Solubility/Bioavailability Enhancement and Modified Release Formulations of Poorly Water-Soluble Drugs

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To my family

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List of abbreviations

A_n	Absorption number
AUC	Area under the curve
BCS	Biopharmaceutical classification system
BP	British pharmacopoeia
CMC	Critical micelle concentration
DCS	Developability classification system
DLS	Dynamic light scattering
D_n	Dissolution number
D_0	Dose number
DSC	Differential scanning calorimetry
FTIR	Fourier transforms infra-red
GAS	Gas antisolvent recrystallization
GIT	Gastro intestinal tract
HLB	Hydrophilic lipophilic balance
HPH	High pressure homogenization
HPMC	Hydroxypropyl methylcellulose
HME	Hot-melt extrusion
LD	Laser diffractometry
Log P	Octanol/water partition coefficient
MCC	Microcrystalline cellulose
MAD	Maximum absorbable dose
MP	Melting point
NLCs	Nanostructured lipid carriers
PBS	Phosphate buffer saline
PCS	Photon correlation spectroscopy
PDI	Polydispersity index
PXRD	Powder x-ray diffraction
RESS	Rapid expansion of supercritical solutions
RT	Room temperature
SD	Standard deviation
SCF	Supercritical fluid technologies
SIWV	Small intestine fluid volume
SITT	Small intestinal transit time
SLS	Sodium lauryl sulfate
SLAD	Solubility limited absorbable dose
TEC	Triethyl citrate
T_g	Glass transition temperature
USP	United states pharmacopeia

Chapter 1. Introduction

1. INTRODUCTION

1.1 Solubility/bioavailability fundamentals

Solubility is generally defined as the concentration of the compound in a solution which is in contact with an excess amount of the solid compound when the concentration and the solid form do not change over time (Sugano et al., 2007; Bosselmann and O. Williams, 2012). Compounds with solubility below 0.1 mg/ml face significant solubilization obstacles and are considered practically insoluble drugs according to United States Pharmacopeia (USP) and British Pharmacopoeia (BP).

The new drug entities with poor aqueous solubility are becoming more prevalent as result of high-throughput screening in drug discovery. Poor aqueous solubility presents significant challenges, as it reduces the oral absorption and bioavailability (Lipinski, 2000). Nevertheless, the required solubility to achieve a good bioavailability must be evaluated in view of both the dose and the permeability.

Maximum absorbable dose (MAD) "derived by Johnson and Swindell, 1996 and later by Curatolo, 1998" presents the required solubility and permeability needed to achieve a good oral absorption. Incomplete absorption should be expected if the dose is higher than the MAD value:

$$MAD = C_s \cdot k_a \cdot SIWV \cdot SITT$$

Where C_s is the solubility (mg/ml) at pH 6.5; k_a is the intestinal absorption rate constant ($^{min\text{-}1}$); SIWV is the fluid volume in the small intestine (ml); and SITT the small intestinal transit time (min).

Solubility, dose and permeability are also the key underlying parameters in Biopharmaceutical classification system (BCS) which groups poorly soluble drugs as class II (poorly soluble and highly permeable) and class IV (poorly soluble and poorly permeable). Drug substances are considered as highly soluble, if the largest strength is soluble in ≤250mL over the physiological pH range from 1.0 to 7.5. Otherwise, the drug substance is considered to be poorly soluble. The highly permeable compounds are defined as those compounds that demonstrate >90 % absorption of the administered dose. Otherwise, the drug substance is considered to be poorly permeable. (Amidon et al., 1995; Benet et al., 2008; Dressman et al., 2001; FDA, 2000).

A further model generates three dimensionless numbers; dose number (D_0), dissolution number (D_n) and absorption number (A_n) for assessment of whether dissolution rate,

solubility and/or permeability are likely to limit the oral absorption in gastro intestinal tract. In this model the drug considers as dissolution rate-limited absorption if the drug particles cannot dissolve completely during the transition time to the absorption site, whereby increasing the dose and/or reducing the particle size to sub-micron range should enhance the absorption. On the other hand if the amount of fluid available in gastrointestinal tract is not enough to dissolve the administered dose, the solubility becomes the rate limiting step in absorption. In the case of permeability-limited absorption, the transition rate of the drug across the gut wall is too slow during the residence time in the absorption site (Oh et al., 1993; Yu, 1999; Takano et al., 2008; Butler and Dressman, 2010).

1.2 Basic factors affecting dissolution rate

Several factors affecting the rate at which the solid dissolves in the solvent are described in Noyes Whitney equation (Noyes and Whitney, 1897):

$$\frac{dc_x}{d_t} = \frac{D.A}{h}(c_s - c)$$

Where, $\frac{dc_x}{d_t}$ gives the dissolution rate and is a function of diffusion coefficient of the solute in solution (D), the surface area of the exposed compound (A), the thickness of the diffusion layer (h), the saturation concentration of the compound in the diffusion layer (C_S) and the concentration in the well-stirred bulk (C).

Diffusion coefficient is constant and cannot be significant altered by modifying the drug structure. Furthermore, changing the agitation speed can alter the thickness of diffusion layer in-vitro but it is not applicable in-vivo. Therefore, improving solubility and increasing surface area of the compounds are the only available variables to enhance the in-vivo dissolution rate of poorly soluble drugs (Williams et al., 2013).

1.3 Basic factors affecting solubility/bioavailability

1.3.1 Physiological factors

Several physiological factors in gastrointestinal tract can cause significant variation in drug absorption related to solubility modification such as; pH, gastric emptying and food.

The pH gradient of gastrointestinal tract from acidic pH in the stomach to acidic–neutral pH in the small intestine to basic pH in the colon has a significant effect on the solubility of ionizable drugs. Basic and acidic drugs are more soluble in pHs where they are in the ionized form.

Gastric emptying is an important physiological factor, which influences the uptake of drug substances from the intestine and subsequently the bioavailability. The gastric mobility varies among species and it is affected by the presence of food.

Food can improve the solubility and the absorption of lipophilic compounds by delaying the gastric emptying, slowing the input into the intestine, stimulating bile salt secretions and altering the pH of the gastro intestinal fluid (Kararli, 1995; Kerns and Di, 2008).

1.3.2 Structural properties

Before reviewing the available strategies to overcome the limitations of low bioavailability, it is useful to briefly discuss the structural properties which may affect the solubility and/or the bioavailability such as; melting point, lipophilicity, pK_a, molecular weight and polymorphism (Kerns and Di, 2008).

Yalkowsky and Banerjee equation demonstrates the effect of melting point and lipophilicity on the aqueous solubility (Kerns and Di, 2008):

$$\text{Log } S = 0.8 - \text{Log } P_{ow} - 0.01(MP-25)$$

Where, S is the solubility, Log P_{ow} is the octanol/water partition coefficient (a measure of lipophilicity), and MP is the melting point (a measure of crystal lattice strength).

In this equation the solubility decreases 10-fold as Log P increases by 1 unit or melting point increases by 100°C.

Henderson-Hasselbalch equation provides insights for the effect of pK_a on the aqueous solubility (Kerns and Di, 2008):

$$S = S_0(1+10^{(pH-pKa)}) \text{ Acid}$$
$$S = S_0(1+10^{(pKa-pH)}) \text{ Base}$$

Where, S is the solubility at a given pH and S_0 is the solubility of the neutral compound (intrinsic solubility).

Different solid states of the material "amorphous, crystalline, different polymorphs, hydrates or solvates" have different thermodynamic solubility values where the higher-energy forms tend to have higher solubility than the lower energy forms (Kerns and Di, 2008).

As molecular weight increases the permeability through the intestinal and blood brain barrier decreases, a larger cavity must be formed in water in order to solubilize the compound, and solubility decreases (Pardridge, 1995; Kerns and Di, 2008).

Some researchers have proposed rules for structural properties of the compounds that have a higher probability to reflect the bioavailability such as; Lipinski rules and Veber rules. According to Lipinski rules (the rule of 5), the poor bioavailability is more likely when the compound has more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight is greater than 500 and the lipophilicity Log P (C Log P) is greater than 5 (or M log P 4.15) (Lipinski et al., 1997). In the case of Veber rules, compounds will have a good oral bioavailability if they have $\leq$ 10 rotatable bonds and polar surface area $\leq$ 140 $A^{\circ 2}$ (or $\leq$ 12 H-bond donors and acceptors) (Veber et al., 2002).

1.4 Formulation approaches for poorly soluble drugs

Several formulation strategies have been employed to overcome the limitations of low dissolution rate and/or solubility that cause the poor absorption and bioavailability including; pH-adjustment, co-solvents, surfactants, cyclodextrin complexes, lipid-based formulations, nanosuspensions and solid solution/dispersion technologies.

1.4.1 pH-adjustment

Improving solubility of poorly water soluble drugs containing ionizable groups can be done either by adjusting the pH of the solution to favor the ionized form whereby the buffer capacity and tolerability of the selected pH are important to avoid drug precipitation, or by using solubilized excipients that modulate the environmental pH (pH-modifier) within the solid dosage form, this pH-modifier should present inside the formulation over the dissolution time of the drug compound, therefore the selection of pH-modifier type and concentration is important. The risk of precipitation upon contact with a pH where the drug is less soluble is the major disadvantage of this method (Vemula et al., 2010; Siepe et al., 2008).

1.4.2 Co-solvents

Co-solvents are mixtures of water miscible solvents, and often used to improve the solubility of poorly aqueous soluble drugs by lowering the polarity of water to a level that more closely reflects the polarity of the nonpolar solute, this reduces the ability of water to squeeze out nonpolar hydrophobic compounds. Co-solvents can be used for drugs that are not suitable for pH-adjustment strategies due to lack of ionizable groups or for drugs that show low affinity for solubilization by surfactants or lipids due to moderate log P (between 1 and 3). Furthermore, co-solvents can be used in combination with other solubilization strategies such as; pH-adjustment, surfactant or lipid-based formulations in order to further enhance the solubility of insoluble compounds. The main disadvantages of this method are the reduction of solubilization power upon dilution and the toxicity level of the used solvents (Millard et al., 2002; Williams et al., 2013).

1.4.3 Surfactants

Drugs entrap within the micelles when the surfactants concentration exceeds the critical micelle concentration, and this increases the apparent aqueous solubility of drugs. Surfactants can be used in combination with other solubilization strategies such as nanosuspension

formulations where reducing the particle size of the drug is essential to enhance the surface area and dissolution rate but this creates high free energy, therefore, surfactant in this case act as stabilizers to prevent precipitation by reducing interfacial tension between drug surface and aqueous phase. Furthermore, Surfactants can be used in combination with co-solvents to reduce susceptibility to precipitation upon dilution.

The factors that should be considered when using surfactants include the potential of surfactant to induce hypersensitivity after parenteral administration and the possibility effects of surfactant on transporter/ metabolic enzyme activity (Kerns and Di, 2008; Williams et al., 2013).

1.4.4 Cyclodextrin complexes

Cyclodextrin are macrocyclic oligosaccharides consisting of a hydrophilic outer surface and a hydrophobic inner cavity where guest molecules having a lipophilic nature can be accommodated (Loftsson and Brewster, 1996). Thus, the drugs get encapsulated in the cavity and results in improved aqueous solubility and enhanced dissolution rates.

The formation of cyclodextrin complexes requires specific molecular properties which may not work for certain compounds, and the toxicity of cyclodextrin complexes at high concentrations which limits the dose level are the major limitations of this method (Kerns and Di, 2008).

1.4.5 Lipid-based formulations

Lipid-based formulations consist of a drug dissolved in a blend of two or more excipients such as triglyceride oils, partial glycerides, surfactants or co-surfactants (Pouton, 2000).

Lipid-based formulations can be solutions, suspensions, emulsions, microemulsions, micellar solutions, liposomes, lipid nanoparticles, or nanoemulsions. Among those formulations solid lipid nanoparticles (SLNs, which possess a solid lipid core) and its second generation nanostructured lipid carriers (NLCs, which possess a core of solid lipid and liquid lipid) have attracted a significant level of interest during the recent years. SLNs and NLCs are mainly produced by high pressure homogenization (HPH), have an average particle size below 500 nm and revealed several advantages such as: controlling the drug release and improving the drug stability. Furthermore, SLNs and NLCs are safe carriers and easily produced on large scale (Müller et al., 2000; Mäder and Mehnert, 2001).

Lipid formulations cannot be used for drugs that have strong intermolecular forces in the solid state (high melting points and crystallinity) which limit the solubility in both water and lipid, these drugs are so-called "brick dust" (Stella and Nti-Addae, 2007; Rabinow, 2004).

1.4.6 Nanosuspensions

Reducing the particle size results in increasing the surface area and therefore increasing the dissolution rate of drugs as described above in Noyes Whitney equation (Noyes and Whitney, 1897). The effect of decreasing the particle size to micrometer range (micronization) on dissolution rate and oral bioavailability improvement has been previously demonstrated with a number of poorly soluble drugs, including oxfendazole (Shastri et al., 1980) and progesterone (Hargrove et al., 1989). Besides providing a large increase in the available surface area and dissolution rate improvement, decreasing the particle size to nanometer range (nanosizing) also increases the equilibrium solubility (Buckton and Beezer, 1992; Müller et al., 1999; Böhm and Müller, 1999). The saturation solubility improvement can be explained by the Kelvin equation (Simonelli et al., 1970) and the Ostwald–Freundlich equation (Müller and Peters, 1998; Florence and Attwood, 1981). The Kelvin equation describes the transformation of molecules from a liquid phase (droplet) to a gas phase, where the vapor pressure of liquid droplets in a gas phase increases with decreasing particle size (increasing curvature of the surface) (Junghanns and Müller, 2008):

$$\boldsymbol{ln}\left(\frac{\boldsymbol{P}}{\boldsymbol{P_0}}\right) = \frac{-\boldsymbol{\gamma} * \boldsymbol{V_L} * \boldsymbol{cos\,\theta}}{\boldsymbol{r_K} * \boldsymbol{RT}}$$

Where, P is the vapor pressure, P_0 is the equilibrium pressure of a flat liquid surface, γ is the surface tension, V_L is the molar volume, cos θ is the contact angle, r_k is the radius of droplets, R is the universal gas constant and T is the absolute temperature.

This equation can be applied to the transition of molecules from a solid phase (drug particle) to a liquid phase (dispersion medium) as this transition is in principal identical to the transition of molecules from a liquid phase (droplet) to a gas phase. The vapor pressure is then equivalent to the dissolution pressure. When the dissolution pressure increases, the equilibrium of molecules dissolving and molecules recrystallizing can be shifted and therefore the saturation solubility increases.

The increase in saturation solubility with decreasing particle size to nanometer range can be explained also by the Ostwald–Freundlich equation (Junghanns and Müller, 2008):

$$\log \frac{C_S}{C_\infty} = \frac{2\sigma V}{2.303RT\rho r}$$

Where, C_s is the solubility, C_∞ is the solubility of infinite radius particles, σ is the interfacial tension substance, V is the molar volume of the material, R is the gas constant, T is the absolute temperature, p is the density of the solid and r is the radius.

In addition to the dissolution rate and solubility improvement, nanoparticles may reduce or eliminate the food effect on the absorption of poorly soluble drugs Class II (poorly soluble and highly permeable). NanoCrystal® dispersion formulations of aprepitant (Wu et al., 2004) and cilostazol (Jinno et al., 2006) eliminate the fed vs. fasted state differences in drug absorption, whereby the mean area under the curve (AUC) values for the fasted and fed nanoparticles formulations were not significantly different.

Nanoparticles with a crystalline character and a size between few nanometer and 1000 nanometer are so-called "nanocrysals". Dispersion of drug nanocrystals in a liquid medium leads to "nanosuspensions" (Müller and Junghanns, 2006).

Nanocrystals tend to agglomerate to reduce the generated extra free energy. The increase in free energy by increasing the surface area can be expressed as below:

$$\Delta G = \gamma_{s/l} * \Delta A$$

Where, $\gamma_{s/l}$ is the interfacial tension between the drug surface and aqueous phase and ΔA is the total surface area of the particles (Verma et al., 2009).

For this reason nanocrystals need to be stabilized, stabilizers adsorb at the interface, reducing the interfacial tension ($\gamma_{s/l}$) and thereby decreasing the total free energy of the system.

Selection of stabilizers and their optimum quantities plays a major role in formulating nanosuspensions, polymers as well as surfactants (nonionic and ionic) can be used as stabilizers, the weight ratio of drug to stabilizer is commonly from 20:1 to 2:1. Inadequate type or too low amount of the stabilizer may cause particles agglomeration due to the high surface energy, while inadequate type or too high amount of the stabilizer promote Ostwald ripening due to the solubility enhancement of small nanometer-sized particles which may solubilize and re-crystallize onto larger particles present in the nanosuspension (Merisko-Liversidge et al., 2003; Peltonen and Hirvonen, 2010; Cerdeira et al., 2010; Merisko-Liversidge and Liversidge, 2011).

1.4.6.1 Preparation of nanosuspensions

Nanosuspensions can be produced by different techniques, the "Bottom-up", "Top-down" and "combination" techniques.

1.4.6.1.1 Bottom-up techniques

Bottom-up techniques are usually referred to as "precipitation techniques" and based on the controlled precipitation or crystallization of drug from a supersaturated solution (D'Addio and Prud'homme, 2011). Hydrosol technique was the first precipitation technique to obtain drug nanocrystals, whereby the lipophilic drug is first dissolved in an organic solvent followed by precipitation i.e. adding an anti-solvent that is miscible with the organic solvent, usually water, then the solvents can be removed by evaporation or lyophilization to obtain drug nanocrystals (Sucker and List, 1988).

Auweter and Horn (Auweter et al., 1998; Auweter et al., 2002) developed another precipitation process to prepare amorphous nanoparticles. Although this can be a successful strategy to increase the saturation solubility, there are concerns regarding the physical stability of these amorphous nanoparticles since the amorphous active have the risk of uncontrolled crystallization during storage which leads to decrease the oral bioavailability.

Another category of bottom-up techniques is known as supercritical fluid technologies (SCF). Supercritical fluids are fluids whose temperature and pressure are greater than the critical temperature and critical pressure and used as an anti-solvent. A solution of the lipophilic drug in an organic solvent is saturated with a supercritical fluid (such as supercritical CO2) thereby decreasing the solubility of the drug in the solvent and consequently causing the drug to precipitate at greatly reduced particle size. The most widely employed methods of SCF are gas antisolvent recrystallisation (GAS) (Krukonis et al., 1991) and rapid expansion of supercritical solutions (RESS) (Pace et al., 1999).

The large-scale production of drug nanocrystals by bottom-up techniques is relative easy. However, there are some major disadvantages of the precipitation techniques including; the drug needs to be soluble in at least one solvent and the later needs to be miscible with at least one anti-solvent, solvent residues need to be removed and the difficulty to avoid crystal growth to the micrometer range in the nanosuspensions during processing or storage.

1.4.6.1.2 Top-down techniques

Top-down techniques involve breaking down larger particles via media milling or high-pressure homogenization (HPH).

In media milling the particles are grinded using high shear forces generated by the movement of the milling beads/pearls, whereas, in high pressure homogenization (HPH) the particles are forced to pass through a tiny gap with high velocity causing the particle size reduction by cavitation.

A) Media milling technology

Historically, Liversidge developed the media milling method for particle size reduction to nanometer range (Liversidge et al., 1992). In brief, media milling involves the continuous stirring and wet milling of aqueous drug slurry with specialized rigid media preferably spherical or particulate in form having an average size less than about 3 mm so-called "beads/pearls". A fine nanosuspension can be obtained for periods ranging from hours to several days, depending on the drug hardness, drug quantity, mill type and batch volume (Gao et al., 2008 and Müller et al., 2000).

The NanoCrystal® technology by élan is the first well developed pearl-milling method for particle size reduction. Various products that use the NanoCrystal® technology (élan nanosystems) are commercially available in the market and listed in **Table 1**. Furthermore, various drug candidates are presently in clinical phases (Shegokar and Müller, 2010).

Drug amount, size, density and number/volume of the milling pearls, milling speed, milling time and temperature are the critical parameters for media milling to obtain optimal products with a narrow particle size distribution and a very little batch-to batch variation. Typically, the drug amount in the milling chamber is in the range from 2 to 30%, beads with diameter ranging from 0.1-3.0 mm are normally used and can be obtained in various materials; e.g. various grade of steel, ZrO_2, $ZrSiO_4$, Al_2O_3, SiO_2, annealed glass, polytetraflouroethylene, and hard polystyrene derivatives. The volume of the milling pearls/beads is up to about 2/3 of the mill volume. The milling times and speeds vary significantly depending on a lot of factors, e.g. the drug hardness and quantities. Low milling temperature reduces the process of aggregation and provides high efficiency of the milling process (Keck and Müller, 2006; Gao et al., 2008; Peltonen and Hirvonen, 2010 and Morales et al., 2012).

Several types and sizes of mills have been reported to be used on the production of nanosuspension including; Nanomill® system (élan Drug Discovery, PA, USA), Dyno-mill® (Willy A. Bachofen, Basel, Switzerland) with chamber size of 150, 300 and 600 mL and Netzsch mills (Netzsch Inc., Exton, PA, USA) with chamber size of 2, 10 and 60 L chamber size.

Contamination of the final product with the potential erosion of the milling vessel or milling beads/pearls, is the major concern surrounding the use of media milling (Peltonen, 2013).

Table 1. Commercially available nanocrystal products applied by NanoCrystal technology (élan nanosystems)

Trade name	**Active pharmaceutical ingredient (API)**	**Therapeutic use**	**Pharma company**	**Administration route**
Avinza®	Morphine sulfate	Psychostimulant drug	King Pharmaceuticals	Oral
Emend®	Aprepitant	Antiemetic	Merck & Co.	Oral
Focalin® XR	Dexmethyl-phenidate HCl	Psychostimulant drug	Novartis	Oral
Megace ES®	Megestrol acetate	Antianorexic	Par Pharmaceutical Companies Inc. (Spring Valley, NY, USA)	Oral
Rapamune®	Rapamycin, Sirolimus	Immunosuppressive	Wyeth Pharmaceuticals	Oral
Ritalin® LA	Methylphenidate HCl	Psychostimulant drug	Novartis	Oral
Tricor®	Fenofibrate	Hypercholesterolemia	Abbott Laboratories	Oral
Zanaflex CapsulesTM	Tizanidine HCl	Muscle relaxant	Acorda	Oral

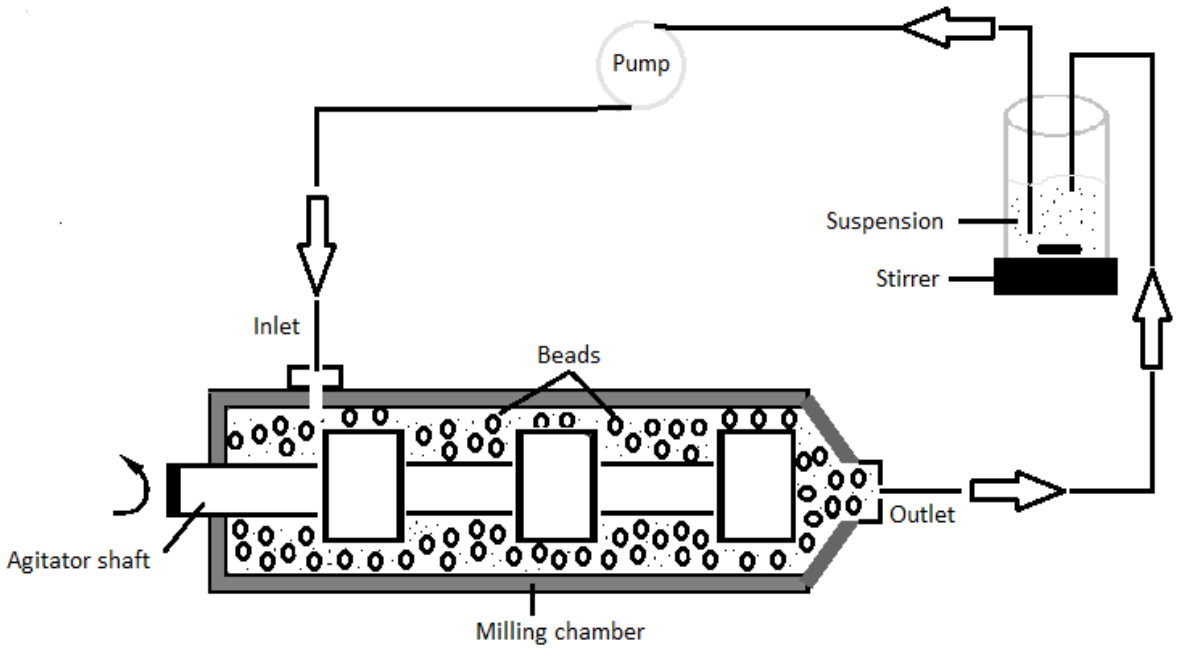


Fig. 1. Schematic drawing of wet-milling process for nanosuspensions preparation

B) High-Pressure Homogenization (HPH)

There are two techniques for the production on nanocrystals by high-pressure homogenization, the mircofluidizer technique e.g. Microfluidizer® (Microfluidics, Newton, USA), and the piston-gap technique e.g. Micron LAB 40 (APV Deutschland GmbH, Unna, Germany).

Mircofluidizer technique is based on the jet stream principle whereby the nanosized particles are generated by a frontal collision of two fluid streams under pressures up to 1700 bar (Bruno and Mcllwrick, 1999). A major disadvantage of microfluidizers is the long production time. In piston-gap homogenization the drug is dispersed in a stabilizer solution and forced through a very tiny gap with an extremely high velocity. The pressure may up to 4000 bar and the diameter of the homogenization gap is from 5 to 20 μm. Nanocrystals are formed in high pressure homogenization due to particle furcating by shock waves, high shear forces, particles collisions and turbulent flow (Peltonen, 2013).

Homogenization pressure, number of homogenization cycle and hardness of the drug are the critical parameters affecting the particle size reduction by high-pressure homogenization (Keck and Müller, 2006).

High pressure homogenization technique has many advantages compared to the other methods, e.g. easy scale up and avoidance of organic solvents. However, it has significant drawbacks including; high energy input, nanoparticles are produced in several production cycles which are very complex and expensive, and possible degradation of the components caused by high temperature or high shear forces (Sutradhar et al., 2013).

1.4.6.1.3 Combination techniques

The combination techniques have been developed to improve the particle size reduction effectiveness, e.g. to obtain a very small nanocrystals below 100 nm, or to overcome the drawbacks of standard nanosuspension processes, e.g. to reduce the number passes through the homogenizer (Junyaprasert and Morakul, 2014). NANOEDGE® is the first combination technology and was developed by Baxter healthcare company, it combines a microprecipitation step (a solvent-antisolvent technique) followed by a high-energy process. H42 is another combination technology where spray-drying and high pressure homogenization are combined. Moreover, H96 combination technology which combines lyophilization and high pressure homogenization, and it considers as the most effective combination technique to produce nanocrystals with particle sizer smaller than 100 nm (Müller et al., 2011).

1.4.6.2 Particle size characterization

1.4.6.2.1 Laser diffractometry (LD)

Laser diffractometry is a widely used particle sizing technique due to its relatively broad particle-sizing range from nanometers up to several millimeters, rapid measurements and repeatability.

Most of LD instruments employ a standard He-Ne laser light source. The measurement of particle size by LD is based on the fact that particles scatter light in all directions as a laser beam passes. Generally large particles scatter light at small angles relative to the laser beam and small particles scatter light at large angles. The measured data is then analyzed to calculate the particle size distribution of the particles based on Fraunhofer diffraction theory or Mie scattering theory. The use of Fraunhofer is suitable for large particles, but for the small particles the Mie theory provides the greatest accuracy.

The Mie theory takes into account the optical properties (refractive index and imaginary component). As the optical properties of many substances is difficult to find in the literature, the user can either measure them or estimate them using an iterative approach based upon the goodness of fit between the modeled data and the actual data collected for the sample.

One should be aware of some major disadvantages by determination of particle size using LD including; solubilization, agglomeration or sedimentation of the sample in the dispersion medium. Furthermore, the laser beam cannot be diffracted if the sample and the dispersion media have the same refractive index (Xu, 2005).

1.4.6.2.2 Photon correlation spectroscopy (PCS)

Photon Correlation Spectroscopy (PCS), also known as dynamic light scattering (DLS), is a technique used to determine the mean particle size diameter (sizing range approximately from 3 nm to 3 μm) and the width of the particle size distribution expressed as polydispersity index (PDI). Usually PCS instruments consist of a laser light which is focused to illuminate a small volume of the sample. When the laser beam passes through a liquid dispersion containing particles in Brownian motion, it experiences fluctuations in its intensity. PCS detects the time-dependent fluctuations in the intensity of scattered light, and analyzes these intensity fluctuations to determine the diffusion coefficients, which in turn yield the particle size through the Stokes-Einstein equation.

The diameter which is measured by PCS is the hydrodynamic diameter and refers to how a particle diffuses within a fluid. The polydispersity index (PDI) values of around 0.10-0.20 indicate a relatively narrow distribution, values of 0.5 and higher are obtained in case of very

broad distributions, and value of 0.0 indicates monodisperse particles population (Müller and Böhm, 1998; Thode et al., 2000 and Kaszuba et al., 2008).

PCS provides an absolute measurement without any need for further information about the composition and the optical properties of the particles in dispersion, measurements are fast, very small quantities of sample can be measured, and the technique is applicable from about 0.003 to several microns. However, if large particles (>3 μm) exist in the dispersion, a complementary analysis with Laser Diffractometry (LD) is recommended in order to corroborate the obtained results.

1.4.6.3 Stabilizers used for nanosuspension

Stabilizers are polymers or surface active agents that adsorb at the interface between the drug particles and water. Polymers and nonionic surfactants stabilize nanosuspensions via steric repulsion while ionic surfactants stabilize nanosuspensions via electrostatic repulsion (Verma et al., 2009).

Steric repulsion is achieved when molecules are adsorbed or attached chemically on the particle surface. Electrostatic repulsion occurs through an electrical double layer surrounding the colloidal particles. Unlike electrostatic stabilizers, polymeric steric stabilizers do not usually destroy the crystal structure of drug particles and their stabilization efficiency is not sensitive to the addition of electrolytes. Furthermore, they provide a greater coverage compare to electrostatic stabilizers. However, steric stabilization is more sensitive to temperature fluctuations than electrostatic stabilization (Peltonen and Hirvonen, 2010 and Shete et al., 2014). Therefore, the combination of steric and electrostatic stabilization known as electrosteric stabilization may provide complementary properties.

Most commonly used polymeric stabilizers include cellulose ethers (e.g. Hydroxypropyl Methylcellulose) and poloxamers. The surfactant excipients can be non-ionic, such as polysorbate, or anionic, such as sodium dodecyl sulfate or sodium docusate. Cationic surfactants are less frequently used (Cerdeira et al., 2010).

Methocel® (Hydroxypropyl Methylcellulose) is a polymeric stabilizer and provides stability via steric stabilization. Besides its high molecular weight, it has too many hydroxyl groups in its structure (**Fig. 2**) which can form lots of hydrogen bonds with drug molecules providing sufficient particle size reduction and stability of nanosuspensions (Esfandi et al., 2014).

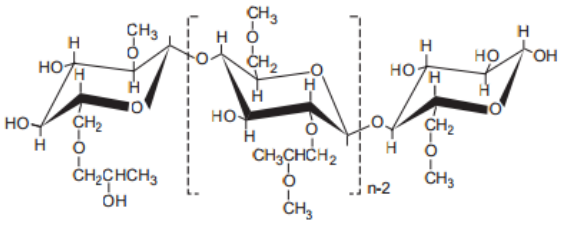


Fig. 2. Chemical structure of Methocel® (Dow, 2002)

Furthermore, Methocel® is a neutral polymer and therefore its stabilizing ability is less dependent on ionic strength changes and pH across the gastro-intestinal tract than ionic polymers or surfactants (Liversidge & Cundy, 1995 and Sepassi et al., 2007).

Lutrol® F68 (Poloxamer 188) is a synthetic block copolymer of ethylene oxide and propylene oxide (**Fig. 3**).

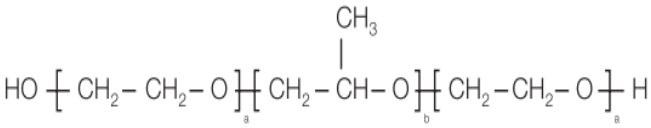


Fig. 3. Chemical structure of Lutrol® F68 (BASF, 2010)

a=80, b=27

It was demonstrated that Lutrol® F68 is an efficient steric stabilizer despite its thin adsorption layer (Jain et al., 2013). It forms monomolecular micelles in liquids at low concentrations; however, multimolecular aggregates are formed at high concentrations.

Lutrol® F68 can be used also to prepare solid dispersions and thereby, improving solubility, absorption and bioavailability of poorly soluble drugs. Lutrol® F68 is also regulatory accepted, even for intravenous administration.

Tween® 80 (polyoxyethlene sorbitan monooleate (**Fig. 4**)) is non-ionic surfactant, above its critical micelle concentration (CMC=0.012 mM in pure water), Tween® 80 exhibits micellar structures. Tween® 80 is frequently used as stabilizer in aqueous formulations of poorly soluble drugs for intravenous administration and as emulsifier for parenteral o/w emulsion products, related to its high hydrophilic lipophilic balance (HLB=15) (Chou et al., 2005 and Huang et al., 2008).

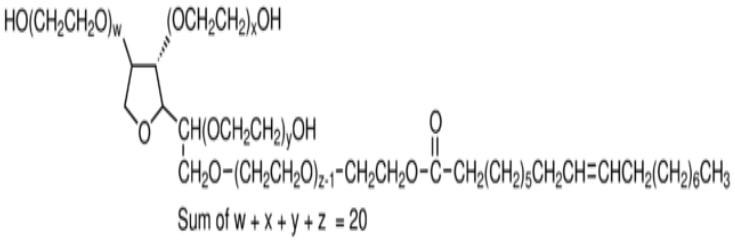


Fig. 4. Chemical structure of Tween® 80

SLS (Sodium lauryl sulfate (**Fig. 5**)) is an anionic surfactant and provides stability via electrostatic repulsion. It has high affinity to adsorb on the particle surface and therefore lowering the surface tension and providing high zeta potential value. The critical micelle concentration of SLS is 7-10 mM and it is a regulatory accepted stabilizer for oral dosage forms (e.g. tablets and capsules) and is therefore suitable to be used in nanosuspensions. SLS is also used as emulsifier for o/w emulsions (HLB=40).

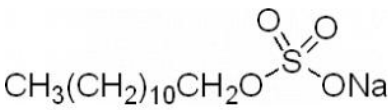


Fig. 5. Chemical structure of SLS

1.4.7 Solid dispersions

Solid dispersion is also a strategy to tackle solubility/dissolution-rate-limited oral absorption by dispersion of one or more active ingredients in an inert carrier at the solid state (Chiou and Riegelman, 1971). Solid dispersions may improve dissolution rate and/or solubility by decreasing particle size, improving wettability, reducing agglomeration, changing physical state of the drug and possibly dispersion on a molecular level (Janssens and Van den Mooter, 2009).

Solid dispersions can be classified in different types based on the amorphous or crystalline state of drug and carrier as described in **Table 2**.

Table 2. Classification for solid dispersions (Dhirendra et al., 2009)

	Eutectic	**Amorphous Precipitation**	**Solid Solution**	**Glass suspension**		**Glassy solid solution**
Drug	Crystalline	Amorphous	Molecular dispersed	Amorphous	Crystalline	Molecular dispersed
Carrier	Crystalline	Crystalline	Crystalline	Amorphous	Amorphous	Amorphous
Phases	2	2	1	2	2	1

Since amorphous carriers with high glass transition temperatures are mostly used to prepare solid dispersions, only three types of the solid dispersions may occur: both glass suspension types and glassy solid solution.

A very stable solid dispersion is achieved if the drug is dispersed as crystals in the amorphous polymer phase since the drug remains in its favored crystalline state. A metastable solid dispersion is obtained if the drug is transformed into its amorphous state but not molecular dispersed in the amorphous polymer phase since the drug may recrystallized during storage or processing. Both those types of solid dispersion are called glass suspension.

The most desired form of solid dispersion for the formulation of poorly soluble drugs is the glassy solid solution, whereby the drug is molecularly dispersed in the amorphous polymer. In this case, the molecularly dispersed drug should be immobilized and the concentration of the drug must be below its saturation solubility in the polymer (Huang and Dai, 2014).

Molecular dispersion represents the ultimate in particle size reduction and after the carrier has dissolved, the drug is present as a supersaturated solution. the capability of drug to maintain dissolved in the supersaturated solution is strongly depends on the polymer characteristics (Leuner and Dressman, 2000).

Typically, the solubility of a drug in a polymer is below 10%, therefore a metastable solid dispersion is commonly obtained if the drug is transformed into its amorphous state. In such system the amorphous drug has a tendency to covert back to its corresponding crystalline form.

Recognizing the concept of molecular mobility by measuring the glass transition temperature (T_g) is known to be the key for stabilization of amorphous drugs. It has been found that amorphous materials should be stored at temperature lower than T_g-50. Therefore, the selection of polymers for amorphous solid dispersion preparation plays an important role in stabilization of the amorphous state. The selecting of high T_g polymers raises the T_g of the overall system so the metastable solid dispersions can be stable at least at room temperature. Furthermore, the polymers which can generate possible interactions with the drug can further improve the physical stability (Lee et al., 2014).

Other important carriers properties for solid dispersion are; solubilizing properties, enough thermal stability and thermoplasticity for systems prepared by hot-stage extrusion, and high solubility in organic solvents for systems prepared by solvent method (Janssens and Van den Mooter, 2009).

Various products containing different drugs and carriers are commercially available in the market and are listed in **Table 3**.

Table 3. Commercially available solid dispersion in the market (Janssens and Van den Mooter, 2009; Tiwari et al., 2009 and Huang and Dai, 2014)

Trade name	**Active pharmaceutical ingredient (API)**	**Carrier**	**Therapeutic class**
Certican	Everolimus	HPMC	Antineoplastic
Cesamet	Nabilone	PVP	Antiemetic
Gris-PEG	Griseofulvin	PEG6000	Antifungal
Incivek	Telaprevir	HPMCAS	Antiviral
Intelence	Etravirine	HPMC	Antiretroviral
Isoptin SR-E	Verapamil	HPC/HPMC	Antianginal, antiarrhythmic, antihypertensive
Kaletra	Lopinavir, ritonavir	PVPVA	Antiretroviral
Kalydeco	Ivacaftor	HPMCAS	Cystic fibrosis transmembrane conductance regulator (CFTR) potentiator
Nivadil	Nilvadipine	HPMC	Antihypertensive
Norvir	Ritonavir	PVP/VA	Antiretroviral
Onmel	Itraconazole	HPMC	Antifungal
Prograf	Tacrolimos	HPMC	Immunosuppressant
Sporanox	Itraconazole	HPMC	Antifungal
Zelboraf	Vemurafenib	HPMCAS	Antineoplastic
Zotress	Everolimus	HPMC	Antineoplastic

1.4.7.1 Preparation of solid dispersions

Various preparation methods for solid dispersions have been reported in literature including; solvent method and fusion method. In solvent method the drug and carrier are dissolved in a common organic solvent, and then the solvent is removed by evaporation. The main advantage of the solvent method is its applicability on heat-sensitive drugs. However, the large volumes of organic solvents used to enable complete dissolution of both drug and polymer and difficult to find a common solvent for both components are the disadvantages of this method (Tiwari et al., 2009). In fusion method, all components are heated above their melting or glass transition temperatures, followed by mixing and cooling (Janssens and Van den Mooter, 2009). The main advantage of fusion method is that no organic solvents are

involved, which reduces production costs and toxicity. However, the thermal degradation of heat-sensitive drugs is the main limitation of this method.

