UNIVERZA V LJUBLJANI
FAKULTETA ZA FARMACIJO

MERCEDES VITEK

# MAGISTRSKA NALOGA

ENOVITI MAGISTRSKI ŠTUDIJSKI PROGRAM FARMACIJA

Ljubljana, 2018

UNIVERZA V LJUBLJANI
FAKULTETA ZA FARMACIJO

MERCEDES VITEK

# PREPARATION OF INDOMETACIN AND COPOVIDONE SOLID DISPERSIONS AND EVALUATION OF THEIR PROPERTIES

# IZDELAVA IN PROUČEVANJE LASTNOSTI TRDNIH DISPERZIJ INDOMETACINA IN KOPOVIODNA

ENOVITI MAGISTRSKI ŠTUDIJSKI PROGRAM FARMACIJA

Ljubljana, 2018

I did the experimental part of my Master's thesis at the Pharmaceutical Institute of the Rheinische Friedrich-Wilhelms-Universität Bonn in Germany at the Department of Pharmaceutical Technology under the supervision of Prof. Dr. Odon Planinšek and Prof. Dr. Karl G. Wagner.

ACKNOWLEDGEMENT

I would like to thank Prof. Dr. Odon Planinšek for the guidance and help during the writing of this Master's thesis. I am very grateful for his quick response, accessibility, and useful advice when I needed it.

Furthermore, I would like to express my gratitude to Prof. Dr. Karl G. Wagner for giving me the amazing opportunity to work in his research group. I am very thankful for his guidance, valuable discussions, and support during my research work in the laboratory.

I would also like to thank the whole research group for providing a very helpful, friendly, and enjoyable work environment. Special thanks go to PhD students Alvaro Lopez and Esther Bochmann for their assistance and guidance during my work, and for kindly sharing their knowledge, experiences, and ideas with me.

Gratitude is extended to my Erasmus friends for all the great memories that we created together. Without them, my stay in Bonn would not have been so pleasant and wonderful.

Moreover, I would also like to thank my friends for lifelong memories and unforgettable student years.

I also wish to thank my friend Tatjana for proofreading this Master's thesis.

Words of appreciation also go to my boyfriend Tadej for his support and words of encouragement all along.

Last but not least, from the bottom of my heart I would like to thank my family – mother Dragica, father Bojan, brother Jernej and his wife Darja, and grandmother Angela – who supported me during my whole life, morally as well as financially. Thank you for your precious love, continuous encouragement, and for always being there for me. Rada vas imam!

STATEMENT

I hereby declare that I have done this Master's thesis independently under the supervision of Prof. Dr. Odon Planinšek and Prof. Dr. Karl G. Wagner.

Ljubljana, 2018 Mercedes Vitek

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ABSTRACT

Poor water solubility of drug molecules often results in low bioavailability. Hence, manufacturing of clinically effective oral dosage forms for such compounds is a challenging and demanding task. One of the possible strategies to improve bioavailability of poorly soluble active pharmaceutical ingredients is the preparation of solid dispersions. The aim of this study was to prepare solid dispersions with poorly water-soluble model drug (indometacin) and with hydrophilic carrier matrix (copovidone) through spray drying and hot-melt extrusion to achieve improved dissolution performance. Moreover, the aim was also to evaluate and compare their properties. The solid dispersions with 10, 20, and 30 percent of indometacin were produced. The obtained products were characterized with respect to their solid-state, solubility, and dissolution properties. X-ray powder diffraction showed that all solid dispersions were completely amorphous, which indicated that both preparation methods were suitable for amorphization of crystalline indometacin. Modulated differential scanning calorimetry showed that there was a good miscibility between the drug and the carrier in each solid dispersion and that no phase separation occurred in any of them. Besides, results also revealed plasticizing effect of the drug on the polymer. Moreover, we found out that regarding the amorphous state no major divergences between spray dried products and extrudates could be seen in terms of glass transition temperatures. Sirius CheqSol experiments indicated that copovidone in solid dispersions effectively inhibited crystallization of the drug in solution. In addition, CheqSol measurements revealed that kinetic solubilities of solid dispersions were lower than kinetic solubility of the pure drug, and that kinetic solubilities of solid dispersions increased with increasing polymer loading. Furthermore, CheqSol determinations revealed that intrinsic solubilites of all solid dispersions were higher than intrinsic solubility of the pure indometacin. Besides, intrinsic solubilities of solid dispersions increased as a function of increasing polymer loading, which indicated that the polymer in solid dispersions played an important role in terms of improved solubility. Dissolution study showed that mostly amorphous pure indometacin did not exhibit improved dissolution behaviour due to supposed recrystallization. Moreover, there was also no large improvement in the dissolution behaviour of physical mixtures. These results indicated that neither the amorphous state of the drug alone nor the presence of highly water-soluble polymer alone were responsible for enhanced dissolution. Furthermore, we observed that indometacin was released only from the dispersions with a high amount of the polymer, namely 10% drug-

loaded spray dried product and 20% or higher drug-loaded extrudates, pointing to the polymer-controlled release mechanism. We assumed that advanced wettability caused by the polymer was one of the crucial reasons for enhanced dissolution of indometacin in our solid dispersions. In addition, the dissolution study also showed that there were differences among the dispersions with improved release of indometacin, namely in the maximal concentration of the released drug, in the time it took these samples to reach their maximal concentration of the released drug, and in the drop of the concentration of the released drug. In conclusion, we can say that the aim of the study was achieved and that above presented observations can serve as a starting point for future research of indometacin and copovidone solid dispersions.

KEYWORDS

indometacin, copovidone, solid dispersions, spray drying, hot-melt extrusion, dissolution enhancement

EXTENDED ABSTRACT IN SLOVENIAN LANGUAGE

RAZŠIRJENI POVZETEK V SLOVENSKEM JEZIKU

Topnost in permeabilnost zdravilne učinkovine (ZU) sta dve ključni lastnosti, ki vplivata na njeno biološko uporabnost. Na osnovi teh dveh lastnosti Biofarmacevtski klasifikacijski sistem deli ZU v štiri razrede. Velika večina novo odkritih ZU se uvršča v razred II, v katerem so slabo topne in dobro permeabilne ZU. Uspešen razvoj formulacije za dostavo ZU s slabo topnostjo je torej eden izmed pomembnih izzivov v sodobni farmacevtski industriji. Obstajajo različni pristopi s katerimi lahko izboljšamo topnost slabo topnih učinkovin. Ena izmed možnosti je izdelava trdnih disperzij.

Trdna disperzija je disperzija ene ali več ZU v inertnem nosilcu, ki je v trdnem agregatnem stanju. Pripravimo jo lahko z raztapljanjem, taljenjem ali kombinacijo obeh postopkov. Ena izmed najpogostejših metod za izdelavo trdnih disperzij je sušenje z razprševanjem, ki je enostopenjski proces, v katerem tekočino pretvorimo v trden produkt. Celoten proces je sestavljen iz treh zaporednih faz: razprševanja tekočine v majhne kapljice, mešanja razpršene tekočine z vročim sušilnim plinom, kar omogoči izhlapevanje topila iz kapljic tekočine in nastanek trdnih delcev, ter ločevanja nastalih delcev od sušilnega medija. Druga pogosto uporabljena metoda za izdelavo trdnih disperzij pa je metoda iztiskanja talin. Gre za neprekinjen proces, pri katerem se vhodna snov zaradi visoke temperature in tlaka zmehča oziroma stali in nato s pomočjo vijakov iztisne skozi odprtino ekstrudorja. Tudi ta postopek lahko razdelimo v tri zaporedne faze: polnjenje ekstrudorja z vhodnim materialom, ki mu sledi mešanje, taljenje in oblikovanje oziroma iztiskanje, na koncu pa dobljene ekstrudate še obdelamo, pri čemer lahko uporabimo različne tehnike.

Glavni namen magistrske naloge je bila izdelava trdnih disperzij s slabo vodotopno modelno ZU in s hidrofilnim nosilcem z metodo sušenja z razprševanjem in z metodo iztiskanja talin, da bi dosegli izboljšano raztapljanje izbrane ZU. Poleg tega smo želeli tudi ovrednotiti in primerjati njihove lastnosti. Izbrana učinkovina je bil slabo vodotopen indometacin, ki se po Biofarmacevtskem klasifikacijskem sistemu uvršča v razred II. Indometacin je šibka kislina s pKa 4,01. Izbran nosilec pa je bil hidrofilen polimer kopovidon. Trdne disperzije so vsebovale 10, 20 in 30 odstotkov indometacina.

Kot je bilo omenjeno, so bile trdne disperzije izdelane z metodo sušenja z razprševanjem in z metodo iztiskanja talin. Poleg tega smo z metodo hitre ohladitve taline pripravili amorfen

čisti indometacin. Kjer je bilo to potrebno, smo vzorce tudi zmleli s kavnim mlinčkom (običajno mletje) ali pa s krogličnim mlinom (kriogeno mletje), tako da smo lahko primerjali vzorce s približno enako velikostjo delcev. V naslednji fazi smo čisto ZU in izdelane trdne disperzije tudi ovrednotili z različnimi analitskimi metodami. Vpliv mletja s kavnim mlinčkom na kristalno čisto ZU, učinkovitost amorfizacije kristalne čiste ZU z metodo hitre ohladitve taline in vpliv sušenja z razprševanjem in hitre ohladitve taline na formulacije v smislu amorfizacije ZU, smo preučili z rentgensko praškovno analizo. Razlike v temperaturah steklastega prehoda amorfne čiste ZU, čistega polimera in trdnih disperzij so bile izmerjene s temperaturno modulirano diferenčno dinamično kalorimetrijo. Izvedli smo dva cikla segrevanja, saj smo na ta način izbrisali termično zgodovino vzorca (učinke izdelave). Tako smo pri drugem segrevanju dobili želene informacije o samem vzorcu brez vpliva metode izdelave. Razlike v kinetičnih in intrinzičnih topnosti čiste ZU in trdnih disperzij smo preučili s Sirius CheqSol metodo. Razlike v raztapljanju oziroma sproščanju kristalne čiste ZU, amorfne čiste ZU, ZU v fizikalnih zmeseh in ZU v trdnih disperzijah pa so bile preučevane s testi sproščanja. Teste sproščanja smo izvajali na miniaturizirani USP 2 napravi v 0,1 M HCl.

Rentgenska praškovna analiza je pokazala, da mletje čiste kristalne ZU s kavnim mlinčkom ni povzročilo amorfizacije ZU ali pa pretvorbe le-te v druge polimorfne oblike. Poleg tega smo ugotovili, da se čisti kristalni indometacin z metodo hitrega ohlajanja taline ni popolnoma pretvoril v amorfno obliko. Dobljeni produkt je bil sicer večinoma amorfen, vendar je vseboval tudi nekaj kristalnih delcev. Rezultati te analize pa so razkrili tudi to, da so bile vse trdne disperzije popolnoma amorfne. Torej sta bili obe metodi izdelave trdnih disperzij primerni za amorfizacijo kristalnega čistega indometacina.
S temperaturno modulirano diferenčno dinamično kalorimetrijo smo za vsako trdno disperzijo določili samo eno temperaturo steklastega prehoda. Na podlagi tega smo sklepali, da je bila mešljivost med ZU in polimerom dobra in da ni prišlo do fazne ločitve. Poleg tega je analiza pokazala tudi to, da je bila temperatura steklastega prehoda vseh trdnih disperzij višja od temperature steklastega prehoda amorfne čiste ZU oziroma nižja od temperature steklastega prehoda polimera. Ugotovili smo tudi, da se je temperatura steklastega prehoda vseh disperzij nižala z naraščajočim deležem ZU. Oba pojava smo pripisali mehčalnemu učinku ZU. Nazadnje smo primerjali tudi temperaturo steklastega prehoda iz obeh ciklov segrevanja in ugotovili, da ni bilo opaznih razlik glede amorfnega

stanja ZU v disperzijah izdelanih s sušenjem z razprševanjem in v disperzijah izdelanih z iztiskanjem talin.

Na podlagi grafov, ki prikazujejo dogajanje v raztopini med izvedbo Sirius CheqSol eksperimentov, smo predpostavili, da je kopovidon učinkovito zavrl kristalizacijo v raztopini oziroma da je imel pomembno vlogo pri stabilizaciji amorfnega stanja ZU v trdnih disperzijah tekom CheqSol meritev. Rezultati, ki smo jih določili s to metodo so pokazali tudi naslednje: kinetična topnost kristalne čiste ZU je bila relativno visoka in višja od kinetičnih topnosti trdnih disperzij. Poleg tega pa smo ugotovili tudi, da so kinetične topnosti indometacina iz disperzij naraščale z naraščajočim deležem polimera. Do teh rezultatov je prišlo zaradi hidrofilnega polimera v formulacijah, saj je le-ta vplival na njihovo topnost in posledično na pH-je precipitacije. Intrinzična topnost kristalne čiste ZU pa je bila nižja od intrinzičnih topnosti trdnih disperzij, kar je bilo pričakovano. Opazili smo tudi, da so intrinzične topnosti disperzij naraščale z naraščajočim odstotkom polimera, kar je kazalo na to, da je imel kopovidon pomembno vlogo pri izboljšanju topnosti indometacina v trdnih disperzijah.

Rezultati študije sproščanja so pokazali, da je bilo raztapljanje večinoma amorfnega čistega indometacina zelo slabo. Predvidevali smo, da je pri testu prišlo do rekristalizacije amorfne čiste ZU. Ta rezultat je pokazal, da samo amorfno stanje indometacina ni bilo dovolj za izboljšanje raztapljanja. Poleg tega tudi fizikalne zmesi niso pokazale izboljšanega sproščanja ZU, kar je vodilo do spoznanja, da tudi prisotnost hidrofilnega polimera ni bila dovolj za spremembo v sproščanju ZU. Izboljšano sproščanje ZU je bilo doseženo samo v nekaterih izdelanih formulacijah, in sicer v tistih disperzijah, ki so vsebovale velik delež polimera: produkt sušenja z razprševanjem z 10% ZU in ekstrudati z 10 in 20% ZU. Na podlagi tega smo sklepali, da je sproščanje indometacina iz naših disperzij potekalo po mehanizmu, ki je nadzorovan s polimerom. Postavili smo tudi predpostavko, da je eden izmed glavnih razlogov za izboljšano sproščanje indometacina izboljšano močenje, ki ga zagotavlja polimer. Poleg tega smo s študijo sproščanja ugotovili tudi, da med trdnih disperzijami z izboljšanim sproščanjem ZU obstajajo razlike v maksimalni koncentraciji sproščene ZU, v času, v katerem so ti vzorci dosegli maksimalno koncentracijo sproščene ZU, ter v padcu koncentracije sproščene ZU.

V zaključku lahko rečemo, da je bil namen magistrske naloge dosežen, rezultati pa predstavljajo izhodišče za nadaljnje raziskave trdnih disperzij indometacina in kopovidona.

KLJUČNE BESEDE
indometacin, kopovidon, trdne disperzije, sušenje z razprševanjem, iztiskanje taline, izboljšano raztapljanje

# LIST OF ABBREVIATIONS

AcOH – acetone

API – active pharmaceutical ingredient

BSC – biopharmaceutical classification system

CheqSol – chasing equilibrium solubility

DMSO – dimethyl sulfoxide

DSC – differential scanning calorimetry

EXT – extrudates

GMP installations – good manufacturing practice installations

HCl – hydrochloric acid

IND – indometacin

KCl – potassium chloride

KOH – potassium hydroxide

KVA64 – Kollidon® VA 64

MeOH - methanol

mDSC – modulated differential scanning calorimetry

PM – physical mixture

SD – spray drying

USP Apparatus – united states pharmacopoeia apparatus

XRPD – X-ray powder diffraction

# 1. INTRODUCTION

Solubility and permeability of the active pharmaceutical ingredient (API) are two crucial factors which influence bioavailability (1). Guideline on the Investigation of Bioequivalence of the European Medicines Agency indicates the following definitions of a highly soluble and a highly permeable substance, respectively. An active substance is regarded as a highly soluble substance when its highest single dose administered as immediate release formulation completely dissolves in 250 mL or less of aqueous media within the pH range of 1-6.8 at 37±1°C. High permeability of a substance is related to its complete absorption, which is considered to be established when the measured extent of absorption is at least 85 % or more (2).

According to the Biopharmaceutical Classification System (BCS), drugs are classified into the following four main classes regarding their solubility and permeability:

- class I – high solubility, high permeability;
- class II – low solubility, high permeability;
- class III – high solubility, low permeability;
- class IV – low solubility, low permeability.

