Master thesis performed at:

GHENT UNIVERSITY

FACULTY OF PHARMACEUTICAL SCIENCES

Department of Pharmaceutics

Laboratory of Pharmaceutical Technology

UNIVERSITY OF HELSINKI

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SOLUBILITY AND PERMEATION STUDIES USING SOLUPLUS® AND HPMC WITH A BCS CLASS II AMORPHOUS DRUG

Hanne VERMEERSCH

First Master of Drug Development

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Co-promoters

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Dr. L. Peltonen

Commissioners

Prof. Dr. B. De Spiegeleer

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May 31, 2016

Promoter

Prof. Dr. C. Vervaet

Author

Hanne Vermeersch

Summary

Indomethacin (IND) is classified in class II of the Biopharmaceutics Classification System (BCS), meaning it exhibits good permeability, but poor solubility. By using the amorphous form of the drug higher apparent solubility can be obtained, but the challenge of crystallization from supersaturated solutions remains. The main aim of this study was to investigate the level and maintenance of supersaturated solutions, with respect to the thermodynamic solubility of crystalline IND, of the amorphous form of the drug. Some experimental conditions of the method were fine-tuned.

Crystalline and amorphous IND were characterised using different analytical techniques, including infrared (IR) spectroscopy, Raman spectroscopy and differential scanning calorimetry (DSC). Crystallization behaviour was studied using polarized light microscopy (PLM) and IR spectroscopy.

During the solubility studies two different polymers (HPMC and Soluplus®) were used in a drug:polymer 1:1 ratio (w/w). The maximum drug concentrations (C_{max}) generated with the suspensions containing the respective predissolved polymer were considerably higher for Soluplus® compared to HPMC. Soluplus® also showed a higher potential in maintaining supersaturated solutions compared to HPMC.

The permeation of IND in the presence of polymer through an artificial membrane as well as the potential impact of permeation on the maintenance of supersaturation was investigated. The concentration of dissolved IND in the donor compartment decreases for each suspension. However, no IND could be measured in the acceptor compartment. The impact of permeation on the maintenance of supersaturation was dependent on the polymer used: for HPMC supersaturation was maintained longer during the permeation studies than during the solubility studies while for Soluplus® the opposite was observed.

Samenvatting

Indomethacine behoort tot klasse II van het Biofarmaceutische Classificatiesysteem (BCS II), wat betekent dat het een goede permeabiliteit, maar een slechte oplosbaarheid vertoont. Door gebruik te maken van de amorfe vorm van het geneesmiddel kan een schijnbaar hogere oplosbaarheid verkregen worden, maar het optreden van kristallisatie vanuit een oververzadigde oplossing blijft een uitdaging. De algemene doelstelling van deze studie was om de fysische stabiliteit van een oververzadigde oplossing van de amorfe vorm van het geneesmiddel te onderzoeken, rekening houdend met de thermodynamische oplosbaarheid van kristallijn indomethacine. Sommige experimentele condities van de methode werden verfijnd.

Kristallijn en amorf indomethacine werden gekarakteriseerd door gebruik te maken van verschillende analytische technieken, waaronder infrarood spectroscopie, Raman spectroscopie en differentiaal scanning calorimetrie. Kristallisatiegedrag werd bestudeerd door gebruik te maken van gepolariseerde lichtmicroscopie en infrarood spectroscopie.

Tijdens de oplosbaarheidstesten werd gebruik gemaakt van twee verschillende polymeren, HPMC en Soluplus®, in een geneesmiddel:polymeer verhouding van 1:1 (m/m). De maximale concentratie aan opgelost indomethacine bereikt vertrekkende van de suspensie die de respectievelijke vooraf opgeloste polymeren bevatte, was beduidend hoger voor Soluplus® dan voor HPMC. Soluplus® was ook beter in staat om de oververzadigde oplossing te stabiliseren, vergeleken met HPMC.

Zowel de permeatie van indomethacine in aanwezigheid van een polymeer door een kunstmembraan, als de impact van permeatie op het behouden van supersaturatie werd onderzocht. De concentratie aan opgelost indomethacine in het donor compartiment daalde voor elke suspensie. Er werd echter geen indomethacine gemeten in het acceptor compartiment. De impact van permeatie op het behouden van supersaturatie hing af van het gebruikte polymeer: voor HPMC werd supersaturatie langer behouden tijdens de permeatie testen dan tijdens de oplosbaarheidstesten, terwijl voor Soluplus® het omgekeerde werd waargenomen.

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Abbreviations

ATR	Attenuated total reflectance
BCS	Biopharmaceutics classification system
CCD	Charge coupled device
CMC	Critical micelle concentration
COX	Cyclooxygenase
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared
HPH	High-pressure homogenization
HPMC	Hydroxypropyl methyl cellulose
IND	Indomethacin
IR	Infrared
PCS	Photon correlation spectroscopy
PLM	Polarized light microscopy
T_c	Crystallization temperature
T_g	Glass transition temperature
XRPD	X-ray powder diffraction

1. INTRODUCTION

1.1. POORLY SOLUBLE DRUGS

Poorly water-soluble drugs are nowadays a great challenge in the pharmaceutical industry. Drugs classified in class II of the BCS exhibit good permeability, but poor solubility and hence poor bioavailability. (1) More than seventy percent of the new chemical entities are Class II drugs. (2) One of the main reasons is the advent of high-throughput technology and the evolution to a target-based drug discovery. (3) For these drugs, solid state modification and the use of certain polymers are interesting approaches to enhance the solubility. The poorly soluble drug investigated in this master thesis is indomethacin. (2)

1.1.1. Indomethacin

Indomethacin is a non-steroidal anti-inflammatory drug (NSAID) with analgesic, antipyretic and anti-inflammatory properties. It prevents the synthesis of prostaglandins by inhibiting the activity of the enzyme cyclooxygenase (COX). Indomethacin is an indole derivative with the IUPAC name 1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid, shown in figure 1.1 It is poorly soluble in water, but slightly soluble in alcohol. The solubility can be affected by the pH of the dissolution medium, because indomethacin is an acidic drug with a pKa of 4.5. (4), (5)

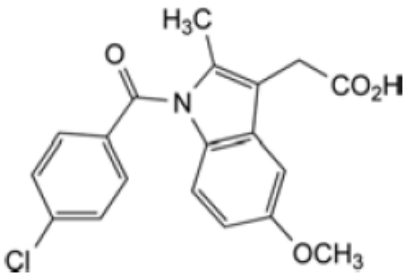

Figure 1.1 - Structure of indomethacin (European Pharmacopeia 8.2: Indomethacin (07/2014:0092) (6)).

Indomethacin has polymorphic properties. Nowadays eight different polymorphic forms are known, namely α, β, γ, δ, ε, ζ, η and a crystal form that is still unnamed. (2) The thermodynamically most stable form is the γ form and the α form is the most commonly

observed metastable form. Depending on the preparation and the storage conditions, amorphous indomethacin often crystallizes to the metastable α and the stable γ form. (2) This will be explained in more detail later on. Indomethacin is sensitive to light, therefore it has to be protected from light during storage.

1.2. FORMULATION STRATEGIES TO IMPROVE AVAILABILITY

Poor solubility can be caused by various physicochemical properties, for instance the complex structure of drugs, high lipophilicity and high molecular weight. In addition to these molecular properties, solid state structure, including crystallinity and polymorphic form, affects apparent (but not thermodynamic) solubility. Therefore, several formulations have been developed that may offer an improvement of bioavailability. Amorphous, nanocrystalline and lipid formulations are examples of possible formulation strategies. Other approaches include complexation of the drug with cyclodextrins, use of surfactants or permeation enhancers and salt formation. (3)

1.2.1. Amorphous formulations

Many compounds can occur in different solid state forms, such as polymorphs, solvates and amorphous form. In amorphous materials, the molecules are disordered, whereas in crystalline material, the molecules are ordered in a crystal lattice. (7) In addition, amorphous solids have a higher energy level than crystalline solids. This results in a higher solubility and dissolution rate of the amorphous form compared to the corresponding crystalline form. This means a supersaturated solution can be achieved with the amorphous form. In the gastrointestinal tract, this will cause a higher concentration gradient resulting in enhanced permeation through the intestinal membrane. Amorphous formulations can thus provide a promising solution for poorly water-soluble drugs. However crystallization to a more stable crystalline form may occur. (4), (8)

Crystallization of amorphous drug consists of two phases: nucleation and crystal growth. Nucleation is a process in which small aggregates are formed. The rate of nucleation depends on the degree of supersaturation. Crystal growth includes dissolved molecules which diffuse from the supersaturated solution and add on to the crystal lattice. Amorphous drug

combined with crystallization inhibitors can provide a solution to delay crystallization and thus to maintain supersaturation, this can be explained by the 'spring and parachute' effect (illustrated in figure 1.2). (4) The metastable amorphous form of the drug generates supersaturated solutions (with respect to the solubility of the crystalline form of the drug) followed by a rapid decline in concentration due to crystallization ('spring'). By adding crystallization inhibitors supersaturation can be maintained for a longer period of time ('parachute'). (4), (9)

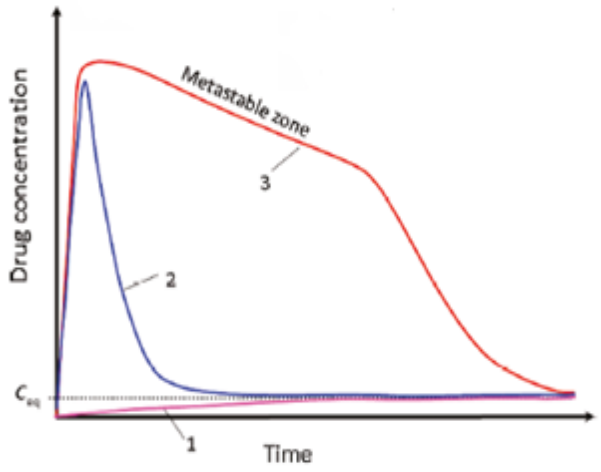

Figure 1.2 – The spring and parachute approach to generate and maintain drug supersaturation for a longer period of time. (1) The crystalline form has a low solubility, (2) the metastable amorphous form generating supersaturated solution, followed by a rapid decrease in concentration, (3) supersaturation is maintained for a longer period of time due to crystallization inhibitors. (N. Babu, 2011 (9)).