Hot-melt extrusion (HME) technique appears to address many of fusion method limitations; therefore, it becomes an attractive alternative to traditional fusion methods. The rotating screws impose intense mixing and agitation and thus de-aggregation of suspended particles in the molten polymer resulting in a more uniform dispersion and the process is continuous and more efficient (Crowley et al., 2007). Furthermore, the residence time of the blend in the extruder is short (up to 2 min) which limit the thermal instability (Breitenbach, 2002).

Generally, the hot-melt extruder consists of single or twin screws inside a stationary cylindrical barrel. The starting material is fed from a hopper directly into the extrusion channel where it is mixed, compressed, melted, and plasticized. After that the blend is pressurized through the die cavity into granules, cylinders, or films (Breitenbach, 2002).

The twin screw extruder (**Fig. 6**) has two screws which can either rotate in the same (co-rotating) or in the opposite (counter- rotating) direction and has several advantages over single screw extruder, such as easier material feeding, high kneading, and dispersing capacities, less tendency to over-heat and shorter transit time.

Counter-rotating twin screw extruders are more utilized in the pharmaceutical industries and can provide very high shear forces as the material is squeezed through the gap between the two screws as they come together. Co-rotating twin screw extruders are very efficient at feeding powders and for staging of downstream unit operations. However, they generally experience lower screw and barrel wear compare to counter-rotating twin screw extruders (Crowley et al., 2007 and Martin, 2013).

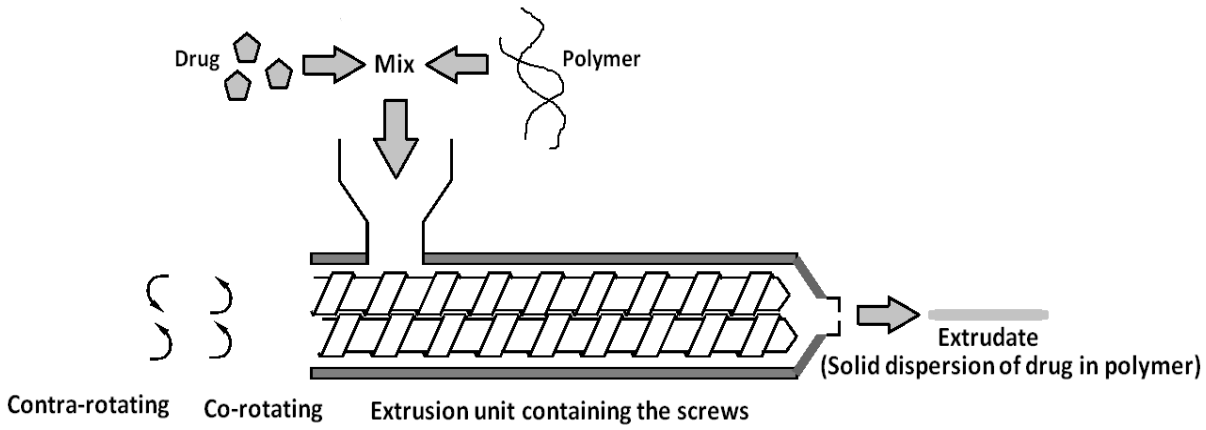


Fig. 6. Schematic drawing of hot-melt extrusion process for solid dispersions preparation

1.4.7.2 Characterization of solid dispersions

Differential scanning calorimetry (DSC), powder x-ray diffraction (PXRD) and infra-red spectroscopy (IR) are the most valuable and widely applied techniques for characterization of solid dispersions at the molecular level owing to their mutually complementary structure-probing capabilities, relative ease of operation and comparatively short analysis time (Lee et al., 2014).

1.4.7.2.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is a valuable technique to understand the thermal properties of materials such as melting and recrystallization. Furthermore it is effective in determining the glass transition temperature of amorphous materials, the miscibility between solid dispersion components and the presence of any phase impurities (Lee et al., 2014).

DSC measures how physical properties of a sample change along with temperature against time. During a change in temperature, DSC determines a heat quantity which is radiated or absorbed excessively by the sample on the basis of a temperature difference between the sample and the reference material (Gill et al., 2010). The sample is sealed into a small aluminum pan. The reference is usually an empty aluminum pan and cover.

During the heating, peaks with positive and negative heat flow difference may be recorded; each peak corresponds to a heat effect associated with a specific process, such as phase transformation, crystallization or melting.

Sample amount and scanning rate are critical parameters for thermal properties determination by DSC, where insufficient sample amount could lead to difficulties in detecting or even an undetectable thermal signal. On the other hand, scanning rate can influence drug recrystallization kinetics, furthermore, increase of scanning rate can provide a better sensitivity for identifying the glass transition event in the DSC thermogram (Liu et al., 2009 and Lee et al., 2014).

1.4.7.2.2 Powder x-ray diffraction (PXRD)

PXRD can be used to confirm the amorphous/crystalline nature of a sample. During X-ray measurement, the sample is exposed to x-rays at various angles; the diffraction patterns produced are then compared with reference standards for identification.

Crystal samples exhibit strong x-ray scattering and generate sharp characteristic peaks at specific collection angles in a diffractogram. While amorphous solids yield diffuse halo patterns due to their lack of long-range order symmetries (Lee et al., 2014).

PXRD is a powerful and rapid (< 20 min) technique and simple in operation. Moreover, the interpretation of data is relatively straight forward. However, the lack of sensitivity is the main drawback of this method (Rendle, 2003).

1.4.7.2.3 Infra-red spectroscopy

Infra-red spectroscopy is well-established technique for compound identification and has been extensively used in the study of intermolecular interactions between solid dispersion components, especially hydrogen bonding (Lee et al., 2014).

During the measurement, the sample is irradiated with a broad spectrum of infrared light, of which a fraction of the light will be absorbed by the sample. The absorption range for FTIR is from 400-4000 cm^{-1}. The region from 400-1000 cm^{-1} is the fingerprint region of the spectrum and the region from 1000-4000 cm^{-1} is defined for specific functional groups (Munson, 2009). For example carbon oxygen double bound absorbs at wavelength around 1800-1600 cm^{-1} whereas, the absorption bands of hydroxyl groups are situated between 3650-3200 cm^{-1}.

The measurements made by infra-red spectroscopy are very accurate and reproducible, and are made in a matter of seconds. Thus, it is a very reliable technique for positive identification of any sample.

1.4.7.3 Combination of solid dispersions with sustained release techniques

Sustained release formulations improve efficacy, reduce side effect, and give more desirable dose regimen (Chen et al., 2010a). However, the release of poorly soluble drugs from the extended release dosage forms is typically not complete without solubilization. Thus, to achieve an extended and complete release of poorly soluble drugs, a combination of solubilization (e.g. by solid dispersion) and retardation is required (Tran et al., 2011 and Li Hong et al., 2013).

The combination of solid dispersion with sustained release techniques can be done either by formulating the solid dispersion in a matrix tablet containing a rate controlling polymer or by directly modifying the release properties of solid dispersion using a rate controlling polymer as a carrier for solid dispersion and then formulating it in a fast disintegrating tablet or in a capsule (Tran et al., 2011).

Matrix based control release tablets are the most popular and commonly used dosage form for control release in pharmaceutical industries because of their pharmaceutical advantages such as, the relative simplicity of process development and scale-up procedures. The matrix tablets can be prepared via wet granulation or by direct compression. The drug release from such systems can occur by several mechanisms such as leaching, diffusion, or erosion, depending on the properties of the rate controlling polymer, therefore, the properties of rate controlling polymers should be carefully considered.

In case of swellable soluble/erodible polymers which are the main focus of this work, they hydrate quickly the tablet surface upon contact with water to form a gelatinous layer, and as the outer gel layer is fully hydrated and dissolved, a new inner layer must replace it. The gel layer controls the in water flux and drug diffusion, and the gel strength is controlled by chemistry, viscosity and concentration of the polymer. Water soluble drugs are released primarily through diffusion through the gel layer. On the other hand, poorly water-soluble drugs are released mainly by the erosion process of the surrounding matrix structure (Maderuelo et al., 2011 and Tran et al., 2011).

The approaches used in this study to extend the release of poorly water-soluble drugs depending on the drug and polymeric carrier properties by hot melt extrusion are presented in **Table 4**.

Table 4. The approaches used in this study to extend the release of poorly soluble by hot melt extrusion

Hot Melt Extrusion			**ER dosage form**
Carrier	**Function**	**Targeted drugs**	
Water insoluble polymer (Kollidon® SR)	Improvement of solubility and release extension	pH-independent poorly soluble drugs	Fast disintegrating tablet
Water insoluble polymer (Kollidon® SR) + water soluble polymer (Soluplus®)			
Water soluble polymer (Soluplus®)	Improvement of solubility and dissolution rate	pH-independent poorly soluble drugs	Erodible matrix tablet
Water soluble polymer (Soluplus®) + enteric polymer (Aqoat® AS-LF or Kollicoat® MAE 100 P)	Improvement of solubility, dissolution rate and pH independent drug release profile	Weakly basic poorly soluble drugs	

1.4.7.4 Carriers used for solid dispersions

Solid dispersions are categorized into 3 different generations depending on the nature of carriers used in solid dispersions preparation; first generation solid dispersions prepared using crystalline carriers, second-generation solid dispersion prepared using amorphous carriers and those prepared using surface-active carriers are referred to as the current generation or third generation solid dispersions (Tran et al., 2011). The following carriers properties should be considered in the preparation of solid dispersions: 1) glass transition temperature (T_g); polymers having high T_g are preferable to have high physical stability as they raise the T_g of the overall system so that the metastable solid dispersions can be stable at least at room temperature, 2) chemical interactions; polymers which can generate possible interactions with the drug can further improve the physical stability (Lee et al., 2014), 3) solubilizing properties, 4) thermal stability and thermoplasticity for systems prepared by hot-stage extrusion, and 5) high solubility in organic solvents for systems prepared by solvent method (Janssens and Van den Mooter, 2009 and Tiwari et al., 2009).

Generally high-molecular-weight amorphous carriers are mostly used to prepare solid dispersions such as: HPMC (Hydroxypropyl Methylcellulose) and PVP (polyvinylpyrrolidone) which are able to reduce the drug recrystallization by increasing the local viscosity in the solid dispersion matrix or by possible generation of intermolecular interactions between polymer and drug and thus restrict the molecular movement and, therefore, enhance physical stability (Williams et al., 2013).

The novel polymer Soluplus® (**Fig. 7**) is a polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer and it acts as a suitable carrier for amorphous solid dispersions, due to its relative high glass transition temperature (T_g of 70 °C) and its amid

structure which provides the drug with hydrogen bond acceptors and this improves the physical stability. Furthermore, it forms micelles in the aqueous solutions above its critical micelles concentration of 7.6 mg/l due to its amphiphilic structure which help to maintain the supersaturation level of the drug in solution.

Hardung et al., 2010 demonstrated the ability of Soluplus® to form glassy solid solutions and to improve the oral bioavailability of many model drugs such as danazol, fenofibrate and itraconazole.

Soluplus® can also be used as a wet or dry binder, solubilizer and stabilizer.

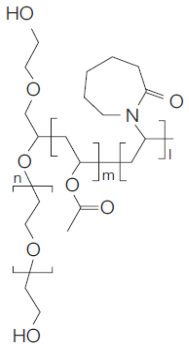


Fig. 7. Chemical structure of Soluplus® (BASF, 2010b)

In case of poorly water-soluble drugs with pH-dependent solubility, ionizable polymers can be used. For example, for weakly basic drugs anionic polymers such as Hydroxypropyl Methylcellulose Acetate Succinate (Aqoat® AS) and Kollicoat® MAE 100 P can be used and for weakly acidic drugs cationic polymers such as Eudragit® E PO can be used (Sarode et al., 2013).

Aqoat® AS (Hydroxypropyl Methylcellulose Acetate Succinate (**Fig. 8**)) is available in six grades which are different in particle sizes and chemical substitution levels. Aqoat® AS has high T_g (120-135 °C) which give excellent physical stability of its solid dispersions. Aqoat® AS forms colloids in the aqueous solutions related to its amphiphilic nature. Furthermore, the charge on the polymer minimizes the formation of large aggregates, allowing drug/polymer colloids to remain stable in the aqueous solutions (pH > 5) (Friesen et al., 2008). Tanno et al., 2004 and Friesen et al., 2008 demonstrated the ability of Aqoat® AS to form amorphous solid dispersion and to enhance the solubility of a poorly-soluble drugs.

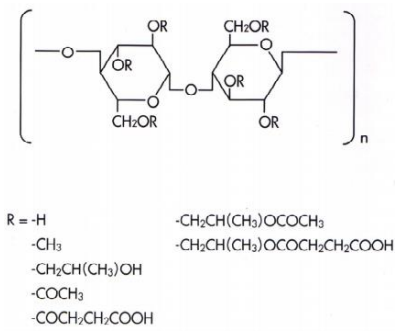


Fig. 8. Chemical structure of Aqoat® AS (ShinEtsu, 2005)

Kollicoat® MAE 100 P (**Fig. 9**) is an anionic co-polymer consists of methacrylic acid and ethyl acylate in ratio of 1:1, it can be used as enteric matrix in solid dispersion formulations or as a film former in enteric coating for solid dosage forms in which the drug being released mainly in intestine. Kollicoat® MAE 100 P also has a high T_g (114 °C) which give excellent physical stability of its solid dispersions.

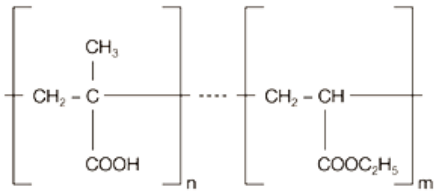


Fig. 9. Chemical structure of Kollicoat® MAE 100 P (BASF, 2010)

Eudragit® E PO (**Fig. 10**) is a poly (butyl methacrylate-co-(2-dimethylaminoethyl) methacrylate-co-methyl methacrylate 1:2:1. It is a cationic copolymer which dissolves in pH less than 5, it is normally used for taste masking and moisture protection, and it can be used also as a carrier in solid dispersion for immediate release or taste-masking formulations. Despite its low T_g (~45 °C), it can reduce the drug recrystallization via hydrogen bonding or ionic interactions and thus provide a good physical stability of amorphous solid dispersions. Kojima et al., 2012 demonstrated the ability of Eudragit® E PO to form an amorphous solid dispersion, enhance the solubility and stabilize the supersaturated solution of mefenamic acid.

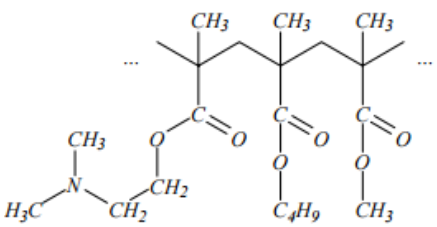


Fig. 10. Chemical structure of Eudragit® E PO (Evonik, 2007)

Water insoluble polymers can also be used as carriers for solid dispersion. However, those systems are maybe not really suitable for poorly water-soluble drugs because the concentration gradient is too low to give adequate drug release.

Kollidon® SR (**Fig. 11**) consists of 80 % polyvinyl acetate (water insoluble polymer), 19% polyvinylpyrrolidone (water soluble polymer), approximately 0.8 % sodium lauryl sulfate (stabilizer) and 0.2 % silica in a physical mixture and is used as an extended release excipient in matrix tablets (Kranz and Wagner, 2006). The sustained release properties of Kollidon® SR are unaffected by pH, ions or salts. It has two glass transition temperatures at 39 and 152 °C and can be used to produce sustained release formulation by direct compresion, roller compaction, wet granulation and extrusion. Özgüney et al., 2009; developed a sustained-release Kollidon® SR mini-matrices for oral delivery of ibuprofen and theophylline by HME.

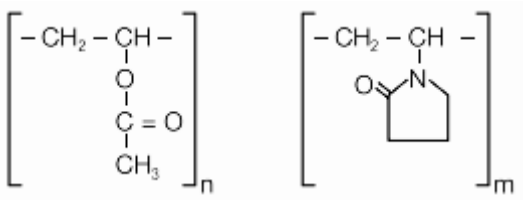


Fig. 11. Chemical structure of Kollidon® SR (BASF, 2008)

1.5 Selection of formulation approaches for poorly soluble drugs based on drug/dosage form specifications

Although many formulation approaches to address the poor bioavailability problem of poorly water-soluble drugs have been published, only few advances have been made to select the best formulation approach based on the drug properties and the required specifications of the final dosage form (**Table 5**). Such formulation selection strategies help to eliminate as many formulation options as possible and therefore faster and more economical pharmaceutical development. Structural properties of the drug molecules such as; melting point, lipophilicity have not only effects on the solubility/bioavailability but also on the formulation selection. Co-solvents and lipid formulations are preferred for drug molecules having high lipophilicity (log p>5), however in case of drug molecules having both high lipophilicity and high melting points, nanosuspensions and solid dispersions by hot melt extrusion are more preferable. This can be due to the reduction in tendency of the drug molecules to dissolve regardless of solvents when the melting point (MP) is high (MP>150 °C), and thus low drug molecules solubility in both water and oil (Rabinow, 2004 and Rowe and Johnston, 2012).

Rabinow (Rabinow, 2004) proposed a decision tree for the selection of formulation approach based on the required dose and few structural properties of the drug molecule such as melting point, log P and the molecular shape. The aim of his work was to show the advantages of nanosuspension formulation over the other formulation approaches, and he concluded that a nanosuspension formulation is the preferred solution for the most challenging compounds that have high log P, high melting point and high dose.

Furthermore, for better formulation selection it is important to distinguish between solubility and permeability limited absorption. If the solubility was identified as the primary limiting factor for absorption, further distinguish between solubility and dissolution rate limited absorption is required. According to Developability classification system (DCS) proposed by (Butler and Dressman, 2010), the solubility limited absorbable dose (SLAD) for highly permeable drugs is expressed as:

$$\boldsymbol{SLAD = S_{si}.V.A_n}$$

Where, S_{si} is the estimate of small intestine solubility, V is the fluid volume (500 mL) and An is the absorption number.

In case of drug molecules with dissolution rate-limited absorption simple solubilization technologies such as micronization or co-solvent may enough to improve the bioavailability

of poorly water-soluble drugs. However, more complex solubilization technologies are needed for drug molecules with solubility-limited absorption. In case of permeability-limited absorption (lower than $1x10^{-4}$ cm/sec), formulations or paracellular permeability enhancers are rarely used to enhance permeability because of toxicity, therefore structure modification is required to improve the permeability (Ward et al., 2000 and Kerns and Di, 2008). However, Roger et al., 2009 demonstrated the ability of lipid nanocarriers to improve the permeability of paclitaxel via active endocytic processes and more particularly via clathrin-dependent and caveolae-dependent transport mechanisms. Particle size reduction to submicron size may also improve the permeability by uptake through the gastrointestinal membrane (Desai et al., 1996).

The required specifications of the final dosage form have also an impact on the formulation selection. Developing a controlled release formulation for a poorly-water soluble drug can be done by carefully selection of the carrier/polymer properties for lipid nanoparticle, polymeric micelles or solid dispersion formulations. In case of nanocrystal, micronization and nanoemulsion formulations further formulation steps are required to achieve a controlled release profile, and thus make them less preferable for controlled release dosage forms (Chen et al., 2011). Nanocrystals are particles made of almost 100% drug and few amount of stabilizers (Müller et al., 2011), therefore, when high drug loading is required, such carrier-free systems are recommended (Rabinow, 2004).

The residual solvent, contamination, agglomeration or presence of microparticles can limit the use of some formulation techniques or method of preparation for intravenous or inhalation administration route (e.g. residual solvent by co-solvent approach or erosion from the milling material by bead milling method for nanocrystal preparation).

Moreover, the thermal/chemical instability of drug molecules can be avoided by carefully selection of proper preparation methods of the elected formulation approaches.

Table 5. Selection of the formulation approach for poorly water-soluble drugs depending on the drug properties and the required specifications of the final dosage form

Drug properties/ final dosage form		**Formulation approaches**							
		Micronization	Co-solvents	Nanoemulsion	Lipid nanoparitcle	Polymeric micelles	Solid dispersion	Nanocrystals	Inclusion complex
↑Log P, > 5			•	•	•	•	•	•	•
↑Melting point, > 150 ˚C			• *	• *	• *	• *	•	•	•
Dissolution rate-limited absorption		•	•						
Solubility-limited absorption				•	•	•	•	•	•
Permeability-limited absorption				•	•			•	
Control release					•	•	•		•
↑Drug loading, > 50%		•						•	
Administration route	Oral	•	•	•	•	•	•	•	•
	Injectable			•	•	•		•	•
	Inhalation			•	•	•		•	•

* Possible only if the drug loading is low

Mainly two poorly soluble drugs were selected for this work and the properties of these drugs are presented in **Table 6**. Itraconazole is an antifungal agent indicated for the treatment of a broad spectrum of fungal infections including: blastomycosis, histoplasmosis and aspergillosis. It has high log P and melting point, a low solubility in pH range from 1-7 and can be classified according to DCS category as solubility-limited absorption drug.

In case of mefenamic acid, it is a nonsteroidal anti-inflammatory drug (NSAID), has a high log P and a high melting point. Furthermore it is has a low solubility in all pH range typical of the gastro-intestinal (GI) tract (pH=1-7). Mefenamic acid located according to DCS category at the borderline between solubility-/dissolution rate-limited absorption.

According to the formulations approach selection of poorly soluble drugs presented in **Table 5**, a lot of formulation approaches can be applied for these both drugs. However, solid solution/ dispersion and nanosuspensions were selected for further distinguish between the both methods with the aim of improving solubility/bioavailability of poorly soluble drugs.

Table 6. Properties of selected poorly soluble drugs

Specification	**Drugs**	
	Mefenamic acid	**Itraconazole**
Water solubility 37°C (USP, µg/ml)	pi, 3 [a]	pi, 0.002 [c]
FaSSIF Solubility 37°C (µg/mL)	36 [b]	0.07 [c]
Single high dose (mg)	250	100
Permeability (cm/s×10^{-4})	14 [b]	>1
BCS category	II	II
DCS category	Solubility-/dissolution rate-limited absorption boundary [b]	Solubility-limited absorption
Melting point, °C	233 [a]	168 [c]
Log P	5.12 [d]	6.2 [e]
pK_a	4.2 [d]	3.7[f]
Dosage	250 mg, 3 times daily	100 mg, twice daily

a) Experimental
b) (Butler and Dressman, 2010)
c) (Ghazal et al., 2009)
d) (Müllertz, 2007)
e) (Yang et al., 2008)
f) (Hong et al., 2006)

pi: practically insoluble

1.6 Research objectives

- To improve the solubility/dissolution rate of poorly soluble drugs using nanosuspension and solid dispersion techniques and to investigate the effect of formulation and process variables on solubility/dissolution rate enhancement and stability.

- To achieve an extended and complete release profile of poorly soluble drugs by combining a release modulating technique with a solubility improvement technique.

- To achieve pH-independent release profiles of ionic poorly soluble drugs with high improvement of solubility and dissolution rate across the gastro intestinal tract and to achieve a pH-independent extended and complete release thereof.

Chapter 2. Materials and methods

2. MATERIALS AND METHODS

2.1 Materials

Model drugs

- Itraconazole, ritonavir (BASF AG, Ludwigshafen, Germany)
- Mefenamic acid (Sigma-Aldrich Chemie GmbH, Steinheim, Germany)

Stabilizers for nanosuspensions

- Hydroxypropyl methylcellulose (HPMC) (Methocel® E5, E15 and E50; Colorcon Ltd, Dartford Kent, UK)
- Poloxamer 188 (Lutrol® F68; BASF AG, Ludwigshafen, Germany)
- Polyoxyethylene sorbitan monooleate (Tween® 80; Sigma-Aldrich Chemie GmbH, Steinheim, Germany)
- Sodium lauryl sulfate (SLS) (Roth GmbH & Co. KG., Karlsruhe, Germany)

Polymers for hot-melt extrusion (HME)

- PEG 6000-vinylcaprolactam-vinyl acetate graft copolymer (Soluplus®), Methacrylic acid-Ethylacrylate 1:1 copolymer (Kollicoat® MAE 100 P), polyvinyl acetate-vinylpyrrolidone co-spray dried (Kollidon® SR) (BASF AG, Ludwigshafen, Germany)
- Hydroxypropyl methylcellulose acetate succinate (Shin-Etsu Aqoat® AS-LF; Shin-Etsu Chemical Co.,Ltd)
- Aminoalkyl Methacrylate Copolymer E (Eudragit® E PO; Evonik Industries AG, Darmstadt, Germany)

Plasticizer for hot-melt extrusion (HME)

- Triethyl citrate (TEC) (Citroflex® 2; Morflex, Greensboro, NC, USA)

Excipients for tableting

- Hydroxypropyl methylcellulose (HPMC) (Methocel® E15, K15M and K100M), Polyethylene oxide (Polyox(TM) WSR Coagulant - LEO NF) (Colorcon Ltd, Dartford Kent, UK)
- Cross linked polyvinylpyrrolidone (Kollidon® CL), Co-processed lactose monohydrate, povidone K30 and crospovidone (Ludipress®) (BASF AG, Ludwigshafen, Germany)
- Microcrystalline cellulose (Avicel® PH 102), Croscarmellosesodium (AC-Di-Sol®) (FMC BioPolymers, Philadelphia, PA, USA)
- Calcium hydrogen phosphate dihydrate (Emcompress®; JRS PHARMA GmbH & Co. KG, Rosenberg, Germany)
- Talc (Luzenac® pharma; Luzenac Europe, Toulouse, France)

Other excipients

- Sodium chloride (NaCl) (Roth GmbH & Co. KG., Karlsruhe, Germany)

2.2 Methods

2.2.1 Nanosuspensions

2.2.1.1 Preparation by wet milling technique

40 g of suspension containing (2, 6 or 10 % w/w) mefenamic acid or (2% w/w) itraconazole was prepared by suspending the drug powder in a stabilizer solution using IKA Ultra-Turrax T25 (Janke & Kunkel GmbH, Staufen, Germany) at 8000 rpm for 10 seconds, then the suspension was milled using Dyno® Mill KDL A (Willy A. Bachofen GmbH, Heldenbergen, Germany) at 3200 rpm milling speed and 0.3 mm yttrium stabilized zirconia grinding beads (YTZ® grinding media; Tosoh Europe B.V., Amsterdam, The Netherlands). The beads: suspension ratio was 75:25. **Table 7-8** summarize all nanosuspensions formulations

Table 7. Formulations of mefenamic acid nanosuspensions

Substance	**Formulation**								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Mefenamic acid	2%	2%	2%	2%	2%	2%	2%	6%	10%
Tween® 80	0.5%	-	-	-	-	-	-	-	-
Lutrol® F68	-	0.5%	-	-	-	-	0.4%	1.2%	2%
SLS	-	-	0.25%	-	-	-	0.1%	0.3%	0.5%
Methocel® E5	-	-	-	0.5%	-	-	-	-	-
Methocel® E15	-	-	-	-	0.5%	-	-	-	-
Methocel® E50	-	-	-	-	-	0.5%	-	-	-
Water	97.5%	97.5%	97.75%	97.5%	97.5%	97.5%	97.5%	92.5%	78.5%

Table 8. Formulations of itraconazole nanosuspensions

Substance	**Formulation**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
Itraconazole	2%	2%	2%	2%	2%	2%
Tween® 80	1.5%	-	-	-	-	-
Lutrol® F68	-	1.5%	0.5%	-	1.2%	-
SLS	-	-	-	0.5%	0.3%	-
Methocel® E5	-	-	-	-	-	1.5%
Water	96.5%	96.5%	97.5%	97.5%	97.5%	96.5%

2.2.1.2 Characterization

2.2.1.2.1 Particle size distribution

Particle size distribution was determined by laser diffractometry (LD) (Mastersizer 2000, Malvern Instruments, UK). The indexes of refraction for mefenamic acid and itraconazole used in this study were 1.693 and 1.678, respectively and the absorption index was 0.001. The sample was added to the dispersion unit containing water until the obscuration was within the range of 4-6%, and then the LD measurement was carried out immediately at 1500 rpm stirring speed. The LD data obtained by averaging of 5 measurements were evaluated using the volume distribution diameters (D50%, D90% and D99%) which indicate the percentage of particles possessing a diameter equal or lower than the given size value.

2.2.1.2.2 Average particle size

The average particle size was determined by photon correlation spectroscopy (PCS) (Zetasizer Nano ZS, Malvern Instruments, UK). PCS measures the mean particle size and the polydispersity index (PDI) which is a measure of the width of the size distribution. The sample was diluted with bi-distilled water to have a suitable scattering intensity, and then the PCS measurement was carried out immediately. The average particle size and PDI values were obtained by averaging of 3 measurements at 25 °C, each measurement consist of 13-15 runs.

2.2.1.2.3 Particle charge (zeta potential)

Zetasizer Nano ZS (Malvern Instruments, UK) was used to measure the zeta potential value by applying Henry equation (Bague et al., 2006):

$$U_E = 2\,\varepsilon z\, f(K_a)/\,3\eta$$

Where, U_E is the electrophoretic mobility, z is the zeta potential, ε is the dielectric constant, η is the viscosity and $f(K_a)$ is the Henry's function which is equal to 1.5 in aqueous media and at a moderate electrolyte concentration, and therefore is referred to as the Smoluchowski approximation. The measurements were performed in distilled water adjusted to a conductivity of 50 μS/cm with sodium chloride solution (0.9 % w/v), and were repeated three times at 25 °C.

2.2.1.2.4 Light microscopy

Light microscopy was performed using Leica Wild M3Z (Leica Microsystems (Schweiz) AG, Heerbrugg, Switzerland). The employed magnification was 40x10 fold. Each sample was investigated 3 times. The crystallinity was examined using polarized light.

2.2.1.2.5 Differential scanning calorimetry (DSC)

Thermal properties of samples were studied using differential scanning calorimetry (DSC) (DSC-822e Mettler-Toledo, Switzerland). 4 mg of the sample was weighed accurately in a 40 µl aluminum pan and sealed. The pan was subjected to a heat rate of 10 ˚C/ min. The melting point (T_m) was determined by the star^e software.

2.2.1.3 Dissolution studies

Specified amount* of drug powder, suspension or nanosuspension were investigated using the USP rotating paddle method (VK 7010, Vankel Technology Group, Cary, USA) in 0.1 N HCl or PBS pH 6.8 for mefenamic acid or itraconazole, respectively, at 37 °C, using 100 rpm stirring speed. At predetermined time points, samples were taken and filtered through a 0.1 µm filter. The amount of dissolved drug was measured UV-spectrophotometrically at 285 or 254 nm for mefenamic acid or itraconazole, respectively.

* Dissolution under sink condition: corresponding to 10 mg mefenamic acid or 2.7 mg itraconazole in 900 ml dissolution medium.

* Dissolution under non-sink condition: corresponding to 50 mg of mefenamic acid or itraconazole in 450 ml dissolution medium.

2.2.2 Solid dispersions

2.2.2.1 Preparation by hot-melt extrusion (HME)

For all formulations, the ingredients were mixed manually using a mortar and pestle for 5 minutes. HME was performed in a twin-screw hot-melt extruder (Minilab HAAKE Rheomex CTW5, Thermo Scientific, Karlsruhe, Germany). Powder blends were fed using force feeder into the preheated barrel.

The first 3 g of extrudates were discarded from each batch and then the extrudates were cut into uniform length of 5 cm, collected in dark glass vials and stored in desiccator at room temperature. The processability parameters of all formulations are presented in **Table 9-11**.

2.2.2.2 Milling and sieving

Milling was performed in the cryo ball mill (Retsch MM 2000 small ball mill, Retsch GmbH, Haan, Germany) using liquid nitrogen. 2-3 g extrudates were filled into 10 ml metal jar supplied with 2 metal balls (10 mm in diameter) and milled with 70-90 stokes/sec for 15-60 seconds.

Milled extrudates were then sieved in a vibratory sieve shaker (Analysette 3 PRO, Fritsch GmbH, Idar-Oberstein, Germany) using sieves 425, 315, 160 µm at amplitude 0.8 mm for 2 min. The size fraction: <160, 160-315, 315-425 µm were used for the further studies.

2.2.2.3 Characterization

2.2.2.3.1 Differential scanning calorimetry (DSC)

Thermal properties of the samples were studied using differential scanning calorimetry (DSC) (DSC-822e Mettler-Toledo, Switzerland). Drug, drug:polymer physical mixture or drug:polymer milled extrudates were weighed accurately in a 40 µl aluminum pans and sealed. The pans were subjected to heat or heat–cool–heat cycle under a nitrogen atmosphere and 10 ˚C/ min scanning rate. The melting point (T_m) and the glass transition temperature (T_g) were determined by the star^e software.

2.2.2.3.1 Powder x-ray diffraction (PXRD)

Drug, drug:polymer physical mixture or drug:polymer milled extrudates were tested using Philips PW 1830 X-ray generator with a copper anode (Cu Kα radiation, λ = 0.15418 nm, 40 kV, 20 mA) fixed with a Philips PW 1710 diffractometer (Philips Industrial & Electro-acoustic Systems Division, Almelo, The Netherlands). The scattered radiation of the samples was detected with a vertical goniometer (Philips PW 1820, Philips Industrial & Electro-

acoustic Systems Division, Almelo, The Netherlands). A scanning rate of 0.02 2θ per sec over the range of 4-40 2θ at ambient temperature was used to determine each spectrum.

2.2.2.3.2 Fourier transforms infra-red (FTIR)

FTIR spectroscopy measurement was performed with an Excalibur 3100 FTIR spectrophotometer (Varian Inc., Palo Alto, USA). The spectra from drug, drug:polymer physical mixture or drug:polymer milled extrudates were obtained in the scan range of 600 to 4,000 cm^{-1} at a resolution of 4 cm^{-1} and average of 16 scans, using a horizontal ATR accessory with a single reflection diamond crystal (Pike Miracle, Pike Technologies, Madison, USA) and Varian software (Resolution Pro 4.0).

2.2.2.4 Aqueous solubility

Excess amount of drugs powder or milled extrudates were suspended in 100 ml 0.1 N HCl, PBS pH 6.8 or (0.15, 0.3, 0.6 % w/v) polymeric solution, and were shaken in vials at 75 rpm using an incubator shaker at 37 ˚C for 24 h or until equilibrium was achieved. (More details for the amount of extrudates added are described in results and discussion). Samples were filtered using 10 µm filter and diluted 10 times with methanol:0.1 N HCl (1:1) solution. The amount of dissolved drugs was measured UV-spectrophotometrically at 258 or 243 for itraconazole or ritonavir, respectively.

Table 9. Summary of the HME processability parameters of all itraconazole formulations

Extrudate			**T Extrusion, °C**	**Screw speed, rpm**	**Torque, Ncm**	**Extrusion rate, g/min**	**Appearance**
Polymer/s	Drug: Polymer/s ratio	Polymer combination ratio					
Soluplus®	1:0.5	/	170	15	-	-	yellow/ semi-transparent
	1:1				6	0.45	yellow/ transparent
	1:2				6	0.45	
	1:5				7-8	0.45	
	1:3		170	15	6-7	0.42	
			170	40	7-8	0.6	
			150	15	13-18	0.44	
			130	15	75-85	0.3	white/opaque
Aqoat® AS-LF	1:3	/	170	15	29-32	0.29	brown/ semi-transparent
Soluplus®: Aqoat® AS-LF	1:3	75:25	170	15	14-16	0.52	yellow/ transparent
		50:50			18-21	0.44	yellow/ transparent
		25:75			26-30	0.37	dark yellow/ semi-transparent
Kollidon® SR	1:1	/	170	15	10-11	0.30	beige/opaque
	1:3				10-12	0.30	beige/opaque
Soluplus®: Kollidon® SR	1:3	50:50	170	15	8-10	0.39	beige/opaque
		25:75			10-12	0.35	beige/opaque
Soluplus®: Kollicoat® MAE100P:TEC	1:3	50:35:15	145	15	6-8	0.7	white/opaque rough surface
Soluplus®	1:1.5	/	170	15	6-8	0.45	yellow/ transparent
Soluplus® extrudates 1:1.5+ Kollicoat® MAE100P:TEC	1:3	50:35:15	145	15	6-8	0.66	white/opaque rough surface

Table 10. Summary of the HME processability parameters of all ritonavir formulations

Extrudate			T Extrusion, °C	Screw speed, rpm	Torque, Ncm	Extrusion rate, g/min	Appearance
Polymer/s	Drug: Polymer/s ratio	Polymers combination ratio					
Soluplus®	1:1.5	/	135	15	7-10	0.5	yellow/ transparent
Soluplus®: Kollicoat® MAE 100 P	1:1.5	75:25	135	15	9-12	0.53	yellow/ semi-transparent
		50:50	142	30	70-100	0.25	yellow
		25:75	145	30	120-150	0.2	dark yellow

Table 11. Summary of the HME processability parameters of all mefenamic acid formulations

Extrudate			T Extrusion, °C	Screw speed, rpm	Torque, Ncm	Extrusion rate, g/min	Appearance
Polymer/s	Drug: Polymer/s ratio	Polymers combination ratio					
Soluplus®	1:3	/	170	15	11-12	0.5	yellow/ transparent
	1:5				11-12	0.51	
Eudragit®EPO	1:1	/	170	15	6-8	0.52	yellow/ transparent
	1:2				6-8	0.52	
	1:3				6-8	0.53	
	1:5				6-7	0.54	
Soluplus®: Eudragit®EPO	1:3	50:50	170	15	9-10	0.5	yellow/ transparent

2.2.2.5 Tableting

Milled extrudates and excipients were gently mixed in the mortar using a plastic card for 5 minutes. Tablets were prepared in the instrumented tablet press EK0 (Korsch AG, Berlin, Germany) equipped with single round punches 8, 10, 12 mm or oval punch 19x11 mm at compression force 5-15 kN. The tablets were further characterized regarding their dimensions and hardness (Multicheck, Erweka GmbH, Heusenstamm, Germany).

2.2.2.6 Dissolution studies

The drug release from different types of tablets was investigated using the USP rotating paddle method (VK 7010, Vankel Technology Group, Cary, USA) either in 900 ml 0.1 N HCl, PBS pH 6.8, PBS pH 5.5, 0.1 N HCl + 0.2 M NaCl or in 750 ml 0.1 N HCl for 2 h followed by pH change to 6.8 using 250 ml 0.2 M tribasic sodium phosphate at 37 °C and 100 rpm stirring speed (unless otherwise noted). At predetermined time points, samples were taken and filtered through 10 µm filters and diluted with methanol. The amount of dissolved drug was measured UV-spectrophotometrically at 258, 243 or 289 nm for itraconazole, ritonavir or mefenamic acid, respectively.

Chapter 3. Results and discussion

3. RESULTS AND DISCUSSION

3.1 Nanosuspensions

3.1.1 Effect of stabilizer type on particle size reduction and zeta potential

Nanocrystals tend to agglomerate to reduce the generated extra free energy. Stabilizers adsorb at the interface between the drug surface and aqueous phase, reducing the interfacial tension and thereby decreasing the total free energy of the system. Therefore, selection of stabilizers (e.g. polymers or surfactants) plays a major role in formulating nanosuspensions. Inappropriate stabilizer may cause particles agglomeration due to the high surface energy or promote Ostwald ripening due to the solubility enhancement of small nanometer-sized particles which may solubilize and re-crystallize onto larger particles present in the nanosuspension. (Merisko-Liversidge et al., 2003; Peltonen and Hirvonen, 2010; Cerdeira et al., 2010; Merisko-Liversidge and Liversidge, 2011).

Particle size reduction from micrometer to nanometer range was successfully obtained by wet milling. The average particle size of all nanosuspension formulations was below 600 nm with 99% of the particles being less than 3.5 μm. All nanosuspension formulations showed sufficient zeta potential values, higher than -48.2 mV in case of electrostatic stabilizer (SLS) and higher than -20 mV in case of steric or electrostatic/steric stabilizers (**Table 12-13**).