Majority of new drug candidates belong to class II (3). Improvement of dissolution rate and solubility of these compounds, respectively, is therefore one of the important challenges for today's pharmaceutical industry.

Dissolution rate of the substance is mathematically described by the modified Noyes-Whitney's equation (Equation *1*):

$$\frac{dC}{dt} = \frac{A \times D \times (Cs - C)}{h} \quad \text{(Equation } 1\text{)}$$

**Equation *1***: Noyes-Whitney's equation (adapted from (4)).
dC/dt – dissolution rate of the substance, A – surface area available for dissolution, D – diffusion coefficient of the substance, Cs – solubility of the substance in the dissolution, C – concentration of the compound in the dissolution medium at the time t, h – thickness of the diffusion layer.

Noyes-Whitney's equation enables an overview of the possible approaches to improve dissolution rate of the compound. One of the options is to increase the surface area available for dissolution by downsizing the particle size, or by improved surface wettability of the substance. Particle size reduction can be achieved, for example, by using nanotechnology or different mills. Wettability can be improved, for instance, by the

addition of a wetting agent. Another possibility to increase the dissolution rate of the compound is to ensure "sink" conditions (5). These conditions are normally established when the volume of dissolution medium is at least 3 to 10 times bigger than the saturation volume (6). The main factors in the human body which affect "sink" conditions are the permeability of mucosa as well as the volume and composition of fluids in the gastrointestinal tract. If a medicine is applied right after a meal, and if the composition of food is favourable for the dissolving of the drug, the dissolution rate is improved and the time available for the substance to dissolve is extended which is also an advantage. A third option for the improvement of the dissolution rate of the substance, which can be discerned from the above mentioned equation, is the increase of the solubility of the drug (5). Various methods are available for this purpose. We can make use of chemical modifications (formation of pro-drugs or salts), physical modifications (crystal engineering (formation of co-crystals), formation of solid dispersions), modifications of solvent compositions (pH adjustment of formulation, use of co-solvents, use of surfactants) or carrier/delivery systems (cyclodextrines, micelles, (micro) emulsions, liposomes) (7).

## 1.1. SOLID DISPERSIONS

A pharmaceutical solid dispersion is a dispersion of one or more active substances in an inert carrier matrix in a solid state. Solid dispersions can be produced by the solvent method, the melting method, or a combination of both (8). The concept of a solid dispersion was presented for the first time in 1961 by Sekiguchi and Obi. They prepared the eutectic mixture of sulfathiazole, which is a poorly water-soluble drug and urea, which was used as a carrier matrix. After oral application the rate of absorption for this dispersion was much higher than for sulfathiazole itself (9). Solid dispersions were originally used only with the purpose of improvement of dissolution properties and consequently bioavailability of poorly water-soluble compounds in immediate release formulations. However, in recent years, the number of studies which are investigating use of solid dispersion technique for preparation of controlled release dosage forms for poorly water-soluble drugs is increasing (10).

### 1.1.1. TYPES OF SOLID DISPERSIONS

The carrier matrix of solid dispersions can be amorphous or crystalline, and the active substance can be dispersed molecularly, in amorphous particles, or in crystalline particles. Based on this, solid dispersions can be divided into four main groups:

- simple eutectic mixtures (crystalline particles of active substance are dispersed in crystalline carrier);
- amorphous precipitations in a crystalline carrier (amorphous particles of active substance are distributed in crystalline carrier);
- glass solutions (active substance is molecularly dispersed in amorphous carrier) and glass suspensions (crystalline or amorphous particles of active substance are dispersed in amorphous carrier);
- solid solutions (active substance is molecularly dispersed in crystalline carrier) (8, 11).

Moreover, solid solutions can be further divided according to their miscibility: continuous solid solutions (active substance and carrier are miscible in all proportions) and discontinuous solid solutions (mixing of both compounds is limited). Beside this criterion for further division of solid solutions, they can also be classified according to the lattice criterion: substitutional solid solutions (solute molecules are substituted for the solvent molecules in the crystal lattice of the solid solvent) and interstitial solid solutions (the interstitial space of the crystal lattice of the solid solvent is occupied with solute molecules) (8).

### 1.1.2. ADVANTAGES AND LIMITATIONS OF PREPARATION AND USE OF SOLID DISPERSIONS

There are many different advantages of the use of the solid dispersion technique. Firstly, the reduction of the particle size of the drug leads to a greater surface area available for the dissolution of the drug, which results in increased dissolution rate of the drug. Secondly, carrier matrix with surface activity can improve wettability of the drug. Higher wettability of the drug results in higher dissolution rate. Thirdly, products of some of the methods for the manufacturing of solid dispersions have a higher degree of porosity, which also increases dissolution rate. Lastly, conversion of a poorly water-soluble crystalline drug into

its amorphous state results in higher solubility of the drug, because there is no need to overcome the crystal lattice energy during the dissolution (11). As mentioned before, higher solubility and dissolution rate of a drug, respectively, lead to the improved bioavailability of a poorly water-soluble drug.

Despite that, the production of solid dispersions was not a very common approach for the improvement of bioavailability of poorly water-soluble drugs in the past because of the following reasons: limitations regarding methods of preparation, low reproducibility of physiochemical properties, difficulties regarding the formulation of solid dispersions into dosage forms, and problems with physical and chemical stability of drugs and vehicles. However, due to the development of certain excipients and technologies, the industrial production and registrations of medicines in the form of solid dispersions are nowadays increasing (12).

### 1.1.3. MANUFACTURING OF SOLID DISPERSIONS

There are three major approaches for the manufacturing of solid dispersions: solvent evaporation, melting, and their combination.

In the solvent evaporation method the solid dispersion is prepared by dissolving the physical mixture of the drug and the carrier in a solvent, followed by evaporation of the solvent from the solution (8). Techniques for the manufacturing of solid dispersions such as oven drying, vacuum drying, rotary evaporation, heating on a hot plate, spray drying, freeze drying, supercritical anti-solvent, co-precipitation, electrostatic spinning, spray freeze drying, ultra-rapid freezing, and fluid-bed coating are founded on this principle.

In the melting method, the active substance and the carrier matrix are molten, the resulting liquid is then cooled or solidified and after this the solid product is crushed, sieved, pulverized, or injection moulded. The following techniques for the production of solid dispersions are based on this approach: ice bath agitation, thin film coating, liquid nitrogen, spray congealing, hot-melt extrusion, Meltrex® (patented solid dispersions production process based on the hot-melt extrusion principle) and melt agglomeration.

The most used techniques for the preparation of solid dispersions are spray drying and hot-melt extrusion due to their high applicability and scalability (13).

## 1.2. SPRAY DRYING

Spray drying is a one step process which converts a feed from a fluid state into a solid state by atomizing it and vaporizing off the solvent (1). It is a widely used technique in many different industrial fields, e. g. food and dairy processing, production of detergents, paints and also in pharmacy (14). In the pharmaceutical industry the spray drying technique has a broad range of applications, for example, particle engineering of bulk active substances and excipients, granulation, encapsulation, preparation of solid dispersions, production of pulmonary formulations, and manufacturing of vitamins and biopharmaceutical products such as peptides and proteins (15, 16).

### 1.2.1. DESCRIPTION OF THE SPRAY DRYING APPRATUS AND THE SPRAY DRYING PROCESS

The spray drying process can be divided into several sequential steps, which are described below. The scheme of a standard spray drying apparatus is presented in Figure 1.

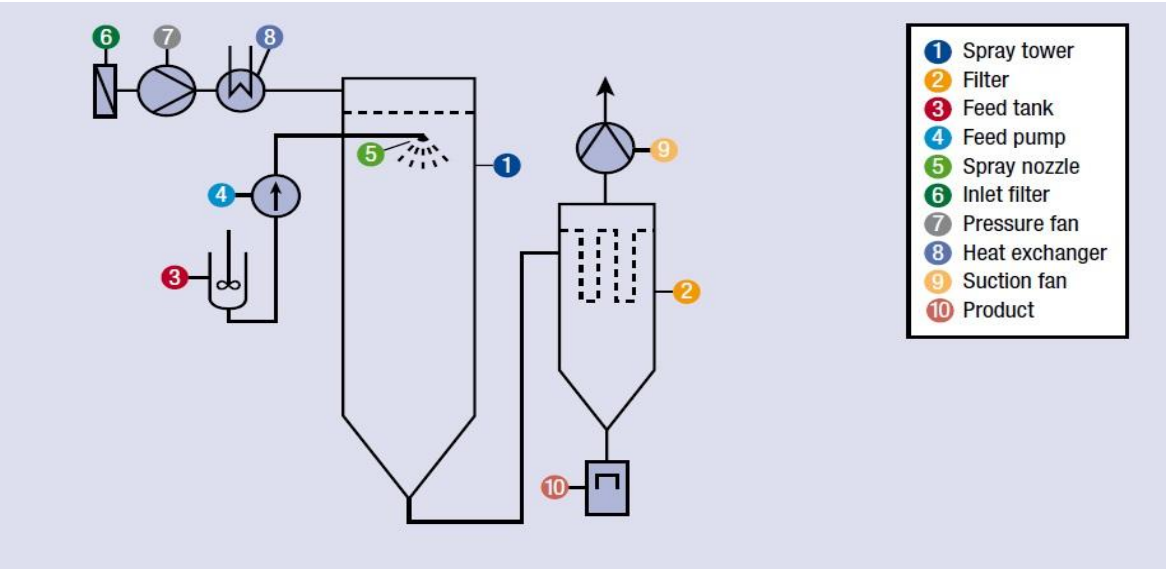



**Figure 1**: Scheme of a standard spray drying apparatus (reproduced from (1)).

At the beginning of the process **the feed is dispersed into small droplets**. The feed can be a solution, emulsion, suspension or dispersion. As the solvent medium, water, organic solvent, or a mixture of both are used. Several types of atomisers can be used for the dispersion of the feed: a pressure nozzle, a two fluid nozzle, a rotary disk atomiser, or an

ultrasonic nozzle. The decision about the selection of the atomiser type depends on the desired amount and the desired characteristics (average particle size and particle size distribution) of the final product.

Further, the **mixing of the spray of the feed and the hot drying medium occurs, which results in the evaporation of the solvent contained in the droplets of the feed**. Air is typically used as the heating medium, but in case of using flammable solvents or oxygen sensitive compounds an inert gas such as nitrogen carries out the role of the drying gas. There are four different ways according to which the spray can come into contact with the drying medium: co-current, counter-current, combined, or disk atomizer/rotary wheel manner. When *co-current flow* is used the droplets are dispersed in the same direction as the flow of the heating medium through the spray dryer. The spray comes into contact with the drying medium when it is the moistest. This manner is used in Figure 1. In case of use of *counter-current flow* the droplets are sprayed in the opposite direction of the flow of the drying gas. The material falls through increasingly hot drying medium into the collection unit. As the particles get very hot during drying, this manner is suitable only for thermally stable compounds. These two methods can also be *combined*. In this case, the heating medium flows from above and the droplets are sprayed upwards, but due to the gravity, which pulls them into the cooler zone, they remain in the hot zone for a short time. And the last way is such that the feed flows onto a rapidly *rotating disk atomizer/rotary wheel* and is converted into a fine mist. The drying gas flows in the same direction as the feed.

Finally, **the product is separated from the drying medium**. Usually cyclone is used for this purpose, but other systems such as electrostatic precipitators, bag filters, or wet collectors are also in use (14).

The properties of the final product strongly depend on the process parameters which we use in spray drying (14). The interplay between process parameters and product characteristics is presented in Table I.

**Table I**: The interplay between process parameters in the spray drying process and product characteristics (adapted from (14)).
↑ - increasing process parameter, / - no influence, ↑ - increasing variable, higher number of arrows represents higher influence, ↓ - decreasing variable, higher number of arrows represents higher influence.

| Product characteristics / Process parameters | **Outlet temperature** | **Humidity in the final product** | **Particle size of the final product** | **Yield** |
|---|---|---|---|---|
| **↑ Aspirator rate** | ↑↑ | ↑↑ | / | ↑↑ |
| **↑ Humidity of drying medium** | ↑ | ↑↑ | / | ↓ |
| **↑ Inlet temperature** | ↑↑↑ | ↓↓ | / | ↑ |
| **↑ Spray gas flow** | ↓ | / | ↓↓↓ | / |
| **↑ Feed rate** | ↓↓ | ↑↑ | ↑ | ↑↓ |
| **↑ Concentration** | ↑↑ | ↓ | ↑↑↑ | ↑ |
| **Organic solvent instead of water** | ↑↑↑ | ↓↓↓ | ↓ | ↑↑ |

## 1.2.2. ADVANTAGES AND LIMITATIONS OF SPRAY DRYING

As it was mentioned at the beginning of this paragraph, spray drying is a widely used technique and is therefore readily available. Moreover, since it is a one step process, it enables production of a powder (for manufacturing tablets) from a liquid in just "one pot". Furthermore, thermal stress is lower during the spray drying process compared to conventional melting methods. In addition, the characteristics of the particles, produced by this technique, can be controlled by process parameters, therefore particles can be designed for certain purposes.

By contrast there are also some disadvantages to this technique. It is not always easy to find a solvent in which a drug and suitable pharmaceutical excipients are soluble. In the case of using an organic solvent, a potential residual of it in the final product can have adverse effects. Besides that, use of organic solvents is not desirable in terms of environmental protection, and, because of this, additional equipment for their recovery is also needed. In addition, organic solvents are usually quite expensive. Moreover, a spray dried product has a high surface area and usually (of course this occurrence also depends

on the hygroscopicity of the formulation) it will easily take up moisture, and this can lead to crystallization of the drug or decomposition of the drug (17).

## 1.3. HOT-MELT EXTRUSION

Hot-melt extrusion is a continuous process where the input material softens and melts under high temperatures and pressures, and is squeezed through an extrusion die by screws. It is a well established production process used in plastic and food industries. Besides, it is a recognized technique for "solving" different pharmaceutical challenges (e. g. poor solubility of the active substance, poor API stability caused by hydrolysis, poor taste of the drug, etc.) in the pharmaceutical industry (17). Final products of the hot-melt extrusion technique can be extruded in the form of tablets, pellets, rods, or they can be grinded and mixed with other excipients for different purposes (18). This technique can be also employed for the manufacturing of implants, stents, and dermal or transdermal patches (17).

### 1.3.1. DESCRIPTION OF THE EXTRUDER SYSTEM AND THE HOT-MELT EXTRUSION PROCESS

The hot-melt extrusion technique consists of three consecutive phases. The details of each phase are described below. The scheme of a typical extruder system is presented in Figure 2.

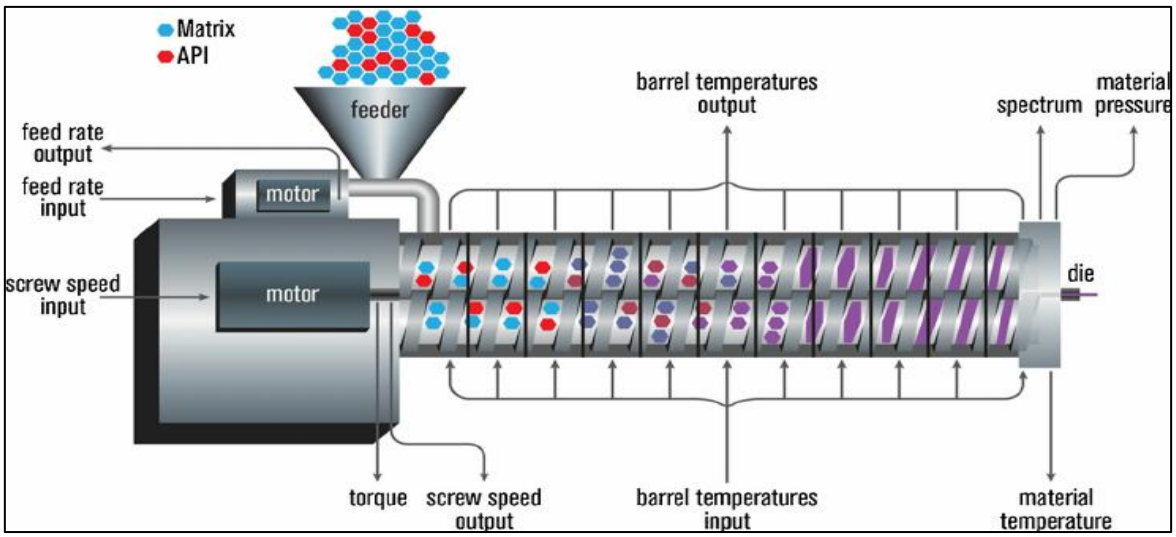



**Figure 2**: Scheme of a typical twin screw extruder system (reproduced from (19)).

In the first phase **the input material is transferred to the barrel**. In the pharmaceutical field the hot-melt extruded systems are composed of drug(s), polymer(s), and optional additives such as plasticizers. A crucial prerequisite of the drug and the polymer to be used is that they are thermally stable at the extrusion temperatures employed. Another important condition connected with the polymer should be also met: appropriate thermoplastic behaviour of the polymer (17). There are two types of feeding units. A *volumetric feeder* discharges a volume of the input material as a function of time (volume per unit time). *Gravimetric feeding systems* are usually composed of a volumetric feeder and a weighing system because they discharge a mass of material as a function of time (mass per unit time) (20). This type of feeding system is typically specified for the Good Manufacturing Practice (GMP) installations (17).