Depending on the potential of the polymer on inhibition of crystallization, supersaturation can be prolonged. (9) The specific mechanism of action of polymers concerning inhibition of crystallization is not fully elucidated, but various mechanisms may play an important role. It is presumable that hydrogen bonds are formed between the polymers and the drug; also hydrophobic interactions between polymer and drug can cause inhibition of crystallization for a certain period of time. Furthermore, polymers can prevent crystal growth by adsorption onto the crystal surfaces. (4) Various types of polymers can have different effects on the physical stability of amorphous drug in aqueous suspension, depending on the strength of the drug-polymer interactions. (10) Excipients used as

crystallization inhibitors during this project are the polymers hydroxypropylmethylcellulose (HPMC) and Soluplus®, illustrated in figure 1.3 a and b respectively. (4)

HPMC is a water-soluble cellulose polymer which enhances the dissolution of poorly water-soluble drugs. (11) It is a pH-independent and hydrophilic polymer that is commonly used as a polymeric carrier to improve solubility. (12) Soluplus® is a more recent polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer. It is a water-soluble copolymer that has been applied to improve the solubility and bioavailability of poorly water-soluble drugs, such as indomethacin. The copolymer is an amphiphilic molecule with both hydrophobic and hydrophilic residues. Soluplus® is a bifunctional polymer acting both as a polymeric carrier and as a solubilizer through the formation of micelles (unlike HPMC which does not form micelles). The mechanism of Soluplus® in enhancing solubilisation by forming micelles is not yet fully elucidated. (13) The advantage of using Soluplus® is that its solubility doesn't change throughout transit in the gastrointestinal tract, considering it is non-ionic and hydrophilic. (14) It has slight surfactant like properties: it improves the wettability of the drug. HPMC as well as Soluplus are besides solubilizers also stabilizers by interacting with drug molecules via hydrogen bonding and van der Waal's forces. (13)

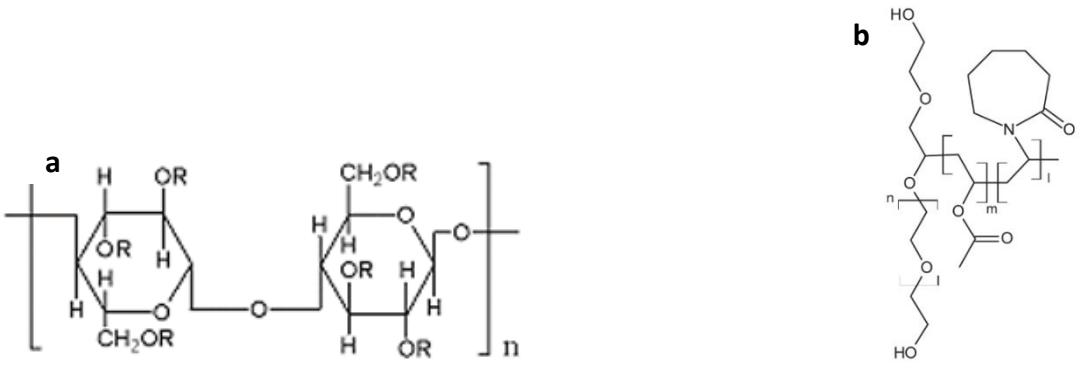

Figure 1.3 – Chemical structure of (a) HPMC and (b) Soluplus®. (M. Raymond, 2009 (15) and S. Tanida, 2016 (13)).

Solid amorphous dispersions can be produced by different pharmaceutical processes, including hot melt extrusion and spray drying. Hot melt extrusion is the process of forcing raw material through a die under increased temperature into a product of uniform shape. (3), (16) Spray drying is a technique that is used to produce dry powder by adding the active

pharmaceutical ingredient (API) to a solution of carrier. This solution is then atomized by forcing it through a nozzle and subsequently the solvent is evaporated. (3), (17)

1.2.2. Nanocrystalline formulations

Nanocrystals are solid nanosized particles covered with a stabilizing agent layer. The particle size from nanocrystals varies usually from 100 nm to 400 nm.

One possibility to improve the dissolution of poorly water-soluble drugs and hence the bioavailability are nanocrystalline formulations. (18) Reduction of the particle size leads to an increased surface area of the drug available for interaction with the solvent. (19) The higher dissolution rate results in more drug in the gastro-intestinal tract and thus in an increased concentration gradient. This enhances the penetration of the drug through the intestinal membrane, but one issue should be taken into account. Faster absorption creates higher maximum serum concentrations (C_{max}) of the drug, which may cause toxic side effects. Lowering the doses can offer a solution in order to prevent the side effects. (18)

As illustrated with the Noyes-Whitney equation (1.1), nanocrystalline formulations result in increased dissolution rate due to the decrease of the particle size (19).

$$\frac{dC}{dt} = \frac{DA\,(Cs-C)}{Vh} \qquad (1.1)$$

Where: *dC/dt*: dissolution rate of the drug particles (mol/ mL*s)

D: diffusion coefficient of the drug (cm^2/s)

A: surface area of the drug particles (cm^2)

V: Volume of the dissolution medium (cm^3)

h: thickness of the diffusion layer (cm)

C_s*:* saturation solubility of the drug (mol/mL)

C: concentration at time t (mol/mL)

The capability of nanocrystalline formulations to enhance the dissolution of a poorly soluble drug in vitro is easy to demonstrate, but in vivo several problems can occur. For

example the drug can precipitate before it is absorbed due to changes in pH or in the ionic environment. (1) The most common problem with nanocrystals is the long-term stability. During storage and processing they tend to aggregate attributed to the decrease in particle size, which creates surfaces with higher energy. Therefore, it is necessary to add stabilizers. Generally stabilizers with amphiphilic properties are used. Since most of the poorly soluble drugs are hydrophobic, amphiphilic stabilizers enhance the wetting of nanocrystals through hydrophilic-hydrophobic interactions. (1), (18) Electrostatic stabilization and steric stabilization are the two primary mechanisms in stabilization of nanocrystalline formulations. Often ionic surfactants are used as electrostatic stabilizers. The major limitation of this technique is the susceptibility of the stabilization effect to environmental factors such as pH changes. Non-ionic surfactants and polymers are used for steric stabilization. They form a physical barrier around the particles to prevent aggregation. Combinations of different stabilizers, especially the combination of a non-ionic stabilizer with an ionic stabilizer, can be advantageous for the long-term stability of nanocrystals. Stabilizers used during this master thesis are Soluplus® (SP) and HPMC. (1), (18), (20)

To produce nanocrystals, both bottom-up or top-down methods can be used. The bottom-up techniques are based on predissolution of molecules in an organic solvent. Then particles are formed by precipitation. The top-down techniques reduce the particle size. The starting point is bulk material and particles with a size around 100 nm can be achieved. Both methods have advantages and disadvantages. With bottom-up methods, controlling of the particle growth can be an issue. On the other hand, very small particles can be achieved. Top-down methods are easy and fast to perform, but require a lot of energy. (18), (21)

The bottom-up processes include antisolvent precipitation and liquid atomization-based methods, such as spay drying and electrospray atomization. Antisolvent precipitation can be performed by dissolving the drug substance and stabilizer in a solvent. After adding the antisolvent, the drug will precipitate. In spray drying, the liquid is atomized into a spray of fine droplets in the drying chamber. The volatile phase evaporates and dry particles are formed. (3), (18) Electrospray atomization is a technique whereby a solution is forced through a needle. Due to the electric potential applied on the tip of the nozzle, charged droplets are formed.

The electric charge generates then electrostatic forces inside the droplets. Once the Coulomb force can overcome the surface tension, the droplets will explode into smaller droplets. (18), (22), (23)

The top-down methods used to produce nanocrystals are milling and high-pressure homogenization (HPH). In the pearl or bead milling technique, the solid drug is dispersed in a medium containing a stabilizer. The stabilizer has the purpose to prevent particle growth during milling. The milling material used are beads made of glass or ceramic. Particles of different size can be achieved by altering parameters, e.g. the size of milling pearls, temperature and milling speed. (18) (24) In high-pressure homogenization, particle size reduction is caused by applying forces under high pressure. HPH techniques include piston gap homogenization and microfluidization, also known as jet-stream homogenization. (24), (25)

1.2.3. Lipid formulations

A third possibility to improve dissolution behaviour of poorly water-soluble, hydrophobic drugs that has gained a lot of interest in recent years are lipid formulations. Lipid formulations are composed of a drug dissolved in a carrier system that consists of a mixture of excipients with different physicochemical properties such as mono- and diglycerides, triglycerides, lipophilic or hydrophilic surfactants and cosolvents. (3), (26) The Lipid Formulation Classification System has been introduced in 2000 the help interpret in vivo studies and subsequently to optimise the formulation for a specific drug. (27) In general, the drug candidates for lipid formulations must be lipophilic.

The limiting step of the bioavailability of poorly water-soluble drugs is the slow dissolution process. The mechanism of lipid formulations is based on the avoidance of this step, preferably by keeping the drug in the dissolved state during its transit in the gastrointestinal tract. An example of such a lipid formulation are self-emulsifying drug delivery systems (SEDDS). SEDDS form fine oil in water emulsions when they are dispersed into aqueous milieu under agitation of the gastro-intestinal tract. The most commonly used dosage form for oral delivery of SEDDS are soft or hard gelatin capsules. (3)

1.3. ANALYTICAL TECHNIQUES

In this project three complementary techniques were used for characterization of the crystalline and amorphous form of indomethacin. Infrared (IR) spectroscopy and Raman spectroscopy are both vibrational spectroscopic techniques. They provide spectra that can be considered as fingerprints of the molecular and even solid state structure of an analyte. The third technique is differential scanning calorimetry (DSC), which is a thermal method often used to study crystalline polymorphs and the amorphous form. Another technique that can be used to characterize polymorphic forms and crystallinity is X-ray powder diffraction.

1.3.1. Infrared spectroscopy

Infrared spectroscopy is a spectroscopic technique used for the identification of compounds. The IR region can be divided in three areas: near IR, mid IR and far IR. The mid IR region, with an electromagnetic radiation between 4000 cm^{-1} and 400 cm^{-1}, is the most frequently used. The electromagnetic radiation is passed through a sample. Absorption of light occurs when the frequency of IR radiation equals the vibrational frequency of a bond. Molecules then change from the ground vibrational energy state (v=0) to an excited energy state (v=1). (28) This change corresponds with an increase of energy defined by equation (1.2) (29). The interaction of energy with materials then results in a spectrum, represented by wavenumber (cm^{-1}) on the x-axis and absorbance on the ordinate. (28)

$$\Delta E = hv \qquad (1.2)$$

Where: E: Energy of the level

h: Planck's constant ($6.625*10^{-34}$ J*s)

v: frequency of the absorbed light (cm^{-1})

IR spectroscopy is one of the most frequently used spectroscopic techniques in pharmaceutics. Different kind of samples, including solid and liquid samples can be analysed. It is also a rather fast and non-destructive method and little sample preparation is required. One of the limitations of this technique is the fact that the molecule has to be active in the IR region. In order for absorption to occur the molecule requires a dipole moment or a net change in dipole moment during the vibration of a molecule. Molecules with a dipole moment

will exhibit IR absorption bands. (28) Molecules that are symmetric, like CO_2, do not have a dipole moment. However, when the molecule induces a change in dipole moment during vibration, absorption will occur. The larger the change in dipole moment, the higher the intensity of the absorption band. (30)

Two kind of spectrometers can be used to detect changes in absorbance, namely dispersive spectrometers and Fourier transform spectrometers. In recent years, Fourier transform infrared spectroscopy (FTIR), illustrated in figure 1.3, has gained more attention due to its superior sensitivity and speed. The basic components of the spectrometer are the radiation source, an interferometer and a detector. The basic Michelson interferometer is the most commonly used interferometer. It consists of a fixed mirror, a moving mirror and a beam-splitter. The latter splits the radiation from the source equally. Half of the IR beam is focused on the fixed mirror, the other half is focused on the moving mirror. After reflection of the two beams, they recombine at the beam-splitter. Depending on the location of the moving mirror, differences in the optic paths are generated. The two beams interfere constructively and therefore lead to a maximum detector response when they are in phase with each other. The beams interfere destructively when they are out of phase with each other. (28), (30)

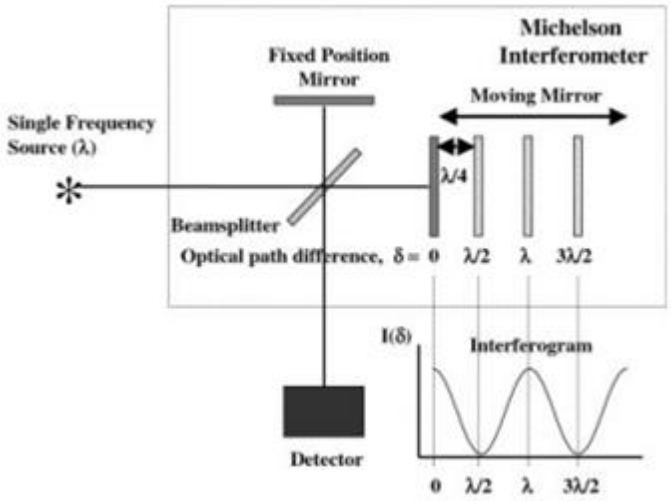

Figure 1.2 – Schematic representation of the operation of a FTIR spectrometer equipped with a Michelson interferometer. The interferogram obtained from a monochromatic source is illustrated (John 2006 (30)).

