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Production of stable bcs class ii drug suspensions by melt emulsification and subsequent incorporation into polymer strip films

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ABSTRACT

PRODUCTION OF STABLE BCS CLASS II DRUG SUSPENSIONS BY MELT EMULSIFICATION AND SUBSEQUENT INCORPORATION INTO POLYMER STRIP FILMS

by
Emanuel Joseph Vizzotti

One of the ways to improve the dissolution rate of poorly water-soluble drugs is to produce fine drug particles with increased surface area. This increase will lead to bioavailability enhancement. However, smaller drug particles are thermodynamically unstable and tend to aggregate or grow. Therefore, proper formulation and process parameters must be chosen to keep the particles small. In this study, fine drug particles of two model drugs, fenofibrate and ibuprofen, are prepared by the melt emulsification technique. The influence of suspension formulation (i.e., the inclusion of polymers and surface-active agents) on particle stability is investigated. Moreover, different agitation techniques during cooling are studied for their impact on the physical stability and recrystallization of molten drug particles. The use of both nonionic surfactant Pluronic F68 and high-intensity ultrasound during cooling produces fenofibrate and ibuprofen suspensions with enhanced short-term physical stability. The optimized fenofibrate suspension is blended with hydroxypropyl methylcellulose film solution and cast into a solid strip film by oven drying. The film is characterized by redispersion and by dissolution rate. Particles are recovered from the films in de-ionized water within 5 minutes with no size increase, and a marked increase in dissolution rate is observed in comparison to as-received particles. The feasibility of incorporating stable drug particles produced by melt emulsification into strip films and consequent enhancement of particle recovery and dissolution rate from these films are successfully demonstrated.

PRODUCTION OF STABLE BCS CLASS II DRUG SUSPENSIONS BY MELT EMULSIFICATION AND SUBSEQUENT INCORPORATION INTO POLYMER STRIP FILMS

by
Emanuel Joseph Vizzotti

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APPROVAL PAGE

PRODUCTION OF STABLE BCS CLASS II DRUG SUSPENSIONS BY MELT EMULSIFICATION AND SUBSEQUENT INCORPORATION INTO POLYMER STRIP FILMS

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I dedicate my thesis work to my family and many friends, with a boundless feeling of gratitude to my loving parents, John Vizzotti and Maria Russo, whose words of encouragement continually ring in my ears. To my sister, Marie, who kept me laughing the whole journey through.

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CHAPTER 1

INTRODUCTION

1.1 Objective

The objectives of this work were to understand the impacts of additive type/concentration and method of agitation during recrystallization on the short-term stability of fenofibrate and ibuprofen suspensions produced by the melt emulsification process. The aim was to then incorporate stable fenofibrate suspensions into the production of polymer strip films as an attractive solid dosage delivery system and characterize the redispersion and dissolution behaviors of these films.

1.2 Background

About 90% of the compounds in pharmaceutical delivery pipelines today exhibit poor water solubility (Page, 2008). This poses a difficulty to the administering of these drugs, since the human body is made up of 60% water. An active pharmaceutical ingredient (API) cannot reach its molecular target in the body if the drug remains undissolved in the gastrointestinal (GI) tract and is ultimately excreted. Simply put, drugs that do not dissolve cannot heal. In this way, solubilization technologies that overcome this issue have become increasingly important to the pharmaceutical industry by allowing for the preparation of effective and commercially viable drugs from actives that would otherwise be impractical due to solubility limitations. Most poorly water-soluble hydrophobic drugs belong to a class [Biopharmaceutics Classification System (BCS) Class II] characterized by limited solubility and dissolution rate, but high permeability through GI membranes (Amidon et al. 1995).