The second phase consists of three crucial steps on which the hot-melt extrusion process is founded: **melting, mixing, and shaping**. All three steps take place in the temperature-controlled barrel with one or two screws (17). Most extruders have a modular design, which enables different screw variations. The configuration of the screw has a great influence on the extrusion process and consequently on the characteristics of the final product. Screw design can be varied by the number and the arrangement of three basic types of screw elements: *flighted elements*, which are responsible for conveyance of the material, *kneading elements* or mixing elements, and *zoning elements*, which isolate two operations (21). According to the number of screws, extruders can be classified as a *single or a twin screw extruder*. The first type is used when melting and extrusion of polymers into continuous shapes is required. Double screw extruder, which is presented in Figure 2, is used for melt-mixing of polymers with additional compounds (compounding), e. g. active substances, and due to this fact it is typically used in the production of pharmaceutical formulations. The two screws can be either *co-rotating* (they rotate in the same direction) or *counter-rotating* (they rotate in the opposite direction).

The barrel is divided into a few heating zones and each zone is equipped with a heater and a cooling system (for the removal of surplus energy). For each part of the barrel a different temperature can be set. Because of the high temperatures the input material starts to melt as soon as it enters (the melting zone of) the barrel. However, the most important reason for the melting of the material is frictional heating within the barrel, and for twin screw extruders, it also melts due to the shearing between the rotating screws, and between the screws and the wall of the barrel (22). The melt then moves in circulatory manner and the

mixing process begins. Some kneading elements of the screws have a stronger *distributive mixing effect* (distribution of solids or fluids in the melt) while others have a more *dispersive effect* (breaking down solids, particles, and droplets). Both types of mixing are compulsory in the manufacturing of solid dispersions. The distributive mixing provides homogeneous distribution of the active substance throughout the whole polymer. The dispersive mixing ensures the breaking down of potential agglomerates. At the end, the melt enters the shaping zone. The function of this zone is to reduce the pulsation flow and to ensure a unified delivery rate through the exit die at the end of the extrusion barrels. Different types of the squeeze nozzle can be used to form the extrudates with desired size and shape.

The last phase of hot-melt extrusion consists of **downstream processing**. There are many different hot-melt extrusion downstream processes and consequently downstream auxiliary components available. The most common hot-melt extrusion downstream techniques are: use of chilled rolls, calendaring, pellet forming, and co-extrusion. The choice mainly depends on whether the cooling rate of the melt is important, what the product will be used for, and how it should look like (17).

Variables in twin screw extrusion can be divided into step changes (i. e. configuration of the screw, die design, and barrel layout) and continuous changes (i. e. feed rate, screw speed, and barrel temperature). Step changes require off-line modification while continuous changes are modified during the running process of extrusion. Feed rate, screw speed, and temperature of the extrusion block are actually the most relevant process parameters (1). Their influence on the residence time of the input material and torque, which are very important system parameters in the hot-melt extrusion process, is presented in Table II.

**Table II**: The interplay between feed rate, screw speed, barrel temperature, and residence time and torque in the hot-melt extrusion process (adapted with changes from (1)).
↑ - increasing process variable, ↑ - increasing system parameter, ↓ - decreasing system parameter.

| System parameters / Process variables | **Residence time** | **Torque** |
|---|---|---|
| ↑ **Feed rate** | ↓ | ↑ |
| ↑ **Screw speed** | ↓ | ↑ |
| ↑ **Barrel temperature** | ↓ | ↓ |

## 1.3.2. ADVANTAGES AND LIMITATIONS OF HOT-MELT EXTRUSION

The first important advantage of hot-melt extrusion technique is that it is a robust and continuous manufacturing process. Secondly, the use of organic solvents is not needed, and because of this many problems connected with these solvents can be avoided. Thirdly, the process is performed in a closed system, which prevents access of moisture and oxygen – potential triggers for degradation of the input material. Furthermore, in comparison with other melting techniques, the residence time of the input material at elevated temperatures is relatively short. Another key advantage to remember is that not only oral drug formulations but also other pharmaceutical forms (e. g. implants, stents, dermal patches, etc.) can be manufactured with this technique.

On the other side, there are also some limitations to this method. One of them is that even though the residence time of the material in the barrel is quite short, this process cannot be used for thermo labile substances. Besides that, the polymer to be used in the process needs to possess the appropriate thermoplastic properties, but unfortunately there is only a limited number of polymers like this. Lastly, high energy input mainly related to great shear forces and high temperatures is also a drawback of hot-melt extrusion technique (17).

# 2. THE AIM OF THE WORK

Poor solubility of many active pharmaceutical ingredients is often the limiting factor regarding their pharmacological activity. There are many approaches to overcome this issue. In this Master's thesis we will focus on one of the possibilities, namely the preparation of solid dispersions. The model compound will be poorly water-soluble drug indometacin and the model polymer will be copovidone. The aim of this study is to prepare indometacin and copovidone solid dispersions through spray drying and hot-melt extrusion to enhance the dissolution of the drug. Moreover, we would also like to evaluate and compare their properties.

We will prepare solid dispersions with three different drug loadings (10, 20, 30 percent of indometacin). In the cases where post-processing through regular milling and cryogenic milling is needed, these two techniques will be used in order to compare the products with a similar particle size. In the next stage, characterization of the pure drug and the formulations will be done through various analytical methods. Influence of the post-processing on the crystalline pure drug, successfulness of the amorphization of the crystalline pure drug through quench cooling, and influence of the spray drying and hot-melt extrusion on the formulations in terms of amorphization of the drug will be investigated through X-ray powder diffraction method. Differences in the glass transition temperatures of the amorphous pure drug, pure polymer, and solid dispersions will be measured by modulated differential scanning calorimetry. Divergences in the kinetic and intrinsic solubilities of the crystalline pure drug and solid dispersions will be studied through Sirius CheqSol method. Differences in the dissolution behaviour of the crystalline pure drug, amorphous pure drug, drug in the physical mixtures, and drug in the solid dispersions will be studied by dissolution tests.

# 3. MATERIALS AND METHODS

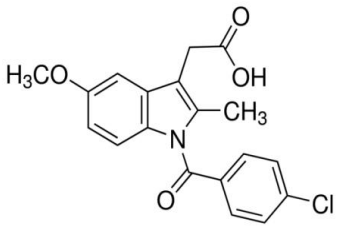
## 3.1. MATERIALS

- Indometacin, Swati Spentose Pvt. Ltd, India
- Kollidon® VA 64, BASF SE, Germany
- Potassium chloride, Sigma Aldrich, Germany
- Standardized 0.5 M Hydrochloric acid solution, VWR Chemicals, France
- Standardized 0.5 M Potassium hydroxide solution, VWR Chemicals, France
- Potassium dihydrogen phosphate, Sigma Aldrich, Germany
- Sodium hydroxide, Merck KGaA, Germany
- Hydrochloric acid fuming 37%, Merck KGaA, Germany
- Methanol for analysis, Merck KGaA, Germany
- Acetone for analysis, Merck KGaA, Germany
- Dimethyl sulfoxide anhydrous, Sigma Aldrich, Germany
- Liquid nitrogen
- Purified water

### 3.1.1. INDOMETACIN

Indometacin, chemically [1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid, is a white or yellow crystalline powder which is practically insoluble in water and sparingly soluble in alcohol (23). Its structural formula and its physical-chemical properties are presented in Table III. According to BCS indometacin belongs to Class II. For these drugs it is common to have a low water solubility and high permeability (24).

**Table III**: Structural formula of indometacin and its physical-chemical properties.

| | |
|---|---|
| **Structural formula** |  (reproduced from (25)) |
| **Molecular weight** | 357,788 g/mol (26) |

| | |
|---|---|
| **pKa** | 4,01 * |
| **Predicted logP** | 4,25 (27, 28) |
| **Predicted water solubility** | 0,0024 mg/mL (27, 28) |
| **Sensitive to light** | Yes (29) |
| **Melting point of crystalline form** | 160,1 °C ** |
| **Glass transition temperature of amorphous form** | 44,0 °C ** |

*This is the value that we got with Sirius UV-metric method. Details of this measurement are described in the subchapter "Sirius UV-metric method and Sirius CheqSol method".

**This is the average value of the three mDSC measurements of crystalline indometacin and amorphous indometacin, respectively, which were performed at the institute by another student (DSC graphs of these determinations are not shown in this thesis). The measurements were performed with Differential scanning calorimeter 2, Mettler Toledo, Germany.

Indometacin is a nonsteroidal anti-inflammatory drug. It has analgesic, anti-inflammatory, and antipyretic properties. It is a nonselective COX (cyclooxygenase) inhibitor. Indometacin is indicated for: moderate to severe rheumatoid arthritis (including acute flares of chronic disease), moderate to severe ankylosing spondylitis, moderate to severe osteoarthritis, acute painful shoulder (bursitis and/or tendinitis) and acute gouty arthritis. Dosage recommendations depend on indication, age of the patient and the patient's health condition. The maximal daily dose of the drug for orally administered immediate-release formulations for adults is 200 mg (30).

### 3.1.2. KOLLIDON® VA 64

Kollidon® VA 64 is a copovidone manufactured by BASF SE, Germany. It is a copolymer of N-vinylpyrrolidone and vinyl acetate in the mass proportion 3:2. The average molecular weight of Kollidon® VA 64 is approximately 45 000 g/mol. Its structural formula is shown in Figure 3. N-vinylpyrrolidone is a water-soluble monomer whereas vinyl acetate is a water-insoluble monomer. However, the ratio of the monomers is balanced in such a manner that the polymer is still readily soluble in water. Besides that, it is also freely soluble in different alcohols such as methanol, ethanol, isopropanol, and not only that, it can also dissolve in methylene chloride and chloroform. Kollidon® VA is described as a white or slightly yellowish free-flowing powder. Since it is an amorphous polymer, it shows a glass transition temperature, which is 107,6 °C (this is the average value of the

three mDSC measurements which were performed at the institute by another student; DSC graphs of these determinations are not shown in this thesis; the measurements were performed with Differential scanning calorimeter 2, Mettler Toledo, Germany) (31, 32).

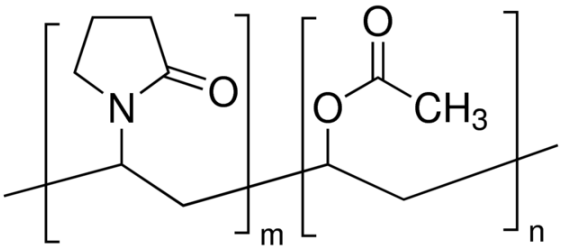



**Figure 3**: Structural form of Kollidon® VA 64 (reproduced from (33)).

In the pharmaceutical industry Kollidon® VA 64 is mostly used as a binder in the manufacturing of granules and tablets by the wet granulation method, and as a dry binder in the direct compression of tablets. Moreover, it is also used as an additional film former in coating on tablets (where it can either work as a protective layer or a sub-coat for tablet cores) or it can be used as a film-forming agent in sprays. Finally, it is also suitable for hot-melt extrusion processes, where it has a role of a solubilizer. In relation to this, the release profile of the extrudates is mostly instant release, but modified release of these formulations is also possible (32).

## 3.2. APPARATUSES

- Analytical balance AG204, Mettler Toledo, Germany
- Overhead stirrer RW 20 DZM, IKA Labortechnik, Germany
- Mini Spray Dryer B-290 Advanced and Inert Loop B-295, Büchi, Switzerland
- Shaker mixer Turbula T2F, Willy A. Bachofen AG Maschinenfabrik, Switzerland
- Twin screw Mini Extruder ZE 12 and Flat-tray feeder ZD 9 FB, Three-Tec, Switzerland
- Universal oven Um, Memmert, Germany
- Coffee bean grinder KSM2, Braun, Germany
- Ball Mill - Mixer mill MM 400, Retsch, Germany
- X-ray powder diffractometer X'Pert, PANalytical, the Netherlands
- Laser diffraction sensor Helos (H0608) & Dry dispersion unit Rodos, Sympatec, Germany

- Upright microscope Leica DM2700 P, Leica Microsystems, Germany
- Sirius T3 instrument, Sirius Analytical, USA
- Miniaturized dissolution apparatus based on USP Apparatus 2, Boehringer Ingelheim Pharmaceuticals, Germany
- UV-visible spectrophotometer Agilent 8453, Agilent Technologies, USA
- Differential scanning calorimeter 2, Mettler Toledo, Germany

## 3.3. METHODS

### 3.3.1. PREPARATION OF SAMPLES

**Preparation of amorphous pure indometacin through quench cooling**

15 g of indometacin was weighed in an oblong container, which was hand-formed from aluminium foil. The container with the pure drug was then carried to the Universal oven Um, which was already heated up to 170°C. During the process we opened the oven a few times in order to visually check viscosity and colour of the API. Transition from crystalline state to amorphous state was determined organoleptically: when it turned into a light orange liquid (this happened after approximately 15 minutes), we took the container with the pure drug out and we placed it on a lab countertop in order for it to cool down (the temperature in the lab was 25°C) and to convert into a solid state (this happened after approximately 1 minute).

Immediately after this, the sample was milled and then analyzed, which is described below. It was important that grinding and analytical processes were performed directly after melt-quenching because amorphous pure indometacin prepared by this method converts back to crystalline form quite quickly.

**Preparation of solid dispersions of indometacin and Kollidon® VA 64 through spray drying**

We prepared 15% feed solutions of indometacin, Kollidon® VA 64, and acetone, each with different mass ratios of the drug and the polymer (IND:KVA64 = 3:7, IND:KVA64 = 2:8, IND: KVA64 = 1:9). Feed solutions were prepared by mixing accurately weighed amounts of API, polymer, and acetone in a beaker for half an hour. Mixing was performed with Overhead stirrer RW 20 DZM. The mass of each component in each solution is presented in Table VI.

**Table IV**: The mass of indometacin, Kollidon® VA 64, and acetone in feed solutions of all spray drying processes.

| | **IND:KVA64 = 3:7** | **IND:KVA64 = 2:8** | **IND:KVA64 = 1:9** |
|---|---|---|---|
| **Mass of IND (g)** | 6,02 | 6,09 | 3,06 |
| **Mass of KVA64 (g)** | 14,1 | 24,1 | 27,0 |
| **Mass of AcOH (g)** | 113 | 170 | 170 |

The spray drying process was performed with Mini Spray Dryer B-290, which is shown in Figure 4, and Inert Loop B-295. Nitrogen was used as a spray gas. The compressor was set to 5 - 6 bar output pressure. The mixing of nitrogen and feed solution was co-current. The two fluid nozzle, with a nozzle tip diameter of 0,7 mm and a nozzle cap diameter of 1,4 mm, was used. The process parameters of all three spray drying processes are presented in Table V. The outlet temperature in the first spray drying process (IND:KVA64 = 3:7) varied from 52°C to 55°C, in the second (IND:KVA64 = 2:8), outlet temperature was kept at 53±2°C, and in the third (IND:KVA64 = 1:9), it varied from 53 to 57°C. All three spray dried samples were manually transferred to plastic vials, sealed with ParaFilm, and wrapped with aluminium foil. They were stored in a desiccator (with silica gel) at 25°C.

**Table V**: Process parameters of all spray drying processes.

| | **IND:KVA64 = 3:7** | **IND:KVA64 = 2:8** | **IND:KVA64 = 1:9** |
|---|---|---|---|
| **Inlet temperature (°C)** | 70 | 70 | 70 |
| **Aspirator rate (% of max. aspirator rate)** | 95 | 90 | 90 |
| **Pump rate (% of max. pump rate)** | 20 | 20 | 20 |
| **Spray gas flow rate (L/h)** | 538 | 538 | 538 |
| **Feed rate of feed solution (mL/min)** | 5,1 | 4,8 | 4,3 |

**Figure 4**: Mini Spray Dryer B-290.
Cyclone for separating particles from gas stream and product collection vessel were wrapped with aluminium foil during each spray-drying process because indometacin is light-sensitive.