1.3.2. Raman spectroscopy

Raman spectroscopy is, besides IR spectroscopy, one of the two main spectroscopic methods used for vibrational analysis. Both techniques are used to provide a fingerprint of molecules by generating spectra. The main difference is that IR spectroscopy is based on absorption of photons with a frequency equal to the vibrational frequency of functional groups, whereas Raman spectroscopy is based on inelastic scattering of monochromatic light. (28) Also different selection rules apply to these techniques, meaning the techniques are complementary. As mentioned earlier, a molecule is IR-active when there is a change in the dipole moment during vibration. On the other hand, only molecules that exhibit a change in polarizability during the vibration are Raman-active. (30)

When a sample is irradiated with monochromatic light, the incident photons are scattered. In the case of elastic or Rayleigh scattering, the molecules transition from the ground state to a virtual excited state and relax back to the original vibrational state. This means they are scattered without exchange of energy. When the molecule relaxes to another state than the ground state, this is known as inelastic or Raman scattering. The photon can be shifted to lower energy (Stokes shift) or to higher energy (anti-Stokes shift), as illustrated in figure 1.3. The difference in energy between incident and scattered photons associated with the transitions between these vibrational energy states is reflected as a change in wavelength (or frequency) in the scattered photon. (28), (30), (31), (32)

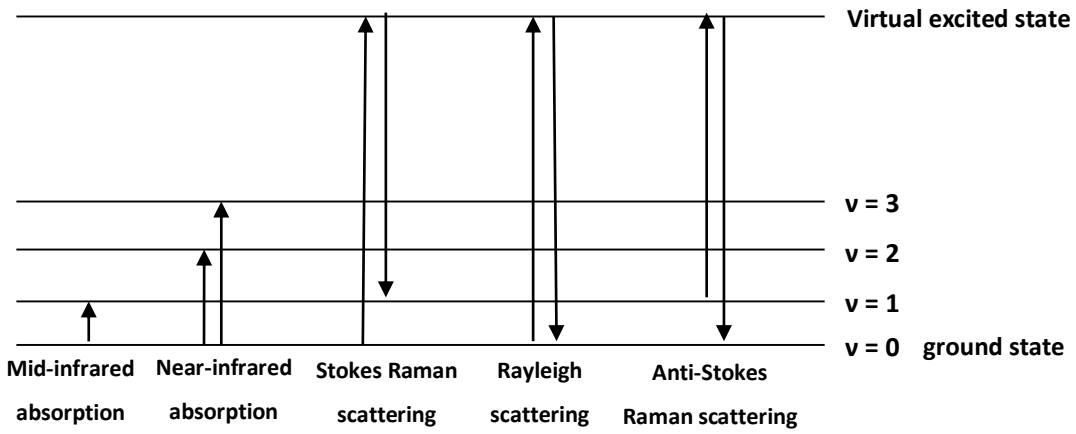

Figure 1.3 - Schematic illustration showing the vibrational energy level transitions of IR absorption and Rayleigh and Raman scattering (John 2006 (30)).

1.3.3. Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a thermal analytical technique, often used for the identification and characterization of polymorphic forms. It measures the heat flow required to maintain the sample and reference at the same temperature, i.e. the enthalpy change is measured. One of the interesting parts of DSC is the fact that only a few milligrams (2-10 mg) of the sample are needed. On the other hand, the sample cannot be reused again because DSC is a destructive technique. (33)

The DSC results are shown in a thermogram whereby heat flow is plotted against temperature. The area under the curve represents the energy required to compensate for the thermal events of the sample. Some instruments represent endothermic processes (melting) as downward curves and exothermic processes (crystallisation) as upwards peaks, while other instruments show it in the opposite way. (33) DSC can be used to evaluate different thermal events including melting, solid-state transitions, crystallization and glass transitions. (34) DSC may therefore be used to differentiate between polymorphs according to their melting point or to examine the transformation of metastable systems. It is essential though to confirm the results of DSC with other characterization techniques such as Raman spectroscopy, IR spectroscopy and X-ray diffraction. (33)

1.3.4. Polarized light microscopy

Polarized light microscopy (PLM) was used during this study to evaluate crystallization behaviour. A polarized light microscope is a microscope that consist of two polarized filters, the polarizer (placed below the sample) and the analyser (placed above the sample). In non-polarized light, the electric field is oscillating in all directions perpendicular to the direction of propagation of light. By passing non-polarized light through the polarizer, a plane of polarized light is produced. If the analyser is aligned perpendicularly to the vibration direction of the polarizer, thus no light is transmitted. (35) Isotropic specimens will not be seen, because they will occur as black spots against a black background, whereas anisotropic specimens will be visible due to double refraction or birefringent. (35), (36) The phenomenon whereby incident ray splits into two different rays propagating in different directions is called birefringence. Many crystals are optically anisotropic and thus exhibit birefringence. This is due to the fact

that crystals consist of molecules arranged in a highly ordered structure, namely a crystal lattice. Since amorphous materials consist of disordered molecules, they are usually isotropic and don't exhibit birefringence.

1.3.5. X-ray powder diffraction

X-ray powder diffraction (XRPD) is another method to investigate polymorphic forms and crystallinity. Practically all crystalline substances exhibit specific x-ray diffraction patterns, because they all possess unique three-dimensional lattice plane spacings. As a result, the incident monochromatic x-ray beam on the crystal planes, is constructively scattered at different angles. The diffraction patterns can be represented by graphs with the scattering angle on the x-axis and the intensity on the y-axis. In this way, XRPD is a valuable analytical technique for the identification of crystalline substances. (31) Amorphous forms will not exhibit specific diffraction peaks in XRPD, because of the absence of periodically arranged lattice planes. (37) Instead, an amorphous 'halo' is observed.

2. OBJECTIVES

The overall aim of this thesis project was to study the level and maintenance of supersaturated solutions (with respect to the thermodynamic solubility) of indomethacin prepared with the amorphous form of the drug, in different environments.

In order to achieve this overall aim, physical characterisation of the formulations was performed. The techniques used included infrared spectroscopy, Raman spectroscopy and differential scanning calorimetry. For establishing the solubility protocol, two different separation techniques, filtration and centrifugation, were studied.

After the first two steps, the effect of two different polymers (HPMC and Soluplus®) on the level and maintenance of supersaturated solutions was investigated.
The link between supersaturation and crystallisation behaviour was studied.

To better mimic in vivo conditions with a rapidly dissolving amorphous drug reaching supersaturation, an artificial membrane was used to create an open environment in which the drug is able to permeate from the system. The effect of this environment on maintaining of supersaturation and permeation was investigated.

3. MATERIALS AND METHODS

3.1. MATERIALS

The γ form of indomethacin was obtained from Orion Pharma (Helsinki, Finland). Potassium phthalate monobasic (Sigma Aldrich, Steinheim, Germany), sodium hydroxide (Eka Nobel, Bohus, Sweden), HPMC E5 (Dow chemical Company, Michigan, USA), Soluplus® (BASF, Ludwigshafen, Germany) and ethanol 99.5% (Altia Oy, Rajamäki, Finland) were used as received.

3.2. METHODS

3.2.1. Preparation of amorphous indomethacin

Amorphous indomethacin was prepared by spreading a thin layer of the crystalline powder on an aluminium pan. Subsequently, the aluminium pan was heated to 165 °C on a hot plate. The melt was then cooled down to room temperature by placing the pan on a cold metal surface.

3.2.2. Analytical techniques

3.2.2.1. Infrared spectroscopy

Infrared spectroscopy was performed with a Bruker Vertex 70 FTIR spectrometer (Bruker Optik, Ettlingen, Germany). An ATR accessory with a single reflection diamond crystal was used. To increase the contact with the ATR crystal, the solid samples were pressed onto the crystal using a clamp. The samples were measured within a spectral range from 1000 cm^{-1} to 1800 cm^{-1} and with a resolution of 4 cm^{-1}. The final spectrum was the mean of 64 scans. To process the data and present the spectra, OPUS software (v. 5.0, Bruker Optik, Ettlingen, Germany) was used. FTIR spectroscopy was performed for both crystalline and amorphous indomethacin.

3.2.2.2. Raman spectroscopy

Raman spectroscopy was performed using a Raman RXNI-785 spectrometer (Kaiser Optical Systems, Ann Arbor, MI, USA). The Raman spectrometer is composed of a laser source with a wavelength of 785 nm and has a laser spot size of 6 mm. Furthermore, the Raman

spectrometer consists of a probe with a nominal focal length of 250 mm and a silicon charge coupled device (CCD) detector. At the beginning of the measurement, a Raman shift calibration was conducted to ensure accurate results. This was accomplished by using a suitable reference, namely cyclohexane. For the measurements of the samples, glass vials were used as sample holders. The spectra were recorded using an exposure time of 1 s and 3 for the settings of accumulations. HoloGRAMS software version 4.1 (Kaiser Optical Systems, Inc., Ann Arbor, MI, USA) was used to collect the spectra.

3.2.2.3. Differential scanning calorimetry

A DSC 823 (Mettler Toledo Inc., Columbus, USA) was used to perform thermal analysis. A few milligrams (2-10 mg) of the sample was placed in an aluminium pan. The sample was spread evenly over the bottom of the pan. The sample pan was then covered with a lid, hermetically sealed with the crimper, placed in the sample holder of the DSC and heated at a rate of 10 °C/min. An empty aluminium pan was used as a reference.

As mentioned earlier, indomethacin is a drug with different polymorphic forms. The α form has a melting point onset of 154-155 °C and the γ form has a melting point onset at 161 °C. (2) Therefore a temperature program from 25 °C to 185 °C was used. The test was carried out under a nitrogen gas flow of 50 mL/min. Thermal events were visualised in a thermogram and analysed with STARe software (Mettler Toledo Inc., Columbus, USA).

3.2.2.4. Polarized light microscopy

During two permeation tests samples were taken and analysed with polarized light microscopy to study crystallization behaviour. The first sample was taken during the permeation test performed after 1h of stirring of amorphous IND. The second sample was taken during the permeation test performed immediately after sample preparation. The samples were taken after 45 min from the donor compartment and centrifuged for 6 minutes at 13.000 rpm. The supernatant was removed and a small amount of the remaining solid was placed on a glass slide. The solids were analysed to see if crystallization had occurred using the Leica DMLB microscope (Leica Microsystems, Wetzlar, Germany) at a magnification of 5x or 10x.

3.2.3. Solubility tests

The solubility tests were performed by a traditional shake flask method based on the article "*Polymer incorporation method affects the physical stability of amorphous indomethacin in aqueous suspension*". (38) Solubility tests were performed for both the γ form and the amorphous form of indomethacin (IND). First reference suspensions were prepared in pH 5.5 phthalate buffer medium. These reference suspensions included (I) crystalline IND (10 mg/mL) without polymer addition, (II) crystalline IND (10 mg/mL) with predissolved HPMC and (III) crystalline IND (10 mg/mL) with predissolved Soluplus®, prepared at a drug-polymer ratio of 1:1 (w/w). These data were used as a reference to help interpret the data of (IV) pure amorphous IND (10 mg/mL) without polymer addition, (V) amorphous IND with predissolved HPMC and (VI) amorphous IND with predissolved Soluplus® (prepared at a drug-polymer ratio of 1:1 (w/w)). The suspensions were stirred with a magnetic stirring bar of 3 cm at 250 rpm. From each suspension, 3 mL sample was taken at different time points (5, 15, 30, 45, 60, 120 and 360 min). Each time a sample was taken, 3 mL of phthalate buffer was added to the suspension to maintain the same volume. Each sample was then immediately filtered using filters with a polyethersulfone membrane and a pore size of 0.2 µm (VWR International, USA). The obtained sample was then diluted with pH 5.5 phthalate buffer and analysed by UV spectrometry (UV- 1600PC, VRW, China) at a wavelength of 320 nm. (38) All solubility tests were performed at room temperature. For each suspension, the measurements were carried out in duplicate.