There are a number of methods that have been developed to improve drug solubility and/or dissolution rates. These include: (a) reduction of drug particle size to increase available surface area for dissolution (Bruno et al. 1996; Merisko-Liversidge et al. 2003; Bhakay et al. 2011), as per the Noyes-Whitney equation (1897); (b) manipulation of solid state of drug substance to improve drug dissolution i.e., by decreasing crystallinity of drug substance through formation of solid solutions/amorphous solids (Zhang et al. 2006; Kim et al. 2008; Shen et al. 2010); (c) solubilization in surfactant systems (Carvalho et al. 2010); (d) formation of water-soluble complexes (Jansook et al. 2010); and (e) use of pro-drug and drug derivatives, such as strong electrolyte salt forms that usually have higher rate of dissolution (Liu et al. 2006). The implications that come with using the two most popular of these methods (i.e., particle size reduction and formation of amorphous solids), including practical limitations, will be assessed in Chapter 4.

1.2.1 Stability of Drug Particle Suspensions

The common physical stability issues related to suspensions include sedimentation/creaming, aggregation, crystal growth and change of crystallinity state (Wu et al. 2011). During the storage of suspensions, particle motion exists due to Brownian motion, convection currents, and sedimentation. Drug particles can either settle down or cream up in a formulation medium, depending on their density relative to the medium. The sedimentation rate is described by Stokes' law (Kim, 2004), which describes the important roles of particle size, medium viscosity, and the density difference between medium and dispersed phase. When the particles settle, a dense mass is formed since there is no association between deflocculated particles. Downward

movement due to gravity and the lateral motion due to Brownian movement facilitate tight packing of larger particles with the smaller particles filling the void spaces. Particles at the bottom of the cake are gradually pressed together by the weight of the ones above. Decreasing particle size is the most common strategy used to reduce particle settling.

The large surface area of nanoparticles, however, gives rise to high total surface energy. This is a thermodynamically unfavorable situation, and particles tend to aggregate in order to minimize their collective surface energy (Merisko-Liversidge et al. 2003). The mechanism of aggregation depends on the density and viscosity of the suspension medium. In the continuum regime, the particle collisions occur due to Brownian motion, which describes the random motion of particles suspended in a fluid (a liquid or a gas) resulting from their bombardment by the fast-moving atoms or molecules in the gas or liquid (Mörters and Peres, 2008). If there is negligible repulsive force between the particles, and the collisions result in the irreversible binding of particles and clusters, the aggregation is called Brownian, since the kinetics of the process are limited by Brownian diffusion ("Hydrodynamic Parameters of Aggregated Suspensions Formed from Colloidal Dispersions"). Aggregation can cause a variety of issues for nanosuspensions including rapid settling/creaming, crystal growth and inconsistent dosing. The most common strategy used to tackle this issue is the introduction of stabilizers, the selection of which is based on their ability to provide wetting to surface of the particles and offer a barrier to prevent nanoparticles from aggregation (Eerdenbrugh et al. 2008).

A graphical summary of the discussed phenomena associated with suspension instability, along with a depiction of the stabilization mechanisms discussed, is shown in

Figure 1.1. There are two main mechanisms through which colloidal suspensions can be stabilized in suspension media: Electrostatic repulsion and steric stabilization (Kim, 2004; Rabinow, 2004; Nutan and Reddy, 2009). These two mechanisms are achieved by the addition of ionic and non-ionic stabilizers into the media, respectively. Stabilization in aqueous media from electrostatic repulsion can be described by the Derjaguin–Landau–Verwey–Overbeek (DLVO) theory (Derjaguin and Landau, 1941; Verwey, 1947). This theory assumes that the forces acting on colloidal particles in a medium include repulsive electrostatic forces and attractive van der Waals forces. The repulsive forces originate from the overlapping of an electrical double layer (EDL) surrounding particles in a medium, thus preventing colloidal aggregation. As its name implies, the EDL consist of two layers: (1) a stern layer consisting of counter-ions attracted toward the particle surface to maintain system electrical neutrality and (2) a Gouy layer, which is essentially a diffusion layer of ions.