## Preparation of solid dispersions of indometacin and Kollidon® VA 64 through hot-melt extrusion

First we prepared three different physical mixtures of indometacin and Kollidon® VA 64. The final mass of each physical mixture was 100 g, they differed from one another only in mass ratios of the drug and the polymer (IND:KVA64 = 3:7, IND:KVA64 = 2:8, IND: KVA64 = 1:9). Each of them was mixed in shaker mixer Turbula T2F for 10 minutes at 50 rpm.

Hot-melt extrusion was performed with a twin screw Mini Extruder ZE 12. The extruder was fed through a flat-tray feeder ZD 9 FB. Both are shown in Figure 5. The feed rate was 2 g/min. The extrusion block was divided into five heating zones ($T_1$, $T_2$, $T_3$, $T_4$, $T_5$) and we could set an optional temperature for each zone. The temperature profile that we used was: $T_1$ = 80°C, $T_2$ = 120°C, $T_3$ = 150°C, $T_4$ = 150°C, $T_5$ = 150°C. It was chosen based on previous experiences of other students at the institute who worked with the same drug and polymer. At the beginning of each extrusion process, screw speed was set to 50 rpm and during this time torque varied from 3 to 4 Nm. After 5 minutes, we set it to 100 rpm and torque then varied from 7 to 8 Nm. The extrusion die was in the shape of a round hole with

a 2 mm diameter. All parameters, which are described in this paragraph, were the same for all three physical mixtures. All samples were stored in plastic bags which were wrapped with aluminium foil and put into a desiccator (with silica gel) at 25°C.

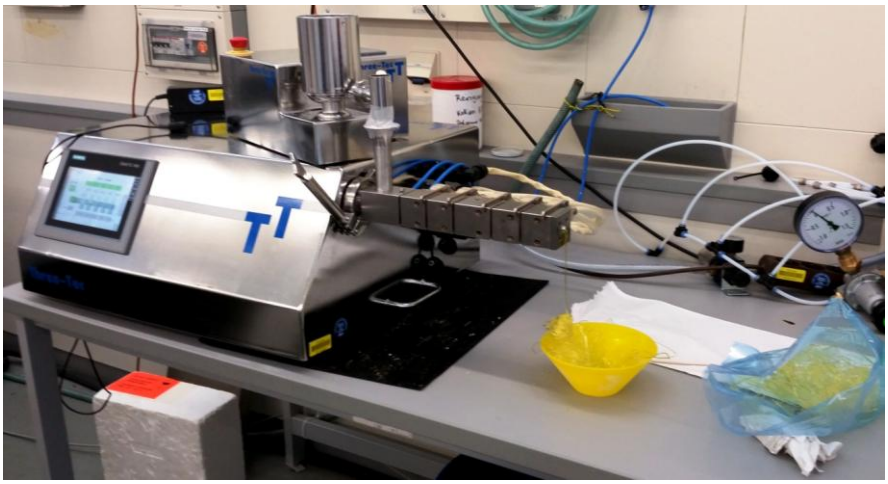


**Figure 5**: Double screw Mini Extruder ZE 12 and Flat-tray feeder ZD 9 FB.
When each extrusion process was finished, we transferred extrudates to plastic bags which were wrapped with aluminium foil because indometacin is light-sensitive.

### 3.3.2. POST-PROCESSING

**Milling of samples with coffee bean grinder**

Pure indometacin was milled for the comparison of the XRPD pattern of the milled drug with the XRPD pattern of the unmilled API.

Quench cooled pure indometacin was not suitable for cryogenic milling (we needed powder for this process), however, grinding with a coffee bean grinder converted it into a powder.

Extrudates were milled with the purpose of converting them into a powder, which we needed for cryomilling and XRPD and mDSC analyses.

We milled physical mixtures with coffee bean grinder because we wanted to compare the XRPD results of the extrudates with the XRPD results of the physical mixtures, and since we expected that milling of the extrudates with coffee bean grinder has at least a small influence on the amorphization of the sample, the only way to do a proper comparison was to mill the physical mixtures too. Another reason for milling the physical mixtures was connected with cryomilling. We wanted all the samples which were cryomilled to have a similar initial particle size. Quench cooled pure indometacin and extrudates were milled

with the coffee bean grinder before cryogenic milling, and consequently the physical mixtures had to be grinded too.

Samples listed above were grinded with coffee bean grinder KSM2. 10 g of each sample was milled for 120 seconds. But because 10 g of each sample of physical mixtures and extrudates was not enough for all the experiments and cryomilling (we needed around 20 g of each of these samples), grinding of these samples with coffee bean grinder had to be done twice. For this reason we milled another 10 g of each sample of the physical mixtures and the extrudates.

**Cryogenic milling of samples with ball mill**

As indicated in the previous paragraph, for some of the samples which were grinded with the coffee bean grinder, the next step of post-processing was cryomilling. These samples were: grinded quench cooled pure indometacin, grinded physical mixtures of all three mass ratios, and grinded extrudates of all three mass ratios. The purpose of cryogenic milling was the particle size reduction. The average particle size of samples influences results of solubility measurements and dissolution studies, and because of this the particle size of the aforementioned samples had to be reduced to the average particle size of the spray dried products, which was approximately 6 μm. Samples listed above were cryomilled with Ball mill – Mixer Mill MM 400, which is shown in Figure 6. Around 10 mL of each of these samples was milled three times for 15 seconds with the frequency of 30/s. The cooling agent we used during the process was liquid nitrogen.


**Figure 6**: Ball mill – Mixer Mill MM 400.

### 3.3.3. ANALYTICAL METHODS

**X-ray powder diffraction**

XRPD patterns of pure indometacin, grinded (with coffee bean grinder) pure indometacin, cryomilled quench cooled pure indometacin, grinded (with coffee bean grinder) physical

mixtures of all three mass ratios, spray dried samples of all three mass ratios, and grinded (with coffee bean grinder) extrudates of all three mass ratios were recorded to check if these samples were amorphous or not.

X-ray powder diffractometer X'Pert was used. A continuous 2θ scan was performed in the range of 3°-45° with the speed of 2,1°/min. The voltage and current applied were 30 kV and 10 mA, respectively. Cu Kα radiation was used.

## Modulated differential scanning calorimetry

Glass transition temperature was determined for spray dried samples of all three mass ratios and for grinded (with coffee bean grinder) extrudates of all three mass ratios. Modulated differential scanning calorimetry (mDSC) measurements for each sample were performed in triplicate, the average values are reported.

mDSC analyses were done with Differential scanning calorimeter 2. 8 to 12 mg of each sample was weighed into an aluminium pan with a perforated lid. The temperature range of the measurements stretched from -20 to 175°C, the heating rate was 2°C/min, modulation amplitude was 0,318°C every 60 seconds. Each sample was subjected to two heating runs.

## Laser diffraction

The purpose of measuring the particle size of the spray dried product with a mass ratio IND:KVA64 = 1:9 was the determination of the average particle size of this sample. This was then the reference value according to which we cryomilled samples which are listed in the paragraph about cryogenic milling.

Laser diffraction sensor Helos (H0608), combined with dry dispersion unit Rodos, was used. The focal length of the lens was 200 mm. The dispersive pressure was 0,5 bar. Three measurements were performed, the average values are reported.

## Light microscopy

Particle size of all cryomilled samples was examined in order to check if these samples were cryomilled to the adequate size or not.

We used Upright microscope Leica DM2700 P. The total magnification of 400 x was used.

## Sirius UV-metric method and Sirius CheqSol method

### Preparation of 0,06 M potassium chloride solution

On Sirius T3 instrument, which is shown in Figure 7, the Sirius UV-metric method and the Sirius CheqSol method typically require 0,15 M potassium chloride (KCl) solution as a bulk medium for titration. But since it is reported that indometacin agglomerates at a high ionic strength of medium/solution (34), we decided to run our experiments in low ionic strength conditions in order to avoid this phenomenon. Instead of 0,15 M KCl solution, we used 0,06 M KCl solution. We chose this ionic strength of the solution based on experiences of researchers from Sirius Analytical Inc, Beverly, MA, USA and from Sirius Analytical Ltd, Forest Row, East Sussex, UK, who also studied pKa value and solubility of indometacin through the same methods (35).

100 mL of the 0,06 M KCl solution was prepared according to the following procedure: first we weighed 0,4473 g of KCl into the 100 mL volumetric flask and then we filled up the volumetric flask with purified water to the mark on the neck of it and mixed its content on a magnetic stirrer.

### Sirius UV-metric method

pKa of pure indometacin was determined through this method.

First we prepared 1 mL of the 10 mM sample solution of indometacin and dimethyl sulfoxide (DMSO was added to help ensure that the drug remained in solution during the titration) according to the following procedure: we weighed 3,6 mg of the API into a small beaker in which we then pipetted (with an automatic pipette) 1 mL of DMSO. After this we pipetted (with an automatic pipette) 10 μL of the sample solution into a glass vial, and this amount/volume of the sample solution was then used for the measurement. The experiment was run at the temperature of 25°C.

Sirius T3 instrument was used. There were two important facts which influenced the settings we chose for the experiment. The first fact was that indometacin is a weak acid, which means that it is more soluble in basic conditions, and because of this we set the direction of the titration from high to low pH. The second fact we had to consider was that indometacin decomposes at a high pH to form p-chlorobenzoic acid and 5-methoxy-2-methyl-3-indoleacetic acid (36). Since the usual pH range in which titrations are performed

on the instrument is between 2 to 12, we had to change the maximal pH of the titration to 9.

### Sirius CheqSol method

Since CheqSol (*Ch*asing *Eq*uilibrium *Sol*ubility) method is not yet a widely known method, a short presentation of it follows. CheqSol method is an acid-base titration method developed by Sirius Analytical and used for measuring *kinetic* (the concentration of the substance in the solution when an induced precipitate first appears) and *intrinsic solubility* (the equilibrium solubility of the unionised/neutral form of the substance at the pH where the substance is fully unionised; this is the pH, which is at least 2 units below the pKa of an acid and at least 2 units above the pKa of a base, respectively) of ionisable drug molecules (37).

The CheqSol method consists of the following stages:

- **Dissolution:** a measured volume of bulk solution is added into a glass vial and after this a measured volume of either base or acid titrant (depending on whether the sample is an acid or a base) is added to adjust the pH of the solution to a pH at which the sample is fully dissolved in its ionized form.
- **Seeking precipitation:** measured aliquots of a titrant which would induce precipitation of the neutral species are added. Occurrence of precipitation is detected by a spectroscopic dip probe.
- **Additional precipitation:** additional aliquots of the same titrant are added until pH value changes for a 0,1 pH unit or until 60 seconds pass in order to ensure that adequate precipitation is present for the next step.
- **Chasing equilibrium:** the solution is twisted from *supersaturated* (the concentration of dissolved neutral species is bigger than the intrinsic solubility; the sample is precipitating) to *subsaturated* (the concentration of dissolved neutral species is smaller than the intrinsic solubility; the sample is dissolving) and back again several times by changing the pH with rotating addition of measured aliquots of acid and base titrant. This way, the solid and the solution are in equilibrium much faster (usually it takes around 20 minutes) than in conventional methods used for solubility determinations, and this is one of the main advantages of this method. Instrument determines the equilibrium pH and uses this value in the procedure of data processing.

- **Redissolution:** the pH is changed to a value at which the sample is fully ionized so that the sample completely dissolves in order to ensure that no crystals or solid residues remain on the instrument
- **Data processing:** software determines kinetic and intrinsic solubility of the sample (38).

Kinetic and intrinsic solubilities of pure indometacin, spray dried samples of all three mass ratios, and cryomilled extrudates of all three mass ratios were determined by this method. Measurements for each sample were performed in triplicate, the average values are reported.

Based on the theoretical drug content, we calculated how much of each sample we needed to weigh in each glass vial, so that we theoretically had 1±0,5 mg of indometacin in each glass vial, and for each measurement, respectively. Titrations of pure indometacin were run in the presence of 50 µL of DMSO (with a DMSO concentration of about 2% v/v), which was added manually into glass vials before the experiment started to aid dissolution of the sample. As described above, it is necessary that the sample is completely dissolved in the first stage of the experiment, but since indometacin does not fully dissolve at pH 9, we had to add cosolvent. *Comer et al.* report that this amount of DMSO should not have a significant effect on the measured solubility values of most compounds (36). All experiments were run at the temperature of 25°C.

The same instrument and the same experimental conditions regarding the direction of the titration and the maximal pH of the titration were used as in the experiment in which the pKa of indometacin was determined (details and reasons for choosing these experimental conditions in both methods were the same and they are described above in the paragraph about Sirius UV-metric method).

**Figure 7**: Sirius T3 instrument.
The sample position in the instrument was covered with a cap during each measurement because indometacin is light-sensitive.

## Dissolution study

### Preparation of 0,1 M hydrochloric acid

At the beginning we prepared 1000 mL of the 0,1 M hydrochloric acid (HCl), which we then used as a component of the solvent which we needed for the standard curve determination and as a dissolution medium for the dissolution testing.

0,1 M HCl was prepared according to the following procedure: first we poured approximately 900 mL of purified water into the 1000 mL volumetric flask, in which we then pipetted (with a graduated pipette) 8,3 mL of 37% (w/w %) HCl. In the end we filled up the volumetric flask with purified water to the mark on the neck of it and mixed its content on a magnetic stirrer.

### Standard curve determination

In our study we have investigated 50 mg immediate-release capsules. If a patient takes a 50 mg capsule with 250 mL of water, the concentration of the API in water is 200 µg/mL. Considering this, we prepared 50 mL of the stock solution with a concentration of 200

µg/mL according to the following procedure: at the beginning we predissolved 100 mg of indometacin in 5 mL of DMSO. 500 µL of this solution was then pipetted (with an automatic pipette) into a 50 mL volumetric flask. Mixture of methanol (MeOH) and 0,1 M HCl, with a mass ratio of MeOH:0,1 M HCl = 3:7 and pH = 1,1, was then poured into the volumetric flask to the mark on the neck of it. Standard solutions with concentrations of 160 µg/mL, 120 µg/mL, 80 µg/mL, 40 µg/mL, and 20 µg/mL were prepared with dilution of the stock solution.

UV-visible spectrophotometer Agilent 8453 (Figure 8) was used for spectrophotometric measurements. The wavelength for detection of drug amount was chosen based on the measurement of absorbance maximums of the API: the highest absorbance maximum of indometacin was measured at the wavelength of 261 nm, and this was therefore the wavelength which was used in all our measurements. Absorption spectra of the sample solutions were measured against the reference solution (aforementioned mixture of MeOH and 0,1 M HCl) using 1,0 mm light-path length cuvettes. From the absorption spectra achieved with standard solutions we drew the standard curve and calculated the equation of the curve and $R^2$, which was 0,9996.

## Dissolution testing

Miniaturized dissolution apparatus based on the USP Apparatus 2 (Figure 8) was used for the dissolution testing. The following samples were tested in the dissolution study: pure indometacin, cryomilled quench cooled pure indometacin, cryomilled physical mixtures of all three mass ratios, spray dried samples of all three mass ratios, and cryomilled extrudates of all three mass ratios. Concentration of dissolved substance was determined for each of them. Dissolution studies for each sample were performed in triplicate, the average values are reported. At the beginning (first few seconds) of each dissolution test the values of measured concentrations of the sample were negative. These negative values were not presented in the graphs.

Considering the fact that the volume of the dissolution medium was 20 mL, we calculated, based on theoretical drug content, how much of each sample we needed to weigh in each capsule, so that we could theoretically achieve the concentration of 200 µg/mL (this is a theoretical concentration of indometacin - 50 mg of indometacin in 250 mL of water - in a digestive system of a patient, which has been already calculated above). As mentioned at the beginning of this paragraph, the dissolution medium was 0,1 M HCl.

The apparatus was covered with aluminium foil during each dissolution test because indometacin is light-sensitive. Parameters used in dissolution testing are presented in Table VI. UV-visible spectrophotometer Agilent 8453 was used to quantify the amount of dissolved indometacin. The wavelength for detection of drug amount was 261 nm. Absorption spectra of the sample solutions were measured against the reference solution (0,1 M HCl) using 1,0 mm light-path length cuvettes.

