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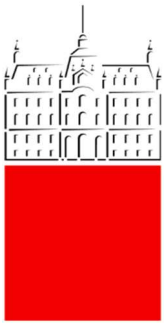

DENIS ĐALAPA

POROUS CALCIUM CARBONATE AS A CARRIER FOR NAPROXEN DISSOLUTION IMPROVEMENT

POROZNI KALCIJEV KARBONAT KOT NOSILEC ZA IZBOLJŠANJE RAZTAPLJANJA NAPROKSENA

Ljubljana, 2015

The research work was carried out at Instituto Andaluz de Ciencias de la Tierra (IACT) – CSIC Granada in Spain under supervision of my co-mentor Prof. Dr. César Viseras Iborra and at Faculty of Pharmacy Ljubljana under supervision of my mentor Prof. Dr. Odon Planinšek.

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STATEMENT

I declare that I have carried out my master thesis independently under the mentorship of Prof. Dr. Odon Planinšek and co-mentorship of Prof. Dr. César Viseras Iborra.

Master degree commission

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API Active pharmaceutical ingredient

ATC Anatomical Therapeutic Chemical

BCS Biopharmaceutics Classification System

CAL1 Commercial ground calcium carbonate sample

CAL2 Pharmaceutical grade of ground calcium carbonate sample

CEP/COS Certificate of suitability

DL Drug load

DSC Differential scanning calorimetry

EDQM European directorate of the quality of medicines

EQ equilibrium method

GCC Ground calcium carbonate

GIT Gastro-intestinal tract

IUPAC International union of pure and applied chemistry

NAP Naproxen

NSAID Nonsteroidal anti-inflammatory drug

PCC Precipitated calcium carbonate

PEG Polyethylene glycol

pH Hydrogen-ion concentration (Alkalinity)

PM Physical mixture

PVP Polyvinylpyrrolidone

SD Solid dispersion

SE solvent evaporation

SEM Scanning electron microscope

Sw Surface area

Tg Glass transition temperature

TGA/DTA Thermo-gravimetric analysis

THF Tetrahydrofuran

UV-Vis Ultraviolet-visible

ABSTRACT

Nowadays, porous carriers are increasingly used as excipients to improve the solubility of poorly soluble drugs, since they allow adsorption of substances in pores in the amorphous forms, which have a better solubility than the crystals itself. The main problem of amorphousness lies in the instability of this form. Therefore, the stability is improved with using porous carriers and consequent formation of solid dispersions, since pores, due to their size, are limiting the formation of crystals of active substances within them.

We tried to utilize all the advantages of a porous carrier, in our case calcium carbonate, in order to improve the solubility of the widely used naproxen drug, which is poorly water-soluble. First, we studied calcium carbonate and its properties through various methods in order to determine its potential as a porous carrier. Naproxen was then adsorbed on the carrier with two different methods, i.e. an equilibrium method and the solvent evaporation method and their combination. Hence, we made physical mixtures for comparison to determine the effectiveness and usefulness of these methods.

Our study has proven that samples of calcium carbonate have the potential to be used as porous carriers. We have determined with the above methods that the drug is absorbed in an amorphous form. Furthermore, a dissolution improvement occurred in comparison with the drug itself, however, the dissolution rate of dispersion decreased with an increasing proportion of active ingredients. The dissolution improvement of methods used for the manufacture of solid dispersions in comparison with physical mixtures is also reduced by increasing the proportion of active ingredients.

Keywords: porous carriers, solid dispersions, calcium carbonate, naproxen, dissolution

POVZETEK

Porozni nosilci se v današnjem času vse bolj uporabljajo kot pomožne snovi za izboljšanje topnosti slabo topnih učinkovin, saj omogočajo, da se te sorbirajo v pore v amorfni obliki, ki je za razliko od kristalov zaradi svoje nestabilnosti bolj topna. Problem amorfne oblike je njena nestabilnost, ki pa jo izboljšamo z uporabo poroznih nosilcev oziroma s tvorbo trdnih disperzij, saj pore zaradi svoje velikosti omejujejo nastajanje kristalov.

V magistrski nalogi smo skušali uporabiti vse prednosti poroznega nosilca, kalcijevega karbonata, za izboljšanje raztapljanja zdravilne učinkovine naproksena, ki je v vodi zelo slabo topen. Najprej smo proučili lastnosti kalcijevega karbonata z uporabo različnih metod za ugotavljanje potenciala kot poroznega nosilca. Nato smo naproksen sorbirali na/v nosilec s pomočjo dveh različnih metod in sicer z ravnotežno metodo in metodo z odparevanjem topila ter še s kombinacijo obeh. Za ugotavljanje učinkovitosti in uporabnosti omenjenih postopkov smo za primerjavo izdelali tudi fizikalne zmesi.

Ugotovili smo, da imajo preiskovani vzorci kalcijevega karbonata potencial za porozne nosilce. Pri uporabi navedenih metod je namreč prišlo do sorbcije zdravilne učinkovine v amorfni obliki in izboljšanja raztapljanja v primerjavi s samo zdravilno učinkovino, in sicer kljub temu, da se s povečevanjem deleža učinkovine njeno raztapljanje slabša. Izboljšanje raztapljanja z uporabljenimi metodami za izdelavo trdnih disperzij, v primerjavi s fizikalnimi zmesmi, prav tako zmanjšuje s povečevanjem deleža učinkovine.

Ključne besede: porozni nosilci, trdne disperzije, kalcijev karbonat, naproksen, raztapljanje.

1. INTRODUCTION

The use of oral medications is the most prevalent forms of intake as it is well known, simple and allows non-invasive dosing with a few drawbacks. In drug design, first-pass metabolism is the most important characteristic. Before absorption, active ingredients must be dissolved in the digestive tract. Since the solubility of the substance in the physiological media is, in addition to its permeability, one of the most important parameters that affect bioavailability, it presents a huge problem for drugs that are poorly water-soluble. Actually large percentages of new drug compounds are poorly water soluble. The bioavailability increase of these compounds represents one of the major challenges in the development of effective drug formulations (1).

1.1 SOLUBILITY AND DISSOLUTION RATE

The intrinsic solubility is a substance property representing its maximum concentration which in a given volume of solvent forms a saturated homogeneous molecular dispersion. The solubility is, therefore, equal to the concentration of saturated solution of a given substance in a given solvent. Solubility can be expressed as an absolute solubility, i.e. the exact concentration of a solute in a solvent in known conditions) or as the degree of solubility (solubility within certain limits), as presented in European Pharmacopoeia and summarized in Table I (2, 3).

Table I: Degrees of solubility at 15–25 °C (2).

Descriptive term	**Approximate volume of solvent in milliliters per gram of solute**			
Very soluble	less than	1		
Freely soluble	from	1	to	10
Soluble	from	10	to	30
Sparingly soluble	from	30	to	100
Slightly soluble	from	100	to	1,000
Very slightly soluble	from	1,000	to	10,000
Practically insoluble	more than			10,000
The term **'partly soluble'** is used to describe a mixture where only some of the components dissolve. The term **'miscible'** is used to describe a liquid that is miscible in all proportions with the stated solvent.				

The dissolution rate is described with a modified Noyes-Whitney equation, in which dC/dt is the dissolution rate of a drug, A is a surface available for dissolution, D diffusion coefficient, Cs drug solubility in a dissolution medium, C the concentration of a drug in time t, and h the thickness of the diffusion layer which is in contact with a dissolving particle surface (4).

$$\frac{dC}{dt} = \frac{D * A * (Cs - C)}{h}$$

Equation 1: The modified Noyes-Whitney equation (4).

Today, poor solubility and a low dissolution rate of active substances in water are one of the main problems in the development processes of pharmaceuticals. The last couple of decades have seen a rapid development of combinatorial chemistry and high-throughput screening methods leading to discovery of more and more potential therapeutic agents with poor water solubility and low dissolution rates (5, 6) were discovered. Approximately 70 % of the newly detected potential active ingredients exhibit poor solubility in water. As much as 40 % of all oral dosage forms with immediate-release currently available on the market, contain a substance which is practically water insoluble (7). Some of the active ingredients with poor solubility are phenytoin, chloramphenicol, carvedilol and naproxen, among many others. Furthermore, one of the main challenges for the pharmaceutical industry remains to develop efficient methods for increasing the solubility and dissolution rates of such poorly water soluble drugs. Enlarged bioavailability of the active substances is the consequence of increased solubility and dissolution rates. This ensures that for the same therapeutic effects, lower dose of the active ingredient is needed, which leads to a better clinical outcome of the treatment and fewer side effects (5, 6, 8).

A poorly water soluble substance is defined as a substance of which less than one part is dissolved in 1,000 parts of water or the solubility of which is less than 100 μg/mL. Moreover, poorly soluble substances may be defined as substances that need more time to dissolve in the gastro-intestinal tract (GIT) in comparison with the duration of their absorption (9). According to the Biopharmaceutics Classification System – BCS, substances are divided in 4 classes depending on solubility and permeability as shown in Figure I (10).

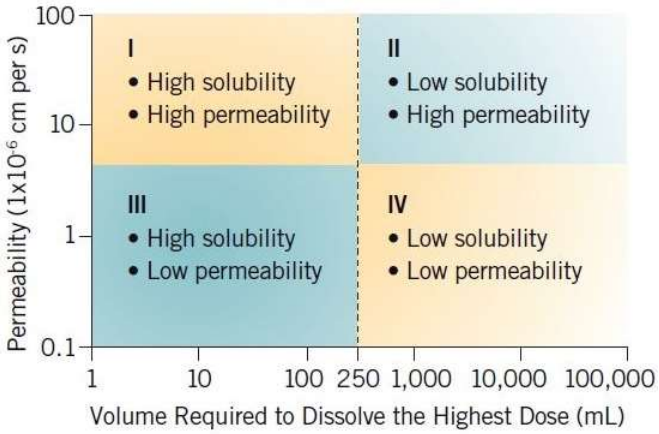


Figure 1: The biopharmaceutics Classification System (11).

1.2 METHODS FOR IMPROVING DISSOLUTION OF POORLY WATER SOLUBLE DRUGS

To improve the dissolution of poorly water-soluble active substances, both chemical and physical methods are being used. They are presented in Figure 2.

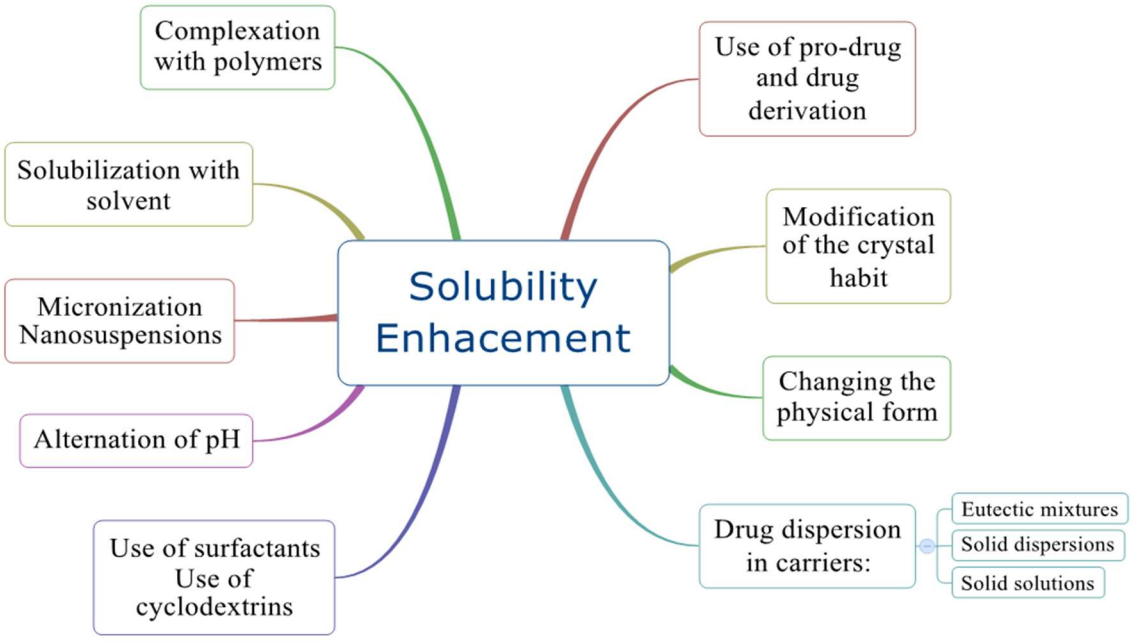


Figure 2: The solubility enhancing techniques.

The most common among the chemical methods are the formation of salt and synthesis of a soluble prodrugs. Physical methods consist of the following: reduction of particle size, modification of crystal habits (polymorphs, pseudopolymorphs and amorphs), complexation, solubilization with the use of a surfactants or cyclodextrins and dispersion of the active

ingredient with excipients (12). The salt forming method is not possible in case of neutral substances as it is not always suitable in case of a weak base or a weak acid. Even if the active substance can be prepared as a salt, it does not mean that this will improve its solubility, as it can convert back to its original form in the gastrointestinal tract. Surfactants and cosolvents liquid formulations are made with the solubilization of active ingredients in organic and aqueous media, thus these are commercially less acceptable as solid dosage forms (13). The dissolution rate of a given substance can be increased by increasing its surface area being available for dissolution. This can be achieved by reducing the size of particles or improving the wetting of the substance. By milling a powdered substance we may reduce particle sizes, whereas the method is limited with size of particles that can be achieved. In some cases, the resulting powder particles tend to agglomerate, which partially eliminates the effects of grinding. Furthermore, powder particles are also problematic to handle and have poor wettability (12, 13).

1.3 THE AMORPHOUS STATE

The amorphous state of solids is composed of disorderly arranged molecules, which do not form the characteristic of crystal grid and therefore has zero crystallinity. Distribution of molecules in the solid amorphous form is not entirely random. Actually it has a specificity of a gaseous state with a certain degree of order between neighboring molecules. Due to the lack of regulation and the absence of a crystal grid compared to the crystal, the amorphous form has higher enthalpy, entropy and Gibbs free energy. Therefore, it is thermodynamically unstable and susceptible to spontaneous transition into a more stable crystalline form (14). Differences between the crystal and the amorphous states are shown in Figure 3.