In case of mefenamic acid, the best particle size reduction was obtained from nanosuspension stabilized by Methocel® E5 followed by the one stabilized by SLS with average particle size of 230 nm and 270 nm respectively, whereas nanosuspension stabilized by Lutrol® F68 resulted in particles size around 300 nm. Methocel® E5 and Lutrol® F68 are steric stabilizers and have many hydroxyl groups enabling them to form hydrogen bonds with the carboxylic acid group presented in the structure of mefenamic acid, thus provide inhibitory effects of nucleation and crystal growth. The difference between the particle size obtained using Methocel® E5 and Lutrol® F68 can be related to variations in the level of hydroxyl group substitution along both polymers. The good particle size reduction obtained by SLS is related to its high affinity to adsorb onto the particle surface leading to a high zeta potential value -51.5 mV, furthermore, SLS provided a large improvement in wettability, where the contact angle between its 0.25% aqueous solution and compressed disc of mefenamic acid decreased to 23±1.5˚ compared to contact angle of 80±2˚ between water and compressed disc of mefenamic acid. These all help to decrease the particle size by preventing nucleation and crystal growth.

In case of itraconazole, the best particle size reduction was obtained from nanosuspension stabilized by the electrostatic stabilizer SLS with average particle size of 227 nm, whereas

slightly bigger particle size around 300 nm was obtained from nanosuspension stabilized by the steric stabilizer Lutrol® F68. This could be due to the better wettability of itraconazole particles when SLS was used. Furthermore the amine groups present in itraconazole structure might have caused attractive interactions with the anionic surfactants SLS.

Surprisingly, mefenamic acid nanosuspension prepared with a combination of the steric stabilizer Lutrol® F68 and the electrostatic stabilizer SLS resulted in slightly larger particles size around 350 nm compared to nanosuspensions stabilized by a single stabilizer. This might be due to the strong interaction between Lutrol® F68 and SLS (Hecht et al., 1995), which might have hindered the physical interactions between the stabilizers and the drug. However, using the same stabilizers combination for the preparation of itraconazole nanosuspension resulted in an average particle size comparable to the one obtained by SLS alone. This could be due to the strong interaction between the itraconazole and SLS. The same finding was obtained by Lee et al., 2008, whereby addition of anionic/cationic surfactant stabilizer to a polymeric stabilizer promoted nanogrinding in some formulations, but caused particles growth in some other formulations.

The largest particle size was obtained from mefenamic acid/itraconazole nanosuspensions stabilized by Tween® 80 which can be related to crystal growth or aggregates formation.

Moreover, the type of stabilizer can affect the width of particle size distribution. Not only smaller particle size but also finer particle size distribution was obtained from nanosuspension stabilized by Methocel® E5 compared to the one stabilized by Tween® 80 (**Fig. 12**).

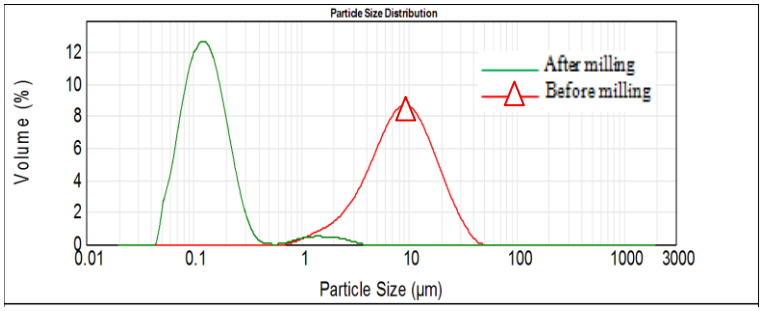
Table 12. Effect of stabilizer type on the efficiency of mefenamic acid nanomilling and zeta potential (all formulations contain 2% mefenamic acid)

Stabilizer, % w/w	Before milling			After 40 min milling					
	Particle size distribution (µm)			Particle size distribution (µm)			Mean particle size (µm)	PDI	Zeta potential (mV)
	D 50	D 90	D 99	D 50	D 90	D 99			
Methocel® E5, 0.5	8.55	19.98	33.43	0.124	0.228	1.98	0.23	0.19	-21.50
SLS, 0.25	15.62	36.27	63.13	0.245	0.771	1.85	0.27	0.23	-51.50
Lutrol® F68, 0.5	8.51	27.22	135.9	0.25	1.09	2.24	0.30	0.19	-23.80
Lutrol® F68, 0.4 + SLS, 0.1	9.93	33.60	52.59	0.35	2.25	3.34	0.35	0.25	-40.80
Tween® 80, 0.5	14.73	39.17	64.36	0.58	2.08	3.23	0.53	0.35	-28.10

Table 13. Effect of stabilizer type on the efficiency of itraconazole nanomilling and zeta potential (all formulations contain 2% itraconazole)

Stabilizer, % w/w	Before milling			After 40 min milling					
	Particle size distribution (µm)			Particle size distribution (µm)			Mean particle size (µm)	PDI	Zeta potential (mV)
	D 50	D 90	D 99	D 50	D 90	D 99			
SLS, 0.5	2.61	4.58	6.72	0.12	0.22	1.32	0.22	0.32	-47.2
Lutrol® F68, 1.5	2.22	4.08	6.07	0.13	0.96	2.56	0.30	0.30	-20.9
Lutrol® F68, 1.2 + SLS, 0.3	3.26	8.60	21.24	0.12	0.26	3.32	0.23	0.34	-38.7
Tween® 80, 1.5	4.19	20.51	58.78	0.15	0.97	2.89	0.41	0.21	-20.7

Methocel® E5



Tween® 80

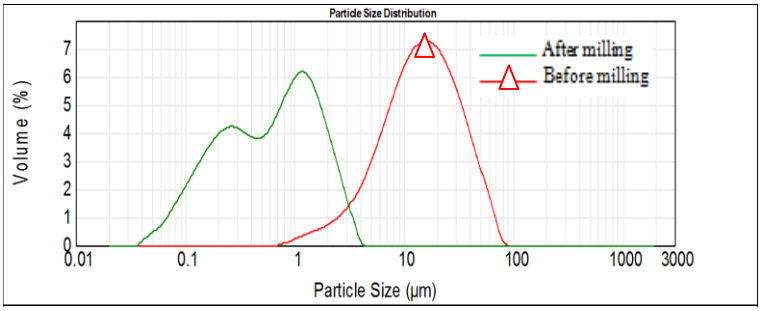


Fig. 12. Volume particle size distribution before and after milling of 2% mefenamic acid suspensions stabilized by different stabilizers

3.1.2 Effect of milling time on particle size reduction

The mean particle sizes along with particle size distribution continuously decreased as effect of milling time for all stabilizers. Milling for 20 minutes was sufficient to obtain nanosuspensions of all formulations with a mean particle size less than 600 nm and D 90 being less than 3 µm, slowly reduction of particle size was obtained by continuous milling thereafter. The PDI tends to decrease as effect of milling time. However, a slight fluctuation in PDI was observed for some formulations which can be due to reversible aggregations (**Fig. 13-16**).

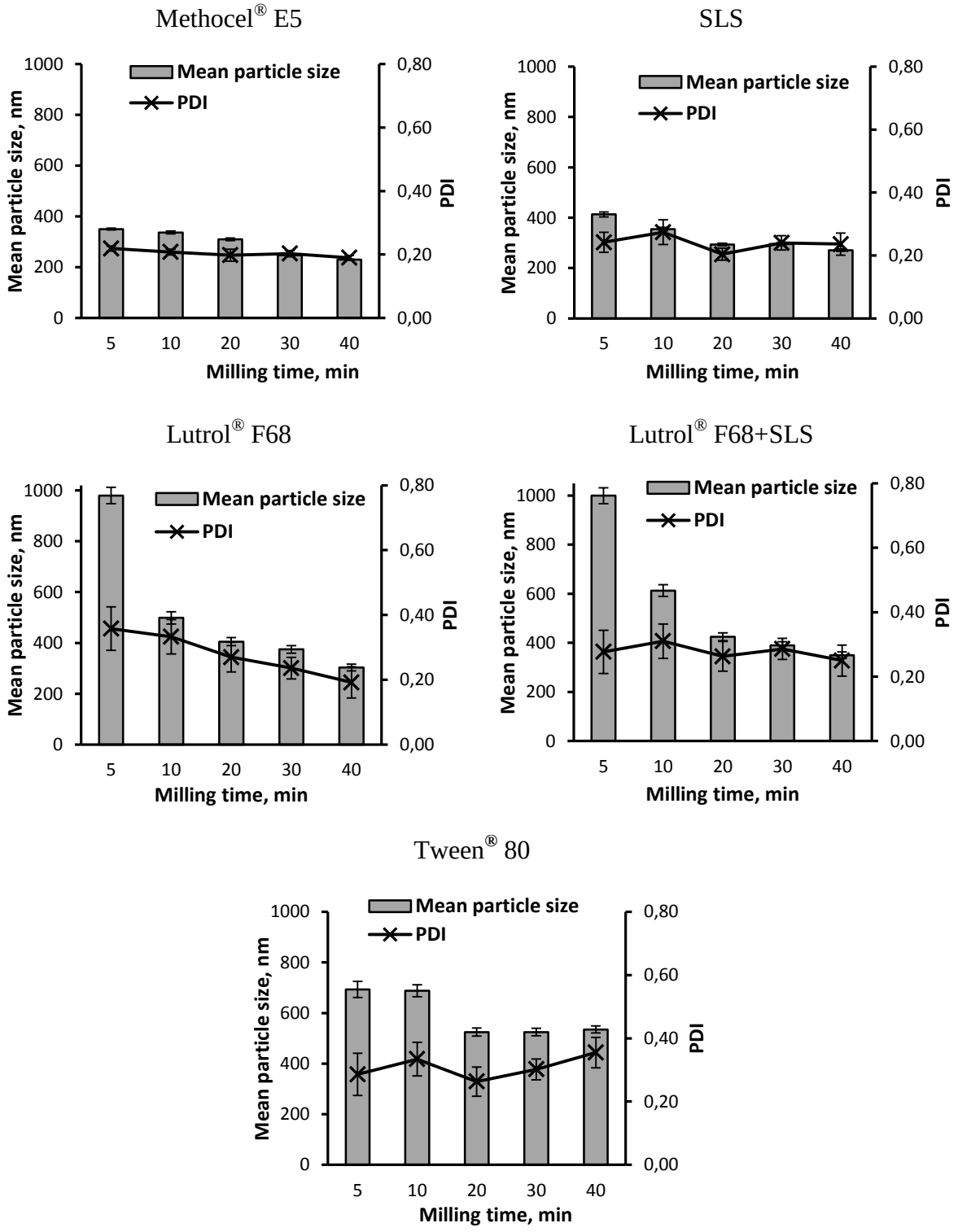


Fig. 13. Effect of milling time on the mean particle size and PDI of 2% w/w mefenamic acid suspensions stabilized with different stabilizers

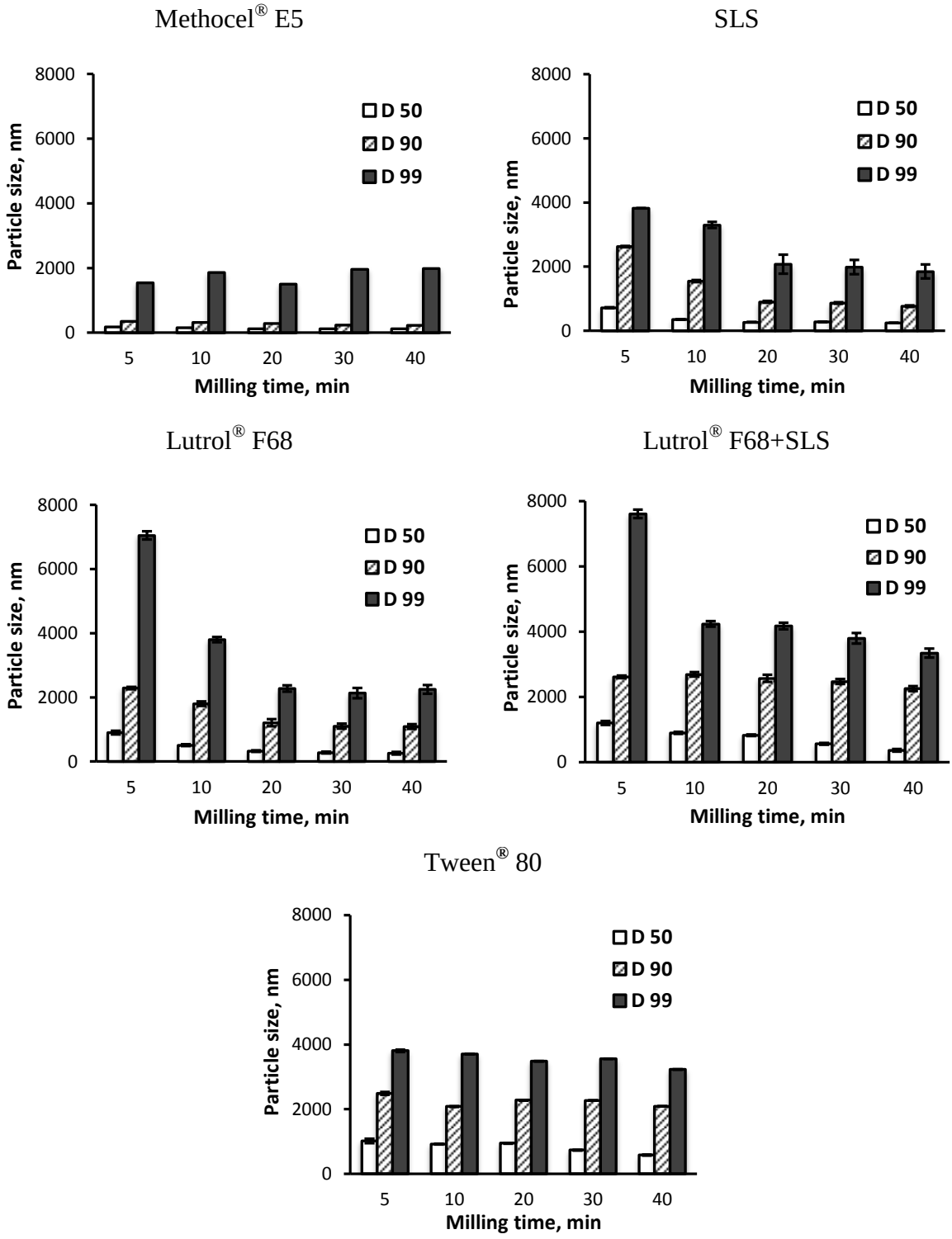


Fig. 14. Effect of milling time on the particle size distribution of 2% w/w mefenamic acid suspensions stabilized with different stabilizers

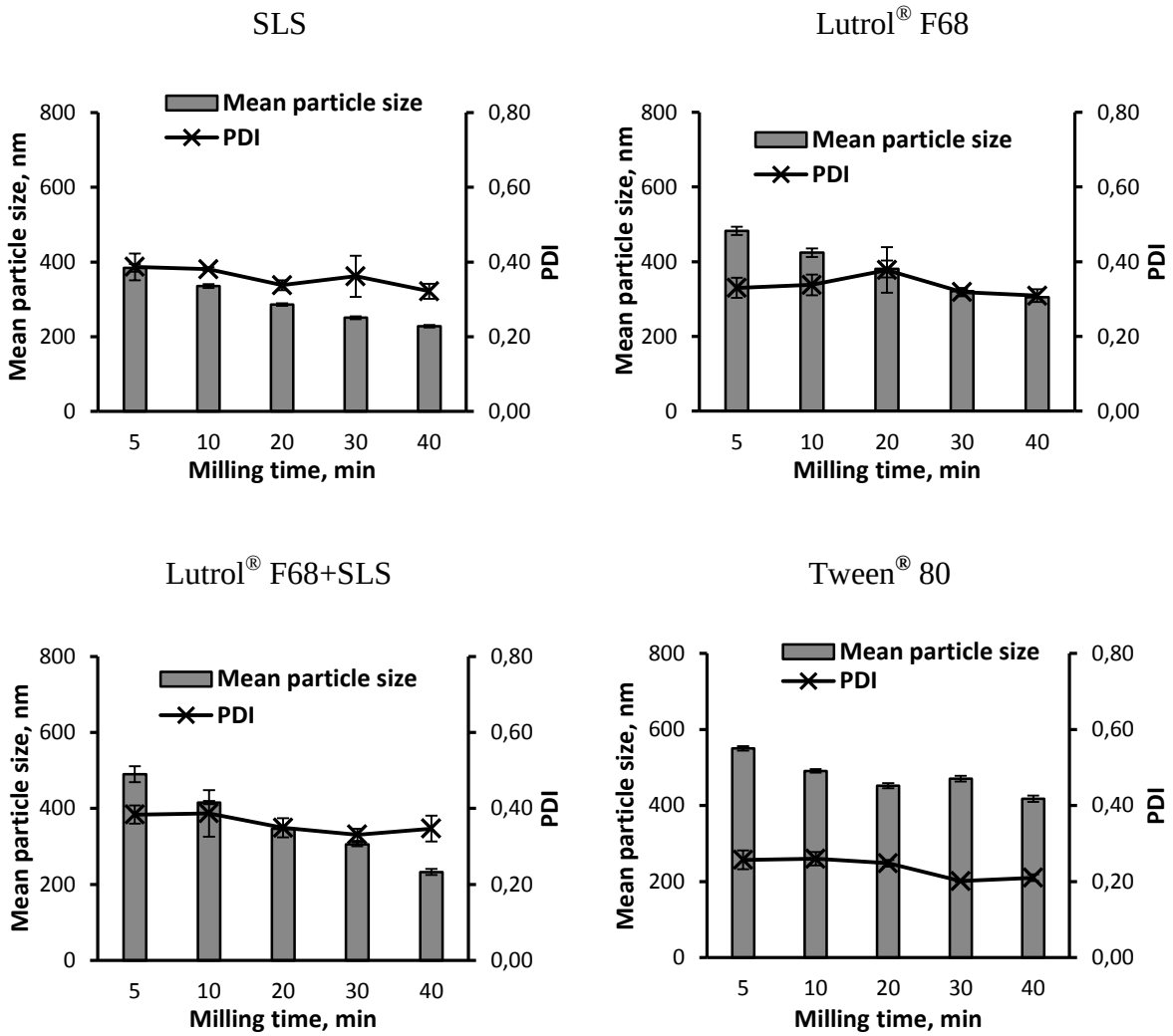


Fig. 15. Effect of milling time on the mean particle size and PDI of 2% itraconazole suspensions stabilized with different stabilizers

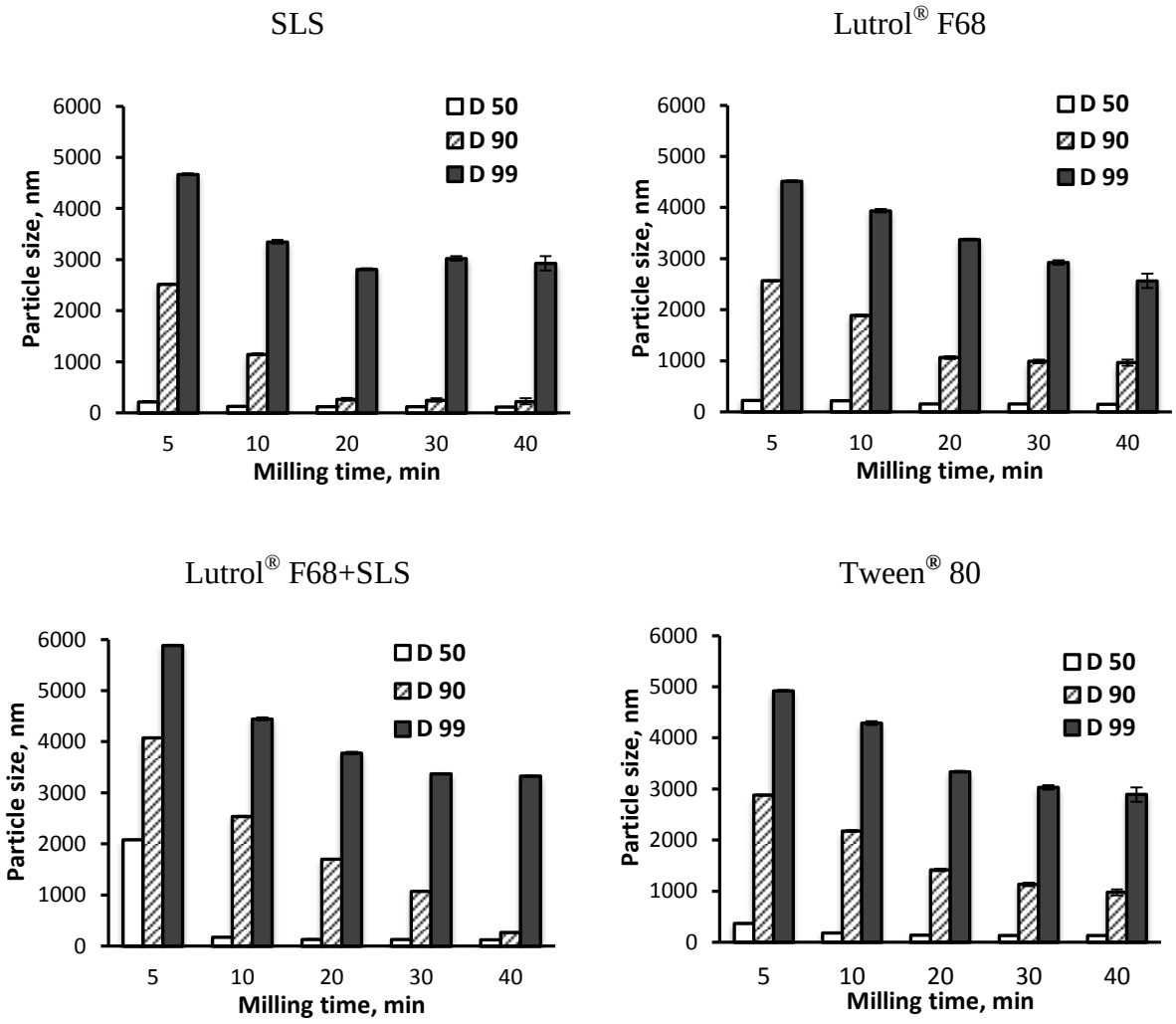


Fig. 16. Effect of milling time on the particle size distribution of 2% itraconazole suspensions stabilized with different stabilizers

3.1.3 Effect of Methocel® viscosity grade on particle size reduction

Different grades of Methocel® (E5, E15 and E50) were used as stabilizers for suspensions in order to evaluate their effect on milling time and particle size reduction.

Longer milling time was required to reduce the particle size of suspensions stabilized by higher viscosity grades of Methocel® compared to the lower viscosity grade. Therefore, larger particle size was obtained after 40 min milling of suspensions containing the higher viscosity grades of Methocel®. This can be attributed to the increase in solution viscosity that influences the movement of the grinding media and therefore the stress number and intensity (Kwade, 1999). A slight fluctuation in PDI was observed from nanosuspension stabilized with the highest Methocel® viscosity grade (Methocel® E50) which can be due to reversible aggregations (**Fig. 17**).

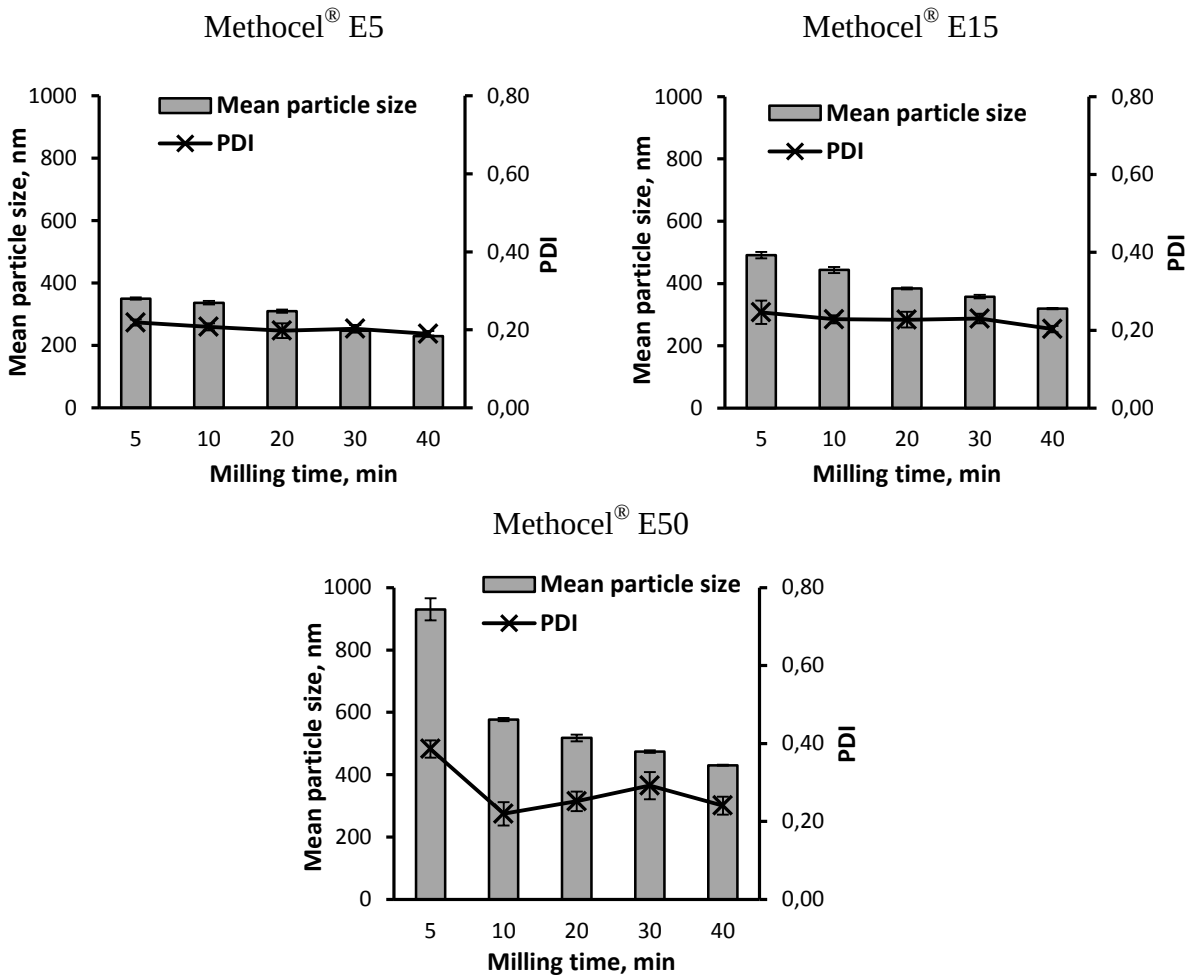


Fig. 17. Particle size reduction profile during milling of 2% w/w mefenamic acid suspensions stabilized by different viscosity grades of Methocel®

3.1.4 Effect of drug loading on particle size reduction

Shorter milling time was required to reduce the particle size of suspension with higher drug loading compared to the lower one (**Fig. 18**). This might be due to the higher probability of the drug particles to be stressed at a grinding media contact when higher drug loading was used. However, after reaching a minimum value, the D 99 of nanosuspensions with the highest drug loading (10% w/w mefenamic acid) started to increase. This could be due to the formation of large energy zones between the particles which were closer to each other at higher drug loading, and therefore the stabilizers tend to diffuse away, and thus the stabilizing effect was lost (Peltonen and Hirvonen, 2010).

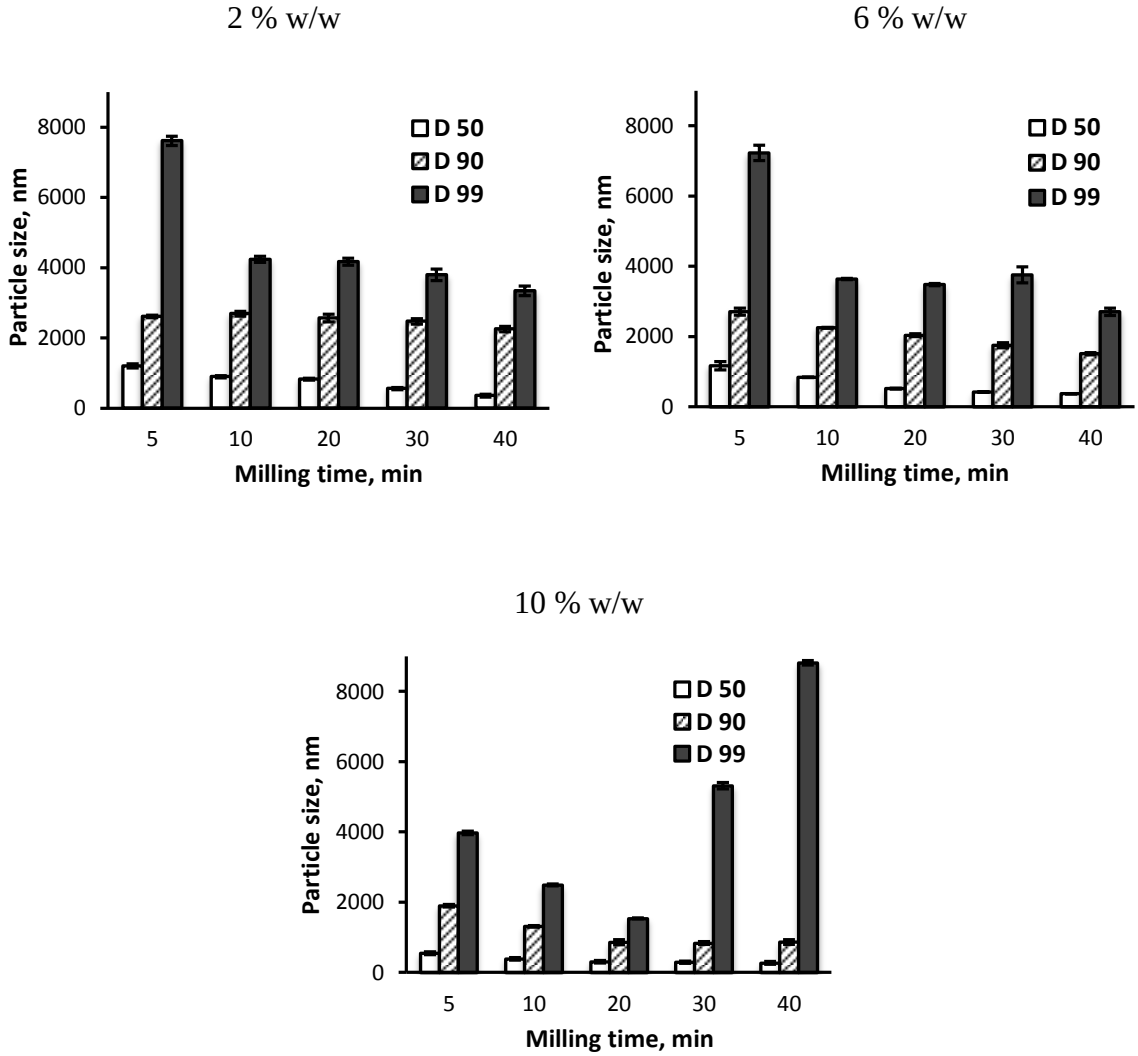


Fig. 18. Particle size reduction profile during milling of mefenamic acid suspension stabilized by Lutrol® F68+SLS at different drug loading

3.1.5 Effect of stabilizer concentration on particle size reduction

During the milling process, drug crystals break into smaller particles and thus generate new surfaces continuously. Therefore, covering these surfaces of nanoparticles during milling process with adequate amount of stabilizer/s is needed to reduce the aggregation of particles and thus to successfully produce a stable nanosuspension (Ghosh et al., 2012).

0.5% Lutrol® F68 was not enough to reduce the particle size to nanometer range, this is due to insufficient polymer concentration to completely cover the surface of the drug particles, this leads to aggregation of these small sized particles, and thus the anticipated benefits of nanomilling was lost (Sepassi et al., 2007). However, A fine nanosuspension with average particle size of 300 nm and D 90 being less than 1000 nm was successfully produced by increasing the concentration of Lutrol® F68 form 0.5 to 1.5% w/w (**Fig. 19**).

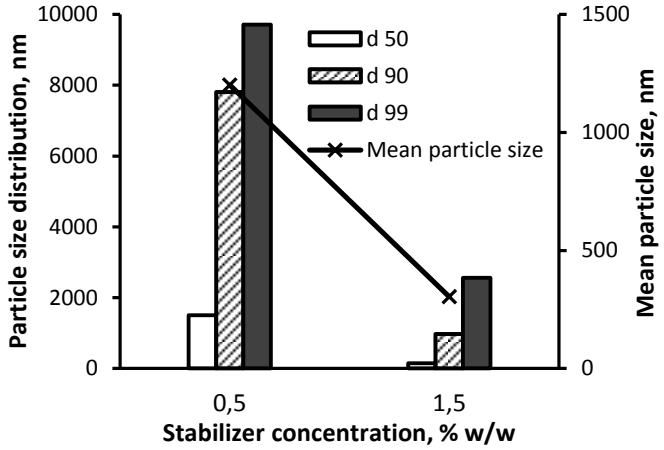


Fig. 19. Effect of stabilizer concentration on particle size reduction after 40 min milling of 2 % w/w itraconazole suspension stabilized by Lutrol® F68

3.1.6 Physical stability of nanosuspensions upon storage

In order to evaluate the potential growth of particle size upon storage and thus identifying the best formulations for both mefenamic acid and itraconazole nanosuspensions, the physical stability of all nanosuspension formulations was evaluated over a period of 3 months at room temperature. At certain time points, samples were taken and evaluated regarding the particle size. All 2% w/w mefenamic acid nanosuspension formulations were relatively stable during at least 3 months storage at room temperature (**Fig. 20**). However, the nanosuspension stabilized by Tween® 80 showed a slight increase in particle size upon storage which was mainly due to crystal growth (**Fig. 21**). Similar to mefenamic acid nanosuspensions, mostly all the 2% w/w itraconazole nanosuspension formulations were stable during at least 3 months storage at room temperature (**Fig. 22**). However, itraconazole nanosuspension stabilized by Tween® 80 showed an increase in particle size from 417 nm to 826 nm within 3 months which was due to both crystal growth and particles aggregation (**Fig. 23**). Moreover, the stability of 2% w/w mefenamic acid nanosuspensions was not affected by the viscosity grade of Methocel® (**Fig. 24**).

Increasing drug loading resulted in less physical stability of particle size upon storage (**Fig. 25**). Furthermore an extreme increase in the mean particle size and a complete phase separation (sedimentation) occurred after 15 days storage of 10% w/w mefenamic acid nanosuspension. This could be due to the higher tendency of particles agglomeration at higher drug loading as the particles were closer to each other.