**Table VI**: Parameters used in dissolution testing.

| **Parameter** | **Concentration, composition, volume, temperature of dissolution medium** | **Rotation speed of vessels** | **Duration of each dissolution test** | **Size and volume of each capsule** |
|---|---|---|---|---|
| | 0,1 M HCl, 20 mL, 37°C | 75 rpm | 180 min | size 4, 0,2 mL |

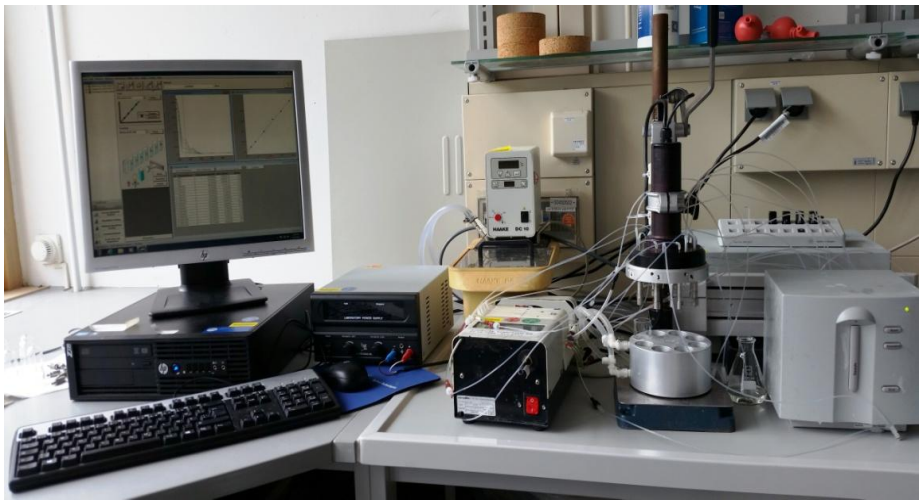


**Figure 8**: Miniaturized dissolution apparatus based on USP Apparatus 2 and UV-visible spectrophotometer Agilent 8453.

# 4. RESULTS AND DISCUSSION

## 4.1. XRPD MEASUREMENTS

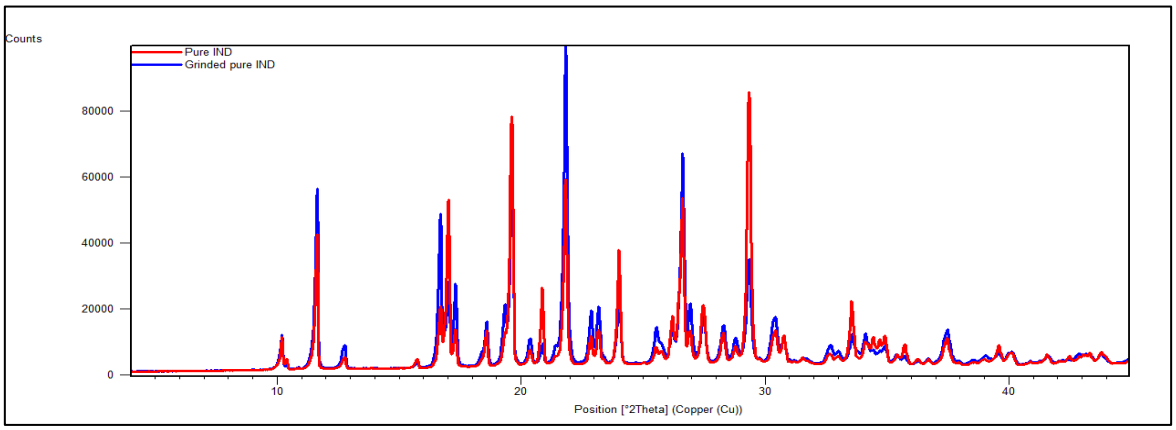



**Figure 9**: XRPD spectra of pure indometacin (red curve) and grinded (with coffee bean grinder) pure indometacin (blue curve).

There were some minor differences in the intensities of XRPD patterns of pure indometacin and grinded (with coffee bean grinder) pure indometacin (Figure 9). Both of them were crystalline. Therefore, grinding with coffee bean grinder (mass and time of grinding is presented in the previous chapter) did not have any crucial effect on indometacin in terms of its potential conversion into amorphous form or other polymorphic forms (Yamamoto reported that he had isolated α, β and γ polymorphs of indometacin (39) whereas Borka and Lin reported that they had found at least four polymorphic solid forms of indometacin (40, 41)).

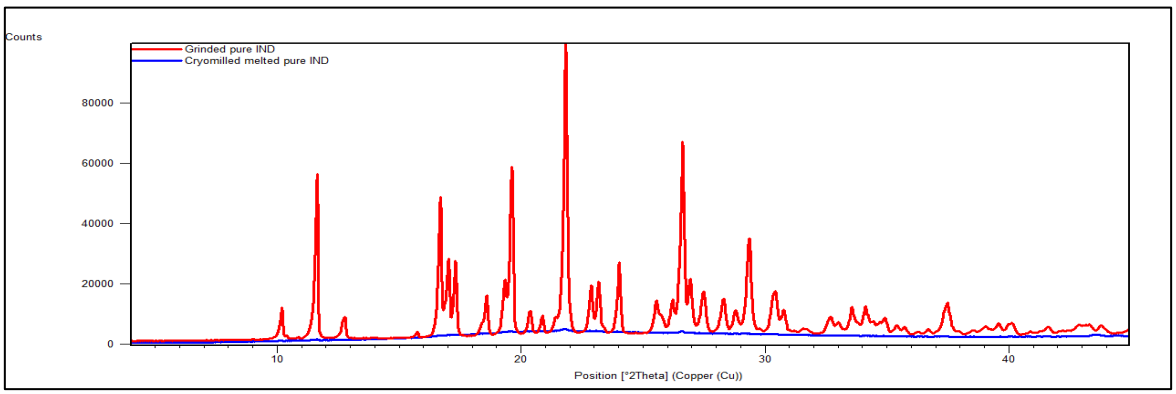



**Figure 10**: XRPD spectra of grinded (with coffee bean grinder) pure indometacin (red curve) and cryomilled quench cooled pure indometacin (blue curve).

A few smaller peaks in the XRPD spectrum of cryomilled quench cooled pure indometacin (Figure 10) indicated that a small percentage of remaining crystals of indometacin was still present in the sample, but most of the sample was amorphous.

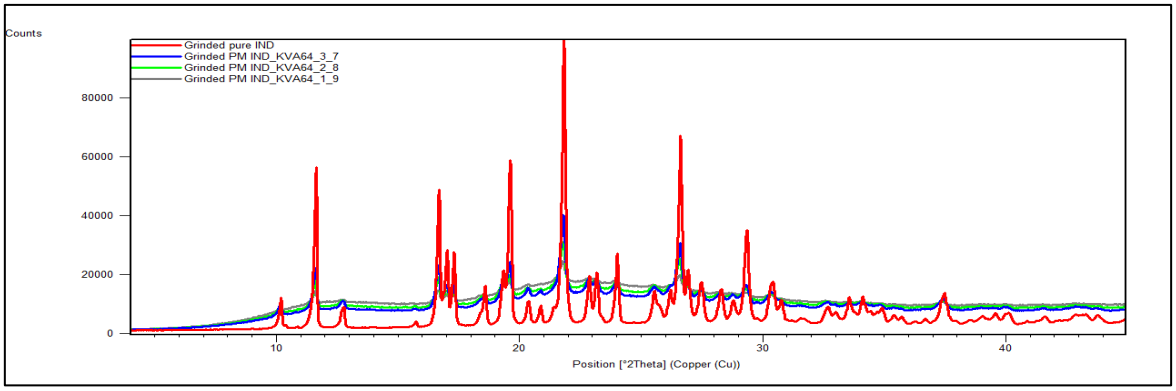



**Figure 11**: XRPD spectra of grinded (with coffee bean grinder) pure indometacin (red curve) and grinded (with coffee bean grinder) physical mixtures of all three mass ratios (blue, green, grey curves).

Comparison of the XRPD spectra of grinded (with coffee bean grinder) pure API and of grinded (with coffee bean grinder) physical mixtures (Figure 11) revealed that the presence of amorphous polymer had a great impact on the shape of curves of physical mixtures. For all physical mixtures, characteristic peaks appeared in the same positions in the XRPD graph as for pure indometacin, but the intensity of these peaks was much lower than the intensity of peaks of pure API. Based on the XRPD spectra from Figure 9, we could say that this was not a result of grinding of these samples with coffee bean grinder. Rather, this phenomenon was a consequence of the fact that physical mixtures consisted of a crystalline active substance and an amorphous polymer. In addition to this, comparison of the intensity of peaks of all physical mixtures showed that intensity was the highest for the physical mixture with the highest percent of indometacin, and the lowest for the physical mixture with the lowest percent of API – actually, characteristic peaks were almost completely absent for this grinded physical mixture. However, when we compared entire curves of all three grinded physical mixtures, we observed that the increase in the percentage of the polymer resulted in the increased extent of the amorphous hallow and the shift of the entire graph towards higher intensities, respectively.

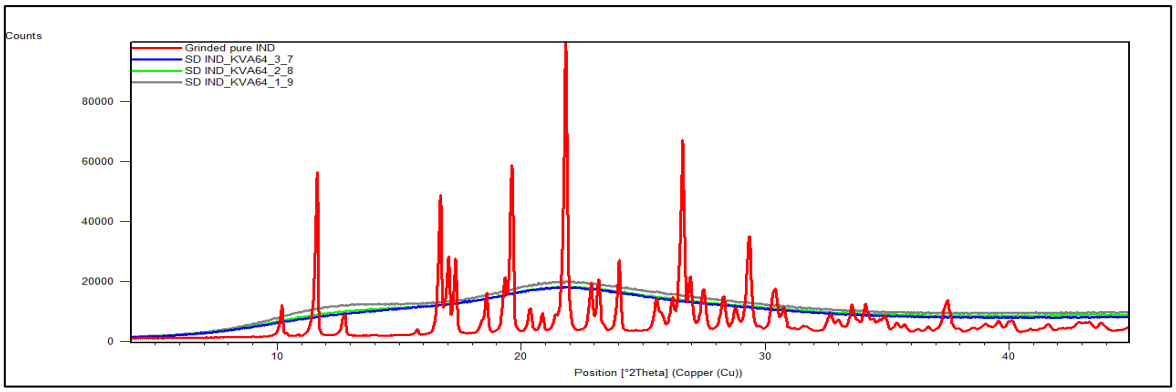



**Figure 12**: XRPD spectra of grinded (with coffee bean grinder) pure indometacin (red curve) and spray dried samples of all three mass ratios (blue, green, grey curves).

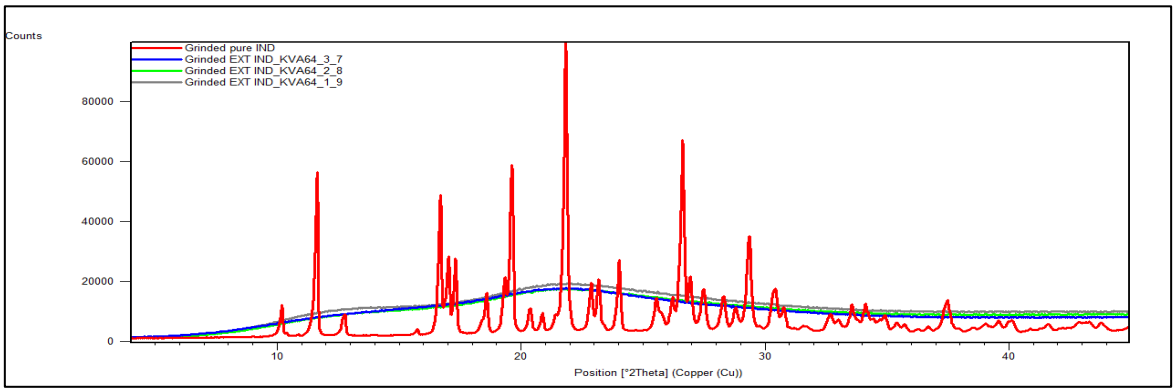



**Figure 13**: XRPD spectra of grinded (with coffee bean grinder) pure indometacin (red curve) and grinded (with coffee bean grinder) extrudates of all three mass ratios (blue, green, grey curves).

Characteristic peaks of indometacin were completely absent in the XRPD spectra of spray dried products of all three mass ratios (Figure 12), which indicates that all three samples were completely amorphous, namely in their active substance and polymer. This phenomenon was attributed to the spray drying process. Further on, comparison of the entire curves of spray dried products of all three mass ratios led to the same finding as for physical mixtures: a higher percent of polymer in the spray dried product resulted in a more pronounced amorphous hallow in the XRPD pattern. The same observations and conclusions, which were presented in this paragraph, were also valid for the grinded (with coffee bean grinder) extrudates of all three mass ratios (Figure 13). The proof that the disappearance of characteristic peaks of indometacin, which happened in the XRPD analysis of grinded extrudates, was not a result of grinding of these samples with coffee bean grinder is the XRPD graph from Figure 9. Hence, this phenomenon was ascribed to the hot-melt extrusion process.

## 4.2. mDSC MEASUREMENTS

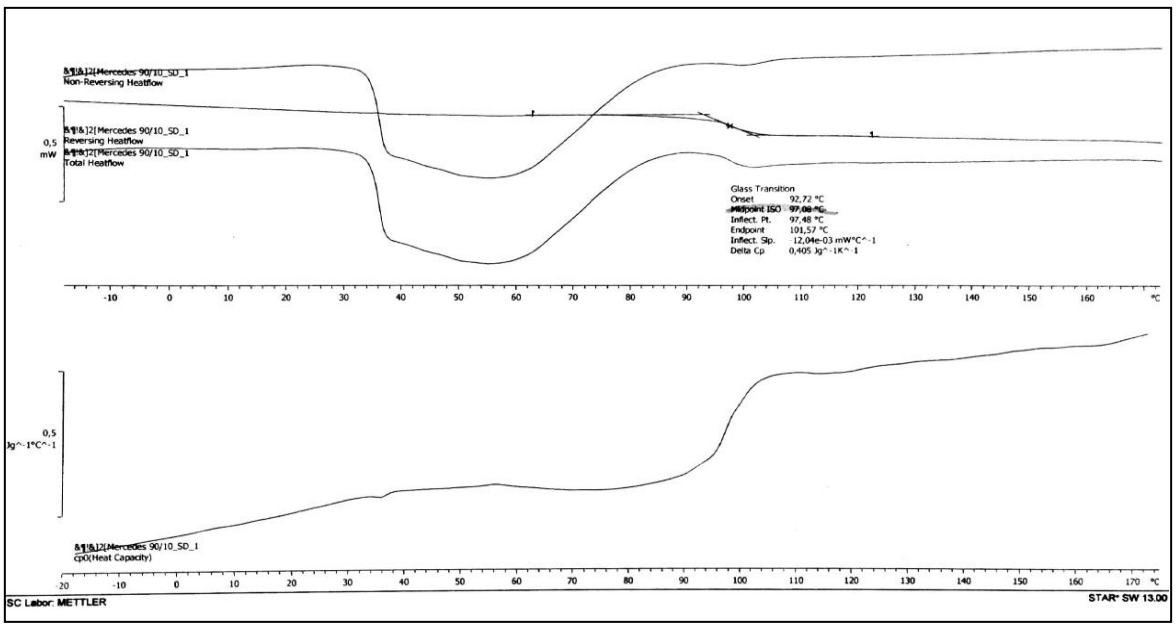



**Figure 14**: DSC plot of the first heating cycle for the spray dried product with a mass ratio IND:KVA64 = 1:9.

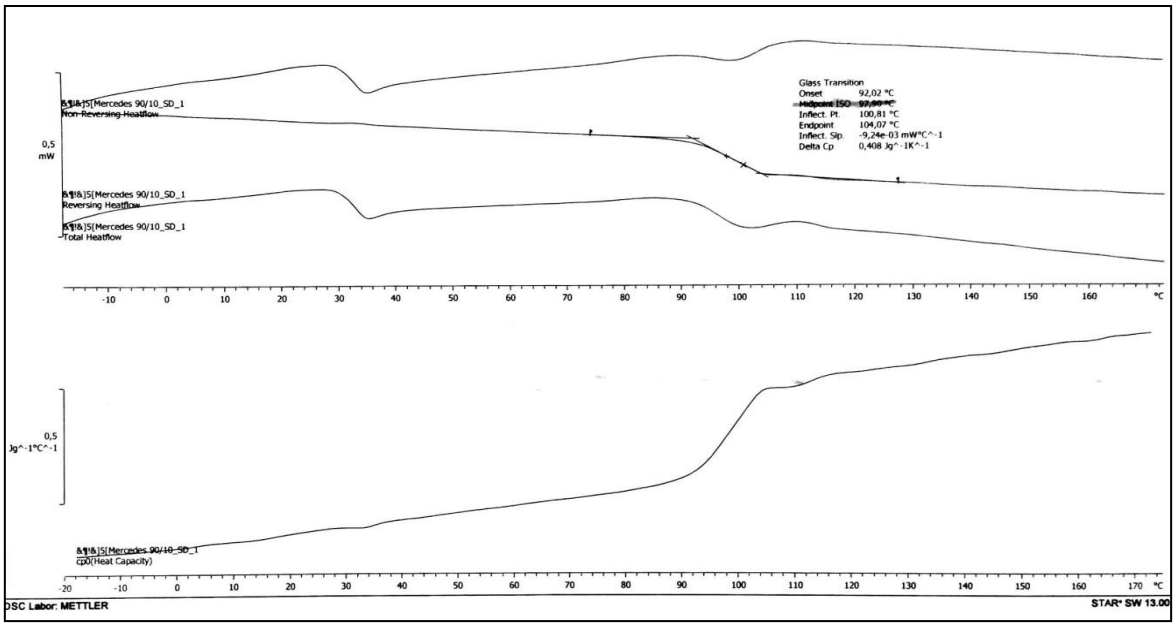



**Figure 15**: DSC plot of the second heating cycle for the spray dried product with a mass ratio IND:KVA64 = 1:9.

Since the shapes of the curves in DSC plots of all solid dispersions were similar, a plot of only one solid dispersion is presented as an example. Figure 14 and Figure 15 show DSC plots of the first and the second heating cycle for the spray dried product with a mass ratio IND:KVA64 = 1:9. The bigger peak between 30 and 70°C from the first DSC plot (Figure

14) and the smaller peak between 30 and 40°C from the second DSC plot (Figure 15) were attributed to the relaxation process related to the stress during production. The small curves around 100°C from the first and from the second DSC plot (Figure 14 and Figure 15) revealed glass transition temperatures from the first and from the second heating cycle, respectively.

