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ANA APAT

CORRELATION BETWEEN INTRINSIC VISCOSITY OF POLYVINYLPYRROLIDONE AND PARTICLE PROPERTIES OF SPRAY DRIED SOLID DISPERSIONS

VPLIV INTRINZIČNE VISKOZNOSTI IZHODNE POLIMERNE RAZTOPINE NA LASTNOSTI DELCEV POLIVINIL PIROLIDONA, PRIDOBLJENIH S SUŠENJEM Z RAZPRŠEVANJEM

Ljubljana, 2014

Master thesis studies were carried out at Faculty of Pharmaceutical Sciences, University of Copenhagen, as part of study exchange programme. I was positioned as a master student at the Pharmaceutical technology group of the Department of Pharmaceutics and Analytical chemistry. My supervisors were prof. dr. Stanko Srčič and prof.dr. Mingshi Yang.

Diplomsko nalogo sem opravljala v okviru študijske izmenjave na Fakulteti za farmacijo, Univerza v Kopenhagnu. Sodelovala sem v skupini za farmacevtsko tehnologijo na Katedri za farmacevtsko tehnologijo in analitsko kemijo. Diplomsko nalogo sem izdelala pod mentorstvom prof. dr. Stanka Srčiča in somentorstvom prof. dr. Mingshi Yang-a.

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I would especially like to thank my family for their unconditional support in achieving my goals, whatever they might be. Thank you.

Statement

Hereby I declare that I have prepared this master thesis independently under supervision of prof. dr. Stanko Srčič and prof. dr. Mingshi Yang.

Izjava

Izjavljam, da sem diplomsko delo samostojno izdelala pod vodstvom mentorja prof. dr. Stanka Srčiča in somentorja prof. dr. Mingshi Yang-a.

Ana Apat

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TABLE OF CONTENT

ABSTRACT

New methods of finding compounds with potential pharmaceutical activity, such as virtual screening and high throughput screening, enable the identification of numerous new potential compounds as drug candidates that have the ability to bind to the target. Many of them commonly exhibit poor water solubility and consequently low bioavailability.

Formulating an amorphous drug as a solid dispersion is one of the successful methods to improve drug dissolution rate. The major disadvantage when formulating an amorphous drug is its thermodynamic instability as it tends to revert back to a more oriented crystalline form, its lowest energy state. Consideration should be given to the choice of carrier, where often polymers are used. The polymer should be highly water soluble and able to interact with the drug in order to inhibit drug movement and stabilize the amorphous drug inside the solid dispersion formed.

In this study, celecoxib, a BCS Class II drug, was examined as it is practically insoluble in water. Polyvinylpyrrolidone (PVP) was used as carrier to enhance the drug dissolution rate. Solid dispersions were prepared by spray drying. The drug-polymer ratio used was either 1:1 or 1:3. With intrinsic viscosity measurements of PVP in various solvents, methanol and methanol:acetone 1:5, were chosen as good and poor solvents for PVP, respectively. The influence of the solvents used for spray drying on particle properties of solid dispersions was investigated.

Dissolution studies in phosphate buffer pH 6.8 exhibited enhanced dissolution rate of celecoxib when formulating it into solid dispersions. Solid dispersions remained stable for a longer period of time compared to pure amorphous celecoxib. Stability studies and possible drug-polymer interactions were examined using XRPD, FTIR and Raman spectroscopy. Particle morphology of solid dispersions was investigated using SEM, whereas residual moisture content was obtained by TGA.

No significant difference in particle morphology, stability and dissolution rate was observed when using different solvents, while solid dispersions prepared from methanol:acetone 1:5 mixture contained a higher residual moisture. The dissolution rate as well as the residual moisture was dependent on the drug-polymer ratio used.

POVZETEK

Z novimi metodami za iskanje spojin s potencialnim farmakološkim učinkom, kot sta virtualno rešetanje in rešetanje visokih zmogljivosti, je število novo odkritih potencialnih učinkovin v zadnjih desetletjih močno naraslo. Mnogo teh učinkovin pa je težko topnih v vodi, kar predstavlja velik problem pri absorpciji iz gastrointestinalnega trakta. Posledično je biološka uporabnost novo odkritih učinkovin majhna.

Ena izmed uspešnejših metod za izboljšanje raztapljanja je formulacija amorfne trdne disperzije. Slabost amorfne oblike učinkovine je njena nestabilnost, saj teži k pretvorbi nazaj v bolj urejeno, kristalno obliko. Pri izdelavi trdne disperzije moramo zato veliko pozornosti nameniti izbiri nosilca, ki je v večini primerov polimer. Izbran polimer naj bi bil sposoben tvoriti vezi z učinkovino, saj bi tako povečal stabilnost njene amorfne oblike, hkrati pa je zaželeno, da je dobro topen v vodi.

V diplomski nalogi je opisana izdelava trdnih disperzij z metodo sušenja z razprševanjem. Kot težko topno učinkovino smo uporabili celekoksib, za nosilec pa je bil izbran polivinil pirolidon (PVP). Z merjenjem intrinzične viskoznosti smo izbrali dobro in slabo topilo za PVP, saj smo predvidevali, da se bodo lastnosti trdnih disperzij razlikovale zaradi uporabe različnih topil. Metanol je bil izbran kot dobro topilo, medtem ko je metanol:aceton 1:5 izkazoval lastnosti slabega topila za PVP. Razmerje med učinkovino in polimerom, ki smo ga uporabili za izdelavo trdnih disperzij, je bilo 1:1 ali 1:3.

Študije stabilnosti in tvorbe vezi v trdni disperziji smo izvedli s pomočjo rentgenske praškovne difrakcije (XRPD), infrardeče spektroskopije s Fourierjevo transformacijo (FTIR) in Raman spektroskopije. Površino delcev trdne disperzije smo preučili z uporabo vrstičnega elektronskega mikroskopa (SEM), količino preostale vlage po sušenju pa z uporabo termogravimetrične analize (TGA).

Test raztapljanja, ki smo ga izvedli v fosfatnem pufru s pH 6,8, je pokazal da se celekoksib iz trdnih disperzij raztaplja hitreje. Prav tako smo ugotovili, da je amorfna oblika celekoksiba v vseh trdnih disperzijah ostala stabilna dlje časa v primerjavi s samim amorfnim celekoksibom.

Pri merjenju hitrost raztapljanja, stabilnosti in preiskovanju površine delcev nismo zaznali nobene razlike, ki bi jo lahko pripisali uporabi različnih topil. Trdne disperzije, ki so bile pripravljene z uporabo slabega topila so izkazovale večjo količino preostale vlage, na količino preostale vlage pa je vplivalo tudi razmerje med učinkovino in polimerom.

Od razmerja med učinkovino in polimerom je odvisna tudi na hitrost raztapljanja trdnih disperzij.

LIST OF ABBREVIATIONS

BCS	biopharmaceutical classification system
c*	critical overlap concentration
CEL	celecoxib
FTIR	Fourier transform infrared spectroscopy
HPMC	hydroxypropylmethycellulose
HPMCas	hydroxypropylmethycellulose acetate succinate
M:A 1:5	methanol: acetone 1:5
MeOH	methanol
PVP	polyvinylpyrrolidone
SEM	scanning electron microscopy
SLS	sodium lauryl sulphate
T_g	glass transition temperature
T_{in}	inlet temperature
T_{out}	outlet temperature
TGA	thermogravimetric analyses
XRPD	X-ray powder diffraction

1 INTRODUCTION

New methods of finding compounds with potential pharmaceutical activity, such as virtual screening and high throughput screening, enable the identification of numerous new potential compounds as drug candidates that have the ability to bind to a specific target. With new targets also being discovered the number of potential drug candidates has risen significantly, but many of them commonly exhibit poor water solubility (1). Solubility of the drug is in addition to its permeability the most important factor for bioavailability of the drug (2). Poorly water soluble compound is defined as a compound of which less than one part dissolves in more than 10000 parts of water, which means that solubility of the compound is less than 100 μg/ml (3).

According to Biopharmaceutical classification system (BCS) compounds are divided into four classes regarding their solubility and permeability (4).

- Class I – Good solubility, good permeability
- Class II – Poor solubility, good permeability
- Class III – Good solubility, poor permeability
- Class IV – Poor solubility, poor permeability

Oral delivery is the easiest way of drug administration, but developing solid state drug formulation represents a big challenge for pharmaceutical industry when dealing with poorly soluble drugs (5). For Class II and IV, different methods can be used for enhancing dissolution rate, oral absorption and therefore bioavailability, like salt formation, pro-drug formation, particle size reduction or solubilization but all of them exhibit some limitations in practical use (5).

Salt formation is a common approach but it cannot be used for neutral compounds and often forming salts may not present desired properties if using week acid or base. It is also possible that the increased dissolution rate will not be achieved due to the conversion of the salt back into its original form, acid or base (6). Another downside is that salt form of the drug must undergo safety studies as it is considered as a new compound, the same goes when formulating pro-drug (7). The surface area available for dissolution can be increased by reducing particle size of the compound, for example using milling, or by improving particle wettability. Drawback with these methods is a limit of how much particle size can be reduced because the surface area may not be sufficiently increased. Very fine powders

also tend to agglomerate, have low flowability and are problematic for handling in production. Solubilization leads to liquid formulation which is less desired with patients than solid form. Solubilization is achieved with use of surfactants and cosolvents or formulation of self-emulsifiying drug delivery systems (7-8). One other possibility is applying the drug right after meal what would extend the time available for the drug to dissolve as it would stay in a stomach longer and also more fluid would be available in the gastrointestinal tract for dissolution. Ensuring sink conditions is also an option (2).

Preparing amorphous form of a drug candidate is also one of the popular approaches for improving the dissolution rate (9). Many researchers have shown that amorphous form exhibit higher solubility compared to crystalline form of the drug as it exist in a metastable state with high free energy (10). As this is not equilibrium state for drug molecule, the drug tends to revert back to its crystalline form rapidly, especially when exposed to humidity, temperature or mechanical stress. Recrystallization could also occur when amorphous drug is exposed to the dissolution medium, what could lead to inhibited dissolution (11-12). Goal is to formulate amorphous drug in a way which would provide better stability, thus decreasing its tendency to recrystallize, but still exhibit enhanced dissolution rate (11). One way this can be achieved is by formulating solid dispersion which is a widely used and one of the most successful techniques for enhancing drug solubility for fast delivery dosage form (13).

1.1 SOLID DISPERSIONS

Solid dispersion is a dispersion consisting of at least two components, e.g. poorly water soluble drug and hydrophilic carrier (5). The carrier should be inert, thermostable, soluble in wide range of solvents and compatible with the drug (14). A carrier with high T_g and possibility to interact with the drug should be used to decrease the tendency of the drug from reverting back to its crystal form (12). Depending on the carrier selection controlled release could also be obtained (15).

1.1.1 TYPES OF SOLID DISPERSIONS

Solid dispersion method was first used in 1960's when Seguchi and Obi prepared eutectic mixtures of sulfathiazole and urea which exhibited faster absorption after oral application than sulfathiazole itself (13).