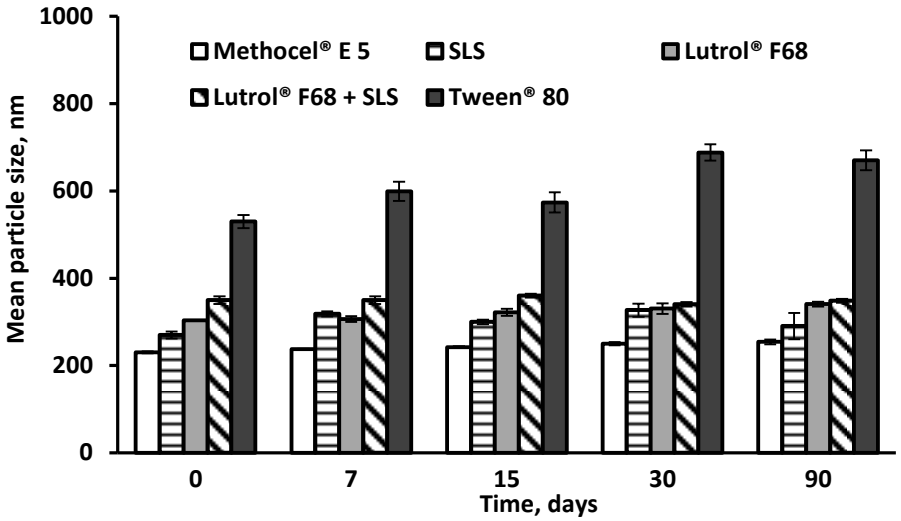


Fig. 20. Effect of storage at room temperature on the mean particle size of 2% w/w mefenamic acid nanosuspensions stabilized by different stabilizers

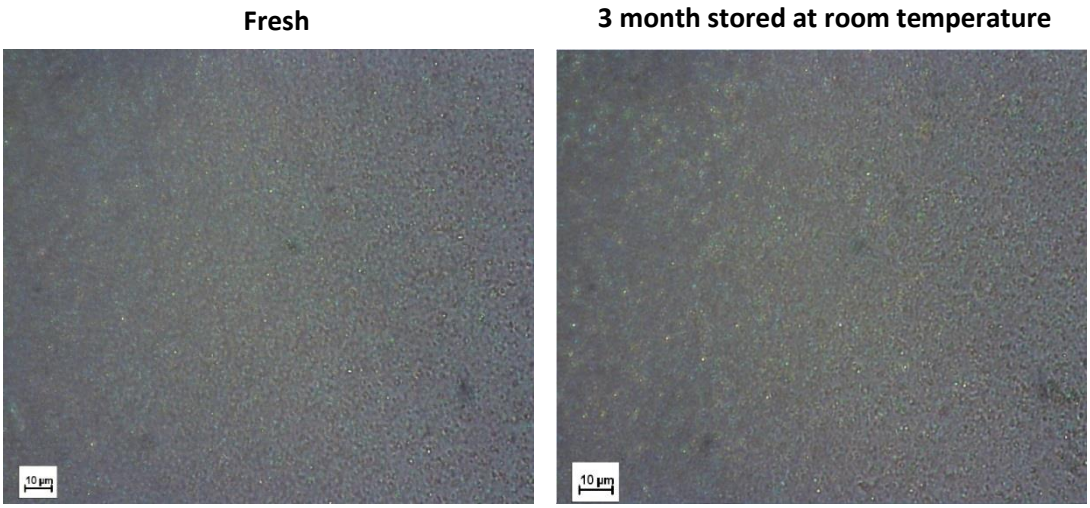


Fig. 21. Polarized light microscopic images of 2% w/w mefenamic acid nanosuspension stabilized with Tween® 80

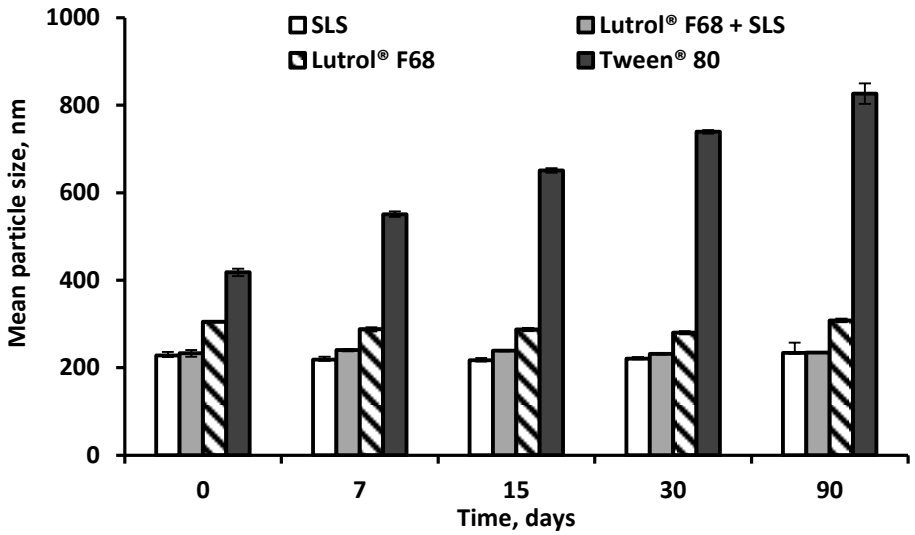


Fig. 22. Effect of storage at room temperature on the mean particle size of 2% w/w itraconazole nanosuspensions stabilized by different stabilizers

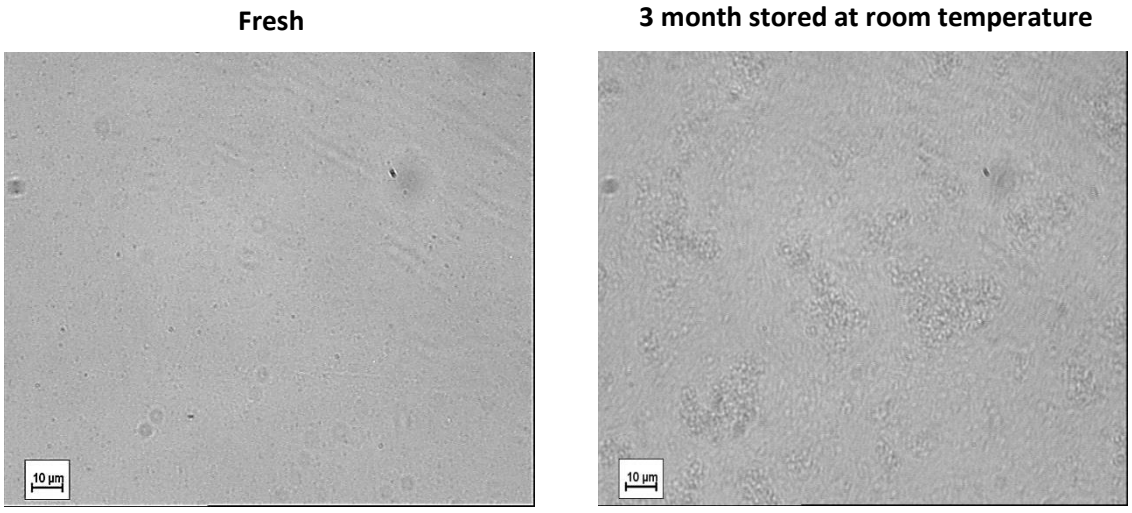


Fig. 23. Light microscopic images of 2% w/w itraconazole nanosuspension stabilized with Tween® 80

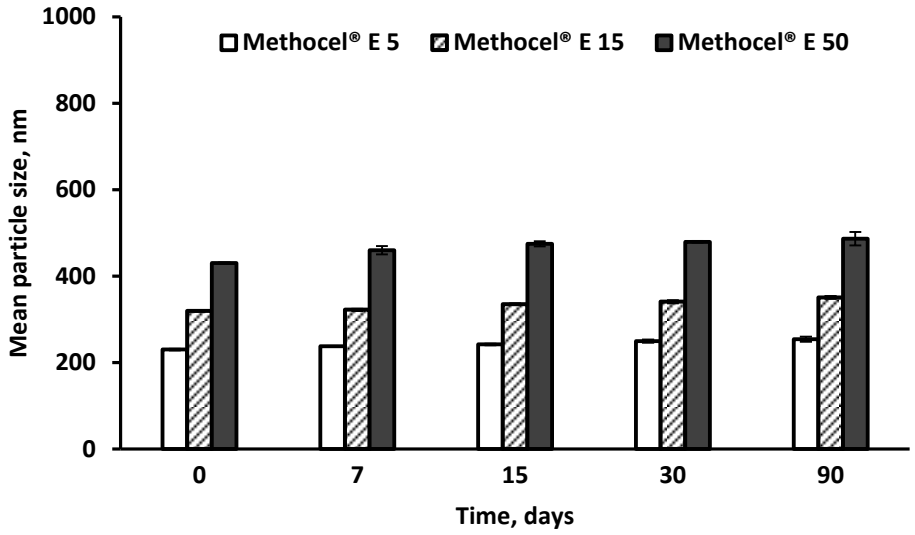


Fig. 24. Effect of storage at room temperature on the mean particle size of 2% w/w mefenamic acid nanosuspensions stabilized by different viscosity grades of Methocel®

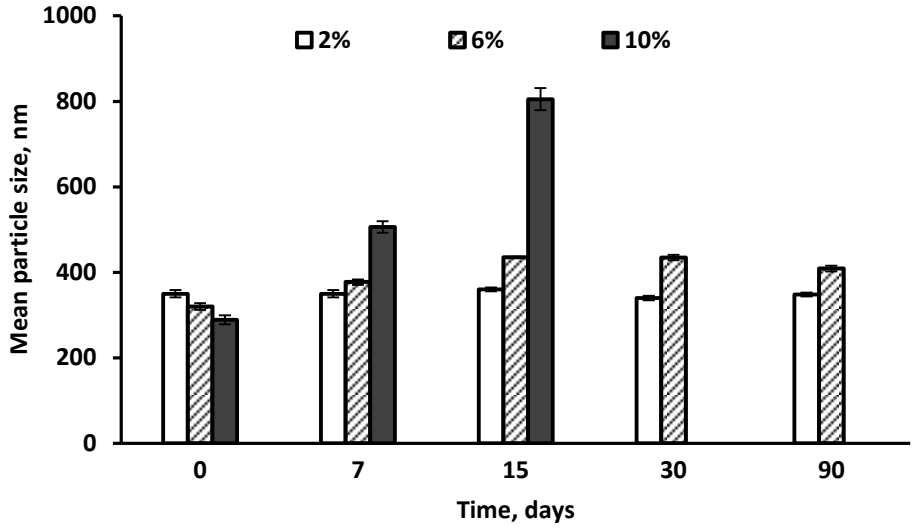


Fig. 25. Effect of storage at room temperature on the mean particle size of 2, 6 and 10 % w/w mefenamic acid nanosuspensions stabilized by Lutrol® F68+SLS

3.1.7 Effect of wet milling process on the solid state of drugs

Transformation of the crystalline state to the amorphous state may occur during wet milling process related to the high mechanical energy input (Sharma et al., 2009). These different solid states have different solubility properties, where the amorphous state has higher solubility compared to the crystalline state of drug. However, the formation of amorphous nanocrystals is undesirable because it causes poor stability (Peltonen and Hirvonen, 2010).

Thermal analysis of the nanosized drugs powder was compared to the unprocessed drugs powder. The nanosized drugs powder was obtained by centrifugation of the nanosuspensions for 30 min at 13000 rpm, the supernatant was discarded and the sediment was dried at 25 °C under vacuum.

The endotherms at 229.5 °C and 169 °C for unprocessed mefenamic acid and itraconazole powder, respectively, indicate their melting points. On the other hand, the DSC thermograms of their nanosized powder showed slight shifts in melting points (from 229.5 °C to 228.9 °C and from 169 °C to 165.5 °C for mefenamic acid and itraconazole, respectively) (**Fig. 26**). This could be attributed to the miscibility of the drugs in the stabilizers (Methocel® E5 in case of mefenamic acid nanosuspension and Lutrol® F68 in case of itraconazole) (Chokshi et al., 2005). In conclusion, it appears that following wet milling process there were no changes in the crystallinity of drugs.

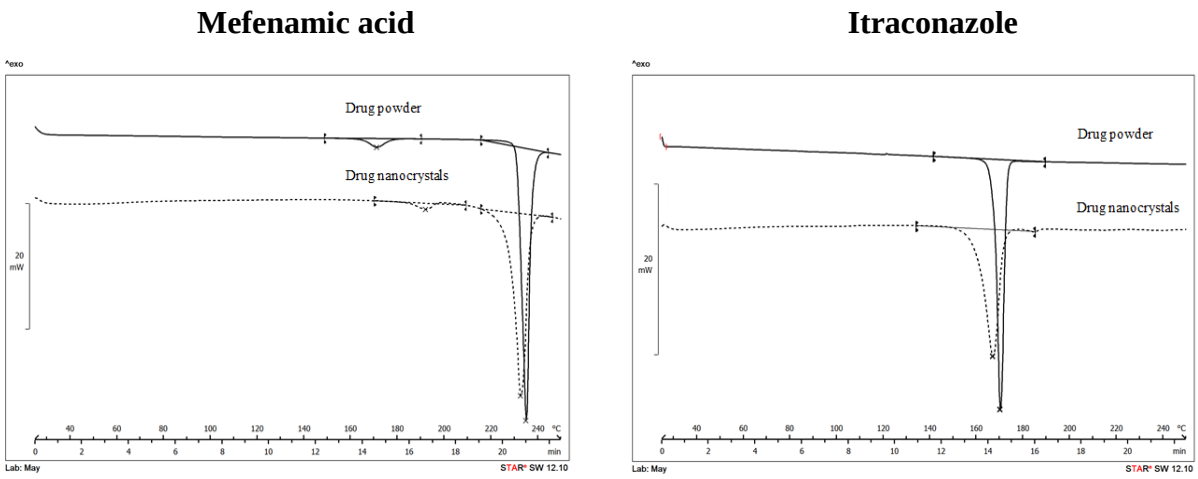


Fig. 26. DSC thermograms of unprocessed drugs powder and drugs nanocrystals

3.1.8 Dissolution studies

Improving dissolution rate and/or solubility of poorly soluble drugs is essential to improve bioavailability. In order to evaluate the effect of nanosizing on improving the dissolution rate of mefenamic acid, dissolution rate under sink condition in PBS pH 6.8 for different mefenamic acid nanosuspension formulations and pure mefenamic acid were compared.

The dissolution rate of pure mefenamic acid was slow, only 70 % drug released within 120 minutes, while significantly improved drug release was obtained from all nanosuspension formulations. Furthermore, the drug release was fast and complete within a few minutes irrespective to the particle size of nanosuspensions (**Fig. 27**). This was due to the increase in surface area and therefore the dissolution rate as described in Noyes Whitney equation.

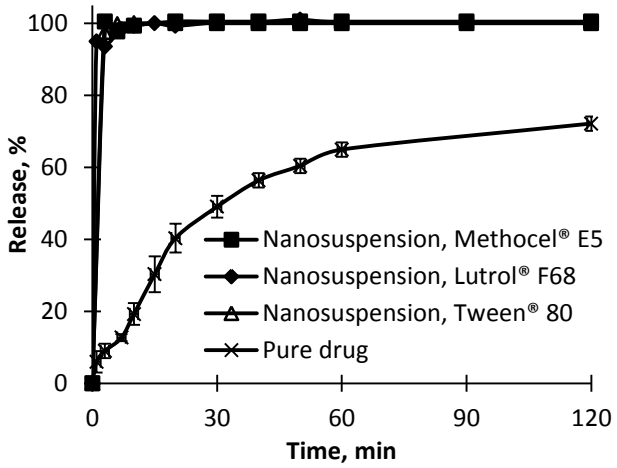


Fig. 27. Dissolution profiles under sink conditions for various mefenamic acid nanosuspension formulations and mefenamic acid as pure drug in PBS pH 6.8

Besides providing a large increase in the available surface area and therefore dissolution rate improvement, decreasing the particle size to nanometer range (nanosizing) also increases the equilibrium solubility. In order to evaluate the effect of nanosizing on improving the solubility of mefenamic acid/itraconazole nanosuspensions, the amount of drug released under non-sink conditions (corresponding to 50 mg drug in 450 ml dissolution medium) from nanosuspension and suspension formulations was compared. In case of mefenamic acid the drug release was performed in PBS pH 6.8. Both solubility and dissolution rate were improved from nanosuspensions compared to suspensions. The increase in mefenamic acid solubility from nanosuspension stabilized by Methocel® E5 was up to 1.5 times which was much higher than nanosuspension stabilized by Tween® 80. Furthermore the supersaturation solubility generated from nanosuspension stabilized by Methocel® E5 was stable within at

least 240 minutes; in contrast to fast decrease in mefenamic acid concentration within 90 min to reach its saturation solubility was from nanosuspension stabilized by Tween® 80 (**Fig. 28**). In case of itraconazole the drug release was done in 0.1 N HCl, both solubility and dissolution rate were improved from nanosuspensions compared to suspensions. The increase in itraconazole solubility was up to 2.3 and 1.8 times from both nanosuspensions stabilized by Lutrol® F68 and Tween® 80, respectively. Furthermore the supersaturation solubility generated from all nanosuspensions was stable within at least 240 minutes (**Fig. 29**). The increase in solubility is related to the particle size reduction to nanometer range as described by Kelvin-Gibbs equation and Ostwald–Freundlich equation. The difference in particle size reduction could be the reason for the higher supersatuation level obtained from different nanosuspensions. In case of itraconazole, Lutrol® F68 provided not only smaller particle size but also higher saturation solubility compare to Tween® 80 which could be also the reason for the higher supersatuation level obtained from nanosuspensions stabilized by Lutrol® F68 compared to Tween® 80.

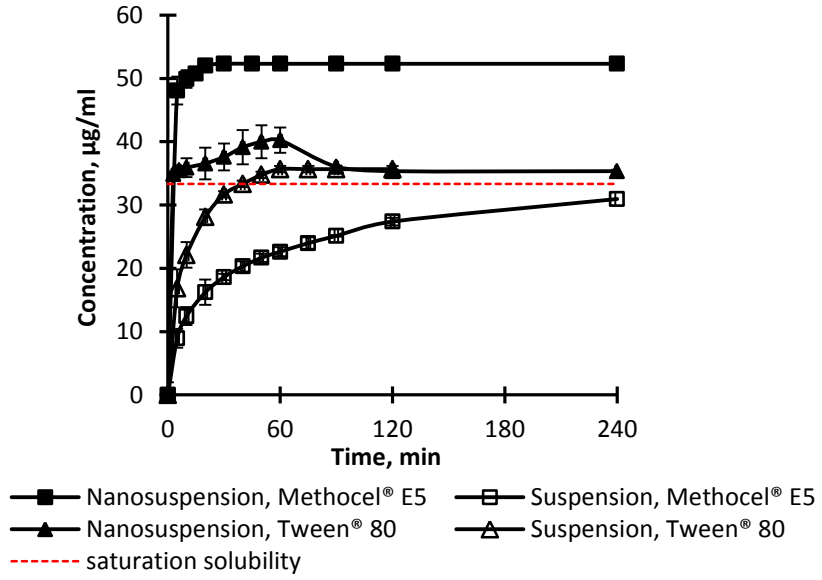


Fig. 28. Comparison between the kinetic solubility of mefenamic acid from various nanosuspension and suspension formulations in PBS pH 6.8

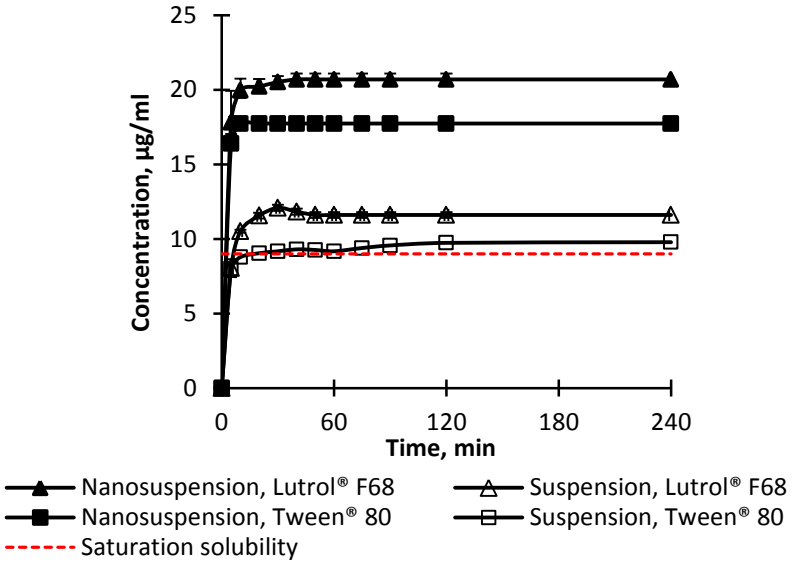


Fig. 29. Comparison between the kinetic solubility of itraconazole from various nanosuspension and suspension formulations in 0.1 N HCl

3.2 Solid dispersions

3.2.1 Hot-melt extrusion of basic poorly soluble drugs (itraconazole, ritonavir)

3.2.1.1 Solid-state characterization

The low solubility of many crystalline drugs can result in low bioavailability. Improving solubility can be done by changing the crystalline state to amorphous state; differential scanning calorimetry (DSC) and X-ray diffractometery were performed to distinguish between crystalline and amorphous solid dispersions.

Itraconazole as a pure drug has a crystalline structure presenting a melting peak at 169 ˚C. A slightly shift in the melting peak was observed from its physical mixtures with polymers. On the other hand, all its milled extrudates with different polymers (e.g. Soluplus®, Kollidon® SR, Soluplus®:Kollidon® SR, Aqoat® AS-LF, Soluplus®:Aqoat® AS-LF, Soluplus®:Kollicoat® MAE 100 P:TEC) did not show this melting peak (**Fig. 30**).

Ritonavir as a pure drug has a crystalline structure presenting a melting peak at 123 ˚C. No shift in the melting peak was observed from its physical mixtures with polymers, and no melting peak was observed from its milled extrudates with Soluplus® or Soluplus®:Kollicoat® MAE 100 P (**Fig. 31**).

Numerous diffraction peaks of itraconazole and ritonavir were observed when these compounds were evaluated individually, indicating the presence of their crystalline nature. On the other hand, their milled extrudates did not show any sign of crystallinity (**Fig. 32-33**).

According to the results obtained by DSC and X-ray diffractometery, amorphous solid dispersions of both drugs were obtained by hot-melt extrusion with all polymers used.

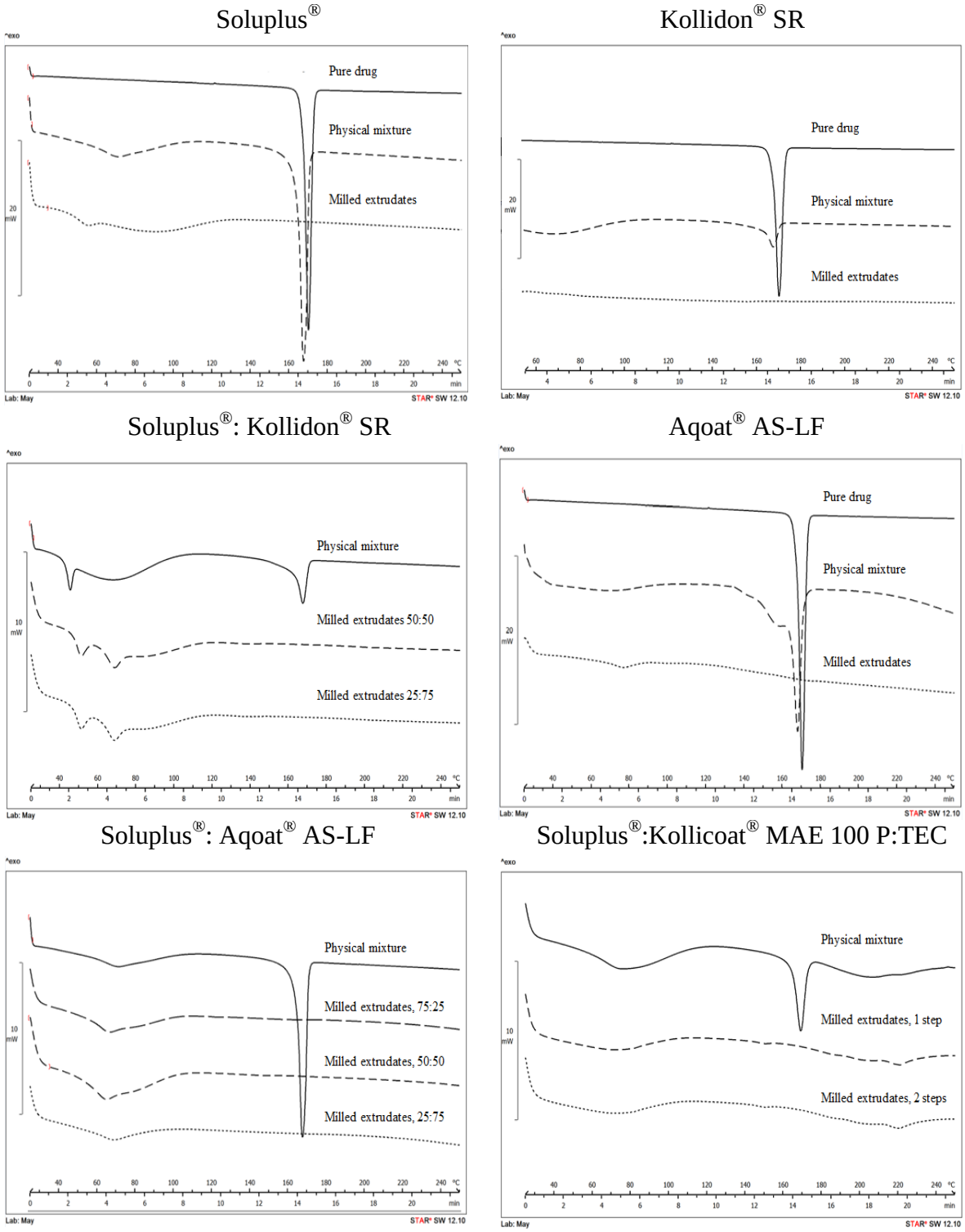


Fig. 30. DSC thermograms of itraconazole, its physical mixtures and milled extrudates with different carriers (Drug:polymer ratio 1:3)

Soluplus® Soluplus®:Kollicoat® MAE 100 P

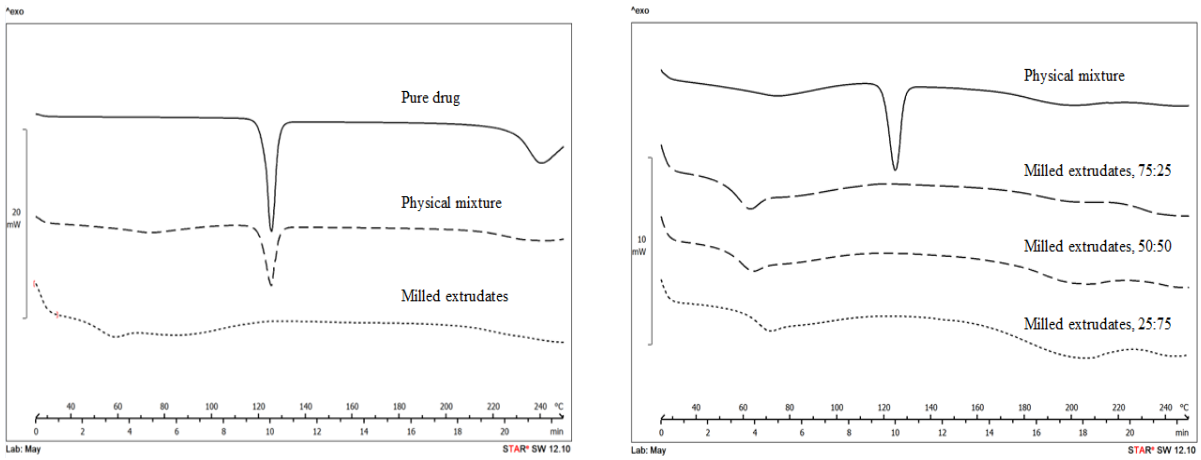


Fig. 31. DSC thermograms of ritonavir, its physical mixtures and milled extrudates with different carriers (Drug:polymer/s ratio 1:1.5)

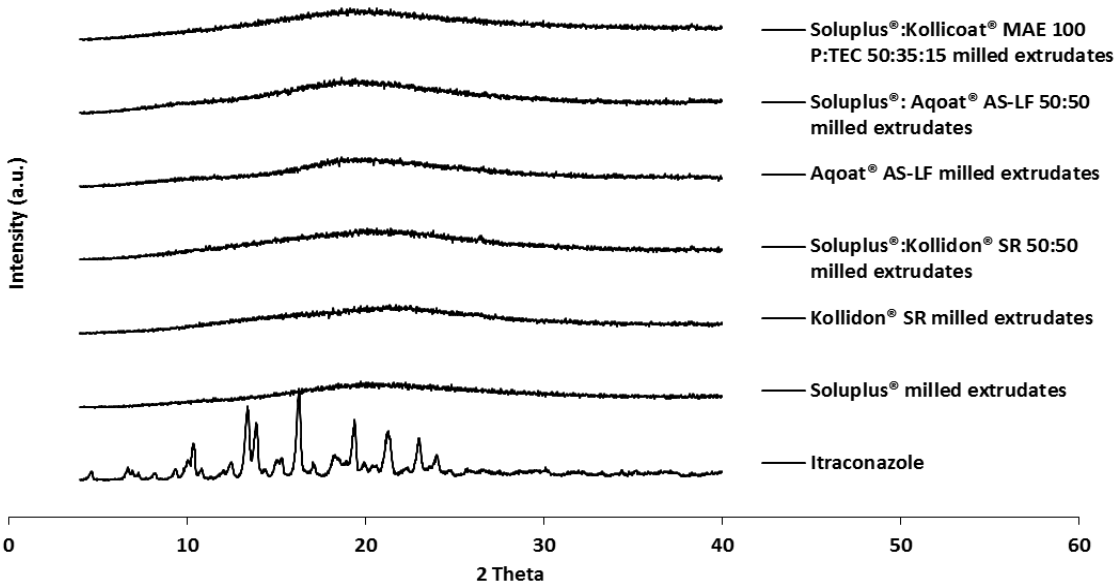


Fig. 32. X-ray diffractograms of itraconazole and its extrudates with different carriers (Drug:polymer/s ratio 1:3)

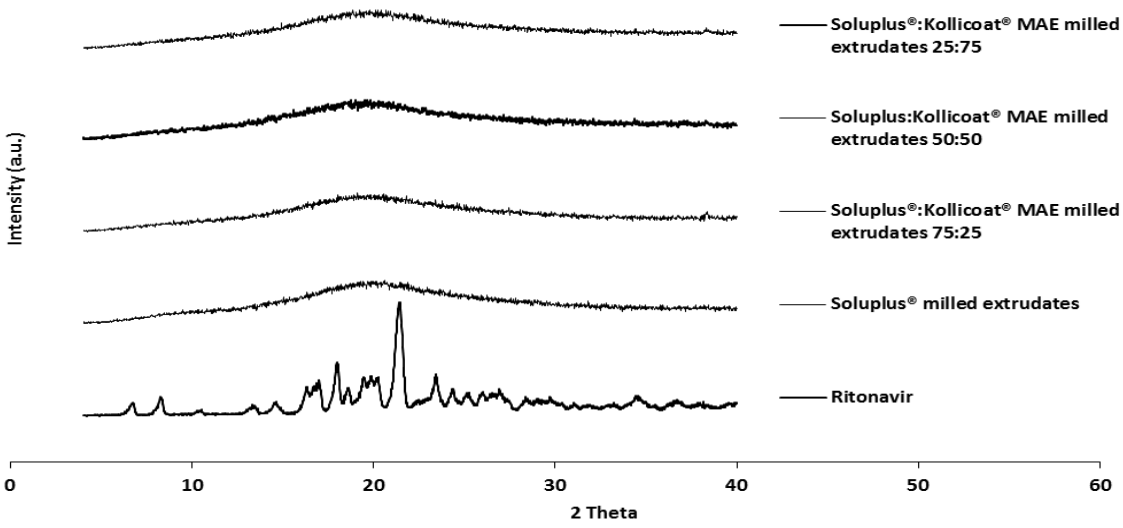


Fig. 33. X-ray diffractograms of ritonavir and its extrudates with different carriers (Drug:polymer/s ratio 1:1.5)

An estimation of the crystallinity of milled extrudates was also done by optical investigation (**Fig. 34-35**), whereby the extrudates were yellow transparent with Soluplus®, beige/opaque with Kollidon® SR and Soluplus®:Kollidon® SR polymers combinations, brown semi-transparent with Aqoat® AS-LF and yellow transparent with Soluplus®:Aqoat® AS-LF combinations confirming the results obtained by DSC and X-ray diffractometery. The extrudates with Soluplus®:Kollicoat® MAE 100 P combinations were opaque with a rough surface, however, no crystals peak were detected by DSC or X-ray diffractometery.

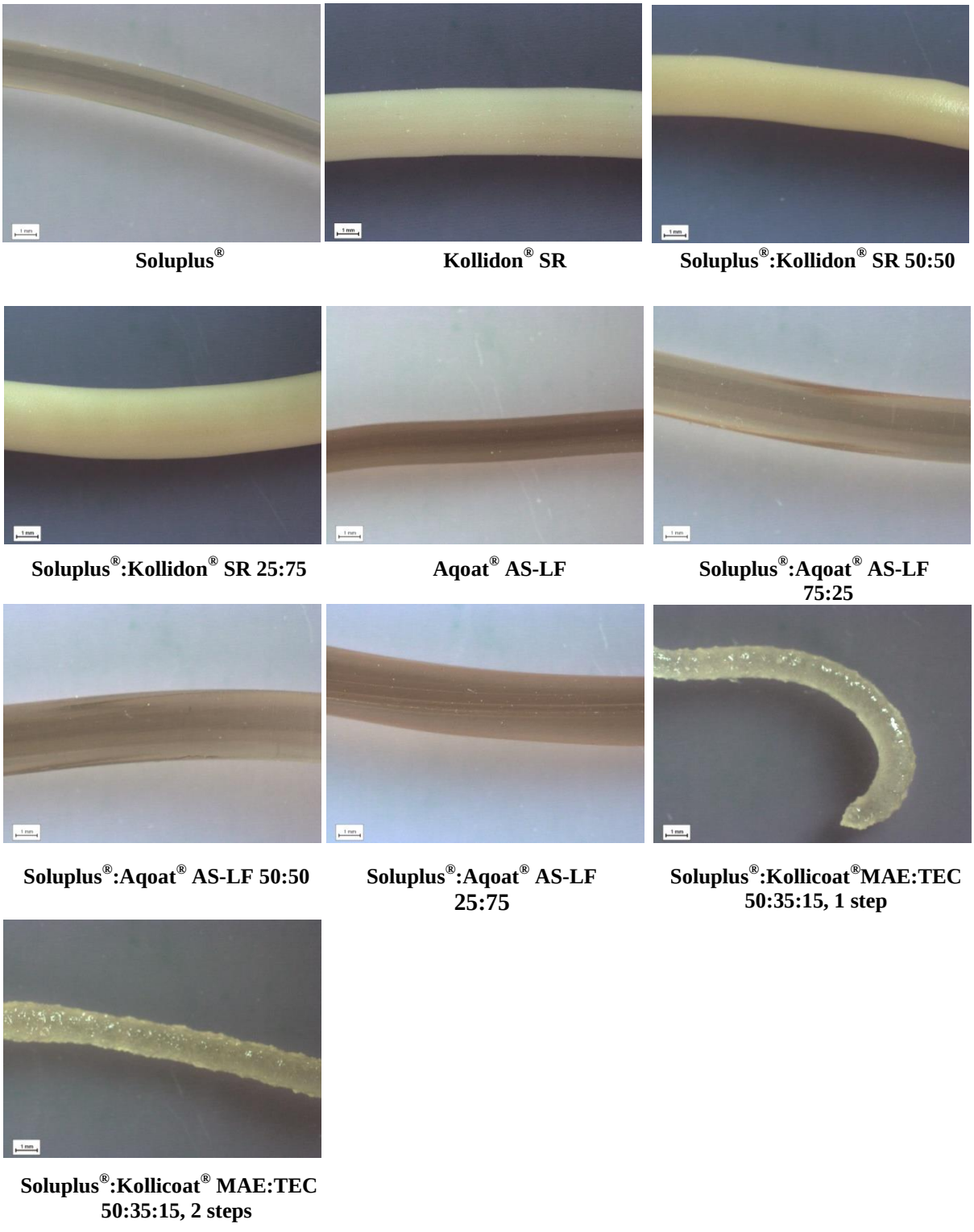
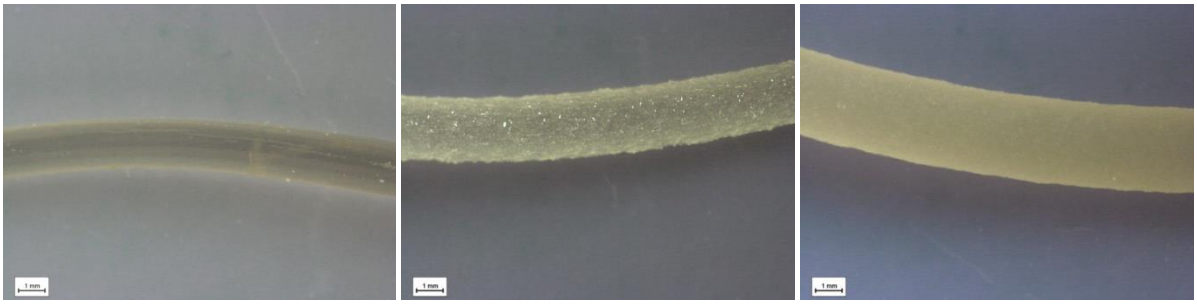
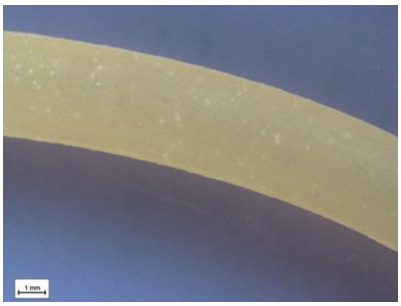


Fig. 34. Macroscopic pictures of itraconazole extrudates with different polymeric carriers (itraconazole:polymer/s ratio 1:3)



Soluplus® **Soluplus®:Kollicoat® MAE 75:25** **Soluplus®:Kollicoat® MAE 50:50**



Soluplus®:Kollicoat® MAE 25:75

Fig. 35. Macroscopic pictures of ritonavir extrudates with different polymeric carriers (ritonavir:polymer/s ratio 1:1.5)

3.2.1.2 Estimation of the apparent solid-state solubility

Different ratios of itraconazole:Soluplus® milled extrudates (1:3, 1:2, 1:1 and 1:0.5) were prepared by hot-melt extrusion, the apparent solid-state solubility of itraconazole in Soluplus® solid dispersion was estimated using DSC and X-ray diffractometery. Itraconazole:Soluplus® milled extrudates 1:3, 1:2 and 1:1 did not show any melting or diffraction peaks. Itraconazole:Soluplus® milled extrudates 1:0.5 exhibited a melting peak corresponding to the crystalline itraconazole (**Fig. 36**), however no diffraction peaks were observed by X-ray (**Fig. 37**) which could be related to low X-ray sensitivity to detect low degree of crystallization (the detection limit of the technique has been reached). These results indicate that the apparent solid-state solubility of itraconazole in the Soluplus® solid dispersion is at least 50%, where itraconazole is present as an amorphous form in the Soluplus® polymeric matrix.

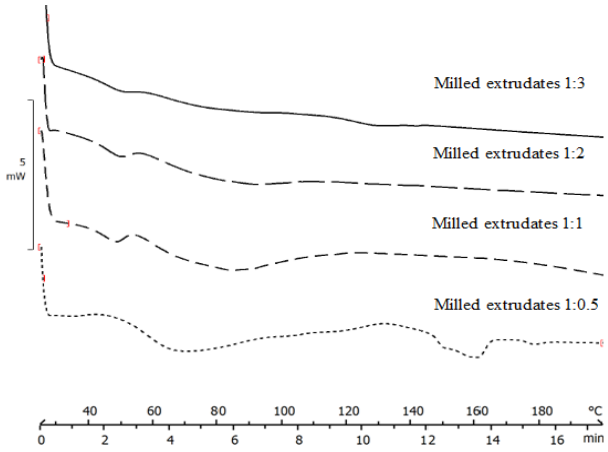


Fig. 36. DSC thermograms of different ratios of itraconazole:Soluplus® milled extrudates

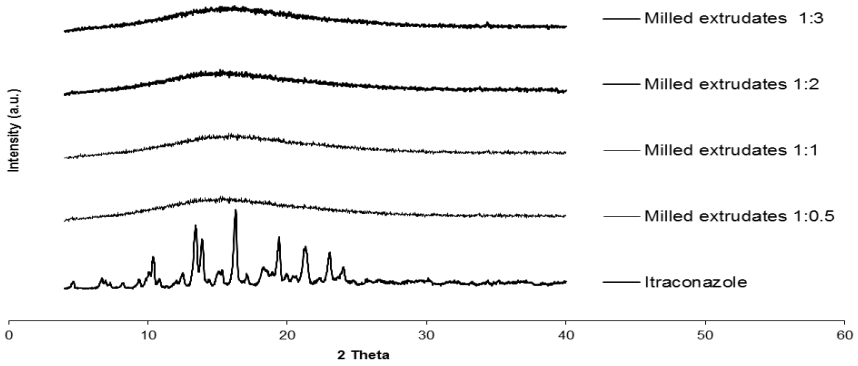


Fig. 37. X-ray diffractograms of itraconazole and different ratios of itraconazole:Soluplus® milled extrudates

3.2.1.3 Estimation of drug-polymer miscibility

Polymeric carriers have been shown to inhibit or delay drug crystallization from solid dispersions by forming a miscible blend (amorphous molecular dispersion) with the drug (Vasanthavada et al., 2005). The glass transition temperature (T_g) can be used as an indicator of interaction between polymer and drug, where the miscible blend shows a single glass transition temperature exists at some immediate value between the T_gs of the both components (e.g. polymer and drug) (Brostow et al., 2008).

The drug-polymer miscibility was studied using DSC, the analysis of DSC curves to obtain the glass transition temperatures was carried out for the second heating run data.

Itraconazole has a glass transition temperature of 59.36 ˚C followed by 2 endothermic peaks. The pure polymers Soluplus®, Aqoat® AS-LF and Kollicoat® MAE 100 P have glass transition temperatures of 73.49, 120.51 and 110.11 ˚C, respectively. The milled extrudates of itraconazole with Soluplus®, Aqoat® AS-LF or Soluplus®:Aqoat® AS-LF 50:50 show a single glass transition temperature at 60.21, 88.57 or 79.86 ˚C, respectively, suggesting a complete miscibility and amorphous molecular dispersions. In the case of milled extrudates of itraconazole with Soluplus®:Kollicoat® MAE: TEC two T_gs were observed, the first T_g appeared at 33.44 ˚C significantly lower than the T_g of itraconazole and the second one appeared around 100 ˚C (**Fig. 38**). Therefore, the combination of Soluplus®:Kollicoat® MAE:TEC maybe not suitable for preparation of a stable amorphous solid dispersion of itraconazole.

The glass transition temperature of ritonavir is 51.08 ˚C, the milled extrudates of ritonavir with Soluplus® has a single glass transition temperature at 58.13 ˚C, suggesting a complete miscibility and therefore an amorphous molecular dispersion. In case of ritonavir milled extrudates with Soluplus®:Kollicoat® MAE 100 P two T_gs were obtained, the first one is at 53.74 ˚C and the second one is around 96.10 ˚C, which may indicate poor miscibility of drug and polymers (**Fig. 39**).