In order to investigate the influence of different parameters and to obtain results comparable to those in the reference article, the experiments were repeated a second time but with different parameters. First, instead of 250 rpm, a higher rotation speed of 400 rpm was applied. Furthermore a larger volume of buffer was used, but the concentration was kept the same and no fresh buffer was added after sampling. All solubility tests were performed at room temperature. For each suspension, the measurements were carried out in duplicate.

Additionally, another series of experiments was carried out to examine the influence of different separation methods on the drug concentration-time profile. During the initial solubility test, filtration was used to separate solids and liquids. These experiments were then

repeated using centrifugation instead of filtration at different speeds (2200 rpm, 3200 rpm, 13000 rpm) during 2 minutes. The suspension that was used to evaluate the influence of various separation methods was the crystalline form of IND (10 mg/mL) with predissolved HPMC (prepared at a drug-polymer ratio of 1:1 (w/w)).

3.2.4. Combined solubility and permeation tests

Solutions were prepared in pH 5.5 phthalate buffer, using the maximum drug concentrations as obtained during the solubility tests. These solutions included (I) γ form of IND, (II) amorphous form of IND, (III) γ form of IND and HPMC, (IV) amorphous form of IND and HPMC, (V) γ form of IND and Soluplus®, (VI) amorphous form of IND and Soluplus®. The in vitro combined solubility and permeation studies were performed using Franz diffusion cells (Crown glass co INC, New Jersey). The donor compartment contained the sample and the acceptor compartment contained the collection medium, in this case pH 5.5 phthalate buffer. A polyvinylidene fluoride, hydrophilic membrane with a pore size of 0.22 µm (Merck KGaA, Darmstadt, Germany) was placed between the two compartments. In addition, a rubber O-ring was placed around the membrane to prevent leaking. The cells, membrane and O-ring were then tightened together by a cell clamp that was placed around them. The contents of both compartments were stirred using magnetic stirring bars of 2 mm. An aliquot of both compartments was collected at different time points (5, 15, 30, 45 and 60 min). Each time a sample was taken, fresh phthalate buffer was added. These aliquots were then diluted and analysed by UV spectrometry (Agilent technologies, USA) at a wavelength of 320 nm. All experiments were carried out at room temperature.

Studies were performed:

1. after 1h of stirring using supersaturated concentrations obtained during the solubility tests for amorphous IND, amorphous IND with predissolved HPMC and amorphous IND with predissolved Soluplus®. All measurements were carried out in duplicate.
2. immediately after sample preparation using concentrations of 5 mg/mL for amorphous IND, amorphous IND with predissolved HPMC and amorphous IND with predissolved Soluplus® . All measurements were carried out in triplicate.

3. after 1h of stirring using concentrations of 5 mg/mL for amorphous IND, amorphous IND with predissolved HPMC and amorphous IND with predissolved Soluplus® . All measurements were carried out in duplicate.

4. RESULTS AND DISCUSSION

4.1. SOLID STATE CHARACTERIZATION

Different techniques were used to characterize crystalline and amorphous indomethacin, including infrared spectroscopy, Raman spectroscopy and differential scanning calorimetry.

4.1.1. Vibrational spectroscopy

Since indomethacin exists as different polymorphic forms, solvates and in the amorphous form, (39) physical characterization of crystalline and amorphous indomethacin was performed, using IR spectroscopy and Raman spectroscopy. IR spectra of crystalline and amorphous indomethacin are shown in figure 4.1 a and b respectively. Raman spectra of crystalline and amorphous indomethacin are shown in figure 4.2 a and b respectively. The spectra obtained with vibrational spectroscopy were later used as a reference to establish crystallisation behaviour.

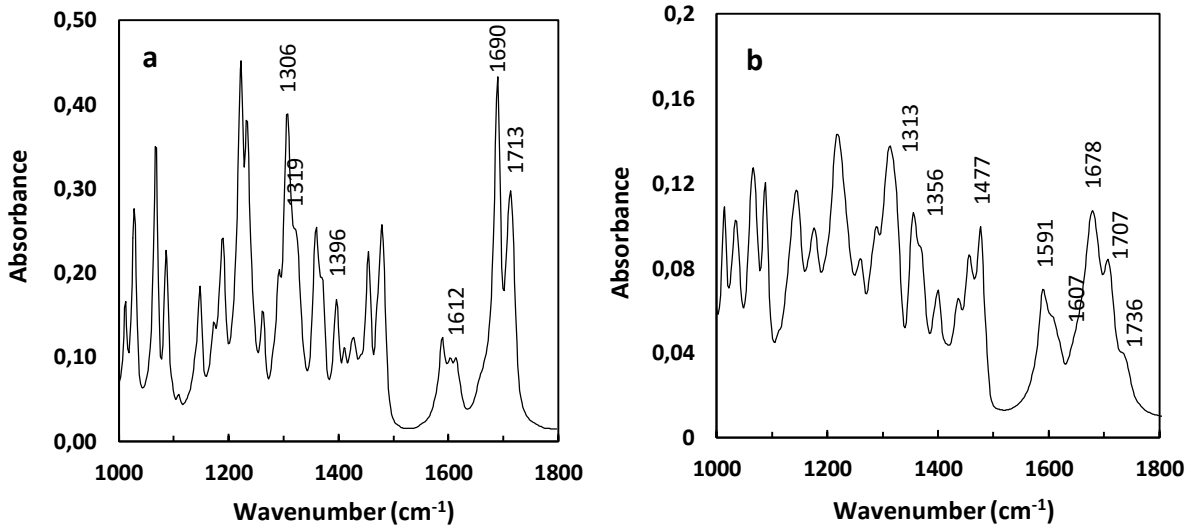

Figure 4.1. – IR spectra of (a) crystalline and (b) amorphous indomethacin over the spectral region 1000 - 1800 cm^{-1}.

When analyzing the IR and Raman spectra of crystalline and amorphous indomethacin, certain differences can be observed. The spectra of the amorphous form contain peaks that are less intense and broader then the γ-crystalline form. This can be attributed to the variation

in molecular orientation and arrangement of amorphous form of indomethacin (compared with the ordered γ-crystalline form). Furthermore, some peaks of the spectra of the amorphous form are shifted compared to those of the crystalline form. (4), (39), (40) As can be noticed in figure 4.2b, a high baseline occurs over which the Raman signal is superimposed, which is due to fluorescence.

For both crystalline and amorphous indomethacin, OH vibrations of the carboxylic acid group are observed between 3400 and 2500 cm^{-1} in the infrared spectra, illustrated in figure 7.1 a and b in the appendix. Peaks associated with CH stretching occur around 3000 cm^{-1}, but are in the infrared spectra predominated by the OH stretching bands. (39)

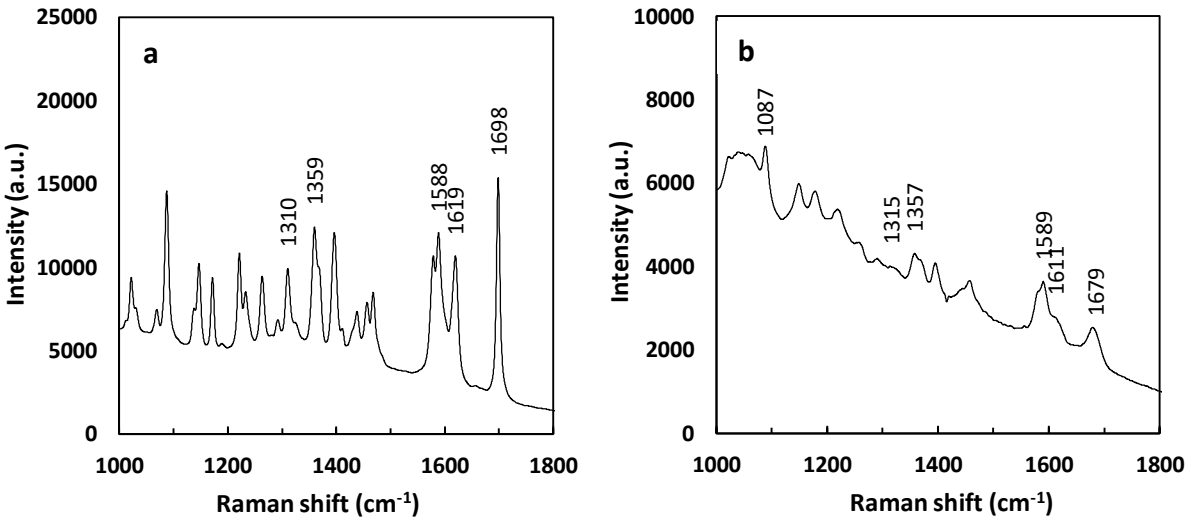

Figure 4.2 – Raman spectra of crystalline (a) and amorphous (b) indomethacin over the spectral region 1000 - 1800 cm^{-1}.

Indomethacin contains two hydrogen bond acceptors, the acid and benzoyl carbonyl groups. The carbonyl stretch is typically observed in the region between 1750 and 1600 cm^{-1}. The most intense peak, associated with the benzoyl C=O stretch of crystalline indomethacin, occurs at 1690 cm^{-1} and 1698 cm^{-1} in the IR spectrum (figure 4.1a) and the Raman spectrum (figure 4.2a), respectively. The peak attributed to the asymmetric acid C=O stretch can be noticed at 1713 cm^{-1} in the infrared spectrum of crystalline indomethacin. No corresponding peak was observed in the Raman spectrum of crystalline indomethacin. (38), (39)

The spectra of amorphous indomethacin are similar to the spectra observed for the γ form of indomethacin. The peaks of the benzoyl carbonyl group are shifted to 1678 cm^{-1} and 1679 cm^{-1} in the IR spectrum (figure 4.1b) and the Raman spectrum (figure 4.2b), respectively. The asymmetric acid C=O stretch was observed in the infrared at 1707 cm^{-1}. No corresponding symmetric acid carbonyl stretch was seen in the Raman spectrum. The shoulder appearing at 1736 cm^{-1} in the infrared spectrum can be assigned to non H-bonded acid stretching. (4), (39) A summary of the peak assignments can be found in Table 4.1.

Table 4.1 – Vibrational peak assignment of the infrared and Raman spectra from crystalline and amorphous indomethacin.

Compound	Wavenumber (cm^{-1})	Raman shift (cm^{-1})	Vibrational assignment
γ-indomethacin	1690 s	1698 s	Benzoyl C=O stretching
	1713 s		Asymmetric acid C=O stretching
Amorphous indomethacin	1678 s	1679 s	Benzoyl C=O stretching
	1707 s		Asymmetric acid C=O stretching
	1736 sh		Non hydrogen acid C=O stretching

S = strong, sh = shoulder

4.1.2. Differential scanning calorimetry

Thermal analysis was performed to characterize crystalline and amorphous indomethacin. The α form of indomethacin has a melting point onset at 154-155 °C and the γ form has a melting point onset at 161 °C. (2) Amorphous indomethacin has a T_g, normally within a range of 42-50 °C. (38) The DSC patterns of crystalline and amorphous indomethacin are shown in figure 4.3 a and b, respectively.

In figure 4.3a, the endothermic peak represents the melting of the γ form of indomethacin at 160.56 °C. As can be noticed in figure 4.3b the glass transition occurs at 47.91 °C. The exothermic peak indicates crystallization, with a peak crystallization temperature (T_c) of 119.72 °C. Two characteristic endothermic melting peaks can be observed at 155.06 °C and 160.82 °C, confirming that crystallization of the amorphous form had occurred earlier. The

peak at 155.06 °C is associated with the α form of indomethacin and the peak at 160.82 °C can be attributed to the γ form.

Looking more into detail to the shift of the baseline at the glass transition temperature, it can be noticed that relaxation of amorphous material has occurred. This may be due to an excessive period of time between the sample preparation of the amorphous form and the actual measurements with DSC. Therefore another thermal analysis of amorphous indomethacin was performed, immediately after sample preparation. The T_g and T_c can be found at 44.47 °C and 127.93 °C respectively, as illustrated in figure 7.2 in the appendix. The peaks at 155.03 °C and 159.30 °C represent the α and the γ form respectively. No relaxation was observed.