Eutectic mixture is composed of two components that are miscible in liquid state but not miscible in the solid state. At the eutectic temperature and corresponding ratio of the two components, they will both start to crystallize out, forming very fine crystal particle dispersion. When the mixture is dissolved in the aqueous medium, it is the increased surface area of the very fine particles that is accountable for enhanced dissolution rate (16). Solid solutions represent a system, where at certain proportions drug can be dissolved in a crystalline carrier. They can be divided depending on miscibility to continuous solid solutions, or to discontinuous solid solutions. Depending on how drug molecules are distributed in the carrier they are divided to substitutional crystalline solid solution, interstitial crystalline solid solution and amorphous solid solution (14). Amorphous solutions, also known as glass solutions, are homogenous systems where carrier is amorphous and drug is molecularly dispersed into it or precipitate in its amorphous form. Often polymers are used as carriers as most of them are amorphous or partially amorphous. Particle size of the drug is reduced. Carrier used should have a high glass transition temperature (T_g) to ensure stabilization of the drug (17).

1.1.2 PREPARATION OF SOLID DISPERSIONS

Fusion or melting method

When using this method, mixtures of drug and carrier are heated above their melting temperature and then cooled rapidly by placing aluminium or steel plates with melted product over ice, dry ice or by blowing cold air over them. Usually eutectic mixtures are used to lower melting temperatures or only carrier is melted and drug is then suspended into it. Rapid cooling is preferred as in this way drug is incorporated in a carrier at an instant. Solid dispersions are hardened so they can be pulverized and used for further formulation (7). Hot melt extrusion is often used for production of solid dispersions. Here, usually twin screw is used for extrusion of physical mixture of drug and carrier at melting temperature. Mixture is exposed to melting temperature for shorter time than in classic melting method. The melt is then cooled rapidly and extruded trough die which gives solid product in desired form (18).

Solvent evaporation method

Solid dispersion is produced by dissolving drug and carrier in a common solvent and then evaporating the solvent to obtain dry particles. Many times it is difficult to find a solvent

that dissolves both, as hydrophobic drug and hydrophilic carrier are used. If that is the case, mixtures of two solvents can be made. Often large amounts of organic solvents are used, so it is important there is no residual solvent present in solid product after drying (16-17). For evaporation of the solvent different techniques can be used like vacuum drying, rotary evaporation, freeze drying, spray drying or combination of spray-freeze drying. After drying, solid dispersions can further be stored in vacuum desiccators to remove residual solvent (7). When freeze drying, the solution is frozen and solvent is then sublimed. This way drug is exposed to almost no thermal stress and when dispersion is solid, there is minimal chance for phase separation. In spray-freeze drying the solvent is sprayed into liquid nitrogen and frozen droplets then undergo sublimation (5). Spray drying will be discussed in details further in the thesis.

Supercritical fluid processing

Fluid is supercritical above its critical temperature and critical pressure, where liquid and gas state exists as one phase. In supercritical state it has compressibility and permeability equal to gas and density as a liquid, which makes it adequate to be used as solvent (15). Carbon dioxide (CO_2) is the most often used supercritical fluid as it is cheap and non-toxic. It has low critical temperature (31.1°C) what is suitable for handling thermo labile compounds. CO_2 is used either as a solvent or anti-solvent (19). In first case, rapid expansion of supercritical solution method is used, where drug and solvent are dissolved in CO_2 and sprayed trough nozzle into expansion barrel, where nanometre sized particles are produced. The problems with this method are low solubility of drug and carrier in CO_2 and insufficiently dried particles. When CO_2 is used as anti-solvent, drug and carrier are dissolved in organic solvent, supercritical fluid is then added to the solution which causes the precipitation of solid dispersion particles that can further be milled if needed. Instead of dissolving drug and carrier in organic solvent, they could be melted and then saturated with CO_2 what would cause to break the melt into particles and cool them (2).

1.1.3 ADVANTAGES AND LIMITATIONS OF SOLID DISPERSIONS

Particles with reduced size, higher porosity and improved wettability are produced that improve dissolution rate of the drug. Carriers used to produce solid dispersions are already known in pharmacy, no safety studies are needed (5). Drugs can be converted from its crystalline to amorphous form, which exhibit higher solubility and dissolution rate due to

its high free energy and metastable state (10). However, frequent problem is instability of solid dispersions, which is why carrier should be chosen with consideration (20).
Reproducing particle properties is hard as any difference made in process conditions may have great influence on properties of solid dispersion. This makes the process very challenging to scale up. When scaling up solvent evaporation methods, special equipment for evaporation and recovery of organic solvents is needed. Therefore, the production can be very expensive. Some solid dispersion particles may be tacky and soft what makes them difficult to pulverize and produce the desired dosage form (7-8).

1.2 SPRAY DRYING

Spray drying technique is employed in many industries, pharmaceutical, chemical, food, textile and others. Products that are used in everyday life have been manufactured using spray dryers, like soups, dry milk powder, detergent, face powder, dyes and similar (21). In pharmaceutical industry this technique is often used to enhance bioavailability of the drug by conversion to its amorphous form. It can also be used for granulation, encapsulation, formulating particles for inhalation or for manufacturing controlled-released particles (22).

Spray drying is a widely used, one step method, where feed solution, suspension or emulsion is atomized into droplets and immediately transformed into dry solid particles by spraying the feed into drying medium, either hot air or inert gas, if using organic solvents (21). Scheme of the process is shown in Figure 1. Thermo labile drugs can be used as the evaporation of the solvent is very fast, which sustains low product temperature (23).

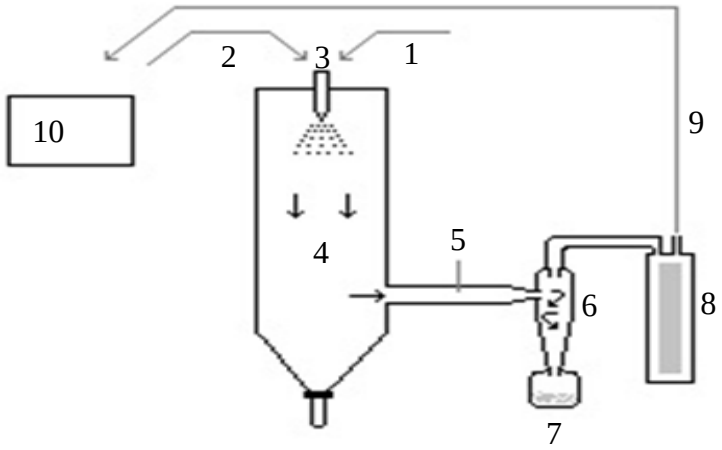

Figure 1 Scheme of spray drying process. 1) feed solution, 2) drying gas - air or nitrogen,

3) two-fluid nozzle, 4) drying chamber, 5) measuring T_{out}, 6) separating the particles in cyclone, 7) collected particles, 8) filter for fine particles, 9) aspirator, 10) inert loop. (22)

Different steps take place during spray drying process:

1. The feed solution is atomized into droplets using a pressure nozzle, two-fluid nozzle, a rotary disk atomizer or an ultrasonic nozzle, depending on desired particle properties (23). (Figure 1, (part 3))
2. Droplets can get in the contact with hot drying medium in four different ways. When using spray drier with co-current flow, the feed and drying medium are sprayed in the same direction, from above of the chamber. (Figure 1, (part 1-3)) In counter-current manner the feed and the drying medium are sprayed in the opposite direction. The feed flows downwards, while the drying medium comes from the bottom. Thermo labile compounds cannot be used here as the particles get very hot during drying. These two methods could be combined. In this case, drying medium flows from above but feed solution is sprayed form the bottom. After short period of time, the drying particles start flowing down the chamber due to gravity, where they are cooled. A rotary disk atomizer can also be used. The feed and drying medium are sprayed in the same direction. When disk rotates the feed is transformed into mist (24).

   The equipment most often used is co-current spray drier.
3. Drying of the feed starts almost instantaneously and is very effective because of the high surface area of the droplets. It is not likely that the particles would be exposed to high temperatures, as evaporation lowers the droplet temperature and the product leaves the drying chamber before its temperature could rise. For control of the particle temperature load, outlet temperature (T_{out}) is measured before particles are collected (24). (Figure 1, (part 5))
4. To recover dry product at the end of process and to separate it from the drying medium often cyclone is used (Figure 1, (part 6)), but bag filters and electrostatic precipitators are also in use (25).
5. Evaporated solvent is then recovered using inert loop if organic solvent is used, or by using humidifier when water is used. (Figure 1, (part 10))

Properties of dry particles depend on spray drying conditions, mainly on inlet temperature (T_{in}), spray air flow, drying gas flow rate and feed rate, as can be seen from Table 1 (24). In addition, density and shape of spray dried particles also depend on spray drying process parameters (26).

Table 1 Influence of spray drying parameters on product temperature, residual moisture, particle size, and yield of solid dispersion.

	↑T_{in}	↑Feed rate	↑Spray air flow	↑Drying gas flow rate	↑c	↑Air humidity	Organic solvent, not water
T_{out}	⇧	⇩	↓	⇧	⇧	↑	⇧
Particle size	/	↑	⇩	/	⇧	/	↓
Residual moisture	⇩	⇧	/	⇧	↓	⇧	⇩
Yield	↑	↑↓	/	⇧	↑	↓	⇧

T_{in} - inlet temperature, c - solid concentration, T_{out} - outlet temperature. / - no influence, ↑ - minor influence, ⇧ - high influence.

1.2.1 ADVANTAGES AND LIMITATIONS OF SPRAY DRYING

Spray drying is a one step process with fast evaporation what makes it adequate for use with thermo labile drugs. Amorphous solid dispersions are produced, which exhibit better dissolution rate (23). Spherical particles can be produced (25). Carriers with high melting temperature can be used what is not desired with melting methods (14).

However, sometimes it is difficult to find a common solvent that would dissolve both, drug and carrier. Large volume of solvent is often needed for dissolution. Organic solvents are expensive and equipment for evaporation and recovery of organic solvents is needed. Residual moisture is often observed with solid dispersion what needs to be avoided, as

organic solvents present can have adverse effects (8). Also, moisture uptake could lead to lowering of T_g, making the dispersion less stable and converting amorphous form back to crystalline (9).

1.2.2 SELECTING CARRIER AND SOLVENT FOR SPRAY DRYING

Choice of polymer and solvent influence the properties of spray dried solid dispersions (27). Often polymer or combination of two polymers is selected as a carrier. Polymer should be inert, safe, compatible with the drug and soluble in a variety of organic solvents. When formulating solid dispersions for enhancing the dissolution rate, polymer should exhibit very good solubility in water and solubilising properties. The choice of polymers has influence on the recrystallization of the drug. Polymers with high T_g are desired as they increase T_g of solid dispersion. This way drug stability is improved by lowering molecular mobility. Also, for enhancing stability of amorphous drug it is desired that polymer can interact with the drug by forming specific hydrogen bonds for example, making the drug mobility or its precipitation from the matrix less possible. It has been showed that the stability of the solid dispersion is in correlation with drug: polymer ratio used (20). Polymers like PVP and HPMC, HPMCas are often used in spray drying for enhancing stability and dissolution rate (16).

Solvent used for spray drying of solid dispersion formulation should dissolve both drug and polymeric carriers. Sometimes combination of two solvents is used to make this possible. Toxic solvents should be avoided as it is possible that some residual solvent is present in solid dispersions after spray drying (14).