The total potential energy of a particle–particle interaction is the sum of the repulsion potential generated from EDLs and the attraction potential due to van der Waals forces (Wu et al. 2011). The attraction potential is determined by the Hamaker constant, particle size, and inter-particulate distance, while the repulsion potential depends on particle size, inter-particulate distance, zeta potential, ion concentration and dielectric constant of the medium. The repulsion potential is extremely sensitive to the ion concentration in the medium; as the ion strength is increased, the thickness of the EDL decreases due to screening of the surface charge (Kim, 2004; Nutan and Reddy, 2009). This causes a decrease in the repulsion potential, increasing the susceptibility of dispersed particles to form aggregates. Zeta potential is the electric potential at the shear

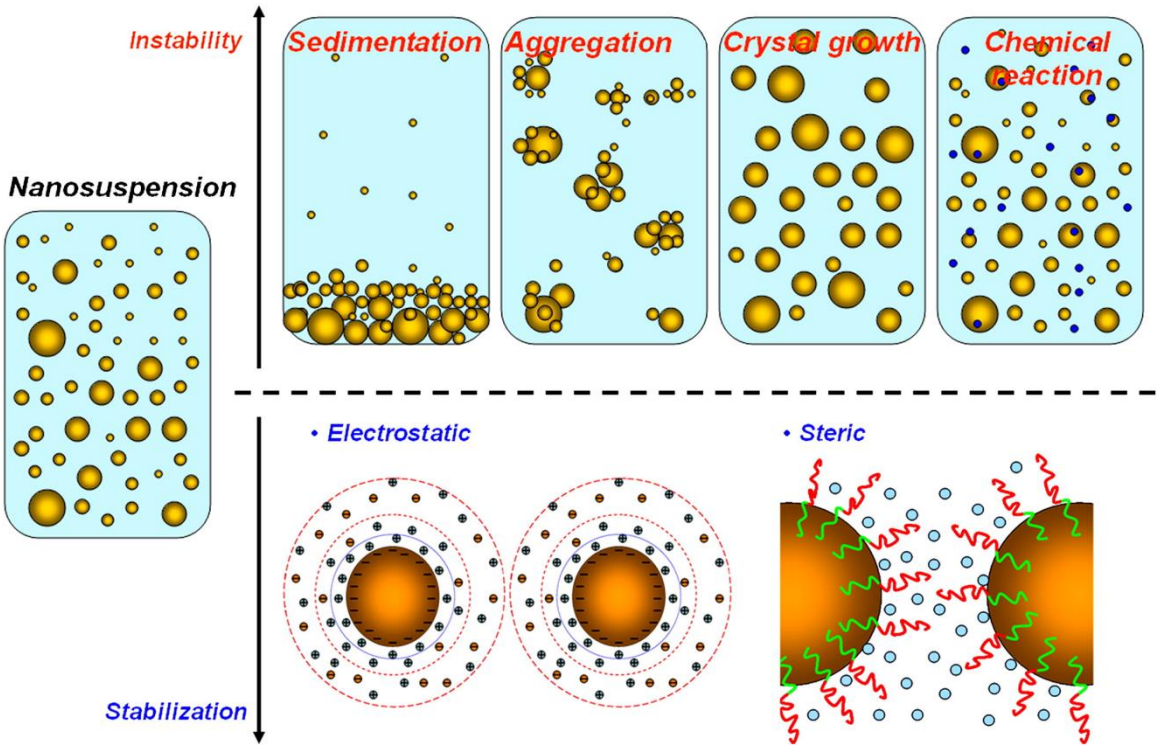

Figure 1.1 Graphical representation of the physical and chemical stability of nanoparticle suspensions. The top pictures present various mechanisms associated with suspension instability; the bottom pictures present methods of promoting suspension stability.

Edited from source: Wu, L., *et al*. "Physical and Chemical Stability of Drug Nanoparticles." *Advanced Drug Delivery Reviews*. 63.6 (2011): 456-69.

plane—the boundary of the liquid layer surrounding particles in the medium. Zeta potential is a key parameter widely used to predict suspension stability; the greater the absolute value of the zeta potential, the more stable the suspension is.