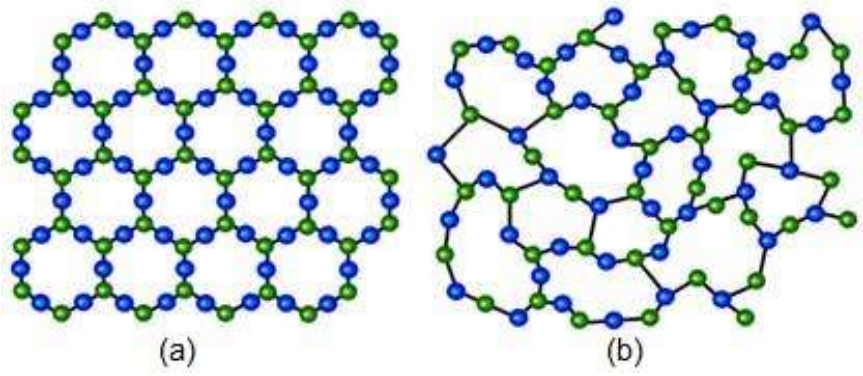


Figure 3: Crystal (a) and amorphous (b) state (15).

The glass transition temperature is one of characteristics of the amorphous form. This is a temperature interval in which the heated substance passes from a solid glassy state into a

softened glassy state which is distinguishable from a liquid due to mobility of molecules (16). Glasses are liquids that are frozen in time when being evaluated (experiment). Irrespective of their thermodynamic instability, they can be stable from a kinetic point of view, but only as long as applicable in pharmaceuticals (17). Movement of molecules below the glass transition temperature is very limited, whereas amorphous materials are relatively stable below the glass transition temperatures, while above them they are vulnerable to mechanical and thermal stresses. Furthermore, molecules are moving slowly below the mentioned temperature, resulting in alteration of properties during aging of an amorphous sample (16).

The advantage of an amorphous substance in comparison with its crystalline form is its improved rate of dissolution. Actually active substances in their amorphous forms are in most cases more soluble and dissolve more rapidly than in their crystalline forms. Sputtering kinetic solubility of the amorphous form in comparison with its crystals can be up to 1,500 times higher. This advantage is of course significant in case of poorly soluble substances and results in higher bioavailability of their amorphous forms (18).

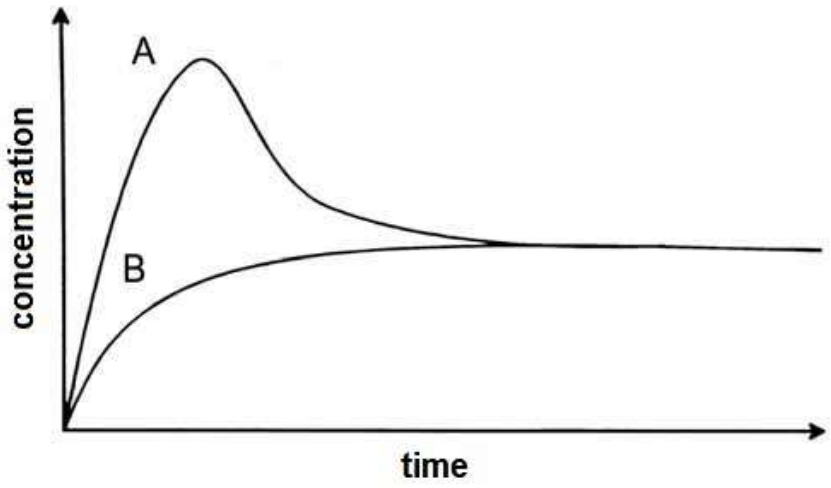


Figure 4: Dissolution rates of amorphous (A) and crystal (B) drug (14).

One of the methods used to stabilize the amorphous state of given drug is the formation of solid dispersions.

1.4 SOLID DISPERSIONS

The term solid dispersion (SD) refers to a group of solid products consisting of at least two different components, generally a hydrophilic matrix and a hydrophobic drug. Such matrix can be either crystalline or amorphous, whereas the drug can be dispersed molecularly, in amorphous particles (clusters), or crystalline particles. Based on their molecular arrangement, six different types of SD can be distinguished, as shown in Table II (19).

Table II: Types of solid dispersions (19).

Solid dispersion type		Matrix *	Drug **	Remarks	No. phases
I	Eutectics	C	C	The type of solid dispersions that was prepared first.	2
II	Amorphous precipitations in a crystalline matrix	C	A	Rarely encountered.	2
III	Solid solutions				
	Continuous solid solutions	C	M	Miscible in all compositions, but never prepared.	1
	Discontinuous solid solutions	C	M	Partially miscible, 2 phases even though a drug is molecularly dispersed.	2
	Substitutional solid solutions	C	M	Molecular diameter of a drug (solute) differs less than 15 % from the matrix (solvent) diameter. In this case the drug and the matrix are substitutional. Can be continuous or discontinuous. When discontinuous: 2 phases even though drug is molecularly dispersed.	1 or 2
	Interstitial solid solutions	C	M	Drug's (solute) molecular diameter is less than 59 % of the 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 the cooling/evaporation rate. Obtained after crystallization of a drug in an amorphous matrix.	2
V	Glass suspension	A	A	Particle sizes of dispersed phase dependent on the cooling/evaporation rate; several solid dispersions are of this type.	2
VI	Glass solution	A	M	Requires miscibility or solid solubility, complex formation upon fast cooling or evaporation during preparation. Several (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.
***: polyethylene glycol
****: polyvinylpyrrolidone

1.4.1 Mechanisms for increased solubility and dissolution rates in solid dispersions

Mechanisms and the dissolution rate of an active ingredient in a solid dispersion are influenced by various factors. Which of them will prevail, depends mainly on the composition and preparation method of a solid dispersions. Molecular dispersion is the last stage in the process of particle size reduction. In this way, the surface area of the particle available for dissolution is increased to the maximum. Consequently, the rate of dissolution and solubility of the drug increases (19). Specific surface area of a drug can be increased by using a porous, on which an substance can be absorbed (20). Better wetting is a large contribution to improvement of dissolution that appears both in the media with surface activity, as well as in those without it, because each drug particle is completely surrounded with a water soluble carrier, which, in contact with water, dissolves rapidly (21). In a solid dispersion a drug is often in an amorphous state. Less energy is required for the dissolution of the active ingredient in an amorphous state, because it is not consumed for degradation of the crystalline structure during the process (19, 22, 23). Furthermore, a lack of aggregation and agglomeration of crystals of a pure hydrophobic drugs plays an important role in increasing the dissolution rate. In a solid disperse it is surrounded by a carrier, which prevents convergence and aggregation of particles. The dissolution rate may also be affected by a solubilization effect of a carrier in microenvironment, which is completely dissolved in the vicinity of the drug (21). The most common interactions between a drug and a carrier are the van der Waals (VDW) forces and hydrogen bonds. They can either increase the release of a drug from the stabilized amorphous form or slow it down by capturing active substance into pores of a carrier (24, 24).

1.4.2 Disadvantages of solid dispersions

Despite intensive scientific research of SDs, they are rarely used in commercial products. The reasons for this are: poorly defined and expensive manufacturing methods involving use of high temperatures or large amounts of organic solvents, low production reproducibility, problems regarding manufacturing of an adequate dosage forms, limitations in the increasing of production batches, and physical and chemical instabilities. The difficulties in designing adequate dosage forms occur due to stickiness, poor flow properties and incompressibility of a given SD, fragmentation of too soft particles and poor disintegration of tablets. The biggest weakness of SDs however is a possibility of conversion an amorphous form of an active substances into a crystal, which is less water soluble (26).

1.4.3 Stability of amorphous solid dispersion and prevention of its crystallization

Drugs in a solid dispersion can change into a less stable crystalline form during manufacturing process or storage, due to mechanical stress, high temperatures or moisture influence (27). Molecular mobility has the greatest role in the stability of an amorphous material. It depends on the composition of the SD, as well as on the manufacturing method and conditions, because it strongly influences the thermal history of the substance (28). SDs with low glass transition temperature (Tg) are the least stable, whereas molecular mobility above this temperature is very high and easily leads to nucleation and crystal growth (29). Even in especially viscous systems under Tg amorphous substances in the SD have sufficient molecular mobility that in pharmaceutically important time can result in nucleation and crystal growth. Therefore, it has become a rule that a SD should remain stable when stored at a temperature of 50 °C lower than the Tg (27, 30). Furthermore, during storage, the mobility of molecules is affected by moisture, since it acts as a softening agent and speeds up crystallization of active substances (29). Moisture is absorbed by numerous polymers, and this may result in separation of phases, crystal growth and conversion of less stable forms of active ingredients into stable ones. These changes are reflected in the reduction of solubility and dissolution rate of an active substance (27).

1.4.4 Solid dispersion production methods

Various preparation methods of solid dispersions have been reported in literature. Thus all deal with a challenge of mixing a matrix and a drug, preferably at molecular level, while knowing that the matrices and drugs are generally poorly miscible. During this demanding process, partial or complete de-mixing and formation of different phases are observed (19).

Methods for making SDs can roughly be divided into the those applying melting, dissolution and other processes, as shown in Figure 5. The first step in melting methods is carried out at elevated temperature, followed by cooling and pulverization of the product. High temperatures which can cause decomposition of active ingredients are the main limitation of these methods. Methods using organic solvents for dissolving active ingredients and carriers, followed by solvent evaporation are carried out at relatively low temperatures and therefore the resulting product is pulverized. The method of solvent evaporation can have excipient undissolved, whereas the active substance precipitates in its pores and is adsorbed to particle surface. In this case, excipients are insoluble hydrophilic materials, for example porous calcium carbonate. Melting by solvent evaporation which is a combination of melting, dissolution methods and co-grinding methods belong to the other methods of solid dispersion production (1, 19, 27, 31, 32).

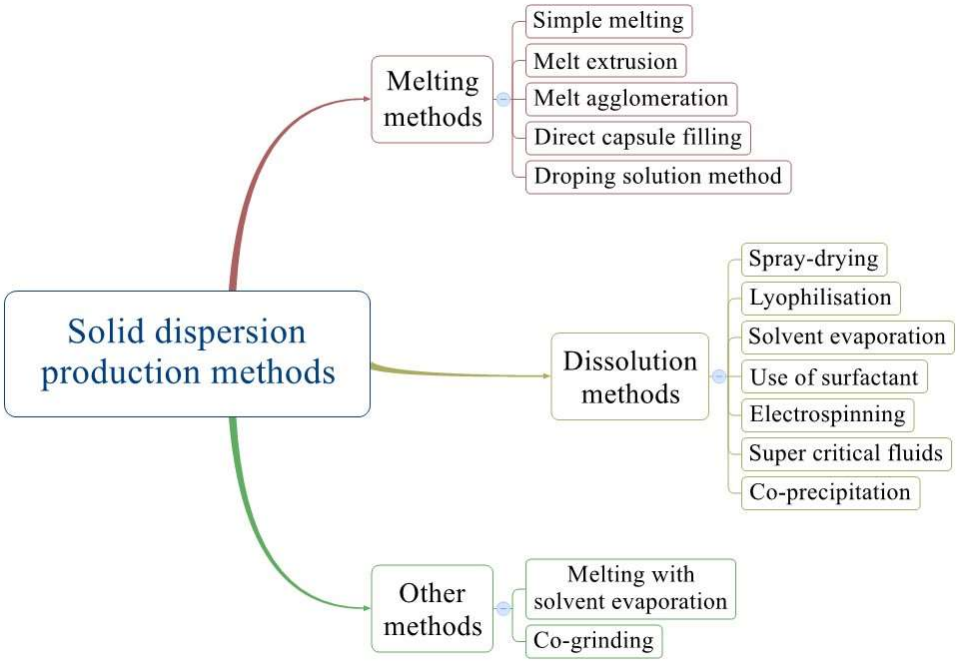


Figure 5: Various solid dispersion production methods.

1.5 POROUS CARRIERS

The basic goal of all drug delivery systems is to provide therapeutic amounts of a given drug to a proper site in the body promptly relief and to maintain its desired concentration. Controlled release delivery of drugs began in the 1970s and has since continued to expand quickly. Different drug delivery systems, such as liposomes, micelles, polymeric micro/nanoparticles and emulsions have shown great promise in controlled and targeted drug delivery. Porous materials are emerging along with these systems as a new category of host-guest systems. Possessing several alternative features, such as high surface area, stable uniform porous structure, tunable pore sizes with narrow distribution and well defined surface properties, greater attention was given to the development of porous materials as modified drug delivery matrices. Owing to their wide range of useful properties, porous carriers have been used in pharmacy for many purposes, including development of novel drug delivery systems, such as sustained drug delivery systems, floating drug delivery systems and for solubility enhancement of poorly water-soluble drugs. These materials contain great numbers of nanopores that allow entrapment of drug molecules. These allows them to adsorb and release drugs in a more reproducible and predictable manner. The application of mesoporous, microporous and nanoporous carriers used for drug delivery is therefore a part of a growing research area (33).

Water insoluble porous carrier materials for pharmaceutical applications are: porous silicon dioxide (Syloid®, Sylysia®), polypropylene foam powder (Accurel®), porous calcium silicate (Florite®), porous ceramics, calcium carbonate ($CaCO_3$) and magnesium aluminometasilicate (Neusilin®). According to the size of pores, the IUPAC nomenclature distinguishes: microporous (0.3 to 2 nm), mesoporous (2 to 50 nm) and macroporous materials (greater than 50 nm) (34). Despite their water insolubility, they can posses either hydrophobic or hydrophilic characters. The hydrophobic character enables them to be used for making floating delivery systems or for sustained release of drugs. On the other hand, their hydrophilic nature enables them to improve the dissolution of poorly water soluble drugs and to be carrier system (35, 3636).

1.5.1 Calcium carbonate

Calcium carbonate is one of the most abundant calcium salts present in the earth's crust. Calcium is an alkaline earth metal with the atomic number 20 and chemical formula Ca^{2+}. It represents about a third of the metals found on the earth and is essential for living organisms. In fact, it is the key component of a balanced diet, as it is crucial for formation of bones and teeth and overseeing important physiological functions (37).

Because of its chemical reactivity with water, pure calcium cannot be found in nature, except in some living organisms, where Ca^{2+} plays an essential role in their cellular physiology. Large quantities of this metallic element are present in carbonate rocks, such as marble, limestone, gypsum and fluorspar, in all of which it represents a fundamental component in the form of salt derived from carbonic acid, i.e. calcium carbonate ($CaCO_3$).

Pure $CaCO_3$ is a white solid at room temperature with a MW of 100 as presented in Figure 6. It occurs as a powder or crystals, is odorless and tasteless and practically insoluble in ethanol (95%) and water. Its solubility in water is increased in presence of ammonium salts or carbon dioxide, while alkaline hydroxides are reducing it. The hexagonal calcite is the most common and stable form of $CaCO_3$.