Properties of solid dispersion may also differ depending on the solvent used as polymer coil behaves differently whether good or poor solvent for the polymer is used. Zhou et al. showed that when spray drying from good solvent, larger particles of polymer were obtained. Difference in particle surface has also been reported (28). Consequently this may affect the ability of polymer to interact and stabilize the drug (29). Al-Obaidi et al. showed that when formulating solid dispersions of griseofulvin, it crystallized out faster when using poor solvent. Smaller particle size was observed when spray drying from a poor solvent. Smaller particles were also obtained when using lower concentrations of feed solution at constant ratio between compounds. Results were attributed to lower viscosity of solution in both cases (29). Paudel and Van den Mooter produced solid dispersions of

naproxen and PVP from different solvents. They reported that when poor solvent for PVP was added to the feed solution, improved physical stability of solid dispersion was observed. They also showed that lower concentration of the drug contained higher amount of residual moisture, what can consequently reduce stability of solid dispersions by weakening of interactions between drug and polymer (20).

1.3 VISCOSITY

Viscosity is one of the most important properties when dealing with polymer solutions (30). It depends on temperature, molecular weight, chain flexibility and concentration of the polymer (31). Polymer-solvent interactions in the solution are important when spray drying as different polymer coil dimensions can affect physical properties of solid dispersions (29).

Viscosity measurements of diluted polymer solutions are usually done with capillary viscometer. From efflux time of pure solvent (t_0) and solution (t), specific viscosity (η_{sp}), which measures the capability of a polymer to increase viscosity of pure solvent upon its addition, is calculated as can be seen from Eq.*1* (31).

$$\eta_{sp} = \frac{t - t_0}{t_0} = \frac{\eta - \eta_0}{\eta_0} = \eta_{rel} - 1 \qquad \text{Equation } 1$$

η_0 and η are kinematic viscosities for pure solvent and solution, respectively. Relative viscosity (η_{rel}) can also be calculated. Intrinsic viscosity ([η]) is then derived by extrapolating η_{sp}/c against c to zero polymer concentration to obtain linear plot. Huggins proposed the following equation for determination of intrinsic viscosity (Eq. *2*), where k_H is Huggins constant, obtained from slope, and c is polymer concentration.

$$\frac{\eta_{sp}}{c} = [\eta] + k_H[\eta]^2 c \qquad \text{Equation } 2$$

Alternative possibility, proposed by Kraemer, is to extrapolate ln η_{rel}/c against c to zero concentration (Eq. *3*), where k_K is the Kraemer constant (30-31).

$$\frac{\ln \eta_{rel}}{c} = [\eta] + k_K[\eta]^2 c \qquad \text{Equation } 3$$

Intrinsic viscosity represents the unique function of size and shape of the polymer coil and its behaviour in solution (31). It is determined at certain temperature for a polymer in a given solvent, independent of concentration (32). Solvent-polymer interactions influence

the conformational properties of the polymer chains. In a good solvent hydrodynamic volume of the polymer coil increases due to expansion of the coil. Additionally, the intrinsic viscosity increases. The opposite happens in a poor solvent for polymer, where polymer-polymer interactions are preferred to polymer-solvent interactions. The polymer coil shrinks, and can completely collapse, and intrinsic viscosity is decreased (33).
It is assumed that when spray drying from good or poor solvent, solid dispersions will have different properties. If good solvent is used for the polymer, it is expected that its coil will be more expanded as less polymer-polymer interaction will occur, thus more polymer molecules will be available to interact with the drug. As a result, differences in stability and dissolution of solid dispersion are expected, when using good solvent compared to spray drying from poor solvent (20, 29).

Another important quantity of polymer solutions is critical overlap concentration (c*), which is derived from intrinsic viscosity. At c* polymer coils make up an interconnected network throughout the whole solvent volume and continuous contact between polymer molecules is present (Figure 2). The concentration of polymer below c* is defined as dilute solution (32). Working with polymer concentrations below c* is desired as there are no continuous polymer-polymer interactions. This way, more polymer molecules are available to interact with the drug.

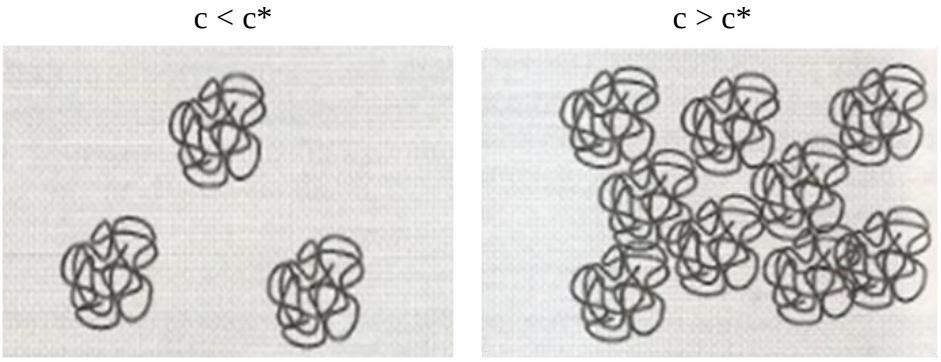

Figure 2 Schematic representation of dilute and semi-dilute solution (32).
c - polymer concentration, c* - critical overlap concentration.

2 AIM OF THE STUDY

The aim of this thesis is to study the influence of different solvents on polymer characteristics and further on how the polymer in combination with process variables influence the particle characteristics, dissolution rate and stability of the spray dried solid dispersion.

- Suitable solvents or mixture of solvents will be chosen, regarding the polymer which is intended for use. Viscosity measurements will be obtained for polyvinylpyrrolidone (PVP) with average Mw 50000 and 360000 in different mixtures of methanol and acetone. The aim is to find good and poor solvent for PVP using intrinsic viscosity values. Once they are obtained the critical overlap concentration (c*) will be calculated, giving the concentration range of PVP for further work.

- Concentrations of PVP above and below c* in different solvents are going to be spray dried. Focus will be on particle shape and residual moisture of spray dried product. Once these properties are obtained, it will be decided if any of the spray drying parameters, like T_{in}, flow rate, drying gas flow rate and others should be changed for the further work when applying drug to the solution.
  Then poorly water soluble drug (celecoxib) will be added to the polymer solution and spray dried in different drug to polymer ratios. The aim is to obtain amorphous solid dispersion.

- Finally, solid dispersions will be analyzed using XRPD to confirm amorphous form, TGA to determine residual moisture, FTIR and Raman to confirm interactions between celecoxib and PVP and SEM to check particle morphology. Dissolution tests of pure celecoxib and solid dispersions will be made in phosphate buffer with pH 6.8. Aim is to confirm better dissolution rate of amorphous solid dispersions compared to pure celecoxib. Enhanced stability of solid dispersions is also expected compared to pure amorphous celecoxib.

3 MATERIALS AND METHODS

3.1 MATERIALS

- Celecoxib was purchased from Dr Reddy, India.
  Celecoxib (CEL) is a nonsteroidal anti-inflammatory drug that belongs to the group of selective cyclo-oxygenase 2 (COX-2) inhibitors. It blocks the COX-2 enzyme, resulting in a reduction in the production of prostaglandins, substances that are involved in the inflammation process. It is used for treatment of rheumatoid arthritis and belongs to BCS class II drugs (34).

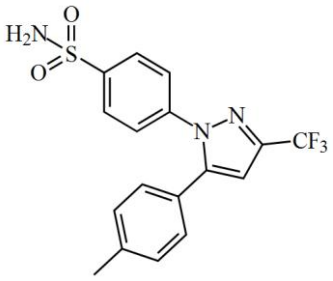

Figure 3 Celecoxib

- Polyvinylpyrrolidone (PVP) is a synthetic polymer that consists of linear 1-vinyl-2-pyrrolidinone groups. PVP is water soluble polymer, often used in pharmaceutical technology as binder, solubilizer, stabilizer and coating agent. PVP powder is white, fine and hygroscopic (35).
  Kollidon® 30 (PVP K30) with average Mw 48000 was purchased from BASF, Germany.
  PVP360 with average Mw 360000 was purchased from Sigma-Aldrich.

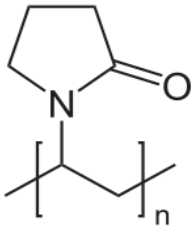

Figure 4 PVP

- Solvents used were bought from Sigma-Aldrich and were of analytical grade:
  - Methanol
  - Acetone
  - Ethanol

- Sodium lauryl sulphate (SLS), Sigma-Aldrich

- Phosphate buffer (pH 6.8)
  - Sodium dihydrogen phosphate, NaH_2PO_4, Sigma-Aldrich
  - Disodium hydrogen phosphate, Na_2HPO_4, Sigma-Aldrich

3.2 INSTRUMENTS

- Analytical balance Mettler AE240, Mettler Toledo Switzerland
- Analytical balance Mettler Toledo XP26 DeltaRange® Excellence Plus, Mettler Toledo Switzerland
- Magnetic stirrer Kika Werke RCT basic, IKA®-Werke GmbH & Co. KG, Germany
- Cannon-Ubbelohde Semi-Micro Viscosimeter, Cannon Instrument Co., USA
- Büchi B-290 spray dryer, Büchi Labortechnik AG, Switzerland
- Büchi Inert Loop B-295, Büchi Labortechnik AG, Switzerland
- X-ray powder diffractometer PANalytical X'Pert Pro diffractometer equipped with a PIXcel detector, PANalytical B.V., The Netherlands;
- Fourier Transform Infrared spectroscope NICOLET 380, smart orbit with diamond crystal and Omnic software, Thermo scientific Inc; USA
- Raman spectroscope Kaiser Optical Systems Inc., USA
- TGA 7, Perkin Elmer, connected to Thermal Analysis Controller TAC 7/DX, controlled by Pyris software, USA
- Scanning electron microscope JSM-5200, JEOL, Japan
- E5200 Auto Sputter Coater, Biorad, UK
- Erweka DT-600, a USP type II apparatus, ERWEKA GmbH, Germany
- UV-spectroscope Evolution 300, Thermo Fisher Scientific, controlled by VisionPro software, UK

3.3 METHODS

VISCOSIMETRIC MEASUREMENTS

Viscosity of PVP K30 and PVP360 in different solvents was measured using a calibrated Cannon-Ubbelohde Semi-Micro Viscosimeter. Samples were prepared by accurately weighing PVP which was then dissolved in a known amount of selected solvent and filtered through 0.22 μm filter before usage. Known volume and concentration of sample was then placed into viscosimeter which was immersed in a bath with constant temperature. The temperature was controlled at 25.0°C. Sample was pumped above a certain mark on viscosimeter and time was measured for the sample to pass from one mark trough another by digital stopwatch. Sample was then diluted directly in viscosimeter with certain amount of solvent used and time was measured again. Every sample was diluted five times. All measurements were done in triplicate.

Kinematic viscosity was then calculated by multiplying the efflux time with a viscosimeter constant that was provided by manufacturer of viscosimeter. Specific and relative viscosities were calculated using equation 1. By plotting specific viscosity to zero polymer concentration, the intrinsic viscosity was derived as proposed by Huggins (Equation 2).

PREPARATION OF PHYSICAL MIXTURES, AMORPHOUS CELECOXIB AND SOLID DISPERSIONS

Physical mixtures were prepared by mixing accurately weighed amounts of drug and polymer. Both compounds were sieved trough sieves #350 before weighing and mixing.

Amorphous celecoxib was prepared by dissolving the drug in a sufficient amount of methanol and spray drying the solution.

Solid dispersions of CEL and PVP were also prepared by spray drying. Different ratios of drug and polymer were used. The required amount of the drug and polymer was dissolved either in methanol or in methanol: acetone 1:5 ratio, representing a good and a poor solvent for PVP, respectively. Solutions were filtered before spray drying.