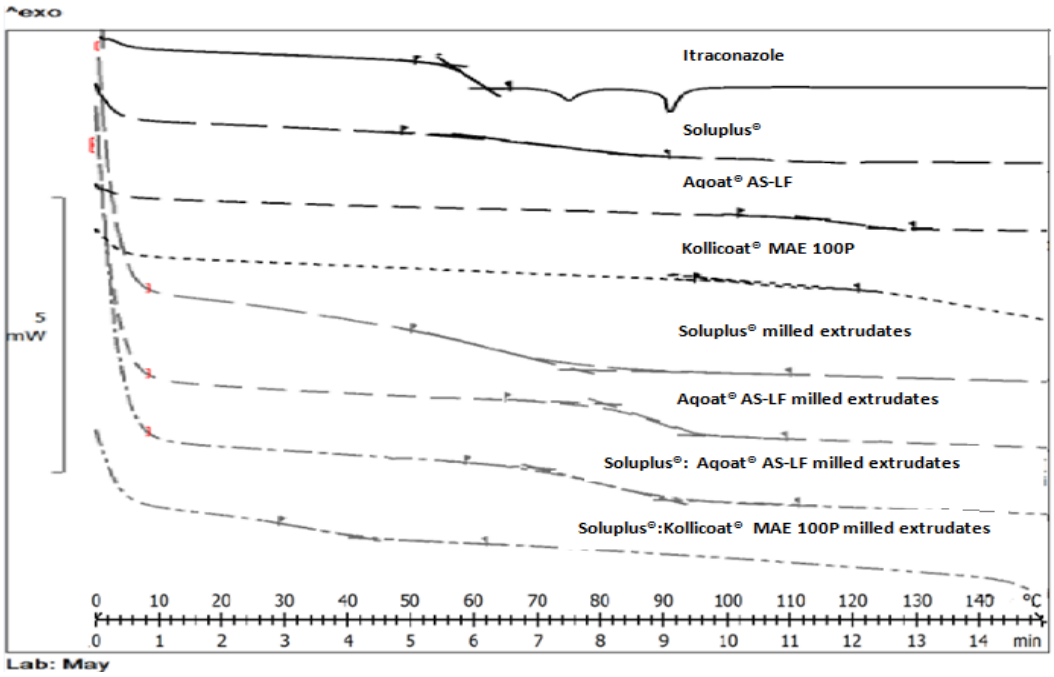


Fig. 38. DSC thermograms of second heating curves of pure itraconazole, pure polymers and their milled extrudates (itraconazole:polymer/s ratio 1:3)

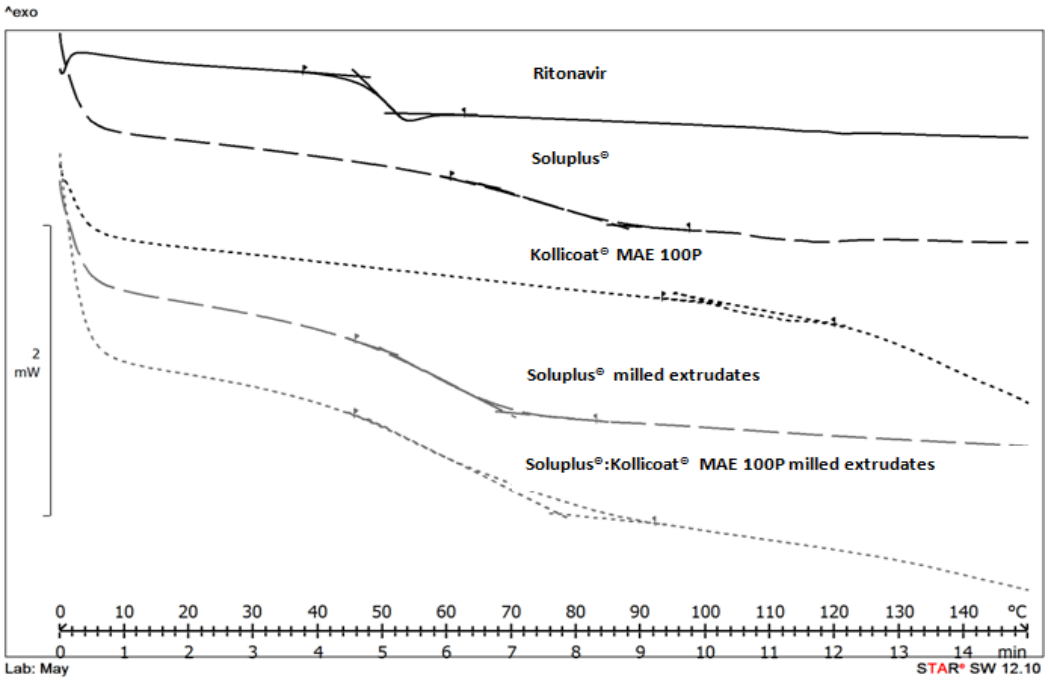


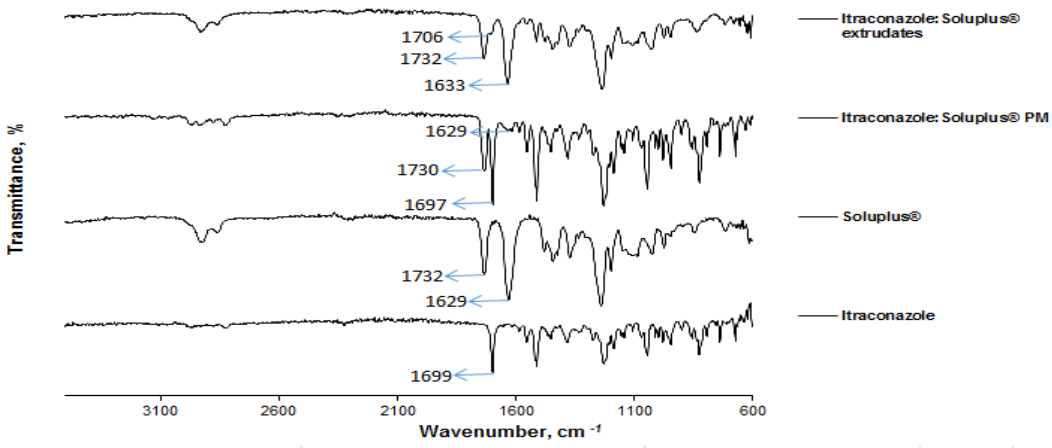
Fig. 39. DSC thermograms of second heating curves of pure ritonavir, pure polymers and their milled extrudates (ritonavir:polymer/s ratio 1:1.5)

3.2.1.4 Evaluation of drug-polymer molecular interaction

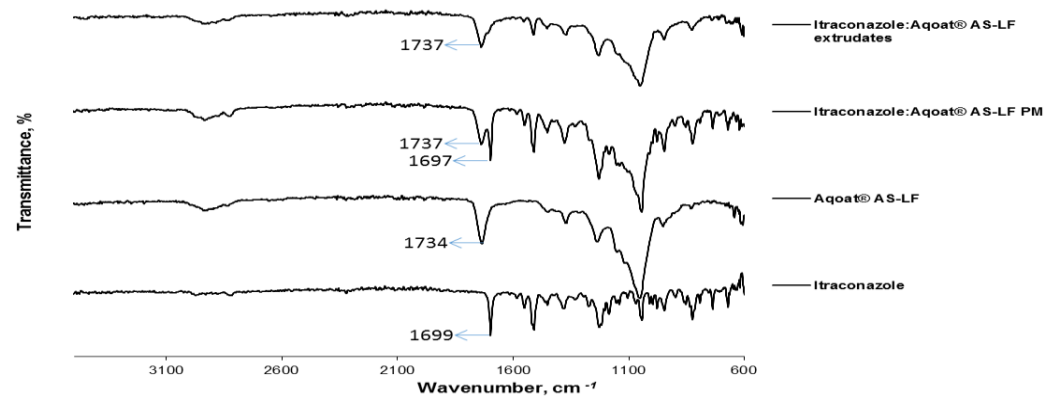
To evaluate the drug-polymer molecular interaction fourier transform infra-red (FTIR) spectroscopy was used. In the spectrum of itraconazole, stretching modes C=O, C=N and C-N were specific for itraconazole and were recorded at approximately 1699, 1512 and 1452 cm^{-1}, respectively. In these wavenumber regions, Soluplus® showed 2 peaks at 1732 and 1629 cm^{-1}, originating from the stretching of ester carbonyl and C=O stretching for tertiary amid respectively, whereas Aqoat® AS-LF showed 1 peak at 1734 cm^{-1} originating from the stretching of carbonyl group. In the spectrum of itraconazole physical mixture with polymers, the same peaks were observed at approximately the same wave numbers as those shown by their individual components. The IR spectra of milled extrudates showed almost the same characteristic peaks for pure polymers. However, the carbonyl group observed in the pure itraconazole was stretched and overlapped with C=O stretching vibration of polymers (**Fig. 40**). Thus, intermolecular interactions between the itraconazole and the polymers could have occurred during hot melt extrusion.

Furthermore, the stretching and overlapping was more significant for Aqoat® AS-LF and Aqoat® AS-LF:Soluplus® polymers combination milled extrudates compared to Soluplus® milled extrudates. This could be derived from stronger intermolecular molecular interaction by hot melt extrusion between itraconazole and Aqoat® AS-LF compare to the one between itraconazole and Soluplus®. These results correlate well with the results obtained by other researchers (Kojima et al., 2012 and Sarode et al., 2013).

Soluplus®



Aqoat® AS-LF



Soluplus®:Aqoat® AS-LF

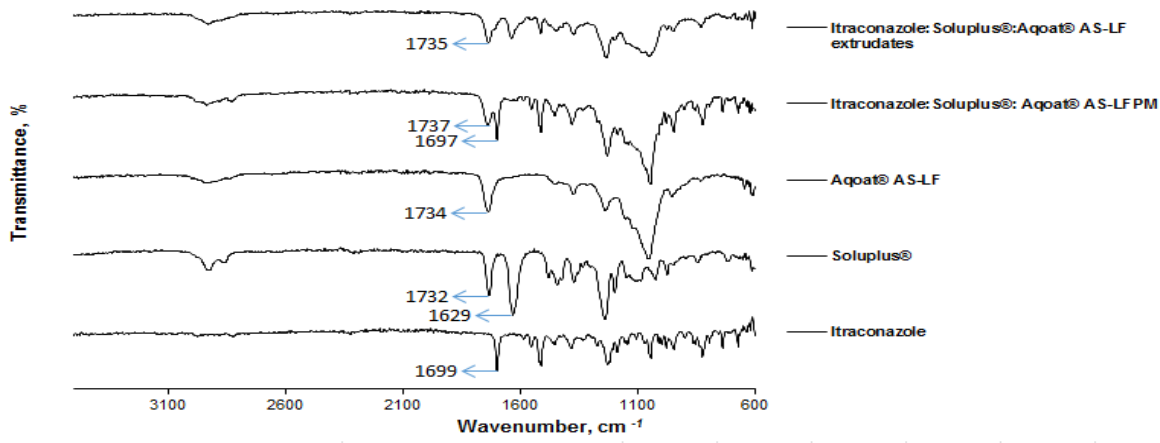


Fig. 40. FTIR spectra of itraconazole, its physical mixture and milled extrudates with different polymers (itraconazole:polymer ratio 1:3)

3.2.1.5 Aqueous solubility study

Itraconazole has a pH-dependent solubility of 9.0 µg/ml in 0.1 N HCl and less than the limit of quantitation (LOQ=3.5 µg/ml) in PBS pH 6.8. The saturation solubility of itraconazole powder increased in polymeric solutions because both Soluplus® and Aqoat® AS-LF act as solubilizers. Supersaturation of itraconazole (considered as the concentration after 24h) was achieved in case of milled extrudates due to molecular dispersion of drug in the carriers achieved by hot-melt extrusion.

In the case of itraconazole:Soluplus® 1:3 milled extrudates, the supersaturation degree in 0.1 N HCl was higher than in PBS pH 6.8, which is due to the basic nature of itraconazole. Itraconazole:Aqoat® AS-LF 1:3 milled extrudates resulted in higher supersaturation degree in PBS pH 6.8 compared to itraconazole:Soluplus® 1:3 milled extrudates. Low solubility was expected from itraconazole:Aqoat® AS-LF 1:3 milled extrudates in 0.1 N HCl and, therefore, was not performed. Furthermore, using Soluplus®:Aqoat® AS-LF polymers combination milled extrudates (drug:polymers ratio 1:3) modified the supersaturation degree in both, 0.1 N HCl and PBS pH 6.8 (**Table 14**).

Table 14. Aqueous solubility of pure itraconazole and itraconazole:carrier 1:3 milled extrudates in both 0.1 N HCl and PBS pH 6.8 mediums

Medium	**(without polymer)**	**0.3% w/v, Soluplus®**	**0.3% w/v, Aqoat® AS-LF**	**0.3% w/v, Soluplus®:Aqoat® AS-LF**		
				75:25	**50:50**	**25:75**
Itraconazole powder saturation solubility, µg/ml, n=2						
0.1 N HCl	9	25.2	ND	ND	ND	ND
PBS pH 6.8	<LOQ	6.2	24	10	18	20
Milled extrudates dissolved after 24 h*±SD, µg/ml, n=2						
0.1 N HCl	-	350±37	ND	760±83	485±55	306±76
PBS pH 6.8	-	200±50	300±75	815±70	600±35	747±190

LOQ: Limit of Quantitation~3.5 µg/ml

* The amount of milled extrudates was selected to keep constant the concentration of polymers in the medium (0.3 % w/v)

ND: Not detected

In order to understand the solubility behavior of itraconazole from milled extrudates, the kinetic solubility of itraconazole:Soluplus® milled extrudates in 0.1 N HCl was evaluated. The results are shown in **Fig. 41 A and B** and were as follows:

- Rapid increase in concentration until maximum (here value after 24 h) – which characterizes supersaturation due to molecular dispersion of drug in the carrier.
- Followed by gradual decrease of itraconazole concentration until plateau – which characterizes solubilization of itraconazole in the corresponding Soluplus® solution.

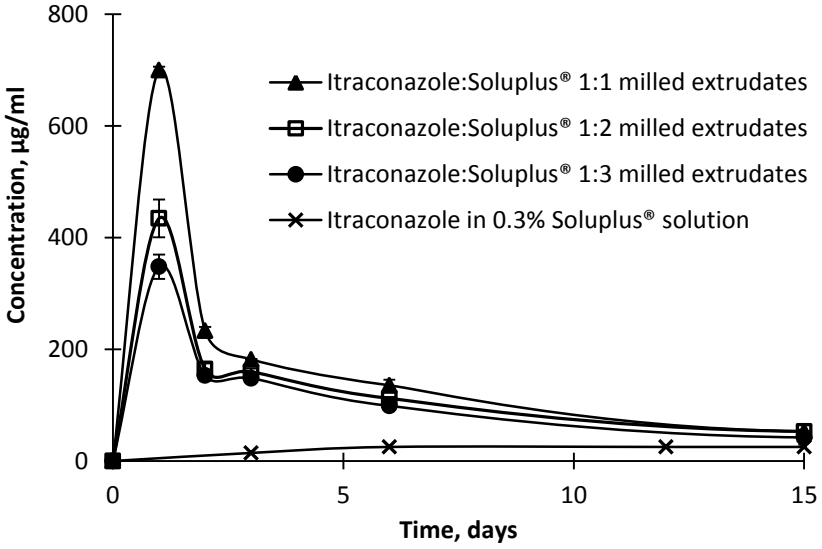
In case of Soluplus® milled extrudates with different itraconazole:Soluplus® ratios e.g. 1:3, 1:2 and 1:1, the supersaturation and the solubilization were characterized according to 2 different approaches as follows:

- The amount of milled extrudates was selected to keep constant the concentration of Soluplus® in the medium (e.g. 0.3 % w/v). In this case, the drug amount in extrudates was increased in order of itraconazole:Soluplus® 1:1 > 1:2 > 1:3. According to this approach:
  - The supersaturation (value after 24 h) increased -700, 435, 350 µg/ml in order of itraconazole:Soluplus® extrudates 1:1 > 1:2 > 1:3, respectively (**Table 15, Fig. 41A**). This can be attributed to higher initial amount of molecularly dispersed itraconazole. However, the percentage of dissolved itraconazole increased in the opposite order: 23.3, 29, 34.5 %, respectively (**Fig. 41B**).
  - The supersaturation (value after 24 h) increased further -467, 656, 718 µg/ml with increasing the extrudates amount in following order of itraconazole:Soluplus® extrudates 1:3< 1:2 ≈1:1, respectively. This is due to higher amount of extrudates associated with higher Soluplus® concentration (e.g. 0.6 % w/v) (**Table 15**, **Fig. 42**).
  - The saturation solubility (concentration at plateau) increased due to solubilization by Soluplus® irrespective of itraconazole:Soluplus® ratio (**Table 15**).
- The amount of extrudates was selected to keep the amount of itraconazole constant (e.g. 150 mg). In this case, the Soluplus® amount decreased in order 1:1< 1:2 < 1:3. According to this approach:
  - The supersaturation (value after 24 h) increased -467, 435, 353 µg/ml in the order of itraconazole:Soluplus® extrudates 1:3 > 1:2 > 1:1, respectively (**Table 15**). This was due to better dispersion of itraconazole in higher ratio of Soluplus® carrier.
  - The saturation solubility (concentration at plateau) was not measured but expected to increase due to solubilization by Soluplus®.

Overall, a supersaturation 40-80 times was achieved from itraconazole:Soluplus® extrudates (**Table 15**).

In the case of ritonavir, the solubility was 1085 µg/ml in 0.1 N HCl and 8.8 µg/ml in PBS pH 6.8. The saturation solubility of ritonavir powder increased in Soluplus® solution to 1095 µg/ml or 175 µg/ml in both 0.1 N HCl or PBS pH 6.8, respectively.

A) amount dissolved



B) % dissolved

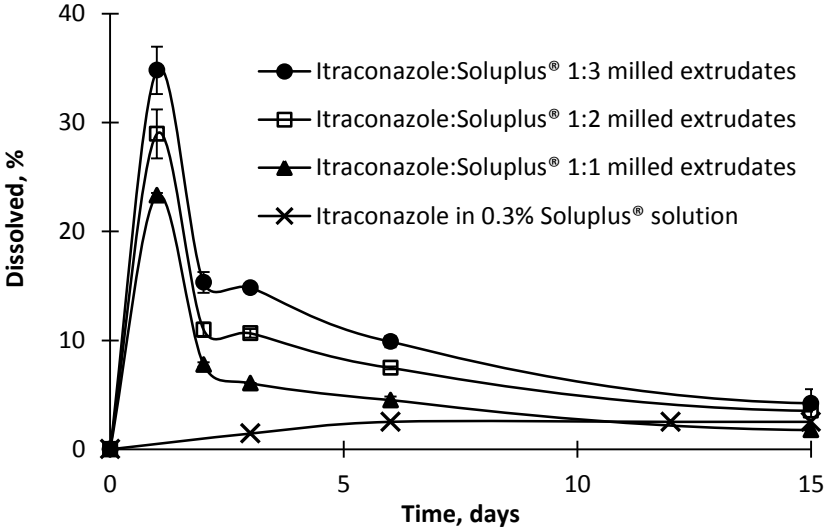


Fig. 41. Kinetic solubility of itraconazole from itraconazole:Soluplus® milled extrudates in 0.1 N HCl

Table 15. Aqueous solubility of itraconazole:Soluplus® milled extrudates in 0.1 N HCl

Itraconazole: Soluplus® milled extrudates	Itraconazole (mg)	Soluplus® (mg)	Solubility (plateau) (µg/ml)	Dissolved after 24 h (µg/ml)	Increase in solubility after 24 h (times)
1:1	150	150	ND	353	39.22
1:2	150	300	ND	435	48.33
1:3	150	450	ND	467	51.88
1:1	300	300	53	700	77.70
1:2	150	300	53	435	48.33
1:3	100	300	43	350	38.88
1:1	600	600	ND	718	79.77
1:2	300	600	ND	656	72.88
1:3	200	600	ND	476	52.88

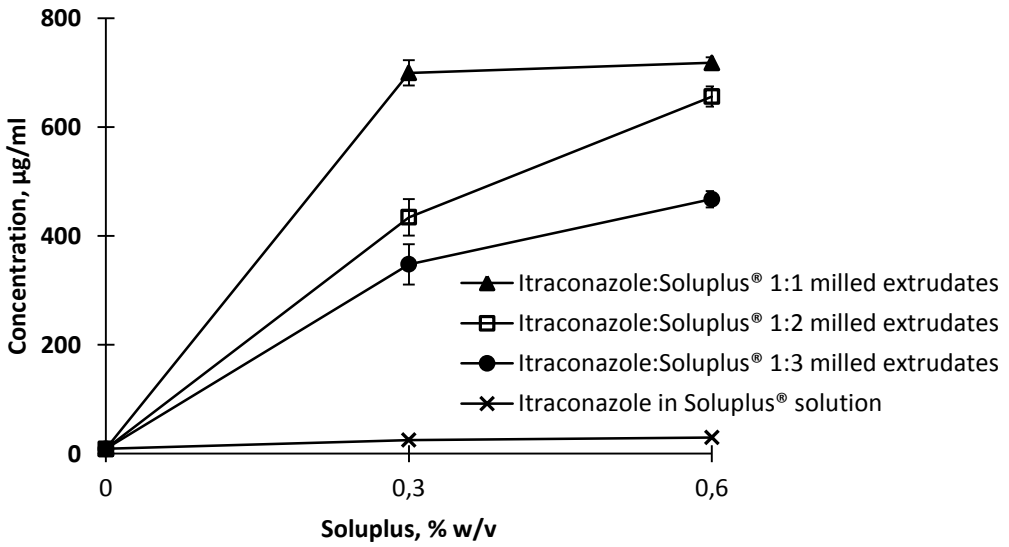


Fig. 42. Effect of the amount of itraconazole:Soluplus® milled extrudates on itraconazole solubility after 24 h in 0.1 N HCl

3.2.1.6 Dissolution studies

3.2.1.6.1 Immediate release formulations of Soluplus® milled extrudates

Effect of solid state on drug release

The generation and maintenance of supersaturated solutions of poorly soluble drugs are essential to improve the bioavailability.

Fig. 43 shows the dissolution profile under non-sink conditions corresponding to the typical dose (100 mg) of itraconazole in 0.1 N HCl from fast disintegrating tablets containing pure itraconazole, itraconazole:Soluplus® physical mixture or itraconazole:Soluplus® milled extrudates. Due to poor solubility, the dissolution of pure itraconazole was very low, only 10 % drug release was obtained within 24 h. A slight release improvement was obtained from physical mixtures. Significantly improved drug release was obtained from itraconazole:Soluplus® 1:3 milled extrudates compared to physical mixture and pure drug. Furthermore, the release from itraconazole:Soluplus® 1:3 milled extrudates was rapid and complete within 30 min, and the supersaturated solution was stable within 24h.

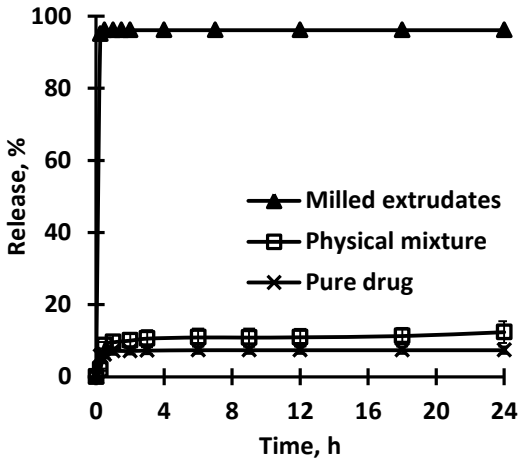


Fig. 43. Comparison of dissolution profiles under non-sink conditions (corresponding to 100 mg drug) in 0.1 N HCl between itraconazole, its physical mixture and milled extrudates with Soluplus® (itraconazole:Soluplus® ratio 1:3)

In the case of ritonavir, fast and complete drug release was obtained within 30 min in 0.1 N HCl irrespective of the solid state (milled extrudates, physical mixture or pure drug). This is due to sink conditions being reached with a typical dose of 200 mg ritonavir (**Fig. 44 A**). Improved drug release in 0.1 N HCl was achieved from ritonavir:Soluplus® 1:1.5 milled extrudates formulated in fast disintegrating tablets compared to physical mixture and pure drug under non-sink conditions, corresponding to 1200 mg ritonavir, and the supersaturated solution was stable within 24h (**Fig. 44 B**).

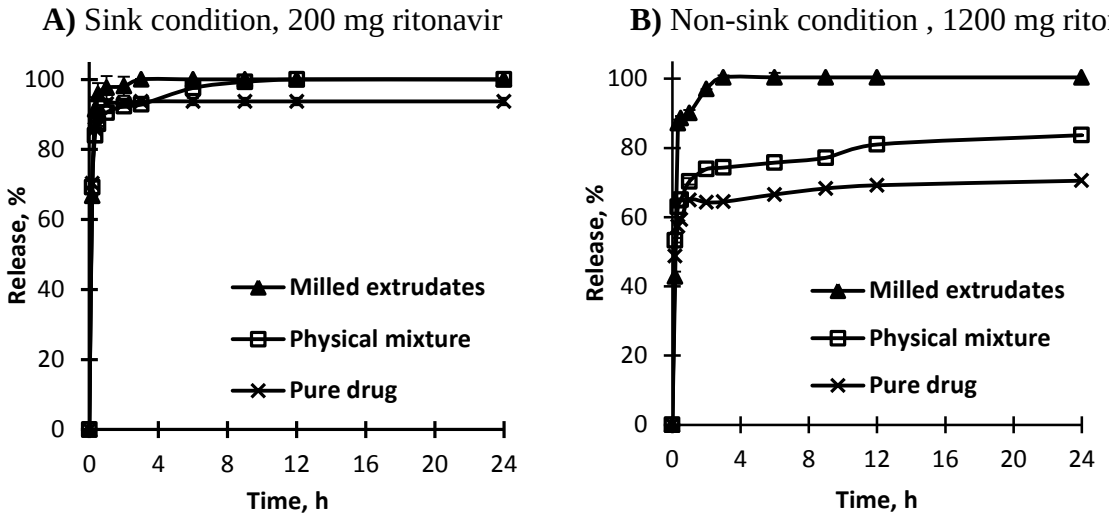


Fig. 44. Comparison of dissolution profiles between ritonavir, its physical mixture and milled extrudates with Soluplus® (ritonavir:Soluplus® ratio 1:1.5)

Effect of formulation and process variables on drug release

Drug:polymer ratio, extrusion temperature and extrusion speed were selected for the evaluation.

A slightly slower itraconazole release rate was obtained from itraconazole:Soluplus® 1:1 milled extrudates compared to itraconazole:Soluplus® 1:2 milled extrudates, which is due to better dispersion of itraconazole in higher Soluplus® amount. However, the effect of drug:polymer ratio became negligible with further increases of the polymer amount. Furthermore, Soluplus® was able to maintain the supersaturation state within at least 24 h from all itraconazole:Soluplus® milled extrudates ratios (**Fig. 45**).

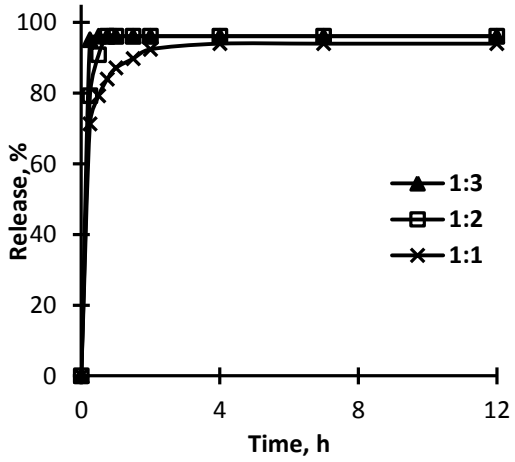


Fig. 45. Effect of itraconazole: Soluplus® milled extrudates ratio on itraconazole release in 0.1 N HCl

The extrusion temperature plays an important role in the preparation of solid dispersions (Shibata et al., 2009).

The dissolution profile of the formulations is dramatically affected by the extrusion temperature, whereby a lower drug release was obtained when the processing temperature was much lower than the drug melting point (**Fig. 46**). This is due to still undissolved drug particles, when low extrusion temperature below the melting point of the drug was used confirmed by DSC (**Fig. 48**).

Increasing screw speed results in a higher shear rate, an improved mixing, a shorter residence time, a lower viscosity and a lower torque (Saerens et al., 2013), this may affect the solid state of the final product and therefore the solubility and drug release. However, an enhanced dissolution profile was obtained irrespective of the extrusion speed (**Fig. 47**). This is caused by the unchanged solid state of the drug as confirmed by DSC (**Fig. 48**).

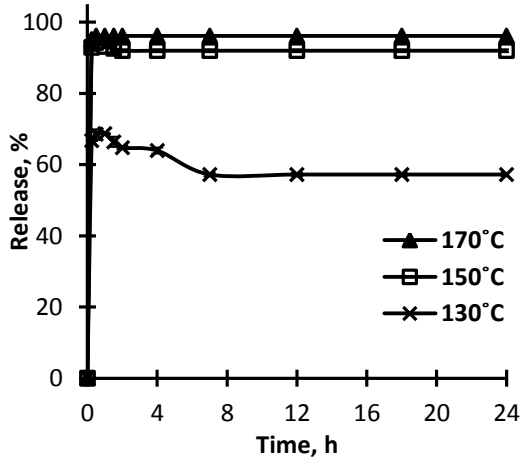


Fig. 46. Effect of extrusion temperature on itraconazole release in 0.1 N HCl from itraconazole:Soluplus® 1:3 milled extrudates (15 rpm extrusion speed)

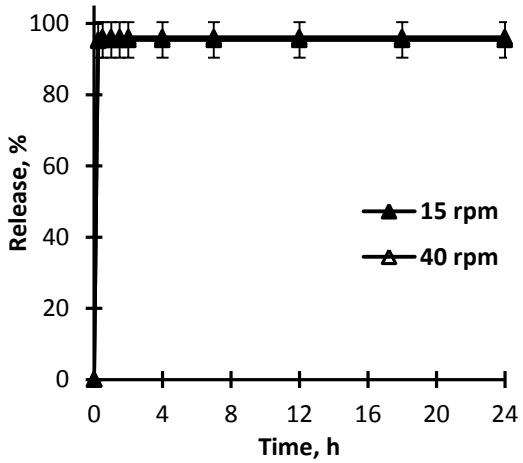


Fig. 47. Effect of extrusion speed on itraconazole release in 0.1 N HCl from itraconazole:Soluplus® 1:3 milled extrudates (170 ˚C extrusion temperature)

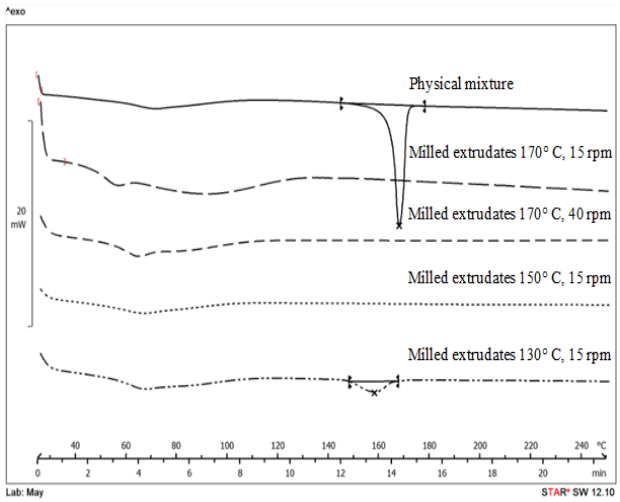


Fig. 48. DSC of itraconazole:Soluplus® 1:3 physical mixture and milled extrudates prepared at different extrusion temperatures and speeds

Effect of extrudates particle size on drug release

Particle size has a pronounced effect on dissolution rate and bioavailability of poorly soluble drugs (Chu et al., 2012). No influence of particle size on the dissolution profiles from itraconazole:Soluplus® 1:3 milled extrudates. This can be attributed to the carrier-controlled release from the molecularly dispersed drug in the amphiphilic co-polymer Soluplus® (Craig, 2002). Moreover, the high improvement of solubility obtained by hot-melt extrusion with the amphiphilic co-polymer Soluplus® may eliminate the effect of particle size on drug release. However, a slower but still complete drug release was obtained from the 5 mm extrudates, due their much smaller surface area compared to the milled extrudates (**Fig. 49**).

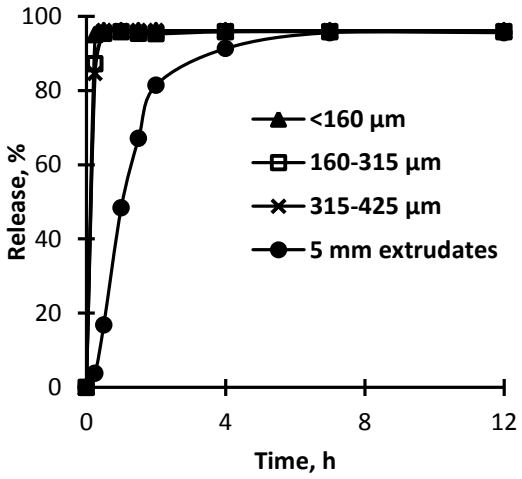


Fig. 49. Effect of extrudates particle size on drug release in 0.1 N HCl from itraconazole: Soluplus® 1:3 milled extrudates

Effect of dose on drug release

The doses of drugs were varied to evaluate the ability of hot-melt extrusion with Soluplus® carrier to generate and maintain different degrees of supersaturated solutions.

Itraconazole is available in 100 mg strength for twice-daily administration. In case of ritonavir, its maximum dose is 600 mg twice a day. In this study, the doses were increased for both itraconazole and ritonavir to 200 mg and 1200 mg, respectively, in order to evaluate the ability to reduce dosing frequency from twice-daily to once daily for further extended release propose.

Fast and complete release was obtained for both drugs from all doses (**Fig. 50**). Furthermore, the supersaturated solutions were stable for more than 24 h. This suggests that the formulation of Soluplus® milled extrudates in extended release dosage forms could be a promising approach to extend the release of poorly soluble drugs.

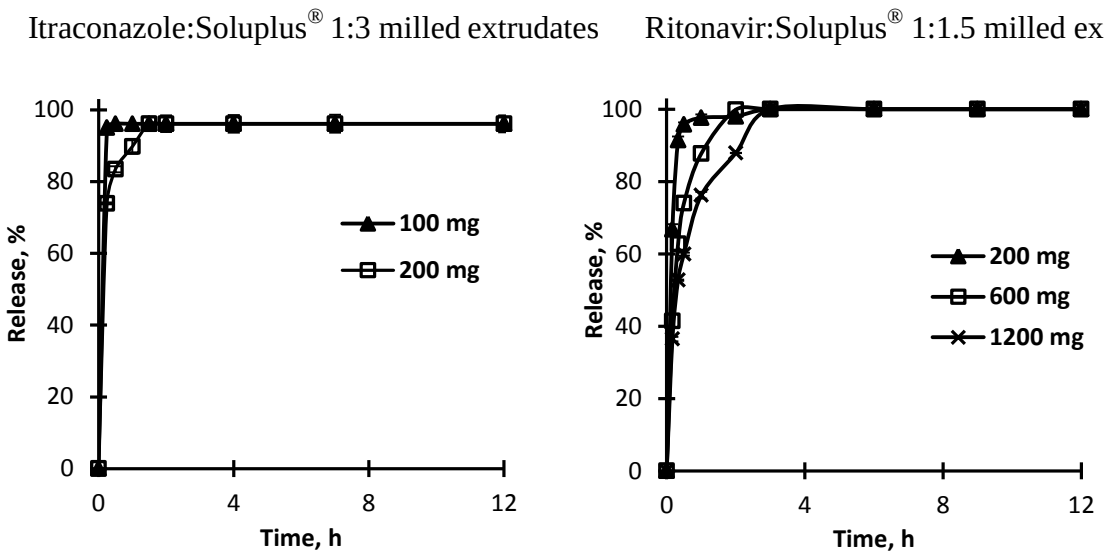


Fig. 50. Effect of dose on drug release in 0.1N HCl from different milled extrudates formulated in fast disintegrating tablets

Effect of pH-change on drug release

pH-change along the gastrointestinal tract affects the absorption of weakly basic drugs as the solubility significantly depends on the pH, this may lead to drug precipitation as the pH increases (Siepe et al., 2008).

The use of hot-melt extrusion to produce amorphous solid dispersions of itraconazole with the aim of improving solubility and dissolution rate in 0.1 N HCl has been reported in numerous reports (Verreck et al., 2003; Rambali et al., 2003). However, few studies have been published with the aim of prolonging the extent of supersaturation following acidic-to-neutral pH-change (Miller et al., 2007; Miller et al., 2008). Furthermore, their results demonstrate that the supersaturated levels were not stable for long time after pH-change, as the drug tends to precipitate as the effect of time.

In this study, the fast disintegrating tablets containing itraconazole:Soluplus® 1:3 milled extrudates were first subjected to 0.1 N HCl for 2 h followed by a pH adjustment to 6.8.

The dissolution testing incorporating a pH change (from pH 1 to 6.8 at 2 h) revealed that Soluplus® milled extrudates could protect the supersaturated solution from drug precipitation once the pH was increased (**Fig. 51**). However, changing pH resulted in higher solution turbidity due to the formation of bigger Soluplus® micelles: 265 nm in PBS pH 6.8 compare to 160 nm in 0.1 N HCl (measured by photon correlation spectroscopy (PCS)). This suggests that Soluplus® milled extrudates in fast disintegrating tablets is the optimum formulation of itraconazole as immediate release dosage form, which can perhaps improve the absorption window in the small intestine.

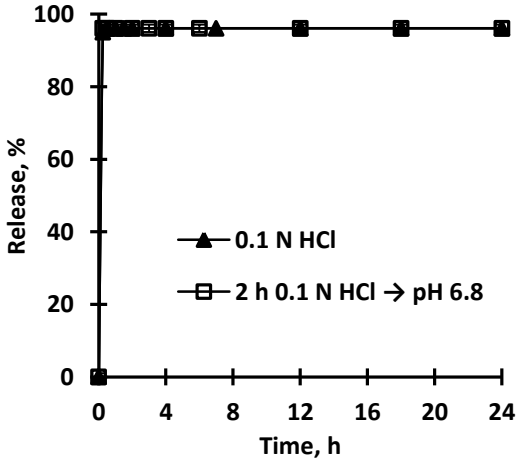


Fig. 51. Effect of pH-change on itraconazole release from itraconazole:Soluplus® 1:3 milled extrudates formulated in fast disintegrating tablets

3.2.1.6.2 Modified release formulations

The slow and incomplete release of poorly soluble drugs make them challenging for extended release formulations (Chen et al., 2010). Therefore, the combination of solubilization and retardation techniques has become an attractive approach to achieve acceptable extended release profiles of poorly soluble drugs (Tran et al., 2011).

3.2.1.6.2.1 Fast disintegrating tablets of water insoluble polymer (Kollidon® SR) milled extrudates

Kollidon® SR is a co-spray dried mixture of 80 % polyvinyl acetate, 19% polyvinylpyrrolidone and approximately 0.8 % sodium lauryl sulfate. It is typically used as a matrix former for extended release tablets (Kranz and Wagner, 2006). Özgüney et al. (2009) developed an extended-release Kollidon® SR mini-matrices for oral delivery of ibuprofen and theophylline by hot melt extrusion.

As shown before, the release from Soluplus® milled extrudates was rapid and complete within 30 minutes in 0.1 N HCl independent of particle size, therefore, an additional formulation step was required to achieve an extended release profile.

The aim of this study was to achieve both solubilization and retardation using a single formulation step. Therefore, itraconazole was extruded with Kollidon® SR using different itraconazole:Kollidon® SR ratios and then formulated into fast disintegrating tablets.

The extent of itraconazole release from itraconazole:Kollidon® SR milled extrudates was significantly improved compared to pure itraconazole, this was related to the formation of amorphous solid dispersions by hot-melt extrusion. Moreover, the release was well retarded and controlled by the Kollidon® SR ratio in the extrudates (**Fig. 52**).

Surprisingly, no drop in dissolution (precipitation) occurred within 24 h which could be attributed to the solubilizing effect of the water soluble polyvinylpyrrolidone and sodium lauryl sulfate.

The release of itraconazole from itraconazole:Kollidon® SR milled extrudates was controlled by the particle size of milled extrudates, whereby decreasing the particle size resulted in faster drug release related to higher surface area (**Fig. 53**).