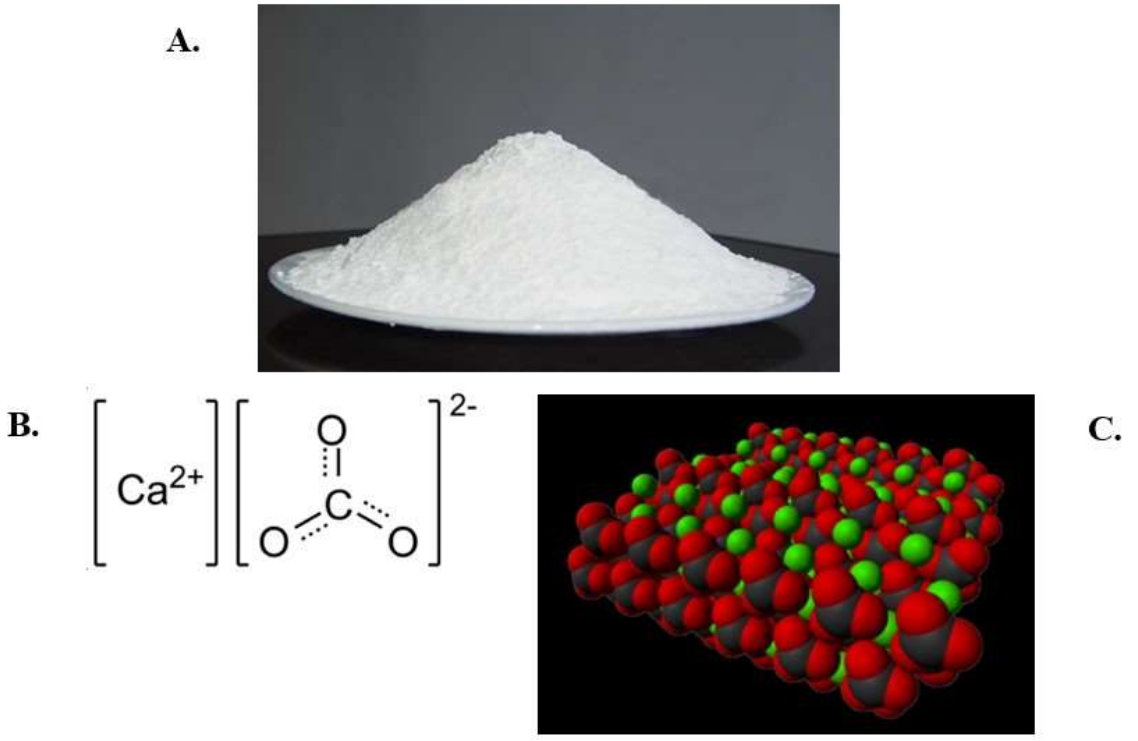


Figure 6: $CaCO_3$ powder (A); chemical formula of $CaCO_3$ (B); molecular 3D structure of $CaCO_3$ (C).

Aqueous solution of $CaCO_3$ (10% w/v) has a pH value of 9.0. Calcium carbonate has an apparent density of 0.8 g/cm^3, a hardness index of 3,0 on the Mohs scale, a decomposition point of 825 °C and a refractive index of 1.59. Its specific surface area is between 6.21 and 6.47 m^2/g and specific gravity 2.7. Calcium carbonate is prepared through double decomposition of calcium chloride and sodium bicarbonate in an aqueous solution. Its density and fineness are governed by concentrations of reacting solutions. Calcium carbonate can also be obtained from naturally occurring minerals like aragonite, calcite, and vaterite. The particles of $CaCO_3$ are characterized by cohesive fluidity. The substance is stable and should be stored in a tightly closed container in a cool, dry place, especially when the temperature is out of 15–25 °C interval, humidity >60 % RH and when storage area is poorly ventilated (38).

1.5.1.1 The history of $CaCO_3$ use

Calcium carbonate was used as early as 40,000 BC. The history of calcium shows how the human race was able to use unique properties of this mineral for various applications ranging from prehistoric cave paintings to production of paper and plastics in the last century. It was detected in almost all prehistoric cave paintings from the period between 40,000 and 10,000 BC, although it was right at the end of this era that the chalk and limestone dust were actually used by "cavemen artists".

In 100 BC the Romans used plaster, stone and gravel for road construction. In particular, gypsum was also used for the production of cosmetics for Roman women.

Plaster was used as soil fertilizer in medieval Britain. Also during this period, plaster was used for medicinal purposes in the fight against scurvy, however probably without success.

Following the rapid increase in the use of brick and stone for construction during the industrial revolution in the 18th and 19th century, the usage of calcium carbonate in lime and paints grew progressively. Greater demand for $CaCO_3$ was also created by the expanding dyeing and printing industry. As a result, its suppliers began to develop methods for the industrial production of $CaCO_3$.

The production of precipitated calcium carbonate (PCC) started in 1841. The first producer was the British company, John E. Sturge Ltd., which treated the residual calcium chlorate from their potassium chlorate manufacture with sodium carbonate and carbon dioxide to form what they called the gypsum precipitate.

In 1898, a new plant was built in Birmingham and was the first to adopt the milk of lime method, a process, still in use today.

The production of $CaCO_3$ increased and this mineral was also used in the manufacture of glass.

In the early 1900s, the modern toothpaste was invented to facilitate the removal of foreign particles and food substances from the oral cavity, as well as to clean teeth. Chalk was commonly used as abrasive in early formulations. Subsequently, in 1930, it was completely replaced by PCC.

In 1940, the outbreak of rickets in Dublin pushed the government to issue a decree providing for the addition of calcium into common bread. Therefore, plaster became the binding agent food additive. It was believed that the reduction in provision of calcium, due to lack of food in times of war, caused rickets in children and the bone disease, osteomalacia, in adults.

Development of various industries and the growing demand for plastics, paints, filler materials and detergents led to an increased use of $CaCO_3$, a material appreciated for its ability to adapt to various requirements due to the fineness of its dust and particle size distribution.

Calcium carbonate was introduced in the production of modern paper for printing in the mid-twentieth century. This opened the way for GCC as well as PCC, both widely used nowadays, as fillers in coated paper and pigments in graphic paper and cardboard.

Since the late 1980's, $CaCO_3$ has been used in environmental protection. The technology of desulfurization of exhaust gases has significantly reduced the phenomenon of 'acid rain' by using a sorbent, usually lime or limestone, to remove sulfur compounds produced in the combustion of fossil fuels.

Since 1995, $CaCO_3$ has also been used for development of 'functional additives', the polyolefins. These were developed for the production of breathable films used by the hygiene market, particularly as diapers.

The $CaCO_3$ industry during the first part of the twenty-first century can be represented by the three 'Es': economy, ecology and energy.

Although its use will decline in some markets, it is certain that $CaCO_3$ will sustain its significant role in everyday products (39).

1.5.1.2 Present use of $CaCO_3$ in pharmaceutical field

Calcium carbonate is most commonly used in the pharmaceutical field as an active ingredient in various formulations of antacids, because it neutralizes acid rapidly, effectively and inexpensively. Calcium-based antacids are available in a wide range of preparations, as tablets, liquids, soft gels, effervescent tablets and chewing gums.

In case of hypocalcaemia, $CaCO_3$ is used in human food as a supplement, especially in osteoporosis treatment, and in cosmetology as an excipient for preparation of tooth pastes and beauty creams.

Other pharmaceutical applications depend on its high calcium content (40% elemental calcium) and adsorbing power, especially when used in powders with a high surface area of particles, as well as its ability to act as a less expensive filler and extension.

Concerns about its biocompatibility within the gastrointestinal tract can be excluded, since $CaCO_3$ is widely used as food additive. Biocompatibility of its porous particles in micrometer and nanometer range is proven by toxicological tests in HeLa cells. Calcium carbonate decomposes very fast under acidic conditions in the stomach, liberating carbon dioxide. When enteric coated capsules or tablet formulations are administered, the enteric chemical decomposition of $CaCO_3$ microparticles might play only a minor role due to a relatively high pH in the small and large intestine, thus most of them will be eliminated through excretion. However, despite of its favorable properties, the available literature dealing with drug loading into porous $CaCO_3$ microparticles is sparse, as low drug-loading capacity limits the use of such carriers. Therefore, $CaCO_3$ particles with larger pore volume are an alternative in providing higher active substance loading capacity.

Drug loading can theoretically be done during the synthesis of the carrier material, but it is more convenient to use prefabricated carrier particles. This allows that poorly water soluble drugs can be loaded by using organic solvents. The most common method is based on adsorption of a drug to carrier particles by impregnating them in a drug solution. However, sufficient payload represents a challenge in the development of the right impregnation method. Sufficient amounts of the drug need to be dissolved in a solvent to be able to obtain its satisfactory load, but unfortunately strong solute–solvent interactions can lead to lower carrier adsorption. The resulting drug load (DL) can vary considerably, therefore representing a major challenge when developing a reproducible impregnation method (40).

The solvent evaporation method, which is usually performed under reduced pressure, is an alternative drug loading method. Direct determination of DL by summing the masses of drug

and carrier material is enabled by continuous removal of the solvent until complete dryness. Otsuka et al. made use of this approach by loading phytonadione into porous silica, resulting in a distinct mode of drug release that was rapid (1 h) initially, but continued slowly over the period of 24 h (41). Sher et al. loaded ibuprofen into macroporous polypropylene microparticles by evaporating methanol or dichloromethane under ambient conditions and investigated the influence of solvent volume on the adsorption mechanism (42). In fact, the feasibility of the solvent-evaporation method for drug loading has been questioned, since it is considered to deposit the drug as a crystalline layer whose properties and composition cannot be reproducibly controlled. However, it is believed that a better understanding of the processes is needed before the solvent-evaporation method can be declared as unsuitable for drug loading in porous carriers (40).

2. RESEARCH OBJECTIVES

A great number of drugs available on the market contain an active ingredient that is poorly soluble in water and has a low dissolution rate. Various approaches to improve these properties exist. One of the most common ones is the production of a solid dispersion, where the use of porous carriers, such as $CaCO_3$ has proven some potential.

Since naproxen is a substance with a good permeability and a low solubility (Class II substance, according to Biopharmaceutics Classification System (BCS)), its dissolution is the limiting factor that determines the speed and extent of absorption of this active ingredient.

Our aim is to investigate whether, by incorporating naproxen, which is practically insoluble in water, into $CaCO_3$, its solubility and dissolution rates will increase.

First, we will determine the properties of various $CaCO_3$ samples (non-commercial ground $CaCO_3$ and pharmaceutical grade $CaCO_3$) with different methods to gather useful information about this excipient for further investigation and its suitability as a porous carrier for the solid dispersions (SD).

Furthermore, we will produce the SD by using two different methods (equilibrium and solvent evaporation) and a combination of both. The success of naproxen entrapment within the excipient will be determined with DSC analysis, which will provide information about the crystallinity of dispersions. The profile of naproxen release from $CaCO_3$ will be determined with a dissolution test, whereas the dissolution rate of our SD will be compared with the release rate of the pure active ingredient, as well as with simple physical mixtures with $CaCO_3$ excipients.

3. MATERIALS AND METHODS

3.1 MATERIALS

For experimental work we used the following:

- **compounds:**
  - Naproxen (Lex, Slovenia),
  - Commercial ground $CaCO_3$ – CAL 1 (TRITURADOS Blanco Macael, Spain),
  - Pharmaceutical grade ground $CaCO_3$ Omyapure 35 OG – CAL 2 (Omya, Switzerland),
  - Hydrochloric acid, HCl (Merck, Germany).
- **solvents:**
  - 96% Ethanol (Pharmachem, Slovenia),
  - Acetone (Merck, Germany),
  - Dioxane (Merck, Germany),
  - Tetrahydrofuran, THF (Carlo Erba, France),
  - Purified water.
- **laboratory equipment:**
  - Glassware (beakers, flasks, tubes, measuring cylinders, sticks etc.),
  - Pipettes,
  - Plastic dropper,
  - Syringes and needles,
  - Plastic weighing boats or dishes,
  - Minisart® RC 25 0,45µm filters (Sartorius, Germany),
  - Millipore Durapore® 0,1µm membrane filters (Merck, Germany),
  - Parafilm (Pechiney Chicago, USA).

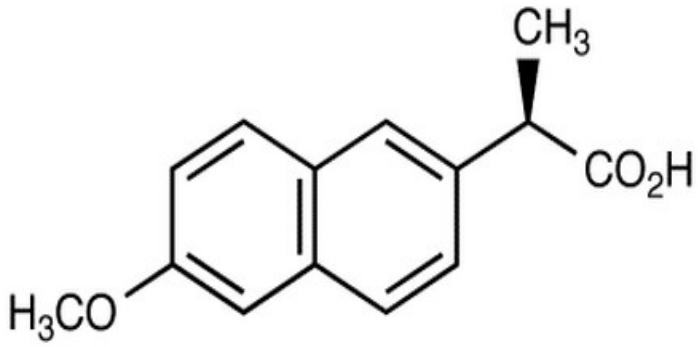
3.1.1 The drug used in experiments

3.1.1.1 Identification

NAPROXEN

IUPAC name: (+)-(S)-2-(6-methoxynaphthalen-2-yl) propanoic acid

Structure:



Molecular formula: $C_{14}H_{14}O_3$

Molecular mass: 230.259 g/mol

Melting temperature: 152–154 °C

Solubility in water: 15.9 mg/L

Description: Naproxen is an odorless, white to off-white crystalline substance. It is lipid-soluble, practically insoluble in water at low pH and freely soluble in water at high pH. Its logarithmic octanol/water partition coefficient of at pH 7.4 is 1.6 to 1.8 (43).

Pharmacotherapeutic group: non-steroidal anti-inflammatory and antirheumatic drug (NSAID), propionic acid derivatives, ATC code: M01AE02 (44).

3.1.2 Porous calcium carbonate

We used a sample of non-commercial ground $CaCO_3$ (CAL 1) from the marble quarries of Macael (TRITURADOS Blanco Macael, SA, Almeria, Spain). This sample was ground by the company in an industrial ball mill and sieved according to grain size through the pneumatic device (cyclone). This sample was compared with a sample of pharmaceutical grade $CaCO_3$ from Omya (Omyapure 35 OG (CAL 2)). The SEM pictures of samples are presented in Figure 7.

Omya is the world's largest producer of natural $CaCO_3$ with production facilities in various countries. The company owns two manufacturing plants, which produce GCC for the pharmaceutical industry. One is based in the US state of Arizona and the second in Europe, France. The processing in its quarries takes place according to good manufacturing practice and the monographs of major pharmacopoeias: EU, Japan and USA. Equipment and production processes meet all the requirements of the ISO 9000 standard. In particular, Omya GCC has obtained the certificate of suitability (CEP/COS) for the production of natural $CaCO_3$ as an active pharmaceutical ingredient (API) by the European Directorate of the Quality of Medicines (EDQM) in accordance with the European Pharmacopoeia.