SPRAY DRYING

Spray drying process was performed using Büchi B-290 spray dryer connected to Büchi Inert Loop B-295, Büchi Labortechnik, Switzerland. Nitrogen was used as atomizing gas

in co-current mode. Solutions containing pure PVP were spray dried at 60°C inlet temperature, feed rate of 3 ml/min and 37 m^3/h of gas flow rate. Solutions containing different ratios of drug and polymer were spray dried at 70°C inlet temperature, 3ml/min feed rate and 37 m^3/h of gas flow rate. Spray dried particles were separated from drying medium using cyclone and collected. Dry product was then manually transferred to glass vials immediately after production. Lid was wrapped using ParaFilm not the get in contact with air humidity and then stored in vacuum desiccator with silica gel for 7 days.

STABILITY STUDIES

To investigate the stability of solid dispersions samples were put in an oven at 40°C for 28 days. All samples were analyzed immediately after production and on different days during storage in the oven. Possible recrystallization behaviour of amorphous CEL dispersed in solid dispersion was analyzed using X-ray powder diffraction, Fourier transform infrared spectroscopy and Raman spectroscopy. Crystalline CEL, as well as PVP and their physical mixtures were also analyzed using this method for further comparison with solid dispersions behaviour.

X-Ray powder diffraction (XRPD)

X-ray powder diffraction patterns were obtained using PANalytical X'Pert Pro diffractometer equipped with a PIXcel detector (PANalytical B.V., The Nederlands). A continuous 2θ scan was performed in a range of 2°–35° using a Cu Kα radiation (λ=1.54187 Å). The Kβ radiation was eliminated by nickel filter. The voltage and current applied were 45 kV and 40 mA, respectively. Each measurement was done with a step size of 0.026° 2θ and at a speed of 0.067°/s. Sample spinning was employed during measurements to avoid preferred orientation effects. Data were collected using X'Pert data collector and were analyzed with X'Pert Highscore plus (both from PANanalytical B.V., The Nederlands)

Fourier transform infrared (FTIR)

FTIR analyses were performed using a NICOLET 380 (Thermo Scientific) smart orbit with diamond crystal and Omnic software. For each spectrum 64 scans were performed at a resolution set to 2 cm^{-1}. The scanning range was from 4000 cm^{-1} to 525 cm^{-1}.

Raman spectroscopy (Raman)

The Raman spectra were obtain using a wide angle (Phat) probe (Kaiser Optical Systems Inc., USA) with a spot size of 3 mm. Pure drug, physical mixtures and spray dried products spectra were collected using a 4s exposure time and 2 accumulations. A total of 8s was used to obtain each spectrum.

THERMOGRAVIMETRIC ANALYSES (TGA)

To determine the content of residual moisture in our samples TGA 7 (Perkin Elmer, USA), controlled by Pyris software was used. Samples were investigated directly after spray drying and after 7 days of post drying in vacuum desiccator. Samples were loaded in a flame-cleaned platinum sample pan and heated from 25 to 200°C at a rate of 10°C/min.

SCANNING ELECTRON MICROSCOPY (SEM)

Scanning electron microscope (JMS-5200, JEOL, Japan) was used to check particle morphology and size of solid dispersions. Samples were mounted on aluminium stubs with double adhesive carbon tape and sputter coated with gold at 20 mA for 120s in argon atmosphere using E5200 Auto Sputter Coater (Biorad, UK) prior to microscopy.

DISSOLUTION STUDY

The dissolution rate of CEL and solid dispersions was studied using Erweka DT-600, a USP type II dissolution test apparatus with paddle stirrer. Samples were placed in a dissolution vessel with 500 mL of 0,01M phosphate buffer (pH 6.8) containing 1.5 % surfactant, sodium lauryl sulphate. The temperature was maintained at 37°C and stirred at 50 rpm. As a sample 10 mg of CEL was accurately weighed as well as corresponding amounts of solid dispersions (it was assumed that 20 mg of solid dispersion should contain 10 mg of CEL, if it was prepared from solution containing CEL and PVP in 1:1 ratio). During dissolution samples of 5 ml were collected at different time intervals and replaced with an equivalent amount of fresh dissolution medium at 37°C. The vessel was covered during the test to minimize the evaporation of dissolution medium.

Dissolution tests were performed in a duplicate.

Buffer solution

Phosphate buffer was prepared by mixing proper amounts of 0,1M Na_2HPO_4 and 0,1M NaH_2PO_4 and diluting it with distilled water to obtain 0,01M buffer. For 100 ml of phosphate buffer with pH 6.8, 28.01 ml of Na_2HPO_4 x $2H_2O$ and 71.99 ml of NaH_2PO_4 x $2H_2O$ was used.

UV spectroscopy

UV spectroscopy was used to quantify the concentration of the drug dissolved using UV spectrophotometer (Evolution 300, Thermo Fisher Scientific, USA). UV spectrum of celecoxib was evaluated in the range of 200-400 nm. Due to the highest molar absorptivity of celecoxib at the wavelength of 253 nm and the high selectivity of this wavelength regarding solvent in the samples the wavelength of 253 nm was selected for detection of drug amount. If necessary, samples were filtered before the analysis trough 0.45 μm filter. Absorption spectra of the sample solutions were measured against the reference solution using 1.0 cm light-path length cuvettes. As the reference pure solvent was used. The spectrum for each sample was collected at room temperature in a triplicate, the average values were reported. The drug content in the unknown samples was calculated based on the absorbance values from the standard curve, which was previously prepared from the absorbance values of known standard solutions, as described below.

Standard curve determination

Standard solutions with known concentrations were prepared by diluting the stock solution. As a solvent phosphate buffer with pH 6.8 was used. The wavelength of 253 was selected for detection. From absorption spectra achieved with standard solutions the standard curve was drawn, linear equation of the curve and R^2 were calculated.

4 RESULTS AND DISSCUSION

4.1 INTRINSIC VISCOSITY

Viscosity measurements were performed to select two solvents, good and poor, which will be further used for spray drying. The kinematic viscosity (η) was calculated by multiplying the average efflux time with viscosimeter constant (0.003559 mm^2/s^2), as specified by viscosimeter manufacturer (36). Then η_{sp} and η_{rel} were obtained as shown in Equation 1. All results can be seen in Table 2.

Intrinsic viscosity of polymer was derived by plotting η_{sp}/c versus c to get the linear relationship, intercept was equal to intrinsic viscosity, [η] as can be seen in Figure 5.

Table 2 Values for PVP concentration (c), average efflux time (t), average kinematic viscosity (η), specific viscosity (η_{sp}) and intrinsic viscosity ([η]) polymer in different solvents.

	PVP K30				
Solvent	c (g/ml)	t (s)	η (mm^2/s)	η_{sp}	[η] (ml/g)
methanol	pure	194,33	0,69	/	/
	0.042	437.82	1.56	1.25	**22.88**
	0.033	382.28	1.36	0.97	
	0.024	320.10	1.14	0.65	
	0.017	277.34	0.99	0.43	
	0.012	252.08	0.90	0.30	
methanol:acetone 1:4	pure	113.44	0.40	/	/
	0.05	266.0	0.95	1.34	**18.38**
	0.04	232.49	0.83	1.05	
	0.033	205.62	0.73	0.81	
	0.025	178.84	0.64	0.58	
	0.018	157.39	0.56	0.39	

	c (g/ml)	t (s)	η (mm^2/s)	η_{sp}	[η] (ml/g)
methanol:acetone 4:1	pure	167.62	0.60	/	/
	0.05	453.79	1.62	1.71	**23.39**
	0.04	384.88	1.37	1.30	
	0.033	340.63	1.21	1.03	
	0.025	288.36	1.03	0.72	
	0.018	250.69	0.89	0.50	
methanol:acetone 5:1	pure	169.22	0.60	/	/
	0.05	466.53	1.66	1.76	**24.92**
	0.04	397.09	1.41	1.35	
	0.033	350.77	1.25	1.07	
	0.025	296.63	1.06	0.75	
	0.018	257.16	0.92	0.52	
ethanol:acetone 4:1	pure	260.75	0.93	/	/
	0.05	747.35	2.66	1.87	**23.67**
	0.04	623.47	2.22	1.39	
	0.033	547.44	1.95	1.10	
	0.025	458.84	1.63	0.76	
	0.018	396.90	1.41	0.52	
PVP360					
methanol	pure	194.33	0,69	/	/
	0.01	784.89	2.79	3.04	**196.55**
	0.008	641.19	2.28	2.30	
	0.0063	521.14	1.85	1.69	
	0.0044	408.20	1.45	1.10	
	0.0027	310.61	1.11	0.60	
methanol:acetone 1:2	pure	119.90	0.43	/	/
	0.01	434.21	1.55	2.62	**184.59**
	0.008	364.91	1.30	2.04	
	0.0063	298.90	1.06	1.49	
	0.0044	236.33	0.84	0.97	
	0.0027	186.18	0.66	0.55	
methanol:acetone 1:5	pure	112.41	0.40	/	/
	0.01	347.95	1.24	2.10	**125.92**
	0.008	291.35	1.04	1.60	
	0.0063	243.51	0.87	1.17	
	0.0044	194.99	0.69	0.73	
	0.0027	156.49	0.56	0.39	

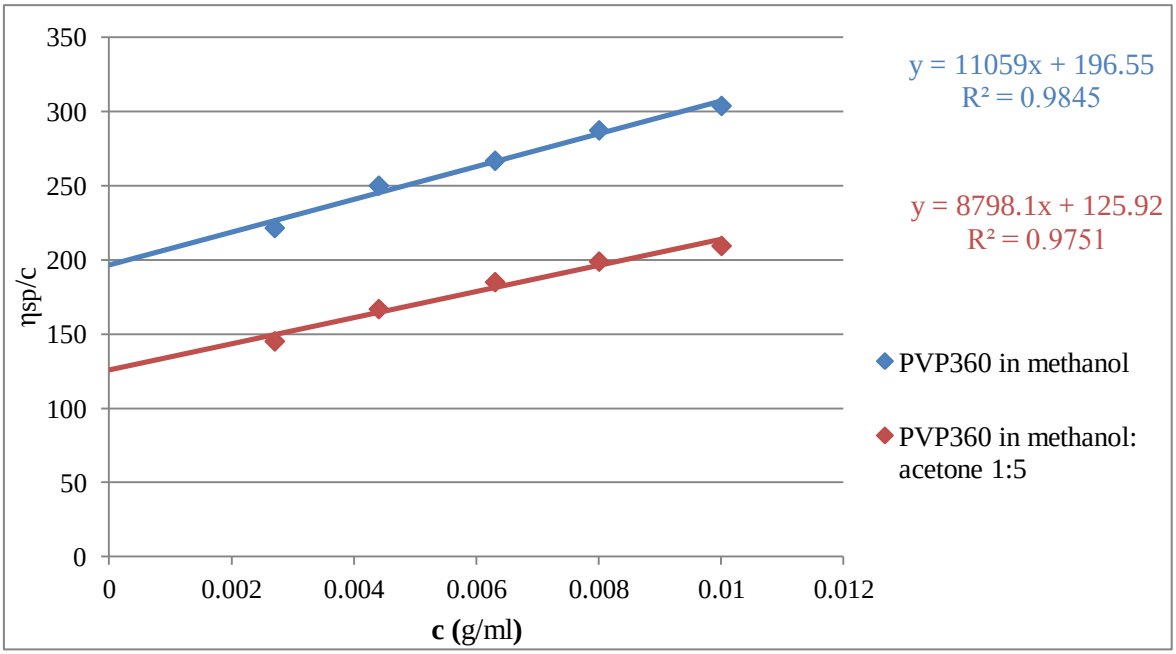

Figure 5 Intrinsic viscosity obtained by extrapolating η_{sp}/c to zero concentration.