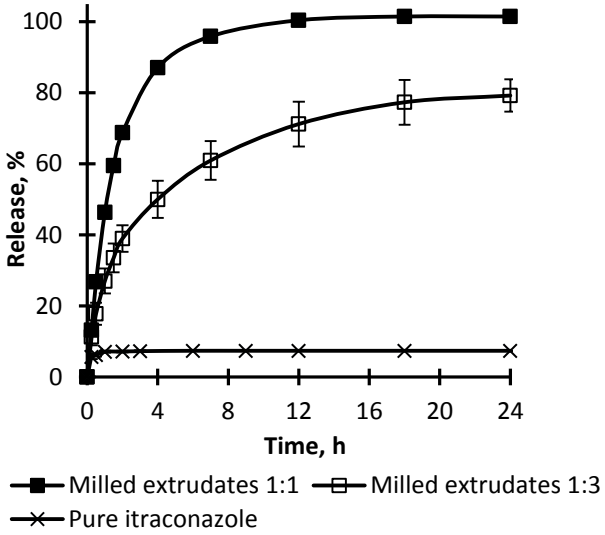


Fig. 52. Comparison of dissolution profiles under non-sink conditions (corresponding to 100 mg drug) between itraconazole:Kollidon® SR milled extrudates (160-315μm) and pure itraconazole

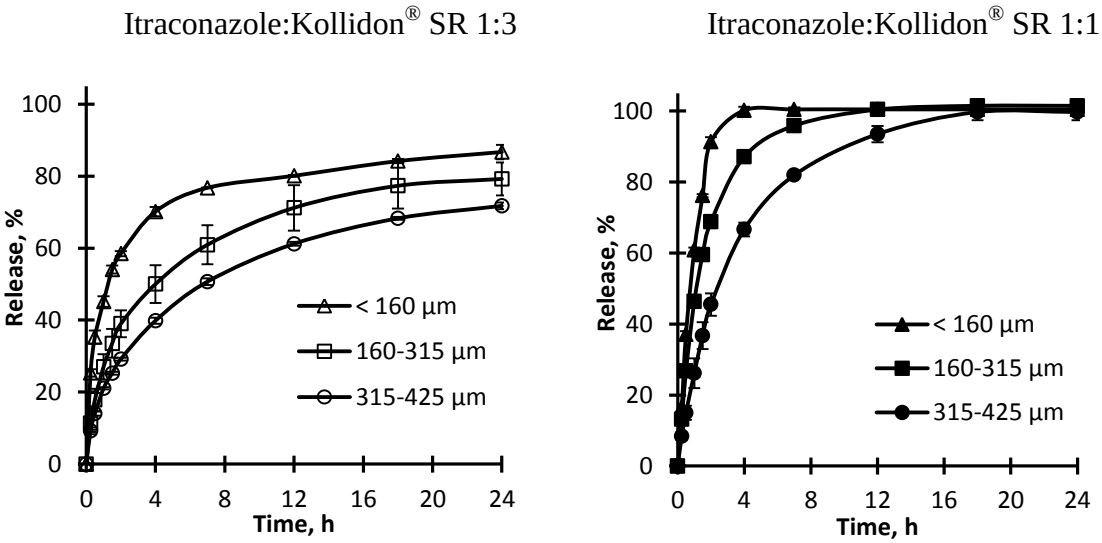


Fig. 53. Effect of particle size on itraconazole release in 0.1 N HCl from Kollidon® SR milled extrudates formulated into fast disintegrating tablets

No itraconazole release was obtained in PBS pH 6.8 in contrast to sustained itraconazole release with T80 of 24 h was obtained in 0.1 N HCl (**Fig. 54**). This is due to the pH-dependent solubility of itraconazole.

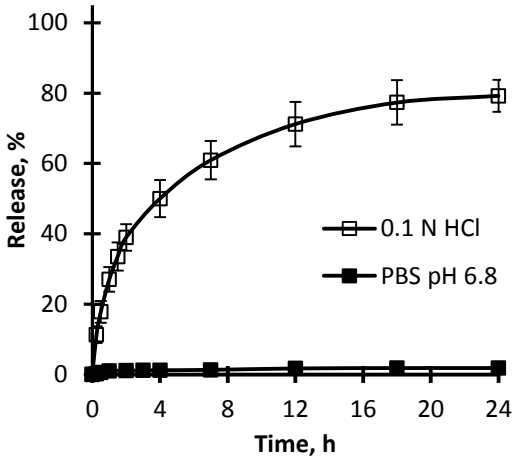


Fig. 54. Effect of pH on itraconazole release from itraconazole:Kollidon® SR 1:3 milled extrudates (160-315 μm) formulated in fast disintegrating tablets

Thus, retardation, solubilization, and stable supersaturated solutions in 0.1 N HCl can be achieved using Kollidon® SR as a carrier for hot-melt extrusion. However, the release is still pH-dependent due to basic nature of itraconazole. Therefore, this approach can be used for extending the release of pH-independent poorly water -soluble drugs.

3.2.1.6.2.2 Fast disintegrating tablets of water soluble (Soluplus®)/insoluble (Kollidon® SR) polymer combination milled extrudates

As shown in the previous section, significantly improved drug release compared to drug powder was obtained from Kollidon® SR milled extrudates. However, the drug release from itraconazole:Kollidon® SR 1:3 milled extrudates was quite slow from all particle sizes, and the extrudates containing lower Kollidon® SR ratio (itraconazole:Kollidon® SR 1:1) were not stable during long term storage at room temperature showing a recrystallization peak in DSC (as shown later in **Fig. 72**).

The aim of this study was to achieve an acceptable extended and complete drug release with improved shelf stability using a single formulation step. Therefore, itraconazole was extruded with a combination of Soluplus® and Kollidon® SR using different Soluplus®:Kollidon® SR combination ratios and then milled and formulated into fast disintegrating tablets.

Extended and complete itraconazole release in 0.1 N HCl was achieved by a single formulation step from Soluplus®:Kollidon® SR extrudates. The release was controlled by the polymers combination ratio and the extrudates particle size. No drop in dissolution (precipitation) occurred within 24 h which attributed to the solubilizing effect of Soluplus® and Kollidon® SR (the water soluble polyvinylpyrrolidone and sodium lauryl sulfate) (**Fig. 55**).

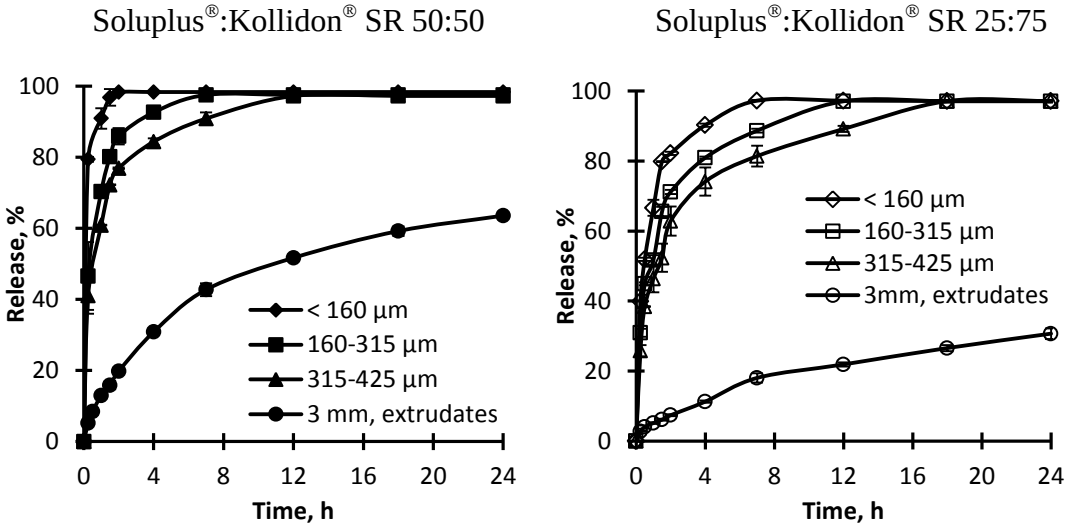


Fig. 55. Effect of particle size on itraconazole release in 0.1 N HCl from Soluplus®:Kollidon® SR extrudates (Drug:polymer ratio 1:3)

No drug release was obtained in PBS pH 6.8 and gradually drop in dissolution occurred by changing the medium pH from pH 1 to 6.8 (**Fig. 56**). This is due to the pH-dependent solubility of itraconazole.

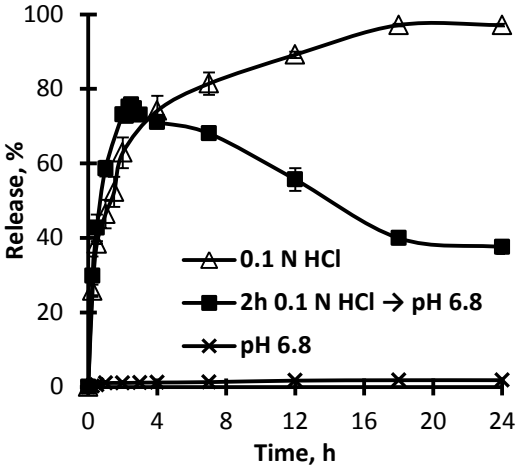


Fig. 56. Effect of pH on itraconazole release from Soluplus®:Kollidon® SR 25:75 milled extrudates (315-425μm) formulated in fast disintegrating tablets

3.2.1.6.2.3 Erodible matrix tablets of water soluble (Soluplus®) milled extrudates

Another possibility to achieve an extended and complete itraconazole release is to include the fast releasing Soluplus® milled extrudates into erodible matrix tablets. In erodible matrix tablets, the drug is entrapped in a polymer which must quickly hydrate the tablet surface upon contact with water to form a gelatinous layer, as the outer gel layer fully hydrates and dissolves, a new inner layer must replace it. The gel layer controls the water flux and drug diffusion, and the gel strength is controlled by chemistry, viscosity and concentration of the polymer (Colombo et al., 2000).

Very slow and incomplete itraconazole release within 24 h was obtained from matrix tablets containing itraconazole:Soluplus® physical mixture due to very low solubility of itraconazole. On the other hand, extended and complete release within 24 h was achieved from matrix tablets containing itraconazole:Soluplus® milled extrudates (**Fig. 57**). Thus, to achieve an extended and complete release of itraconazole, a combination of solubilization (e.g. by HME) and retardation is required.

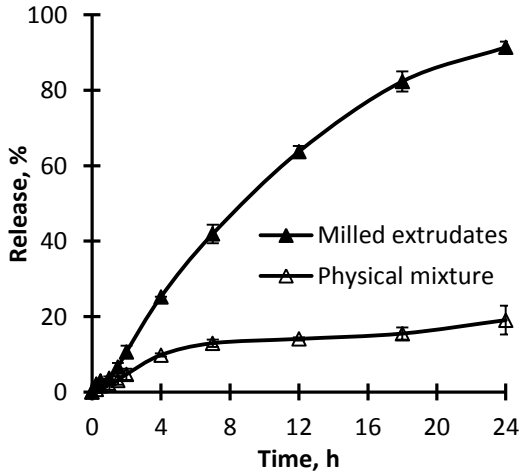


Fig. 57. Comparison of itraconazole release profiles in 0.1 N HCl between itraconazole: Soluplus® 1:3 physical mixture and milled extrudates formulated in directly compressed erodible matrix tablets with 15% Methocel® K15M

Furthermore, erodible matrix tablets of Soluplus® milled extrudates containing two different matrix formers (Hydroxypropyl methylcellulose (Methocel®) or Polyethylene oxide (Polyox(TM))), two different viscosity grads of Methocel® (K15M or K100M) or two different Methocel® K15M concentrations were studied.

Extended and complete release within 24 h in 0.1 N HCl was achieved when 15 % Methocel® matrix former was used. No differences in release profiles from matrix tablets containing Methocel® K15M or K100M were observed; similar results were obtained by (Ford et al.,

1985 and Franz et al., 1987). As the amount of Methocel® K15M was increased to 25 %, the release of drug was decreased due to the formation of a thicker gel layer and subsequently slower erosion. Very strong retardation was obtained with 15 % Polyox(TM) coagulant LEO due its high molecular weight and slow erosion (**Fig. 58**). The release became slightly faster and more linear by decreasing the extrudates portion in the tablets (60 vs. 40 %) (**Fig. 59**), this is due to higher amount of water-soluble excipients which enhances the hydration of the polymer in the matrix tablet.

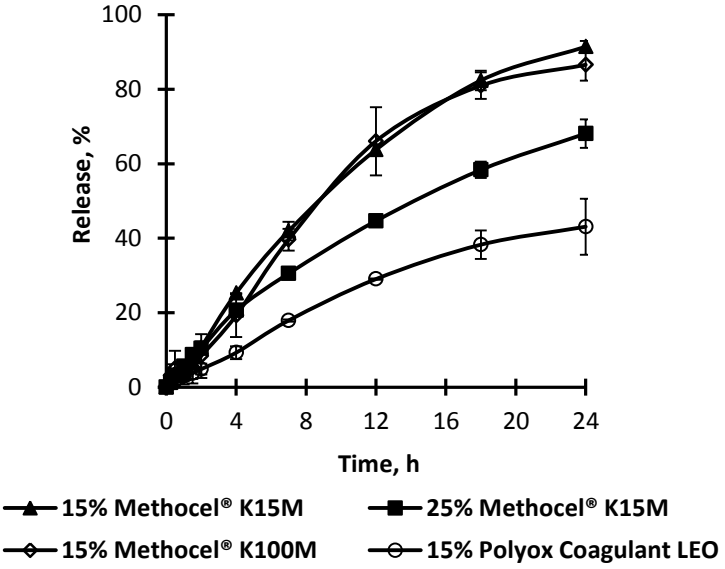


Fig. 58. Effect of matrix former on itraconazole release in 0.1 N HCl from itraconazole:Soluplus® 1:3 milled extrudates formulated into matrix tablets (60% Soluplus® milled extrudates)

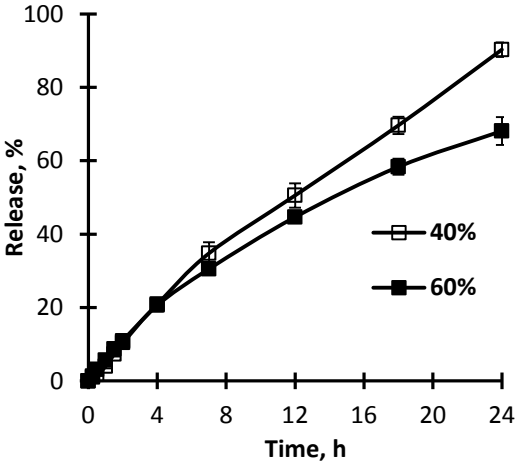


Fig. 59. Effect of extrudates amount in the tablet on itraconazole release in 0.1 N HCl from itraconazole:Soluplus® 1:3 milled extrudates formulated into matrix tablets (25% Methocel® K15M)

The release from all erodible tablets was strongly dependent on the stirring speed; the higher stirring speed resulted in a faster release rate (**Fig. 60**). This is due to the erosion driven release mechanism of the matrix.

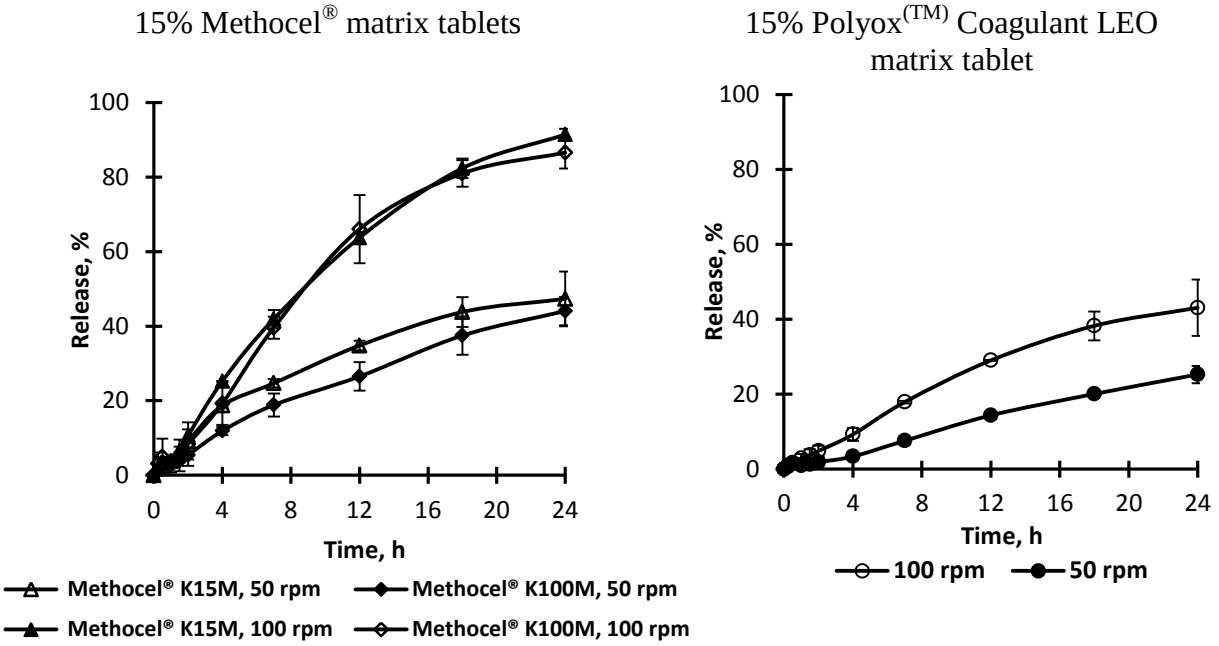


Fig. 60. Effect of stirring speed on itraconazole release in 0.1 N HCl from itraconazole:Soluplus® 1:3 milled extrudates formulated into matrix tablets

The release from all erodible tablets was dependent on the ionic strength of the medium. As the ionic strength increased in the medium, the release from matrix tablets became slower (**Fig. 61**). This is due to the slower polymer hydration and erosion (Kavanagh and Corrigan, 2004).

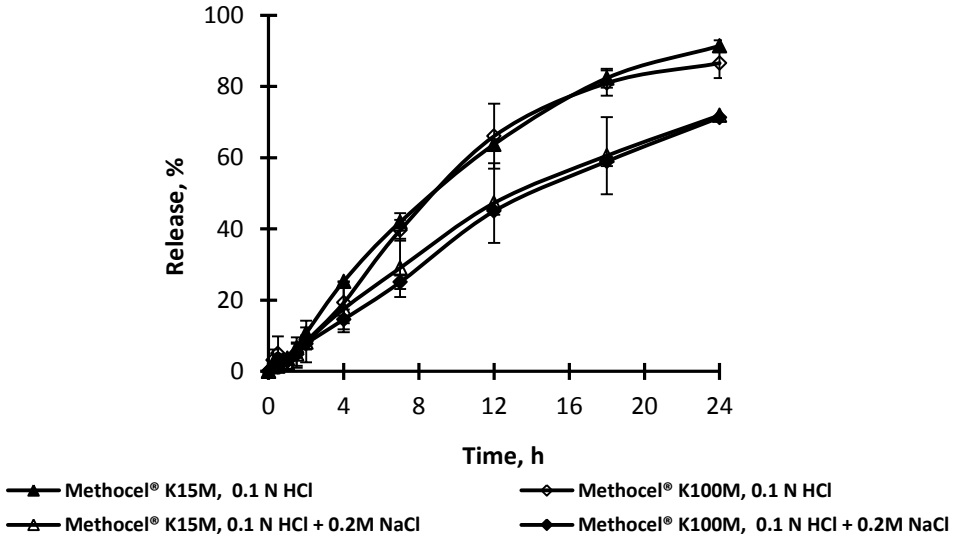


Fig. 61. Effect of ionic strength of the medium on itraconazole release from itraconazole:Soluplus® 1:3 milled extrudates formulated into matrix tablets (15 % Methocel® matrix tablets)

As the solubility of weakly basic drugs depends significantly on the pH of dissolution medium, increasing pH along the gastrointestinal tract may lead to incomplete drug release from extended release dosage forms. As shown before in **Fig. 51**, Soluplus® could protect the supersaturated solution from drug precipitation once pH was increased. However, the drug percentage released during the first 2-5 h from extended release matrix tablets was low (**Fig. 58**). Therefore, it is important to investigate the ability of drug to release further in PBS pH 6.8 from extended release matrix tablets. No itraconazole release was obtained in PBS pH 6.8 in contrast to complete release within 24 h was achieved in 0.1 N HCl (**Fig. 62**). This due to still pH-dependent solubility of itraconazole, even after the significant solubility and release improvement obtained by formation of amorphous molecular dispersion (**Table 14** and **Fig. 63**). Therefore, this approach could be useful for drugs with pH-independent solubility. However, in case of basic poorly soluble drugs, the solubility has to be improved further in PBS pH 6.8 in order to achieve a complete drug release from extended dosage forms.

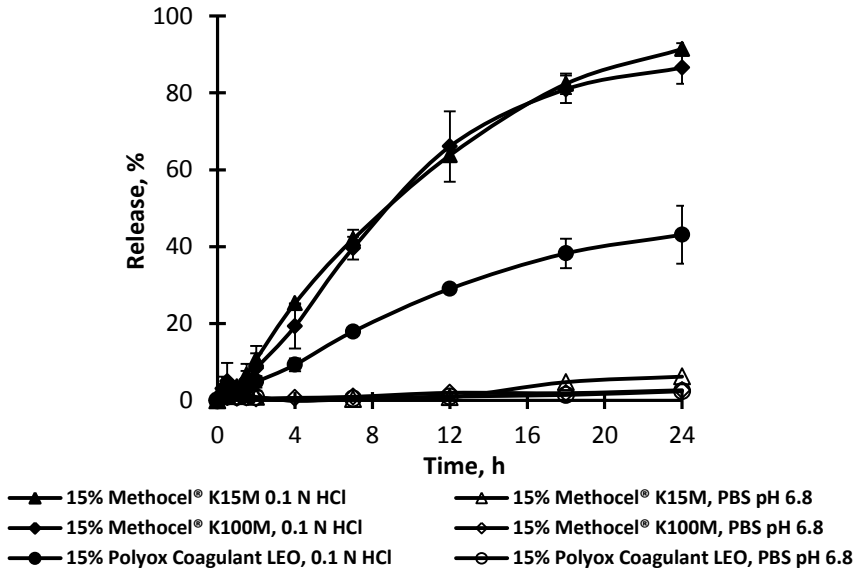


Fig. 62. Effect of pH on itraconazole release from itraconazole:Soluplus® 1:3 milled extrudates formulated into matrix tablets (15 % matrix former)

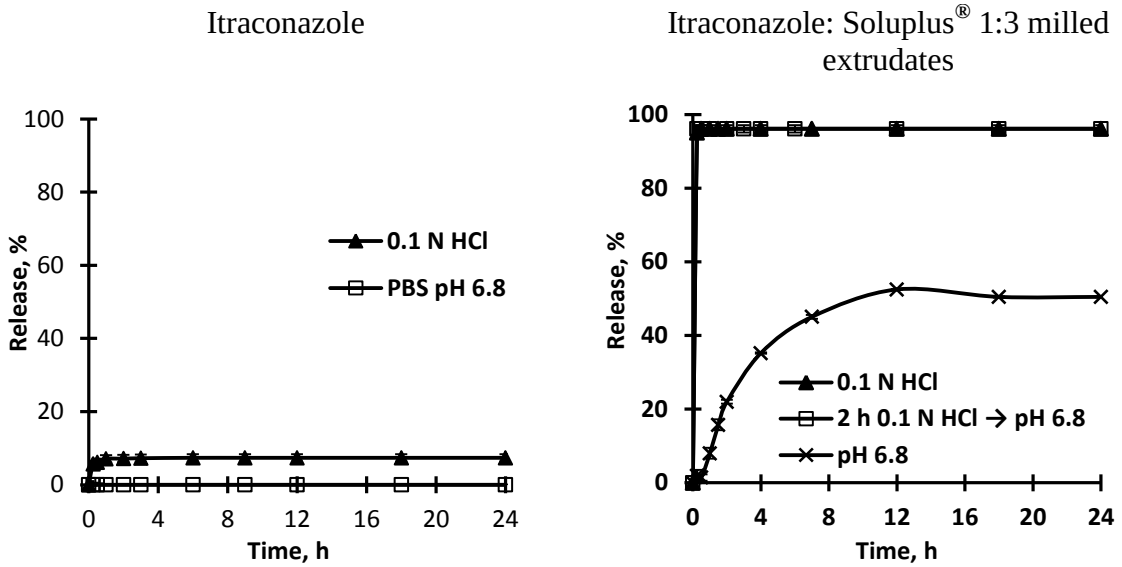


Fig. 63. Effect of pH on itraconazole release from fast disintegrating tablets

Several researchers have successfully enhanced the release of weakly basic drug compounds using pH modifiers inside a matrix system which reduce the micro-environmental pH and thereby the drug release is improved (Streubel et al., 2000; Siepe et al., 2006).

The incorporation of enteric polymers enhanced the drug release in PBS pH 6.8 (**Fig. 64**). However, the drug release was still slow for extended release purposes (40% drug release in 24h).

Therefore, using enteric polymers as carriers for solid dispersion maybe required to obtain further enhancement of solubility and dissolution rate in PBS pH 6.8.

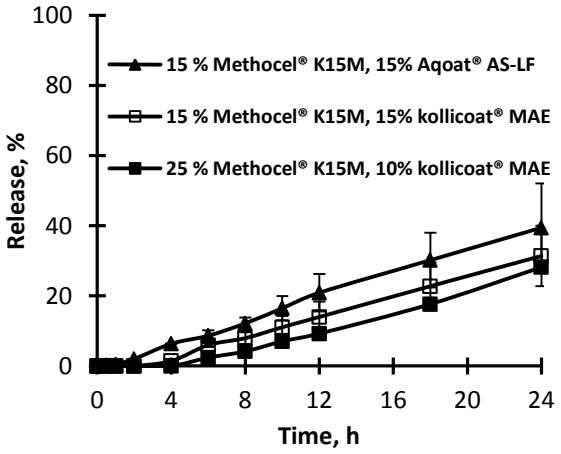


Fig. 64. Effect of enteric polymers as additive excipients on itraconazole release in PBS pH 6.8 from itraconazole:Soluplus® 1:3 milled extrudates formulated into matrix tablets

3.2.1.6.2.4 Erodible matrix tablets of water soluble (Soluplus®)/enteric (Aqoat® AS-LF or Kollicoat® MAE 100 P) polymer combination milled extrudates

As shown before, rapid and complete itraconazole release within 30 minutes in 0.1 N HCl in contrast to slow and incomplete itraconazole release in PBS pH 6.8 was obtained from Soluplus® milled extrudates formulated in fast disintegrating tablets, no drop in dissolution occurred by changing pH medium from pH 1 to 6.8, Soluplus® could protect the supersaturation level. No drug release in PBS pH 6.8 in contrast to complete release within 24 h in 0.1 N HCl was obtained from Soluplus® milled extrudates formulated into erodible matrix tablets due to the pH-dependent solubility of itraconazole. Slightly improved drug release in PBS pH 6.8 using enteric polymers as additive for the erodible matrix tablets containing Soluplus® milled extrudates but release was still too slow for extended release purposes.

Therefore, the objective of this study was to investigate approaches for extended, complete and pH-independent release profile of pH-dependent poorly water-soluble drugs by hot melt extrusion using Soluplus® in combination with enteric polymers Aqoat® AS-LF or Kollicoat® MAE 100 P and the formulation of extended release erodible matrix tablets thereof.

Aqoat® AS-LF milled extrudates formulated into fast disintegrating tablets

The release of itraconazole in 0.1 N HCl from Aqoat® AS-LF milled extrudates was very slow and incomplete; this was related to the acidic nature of Aqoat® AS-LF. However, the drug release in PBS pH 6.8 was improved by Aqoat® AS-LF milled extrudates, whereby the release was rapid and complete within 30 min. On the other hand, the supersaturated solution was not stable and gradual precipitation of itraconazole occurred within 24h (**Fig. 65**). The particle size of precipitated drug after 24 h dissolution was around 500 nm (measured by photon correlation spectroscopy (PCS)).

The higher supersaturation degree of itraconazole in PBS pH 6.8 obtained from Aqoat® AS-LF milled extrudates compared to Soluplus® milled extrudates was related to the acidic nature of Aqoat® AS-LF and to the stronger intermolecular molecular interaction by hot melt extrusion between itraconazole and Aqoat® AS-LF compared to the one between itraconazole and Soluplus® confirmed by FTIR (**Fig. 40**).

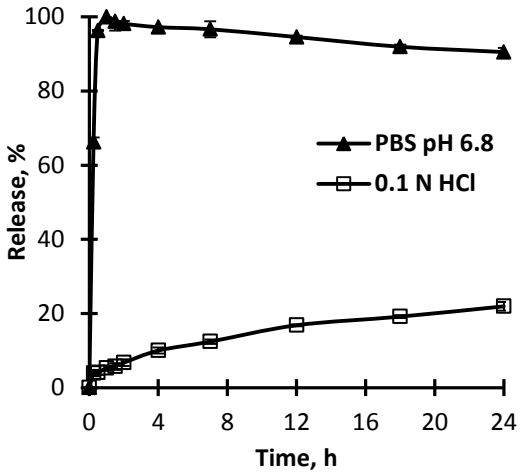


Fig. 65. Effect of pH on itraconazole release from Aqoat® AS-LF milled extrudates formulated in fast disintegrating tablets (drug:polymer ratio 1:3)

Soluplus®:Aqoat® AS-LF milled extrudates formulated into fast disintegrating tablets

To overcome the challenges of pH-dependent itraconazole release from both Soluplus® and Aqoat® AS-LF milled extrudates, extrudates containing different combination ratios between Aqoat® AS-LF and Soluplus® were prepared and evaluated regarding the drug release in both PBS pH 6.8 and 0.1 N HCl. Hot-melt extrusion using combination of Soluplus®:Aqoat® AS-LF adjusted the release of itraconazole in both PBS pH 6.8 and 0.1 N HCl mediums, whereby the release was affected by the ratio of polymer combination. Furthermore, no decrease in dissolution was observed within 24 h which is due to solubilizing effect of Soluplus® (**Fig. 66**). Almost pH-independent release of itraconazole was achieved using Soluplus®:Aqoat® 75:25 (**Fig. 66 A**).

The effect of polymers combination ratio on itraconazole release in 0.1 N HCl and PBS pH 6.8 is summarized in **Fig. 67.** Increasing Aqoat®AS-LF ratio in the combination resulted in slower itraconazole release in 0.1 N HCl and faster itraconazole release in PBS pH 6.8 related to the acidic nature of Aqoat®AS-LF.

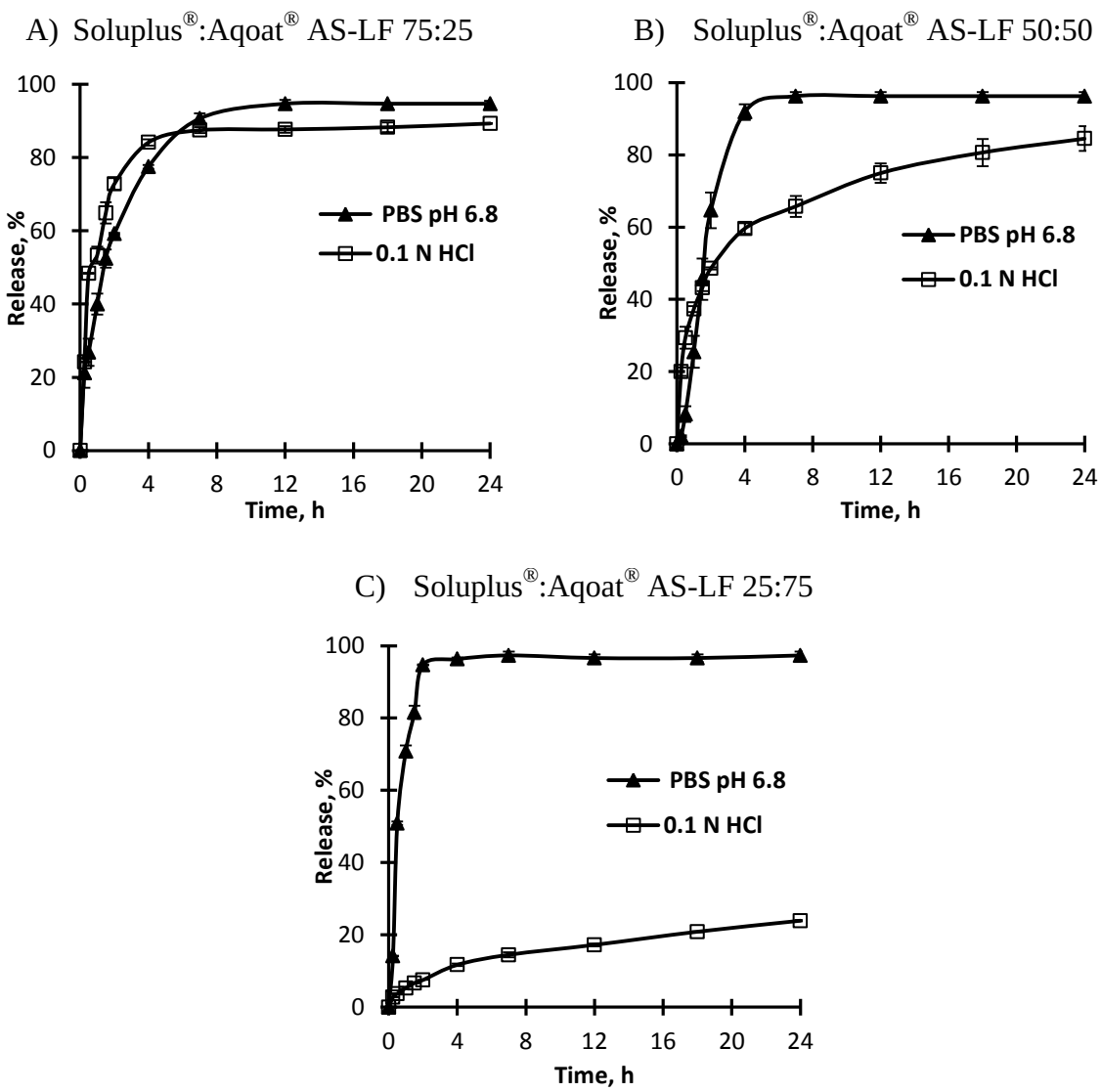


Fig. 66. Effect of pH on itraconazole release from Soluplus®:Aqoat®AS-LF milled extrudates (<315µm) formulated in fast disintegrating tablets (drug:polymer ratio 1:3)

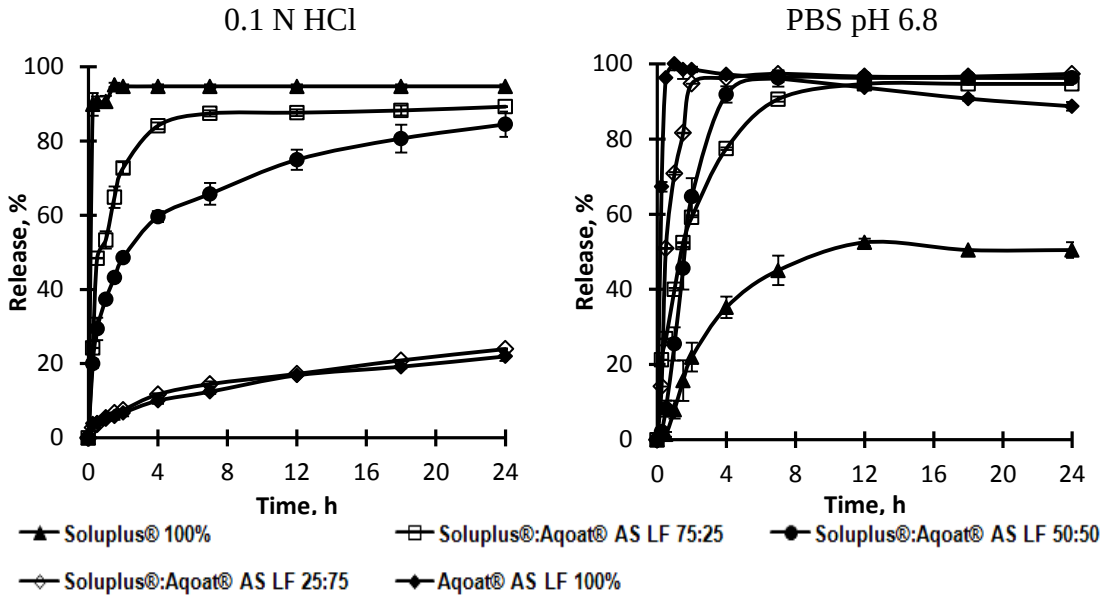


Fig. 67. Effect of polymers combination ratio on itraconazole release from Soluplus®:Aqoat®AS-LF milled extrudates (<315µm) formulated in fast disintegrating tablets (drug:polymer ratio 1:3)

Soluplus®:Kollicoat® MAE 100 P milled extrudates formulated into fast disintegrating tablets

In order to compare the effect of enteric polymer type on the drug release, Kollicoat® MAE 100 P was extruded in combination with Soluplus® and TEC (plasticizer) at 145 ˚C and 15 rpm speed (1 step extrusion) or itraconazole was extruded first with Soluplus® at 170 ˚C and the resulted extrudates were extruded again with Kollicoat® MAE 100 P and TEC at 145 ˚C (2 steps extrusion). These different extruding procedures were done to protect Kollicoat® MAE 100 P from degradation at temperature higher than 150 ˚C.

Fast and incomplete (leveled off at 80%) vs. slow and incomplete (max. 30% followed by precipitation) itraconazole release in 0.1 N HCl vs. PBS pH 6.8, respectively was obtained from 1 step extrusion of Soluplus®:Kollicoat® MAE 100 P:TEC at 145 ˚C (**Fig. 68**). This may be due to still undissolved drug particles as result of low extrusion temperature (below melting point of itraconazole 168 ˚C). The presence of undissolved drug particles can be confirmed by optical investigation of extrudates (**Fig. 34**), where the extrudates were opaque with a rough surface. However, no crystals peak was observed by differential scanning calorimetry **(Fig. 30**) and X-ray diffractometery (**Fig. 32**).

Improved extent of drug release in both PBS pH 6.8 and 0.1 N HCl was achieved from 2 steps extrusion (**Fig. 68**). This was due to better solubilization of itraconazole when it was

extruded first with Soluplus® at 170˚C (higher than its melting point 168˚C) and then extruded again with Kollicoat® MAE 100 P:TEC at 145 ˚C.

As conclusion, Soluplus®:Kollicoat® MAE 100 P:TEC milled extrudates resulted in less supersaturation degree in PBS pH 6.8 compared to Soluplus®:Aqoat® AS-LF milled extrudates, this could be related to the extrusion procedures used to protect Kollicoat® MAE 100 P from degradation.