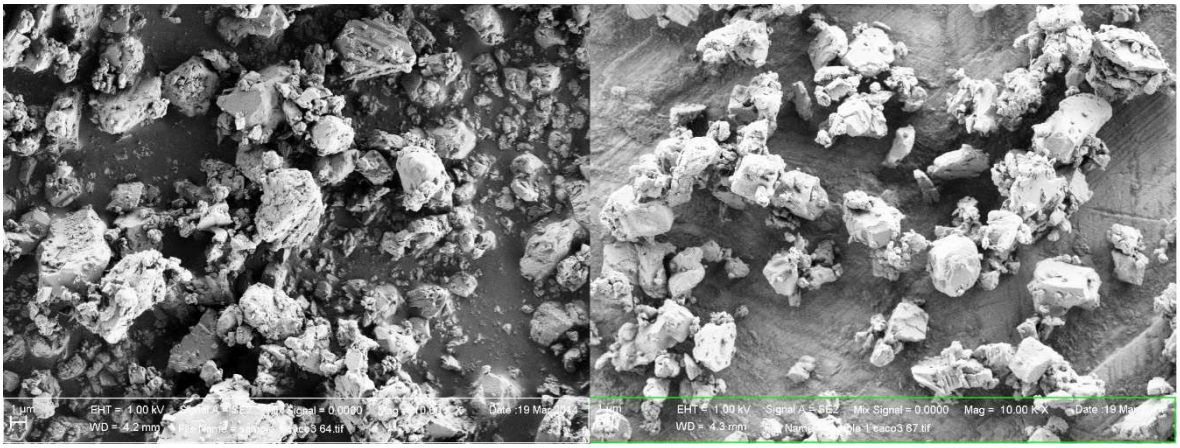


Figure 7: SEM pictures of CAL1 (left) and CAL2 (right).

3.2 EQUIPMENT

Equipment used during experimental work:

- Scale, Mettler Toledo XS 205 Dual range, Switzerland,
- Scale, Mettler Toledo AG 285, Switzerland,
- Helium pycnometer, Micrometrics AccuPyc 1330, USA,
- Thermo balance, Shimadzu 50H, Japan,
- Particle size analyzer, Micromeritics Sedigraph 5100, USA,
- Porosymeter, Micromeritics Autopore III 9410, USA,
- Field emission scanning electron microscope, SEM Supra 35 VP, Carl Zeiss, Germany,
- Differential scanning calorimeter, Mettler Toledo DSC1, STAR software v9.30, Switzerland,
- pH meter, Mettler Toledo Seven Compact, Switzerland,
- Ultrasonic bath, Sonis 4, Iskra pio, Slovenia,
- Magnetic mixer, RTC basic, IKA, Germany,
- Waterbath, Julabo ED, Germany,
- Rotavapor, Buchi R-114, Sigma-Aldrich, USA,
- Waterbath, Buchi B-480, Sigma-Aldrich, USA,
- Vacuum pump, Buchi Vac V-500, Sigma-Aldrich, USA,
- Vacuum controller, Buchi Vacuum controller B-721, Sigma-Aldrich, USA,
- Dissolution tester, Erweka DT6, Germany,
- Spectrophotometer, Hawlett Packard 8453, UV-visible spectroscopy system, Germany,
- Heating and drying oven, Heraeus, Germany.

3.3 METHODS USED FOR DETERMINATION OF CALCIUM CARBONATES

3.3.1 Density

We measured the densities of our excipients (CAL1, CAL2) with a Micrometrics AccuPyc 1330 helium pycnometer. The cell of the apparatus was weighted and then filled up to about 2 thirds of its volume (approximately 4 g of each sample were weighted accurately) and then weighted again. In the end, the cell was put into the pycnometer and density measured.

3.3.2 Thermogravimetric analysis (TGA)

By using the thermogravimetric analysis, we weighted approximately 50 mg of each sample in the aluminium oxide carrier, each was then heated in the range of 30–950 °C at a 10 ºC/min rate in a nitrogen atmosphere. The Shimadzu® 50H thermogravimeter was used for all analyses.

3.3.3 Size distribution of particles

The $CaCO_3$ particle size distribution was determined using a laser granulometer (Micromeritics Sedigraph 5100), which measures the intensity of the radiation diffracted by particles suspended in a liquid solution of pyrophosphate in water.

3.3.4 Granular density and pore diameters

The porosimetric analysis was carried out using the Micromeritics AutoPore III 9410 porosimeter, capable of measuring pores with diameters between 0.003 and 360 µm. Approximately 1 g of each sample was dried for 24 hours at 95 °C and subsequently analyzed. Porosity was calculated according to Equation 2.

$$\varepsilon = \left(\frac{(\rho_r - \rho_g)}{\rho_r} \right) \cdot 100\,\%$$

Equation 2: Porosity calculation (ε= porosity; ρ_r= real density; ρ_g=grain density).

3.3.5 Textural features of particles (SEM)

Textural features of particles were assessed by using the field emission scanning electron microscope (SEM Supra 35 VP-Carl Zeiss, Germany). Samples of excipients were glued on a biadhezive lateral carbon tape and evaluated. Measurements were carried out at 1 kV voltage using electronic canon and a secondary SE2 electron detector. In order to get better results, measurements were made at two different magnifications, for comparison.

3.4 METHODS FOR PREPARATION OF SOLID DISPERSIONS (SD)

3.4.1 Preparation of SD with adsorptive equilibrium

Dispersions made with adsorptive equilibrium were prepared by mixing naproxen and excipients in a solvent. Solvents must dissolve the drug while at the same time excipients must be insoluble in them. Dispersions were mixed in Erlenmayer flasks which were then covered with parafilm in order to prevent solvent evaporation. Further on, the flasks were left on a magnetic stirrer for 3 hours to obtain chemical equilibrium. After that, the mixtures were filtered through Millipore Durapore® 0,1μm membrane filters and dried for 24 hours at 40 °C.

In order to select solvents with best possible properties for preparation of SD we tested 4 different organic ones. The solubility of naproxen in 4 different organic solvents are shown in Table III.

Table III: Solubility of naproxen in different organic solvents.

Solvent	Dipole moment	Solubility M (mol/L)
Ethanol	1.70	0.277
Dioxane	0.45	1.379
Acetone	2.88	0.726
THF	1.63	1.481

Table IV shows the amounts of naproxen, excipients and solvents used for preparation of SD. The amounts depend on the naproxen solubility in these solvents.

Table IV: Parameters for preparing SD by equilibrium method.

Solvent	Solvent volume (mL)	Amount of naproxen (g)	Amount of excipient (g)
Ethanol	50	2	4
Dioxane	25	5	2
Acetone	25	2.5	1
THF	25	5	2

3.4.2 Preparation of SD prepared with solvent evaporation

For the preparation of SD with solvent evaporation under reduced pressure we used ethanol. Namely it was proven to be the best candidate for the production of amorphous structures of naproxen, able to precipitate in pores of a hydrophilic frame, in order to increase its dissolution properties in combination with its higher surface area.

Table V: Parameters used for preparation of SD with solvent evaporation.

Dispersion	Amount of naproxen (g)	Amount of excipients (g)	Solvent volume (mL)
5% dispersion	0.2105	4	50
20% dispersion	1	4	50

Amounts of naproxen shown in table V were dissolved in ethanol and mixed in a flask for few minutes. Afterwards, the excipients were added and mixed. The flask was attached to the Buchi R-114 rotavapor and partly sunk into the Buchi-480 waterbath heated to 40 °C. The parameters applied in the solvent evaporation procedure are shown in Table VI. Pressure was controlled with the Buchi Vac V-500 vacuum pump.

Table VI: Parameters for decreasing pressure.

Pressure (mbar)	Time (min)
150	30
100	30
50	60

To remove any residual solvent, SD were dried out after solvent evaporation in an oven for 60 min at 40 °C. Dry SD were gently crushed in a mortar in order to obtain fine powder for subsequent analyses.

3.4.3 Preparation of SD prepared with a combination of adsorptive equilibrium and solvent evaporation method

The two methods were combined in order to gain benefits from both of them. Dispersions prepared with equilibrium adsorption were used as basics. After measuring the entrapped amount of naproxen in SD, we added proper amounts of the drug to prepare 10% dispersions. Ethanol volume was calculated to obtain saturated solution of naproxen. Amounts of all ingredients used are presented in Table VII. The solvent evaporation method process was the same as described beforehand.

Table VII: Parameters for preparation of SD with combination of two methods.

Sample	Amount of SD prepared with the equilibrium method (mg)	Amount of naproxen (mg)	Ethanol volume (mL)
CAL1+10%NAP	2,000	236.8	5
CAL2+10% NAP	2,000	224.8	5

3.5 METHODS FOR EVALUATION OF DISPERSIONS

3.5.1 DSC

The DSC analyses were carried out to study the crystallinity of naproxen and the prepared SD. Thermal analyses were performed using a Mettler-Toledo DSC1 differential scanning calorimeter, equipped with the STARe Software v9.30. We precisely weighted approximately 5 g of the active substance and 10–15 mg of prepared dispersions in 40 mL pots. Subsequently, the samples were hermetically closed, heated from -10 to 180 °C at a rate of 10 °C/min. Measurements were performed in a nitrogen atmosphere at a flow rate of 50 mL/min, whereas the apparatus was calibrated with indium.

To determine amounts of naproxen in its amorphous state we used DSC curves, as they present enthalpy changes of samples from which parts of crystallinity can be calculated. In order to obtain proper results, we used the correlation between the pure drug and SD. The following equation was used for its determination:

$$w(\%) = \left(1 - \frac{\left(m_1 \cdot \frac{(\Delta H_2)}{(\Delta H_1)}\right)}{m_2}\right) \cdot 100\,\%$$

Equation 3: Percentage of drug in the amorphous state: m_1= mass of naproxen itself; m_2= mass of naproxen present in dispersions; ΔH_1= enthalpy change for naproxen itself; ΔH_2=enthalpy change for naproxen present in dispersions.

3.5.2 Textural features of particles (SEM)

Textural features of particles were assessed with field emission scanning electron microscope (SEM Supra 35 VP), as described in Materials a Methods (Section 3.3.5).

3.5.3 Dissolution test

3.5.3.1 Calibration curve of naproxen

To obtain the calibration curve we prepared a solution containing 11.2 mg/L of naproxen and performed measurements with a spectrophotometer (Hawlett Packard 8453) at 230 nm. The R^2 for absorbance fluctuating between 0 and 1.4, was 0,9989.

3.5.3.2 Solubility of naproxen

Solubility of naproxen was assessed with a 24 hours mixing of the drug in our selected dissolution media with and without surfactant; 250 mg of naproxen were mixed with 100 mL of dissolution medium with 0.3 % and 1 % surfactant and without it.

3.5.3.3 Dissolution test

Dissolution test was performed in accordance to Ph. Eur 8th Ed., Chapter 2.9.3, using the Erweka DT 6 device with paddles (2). The test was performed with two samples of each SD and two of the active ingredient (naproxen) itself. The amount of SD used in the test contained 10 mg of naproxen. As a release medium 0.063 M of HCl was used. The medium volume was 900 mL, the temperature 37.0±0.5 °C and the paddle speed 50 rpm. Samples with a volume of 10 mL were collected manually and the sampling times were: 5, 10, 15, 20, 30, 45, 60, 120 and 180 min. As they were not replaced with new media, this has to be taken into account when calculating the total amount of dissolved substance in a particular time point. Samples were taken into plastic syringes and immediately filtered through Minisart® RC 25 0.45 µm filters. Dissolution medium was used as a blank. The absorbance of each filtered sample and blanks was determined by UV-Vis spectrophotometry at 230 nm. On the basis of the calibration line (see section 3.5.3.1), we determined the concentration of naproxen in each sample tested.

4. RESULTS AND DISCUSSION

4.1 DETERMINATION OF CALCIUM CARBONATE SAMPLES

4.1.1 Density

Helium pycnometer was used to measure the true density of $CaCO_3$ samples. The results presented in Table VIII. are averages of 3 consecutive measurements for each sample.

Table VIII: Real density of samples of $CaCO_3$ obtained with helium pycnometer.

Sample	Average density (g/cm^3)	SD (g/cm^3)
CAL 1	2.7320	0.0007
CAL 2	2.7100	0.0007

We see that the density of both samples is very similar and corresponds to the true density of calcite modification of $CaCO_3$, with the natural sample having a bit higher density than the pharmaceutical sample (45).

4.1.2 Thermogravimetric analysis

Results of thermogravimetric analyses are presented in Figure 8.

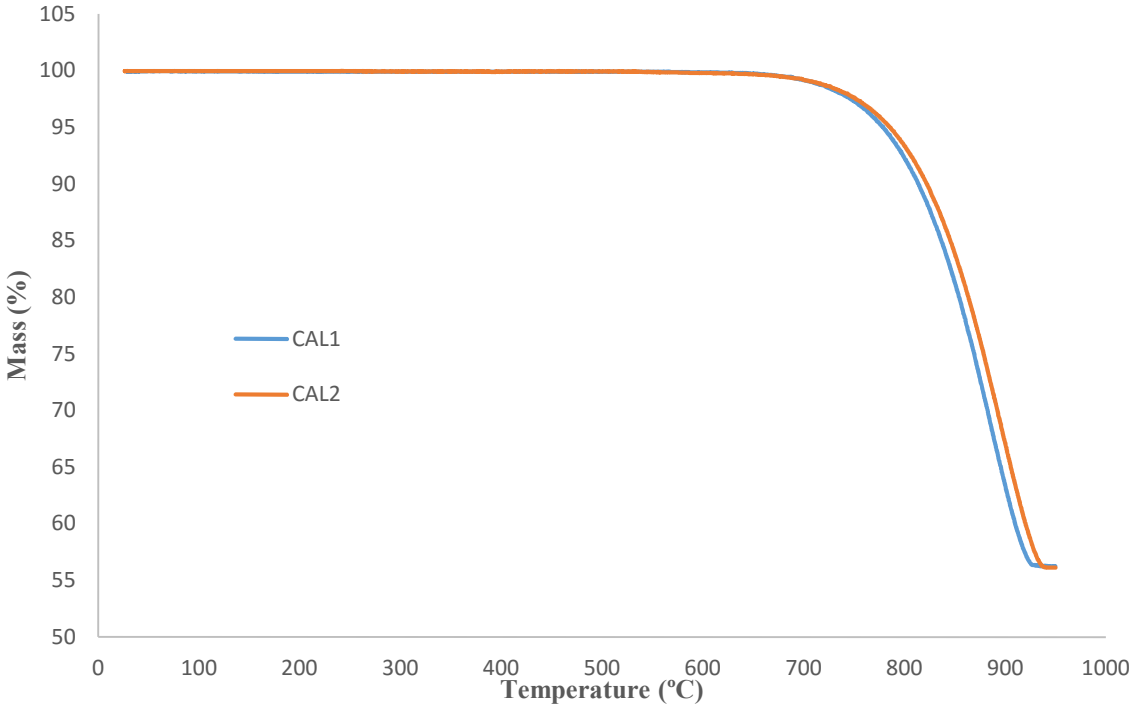


Figure 8: TGA curves of the two tested samples of $CaCO_3$.