As it was discussed in the introduction, intrinsic viscosity can also be obtained by extrapolating ln η_{rel}/c versus c to zero concentration. Both possibilities are compared in Figure 6.

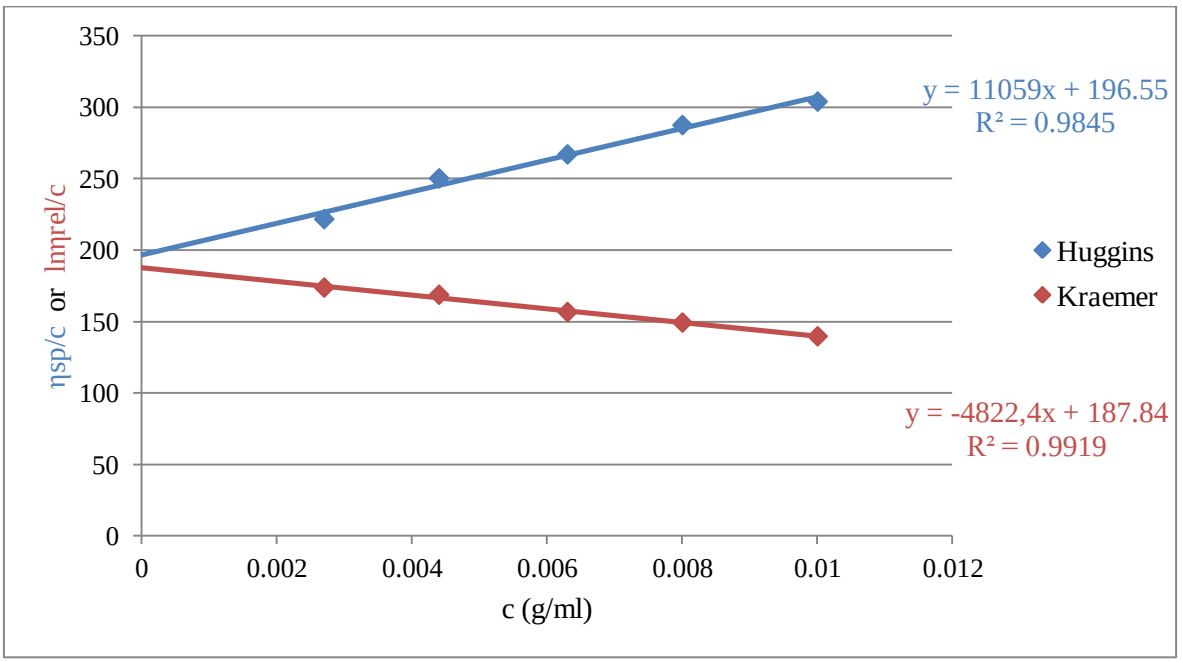

Figure 6 Intrinsic viscosity calculated on the basis of methods from Huggins or Kraemer

It can be seen that similar values for [η] are obtained with both methods. If values differ from each other, one could calculate the average [η] and use it for further calculations. In this thesis, [η] was derived only as proposed by Huggins.

As can be seen from Table 2, not much difference was seen in the values for the intrinsic viscosity for the PVP K30. Therefore, it was decided to use PVP360 with higher molecular weight, as the intrinsic viscosity depends on the size of the polymer. The observed differences were more distinct.

The viscosity measurements for PVP were made as a comparison to viscosity measurements for polyvinyl acetate (PVAc) with Mw 100000 from a similar study, as properties of both polymers in different solvents were compared. Based on the results for intrinsic viscosity of PVAc, the viscosity of PVP360 was measured only in three solvents – methanol, methanol: acetone 1:5 and methanol: acetone 1:2 ratio as those were solvent from which two, good and poor solvent for each polymer, would be selected for comparison. The values of [η] for PVAc in these three solvents were 38.1 ml/g, 65.04 ml/g and 62.08 ml/g, respectively.

It was assumed that by adding acetone to the solvent mixture, less quality solvent for PVP will be obtained, because PVP is soluble in methanol and only slightly soluble in acetone. From [η] values it can be concluded that methanol (MeOH) is a good solvent and methanol: acetone 1:5 (M:A 1:5) is a poor solvent for PVP. In a good solvent polymer coil is more expanded (higher [η]) compared to poor solvent, where polymer exist in folded formation (lower [η]) due to more favourable polymer-polymer interactions. Therefore it was expected to observe better product properties when MeOH is used for spray drying, compared to solutions made with M:A 1:5.
Further work was done using PVP360, which will be referred to as PVP when discussing other results.

Critical overlap concentration

From obtained [η], the c* for PVP in both solvents was calculated as follows:

$$c^* = 1/[\eta] \quad (g/ml) \qquad \text{Equation } 4$$

The c* value for MeOH and M:A 1:5 is 0,005 g/ml and 0,008 g/ml, respectively.
Below these values a dilute solution with no contact between polymer molecules exist and above there is semi-dilute region where polymer molecules can get in continuous contact with each other (32).

4.2 SPRAY DRYING OF PURE POLYMER

Spray drying of pure PVP from MeOH and M:A 1:5 was conducted to see if there were any differences in particle properties after spray drying, regarding the use of different solvents and concentrations (Table 3). The inlet T was adjusted to 60°C, giving outlet T of 42±1°C.

Table 3 PVP solutions used for spray drying

SOLVENT	CONCENTRATION OF PVP (w/v%)	YIELD %
MeOH	0.5 (c*)	45
	1.0	63
M:A 1:5	0.8 (c*)	67
	1.25	63

It was decided to spray dry the concentrations above and at c*, but not below, as concentrations were already very low. At concentrations above c* more polymer-polymer interactions occur, what can result in formation of fibres, which is not desired for further incorporation of the drug in the polymer.

Thermogravimetric analyses

TGA was used to determine the amount of residual moisture in spray dried PVP. The residual moisture varied as can be seen from Table 4. Before spray drying PVP contained 5.57 % of moisture.

Table 4 Results of thermogravimetric analyses

SOLVENT	CONCENTRATION OF PVP (w/v%)	RESIDUAL MOISTURE %
MeOH	0.5 (c*)	6.30
	1.0	8.60
M:A 1:5	0.8 (c*)	9.60
	1.25	13.20

Samples spray dried from M:A 1:5 mixture contained a higher amount of residual moisture compared to the ones that were spray dried from methanol. Additionally, with higher concentration of PVP in solution the residual moisture increased as PVP is hygroscopic polymer.

Residual moisture was tried to be minimized by spray drying 0.5 % PVP in MeOH at higher inlet temperatures to see if any improvements can be achieved.

Table 5 Spray drying of PVP at higher T_{in}.

T_{in} (°C)	T_{out} (°C)	Residual moisture (%)	Yield (%)
70	46	5.85	67
75	48	7.27	45
85	57	8.27	37

It was expected that when spray drying with higher T_{in} the amount of residual moisture in spray dried PVP would decrease (Table 1). The experiment values did not comply with expectations. Sample spray dried at 70°C exhibited the lowest residual moisture (Table 5). Even though the samples were protected with ParaFilm right after spray drying it is possible that air humidity that came in contact with the sample when weighing it and preparing it for TGA could affect the amount of residual moisture found in the samples as they were not produced on the same day. Additionally, with increasing T_{in} decrease in yield was observed. According to the amount of residual moisture determined after spray drying of pure polymer it was decided that further samples will be spray dried at 70°C and

collected product will then be stored in vacuum desiccator for 7 days in desire to reduce residual moisture.

Scanning electron microscopy

The recorded SEM pictures of spray dried PVP are shown in Figure 7.

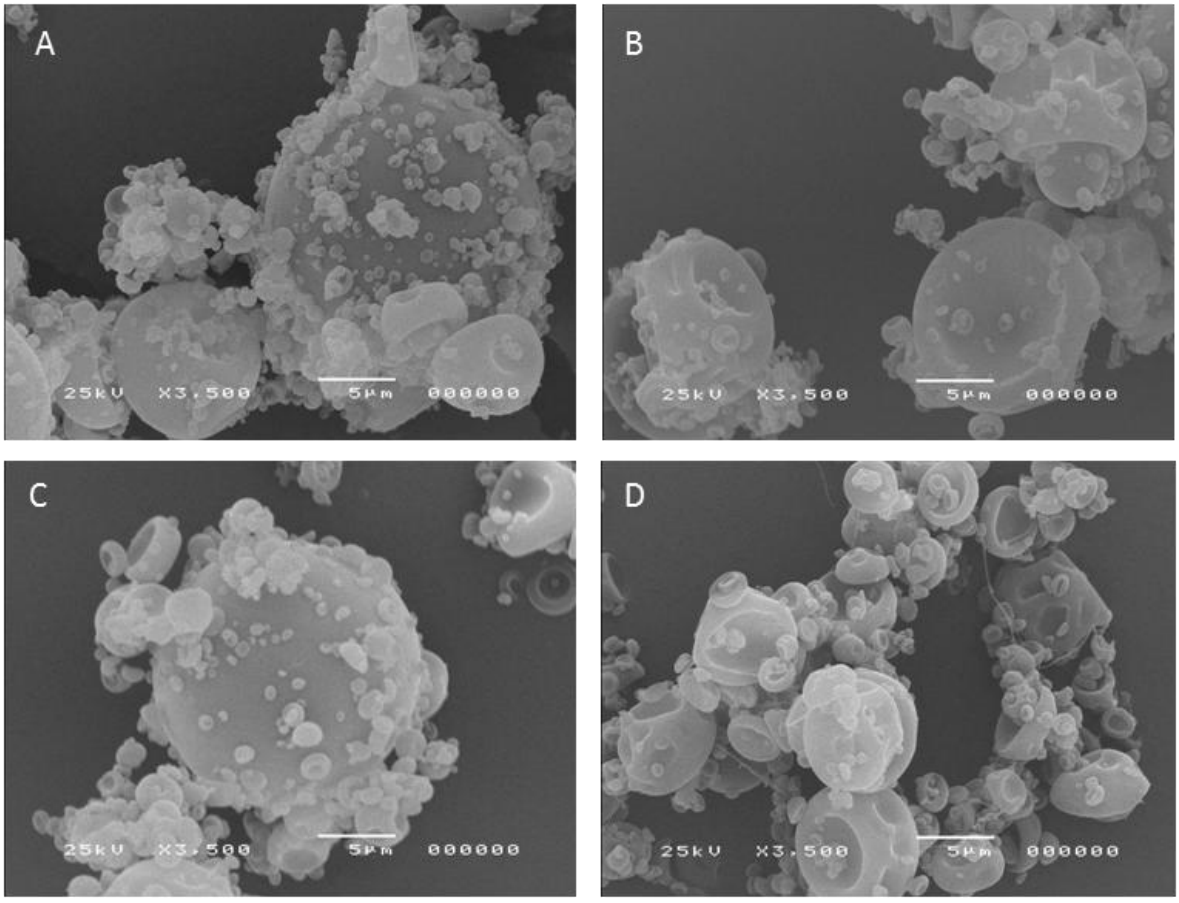

Figure 7 SEM pictures. (A) 0.5 % PVP in MeOH (c*), (B) 0.8 % PVP in M:A 1:5 (c*), (C) 1.0 % PVP in MeOH, (D) 1.25 % PVP in M:A 1:5

It was expected to get round, spherical particles when spray drying from good solvent and particles of different, collapsed shape in poor solvent. However, spray dried particles exhibited collapsed form in all of the samples, regardless of the solvent and concentration used. The collapsed particle formation observed could be explained by early formation of shell and slow migration of the polymer during drying. Because of high viscosity of droplets their shell could collapse before completely dried. It is also possible there were difficulties in vapour flow inside the droplet (37-39).