Steric stabilization is usually provided by the utilization of non-ionic stabilizers like neutral polymers and polymeric surfactants. As these stabilizers hydrate and dissolve in the suspensions, they begin to adsorb onto the particle surfaces through an anchor segment that strongly interacts with the dispersed particles, while the well-solvated tail segment extends into the bulk medium. Non-adsorptive steric stabilization is also a possible mechanism. As two colloidal particles approach each other, the stabilizing

segments may interpenetrate, squeezing the bulk medium molecules out of the inter-particulate space. This interpenetration is thermodynamically disfavored when a proper solvent is used as the bulk medium to stabilize the tail (Wu et al. 2011). Accordingly, provided that the stabilizers can be absorbed onto the particle surface through the anchor segment, strong enthalpic interaction (good solvation) between the solvent and the stabilizing segment of the stabilizer is the key factor to achieve steric stabilization and prevent particles from aggregation in the medium (Kim, 2004; Nutan and Reddy, 2009). In addition to solvation, the stabilizing moiety (part of the molecule) needs to be sufficiently long and dense to maintain a steric barrier that is capable of minimizing particle–particle interaction to a level that the van der Waals attractive forces are less than the repulsive steric forces (Derjaguin and Landau, 1941; Verwey, 1947; Choi et al. 2005). It has been shown that combination of both electrostatic and steric stabilization mechanisms can be very beneficial in achieving stable colloidal suspensions (see Bilgili and Afolabi, 2012 and references cited therein). In addition, the combination of a non-ionic stabilizer with an ionic stabilizer reduces the repulsion between ionic surfactant molecules, leading to closer packing of the stabilizer molecules (Rabinow, 2004).

Crystal growth in colloidal suspensions is generally known as Ostwald ripening and is responsible for changes in particle size and size distribution (Voorhees, 1985). Ostwald ripening is originated from particles solubility dependence on their size; small particles have higher saturation solubility than larger particles, creating a drug concentration gradient between the small and large particles. As a consequence, molecules diffuse from the areas of higher concentration surrounding small particles to areas around larger particles with lower drug concentration. This diffusion process

continues until all the small particles are dissolved, essentially making Ostwald ripening a process where large particles grow at the expense of smaller particles (Wu et al. 2011). This consequentially leads to a shift in the particle size distribution of a colloidal suspension to a higher range.

1.2.2 Melt Emulsification

The technique for solubility enhancement that this thesis work focuses on is a particle engineering method known as melt emulsification (ME). This is a well-known process in food and pharmaceutical processing literature (Mehnert and Mäder, 2001; Kocbek et al. 2006; Nori et al. 2009; Köhler et al. 2011). To summarize, a suspension containing large particles is heated to temperatures above the melting point of the disperse phase– in this case, a drug compound. Since the molten drug is immiscible with the surrounding water, an oil-in-water (o/w) emulsion is formed. This hot emulsion is exposed to some mechanical disturbance (e.g., high-intensity ultrasound) that leads to droplet breakup. Droplet sizes in the nanometer range can be obtained if the applied disturbance is intense enough. Subsequent cooling of the emulsion eventually leads to the recrystallization of the oil droplets to solid particles. In this study, fenofibrate (FNB) and ibuprofen (IBU) were chosen as model poorly water-soluble BCS class II compounds because they exhibit melting temperatures below the boiling point of water and can thus be emulsified in aqueous systems. Fenofibrate is known to reduce the cholesterol level in the blood and exhibits solubility in de-ionized water of just 0.8 mg/L at room temperature (Jamzad and Fassihi, 2006). The drug can crystallize in two polymorphs, the stable form I (Henry et al. 2003) and the metastable form II (Di Martino et al. 2000). Ibuprofen is a non-steroidal anti-inflammatory drug (NSAID) derivative of propionic acid used widely as an analgesic

and as an antipyretic, and it is also used for relief of symptoms of rheumatoid arthritis and osteoarthritis. It exhibits solubility in deionized water of 11.4 mg/L at room temperature (Garzón and Martínez, 2004). The drug is currently available as a racemic mixture of S(+)-Ibuprofen and R(−)-Ibuprofen; S(+)-Ibuprofen is the pharmacologically active form. The racemic mixture is structurally isomorphic and there is no true crystalline polymorphism (Dudognon et al. 2008).