The analysis shows a high thermal stability of $CaCO_3$ samples up to 650 °C. Curves show significant change in slope in the interval between 650 °C and 950 °C, with a loss of almost

45% of the initial mass, which is in accordance with literature data (46). The calcination reaction of $CaCO_3$ results in the release of CO_2 and the consequent formation of CaO as the final residue. The mass losses of each sample and the peaks measured for derivation of TGA curves are presented in Table IX.

Table IX: TGA results of Calcium carbonate samples.

Sample	Mass loss (%m/m)	
	≤650 °C	>650 °C
CAL1	0.43	43.23
CAL2	0.19	43.70

Results show slightly greater mass loss for CAL1 in the temperature interval between 0 °C and 650 °C which is probably result of more impurities present in the non-commercial sample.

4.1.3 Particle size distribution

Table X presents granulometric parameters obtained by laser diffractometry (average values of three measurements and standard deviations). The calculation of average particle size (geometric mean diameter (dg)) was carried out using log-normal transformation of the frequency data obtained. The cumulative representation of data allowed us to calculate the mode values for each sample, as well as average diameters of 95 % of the particles (undersize curve).

Table X: Parameters of particle size distribution of $CaCO_3$ samples.

Sample	Frequency curve		Cumulative curve		
	d_g (µm)	SD	Mode (µm)	SD	95%<∅(µm)
CAL1	5.21	0.18	5.94	0.21	10.81
CAL2	2.82	0.14	2.82	0.11	11.32

Both samples contain micronized particles, with CAL2 having smaller average diameters than CAL1, although the undersize curves of both samples are similar.

4.1.4 Granular density and pore dimensions

The porosimetric analysis with the Hg porosimeter allowed us to measure the granular density (ρ_g) and porosity (ε) as well as the surface area per mass unit (Sw) of the two samples (Table XI). The porosity was calculated using the true density (ρ_r) measured earlier with the helium pycnometer (2.7 g/cm^3) and the Equation 2.

Table XI: Results of Hg-porosimetry of $CaCO_3$ samples.

Sample	ρ_g (g/cm^3)	ε (%)	Sw (m^2/g)
CAL1	0.836	69.0	4.94
CAL2	0.949	64.9	82.66

The data analysis shows that the porosity was very high in both samples, although slightly higher in CAL1. Furthermore, the resulting surface area was substantially higher in CAL2, which could be the result of very small particle sizes present in this sample. Granular density, which represents the ratio between the mass of the solid and its true volume added to the average value of intraparticular pores volume, was similar in both samples.

Graphs showing results of porosimetry of both samples, are presented in Figures 9 and 10.

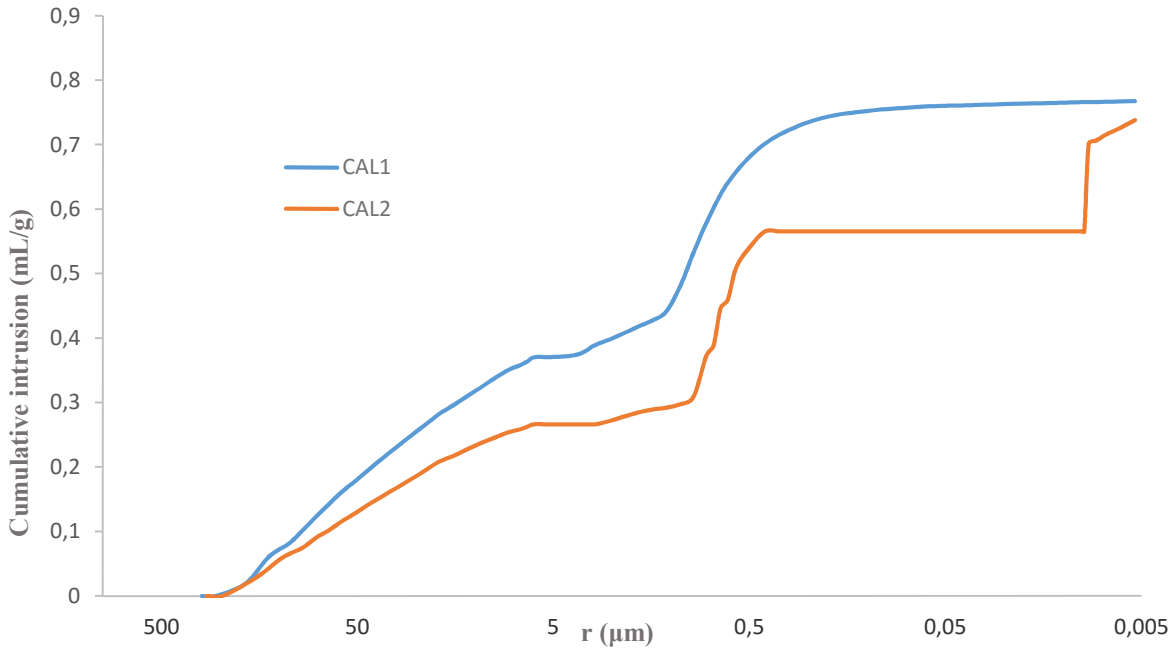


Figure 9: Cumulative intrusion vs. pore diameter (Hg porosimetry).

In Figure 9, increases in the curve slopes correspond to the values of relative pressure of mercury which occurs at significant intrusion of the element, and are therefore related to sizes

of pores. Furthermore, both samples showed interparticle porosity which is represented by the increase of progressive curves between 160 µm and 6 µm, as well as the second increment corresponding to the intraparticle porosity.

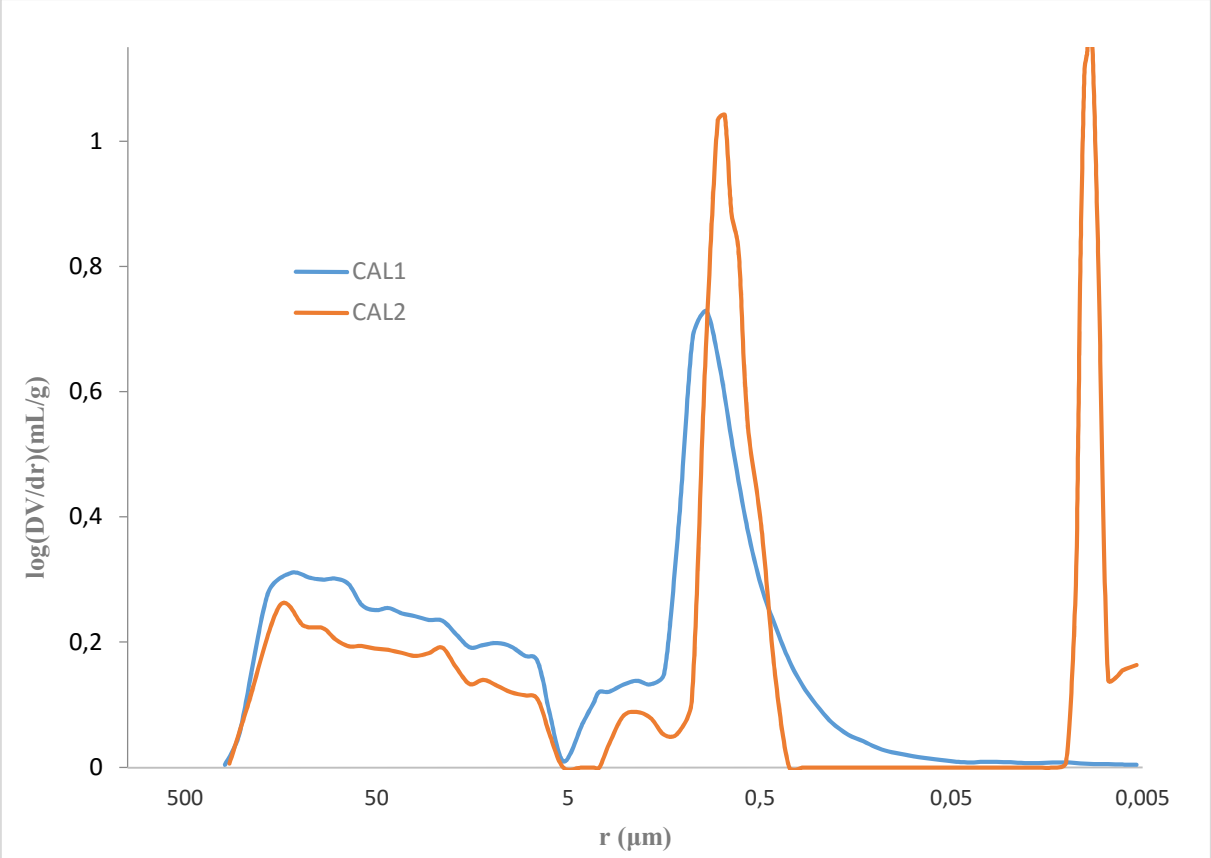


Figure 10: Local curve relative mercury intrusion vs. pore diameter.

Intraparticle porosity of both sample is presented in Figure 10. Greater volume of smaller pores in CAL2 is another indicator of a notably higher surface area, in comparison to CAL1. Table XII presents average pore diameters for both samples. The first curve slope increase shows diameters of interparticle pore dimensions, while the second one represents diameters of intraparticle pores.

Table XII: Pore diameters of both samples.

Sample	Interpaticular pores d (µm)	Intraparticular pores d (µm)
CAL1	110	0.9
CAL2	120	0.8

4.1.5 Textural features of $CaCO_3$ particles

The scanning electron microscopy analysis allows both, the observation of particle morphology and measurement of particle size.

Images from the electron microscopy (SEM) of our the $CaCO_3$ samples at two different magnifications are presented in Figure 11 and 12. Photomicrographs presented allow for comparisons of the similarities and differences of samples.

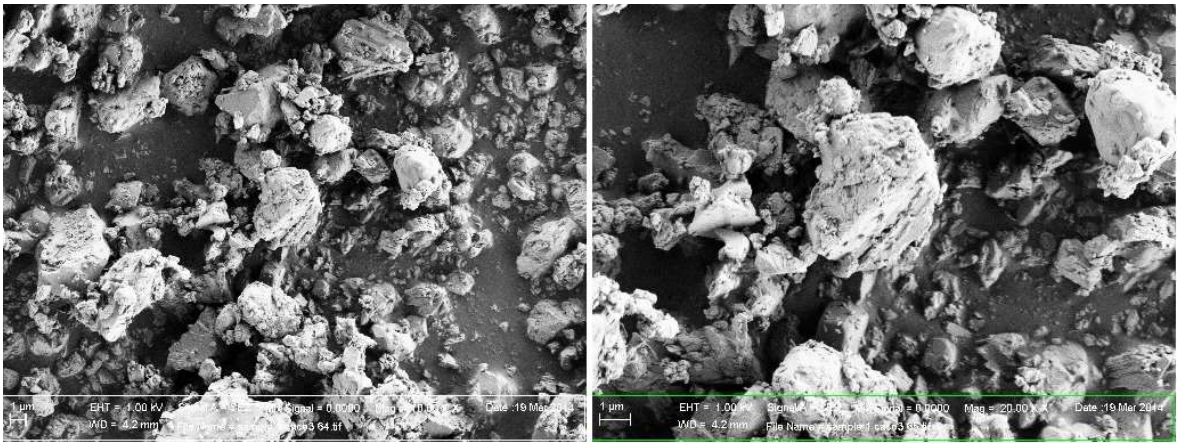


Figure 11: SEM photos of CAL1; magnifications 10 K X (left) and 20 K X (right).

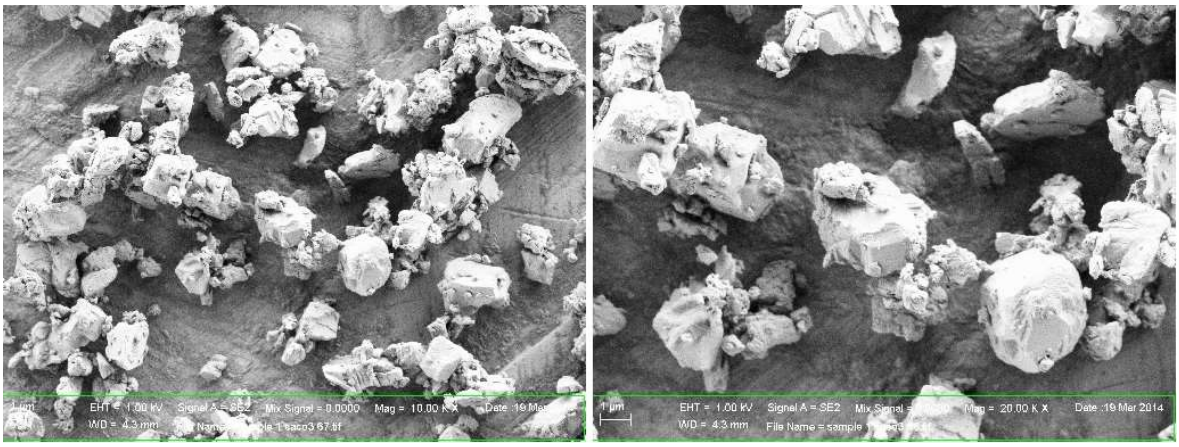


Figure 12: SEM photos of CAL2; magnifications 10 K X (left) and 20 K X (right).

As shown in SEM photos, the CAL2 sample has a greater dimensional uniformity and only little or no presence of big particles, while the CAL1 contains a broader range of particle sizes.

Regarding the morphology of particles in aggregates, CAL1 can be ascribed to the granular-modular type (irregularly shaped and roundish particles), whereas thus are of a more roundish shape, in, and they seem to be less aggregated.

4.2 EVALUATION OF SOLID DISPERSIONS

4.2.1 SD prepared by the equilibrium adsorption method

The equilibrium adsorption method provides the best proximity of the carrier which is then capable to entrap the drug into pore surfaces, however also large amounts of drug get lost during the process. Therefore, we used this method to observe how naproxen reacts with the carrier and if the usage of different solvents affects the adsorbed amounts of the drug. As already mentioned before, we used 4 different solvents, ethanol, acetone, dioxane and THF for preparation of SD.