Some fibres were present in the sample spray dried from 1.25 % PVP in M:A 1:5 (Figure 7, D), indicating that this concentration could be too high for further use. It is possible that this occurred because of difficulty in droplet formation when using polymer with high Mw and concentrations above c* for spray drying.

4.3 SPRAY DRYING OF DRUG AND POLYMER

Solutions containing CEL and PVP in 1:1 and 1:3 ratios were spray dried at T_{in} of 70°C, giving T_{out} of 50±1°C. Gas flow rate was set to 37 m^3/h and feed rate was 3 ml/min. Yield varied from 67 %-77 %. As can be seen from Table 6 the PVP content was either above or under the c* of PVP in both solvents. In this way, possible differences in spray dried samples from concentrated and dilute solutions were investigated.

Table 6 Solutions with different PVP to CEL ratio were used for spray drying. Eight samples were planned to be spray dried as can be seen. Only seven were carried out due to lack of time.

Sample	CEL:PVP	PVP content (w/v%)	Solvent
1	1:1	1,05	MeOH
2	1:3	1,05	MeOH
3	1:1	1,05	M:A 1:5
4	1:3	1,05	M:A 1:5
5	1:1	0,25	MeOH
6	1:3	0,25	MeOH
7	1:1	0,25	M:A 1:5
8	1:3	0,25	M:A 1:5

Thermogravimetric analyses

Residual moisture of solid dispersions was measured right after spray drying and after 7 days storage in vacuum desiccator (Table 7).

Again, it was observed that more residual moisture remained in solid dispersions spray dried from M:A 1:5 as well as in samples that contained higher amount of PVP. With higher drug load the amount of residual moisture decreased probably because more polymer-drug interactions occurred, leaving less polymer molecule available to take up moisture.

Table 7 The amount of residual moisture determined with TGA right after spray drying and following the 7 days post drying in vacuum desiccator.

CEL:PVP (w/v%)	Solvent	After spray drying (%)	7 days in vacuum (%)
1.05:1.05	MeOH	2.0	2.0
0.35:1.05	MeOH	4.17	4.14
1.05:1.05	M:A 1:5	2.91	1.31
0.35:1.05	M:A 1:5	4.70	3.18
0.25:0.25	MeOH	1.33	1.82
0.25:0.25	M:A 1:5	2.53	2.14
0.08:0.25	M:A 1:5	4.54	4.90

As it was discussed before, presence of residual moisture in solid dispersion could lead to instability and precipitation of the drug from solid dispersion. According to this, solid dispersions prepared from M:A 1:5 were expected to be less stable as they contain more residual moisture.

Scanning electron microscopy

SEM pictures (Figure 8) exhibited collapsed particles regardless of solvent and concentration used also when celecoxib was added to the feed solution. Solid dispersion containing 1.25 % CEL: 1.25 % PVP in M:A 1:5 contained some fibres as can be seen in Figure 8 (G).

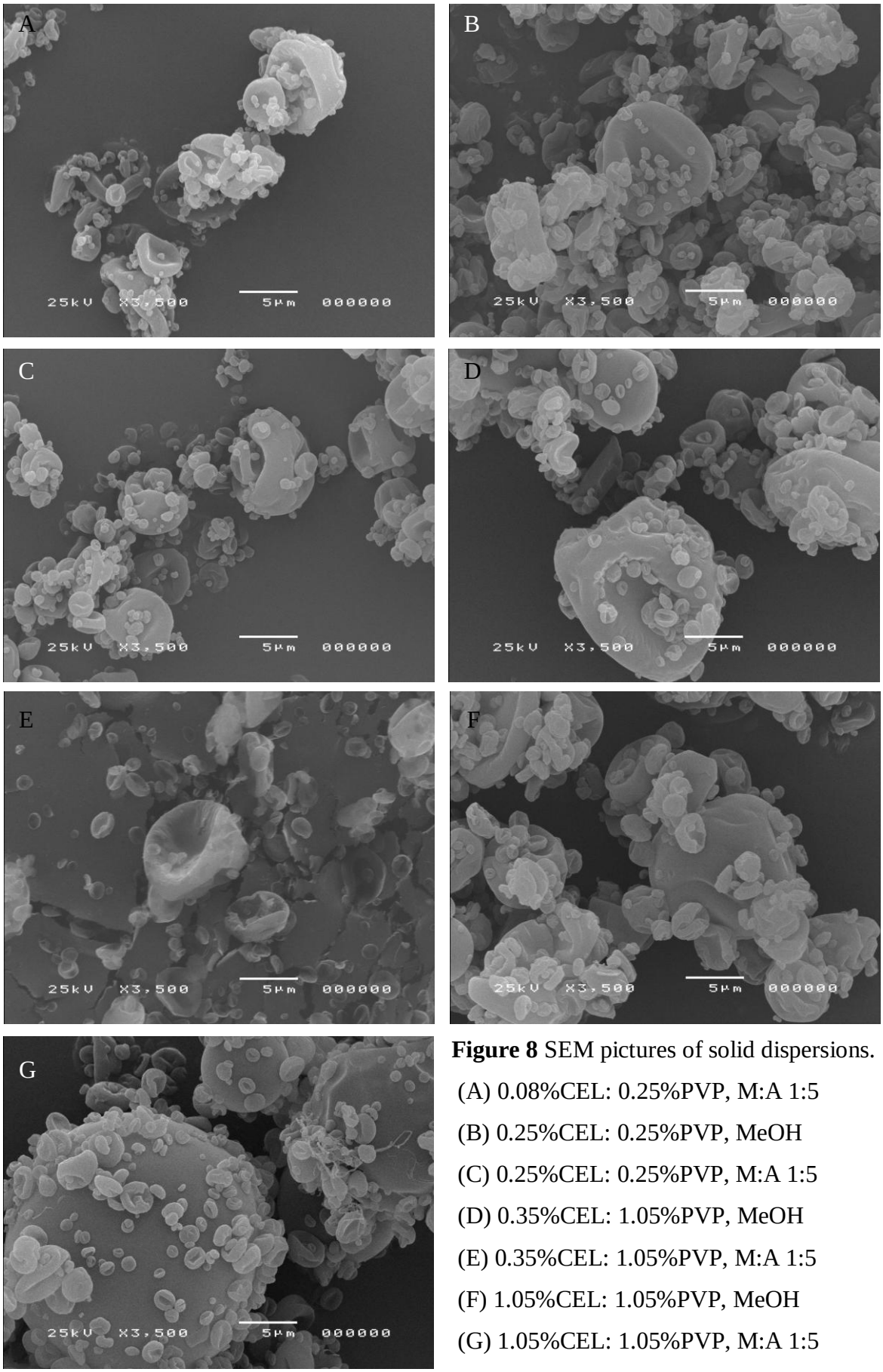

Figure 8 SEM pictures of solid dispersions.

(A) 0.08%CEL: 0.25%PVP, M:A 1:5

(B) 0.25%CEL: 0.25%PVP, MeOH

(C) 0.25%CEL: 0.25%PVP, M:A 1:5

(D) 0.35%CEL: 1.05%PVP, MeOH

(E) 0.35%CEL: 1.05%PVP, M:A 1:5

(F) 1.05%CEL: 1.05%PVP, MeOH

(G) 1.05%CEL: 1.05%PVP, M:A 1:5

4.3.1 STABILITY STUDIES

X-Ray powder diffraction (XRPD)

The diffractograms of pure compounds, physical mixtures and solid dispersion are shown in Figure 9.

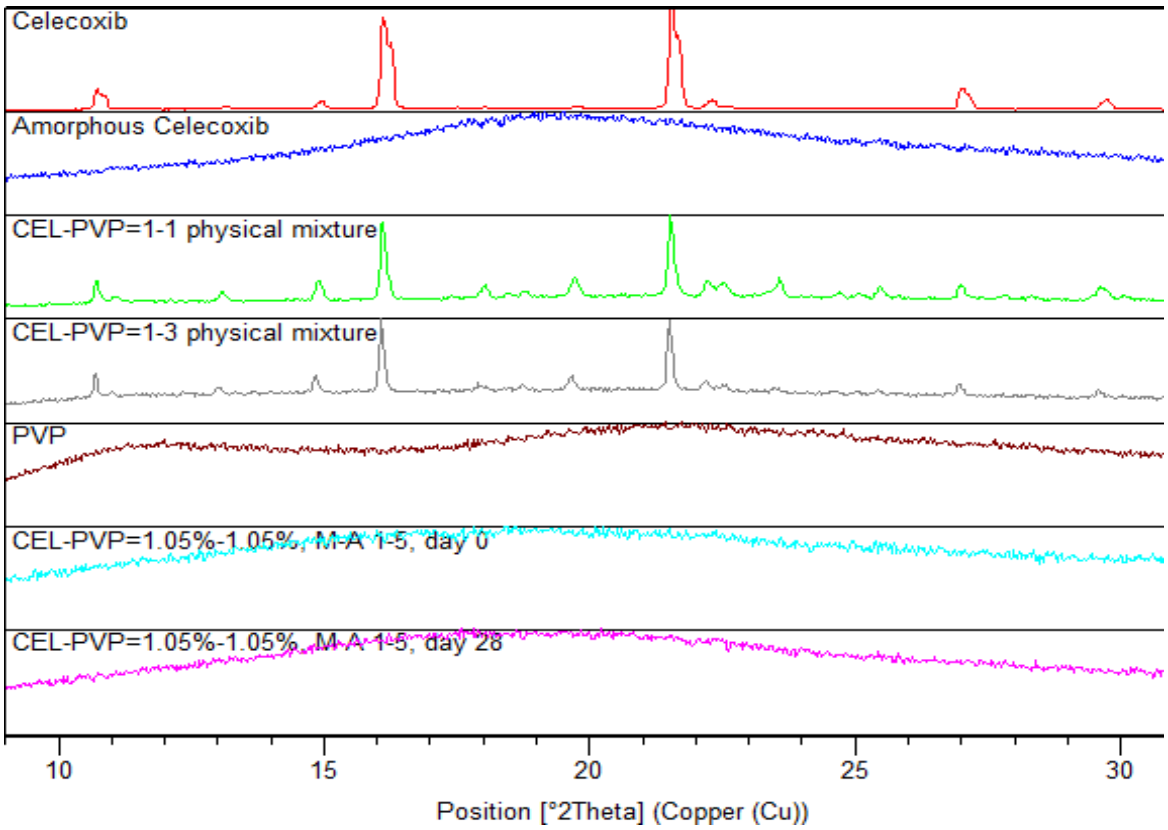

Figure 9 X-ray diffractograms of CEL, amorphous CEL, physical mixtures, PVP and solid dispersion formulated from 1.05 % CEL: 1.05 % PVP in M:A 1:5 at day 0 and day 28.