**Table VII**: Average values of glass transition temperatures of the first and the second heating cycle for spray dried products and extrudates of all three mass ratios determined by modulated differential scanning calorimetry.

| | **Glass transition temperature from the first heating cycle (°C) – average values** | **Standard deviation** | **Glass transition temperature from the second heating cycle (°C) – average values** | **Standard deviation** |
|---|---|---|---|---|
| **SD IND:KVA64 = 3:7** | **82,4** | 0,370 | **84,0** | 0,712 |
| **SD IND:KVA64 = 2:8** | **91,7** | 0,352 | **89,6** | 0,655 |
| **SD IND:KVA64 = 1:9** | **97,8** | 0,667 | **97,1** | 1,056 |
| **EXT IND:KVA64 = 3:7** | **85,9** | 0,291 | **83,3** | 0,204 |
| **EXT IND:KVA64 = 2:8** | **91,2** | 0,616 | **91,2** | 0,482 |
| **EXT IND:KVA64 = 1:9** | **97,7** | 0,320 | **98,3** | 0,471 |

Table VII shows glass transition temperatures of both heating cycles for all solid dispersions. The first heating cycle was used to measure characteristics of the sample, including those related to the manufacturing process. At the end of the first heating cycle the material was heated over its glass transition temperature in order to erase its thermal

history. This way the influence of the production step was nullified, and the glass transition temperature of the second heating cycle reflected only the properties belonging to the material alone.

If we first focus only on the glass transition temperatures of the second heating cycle for all the samples, there are some important observations and conclusions which can be drawn from them. Firstly, each sample exhibited only one glass transition temperature, which indicated that miscibility between drug and polymer in each solid dispersion was high and that no phase separation occurred in any of them (42). Secondly, comparison of glass transition temperatures of our solid dispersions with the glass transition temperature of polymer, which is 107,63°C (see subchapter "Kollidon® VA 64"), revealed that glass transition temperatures of all solid dispersions were lower compared to the glass transition temperature of Kollidon® VA 64. This was attributed to the plasticizing effect of drug on polymer (42). In addition, we also observed that the glass transition temperatures of all samples decreased as a function of increasing drug loading. This was again ascribed to the plasticizing effect of the API.

Furthermore, if we now focus on the comparison of the glass transition temperatures from both heating cycles, this reveals how the production processes influenced our samples. As it can be seen from Table VII, in the case of spray dried products there was no major difference in glass transition temperatures from both heating cycles, whereas in the case of extrudates there was a small difference in both glass transition temperatures. To be specific, glass transition temperature from the second heating cycle was on average one degree lower than glass transition temperature from the first heating cycle. We assume that this was due to the fast cooling to which extrudates were exposed after they left the extruder, resulting in tensed polymeric structures. Based on these results we concluded that in terms of glass transition temperatures there were no major differences between the amorphous state of the spray dried samples and the amorphous state of the extrudates.

## 4.3. LASER DIFFRACTION MEASUREMENTS

Laser diffraction method was used to determine the average particle size of the spray dried product with a mass ratio IND:KVA64 = 1:9 and this was then the reference value according to which we cryomilled samples, which are listed in the subchapter "Cryogenic milling of samples with ball mill". The reason for cryomilling is also presented in this

subchapter. Results of the particle size measurements with laser diffraction method are presented in Table VIII.

**Table VIII**: Results of the particle size measurements with a laser diffraction method for the spray dried product with a mass ratio IND:KVA64 = 1:9.
D10/D50/D90 – 10/50/90 % of all particles in the sample are smaller than this diameter.

| | Average value (µm) | Standard deviation |
|---|---|---|
| **D10** | 1,32 | 0,0306 |
| **D50** | 4,68 | 0,0451 |
| **D90** | 11,62 | 0,173 |
| **Volume based mean diameter** | **5,88** | 0,0757 |

## 4.4. SIRIUS UV-METRIC AND SIRIUS CHEQSOL MEASUREMENTS

pKa of indometacin was required for Sirius CheqSol solubility determinations. A value of 4,01, I = 0,06 M, 25°C was determined through Sirius UV-metric method.

Figure 16 and Figure 17 show CheqSol graphs that represent changes in concentration of neutral species that occurred when kinetic and intrinsic solubilities of a pure indometacin and a spray dried product with a mass ratio IND:KVA64 = 1:9, respectively, were measured by a CheqSol method. The blue star indicates the beginning of the experiment. The red triangles signify the addition of acid (HCl) titrant, the blue triangles indicate the addition of basic (KOH) titrant, and the black circle symbolizes the state when no titrant is added. The pink circle denotes the onset of precipitation.

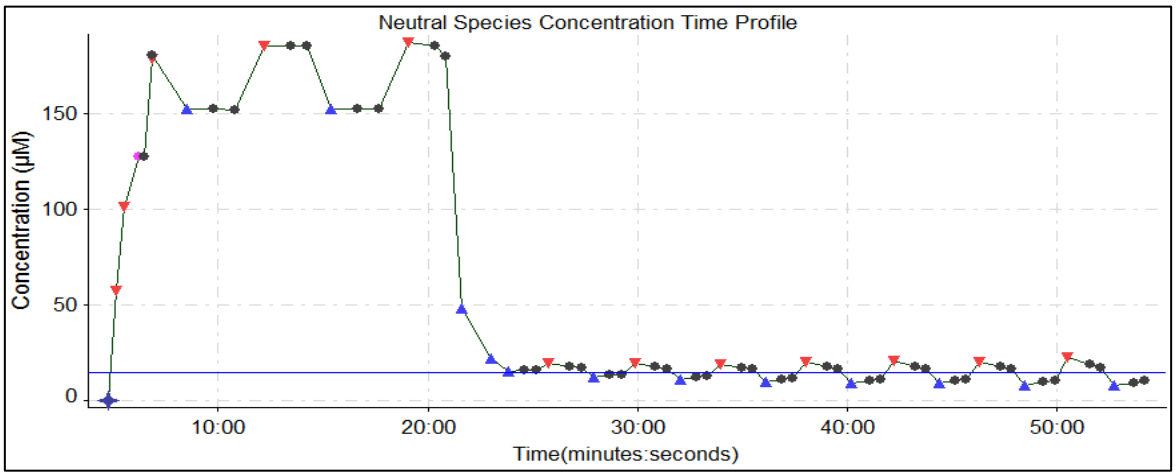



**Figure 16**: CheqSol experiment graph: neutral species concentration profile for pure indometacin.

Graph from Figure 16 indicates that changes in concentration of neutral species occurred after the onset of precipitation of pure indometacin (pink circle). We predicted that indometacin initially precipitated into a more soluble metastable crystalline form. However, since metastable form of any drug is thermodynamically not stable, a conversion into a thermodynamically more stable form usually occurs (39). The change in neutral species concentration that happened 20 minutes after our experiment began was attributed to this occurrence. We suggested that the initial metastable crystalline form (plateau on the left side of the graph) of indometacin converted into a more stable crystalline form (plateau on the right side of the graph). Similar observations and findings have been already reported by *Comer et al.* (36).

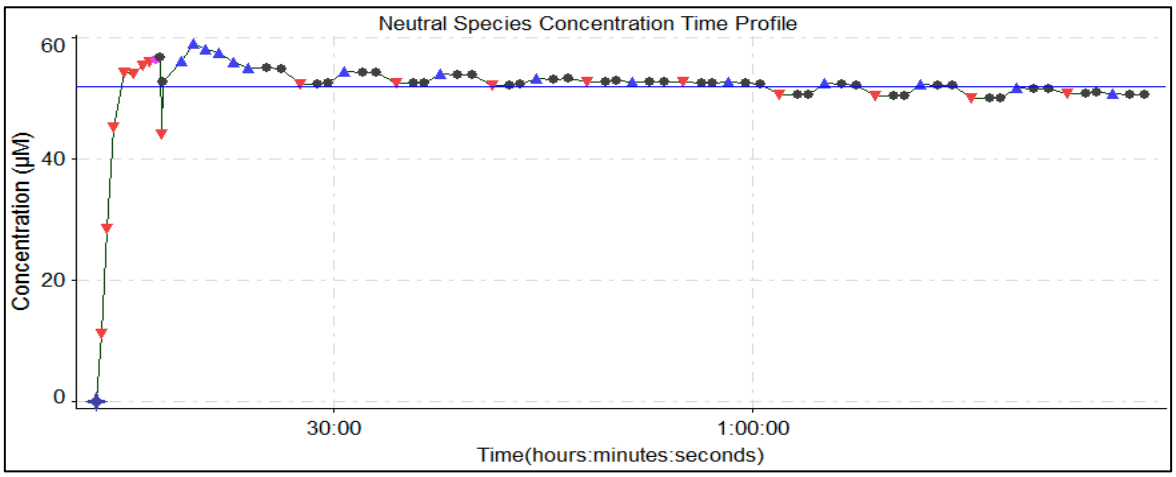



**Figure 17**: CheqSol experiment graph: neutral species concentration profile for spray dried product with a mass ratio IND:KVA64 = 1:9.

Since the shapes of the curves in CheqSol graphs of all solid dispersions were almost identical (with the exception of one spray dried product, which is explained hereinafter), a graph of only one solid dispersion is presented (Figure 17) and is used as an example to show what was happening during CheqSol experiments of all solid dispersions. Graph from Figure 17 reveals that after the onset of precipitation and during chasing equilibrium, respectively, no major changes in the concentration of the neutral species occurred. Therefore, we suggest that in the case of solid dispersions there was no crossing between different forms of indometacin, even though amorphous state of the drug has a higher free energy and because of this it tends to spontaneously transform into more stable crystalline form (43). Inhibition of the crystallization of the drug in solution was probably achieved due to the polymer. Based on these findings, we concluded that the polymer played an

important role in terms of the stabilization of amorphous state of the drug in solid dispersions during CheqSol experiments.

Numerical results of CheqSol experiments for pure indometacin, spray dried products of two mass ratios, and cryomilled extrudates of all three mass ratios are presented in Table VIII. Results of the spray dried product with a mass ratio IND:KVA64 = 2:8 sample are not presented because unexplained extreme deviations in the graphs that we got for this sample occurred, and due to this, software was not able to convert them into numerical values. Measurements of this sample were performed six times, but none of them was successful.

**Table IX**: Average values of precipitation pH-s and kinetic and intrinsic solubilities of pure indometacin, spray dried products of two mass ratios, and cryomilled extrudates of all three mass ratios determined by CheqSol method.

| | **pH of precipitation – average values** | **Standard deviation** | **Kinetic solubility – average values (µg/mL)** | **Standard deviation** | **Intrinsic solubility – average values (µg/mL)** | **Standard deviation** |
|---|---|---|---|---|---|---|
| **Pure IND (crystalline)** | **5,48** | 0,148 | **36,5** | 8,11 | **4,44** | 0,922 |
| **SD IND:KVA64 = 3:7** | **3,76** | 0,392 | **25,3** | 10,2 | **15,2** | 0,721 |
| **SD IND:KVA64 = 2:8** | **/** | / | **/** | / | **/** | / |
| **SD IND:KVA64 = 1:9** | **4,69** | 0,175 | **20,0** | 0,167 | **18,0** | 0,822 |
| **Cryomilled EXT IND:KVA64 = 3:7** | **3,57** | 0,172 | **16,3** | 0,483 | **16,3** | 0,483 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Cryomilled EXT IND:KVA64 = 2:8** | **4,39** | 0,962 | **15,7** | 1,60 | **17,9** | 0,524 |
| **Cryomilled EXT IND:KVA64 = 1:9** | **4,70** | 0,380 | **18,5** | 3,71 | **21,3** | 1,33 |

Interestingly, the data from Table VIII reveal that the kinetic solubility of pure indometacin was relatively high. Since standard deviation for this value was also high, we knew that this result was not very reliable. Nevertheless, we could observe that kinetic solubilities of solid dispersions were certainly lower than kinetic solubility of the pure drug. According to the definition, kinetic solubility is the concentration of the neutral species of the drug at the onset of precipitation (37). As can be seen from Table VIII, indometacin from all solid dispersions precipitated at a lower pH in comparison to the pure indometacin. This phenomenon was related to the presence of hydrophilic polymer in solid dispersions. However, since indometacin is a weak acid, it is less soluble at a lower pH, and this led to the apparent decrease in kinetic solubility in case of solid dispersions. In addition, data from Table VIII also showed that the increase in the kinetic solubilities of solid dispersions correlated with the increase in polymer loading. This observation can be again clarified with the above presented explanation, because the precipitation pH also increased with the polymer loading.

Regarding the intrinsic solubilities, which are also presented in Table VIII, we see that intrinsic solubilites of all solid dispersions were higher than intrinsic solubility of the pure indometacin. According to the definition, intrinsic solubility is the equilibrium solubility of the neutral form of the drug at a pH where the drug is fully unionised (37). Since formulation of poorly soluble drugs as solid dispersions is one of the strategies to improve their solubility, this result was expected and understood. Furthermore, results presented in Table VIII also showed that intrinsic solubilities of solid dispersions increased as a function of increasing polymer loading, which indicated that the polymer in solid dispersions played a key role in terms of improved solubility.

## 4.5. DISSOLUTION STUDY

Miniaturized dissolution apparatus based on the USP Apparatus 2 was used in the dissolution study. The dissolution medium was 0,1 M HCl. The maximal theoretical concentration of dissolved indometacin was 200 µg/mL.