Table XIII: Amounts of naproxen adsorbed on two different $CaCO_3$ samples, using 4 different solvents.

Sample	Solvent	w (%)	$w_{amorphous}$ (%)
CAL1+NAP	Ethanol	1.40	100
CAL1+NAP	Dioxane	4.05	60
CAL1+NAP	Acetone	3.95	76.1
CAL2+NAP	Ethanol	1.89	100

Figure 13 presents DSC curves of SD in all solvents except THF, because we were unable to recover the samples from these suspensions. The melting point of naproxen was lower in all dispersions in comparison to the substance itself, most probably due to small particle sizes. Dispersion curves prepared with ethanol did not have endothermic peaks, therefore we can assume that the adsorbed drug was in amorphous state. Using Equation 3 and analysing these curves, we calculated the amounts of amorphous naproxen absorbed on the carrier. The results are presented in Table XIII. The amorphous state of poorly soluble drugs is one of key characteristics for their improved dissolution properties.

The results indicate higher values for the adsorbed drug in samples prepared with dioxane and acetone, due to higher solubility of naproxen in these two solvents: ethanol (0,277M), acetone (0,726M) and dioxane (1,379M), although they were lower than expected. Furthermore, the percentage of naproxen in an amorphous state was lower in acetone and dioxin. Therefore, we can assume that this is a consequence of a higher amounts of dissolved drug residual in wet products, which crystallize during the drying process. This may also be the reason for a higher drug percentage content calculated for our SD. Thus, we concluded that the amount of the

adsorbed naproxen was too low for preparation of decent usable SD, therefore we applied another SD preparation method as described below.

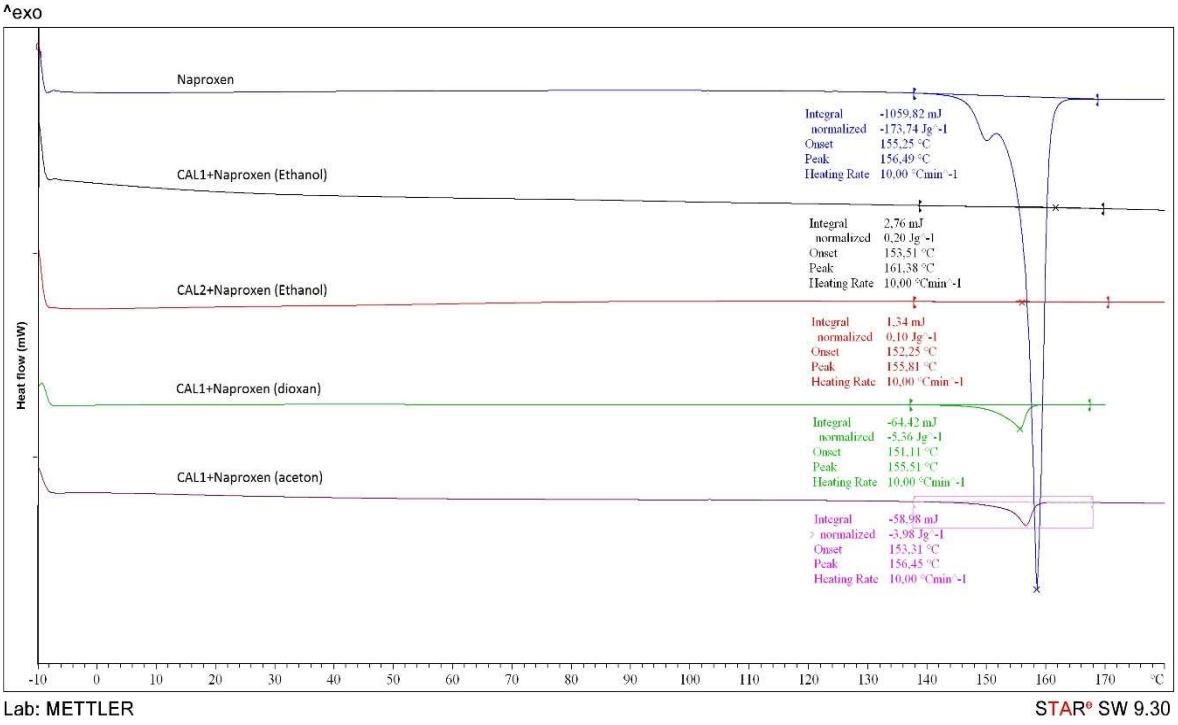


Figure 13: DSC curves of SD samples prepared with different solvents.

4.2.2 Solvent evaporation method

Ethanol was chosen for the solvent evaporation method because of its ability to bring naproxen, as shown in the previous experiment, into an amorphous state. As we wanted to see a difference after adding higher amounts of the to the carrier, we prepared 2 different, namely 5% and 20% SD. Furthermore, we also prepared simple physical mixtures by only mixing naproxen with the carrier to compare them with SDs and examine potential benefits of the evaporation method.

4.2.2.1 5% solid dispersions

Figure 14 presents DSC curves of 5% naproxen dispersions prepared with solvent evaporation and physical mixing. The endothermic peak of naproxen in SDs prepared with the evaporation method was significantly smaller in comparison to that in physical mixtures. The reason for this is that the SD prepared with solvent evaporation contain naproxen in the amorphous state. Because of that a better dissolution is expected, this represents an advantage compared to physical mixtures.

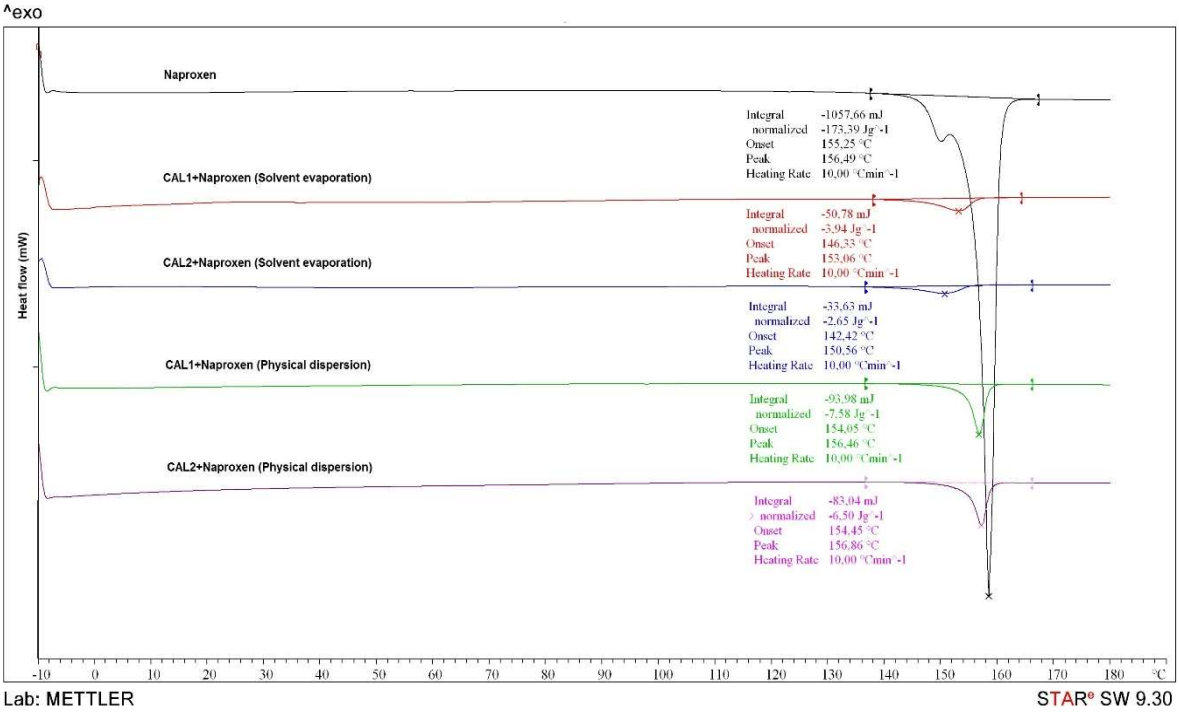


Figure 14: DSC curves of 5% naproxen dispersions and physical mixtures.

Percentage of naproxen in various amorphous state in various preparations are presented in Table XIV.

Table XIV: Amounts of naproxen in the amorphous state within various types of its 5 % dispersions.

Sample	$W_{amorphous\ state}$ (%)
CAL1+5%Naproxen (solvent evaporation)	78.6
CAL2+5%Naproxen (solvent evaporation)	85.3
CAL1+5%Naproxen (physical dispersion)	0
CAL2+5%Naproxen (physical dispersion)	0

Results show slightly higher amounts of naproxen in amorphous state within CAL2, which could be the consequence of smaller pores and because of that of greater surface area available for amorphous drug adsorption on this excipient. In addition, this higher value should also provide improved dissolution rates, with amorphous drug should be more soluble than the crystal one.

4.2.2.2 20% naproxen dispersions

As in previous case, the endothermic peak (melting enthalpy) of naproxen was significantly smaller in SDs prepared with the evaporation method, compared to adequate simple physical dispersions. DSC curves for 20% dispersions are presented in Figure 15.

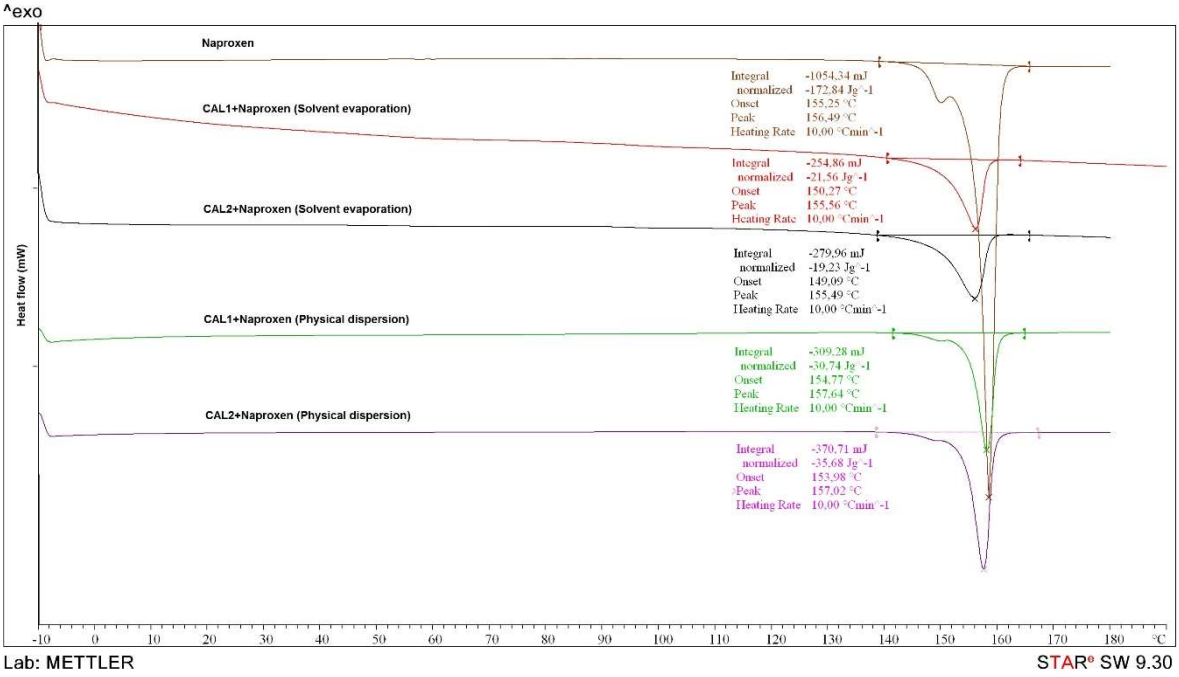


Figure 15: DSC curves of 20% naproxen SDs and physical mixtures.

Amounts of naproxen present in amorphous state in compared dispersions are presented in Table XV.

Table XV: Percentages of naproxen in amorphous state within various types of its 20% dispersions.

Sample	$W_{amorphous\ state}$ (%)
CAL1+20%Naproxen (solvent evaporation)	67.8
CAL2+20%Naproxen (solvent evaporation)	76.7
CAL1+20%Naproxen (physical dispersion)	0
CAL2+20%Naproxen (physical dispersion)	0

We observed that with an increasing amount of naproxen being mixed with excipients, a slightly lower percentage of the amorphous state was obtained, although a significantly higher amount of the drug was in that state in comparison with dispersions prepared with adsorption method and with 5% dispersion produced with the solvent evaporation.

4.2.3 The use of equilibrium adsorption and solvent evaporation for preparation of SDs

In order to improve the results regarding the content of amorphous naproxen in $CaCO_3$ SDs, we combined both previously described methods. Therefore, we first prepared SDs with the equilibrium method to induce amorphous state of naproxen present and then added additional amounts of the drug to achieve 10% SDs, using the solvent evaporation method. DSC curves of such SDs are presented in Figure 16.

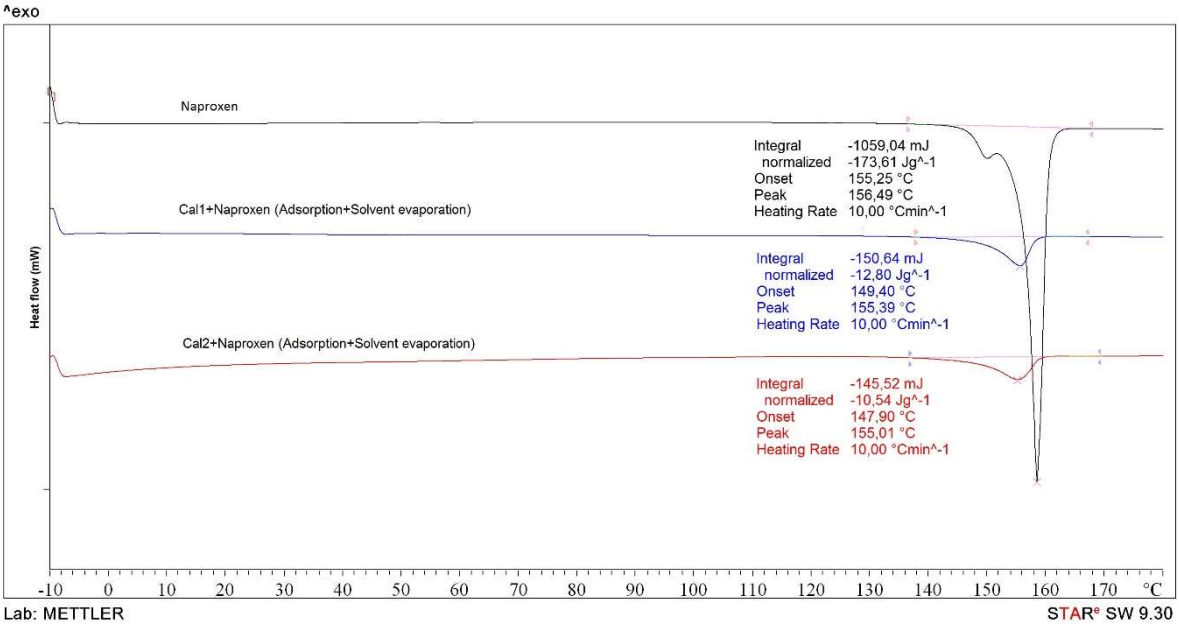


Figure 16: DSC curves of 10% naproxen SDs prepared with the combination of equilibrium and solvent evaporation methods.