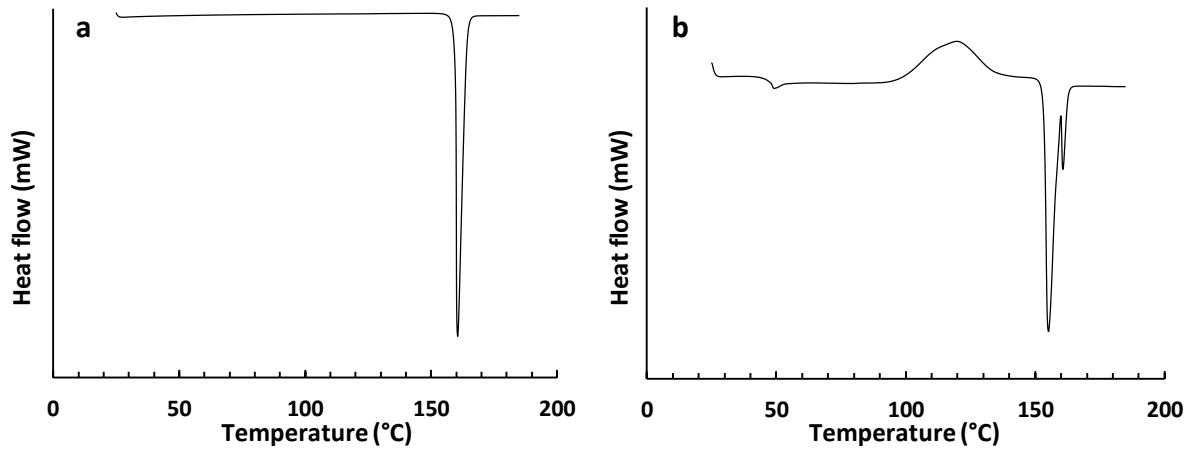

Figure 4.3 – DSC thermograms of (a) crystalline and (b) amorphous indomethacin. A heating rate of 10 °C/min and a temperature program of 25 – 185°C was used.

4.2. SOLUBILITY TESTS

4.2.1. Effect of polymer addition

The solubility tests were performed based on the shake flask technique and the article "*Polymer incorporation method affects the physical stability of amorphous indomethacin in aqueous suspension*". (38) Also different separation methods (filtration and centrifugation) were investigated for establishing the solubility protocol. Initially, the concentration-time profile of the γ form of IND without addition of polymers at pH 5.5 was evaluated. These

results were used as a reference to help interpret the data generated with the amorphous form of IND.

The results obtained for the solubility tests performed at a rotation speed of 250 rpm are illustrated in figure 7.3 in the appendix. Supersaturated solution (~132 µg/mL) with respect to the thermodynamic solubility of the crystalline form was generated within 5 minutes for the pure amorphous IND suspension (figure 7.3b). This was then immediately followed by a decrease in concentration, indicating the onset of crystallization.

The suspensions with predissolved HPMC became supersaturated (with respect to the crystalline form in buffer) within 5 minutes. The maximum drug concentration (C_{max}) generated was around 472 µg/mL, which was considerably higher than the C_{max} generated with the suspensions containing no predissolved polymer. Supersaturation was maintained even after 1h, but was then followed by a reduction in dissolved concentration due to crystallization.

Also with addition of the polymer Soluplus®, high concentration values after fast dissolution were generated within 5 minutes and the concentrations continued increasing until 120 min. The maximum drug concentration obtained (~864 µg/mL) was 6-7 times higher than the one obtained with amorphous IND suspensions without polymers, and approximately 2 times higher than the C_{max} generated with HPMC. These results can be explained by the fact that besides the common mechanism of action of both polymers (acting as a polymeric carrier), Soluplus® has additional mechanisms to enhance solubility, for example formation of micelles and improved wetting. Supposedly these micelles incorporated a large part of the drug molecules. The concentration of Soluplus® used during this study was above the critical micelle concentration (CMC), which is 0.0007% (w/v). (14)

The difference in maintaining supersaturated solutions between HPMC and Soluplus® can depend on a number of factors. Presumably one of the key factors is the strength of the interaction between the drug and the polymer. Another possibility could be the difference in antiplasticising effect of the polymers. (38)

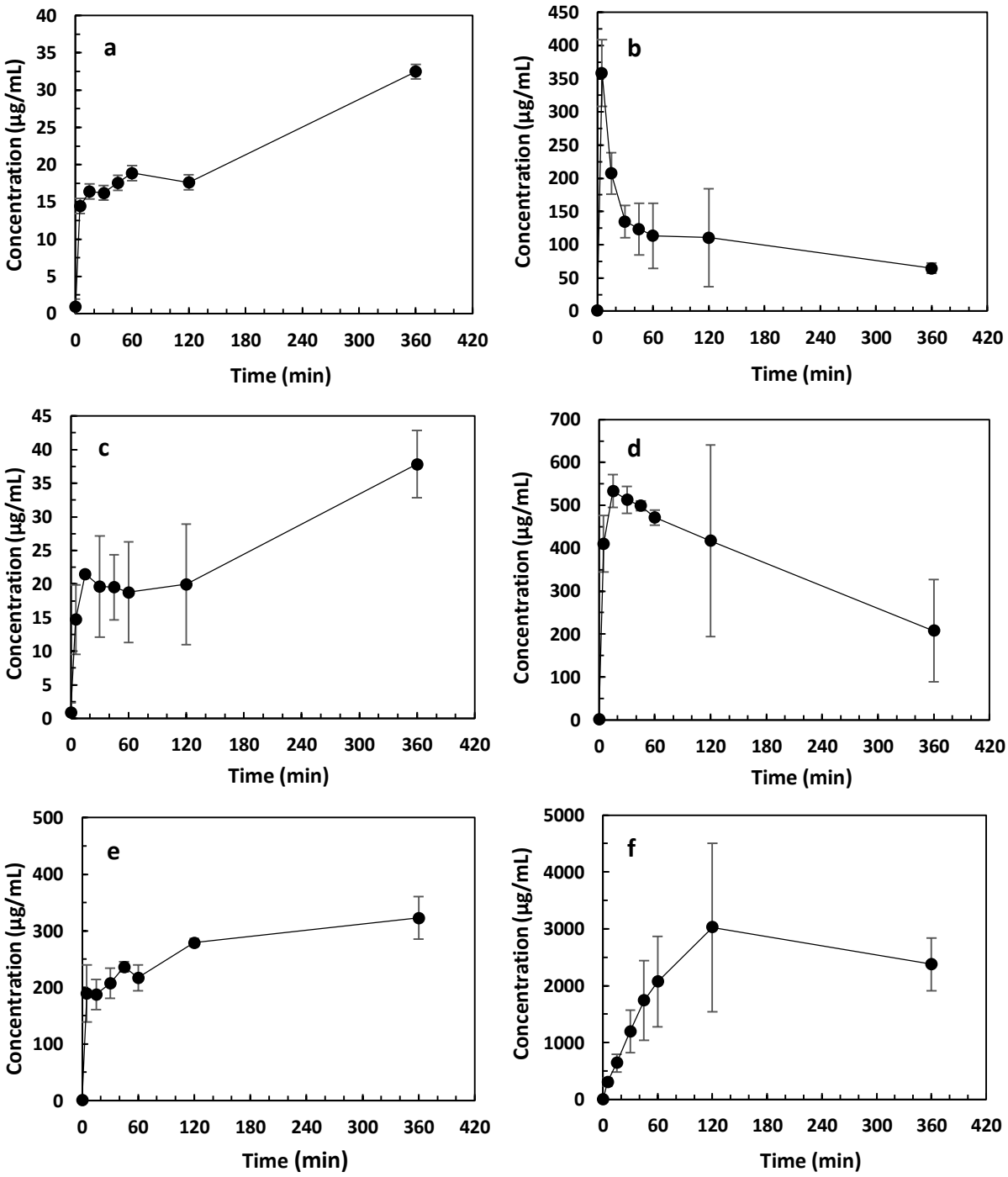

Figure 4.4 – Effect of polymer addition (in a 1:1 ratio drug:polymer (w/w)) on level and maintenance of supersaturated solutions of amorphous IND. The suspensions were stirred at 250 rpm and 25 °C. Concentration-time profiles of crystalline IND without polymer addition (a), amorphous IND without polymer addition (b), crystalline IND with predissolved HPMC (c), amorphous IND with predissolved HPMC (d), crystalline IND with predissolved Soluplus® (e), amorphous IND with predissolved Soluplus® (f). Each bar represents the mean ± SD (n = 2).

To obtain similar results to those in the reference article, the solubility tests were performed a second time with a rotational speed of 400 rpm instead of 250 rpm (figure 4.4). In line with previous results, supersaturated solutions were generated within 5 min for all suspensions and in addition also the maintenance of these supersaturated solutions is comparable. The main difference can be noticed in the maximum drug concentrations that were achieved. The maximum drug concentration obtained with suspensions containing predissolved Soluplus® were approximately 3 times higher for the test performed at 400 rpm (figure 4.4f) compared to the same test performed at 250 rpm (figure 7.3f) and almost 24 times higher than the C_{max} of pure amorphous IND in aqueous suspension stirred at 250 rpm (figure 7.3b). It can be concluded that both polymer addition and the type of added polymer have an influence on the level of supersaturated solutions. (38)

Additional experiments could focus on the impact of the polymer:drug ratio on the level and maintenance of supersaturated solutions (solubility tests with a drug-polymer ratio of 1:3 (w/w)). Presumably the level of supersaturation will be increased and the maintenance will be prolonged. Another approach is to perform these tests at different pH values to improve the correlation with in vivo conditions.

4.2.2. Effect of different separation methods

Another series of solubility tests was carried out to examine the influence of different separation methods on the drug concentration-time profile. During the solubility tests performed earlier, filtration was used to separate solids and liquids. These tests were repeated another time for the suspension containing the γ form of IND with predissolved HPMC, using centrifugation instead of filtration.

Comparing the two methods (figure 4.5), it can be noticed that the shape of the curve is approximately the same for centrifugation and filtration. However, the maximum drug concentrations achieved vary not only for the different separation methods, but also for different rotation speeds used during centrifugation. As illustrated in figure 4.5 (a, b and c), C_{max} decreases with increasing speed of centrifugation. This is most likely due to the fact that with low centrifugation speed, no clear supernatant was obtained. Thus for the tests

performed at a centrifugation speed of 2200 and 3000 rpm, there were presumably still some undissolved particles present in the supernatant which led to higher measured concentrations. For the sample that was centrifuged at 13 000 rpm a clearer supernatant was obtained, but the concentrations remained still higher compared those obtained with filtration. A possible explanation for this phenomenon may be that there were still nanosized particles present in the supernatant, which caused higher absorbance and thus higher measured concentrations. In addition, there could be still some dissolution taking place during centrifugation. On the other hand, there may be adsorption of the drug to the filter during filtration causing lower concentrations.