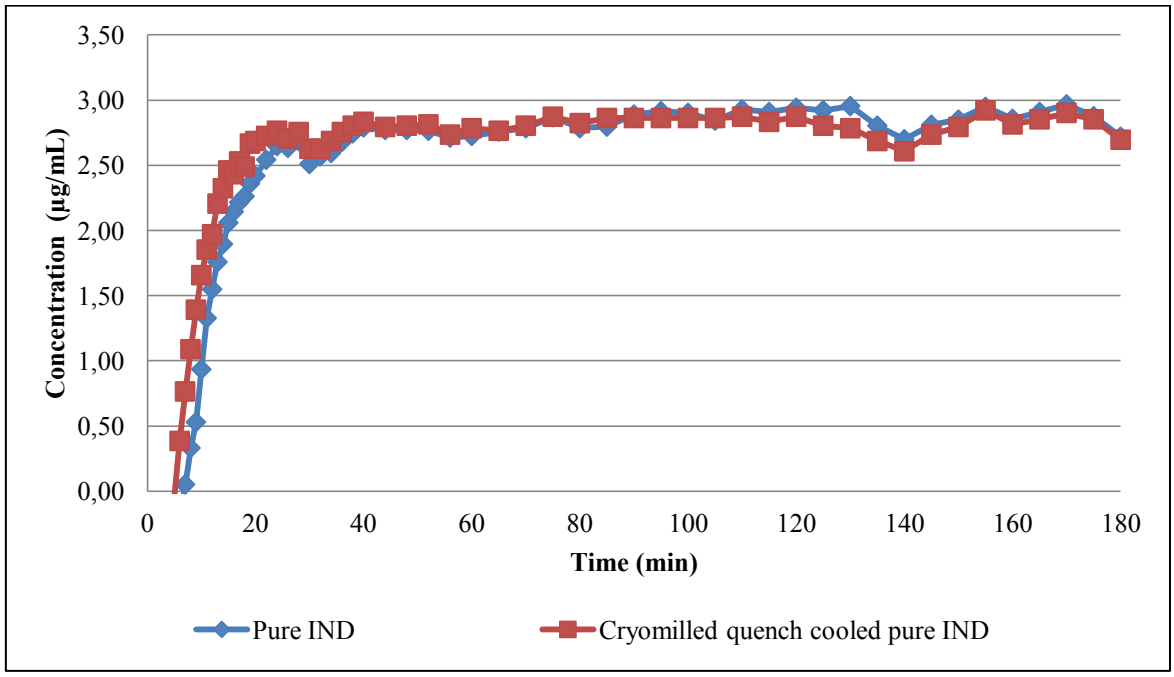



**Figure 18**: Dissolution profiles of pure indometacin and cryomilled quench cooled pure indometacin.

Since indometacin is a weak acid we expected that its crystalline form would exhibit very low dissolution at the pH of the chosen dissolution medium. Results of the dissolution test confirmed this expectation (Figure 18). In contrast, since XRPD analysis of the cryomilled quench cooled pure drug showed that this sample was mostly amorphous (see Figure 10 and text below the figure), we expected that it would exhibit enhanced dissolution rate compared to the pure drug in crystalline form, but the dissolution study did not confirm this assumption. Interestingly, dissolution of this sample was very poor. Moreover, the overall dissolution performance of it was actually comparable to the dissolution behaviour of pure indometacin in a crystalline form (Figure 18). These results indicated that the amorphous state of indometacin alone was not controlling the improvement of the dissolution of the drug.

Similar results have already been reported in the literature. Greco *et al.* examined how different processing methods and the presence of crystalline form of the drug affect

dissolution performance of amorphous indometacin. Compacts of amorphous indometacin, prepared by various methods of amorphization (including melt quenching followed by cryogenic milling), exhibited a decrease in the dissolution rate soon after the dissolution test started. This phenomenon was attributed to a solution mediated phase transformation to less soluble crystalline forms of indometacin. Moreover, the research group also found out that even a small fraction of crystalline impurity led to a faster recrystallization of the samples (44). Based on these findings, we suggest that cryomilled quench cooled pure indometacin (mostly amorphous indometacin), which we used in our study, also might have recrystallized during the dissolution test.

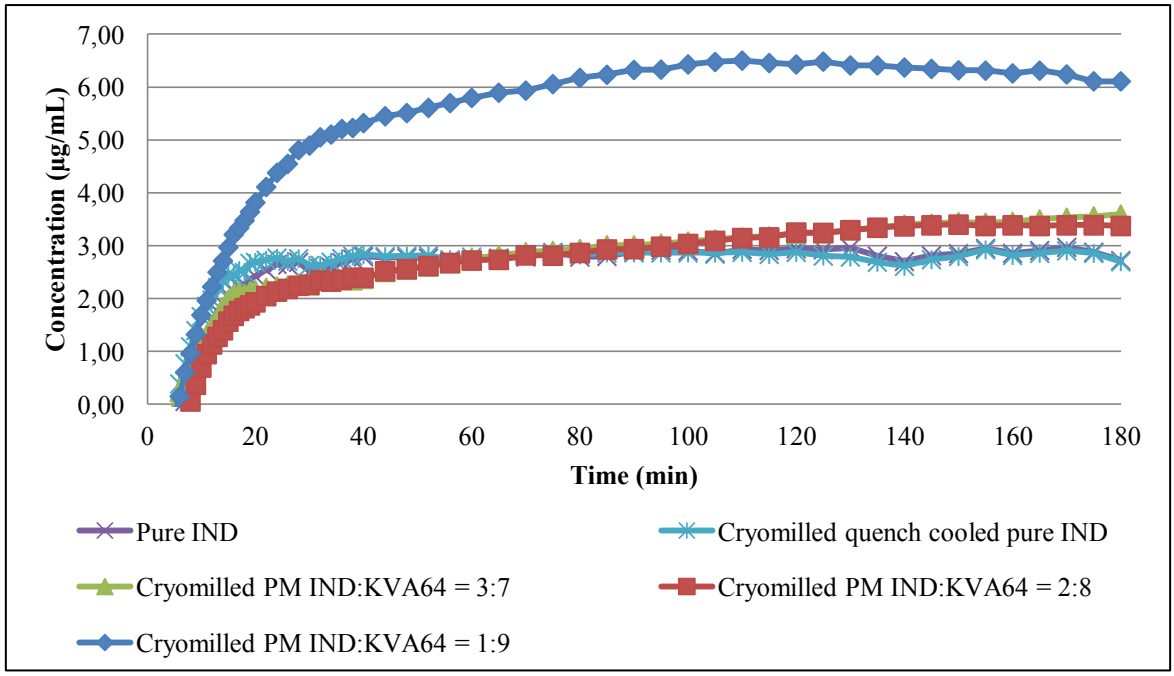



**Figure 19**: Dissolution profiles of pure indometacin, cryomilled quench cooled pure indometacin, and cryomilled physical mixtures of all three mass ratios.

Regarding the dissolution of physical mixtures, which is presented in Figure 19, a very slow release of indometacin was obtained for all three of them. Dissolution profiles of cryomilled physical mixtures with a mass ratio IND:KVA64 = 3:7 and with a mass ratio IND:KVA64 = 2:8 were very similar to the dissolution profiles of pure API (crystalline form of IND) and cryomilled quench cooled pure API (mostly amorphous form of IND). Cryomilled physical mixture with a mass ratio IND:KVA64 = 1:9 exhibited slightly higher concentration of dissolved drug, but the ascent was not significant. Since there was no large improvement in dissolution of all three physical mixtures, which consisted of

crystalline indometacin and hydrophilic polymer, we proved that the presence of highly water-soluble polymer was also not responsible for the change in the dissolution behaviour of the API.

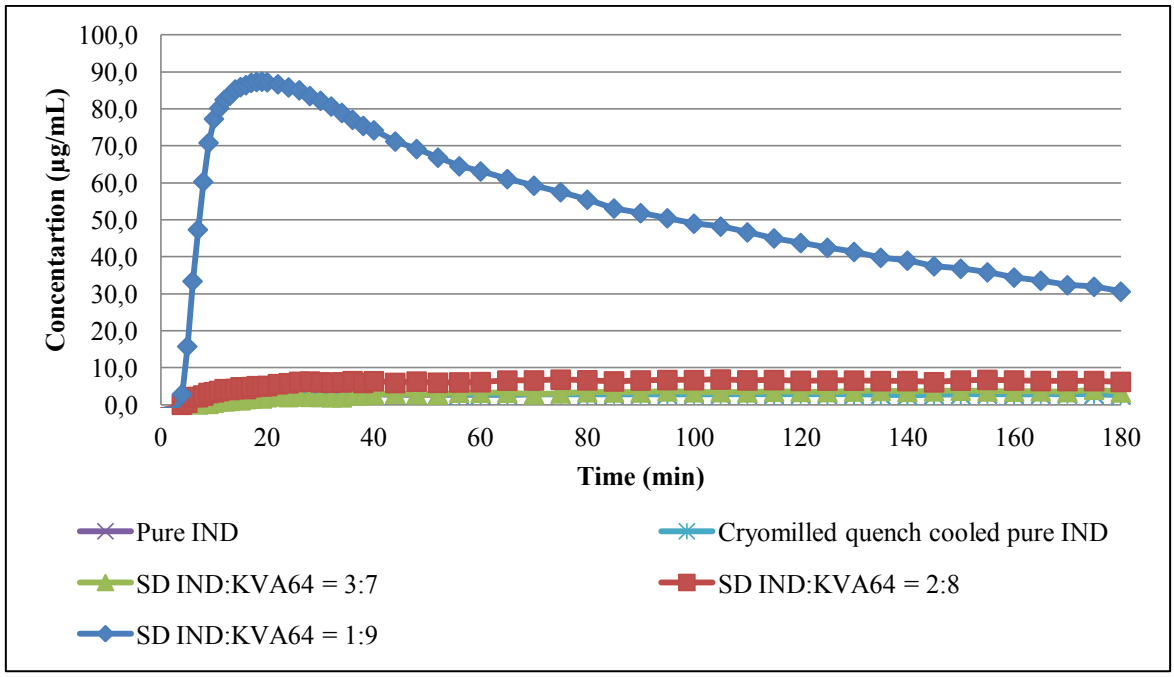



**Figure 20**: Dissolution profiles of pure indometacin, cryomilled quench cooled pure indometacin, and spray dried samples of all three mass ratios.

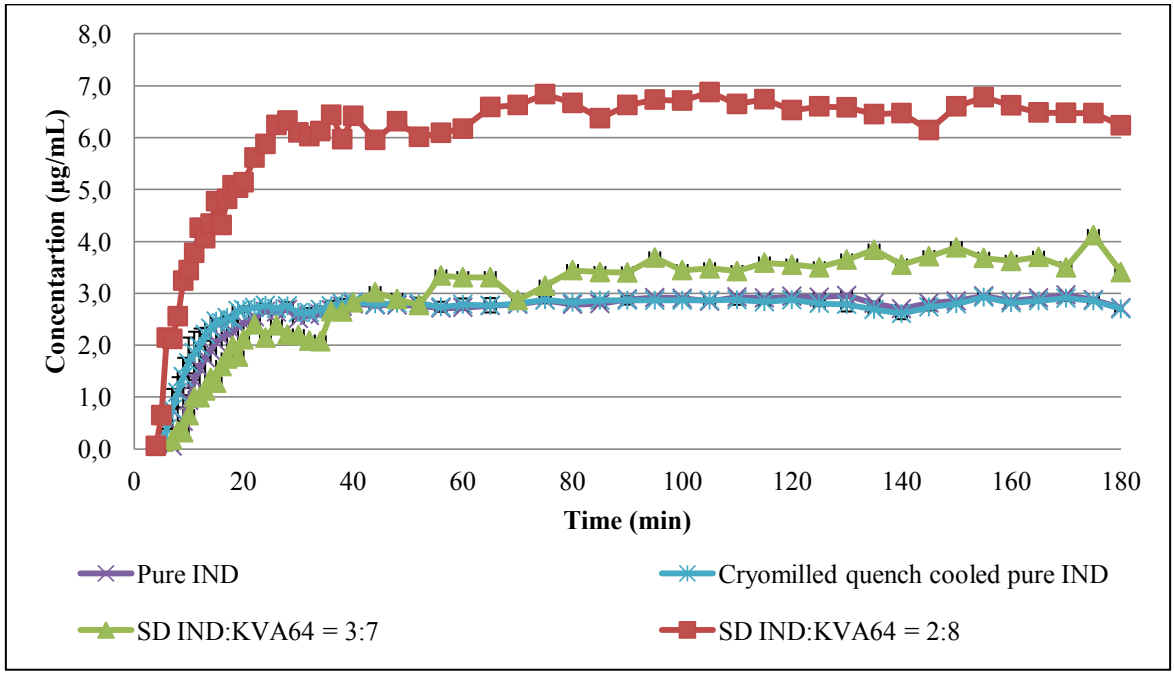



**Figure 21**: Enlarged part of the figure 20, namely the dissolution profiles of pure indometacin, cryomilled quench cooled pure indometacin, and spray dried samples with mass ratios IND:KVA64 = 3:7 and IND:KVA64 = 2:8.

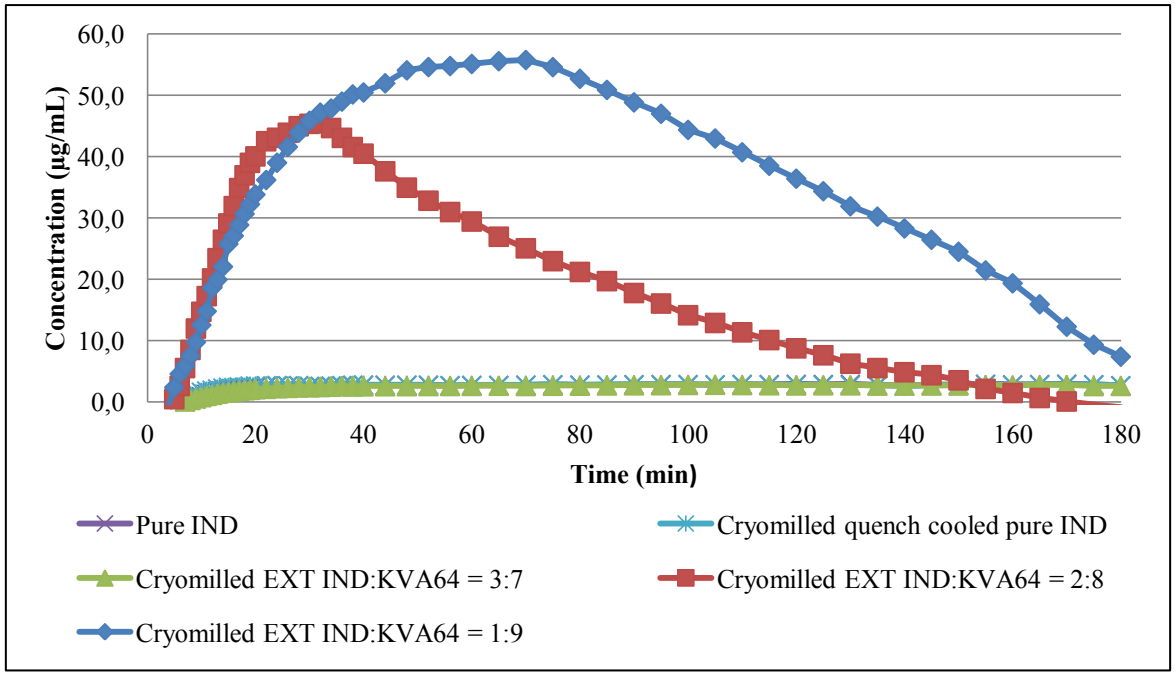



**Figure 22**: Dissolution profiles of pure indometacin, cryomilled quench cooled pure indometacin, and cryomilled extrudates of all three mass ratios.

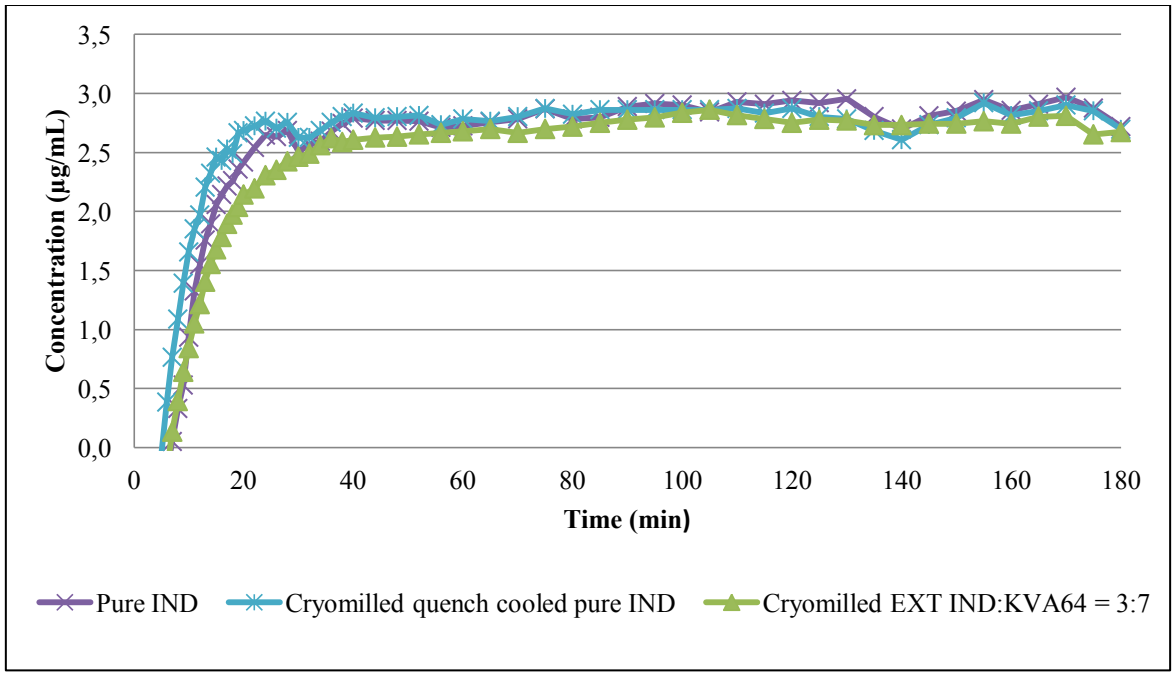



**Figure 23:** Enlarged part of the figure 22, namely, pure indometacin, cryomilled quench cooled pure indometacin, and cryomilled extrudates with a mass ratio IND:KVA64 = 3:7.