The amounts of naproxen in the amorphous state, produced by combination of both methods, are presented in Table XVI.

Table XVI: Percentages of naproxen in the amorphous state in 10% SDs prepared with equilibrium and solvent evaporation methods.

Sample	$W_{amorphous\ state}$ (%)
CAL1+10%Naproxen (adsorption + solvent evaporation)	61.8
CAL2+10%Naproxen (adsorption + solvent evaporation)	73.2

The percentage of naproxen in its amorphous state in within SDs produced with combination of both methods was lower than in SDs prepared only with the solvent evaporation. Therefore,

we can assume that the amorphous crystals, obtained with the adsorption method, have not induced further amorphous growth when applying the other method, as expected, it is obvious that we did not manage to increase the amount of naproxen in its amorphous state by combining these two different methods to the extent which would be higher than that within SD prepared with the solvent evaporation method.

4.2.4 Textural features of prepared solid dispersions

In order to gather more precise information about the SDs, we made their SEM pictures. SEM pictures of pure naproxen are presented in Figure 17.

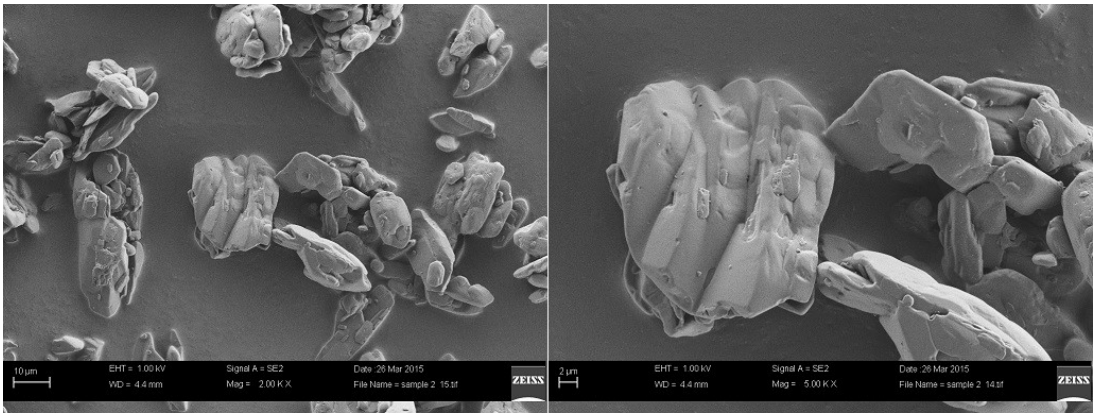


Figure 17: SEM pictures of naproxen; magnifications 2 K X (left) and 5 K X (right).

Figure 18 presents SDs prepared with the equilibrium method.

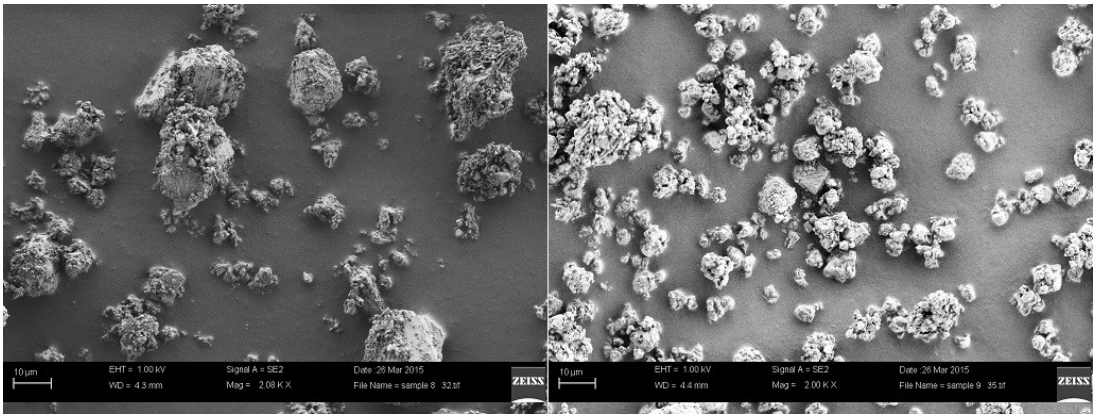


Figure 18: SEM pictures of SD prepared with the equilibrium method CAL1 (left) and CAL2 (right); both magnifications 2 K X.

We see that naproxen crystals are not present on the surface of samples, which is a confirmation for its successful sorption into the pores of the carriers. A comparison of SEM images of SDs containing different amounts of naproxen, prepared with the solvent evaporation and the

combination of equilibrium and solvent evaporation methods, are presented in Figure 19. In SDs produced with solvent evaporation and the combination of both methods, we observed absence of naproxen crystals in particles, while in simple physical mixture such crystals were present.

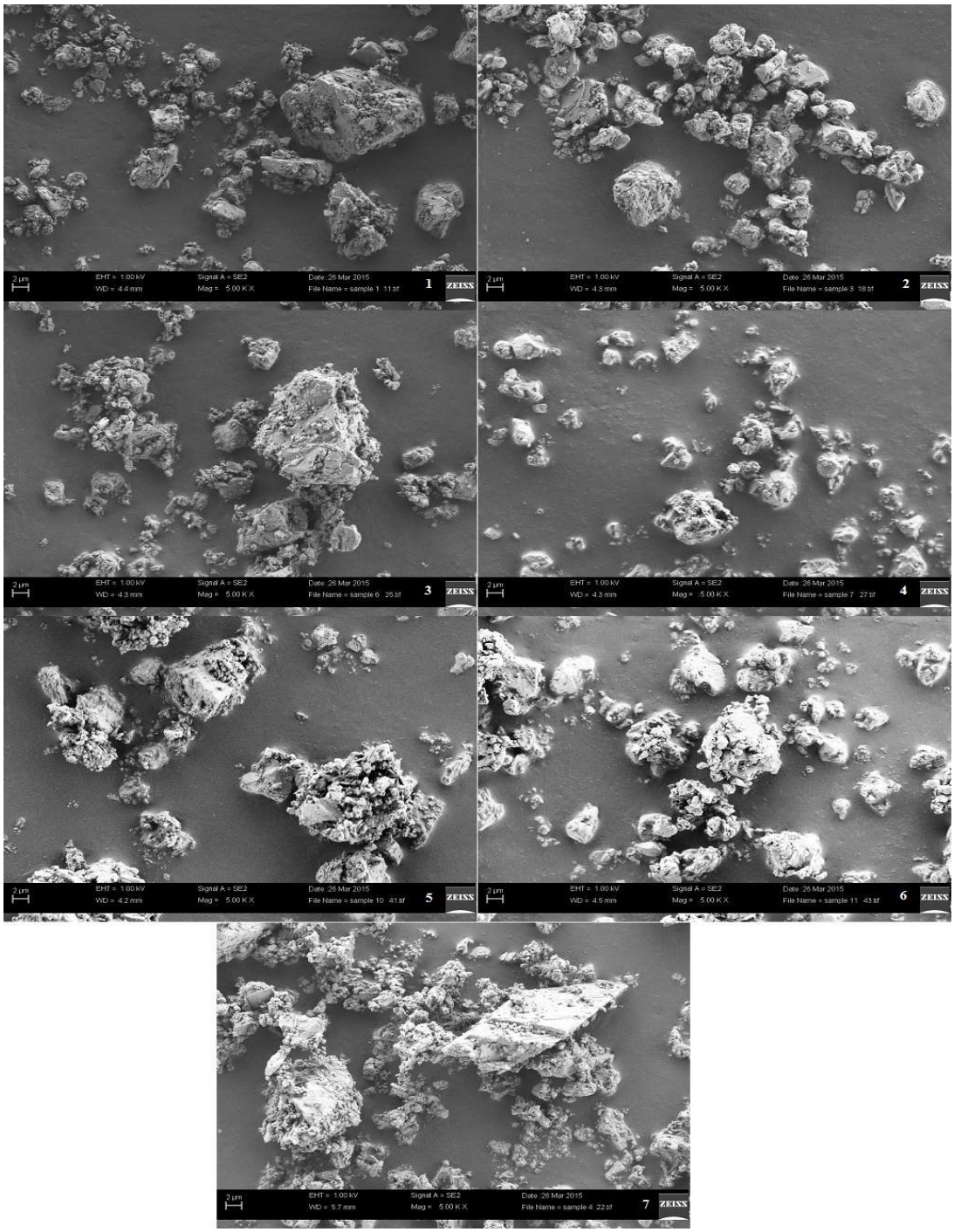


Figure 19: SEM pictures of SDs: 1-CAL1+5% NAP(SE); 2-CAL2+5% NAP(SE); 3-CAL1+20% NAP(SE); 4-CAL2+20% NAP(SE); 5-CAL1+10% NAP(EQ+SE); 6-CAL2+10% NAP(EQ+SE);7-CAL1+5% NAP(PM)); all magnifications 5 K X; NAP= naproxen, EQ= equilibrium method, SE= solvent evaporation method.

4.3 DISSOLUTION TESTS

4.3.1 Dissolution of naproxen from 20% SDs prepared with solvent evaporation

In order to assess potential dissolution benefits of SDs prepared with a porous excipient and the solvent evaporation method, we carried out dissolution tests for both SDs and naproxen itself. In the first dissolution test an equivalent amount of naproxen (NAP) was used as in some drugs available on the market, i.e. 250 mg.

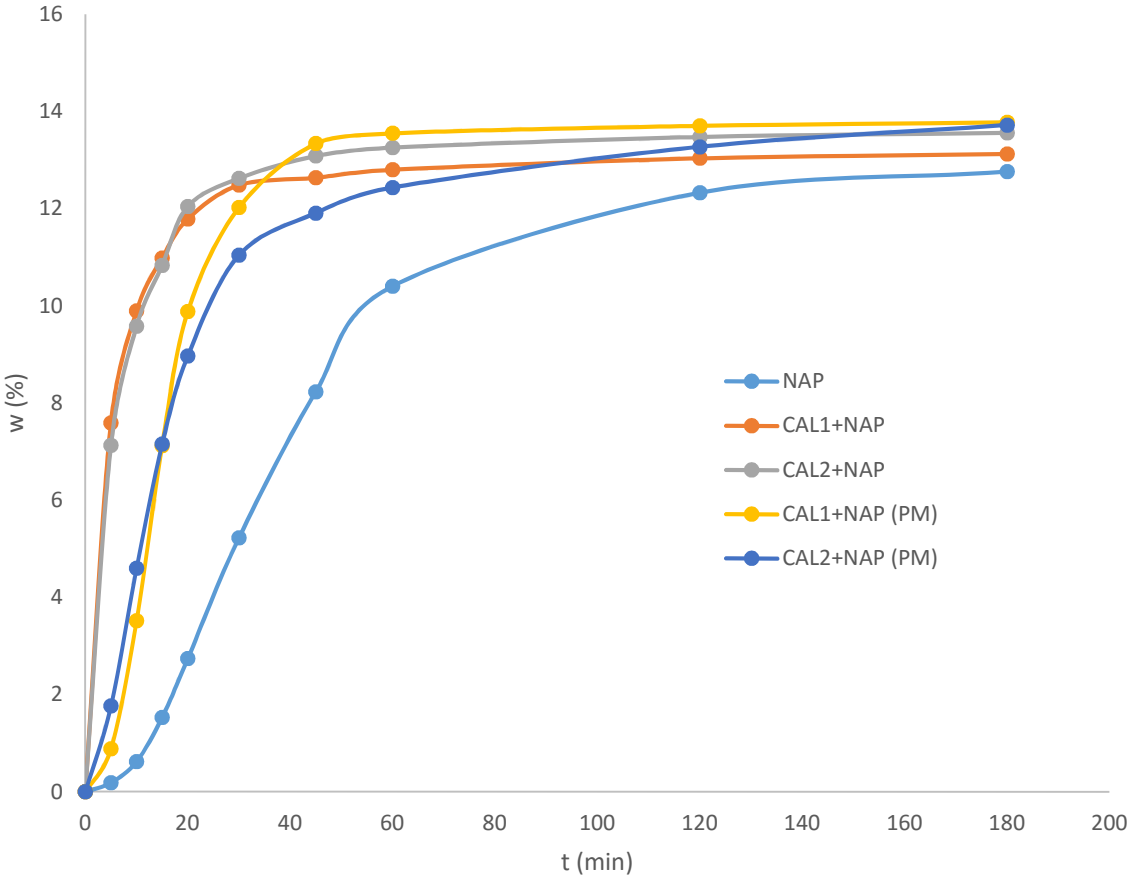


Figure 20: Dissolution curves of naproxen from 20% SDs prepared with the solvent evaporation method and from 20% physically mixed dispersions.

Curves presented in Figure 20 show increased drug dissolution of all SDs in comparison to the drug itself. Dispersions prepared with the solvent evaporation method demonstrate improved release naproxen during the first half an hour, in comparison with simple physically mixed dispersions. The larger surface area of naproxen and its amorphous state in SDs prepared with the solvent evaporation method could be the reason for that. The released amount of the drug after three hours was however very similar for both types of preparations and for naproxen itself.

Table XVII: Dissolution rate in the first 30 minutes of the test.

	Dissolution rate (mg/Lmin)				
Time\sample	NAP	CAL1+NAP	CAL2+NAP	CAL1+NAP (PM)	CAL2+NAP (PM)
0–15 min	0.28	2.01	2.04	0.86	1.25
15–30 min	0.64	0.28	0.36	1.03	0.72

Dissolution rates of SDs prepared with the solvent evaporation was significantly greater than of the drug itself and physical mixtures, during the first 15 minutes, when they almost already hit the "plateau" of all naproxen released.