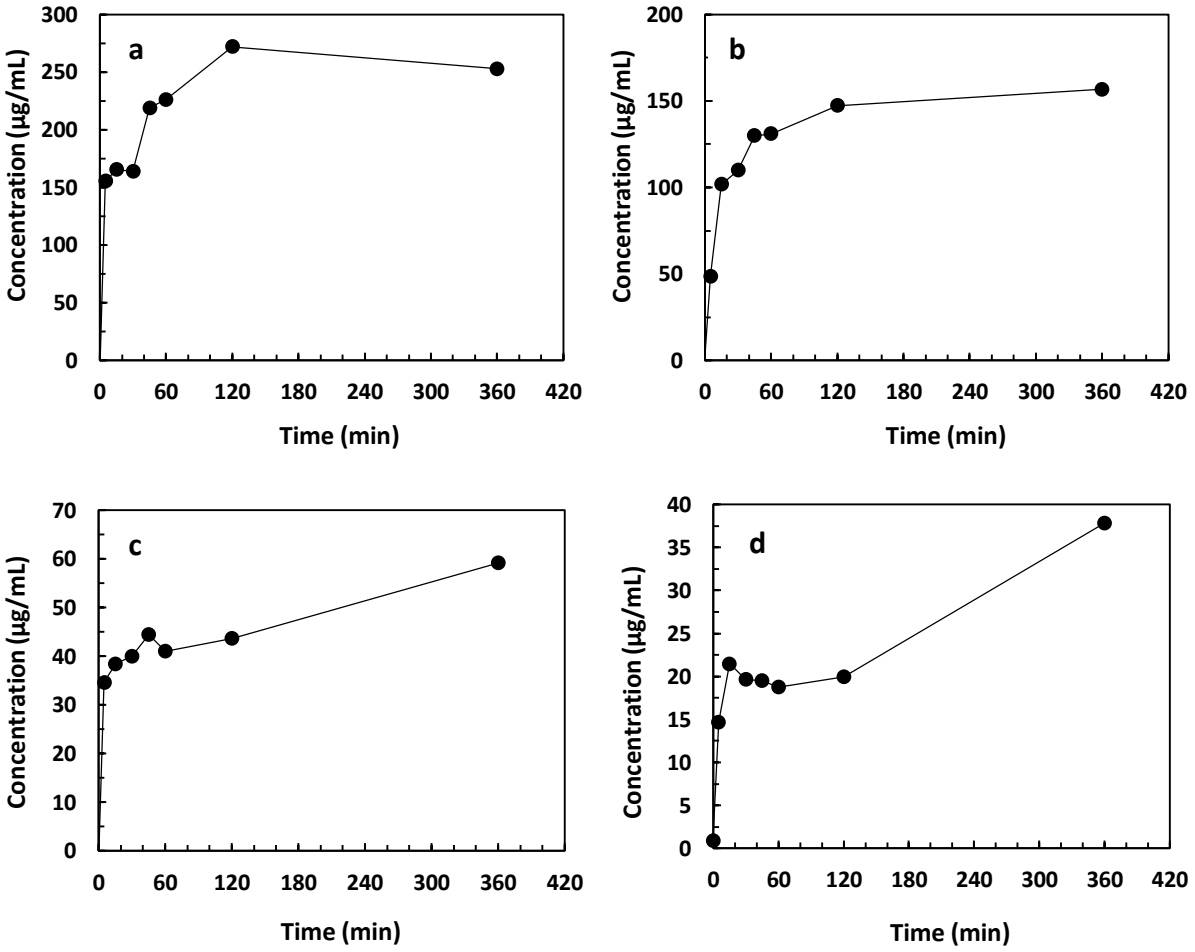

Figure 4.5 – Effect of different separation methods on concentration-time profile: centrifugation at 2200 rpm (a), 3000 rpm (b), 13000 rpm (c) and filtration (d).

4.3. COMBINED SOLUBILITY AND PERMEATION TESTS

The combined solubility and permeation tests were executed three times, each time under different conditions. Initially, the tests were performed using the supersaturated conditions obtained from previous solubility tests (performed at rotation speed of 400 rpm). Unfortunately even after 1h of stirring, no solution could be obtained. Therefore the permeation tests had to be performed with the resulting suspensions. The second series of experiments was carried out after 1h of stirring, using concentrations of 5 mg/mL for both polymer and drug.

As mentioned earlier for the solubility tests performed at 400 rpm crystallization already occurs after 5 minutes and after 30 minutes for the pure amorphous suspension and for the suspension containing amorphous IND with predissolved HPMC, respectively. This means that crystallization has already occurred before the start of the permeation tests. Therefore another series of permeation tests was carried out immediately after sample preparation of the suspensions. Concentrations of 5 mg/mL were used for drug and polymer.

As illustrated in figure 4.6, the concentrations of dissolved IND in the donor compartments decrease for the suspensions containing pure amorphous IND (figure 4.6a), amorphous IND with predissolved HPMC (figure 4.6b) and amorphous IND with predissolved Soluplus (figure 4.6c). However, the concentrations of the acceptor compartment remained around zero for all the suspensions during the whole test. This could be attributed to several reasons. As illustrated in figure 4.6d, the concentration of dissolved amorphous IND decreases. Amorphous indomethacin may have crystallized and therefore have caused a decrease in solubility. The low concentration of IND in the acceptor compartment could also be due to an interaction between IND and the PVDF membrane. Another explanation could be that the polymer and the PVDF membrane interact, hence not allowing the drug-polymer complex to permeate.

Furthermore, it can also be noticed that the concentration of the donor compartment has dropped more rapidly for the suspensions containing predissolved Soluplus®. One of the explanations could be that predissolved Soluplus® showed a stronger increase in

supersaturated concentrations compared to HPMC. This could make the Soluplus® based supersaturated solution more vulnerable for crystallization or precipitation e.g. by the presence of the membrane. If Soluplus® would then interact with the membrane, there would be less dissolved polymer available in the donor compartment as a polymer carrier and for the formation of micelles, causing concentrations of amorphous indomethacin to decrease more rapidly.

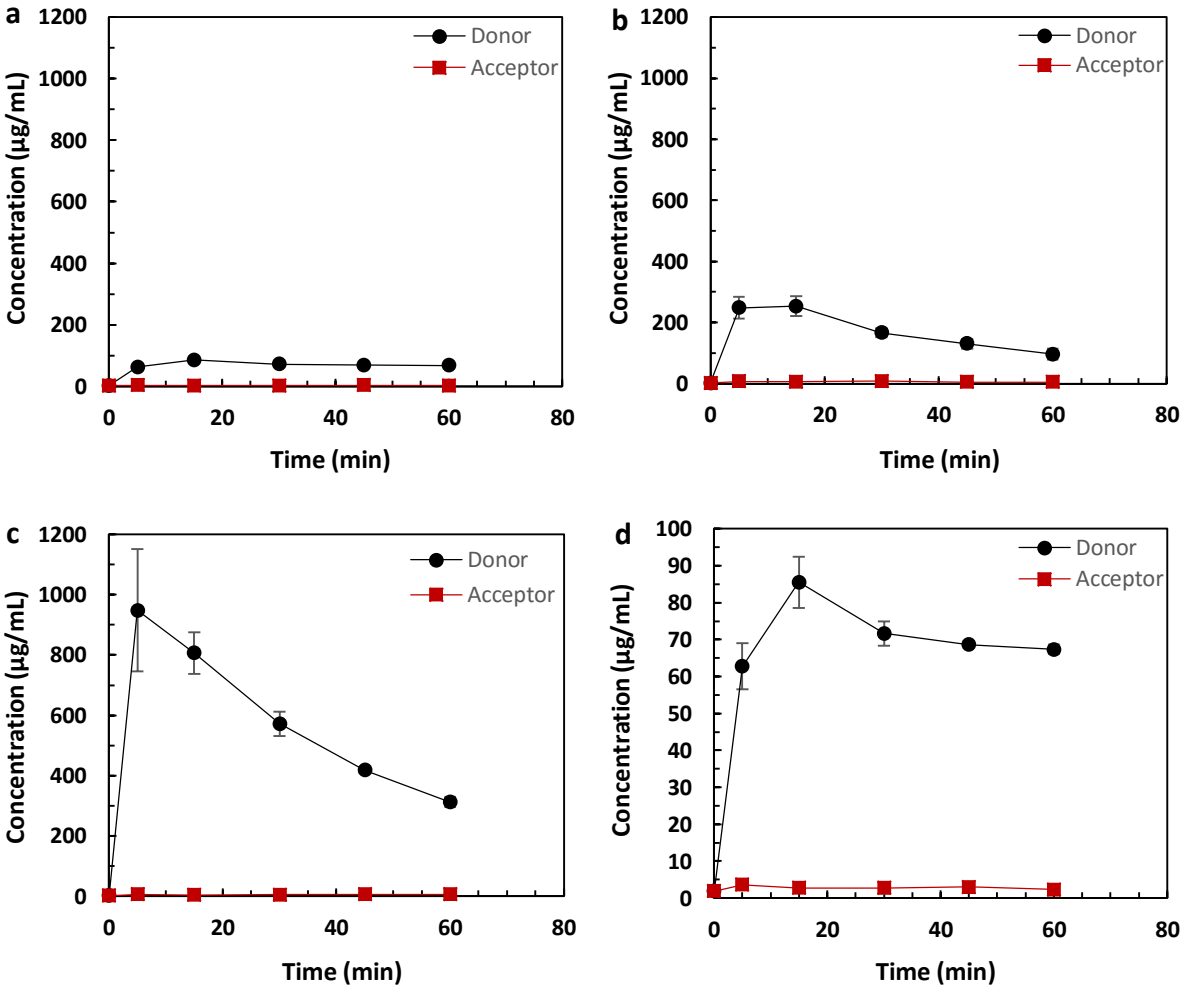

Figure 4.6 – Permeation studies performed after 1h of stirring using supersaturated solutions of (a) pure amorphous IND, (b) amorphous IND with predissolved HPMC, (c) amorphous IND with predissolved Soluplus®, (d) amorphous IND (using another scale). Each bar represents the mean ± SD (n = 2).

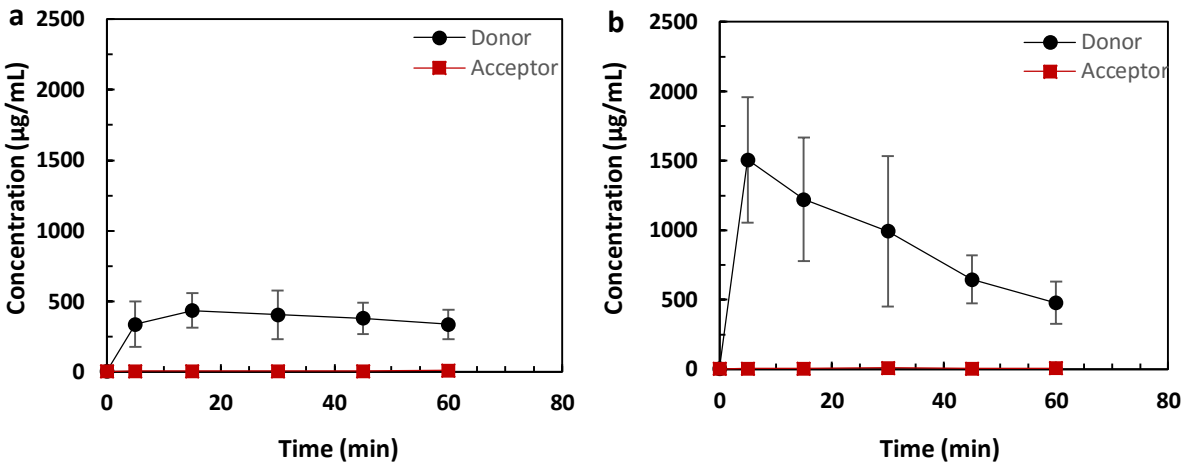

Figure 4.7 – Permeation studies performed after 1h of stirring. Following suspensions (5 mg/mL) were analysed: (a) amorphous IND with predissolved HPMC, (b) amorphous IND with predissolved Soluplus®. Each bar represents the mean ± SD (n = 2).

According to the graphs shown in figure 4.7, a supersaturated solutions is maintained for both polymers. For Soluplus® the concentration is considerably higher than for HPMC but C_{max} is maintained longer for HPMC (30 min) compared to Soluplus® (15 min). Since IND is a BCS class II drug and thus shows good permeability, this could be sufficient to increase bioavailability. This should however be evaluated and eventually confirmed in in vivo experiments (animals, human). With respect to the permeation part of the study, no drug could be measured in the receptor compartment. It has to be further studied if this is due to the experimental setting, making this membrane not a good simulation for the in vivo situation, or if this is due to other reasons (interaction drug/membrane, polymer/membrane …).