The XRPD pattern of CEL shows characteristic peaks between 10-30°2θ. These peaks were absent in spray dried celecoxib and in solid dispersions which indicates that the amorphous form was obtained when spray drying in all the samples created. The pure amorphous drug started to recrystallize already at day 4, which was confirmed by the FTIR (Figure 11) and Raman spectra (Figure 15). Only one of the spray dried samples is showed at day 0 and day 28 to prove the solid dispersion remained stable, but all seven samples remained amorphous from day 0 throughout the day 28, indicating that stability of CEL in solid dispersion is improved when compared to pure amorphous CEL. Again, no difference

was seen when comparing solid dispersion spray dried from different concentrations and solvents.

Fourier-transform infrared spectroscopy

FTIR was used to investigate differences between crystalline and amorphous celecoxib and to define if there are any molecular interactions occurring between celecoxib and PVP in solid dispersion.

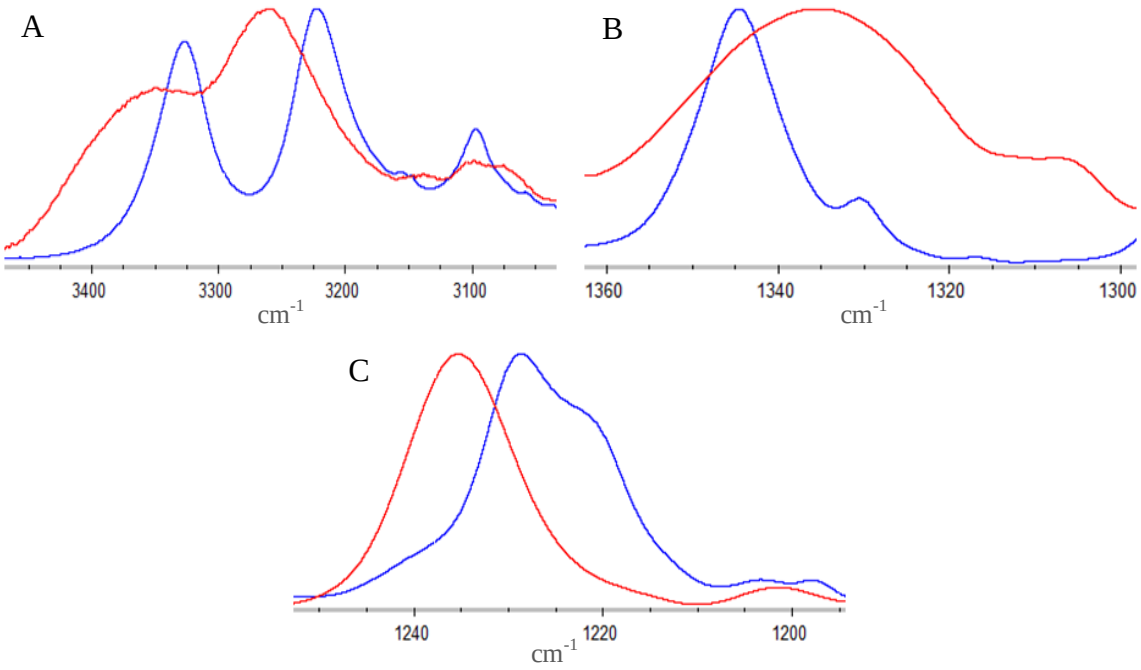

Figure 10 FTIR spectra of crystalline celecoxib (blue) and amorphous celecoxib (red). (A) NH_2 streching of SO_2NH_2 group, (B) S=O band stretching, (C) C-F streching

In Figure 10 the spectra of crystalline and amorphous celecoxib was compared. The N-H stretching vibration of -SO_2NH_2 group was observed as a sharp doublet at 3328 cm^{-1} and 3224 cm^{-1} in crystalline CEL which was shifted to a higher wavenumber of 3351 cm^{-1} and 3261 cm^{-1} and was also much broader for amorphous CEL (A).

The S=O stretching vibration band observed at 1344 cm^{-1} for crystalline CEL broadened and shifted to lower wavenumber of 1335 cm^{-1} for amorphous CEL (B).

C-F stretching vibration band of crystalline CEL at 1228 cm^{-1} shifted to a higher wavenumber of 1235 cm^{-1} for amorphous CEL (C).

Similar shifts have been observed in various studies (40-43). From changes in shape and shifts to different wavelength it can be concluded that the bonding between different CEL molecules comprise mostly of $-NH_2$ bonding with $-SO_2$.

The shifts to higher wavenumbers and the broadening could indicate the weakening of H-bonding strength for –N-H and for -C-F in the amorphous form, whereas a shift to lower wavenumber indicates greater strength for H-bonding of S=O in the amorphous form of celecoxib (40,43).

The spectra of CEL, amorphous CEL, PVP, physical mixtures and solid dispersions are showed in Figure 11.

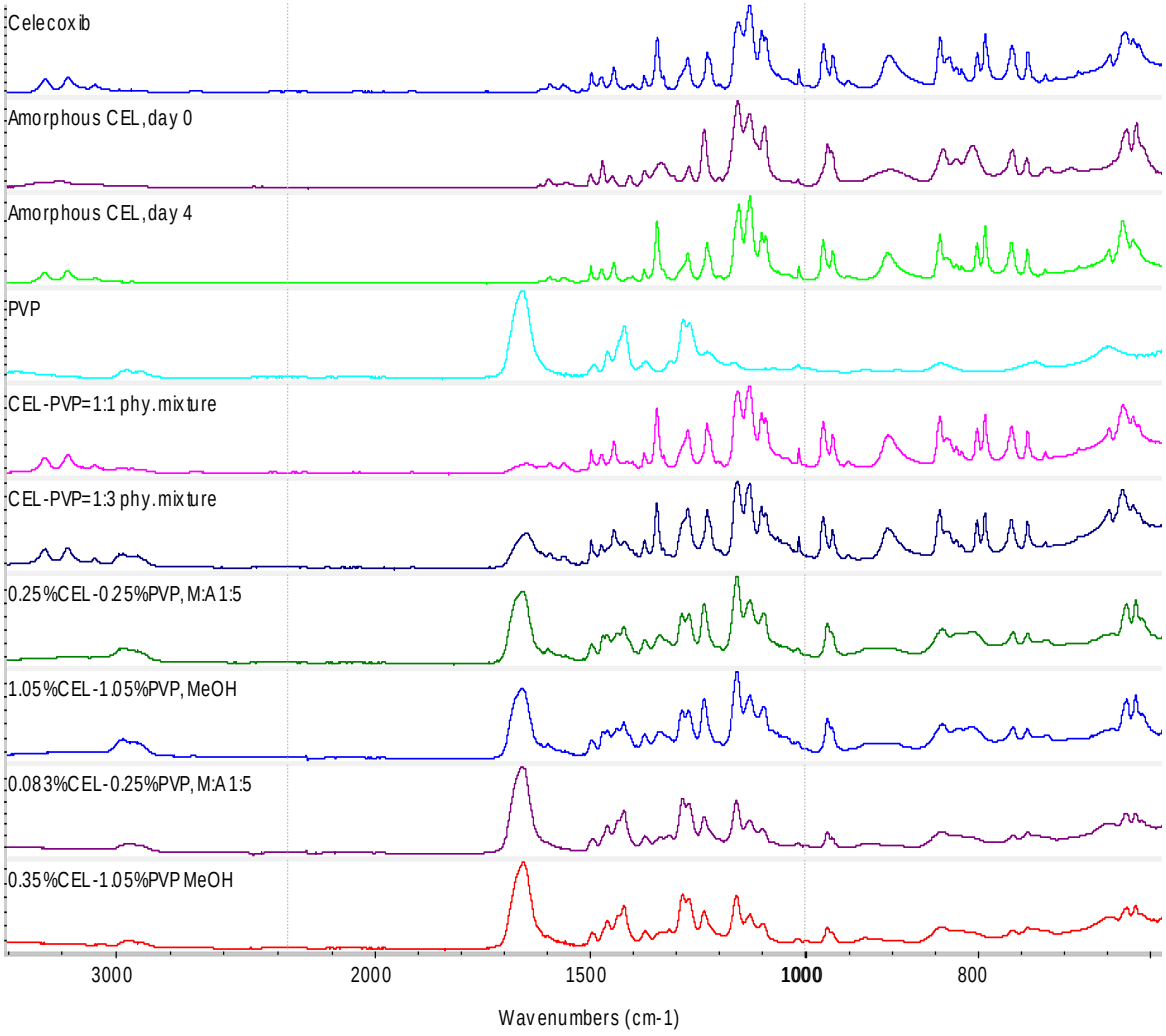

Figure 11 FTIR spectra of CEL, amorphous CEL, physical mixtures and solid dispersions.

From the spectra it can be seen that at day 4 amorphous form already showed sharp peaks comparable to the ones of crystalline CEL, which indicates that the amorphous form has already started to recrystallize. FTIR spectra of physical mixtures exhibit peaks that are specific for crystalline CEL and PVP. They differ from the samples that were spray dried, confirming the formation of solid dispersion.

The aim of FTIR investigation was to determine if any interactions occur between CEL and PVP in solid dispersions. Celecoxib has proton acceptor groups (-SO_2, -CF_3, -N on pyrazole) and a proton donor group (-NH_2 from sulphonamide). NH_2 is a possible site for H-bonding interaction with proton acceptor group (C=O, peak at 1654 cm^{-1}) of PVP. The possibility of this interaction has previously been confirmed and described as a shift for C=O stretching vibration band to lower wavenumbers (40-43).

FTIR spectrum of amorphous CEL was compared to spectra of solid dispersions. In Figure 12 the comparison of significant bonds reported to be eligible for interaction of PVP and CEL can be seen. Only small shift in the band vibrations corresponding to C=O of solid dispersion (1657 cm^{-1}) was observed when compared to spectrum of pure PVP (1654 cm^{-1}) (Figure 12, B). The –NH_2 doublet seen in amorphous CEL (Figure 12, A) disappears in all spectra of solid dispersions, what could be attributed to high percentage of residual moisture present, probably causing the overlap of -OH peak in spectra.
Also throughout the whole spectrum of solid dispersion only small shifts have been observed (up to 2 cm^{-1}) when compared to amorphous CEL and PVP.
Considering the resolution was set to 2.0 cm^{-1} when FTIR spectra were taken it cannot be confirmed that H-bonding has occurred between CEL and PVP in solid dispersion. The same was observed for all solid dispersions regardless of solvent and concentration used.