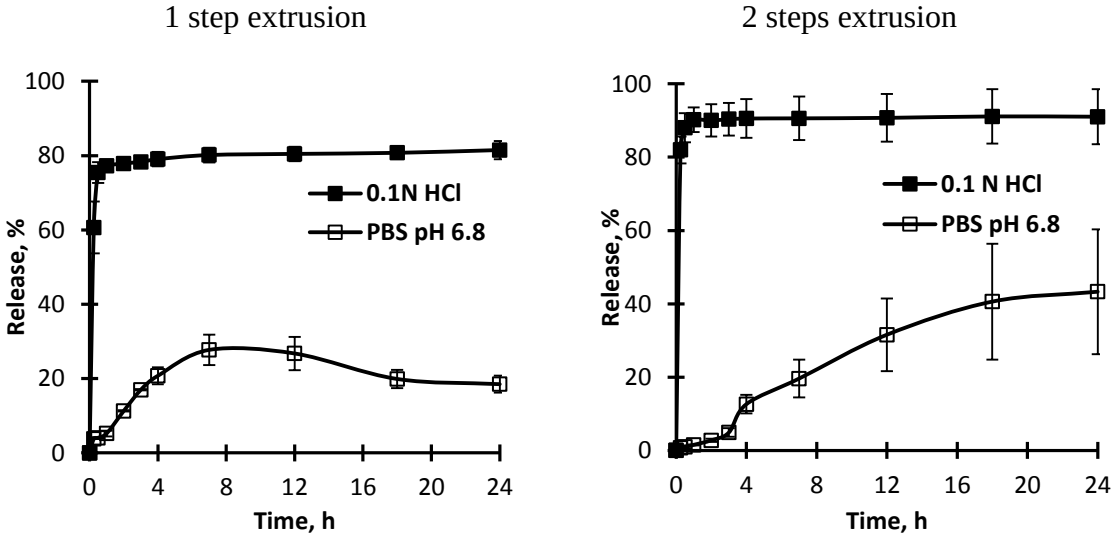


Fig. 68. Effect of pH on itraconazole release from Soluplus®:Kollicoat® MAE:TEC 50:35:15 milled extrudates (<315µm) formulated into fast disintegrating tablets (drug:polymer ratio 1:3)

In order to investigate the ability of Soluplus®:Kollicoat® MAE 100 P to improve the solubility and to provide a pH-independent release of basic poorly soluble drugs which have low melting points, ritonavir (melting peak at 123 ˚C) was extruded with these polymers combination and the effect of pH on drug release from Soluplus®:Kollicoat®MAE milled extrudates formulated in fast disintegrating tablets was evaluated.

Hot-melt extrusion using combination of Soluplus®:Kollicoat® MAE 100 P adjusted the release of ritonavir in both PBS pH 6.8 and 0.1 N HCl mediums, whereby the release was affected by the ratio of polymers combination. However, the release of ritonavir in PBS pH 6.8 was not complete, even from extrudates containing the highest amount of Kollicoat® MAE 100 P (Soluplus®:Kollicoat® MAE 100 P 25:75) (**Fig. 69**).

As conclusion, Soluplus®:Aqoat® AS-LF milled extrudates resulted in higher supersaturation degree in PBS pH 6.8 compared to Soluplus®:Kollicoat® MAE 100 P milled extrudates which can be attributed to better miscibility obtained from Soluplus®:Aqoat® AS-LF milled

extrudates compared to Soluplus®:Kollicoat® MAE 100 P milled extrudates confirmed by T_gs measurements (**Fig. 38-39**), whereby Soluplus®:Aqoat® AS-LF milled extrudates showed a single glass transition temperature indicating a miscible blend, whereas Soluplus®:Kollicoat® MAE 100 P milled extrudates showed 2 glass transition temperatures indicating poor miscibility of drug with polymers.

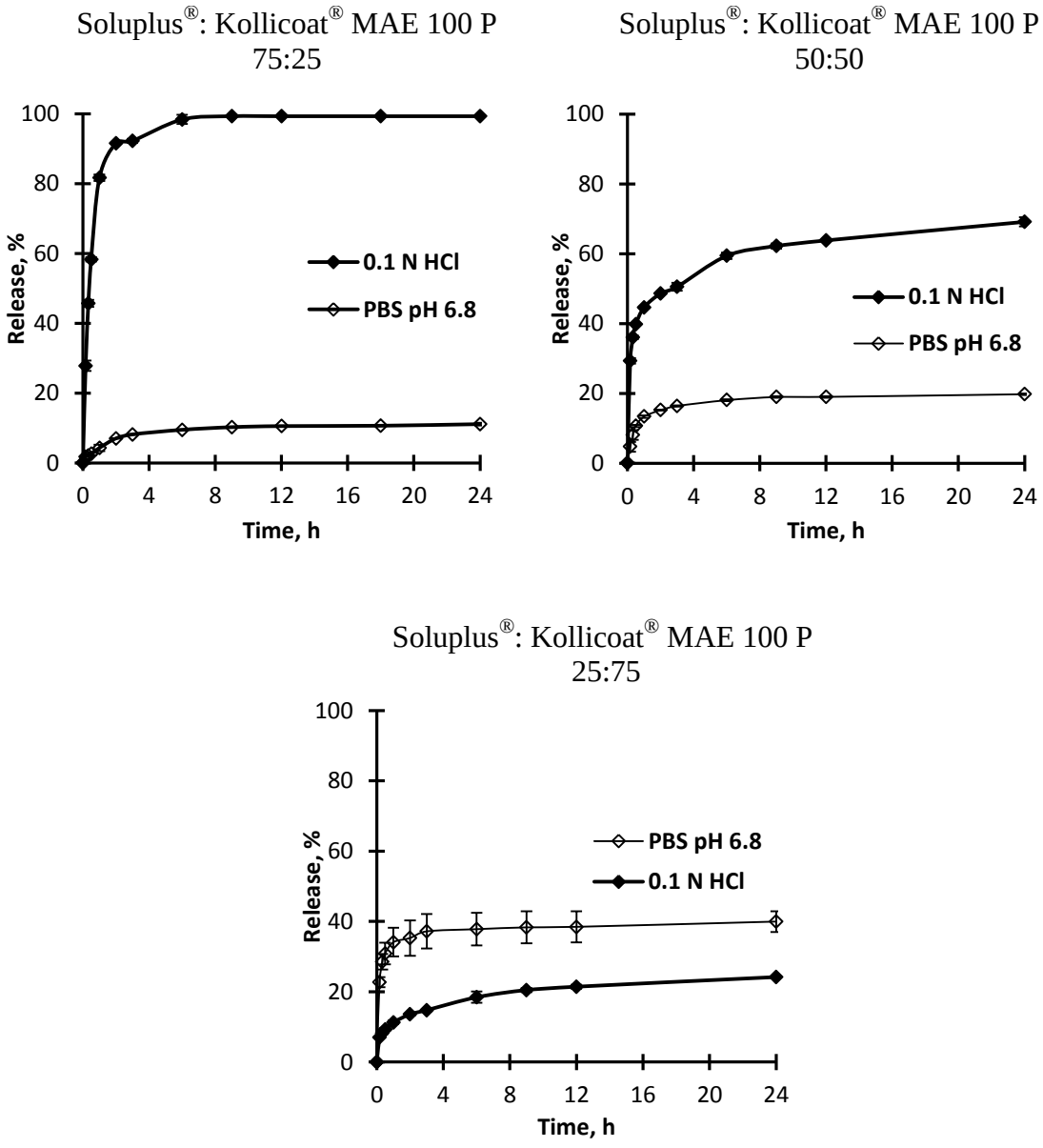


Fig. 69. Effect of pH on ritonavir release from Soluplus®:Kollicoat®MAE 100 P milled extrudates formulated in fast disintegrating tablets (drug:polymer ratio 1:1.5)

Polymeric micelles properties during dissolution studies

Since the supersaturated solutions obtained during the dissolution testing were a bit turbid due to the micelles formation and the micelles containing itraconazole showed strongly adsorption to the filters having different pore sizes or types (cellulose acetate (0.25 and 0.45 µm) or polyester 0.2 µm (chromafil® PET-20/25), it was obligatory to use glass filters with big pores (10 µm) during dissolution studies. Therefore, particle size analysis was performed during dissolution studies directly after sample filtration to ensure that the drug/polymer colloids are in nanometer range during at least 24 h. Dissolution was carried out for all formulations in PBS pH 6.8, however, in case of Soluplus® supersaturated solution was first obtained in 0.1 N HCl and then the pH was changed to 6.8.

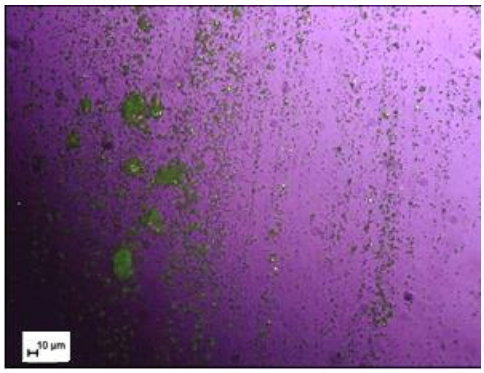
The micelle size was affected by the ratio of polymer combination. The smallest micelles size was obtained from supersaturated solution with Soluplus®, slightly bigger micelles size was obtained from supersaturated solution containing Soluplus® in combination with Aqoat®AS-LF, and the biggest micelles size was obtained from supersaturated solution with Aqoat®AS-LF. The micelles size increased further after 24h, however, the supersaturated solution can still be considered stable as the colloids size is still in nanoscale and it doesn't convert to precipitate (**Table 16**). In case of Aqoat®AS-LF, it forms colloids with size larger than 500 nm which should be considered as precipitation according to Friesen et al., 2008, and this explains the slightly drop in its dissolution curve within 24h (**Fig. 65**).

Table 16. Average micelle size of supersaturated solution with different Soluplus®:Aqoat®AS-LF ratios

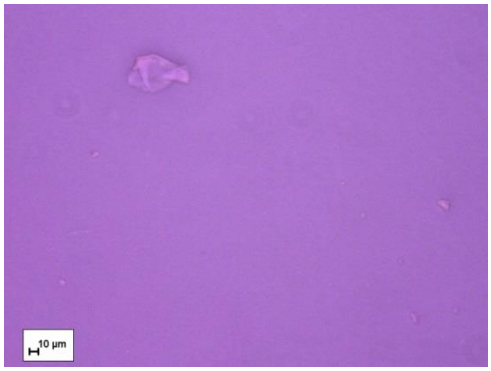
Name	**Average micelles size, nm**		
	pH=1	**pH=6.8**	
	24h	**3h**	**24h**
Soluplus®	160	179.03±3.02	265.66±12.31
Soluplus®:Aqoat®AS-LF, 75:25	-	210.10±5.65	304.97±8.02
Soluplus®:Aqoat®AS-LF, 50:50	-	244.21±9.50	398.08±7.46
Soluplus®:Aqoat®AS-LF, 27:75	-	269.83±8.25	415.73±5.89
Aqoat®AS-LF	-	306.88±12.93	510.01±14.31

Furthermore, the crystallinity in the micelles after 24 h dissolution was evaluated using polarized light microscope. It was estimated that no drug crystallinity in the micelles occurred after 24 h dissolution from milled extrudates. However, further detection of the crystallinity by small angel X-ray scattering would be required as the micelles were too small to be clearly detected by microscope (**Fig. 70**).

Itraconazole



Itraconazole: Soluplus® 1:3 milled extrudates after 24 h dissolution



Itraconazole: Aqoat®AS-LF 1:3 milled extrudates after 24 h dissolution

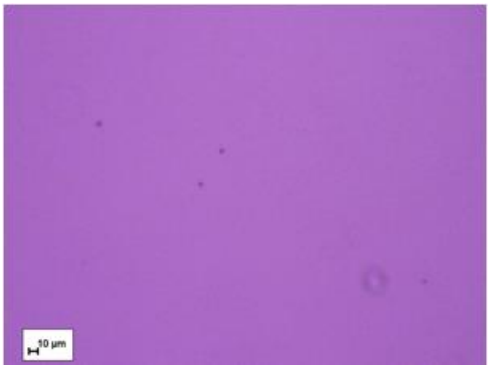


Fig. 70. Polarized light microscopic images of itraconazole powder and milled extrudates after 24 h dissolution

Erodible matrix tablets of Soluplus®:Aqoat® AS-LF milled extrudates

As shown before in **Fig. 66 A**, almost pH-independent release of itraconazole was obtained from Soluplus®:Aqoat® AS-LF 75:25 milled extrudates. Therefore, these milled extrudates were formulated into erodible matrix tablets containing 15% Methocel® K15M and evaluated regarding the effect of pH on drug release.

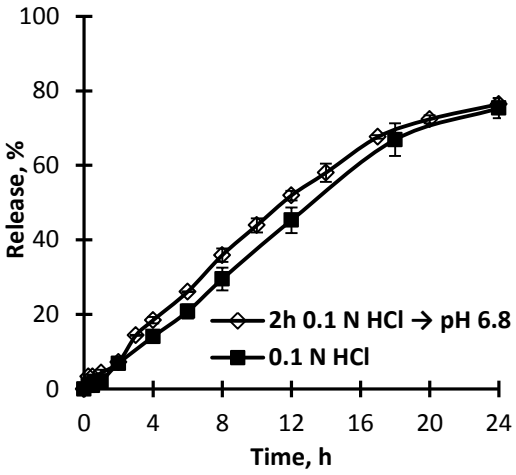


Fig. 71. Effect of pH on itraconazole release from Soluplus®:Aqoat®AS-LF 75:25 milled extrudates (<315µm) formulated in matrix tablet containing 15 % Methocel® K15M

An extended drug release (T80 of 24h) was achieved by formulating the milled extrudates containing Soluplus®:Aqoat® AS-LF 75:25 in erodible matrix tablets whereby the release was also not affected by the change of pH (**Fig. 71**).

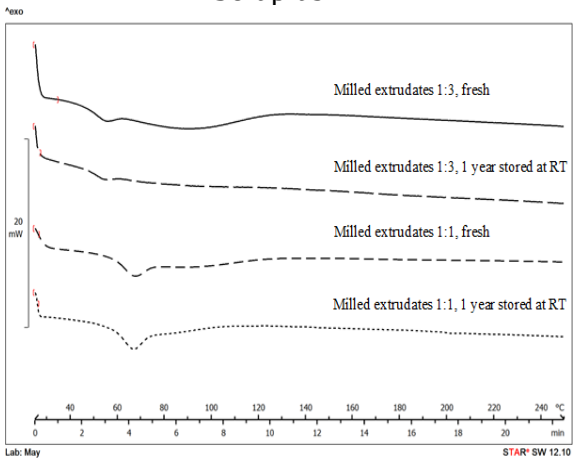
Therefore, this approach can be used for extending the release of pH-dependent basic poorly water-soluble drugs.

3.2.1.7 Effect of storage conditions on physical stability and drug release

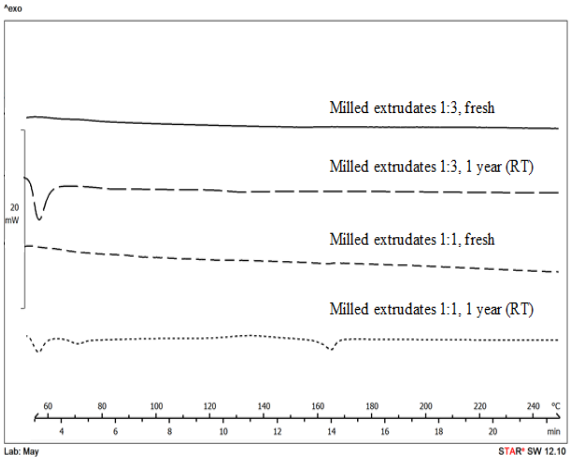
The amorphous state has high energy and will always move toward the lowest energy crystalline state. Solid dispersion technology has been extensively utilized to stabilize amorphous materials by using appropriate carriers (Chiou and Riegelman, 1971).

Milled extrudates were stored in closed vials at room temperature for 1 year. No crystal peaks were observed by both DSC and X-ray diffractometery from all milled extrudates (drug:polymer 1:3). Furthermore, no crystal peak was observed from Soluplus® milled extrudates containing the higher drug loading (itraconazole:Soluplus® 1:1) indicating essential stability of the amorphous solid dispersions upon storage at room temperature. In case of Kollidon® SR milled extrudates containing the higher drug loading (itraconazole:Kollidon® SR 1:1) a crystal peak was observed after 1 year storage at room temperature indicating instability problem, however, no diffraction peaks were observed by X-ray. This could be related to low X-ray sensitivity to detect the low degree of crystallization (**Fig. 72-76**). DSC and X-ray analysis results correlate well with the dissolution studies, as the dissolution profile of itraconazole was not affected by long term storage at room temperature either from milled extrudates (drug:polymer 1:3) or from Soluplus® extrudates containing higher drug loading (itraconazole:Soluplus® 1:1). On the other hand slower rate and extent of itraconazole release was obtained from Kollidon® SR milled extrudates containing higher drug loading (itraconazole:Kollidon® SR 1:1) (**Fig. 77**).

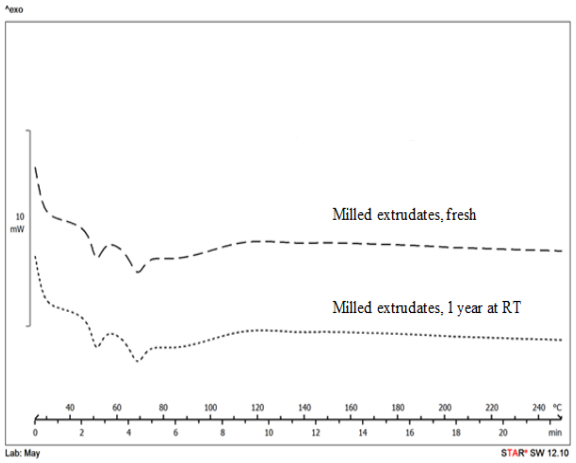
Soluplus®



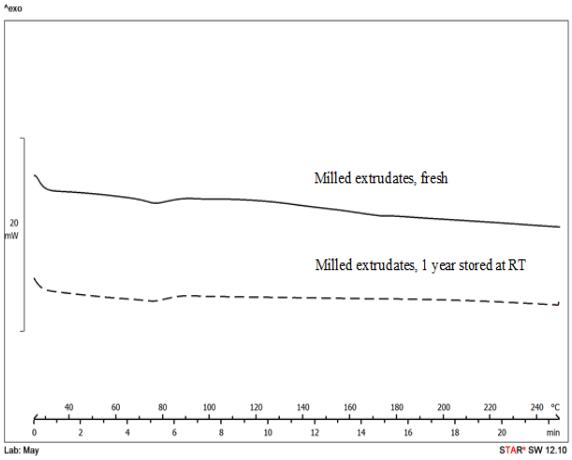
Kollidon® SR



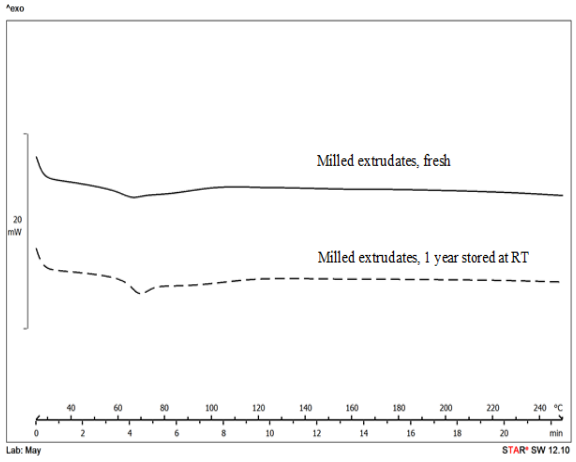
Soluplus®:Kollidon® SR 50:50



Aqoat® AS-LF



Soluplus®:Aqoat® AS-LF 50:50



Soluplus®:Kollicoat® MAE 100 P:TEC 50:35:15

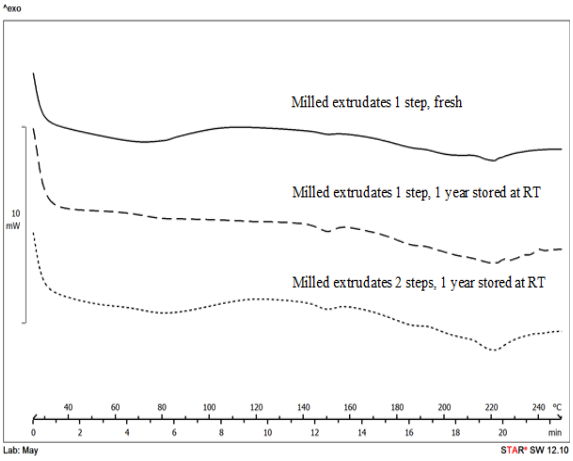


Fig. 72. DSC thermograms of itraconazole milled extrudates with different carriers before and after 1 year storage at room temperature (RT)

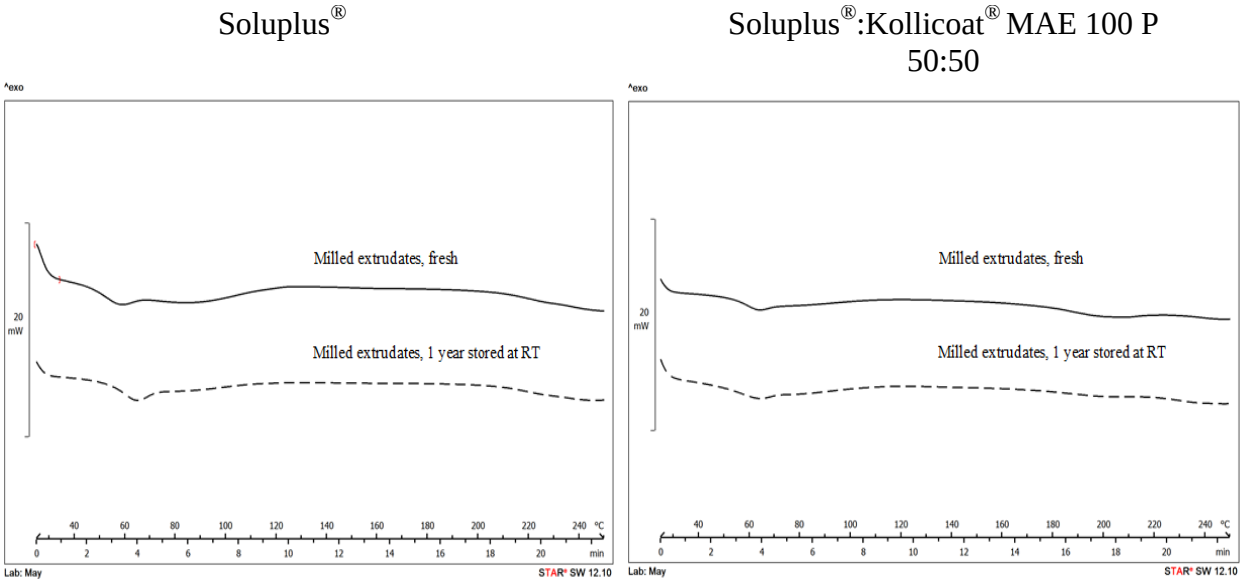


Fig. 73. DSC thermograms of ritonavir milled extrudates with different carriers before and after 1 year storage at room temperature (RT) (ritonavir:polymer ratio 1:1.5)

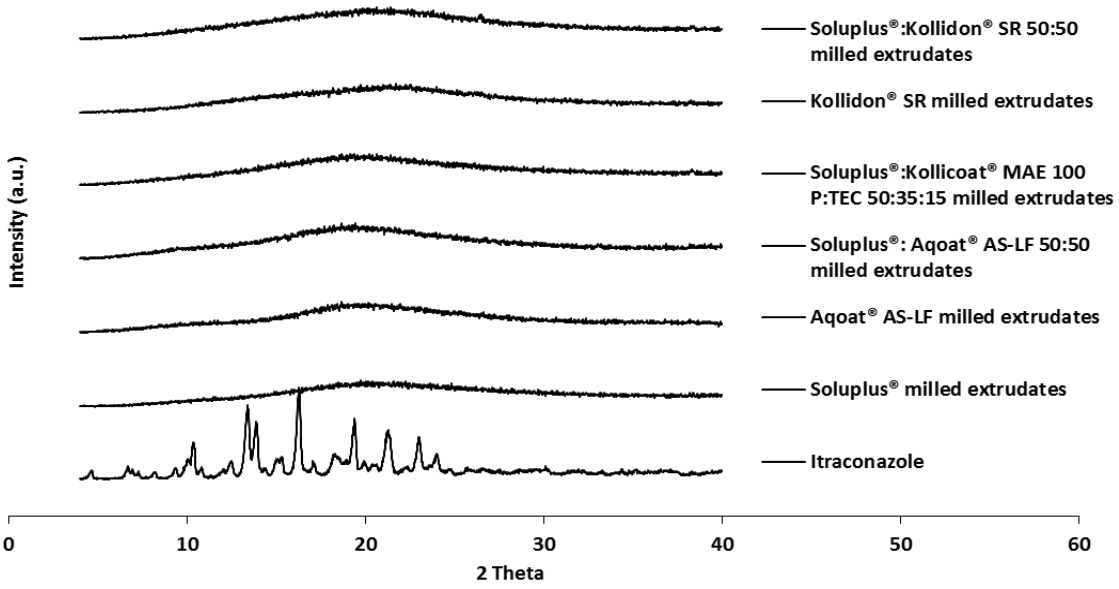


Fig. 74. X-ray diffractograms of itraconazole and its milled extrudates with different carriers after 1 year storage at room temperature (RT), (itraconazole:polymer ratio 1:3)

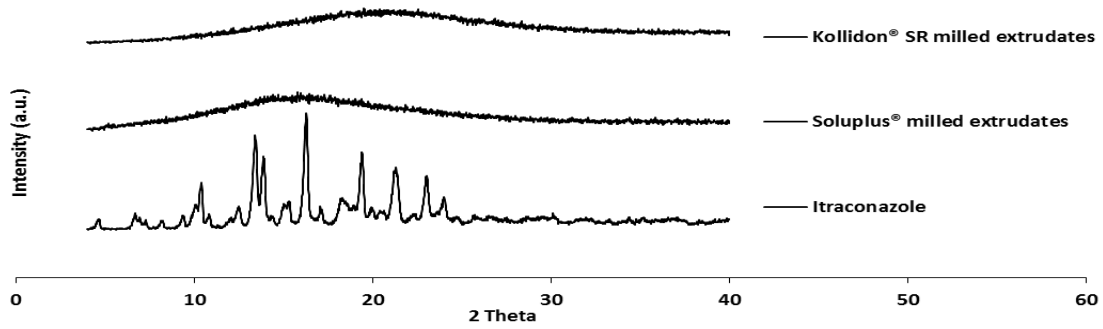


Fig. 75. X-ray diffractograms of itraconazole and its milled extrudates with different carriers after 1 year storage at room temperature (RT), (itraconazole:polymer ratio 1:1)

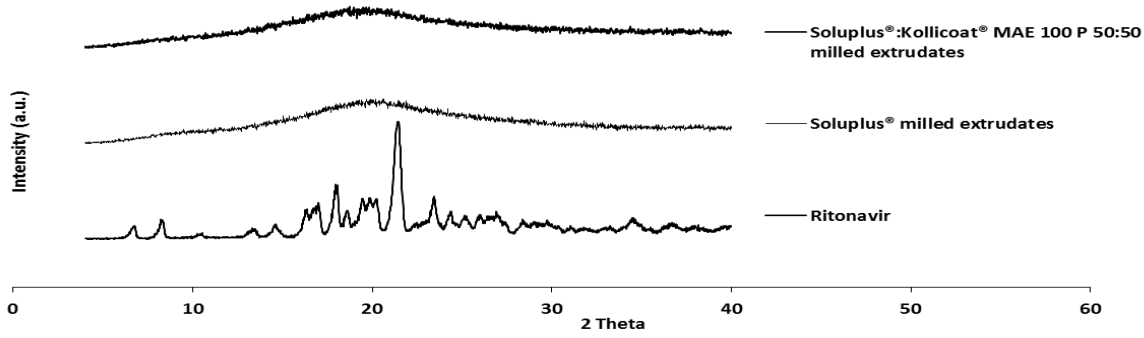


Fig. 76. X-ray diffractograms of ritonavir and its milled extrudates with different carriers after 1 year storage at room temperature (RT) (ritonavir:polymer ratio 1:1.5)

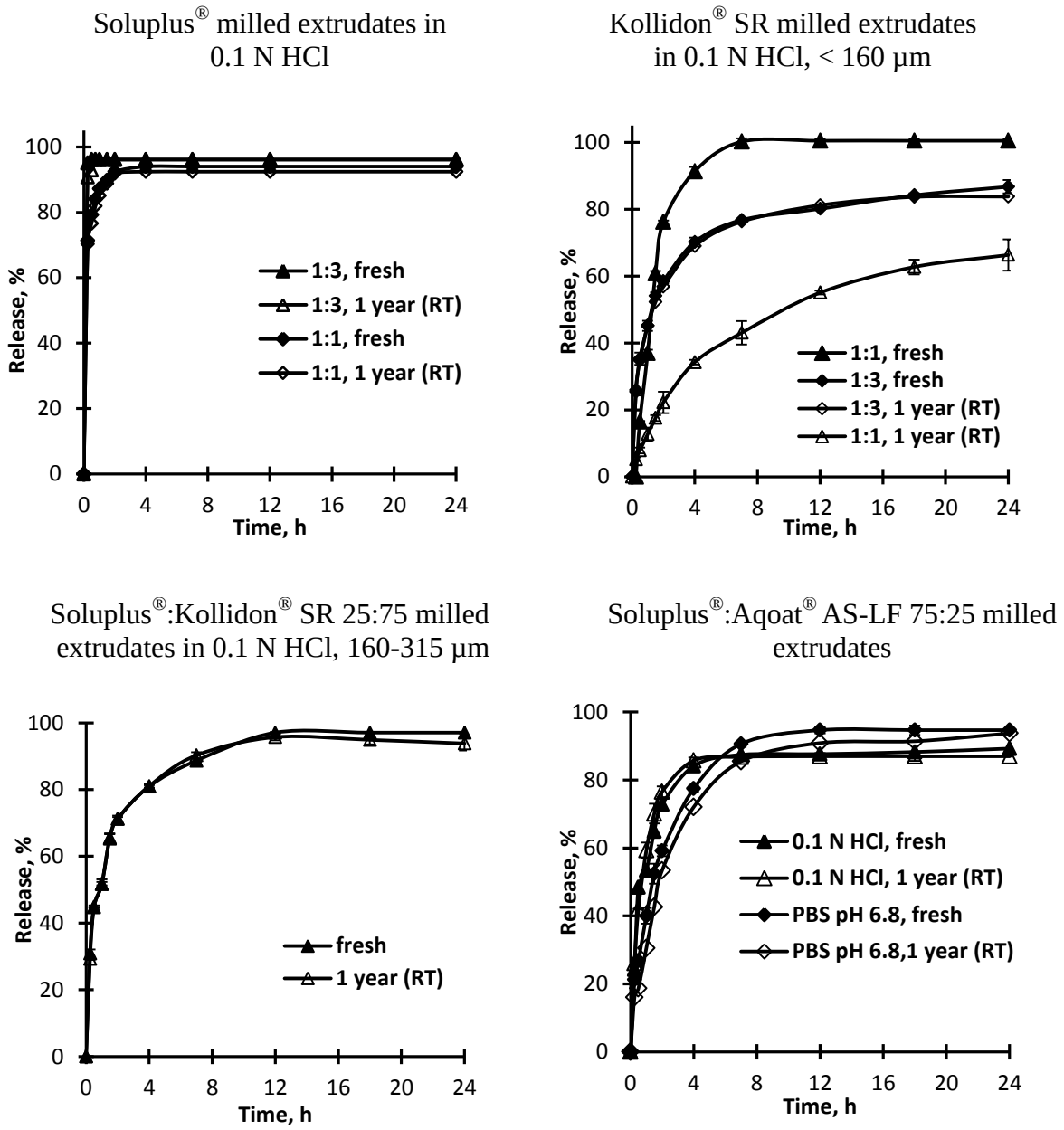


Fig. 77. Stability of itraconazole release upon storage at room temperature from its milled extrudates formulated into fast disintegrating tablets

Furthermore, different ratios of itraconazole:Soluplus® milled extrudates (1:3, 1:2, 1:1) were stored at accelerated conditions (40 ˚C, 75% RH). Itraconazole recrystallized after certain time storage at accelerated storage conditions observed by DSC. Moreover, the recrystallization occurred after 12, 9, 4 weeks for itraconazole:Soluplus® milled extrudates (1:3, 1:2, 1:1), respectively (**Fig. 78**). On the other hand, the rate and extent of itraconazole release was decreased from all milled extrudates ratios (**Fig. 79**). The recrystallization can be attributed more to the high humidity effect rather than the high temperature as the samples were stored at temperatures below their plasticized T_gs and therefore the molecular mobility

expected to be low. However, upon exposure to moisture, the mixtures were plasticized, and therefore the molecular mobility was maybe increased and consequently recrystallization occurred (Vasanthavada et al., 2005; Huang and Dai, 2014).

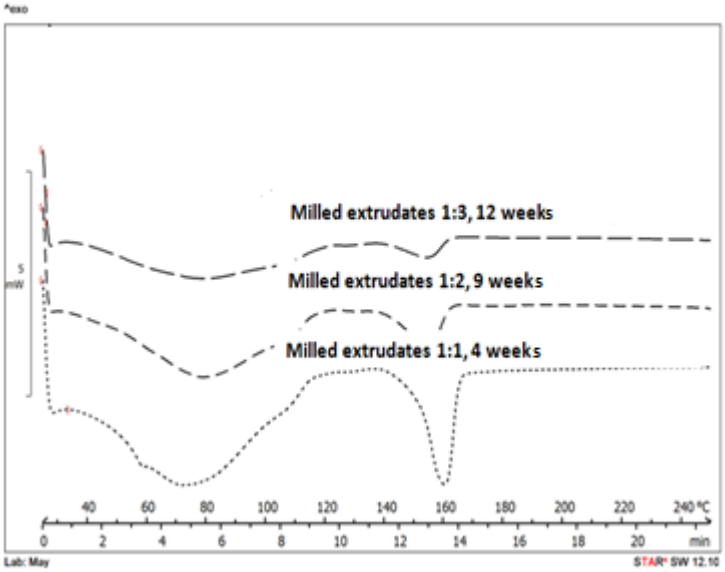


Fig. 78. DSC thermograms of different itraconazole:Soluplus® milled extrudates ratios after storage at accelerated condition (40 ˚C, 75% RH)

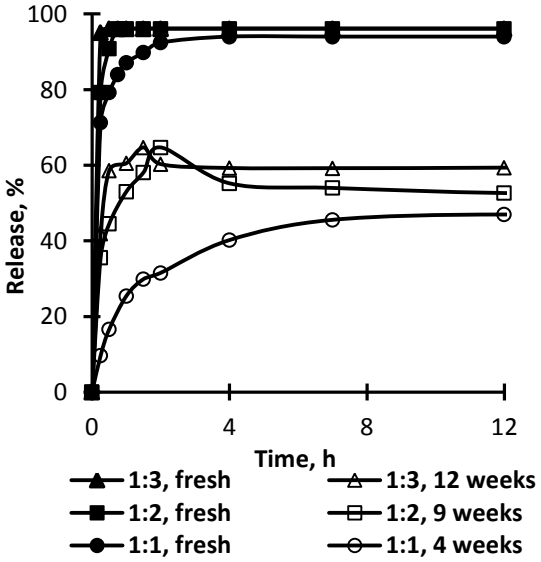


Fig. 79. Stability of itraconazole release upon storage at accelerated conditions (40 ˚C, 75% RH) from its milled extrudates containing different itraconazole:Soluplus® ratios

3.2.2 Hot-melt extrusion of an acidic poorly water-soluble drug (mefenamic acid)

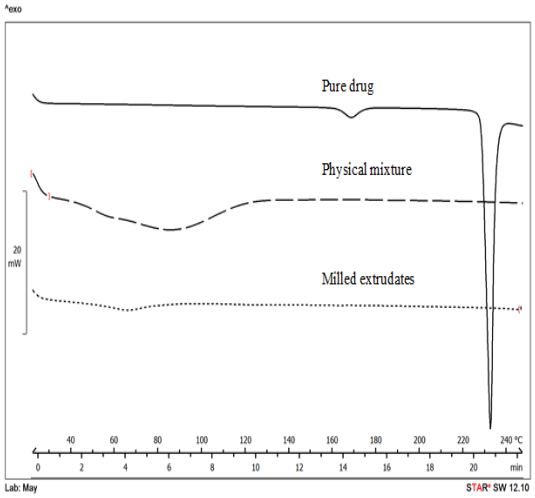
3.2.2.1 Solid-state characterization

Mefenamic acid has two polymorphic forms (Panchagnula et al., 2004), and therefore its DSC thermogram contains two endothermic peaks, the first endothermic peak (at 170.7 °C) corresponds to its transition from the polymorphic form I to the polymorphic form II and the second endothermic peak (at 229.5 °C) corresponds to the melting point of polymorphic form II. No melting peaks were observed by DSC from mefenamic acid physical mixture and milled extrudates with different polymers. Probably, the drug was dissolved in the molten polymer/s before reaching its melting point; therefore, DSC could not be used for the clarification of physical state of mefenamic acid in extrudates (**Fig. 80**).

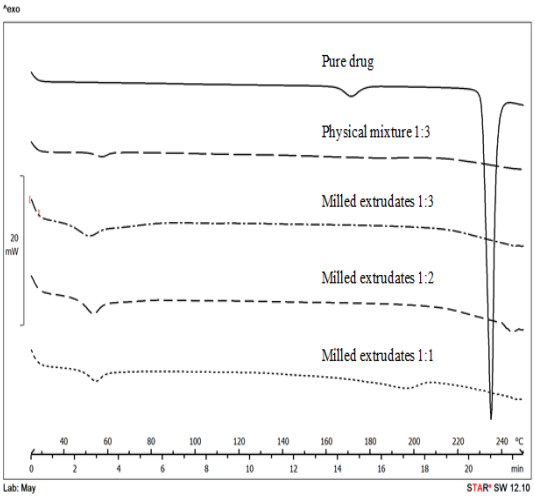
Mefenamic acid as a pure drug has numerous diffraction peaks indicating its crystalline nature, the diffraction peaks of mefenamic acid were also observed (in lower intensity) from its physical mixture with all polymers. On the other hand its milled extrudates with up to 33% drug loading did not show any crystallinity (**Fig. 81**).

According to the results obtained by X-ray diffractometery, amorphous solid dispersions of mefenamic acid with up to 33% drug loading could have been obtained by hot-melt extrusion.

Soluplus®



Eudragit® EPO



Soluplus®: Eudragit® EPO 50:50

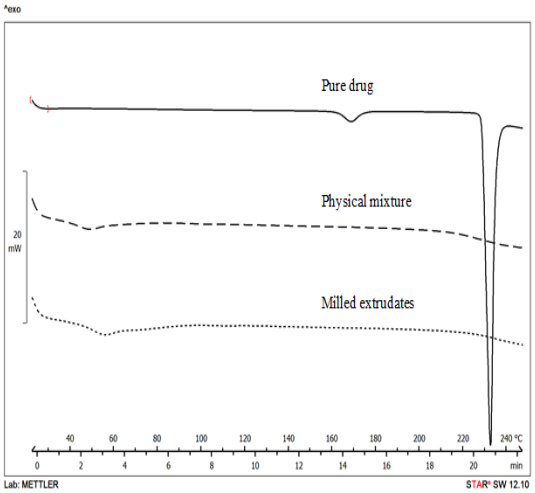
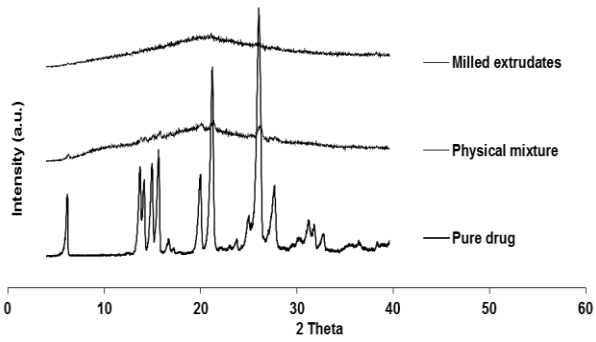
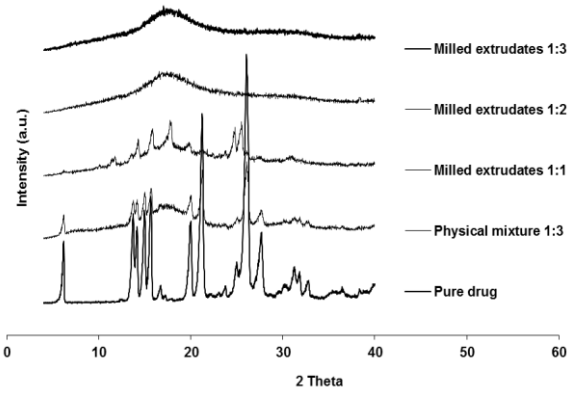


Fig. 80. DSC thermograms of mefenamic acid, its physical mixtures and milled extrudates 1:3 (unless otherwise noted) with different carriers

Soluplus®



Eudragit® EPO



Soluplus®: Eudragit® EPO 50:50

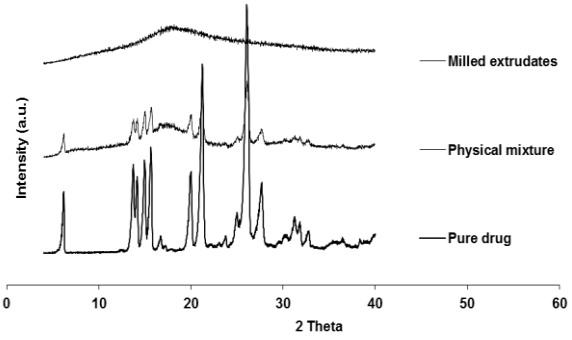


Fig. 81. X-ray diffractograms of mefenamic acid, its physical mixtures and milled extrudates 1:3 (unless otherwise noted) with different carriers

3.2.2.2 Dissolution studies

3.2.2.2.1 Effect of pH on drug release

As shown before, solubility and dissolution rate enhancement of basic poorly soluble drugs in various dissolution mediums were obtained by hot-melt extrusion and were affected by the ionic properties of the extruded polymers.