4.3.2 Naproxen solubility

In order to create sink conditions for our dissolution test, we performed experiments in an acid dissolution media (HCl, pH 1.2), with and without surfactant (0.3% Sodium lauryl sulfate (SLS)). Surfactant was added for a better imitation of naproxen dissolution in the gastrointestinal tract (GIT). The results are presented in Table X.

Table XVIII: Solubilities of naproxen.

Sample	Solubility (mg/L)
Naproxen itself	40.0
Naproxen + 0.3% SLS	39.2

The solubility of naproxen was almost identical in both cases. We also added higher amounts of SLS (1%), but the lack of solubility improvement was still present. Therefore, we decided to use only dissolution media without any surfactants.

4.3.3 Dissolution tests of SDs prepared with the solvent evaporation method (sink)

To obtain more relevant results in our dissolution tests the sink conditions were applied. We used ¼ of the soluble amount of naproxen in all tests (10 mg of naproxen in 900 mL of dissolution media), and only changed the amounts of whole dispersions. The sink condition may be defined as a 3x larger volume of dissolution medium, with or without surfactant, needed to provide complete dissolution of the expected amount of the drug present.

4.3.3.1 Dissolution test of 5% naproxen dispersions

We used approximately 2.5x higher percentage of naproxen for the preparation of the first dispersion, than it was present in SDs produced by using equilibrium method. Physically mixed dispersions were also prepared with the same proportions of both components for comparisons. Also, the same amount of pure naproxen was also tested in parallel. The results are presented in Figure 21.

Dissolution curves of SDs provide better dissolution rates than the drug itself. In addition, differences between both types of SDs were also obvious. Namely dispersions prepared with solvent evaporation had better dissolution rates than simple physical mixtures. During the first 40 minutes, 100 % of naproxen was released from both types of SDs. The dispersion with CAL2 had the fastest total release time (approximately 30 min). The final release of naproxen was much higher from both, solid dispersions and physical mixtures, in comparison with the drug itself.

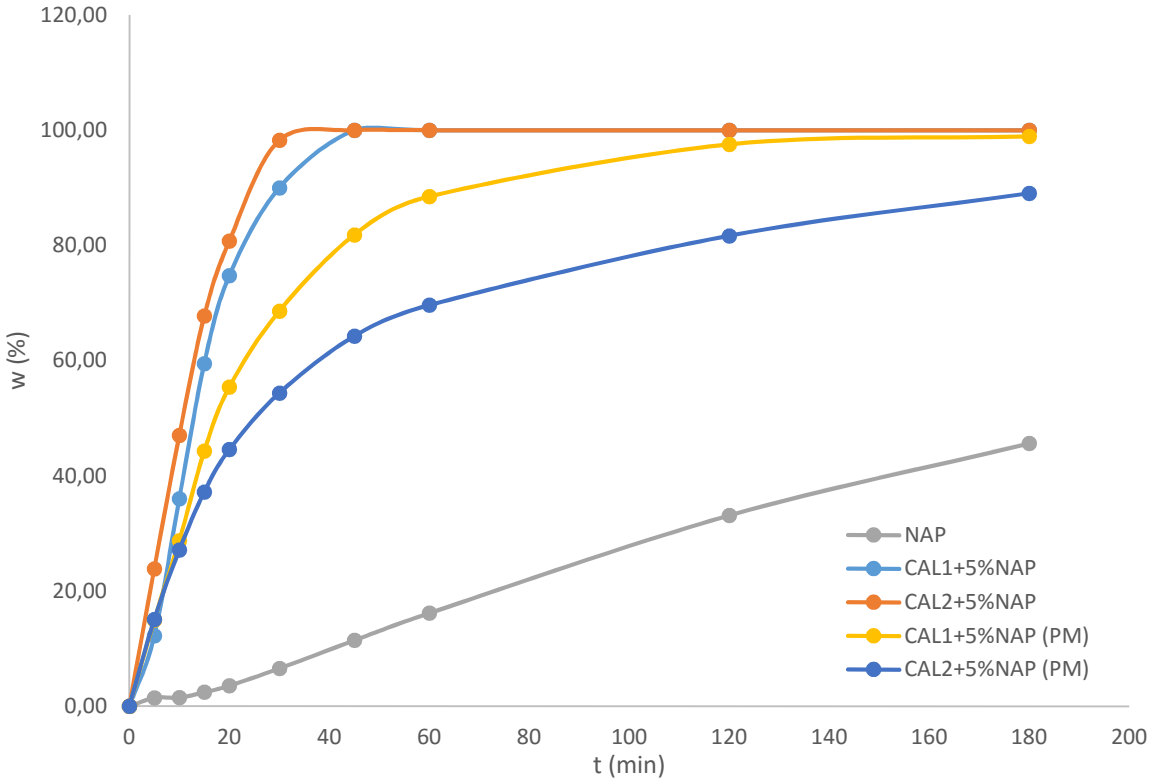


Figure 21: Dissolution curves of naproxen itself, 5% dispersions prepared with the solvent evaporation method and 5% physically mixed dispersions, at sink conditions.

4.3.3.2 *Dissolution test of 20% naproxen dispersions*

We used approximately 10x higher percentage of naproxen for the preparation of the second dispersion than it was present in SDs produced by using with the equilibrium method. Physically mixed dispersions were also prepared with the same percentages of both components for comparisons. Dissolution results are presented in Figure 22.

As curves show, dissolution rates were drastically lower in all SDs in comparison with 5% dispersions, but still greater than the dissolution of the drug itself. Also, differences between SDs prepared with the solvent evaporation method and physically mixed dispersions, which were observed in 5% dispersions, were gone. Especially the curve of CAL2+naproxen prepared with solvent evaporation was much lower after 45 minutes than that of physically mixed dispersions. The reason for this could be, that a significant amount of naproxen crystalized on the surface of $CaCO_3$ thereby prevent the approach of the dissolution media to our excipient. Because of that $CaCO_3$ disintegration could be limited. Nevertheless, the total amount of naproxen released was still higher in all SDs as compared to the drug itself, but also slightly lower in physical mixtures and significantly lower in SDs made with solvent evaporation, especially in the CAL2 samples. The Reasons for that might be the same as mentioned above.

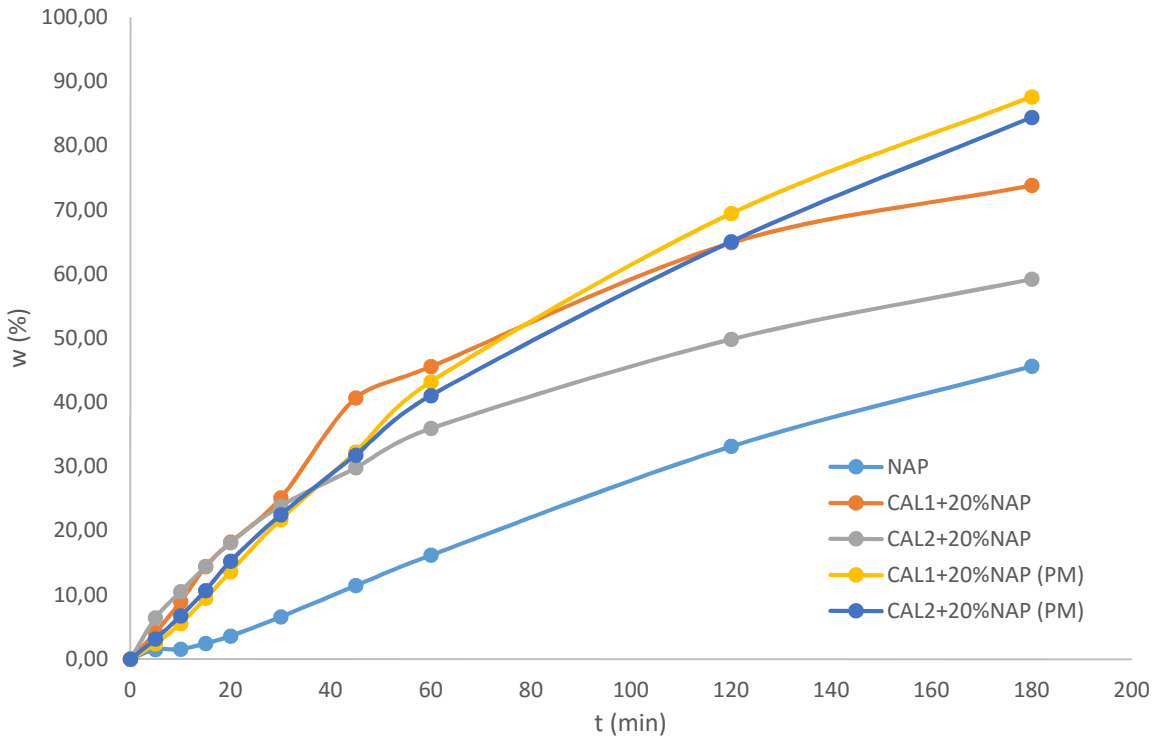


Figure 22: Dissolution curves of naproxen itself, 20% dispersions prepared with the solvent evaporation method and 20% physically mixed dispersions, at sink conditions.

4.3.4 Dissolution tests of dispersions prepared with equilibrium adsorption and solvent evaporation (sink conditions)

4.3.4.1 Dissolution test of 10% naproxen dispersions

In order to gain benefits from both methods, we used SDs prepared with equilibrium adsorption and tried to adsorb some more naproxen onto the surface of our excipients, using solvent evaporation. We were hoping that the presence of the amorphous drug in pores will induce additional growth of amorphous naproxen on the excipient's surface. Therefore, saturated solution of naproxen was used this time to prevent dissolution of the existing amorphous drug from pores. The results are presented in Figure 23.

We observed lower dissolution rates for this type of SDs. As compared to those containing 5% of naproxen, but also increased dissolution rates in comparison with 20% SDs. CAL1 showed similar results in vase of SDs made with the combination of both methods, compared to physically mixed dispersions, while CAL2 expressed a slightly better performance in case of SDs prepared the combination of both methods. CAL1 samples dissolution rate results during the first 30 minutes of the test were significantly better in comparison with CAL2 containing samples, which was also true for the total amount of the drug released. The rates and amounts of naproxen released from all SDs were still significantly higher in comparison with the drug itself.

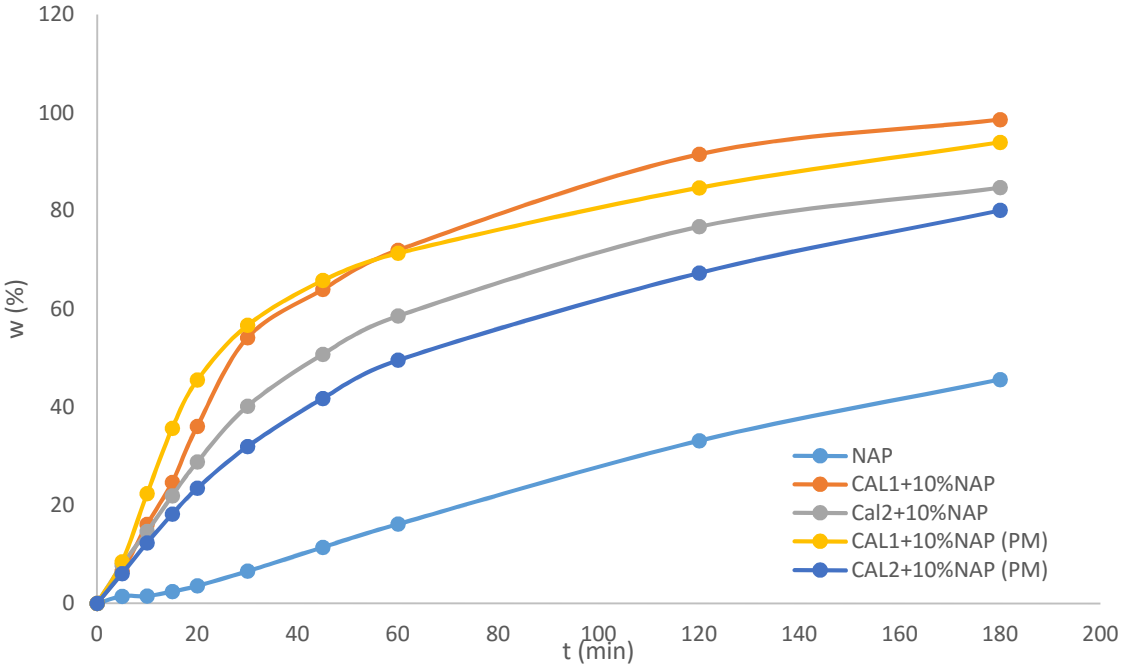


Figure 23: Dissolution curves of naproxen itself, 10% dispersions prepared with the combination of equilibrium adsorption and solvent evaporation method and 10% physically mixed dispersions, at sink conditions.

5. CONCLUSION

The objective of this master's thesis was to evaluate the properties of two $CaCO_3$ (CAL1 and CAL2) samples and their potential suitability as porous carriers used for improvement of dissolution of the poorly water soluble active pharmaceutical ingredient naproxen. Furthermore, our aim was also to maximize the adsorption of naproxen the carriers in the highest possible proportions of its amorphous state, as this enables more rapid dissolution of poorly soluble drugs. For this purpose we prepared solid dispersions using different methods. From obtained results we can conclude the following:

- Calcium carbonate samples showed potential as porous carriers, due to their high porosity and large surface area.
- The equilibrium method used for preparation of solid dispersions (SDs) did not prove to be sufficient enough for entrapment of significant amounts of naproxen (1.4 % with CAL1 and 1.89 % with CAL2).
- Solid dispersions prepared with the solvent evaporation method provide a higher percentages of naproxen in amorphous form, which is requested for the improvement of its dissolution rate.
- When both, the equilibrium and the solvent evaporation method were combined, the percentages of the amorphous naproxen did not increase in comparison with the use of the solvent evaporation alone.
- All prepared SDs had a significant positive impact on naproxen dissolution. However, with increasing percentages of the drug incorporated into SDs, a decrease of its dissolution rate occurred, although it was still considerably higher than the rate of naproxen itself.
- Differences in dissolution rate between SDs prepared with the solvent evaporation and simple physical mixtures occurred at lower percentages of naproxen and were in favor of dispersions produced with the use of the solvent evaporation. These differences disappeared with the increasing amounts of the drug.

Our findings are promising for future developments and generate several options for further investigations, where other types of $CaCO_3$ could be tested. Potential candidates are precipitated and functionalized calcium carbonates which are prepared in a way to have greater porosity and surface areas, therefore for enabling even greater dissolution improvements for poorly water soluble drugs.

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