Dissolution kinetics of all three spray dried products are depicted in Figure 20 and Figure 21. Spray dried formulations with a mass ratio IND:KVA64 = 3:7 and with a mass ratio IND:KVA64 = 2:8 showed very slow release of the API. Dissolution behaviour of spray

dried product with the highest drug loading was comparable to dissolution performances of pure indometacin (crystalline form of IND) and cryomilled pure indometacin (mostly amorphous form of IND). Dissolution behaviour of formulation with a mass ratio IND:KVA64 = 2:8 was slightly better, but still negligible. In contrast, spray dried formulation with a mass ratio IND: KVA64 = 1:9 showed a fast and highly enhanced initial drug release followed by a slow decrease in drug concentration.

The results of the dissolution study of all three extrudates are represented in Figure 22 and Figure 23. The concentration of released indometacin from cryomilled extrudates with a mass ratio IND: KVA64 = 3:7 was very low. The dissolution profile of this sample was also similar to the dissolution profiles of pure indometacin (crystalline form of IND) and cryomilled pure indometacin (mostly amorphous form of IND). In contrast, the other two cryomilled extrudates exhibited a fast and greatly enhanced dissolution. Interestingly, the shape of their curves differed. Cryomilled extrudates with a mass ratio IND:KVA64 = 1:9 reached the maximum concentration later and their drop in drug concentration was much slower than for cryomilled extrudates with a mass ratio IND:KVA64 = 2:8. Besides, among all the extrudates, the ones with the lowest drug loading reached the highest maximum concentration of released indometacin. However, among all the samples tested in this dissolution study, the spray dried sample with a mass ratio IND: KVA64 = 1:9 reached the highest concentration of released drug.

From the results presented above, we concluded that the drug-polymer ratio had a great impact on dissolution behaviour of indometacin in solid dispersions. The drug was released only from formulations with a high amount of highly water-soluble polymer, pointing to a polymer-controlled release mechanism (45).
Similar findings have been previously observed. *Tres et al.* examined the impact of the drug-polymer ratio on the dissolution behaviour of indometacin-Kollidon® VA 64 extrudates. Compacts of extrudates with different drug loading showed different dissolution properties. Only the 5 % extrudates were completely dissolved, whereas the 15% and 30% extrudates did not entirely dissolve. The research group found that the 5 % extrudates remained fully hydrated during the entire dissolution experiment, and that explained their good dissolution performance. In contrast, they got different observations for the 15% and 30% extrudates, which clarified their poor dissolution behaviour. They

found that Kollidon® VA 64 depleted during the dissolution test and that an amorphous and hydrophobic drug-rich shell was formed *in situ* (46). These observations led us to the assumption that enhanced wettability caused by Kollidon® VA 64 was one of the crucial mechanisms according to which dissolution performance of our solid dispersions was improved, and because of this a sufficient amount of polymer in our products was needed for the advanced dissolution of indometacin.

# 5. CONCLUSION

Within the scope of this study, we prepared indometacin and copovidone solid dispersions with three different drug loadings through spray drying and hot-melt extrusion, and we evaluated their properties through various analytical methods. During our work we came to the following observations, which can serve as a starting point for future research of indometacin and copovidone solid dispersions.

X-ray powder diffraction method showed that all solid dispersions of indometacin and copovidone prepared by spray drying and hot-melt extrusion technique were completely amorphous. Therefore, we can conclude that these two methods were suitable for the amorphization of crystalline indometacin.

Single glass transition temperatures, which were determined through modulated differential scanning calorimetry, indicated that miscibility between the drug and the polymer in each solid dispersion was good and that no phase separation occurred in any of them. Besides this, we observed that glass transition temperatures of all solid dispersions were higher compared to the glass transition temperature of indometacin, and lower compared to the glass transition temperature of copovidone. This was attributed to the plasticizing effect of indometacin on the polymer. In addition, we also noticed that the glass transition temperatures of all samples decreased as a function of increasing drug loading. This was again ascribed to the plasticizing effect of the drug. Lastly, comparison of the glass transition temperatures from both heating cycles showed that in terms of glass transition temperatures there was no important difference regarding the amorphous state of the spray dried samples and the extrudates.

Sirius CheqSol method revealed that during the Sirius CheqSol measurement pure crystalline indometacin initially precipitated into a more soluble metastable crystalline form, which persisted for a short time before it converted to a more stable crystalline form. This occurrence was not observed in any of the solid dispersions. An explanation for this effect was that the polymer probably inhibited the crystallization of the drug in solution. Based on this finding, we suggested that the polymer played a key role in terms of the stabilization of the amorphous state of the drug in solid dispersions during CheqSol experiments. Regarding the kinetic solubilities, we observed that the kinetic solubility of pure indometacin was relatively high and that kinetic solubilities of solid dispersions were lower than kinetic solubility of the pure drug. Besides, kinetic solubilities of solid

dispersions increased with increasing polymer loading. These phenomena were explained with the polymer effect and the pH-s of precipitation. As for intrinsic solubilities, we observed that intrinsic solubilites of all solid dispersions were higher than the intrinsic solubility of pure indometacin, which was expected. In addition, intrinsic solubilities of solid dispersions increased as a function of increasing polymer loading, which indicated that the polymer in solid dispersions played an important role in terms of improved solubility.

The dissolution study showed that crystalline pure indometacin and mostly amorphous pure indometacin exhibited almost identical dissolution behaviour. A plausible explanation for this was that recrystallization of the amorphous indometacin occurred during the dissolution testing. In addition, there was also no large improvement in the dissolution of all three physical mixtures, which indicated that the presence of a highly water-soluble polymer was also not enough for the change in the dissolution behaviour of the API. Further, dissolution study showed that indometacin was released only from the dispersions with a high amount of the polymer, namely 10% drug-loaded spray dried product and 20% or higher drug-loaded extrudates. The fact that the API was released only from the dispersions with a high amount of the polymer pointed to the polymer-controlled release mechanism. Besides, we assumed that advanced wettability caused by the polymer was one of the crucial reasons for enhanced dissolution of indometacin. Moreover, the dissolution study also revealed that there were differences among the formulations with improved release of the API, namely in the maximal concentration of the released drug, in the time it took these samples to reach their maximal concentration of the released drug, and in the drop of the concentration of the released drug.

To conclude, we can say that the aim of the study was achieved. We prepared indometacin and copovidone solid dispersions with improved dissolution behaviour through spray drying and hot-melt extrusion. Moreover, we have also successfully evaluated and compared some of their properties.

# 6. REFERENCES


1. Reintjes T (Editor): Solubility Enhancement with BASF Pharma Polymers, Solubilizer Compendium, BASF SE, Pharma Ingredients & Services, Lampertheim, 2011: 9-38.
2. Guideline on the Investigation of Bioequivalence. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500070039.pdf. Available on: 23.5.2018.
3. Page S R: Presentation at CRS Meeting July 12.-16., 2008.
4. Noyes A A, Whitney W R: The Rate of Solution of Solid Substances in Their Own Solutions. J Am Chem Soc 1897; 19: 930-4.
5. Leuner C, Dressman J: Improving Drug Solubility for Oral Delivery Using Solid Dispersions. Eur J Pharm Bipharm 2000; 50: 47-60.
6. European Pharmacopeia, 9th Edition, Council of Europe, Strasbourg, 2016: Volume I: 761.
7. Verma S, Rawat A, Kaul M, Saini, S: Solid Dispersion: A Strategy for Solubility Enhacement. Int J Pharm Tehnol 2011; 3: 1062-99.
8. Chiou W L, Riegelman: Pharmaceutical Applications of Solid Dispersion Systems. J Pharm Sci 1971; 60: 1281-1302.
9. Sekiguchi K, Obi N: Studies on Absorption of Eutectic Mixture I. A Comparison of the Behaviour of Eutectic Mixture of Sulfatiazole and that of Ordinary Sulfatiazole in Man. Chem Pharm Bull 1961; 9: 866-72.
10. Giri T K, Kumar K, Alexander A, Ajazuddin, Badwaik H, Tripathi D K: A Novel and Alternative Approach to Controlled Release Drug Delivery System Based on Solid Dispersion Technique. Bulletin of Faculty of Pharmacy, Cairo University 2012; 50: 147-59.
11. Dhirendra K, Lewis S, Udupa N, Atin K: Solid Dispersions: A Review. Pak J Pharm Sci 2009; 22: 234-46.
12. Planinšek O. Sodobni pristopi k izdelavi trdnih disperzij z izboljšano biološko uporabnostjo učinkovin. Farm Vest 2009; 60: 169-75.
13. Vo C L, Park C, Lee B J: Current Trends and Future Perspectives of Solid Dispersions Containing Poorly Water-Soluble Drugs. Eur J Pharm Biopharm 2013; 85: 799-813.

14. Training Papers Spray Drying. Available from: https://static1.buchi.com/sites/default/files/downloads/Set_3_Training_Papers_Spray_Drying_en_01.pdf?996b2db24007502bd69c913b675467cfc63880ba. Available on: 31. 5. 2018.
15. Re M-I: Formulating Drug Delivery Systems by Spray Drying. Dry Techol 2006; 24: 433-46.
16. Paudel A, Worku Z A, Meeeus J, Guns S, Van den Mooter G: Manufacturing of Solid Dispersions of Poorly Water Soluble Drugs by Spray Drying: Formulation and Process Considerations. Int J Pharm 2013; 453:253-84.
17. Kolter K, Karl M, Gryczke A: Hot-Melt Extrusion with BASF Pharma Polymers, Extrusion Compendium, 2nd Revised and Enlarged Edition, BASF SE, Pharma Ingredients & Services, Ludwigshafen, 2012: 10-83.
18. Verhoeven E, De Beer T, Schacht E, Van den Mooter G, Remon J P, Vervaet C: Influence of Polyethylene Glycol/Polyethylene Oxide on the Release Characteristics of Sustained-Release Ethylcellulose Mini-Matrices Produced by Hot-Melt Extrusion: In Vitro and In Vivo Evaluations. Eur J Pharm Biopharm 2009; 72: 463-70.
19. Patil H, Tiwari R, Repka M A: Hot-Melt Extrusion: From Theory to Application in Pharmaceutical Formulation. AAPS Phram Sci Tech 2016; 17: 20-42.
20. Volumetric and Gravimetric Feeder. Available from: https://www.gea.com/en/products/gea-volumetric-feeder.jsp. Available on: 4. 6. 2018.
21. Ghebre-Sellassie I, Martin C E, Zhang E, DiNunzio J (editors): Pharmaceutical Extrusion Technology, Second Edition, CRS Press, Taylor & Francis Group, Boca Raton, Florida, 2018: 10-12.
22. Hot Melt Extrusion. Available from: https://www.particlesciences.com/news/technical-briefs/2011/hot-melt-extrusion.html. Available on: 4. 6. 2018.
23. European Pharmacopeia, 9th Edition, Council of Europe, Strasbourg, 2016: Volume II: 2759.
24. Vranić E, Uzunović A: Dissolution Studies of Physical Mixtures of Indomethacin with Alpa- and Gamma-Cyclodextrins. Bosn J Basic Med Sci 2010; 10: 197-203.

25. Indomethacin. Available from: https://www.sigmaaldrich.com/catalog/product/sigma/i7378?lang=en&region=SI. Available on: 10. 6. 2018.
26. Indomethacin. Available from: https://www.drugbank.ca/drugs/DB00328. Available on: 10. 6. 2018.
27. Tetko I V, Gasteiger J, Todeschini R, Mauri A, Livingstone D, Ertl P, Palyulin V A, Radchenko E V, Zefirov N S, Makarenko A S, Tanchuk V Y, Prokopenko V V: Virtual Computational Chemistry Laboratory - Design and Description. J Comput Aid. Mol Des 2005; 19: 453-63.
28. VCCLAB, Virtual Computational Chemistry Laboratory. Available from: http://www.vcclab.org/lab/alogps/. Available on 10. 6. 2018.
29. Sunshine I (Editor): CRC Handbook of Analytical Toxicology, The Chemical Rubber Co, Cleveland, 1969: 60.
30. Indometacin Dosage and Administration. Available from: https://www.drugs.com/pro/indometacin.html#s-34068-7. Available on: 30.6.2017.
31. Reintjes T (Editor): Solubility Enhancement with BASF Pharma Polymers, Solubilizer Compendium, BASF SE, Pharma Ingredients & Services, Lampertheim, 2011: 112-7.
32. Kolter K, Karl M, Gryczke A: Hot-melt Extrusion with BASF Pharma Polymers, Extrusion Compendium, 2nd Revised and Enlarged Edition, BASF SE, Pharma Ingredients & Services, Ludwigshafen, 2012: 84-6.
33. Poly(1-vinylpyrrolidone-*co*-vinyl acetate). Available from: https://www.sigmaaldrich.com/catalog/product/aldrich/190845?lang=en&region=SI. Available on: 10. 6. 2018.
34. Fini A, Fazio G, Feroci G: Solubility and Solubilisation Properties of Non-steroidal Anti-inflammatory Drugs. Int J Pharm 1995; 126: 95-102.
35. The Intrinsic Solubility of Indometacin. Available from: https://www.slideshare.net/jonmole/sirius-aaps2010-the-intrinsic-solubility-of-indometacin. Available on: 16.6.2017.
36. Comer J, Judge S, Matthews D, Towes L, Falcone B, Goodman J, Dearden J: The Intrinsic Aqueous Solubility of Indomethacin. ADMET & DMPK 2014; 2: 18-32.
37. Box K, Comer J E, Gravestock T, Stuart M: New Ideas About the Solubility of Drugs. Chem Biodivers 2009; 6: 1767-88.

38. Stuart M, Box K: Chasing Equilibrium: Measuring the Intrinsic Solubility of Weak Acids and Bases. Anal Chem 2005; 77: 983-90.
39. Yamamoto H: A New Syntheses of 1-(p-chlorobenzoyl)-5-methoxy-3indolylacetic Acid and its Polymorphism. Chem Pharm Bull 1968; 16: 17-9.
40. Borka L: The Polymorphism of Indomethacine. New Modifications, Their Melting Behaviour and Solubility. Acta Pharm Suecica 1974; 11: 295-303.
41. Lin S-Y: Isolation and Solid-State Characteristics of a New Crystal Form of Indomethacin. J Pharm Sci 1992; 81: 572-6.
42. Li Y, Pang H, Guo Z, Lin L, Dong Y, Li G, Lu M, Wu C: Interactions Between Drugs and Polymers Influencing Hot Melt Extrusion. J Pharm Pharmacol 2014; 66: 148-66.
43. Hilfiker R (Editor): Polymorphism: In the Pharmaceutical Industry, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2006: 287.
44. Greco K, Bogner R: Crystallization of Amorphous Indomethacin During Dissolution: Processing and Annealing. Mol Pharmaceutics 2010; 7: 1406-18.
45. Craig D Q: The Mechanisms of Drug Release from Solid Dispersions in Water-Soluble Polymers. Int J Pharm 2002; 231: 131-44.
46. Tres F, Treacher K, Booth J, Hughes L P, Wren S A C, Aylott J W, Burley J C: Indomethacin-Kollidon VA 64 Extrudates: a Mechanistic Study of pH-dependent Controlled Release. Mol Pharmaceutics 2016; 13: 116-75.