In this study mefenamic acid as a weakly acidic drug was extruded either with Soluplus® as a neutral polymer, with Eudragit® EPO as a cationic polymer or with Soluplus®: Eudragit® EPO polymer combination in order to investigate the ability of milled extrudates containing different polymers properties to improve the mefenamic acid release at various pHs.

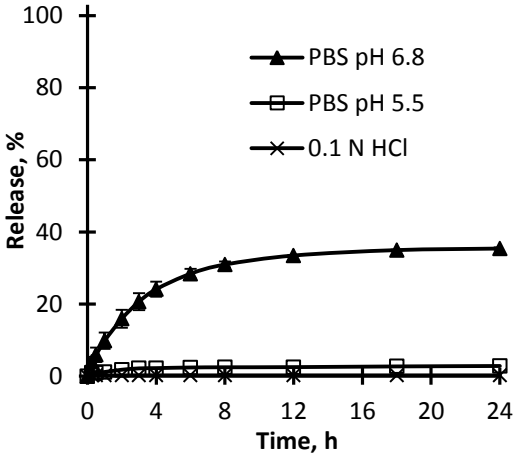
The release of mefenamic acid as a pure drug was very slow and incomplete within 24 h due to its very low solubility. Furthermore, slower release was obtained in lower pH due to pH-dependent solubility of mefenamic acid (**Fig. 82 A**).

The release of mefenamic acid from Soluplus® milled extrudates in different pHs was variable and depended on the weakly acidic nature of mefenamic acid, whereby significantly improved and complete drug release within 15 min was obtained in PBS pH 6.8, improved but still incomplete drug release was obtained in PBS pH 5.5 and no release was obtained in 0.1 N HCl (**Fig. 82 B**).

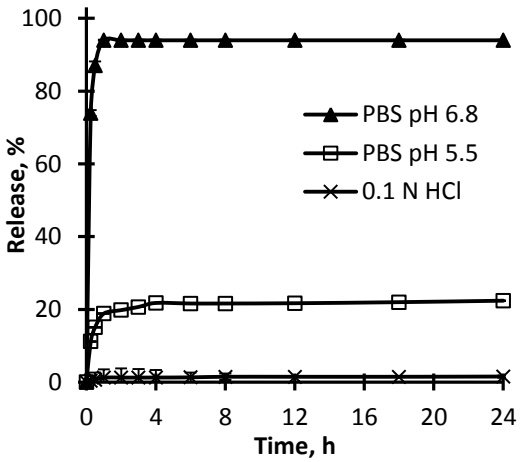
In case of Eudragit® EPO milled extrudates the release of mefenamic acid in different pHs was depended on both the weakly acidic nature of mefenamic acid and the cationic nature of Eudragit® EPO, whereby no release improvement was obtained in PBS pH 6.8, significantly improved and complete drug release within 15 min was obtained in PBS pH 5.5, and improved drug release was obtained in 0.1 N HCl. However, the rate and extent of drug release in 0.1 N HCl was much lower compare to PBS pH 5.5, due to the higher solubility of mefenamic acid in the higher pH where the polymer can still dissolve (**Fig. 82 C**).

Hot-melt extrusion using a combination of Soluplus® and Eudragit® EPO adjusted the release of mefenamic acid in both PBS pH 6.8 and PBS pH 5.5 mediums. However, the release in both release media was incomplete and leveled off at 50% (**Fig. 82 D**).

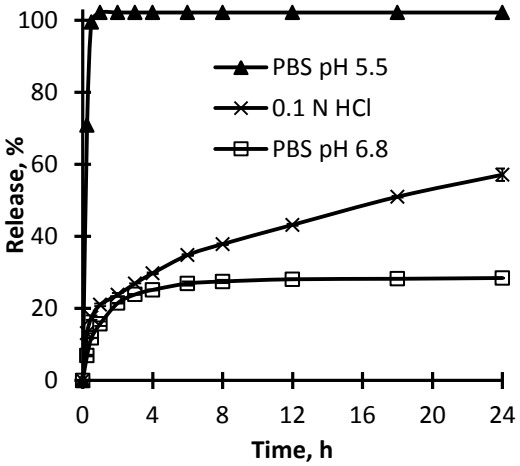
A) Pure mefenamic acid



B) Soluplus® milled extrudates



C) Eudragit® EPO milled extrudates



D) Soluplus®:Eudragit® EPO 50:50 milled extrudates

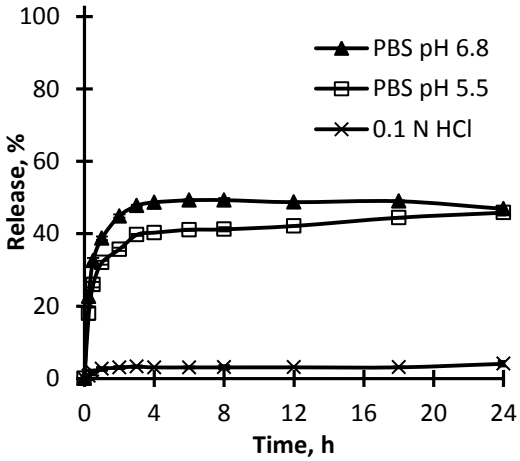


Fig. 82. Effect of pH on mefenamic acid release under non-sink conditions corresponding to 100 mg mefenamic acid from fast disintegrating tablets (mefenamic acid: polymer ratio 1:3)

3.2.2.2.2 Effect of solid state on drug release

The comparison for Soluplus® milled extrudates was performed in PBS pH 6.8, whereby the dissolution of pure mefenamic acid was very slow and incomplete within 24 h due to its poor solubility, almost no release improvement was obtained from physical mixtures compared to the pure drug and significantly improved drug release was obtained from milled extrudates compared to physical mixture and pure drug which can be mainly attributed to the amorphous transformation of mefenamic acid by hot melt extrusion. Furthermore, the release was fast and complete within 30 min, and the supersaturated solution was stable within 24h (**Fig. 83 A**).

In case of Eudragit® EPO milled extrudates the comparison was performed in PBS pH 5.5. Almost no drug release was obtained from pure mefenamic acid, a slight release improvement was obtained from physical mixture compared to the pure drug and significantly improved drug release was obtained from milled extrudates compared to physical mixture and pure drug due to the amorphous transformation of mefenamic acid by hot melt extrusion. Furthermore, the release was rapid and complete within 15 min, and the supersaturated solution was clear and stable within 24h (**Fig. 83 B**).

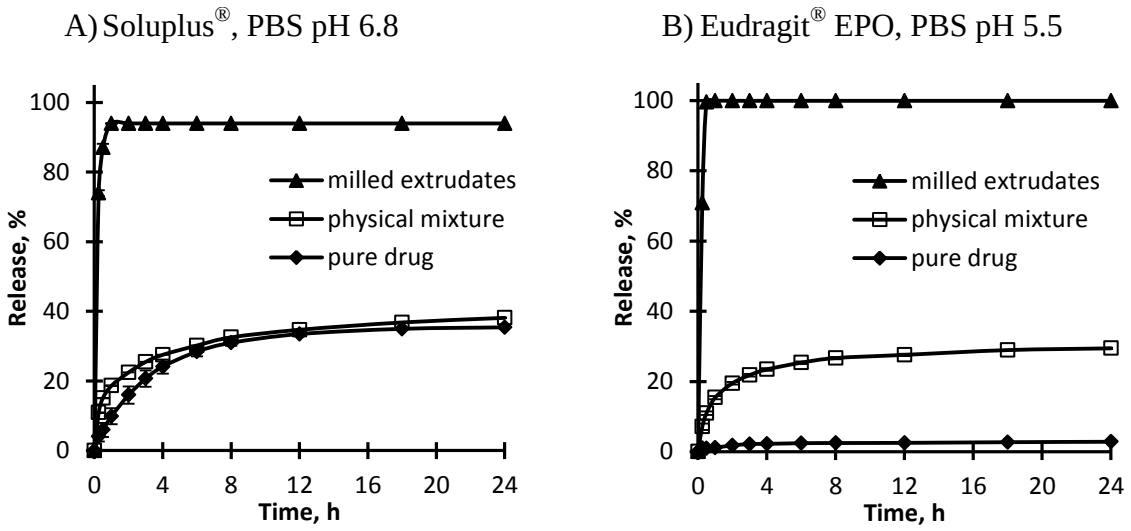


Fig. 83. Comparison of dissolution profiles under non-sink conditions (corresponding to 100 mg drug) between mefenamic acid as a pure drug, its physical mixture and milled extrudates formulated into fast disintegrating tablets

3.2.2.2.3 Effect of dose on drug release

In order to evaluate the ability of hot-melt extrudates with different carriers to generate and maintain different degrees of supersaturated solutions, the release of mefenamic acid from different doses was studied.

In case of Soluplus® milled extrudates, incomplete drug release in PBS pH 6.8 was obtained by increasing the dose to 200 mg and 500 mg. Moreover, 250 mg (50 % of the dose 500 mg) was the maximum drug release achieved from Soluplus® milled extrudates indicating its solubility (**Fig. 84 A**).

In case of Eudragit® EPO milled extrudates, increasing the dose up to 500 mg resulted in slightly slower but still complete drug release in PBS pH 5.5 (**Fig. 84 B**). Thus, Eudragit® EPO milled extrudates exhibited a greater degree of supersaturation compared to Soluplus® milled extrudates in their favorable dissolution media. This could be attributed to stronger ionic interactions between mefenamic acid and Eudragit® EPO that could have been occurred during hot melt extrusion. Moreover, the supersaturated solutions obtained from both Soluplus® and Eudragit® EPO milled extrudates were stable within 24 h.

A) Soluplus® milled extrudates, PBS pH 6.8

B) Eudragit® EPO milled extrudates, PBS pH 5.5

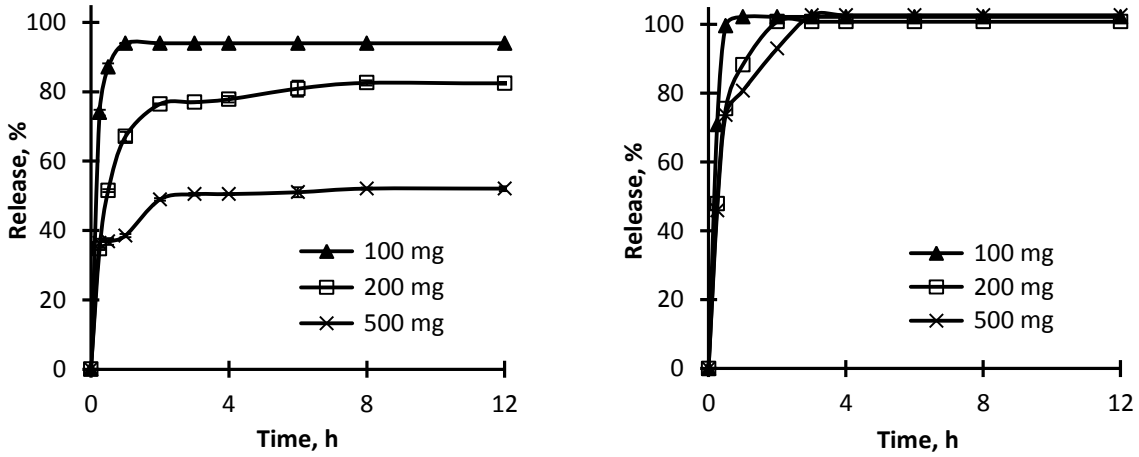


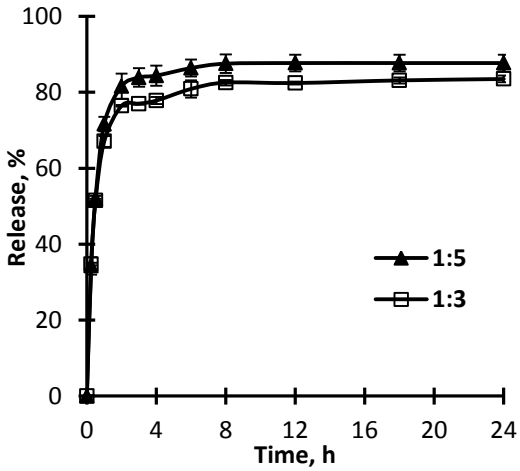
Fig. 84. Effect of dose on mefenamic acid release from milled extrudates formulated into fast disintegrating tablets (mefenamic acid: polymer ratio 1:3)

3.2.2.2.4 Effect of drug:polymer ratio on drug release

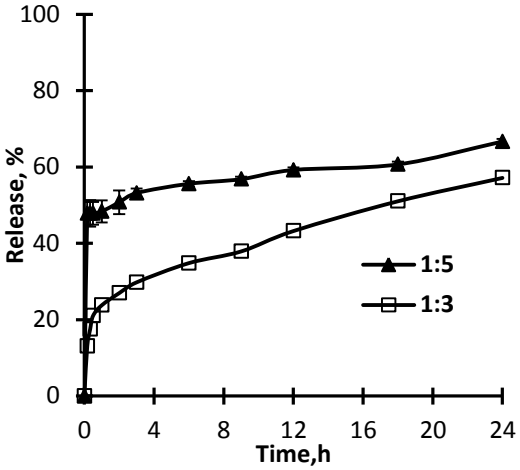
In case of Soluplus® milled extrudates, almost no improvement of drug release in PBS pH 6.8 was obtained by increasing the polymer ratio (mefenamic acid:Soluplus® 1:5) (**Fig. 85 A**). This can be attributed to the formation of molecular dispersion even with lower polymer ratio (mefenamic acid:Soluplus® 1:3) and therefore the supersaturation solubility limit is reached.

In case of Eudragit® EPO milled extrudates, the rate and extent of drug release in 0.1 N HCl was improved by increasing polymer ratio (mefenamic acid:Eudragit® EPO 1:5) (**Fig. 85 B**). This is due to better dispersion of mefenamic acid in higher amount of Eudragit® EPO carrier. Furthermore, less polymer ratio (mefenamic acid:Eudragit® EPO 1:1) resulted in slow and incomplete drug release in PBS pH 5.5 as the drug remained partially in the crystalline form which was confirmed by X-ray, and the supersaturated solutions generated from extrudates containing low polymer ratio (mefenamic acid:Eudragit® EPO 1:2 and 1:1) were not stable as the drug tends to precipitate as effect of time (**Fig. 85 C**).

A) Soluplus® milled extrudates
(200 mg drug, PBS pH 6.8)



B) Eudragit® EPO milled extrudates
(100 mg drug, 0.1 N HCl)



C) Eudragit® EPO milled extrudates
(100 mg drug, PBS pH 5.5)

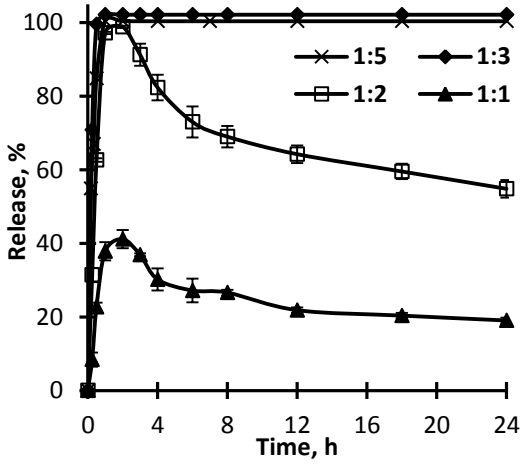


Fig. 85. Effect of polymer ratio on mefenamic acid release from different milled extrudates formulated into fast disintegrating tablets

3.2.2.2.5 Effect of long term storage on drug release

As illustrated before, DSC could not be used for clarification of the physical state of mefenamic acid in extrudates. Furthermore, the sensitivity of X-ray is low (< 5 % present crystals could not be recognized). Therefore, using these methods to study the effect of storage condition on physical stability of mefenamic acid milled extrudates is not useful. Therefore the stability of mefenamic acid release upon storage at room temperature was studied.

A slight decrease in rate and extent of drug release was obtained from Soluplus® milled extrudates after 1 year storage at room temperature. On the other hand the drug release profile of mefenamic acid was not affected by long term storage at room temperature from Eudragit® EPO milled extrudates (**Fig. 86**). These results indicate better stability of the amorphous state of mefenamic acid in its solid dispersion with Eudragit® EPO compared to Soluplus®.

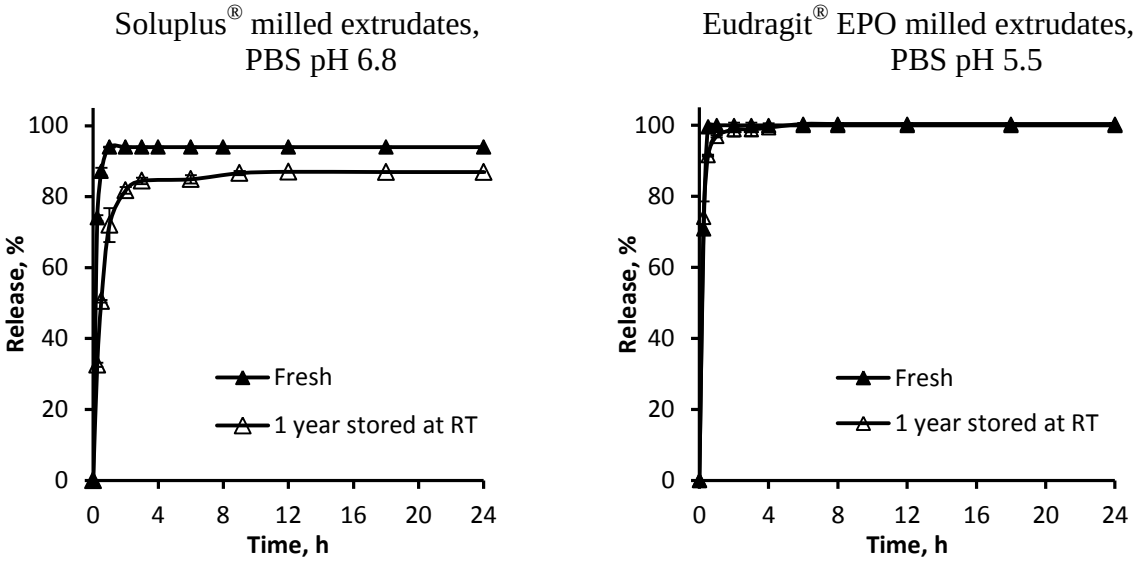


Fig. 86. Stability of mefenamic acid release upon 1 year storage at room temperature from its milled extrudates formulated into fast disintegrating tablets (mefenamic acid: polymer ratio 1:3)

Chapter 4. Summary

4. SUMMARY

New drug entities with poor aqueous solubility are becoming more prevalent as result of high-throughput screening in drug discovery. Poor aqueous solubility presents significant challenges, as it reduces the oral absorption and bioavailability. Several formulation approaches have been employed to overcome the limitations of low dissolution rate and/or solubility including; pH-adjustment, co-solvents, surfactants, inclusion complexes, lipid-based formulations, nanosuspensions and solid solutions/dispersions. In this study a decision table for the selection of formulation approach based on the drug properties and the required specifications of the final dosage form was proposed. Among these formulation approaches nanosuspension and solid solution/dispersion were selected to further distinguish between these methods with the aim of improving solubility/bioavailability of the poorly water-soluble drugs; itraconazole and mefenamic acid.

Nanosuspension formulation approach relies on reducing the particle size to nanometer range (nanosizing) to improve the equilibrium solubility. In this study, particle size reduction from micrometer to nanometer range was successfully obtained by wet milling technique. The average particle size of all nanosuspension formulations was below 600 nm with 99% of the particles being less than 3.5 μm. All nanosuspension formulations showed sufficient zeta potential values, higher than -48.2 mV in case of electrostatic stabilizer (SLS) and higher than -20 mV in case of steric or electrostatic/steric stabilizers. The best particle size reduction was achieved using Methocel® E5 and SLS as stabilizers for both mefenamic acid and itraconazole nanosuspensions, respectively. The mean particle sizes along with particle size distribution were continuously decreased as effect of milling time. Longer milling time was required to reduce the particle size of suspensions stabilized by higher viscosity grades of Methocel® compared to the lower viscosity grades. On the other hand, shorter milling time was required to reduce the particle size of suspension with higher drug loading compared to the lower one. All nanosuspension formulations, except nanosuspension stabilized by Tween® 80, were stable during at least 3 months storage at room temperature. No changes of the drugs crystallinity were observed following wet milling process. Significantly improved drug release under sink conditions was obtained from all nanosuspension formulations. The drug release was fast and complete within few minutes irrespective of the particle size of nanosuspensions. Furthermore, the solubility was improved from nanosuspensions compared to suspensions. The increase in solubility was up to 1.5 and 2.3 times from both mefenamic acid and itraconazole nanosuspensions, respectively.

Solid dispersion formulation approach improves dissolution rate and/or solubility by decreasing particle size, improving wettability, reducing agglomeration, changing physical state of the drug and possibly dispersion on a molecular level. In this study, amorphous solid dispersions with up to 50% drug loading were obtained by hot-melt extrusion using different polymeric carriers. The milled extrudates of itraconazole with Soluplus®, Aqoat® AS-LF or Soluplus®:Aqoat® AS-LF showed single glass transition temperatures by differential scanning calorimetry and intermolecular interactions by fourier transforms infra-red, suggesting a complete miscibility and amorphous molecular dispersions were obtained.

The solubility was significantly improved from milled extrudates (e.g. 40-80 times from Soluplus® milled extrudates) due to amorphous transformation or molecular dispersion of drug in the carriers achieved by hot-melt extrusion. Significant release improvement was obtained from all milled extrudates compared to physical mixtures and pure drugs. Lower extent of drug release was obtained when the processing temperature was much lower than the drug melting point.

In case of Soluplus® extrudates containing a basic poorly water-soluble drug, the drug release was improved by increasing polymer ratio due to better dispersion of drug in higher amount of carrier. The carrier controlled the release from the molecularly dispersed drug in the amphiphilic co-polymer Soluplus®, therefore, the particle size of extrudates had no influence on drug release. Furthermore, doubling the dose resulted in slightly slower but still complete drug release, the supersaturated solutions were stable for more than 24 h and were not affected by changing the medium-pH from 1 to 6.8, these results suggest to formulate Soluplus® milled extrudates in extended release dosage forms as a promising approach for extending the release of poorly water-soluble drugs.

In case of Kollidon® SR and Kollidon® SR:Soluplus® extrudates containing a basic poorly water-soluble drug, extended and complete drug release in 0.1 N HCl was achieved by a single formulation step and was controlled by the polymer ratio, the polymer combination ratio and the particle size of extrudates. No drug precipitation occurred within 24 h. Thus, solubilization, retardation, and stable supersaturated solutions were achieved by formulating Kollidon® SR or Soluplus®:Kollidon® SR milled extrudates in fast disintegrating tablets.

Extended and complete drug release in 0.1 N HCl was also achieved by formulating Soluplus® milled extrudates in hydrophilic matrix tablets whereby the release was controlled by the type of matrix former and its percentage in the tablets. However, slow and incomplete drug release in PBS pH 6.8 was obtained from all previous extended release formulations. This is due to pH-dependent solubility of the drug, even after the significant solubility and

release improvement obtained by the formation of amorphous solid dispersions. A slight release improvement in PBS pH 6.8 was achieved using enteric polymers as additive for the erodible matrix tablets containing Soluplus® milled extrudates but the release was still too slow for the extended release purposes.

Hot-melt extrusion using combinations of Soluplus®:enteric polymer adjusted the release of basic poorly water-soluble drugs in both PBS pH 6.8 and 0.1 N HCl media, whereby the release was affected by the ratio of polymers combination. Furthermore, extended and complete release profile of basic poorly soluble drugs was achieved by formulating Soluplus®:enteric polymers milled extrudates in erodible matrix tablets where the release was also not affected by pH-changing.

In case of hot-melt extrusion with acidic poorly water-soluble drugs, the solubility and dissolution rate enhancement in various dissolution mediums were also affected by the ionic properties of the polymeric carriers, whereby Soluplus®:cationic polymer could be considered as a promising polymer combination to improve solubility and dissolution rate and to achieve pH-independent release profile of acidic poorly water-soluble drug by hot-melt extrusion.

Differential scanning calorimetry and X-ray diffractometery data demonstrated essential stability of mostly all amorphous solid dispersions upon storage at room temperature indicated as well by unchanged release profile. However, drug recrystallization occurred after a certain storage time at accelerated conditions (40 ºC, 75 % RH) which resulted in a slower and incomplete drug release.

As conclusion, both nanosuspensions and amorphous solid dispersions could potentially lead to solubility/dissolution rate enhancement of poorly water-soluble drugs. However, amorphous solid dispersions resulted in a much higher solubility improvement compared to nanosuspensions. In addition, amorphous solid dispersions were able to improve the solubility and dissolution rate of ionic poorly-water soluble drugs in the unfavorable pH conditions by using the polymers counter-ionic to these drugs. Furthermore, developing a controlled release formulation for a poorly-water soluble drug using amorphous solid dispersion can be easily done by a careful selection of the polymeric carrier properties (e.g. water insoluble polymer). Therefore, amorphous solid dispersions were the superior option for solubility/dissolution rate enhancement of ionic poorly water-soluble drugs having a very short half-life.

Kapitel 5. Zusammenfassung

5. ZUSAMMENFASSUNG

Neue Wirkstoffe mit geringer Wasserlöslichkeit werden aufgrund des Hochdurchsatz-Screenings in der Wirkstoffentwicklung immer häufiger. Durch die schlechte Wasserlöslichkeit ergeben sich jedoch erhebliche Herausforderungen, wie die verminderte orale Resorption und Bioverfügbarkeit. Um die Probleme der geringen Löslichkeit und der langsamen Auflösungsgeschwindigkeit zu überwinden, wurden verschiedene Formulierungsmethoden entwickelt. Als Beispiele sind hier Anpassung des pH-Wertes, Co-Lösemittel, Tenside, Einschlusskomplexe, lipidbasierte Formulierungen, Nanosuspensionen und feste Lösungen bzw. Dispersionen zu nennen. In dieser Studie wurde eine Entscheidungstabelle erstellt für die Auswahl einer geeigneten Formulierungsmethode basierend auf den Wirkstoffeigenschaften und den Anforderungen an die Darreichungsform. Von diesen Formulierungsmethoden wurden Nanosuspensionen und feste Lösungen/Dispersionen ausgewählt um diese weiter zu charakterisieren, mit dem Ziel die Löslichkeit und Bioverfügbarkeit der schlecht löslichen Wirkstoffe Itraconazol und Mefenaminsäure zu verbessern.

Bei Nanosuspensionen wird durch die Reduktion der Partikelgröße in den Nanometer-Bereich die Sättigungslöslichkeit erhöht. In dieser Studie wurde die Verkleinerung der Partikel aus dem Mikrometer- in den Nanometerbereich durch ein Nassmahlverfahren erzielt. Die durchschnittliche Partikelgröße von allen Nanosuspensions-Formulierungen war unter 600 nm, wobei 99% der Teilchen kleiner waren als 3,5 µm. Alle Formulierungen wiesen ein ausreichendes Zeta-Potential auf. Mit SLS als elektrostatischem Stabilisator lagen die Werte über -48,2 mV, bei sterischer oder elektrostatisch/sterischer Stabilisierung über -20 mV. Die beste Reduktion der Partikelgröße wurde mit Methocel® E5 und SLS als Stabilisatoren erzielt. Dies gilt sowohl für Mefenaminsäure- als auch für Itraconazol-Nanosuspensionen. Die mittlere Partikelgröße, sowie die Partikelgrößenverteilung wurden durch längere Mahlzeiten kontinuierlich verringert. Längere Mahlzeiten waren erforderlich für Suspensionen, die mit Methocel®-Typen höherer Viskosität stabilisiert wurden, im Vergleich zu denen mit einem Methocel®-Typen mit geringerer Viskosität. Andererseits konnte die benötigte Mahlzeit zur Verringerung der Partikelgröße verkürzt werden, wenn die Wirkstoffkonzentration angehoben wurde. Alle Nanosuspensionen, ausgenommen derer, die mit Tween® 80 stabilisiert wurden, waren während der Lagerung bei Raumtemperatur für mindestens drei Monate stabil. Nach der Nassmahlung wurde keine Veränderung der Kristallinität der Wirkstoffe festgestellt. Die Wirkstofffreisetzung unter Sink-Bedingungen war bei allen Nanosuspensionsformulierungen signifikant verbessert.

Die Wirkstoffe wurden schnell und vollständig innerhalb weniger Minuten freigesetzt, unabhängig von der Partikelgröße der Nanosuspensionen. Des Weiteren war die Löslichkeit der Nanosuspensionen im Vergleich zu den Suspensionen erhöht. Die Löslichkeit der Nanosuspensionen von Mefenaminsäure war bis zu 1,5-mal und die von Itraconazol bis zu 2,3-mal höher.

Bei festen Dispersionen ist die Löslichkeit und die Lösungsgeschwindigkeit erhöht durch Partikelgrößenverringerung, verbesserte Benetzbarkeit, Verhinderung von Agglomeration, Modifizierung des physikalischen Zustandes des Wirkstoffes und potenziell auch durch eine Dispergierung auf molekularer Ebene. In dieser Studie wurden amorphe feste Dispersionen mit bis zu 50% Wirkstoffbeladung mittels Schmelzextrusion mit verschiedenen Polymeren als Trägern hergestellt. Die gemahlenen Extrudate, die Itraconazol mit Soluplus®, Aqoat® AS-LF oder Soluplus®:Aqoat® AS-LF enthielten, zeigten beim DSC eine einzige Glasübergangs-Temperatur und im FTIR-Spektrum intermolekulare Interaktionen. Dies lässt auf eine vollständige Mischbarkeit schließen und deutet darauf hin, dass molekulare Dispersionen erzielt wurden.

Die Löslichkeit von gemahlenen Extrudaten war signifikant verbessert (z.B. 40 – 80-mal bei gemahlenen Soluplus®-Extrudaten), da die Wirkstoffe durch die Schmelzextrusion amorph bzw. molekulardispers im Träger vorlagen.

Eine signifikante Verbesserung der Freisetzung wurde bei allen gemahlenen Extrudaten im Vergleich zu den physikalischen Mischungen und den reinen Wirkstoffen erreicht. Eine geringere Wirkstofffreisetzung war zu verzeichnen, wenn die Prozesstemperatur deutlich unter dem Schmelzpunkt des Wirkstoffes lag.

Bei Soluplus®-Extrudaten mit einem schwer löslichen, basischen Wirkstoff konnte die Freisetzung verbessert werden, wenn der Anteil des Polymers erhöht wurde. Dies liegt an der daraus resultierenden besseren Dispersion des Wirkstoffes im Träger. Der Träger steuerte die Freisetzung des molekular dispergierten Wirkstoffes im amphiphilen Co-Polymer Soluplus®. Daher war kein Einfluss der Teilchengröße des Extrudates auf die Wirkstofffreisetzung zu beobachten. Die Verdopplung der Wirkstoffdosis führte zu einer etwas langsameren, aber immer noch vollständigen Wirkstofffreisetzung. Die übersättigten Lösungen waren für mehr als 24 Stunden stabil und auch die Änderung des pH-Wertes des Mediums hatte keinen Einfluss. Diese Ergebnisse machen gemahlene Schmelzextrudate mit Soluplus® zu einer vielversprechenden Formulierungsmethode um für schwerlösliche Wirkstoffe Arzneiformen mit retardierter Freisetzung herzustellen.

Bei Kollidon® SR und Kollidon® SR:Soluplus®-Extrudaten mit schlecht löslichen, basischen Wirkstoffen konnte eine verlängerte und komplette Wirkstofffreisetzung in 0,1 N HCL mit nur einem Herstellungsschritt erzielt werden. Die Freisetzung wurde durch den Polymeranteil, das Verhältnis der beiden Polymere und die Partikelgröße des Extrudates gesteuert. Innerhalb von 24 Stunden trat kein Abfall der Wirkstoffauflösung auf. Solubilisierung, Retardierung und stabile übersättigte Lösungen konnten durch die Herstellung von schnell zerfallenden Tabletten aus gemahlenen Kollidon® SR- oder Soluplus®:Kollidon® SR-Extrudaten erreicht werden.

Eine retardierte und vollständige Wirkstofffreisetzung in 0,1 N HCl konnte auch bei hydrophilen Matrixtabletten erzielt werden, die gemahlene Soluplus®-Extrudate enthielten. Die Freisetzung wurde dabei von der Art und dem Anteil des Matrixbildners kontrolliert. Allerdings war die Freisetzung von allen bisherigen retardierten Formulierungen in Phosphatpuffer pH 6.8 langsam und unvollständig. Dies liegt an der pH-abhängigen Löslichkeit des Wirkstoffes, die selbst nach der signifikanten Verbesserung der Löslichkeit und Freisetzung durch die Bildung von amorphen festen Dispersionen erhalten bleibt. Eine geringe Verbesserung der Freisetzung in Phosphatpuffer pH 6,8 konnte beobachtet werden durch den Zusatz von magensaftresistenten Polymeren in den erodierenden Matrixtabletten, welche gemahlene Soluplus®-Extrudate enthielten. Jedoch war auch hier die Freisetzung für eine Retardformulierung zu langsam.

Durch die Verwendung von Mischungen aus Soluplus® und magensaftresistenten Polymeren bei der Schmelzextrusion konnte die Freisetzung von schwerlöslichen, basischen Wirkstoffen sowohl in Phosphatpuffer pH 6,8 als auch in 0,1 N HCl angepasst werden, wobei die anteilige Zusammensetzung der Polymere die Freisetzung bestimmte. Des Weiteren konnten retardierte und vollständige Freisetzungsprofile von schwerlöslichen, basischen Wirkstoffen erzielt werden, wenn erodierende Matrixtabletten aus den gemahlenen Extrudaten mit Soluplus® und magensaftresistenten Polymeren hergestellt wurden. Die Freisetzung war von der pH-Wert Veränderung des Mediums nicht beeinflusst.

Bei der Schmelzextrusion mit schwach sauren Wirkstoffen war die Steigerung der Löslichkeit und Lösungsgeschwindigkeit in verschiedenen Freisetzungsmedien auch von den ionischen Eigenschaften der polymerischen Träger abhängig. Die Mischung aus Soluplus® und einem kationischen Polymer kann für schwach saure Wirkstoffe als vielversprechende Formulierung betrachtet werden um mithilfe der Schmelzextrusion eine Verbesserung der Löslichkeit und Lösungsgeschwindigkeit sowie pH-unabhängige Freisetzungsprofile zu erreichen.

DSC und Röntgendiffraktometrie zeigten, dass fast alle amorphen festen Dispersionen während der Lagerung bei Raumtemperatur stabil waren. Dies wurde auch durch unveränderte Freisetzungsprofile bestätigt. Jedoch konnte eine Rekristallisierung des Wirkstoffs nach bestimmten Lagerzeiten unter verschärften Bedingungen (40°C, 75 % relative Luftfeuchte) festgestellt werden. Dies drückte sich in einer langsameren und unvollständigen Wirkstofffreisetzung aus.

Zusammenfassend kann gesagt werden, dass sowohl Nanosuspensionen als auch amorphe feste Dispersionen die Löslichkeit und Lösungsgeschwindigkeit von schwer löslichen Wirkstoffen potentiell erhöhen können. Allerdings wiesen amorphe feste Dispersionen eine erheblich höhere Löslichkeitsverbesserung auf als Nanosuspensionen. Zusätzlich konnten amorphe feste Dispersionen die Löslichkeit und Lösungsgeschwindigkeit von schwerlöslichen, ionischen Wirkstoffen bei ungünstigen pH-Werten verbessern, wenn ein Polymer mit entgegengesetzter Ladung verwendet wurde. Darüber hinaus können amorphe feste Dispersionen verwendet werden, um für schlecht wasserlösliche Wirkstoffe Formulierungen mit kontrollierter Freisetzung zu entwickeln durch eine sorgfältige Auswahl der Eigenschaften des polymeren Trägers (z.B. wasserunlösliche Polymere). Daher waren amorphe feste Dispersionen die überlegene Methode um die Löslichkeit und Lösungsgeschwindigkeit von schwerlöslichen, ionischen Wirkstoffen mit sehr kurzer Halbwertszeit zu verbessern.

Chapter 6. References

6. REFERENCES

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Chapter 7. Publications and presentations

7. PUBLICATIONS AND PRESENTATIONS

Research publications:

- Darwich, M. and Bodmeier R. A comparison study between nanosuspension and amorphous solid dispersion technologies based on improving the solubility/dissolution rate of poorly soluble drugs. (In preparation)
- Darwich, M.; Dashevskiy, A.; Kolter, K. and Bodmeier R. Extended release formulations for poorly soluble drugs prepared by hot-melt extrusion with Kollidon® SR or Kollidon® SR:Soluplus® combinations. (In preparation)
- Darwich, M.; Dashevskiy, A.; Kolter, K. and Bodmeier R. Improving solubility of basic poorly soluble drug by hot-melt extrusion with Soluplus® or Soluplus®:Aqoat® AS-LF combinations and formulation of extended release matrix tablets thereof. (In preparation)
- Darwich, M.; Dashevskiy, A.; Kolter, K. and Bodmeier R. Hot-melt extrusion of pH-dependent poorly soluble drugs; Effect of polymer selection on improving solubility and dissolution rate in various $pH_{S.}$ (In preparation)

Poster presentations:

- Darwich, M.; Dashevskiy, A.; Kolter, K. and Bodmeier R. Hot melt extrudates of poorly soluble drugs and formulation of extended release tablets thereof. PBP world meeting, April 2014, Lisbon, Portugal, poster 126
- Darwich, M.; Dashevskiy, A.; Kolter, K. and Bodmeier R. Extended release formulations of poorly soluble drugs prepared by hot melt extrusion based on Soluplus® and retarding excipients. AAPS Annual Meeting and Exposition, July 2014, Chicago, USA, poster W5130
- Darwich, M.; Dashevskiy, A. and Bodmeier R. Improving solubility and dissolution rate of mefenamic acid using hot-melt extrusion with Eudragit® EPO. AAPS Annual Meeting and Exposition, October 2015, Orlando, USA, poster W4213
- Darwich, M.; Dashevskiy, A. and Bodmeier R. Solubility/dissolution rate enhancement of mefenamic acid by preparation of nanosuspensions using media milling technique. AAPS Annual Meeting and Exposition, October 2015, Orlando, USA, poster T3091

Chapter 8. Curriculum vitae

8. CURRICULUM VITAE

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