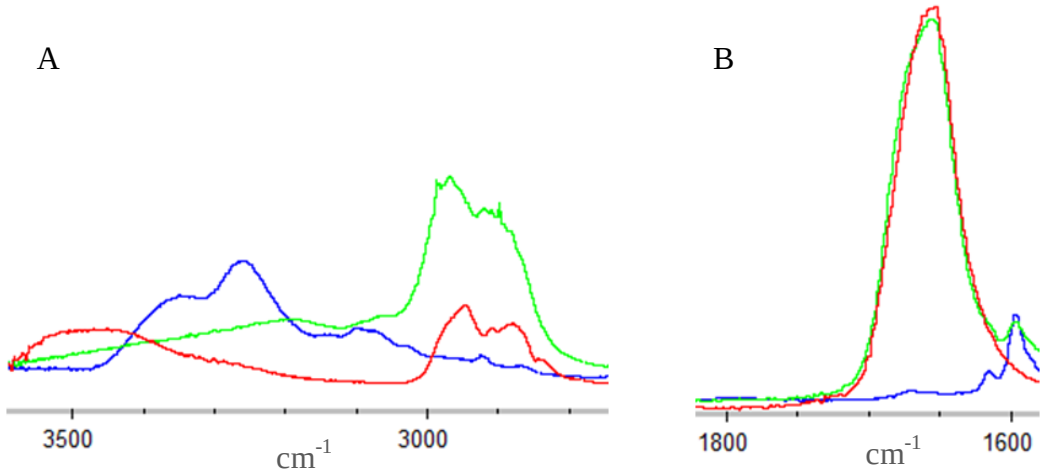

Figure 12 Comparison of spectra for PVP (red), amorphous CEL (blue) and solid dispersion (green). A- NH_2 stretching, B- C=O stretching

In Figure 13 it is shown that spectrum of solid dispersion stayed the same throughout 28 days storage at 40°C, proving that drug has not started to recrystallize. It can be concluded that solid dispersion remained stable. Results confirm what was already observed with XRPD. The same was true for all samples prepared, regardless of CEL:PVP ratios and solvent used.

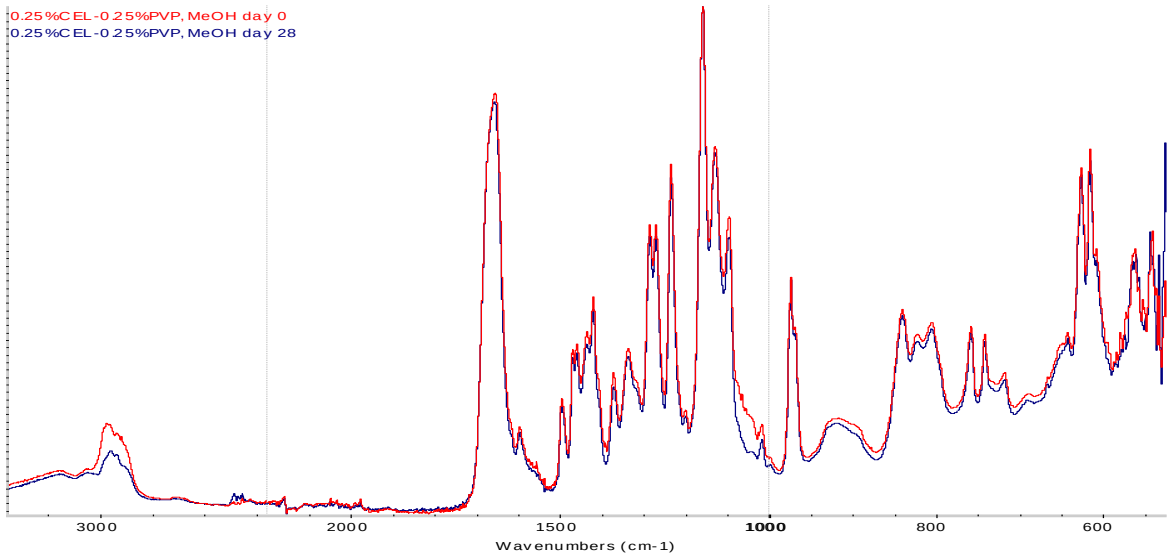

Figure 13 Overlaying spectra of solid dispersion at day 0 (red) and day 28 (blue).

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As no interactions between CEL and PVP could be confirmed, it was concluded that the higher stability of solid dispersions is the consequence of large PVP molecules entrapping the drug inside their chains, thus disabling molecular movements and consequently stabilizing amorphous form.

Raman spectroscopy

Raman was also used to confirm the formation of amorphous CEL and to detect possible interactions between CEL and PVP in solid dispersions. Raman spectra are shown in Figure 14 and 15. The results are comparable to the ones obtained using FTIR.

It was again observed that peaks of pure CEL after spray drying shifted to different wavelengths compared to crystalline form, thus confirming the formation of amorphous CEL. In Figure 14 shift and broadening for $–SO_2$ bond from 1160 cm^{-1} to 1157.4 cm^{-1} as well as shifts in other bonds are shown.

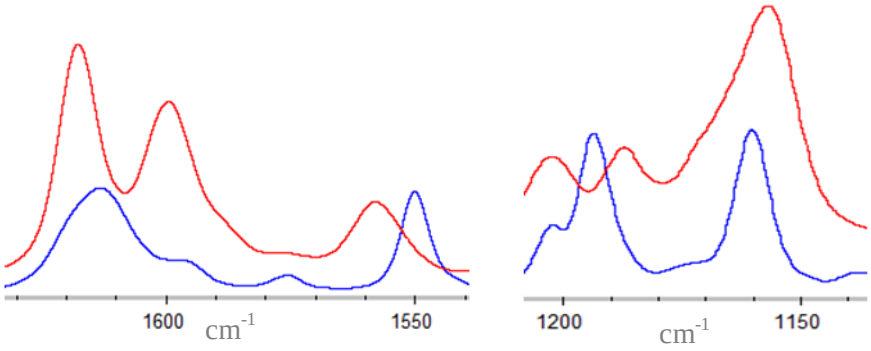

Figure 14 Shifts in Raman spectra. Crystalline CEL (blue), amorphous CEL (red)

It was confirmed that pure amorphous CEL started to recrystallize already at day 4 as can be seen in Figure 15. Again, no significant shifts were seen for solid dispersions in comparison to amorphous form of CEL, applying that no interaction occurred between CEL and PVP during spray drying. When spectra from day 0 and day 28 were aligned, no significant differences could be observed.

Figure 15 Raman spectra of pure and amorphous CEL, physical mixtures and solid dispersions

Again, it was confirmed that no difference in stability of solid dispersions was seen when either different concentrations or solvents were used throughout 28 days of stability studies.

4.4 DISSOLUTION STUDIES

Dissolution studies were done in phosphate buffer with pH 6.8 containing 1.5 % SLS at 37°C.

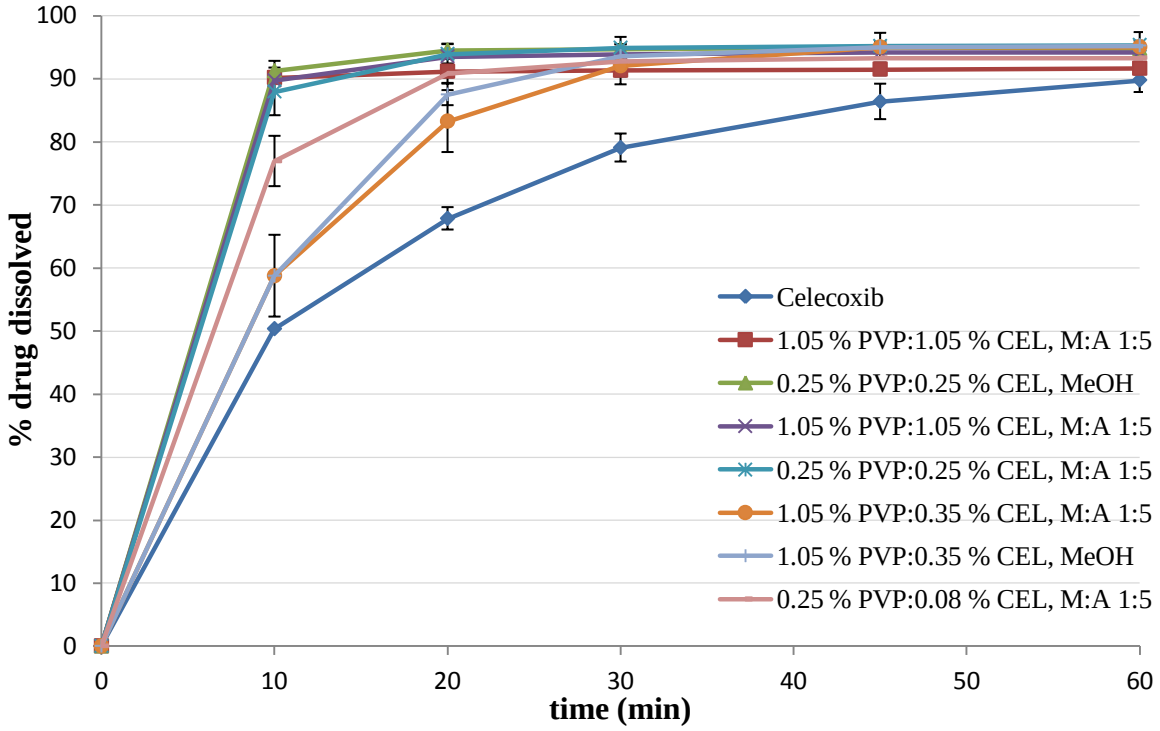

Figure 16 Dissolution rate of pure celecoxib and prepared solid dispersions

The dissolution rate of CEL from all solid dispersions is faster when compared to pure CEL. This was expected as PVP has good water solubility and is often used as solubilizer. The only difference that was observed in dissolution test is that the solid dispersions where drug to polymer ratio was 1:3, exhibit a bit slower dissolution in first 20 minutes compared to solid dispersions with 1:1 ratio, but still achieve complete dissolution faster than pure CEL. (Figure 16) This is probably caused by higher amount of PVP that has to be dissolved first in order to allow the dissolution of CEL that is entrapped in its coil.

There was no difference observed in dissolution rate of CEL from solid dispersions regarding the use of different solvents for spray drying.

5 CONCLUSION

The effect of solvent and concentration of polymer on properties of solid dispersions was studied. Seven different solid dispersions containing celecoxib and PVP were prepared. Using XRPD, FTIR and Raman it was proved that celecoxib was dispersed in PVP carrier in an amorphous state. All of them have exhibited better stability and solubility properties compared to pure amorphous celecoxib.

The stability of amorphous CEL was improved by formulating it with PVP. The stability studies showed that amorphous solid dispersions were stable for 28 days compared to pure amorphous CEL, which was recrystallizing already at day 4. All of the solid dispersion samples remained in amorphous form for 28 days, no difference in stability was seen due to use of different solvent and concentration of PVP. If there would be enough time for longer stability studies, some difference may be observed regarding the use of different solvents. Higher residual moisture present in solid dispersions prepared from M:A 1:5 would probably lead to lower stability of solid dispersion and thus recrystallizing of CEL would start faster compared to the solid dispersions prepared from MeOH.

By formulating celecoxib into solid dispersions, its dissolution rate was also enhanced. This can be attributed to the amorphous state of drug in solid dispersion and use of PVP as a carrier, as it is a good water soluble polymer and often used as solubilizer for poorly water soluble drugs. There were no differences seen in dissolution rates when using different solvents to dissolve the starting material. A slightly slower dissolution rate was observed at the beginning of dissolution test for solid dispersions containing higher amount of PVP. Unfortunately, one sample with higher PVP content could not be prepared, which could have given us an idea, if choice of solvent may attribute to the dissolution rate when higher amount of PVP is used for formulation of solid dispersions.

It can be concluded that using PVP as a carrier when formulating solid dispersions increases the stability of amorphous drug as well as enhances the dissolution rate of celecoxib. This effect is attributed to large PVP molecule entrapping the drug inside its chain, inhibiting the movement needed for recrystallization, as no interactions between drug and polymer could be confirmed using FTIR and Raman.

It would be interesting to see if use of PVP K30 with smaller Mw that was first intended to use, would exhibit different properties.

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