When comparing the graphs of the permeation tests (figure 4.7 b and c) with the graphs of the solubility tests (figure 4.4 d and f), a difference in maintenance of supersaturation can be noticed. During the permeation tests with predissolved HPMC, supersaturation is maintained for a longer period of time, whereas for the solubility tests with predissolved HPMC the concentrations start to decrease after 15 min. Another phenomenon can be seen for Soluplus®, for the permeation tests the concentrations dropped after 5 min, whereas for the solubility test with predissolved Soluplus®, supersaturation is maintained up

till 120 min. This suggests that permeation could have an influence on the maintenance of supersaturation and thus on crystallization behaviour.

As illustrated in figure 4.8, similar results were obtained with the exception that the concentrations are much lower compared to the permeation tests that were performed after 1h of stirring. This is of course correlated to a different sample preparation, namely that the tests were performed immediately after preparation of the suspensions.

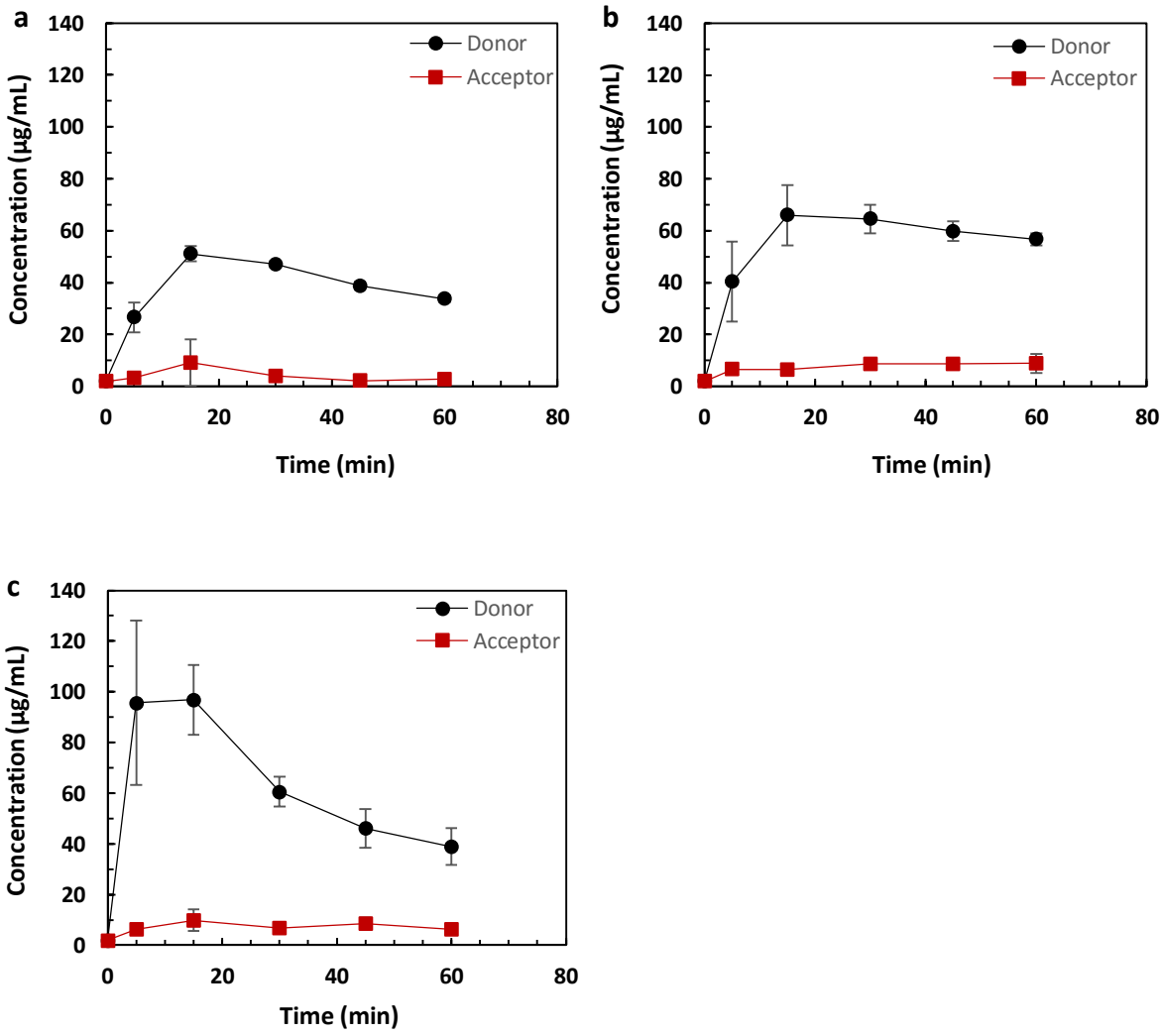

Figure 4.8 – Permeation studies performed immediately after sample preparation. Following suspension (5 mg/mL) were analysed: (a) pure amorphous IND, (b) amorphous IND with predissolved HPMC, (c) amorphous IND with predissolved Soluplus®. Each bar represents the mean ± SD (n = 3).

4.4. EVALUATION OF CRYSTALLIZATION BEHAVIOUR

4.4.1. Polarized light microscopy

The results of PLM are shown in figure 4.9. The PLM images of crystalline and amorphous IND can be seen in figure 4.9 a and b, respectively. For amorphous IND darker spots are visible, no birefringence occurs considering amorphous materials are disordered structures. As illustrated in figure 4.9c, the sample that was stirred for 1h seems to be more birefringent than the amorphous sample, which indicates that crystallization has occurred. Limited sample could be obtained from the permeation test that was performed immediately after sample preparation of amorphous IND. Nevertheless, there are some crystalline particles visible, suggesting crystallization.

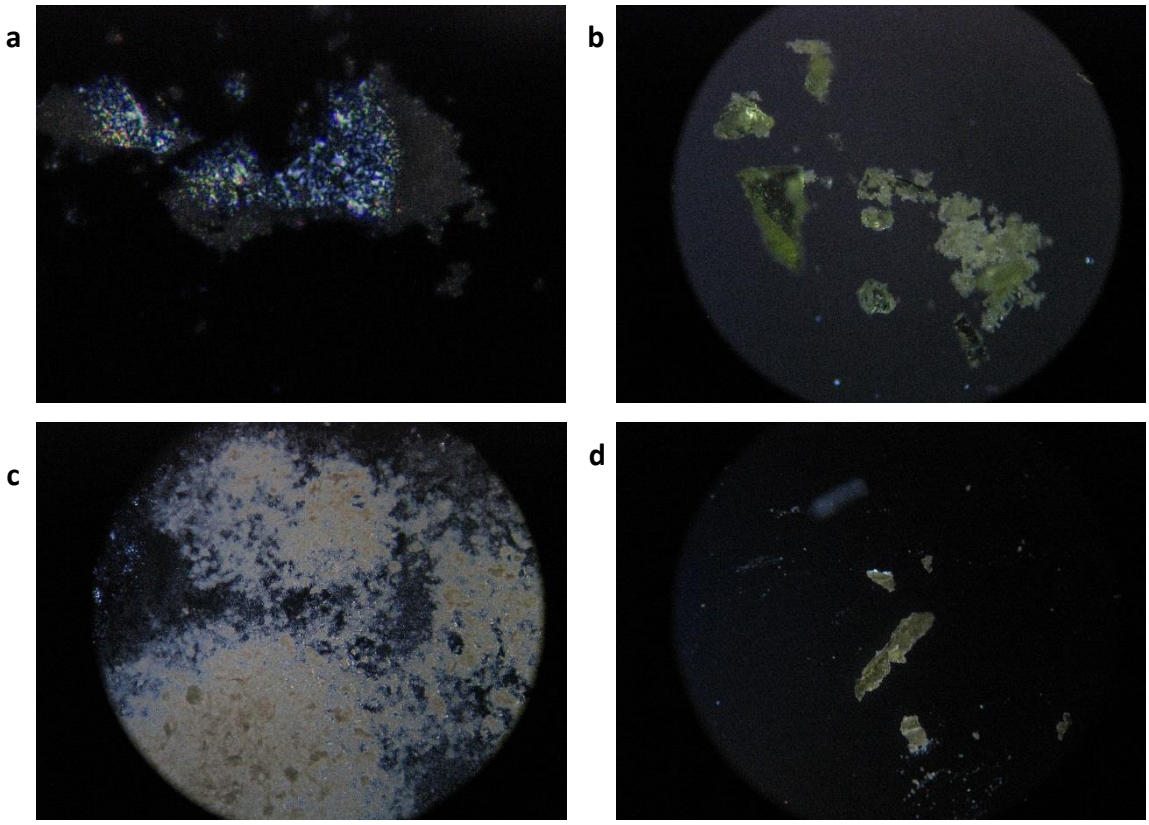

Figure 4.9 - PLM images of (a) crystalline IND, (b) amorphous IND, (c) sample taken from the donor compartment during the permeation test performed after 1h of stirring of amorphous IND (d) sample taken during permeation test performed immediately after sample preparation of amorphous IND.

4.4.2. Infrared spectroscopy

A sample was taken (after 45 min) during the permeation test performed after 1h of stirring. Subsequently, this sample was analysed with IR spectroscopy to study crystallization behaviour. Comparing the spectrum shown in figure 4.10 with reference spectra, it can be concluded that amorphous IND has crystallised to the α form. The peaks attributed to benzoyl C=O stretching can be noticed at 1649 and 1680 cm^{-1}. The peaks asociated with acid C=O stretching can be seen at 1690 and 1735 cm^{-1}. (2)

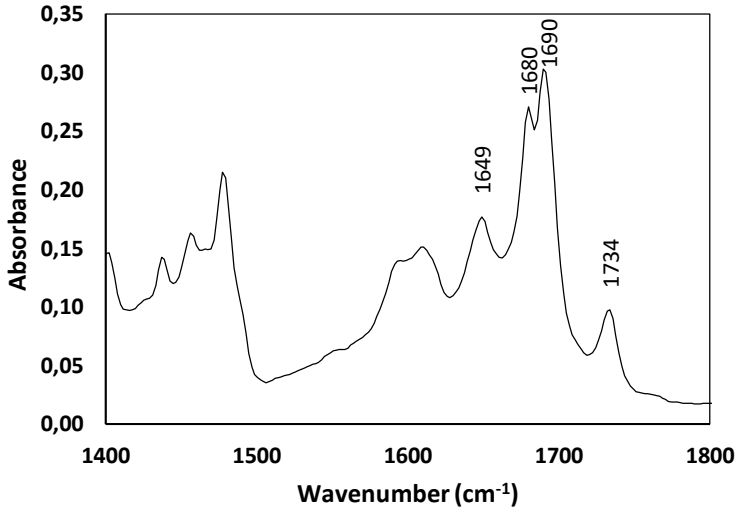

Figure 4.10 – IR spectroscopy analysis of the crystallization behaviour during permeation test of amorphous IND suspension after 1h of stirring.

5. CONCLUSION

During this project, the level and maintenance of supersaturated solutions (with respect to the thermodynamic solubility of crystalline indomethacin (IND)) of amorphous IND was studied. Different analytical techniques were used for the physical characterization of crystalline and amorphous IND.

It can be concluded that both polymer addition and polymer type have an influence on the level and maintenance of supersaturation. After adding HPMC higher supersaturated solutions were generated and supersaturation was maintained for longer periods compared to pure amorphous IND. The supersaturation effect was even more pronounced for Soluplus®: both the maximum drug concentration and the maintenance of the supersaturation were considerably higher compared to the pure amorphous IND and compared to HPMC. This may be due to strong drug-polymer interactions as well as micellar interactions in the case of Soluplus®. This study also showed that different separation methods (filtration and centrifugation) have an impact on the concentration measured and thus are of importance for the solubility tests.

