surface area confirmed that the largest size fraction also had the lowest surface area (table 3).

The difference in dissolution rate between size fraction <50 μm and 50-100 μm was difficult to distinguish since the dissolution profiles overlapped (figure 7). It should be noted that the differences in external surface area was small and that a larger difference in particle size would have been more interesting to evaluate. When comparing the external surface area of the two size fractions, the smaller one (<50 μm) showed a slightly higher value (8.39 m² g⁻¹ versus 8.34 m² g⁻¹). These size fractions were achieved through a sieving procedure and it is possible that both size fractions consisted of a drug-carrier complex in close proximity to 50 μm, consequently no difference in dissolution rate could be obtained.
4.6 Future considerations

In this work a sparingly soluble drug was used. Other drugs were initially subject for evaluation, though due to lack of analysis equipment (i.e. High Performance Liquid Chromatography) they were not able to be detected. It is therefore desirable to investigate this drug delivery system using other, less soluble, drugs. The porosity of the drug-carrier complex and the particle size of the drug were of insignificant importance in abdominal pH-value (1.0) but relevant to the pH-value corresponding to that of the small intestine (pH 6.8). To obtain a faster dissolution it is desirable to reduce the size of the drug-carrier complex and to use the solvent evaporation method as the mixing method. This will increase the external surface area of the drug-carrier complex and provide a more even dispersion of the drug.

5. Conclusion

This work showed that the dissolution rate was not reduced using calcium sulfate as a carrier. Since there was no difference in the dissolution rate between the powder mixtures and the controls it can be assumed that the drug-carrier complex undergoes a fast dissolution at gastric pH and therefore enables a fast release of the model drug. Additional tests on drug substances with poorer solubility than the model substance are yet to be evaluated.

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7. References