Furthermore, it can be concluded that during the permeation tests the concentrations of dissolved IND decreased in the donor compartment, but remained low in the acceptor compartment. Further tests need to be performed to confirm if this is be due to crystallization of the amorphous form, interaction between IND and the PVDF membrane, interaction between polymer and PVDF membrane or other possible explanations. This study also demonstrated that permeation can have an influence on the maintenance of supersaturation and hence crystallization. For HPMC supersaturation was maintained longer during the permeation studies than during the solubility studies while for Soluplus the opposite was observed. Further tests need to be performed to confirm this hypothesis.

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7. APPENDIX

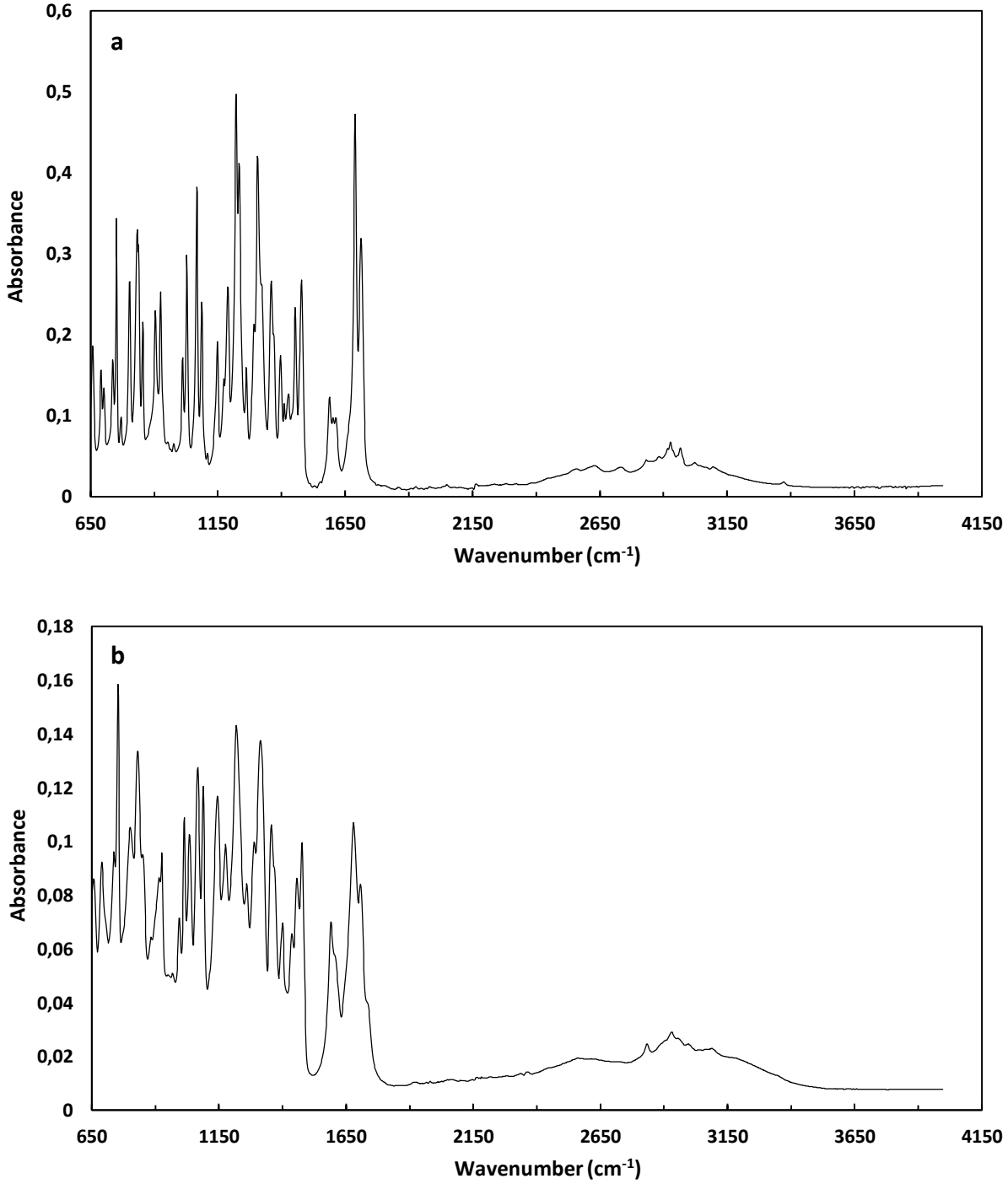


Figure 7.1 – IR spectra of crystalline (a) and amorphous (b) indomethacin over the spectral region 650 – 4000 cm^{-1}.

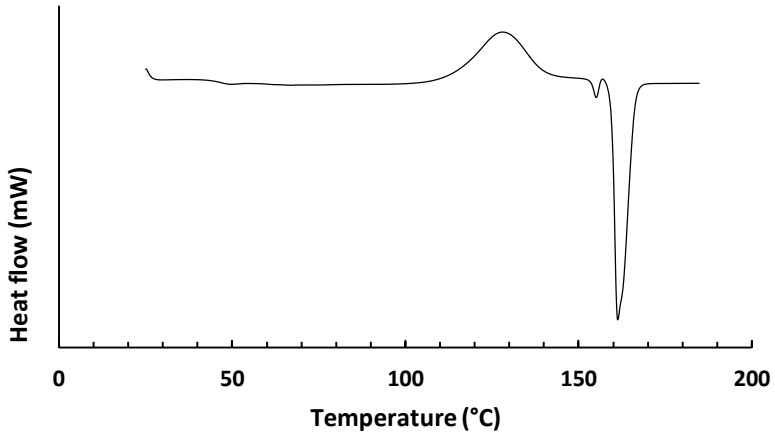
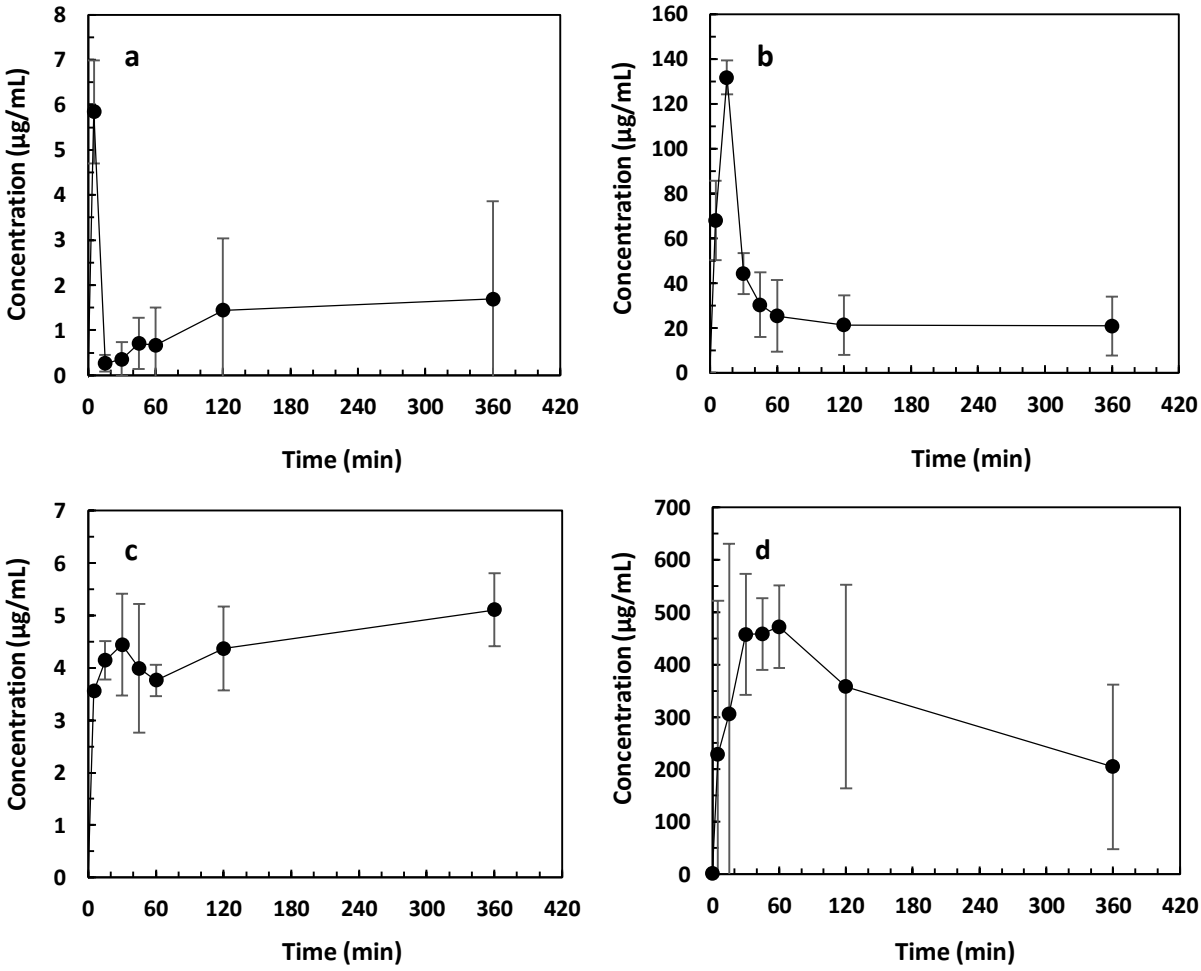


Figure 7.2 – DSC thermogram of amorphous indomethacin. A temperature program of 25 – 185°C was used.



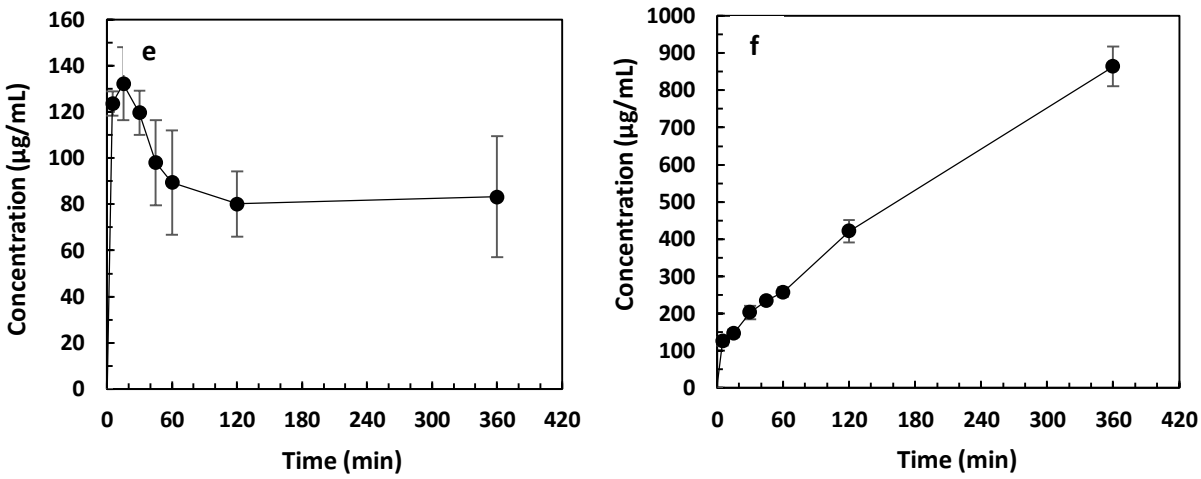


Figure 7.3 – Effect of polymer addition (in a 1:1 ratio drug:polymer (w/w)) on level and maintenance of supersaturated solution of amorphous IND. The suspensions were stirred at 250 rpm and 25 °C. Concentration-time profiles of crystalline IND without polymer addition (a), amorphous IND without polymer addition (b), crystalline IND with predissolved HPMC (c), amorphous IND with predissolved HPMC (d), crystalline IND with predissolved Soluplus® (e), amorphous IND with predissolved Soluplus® (f). Each bar represents the mean ± SD (n = 2).