In pharmaceutical literature, the ME process is well described for the production of solid-lipid nanoparticles (Mehnert and Mäder, 2001). Another application of the method allows for the manufacture of ibuprofen nanoparticle suspensions (Kocbek et al. 2006). Emulsification of a hot pre-emulsion was performed in a high pressure homogenizer at 1000 bar for 5 cycles, followed by rapid cooling in an ice bath. Particle sizes of 170 nm in a 0.5% (w/w) ibuprofen suspension were obtained with this ME process using the non-ionic surfactant Tween 80 as a stabilizer. Huang et al. (2009) investigated the formation of fine FNB powder by another ME process variation. In that study, emulsification of a hot dispersion was carried out by magnetic stirring in the presence of Pluronic F127 (10% w/w wrt drug) as a stabilizing agent. The achieved particle sizes ranged from 1-3 μm with a final suspension solid content of about 2% (w/w). The suspension was filtered and dried immediately after the solidification step. Notable improvement to the dissolution behavior was observed for fine FNB powder when compared to as-received micronized powder (Huang et al. 2009). Li et al. also studied the ME process with FNB as the model drug. A high pressure homogenizer was used for emulsification of suspensions with a solid content of 0.5% (w/v). It was shown that a mixture of polymeric surfactant (Poloxamer 188) and synthetic steric polymer

(PVP K30) was most ideal for stabilizing the molten droplets. Rapid cooling of the hot emulsion in an ice bath resulted in particle sizes of about 300 nm, and strong dissolution rate enhancement was obtained via the produced nanosuspensions. A pharmacokinetic study in rats revealed greater bioavailability of FNB particles with sizes in the nanometer range (Li et al. 2009). In a recent study by Knieke et al. (2013b), drug suspensions with loadings up to 30% (w/w) were produced by a high-intensity ultrasound- (HIU) based ME process. The particles exhibited median sizes down to 150 nm and did not require energy inputs as high as other studies have reported. A variety of stabilizers having different chemical nature were investigated and it was shown that, in general, non-ionic surfactants perform better than pure polymers in order to achieve smaller particle sizes (Knieke et al. 2013b). The current study presented here is a continuation of the latter of these studies.

1.3 Problem Definition and General Solution Strategies for ME-based Suspensions

The physical stability of suspensions produced by an ME process is not reported in literature. Since disperse systems in general, and suspensions in particular, are unstable in the thermodynamic sense (Zatz, 1985), one must understand that "physical stability" or, shortly, "stability" refers to the time-invariance of critical suspension properties (e.g., particle size distribution) over some period of time (e.g., 7 days), which may be required for a specific industrial application. Stability is a critical aspect in ensuring the safety and efficacy of drug products. Aggregated particles may not dissolve fast enough *in vitro* and *in vivo*, thus somewhat nullifying the effectiveness of producing fine particles via any particle engineering technique. Similarly, in intravenously administered nanosuspensions, the formation of larger particles (> 5 μm) could lead to capillary embolism (Patravale et

al. 2004). Therefore, drug particle size distribution needs to be closely monitored during storage.

The particle sizes presented in aforementioned ME studies were measured right after the cooling step (Kocbek et al. 2006; Li et al. 2009; Knieke et al. 2013b); thus, physical stability of produced suspensions was not investigated. In this thesis, it is shown that (a) ME-based suspensions can exhibit significant particle size growth during storage and (b) through proper adjustment of process conditions and selection of additive type/concentration, enhanced physical stability can be realized.

Applying high-intensity ultrasound to crystallizing systems offers significant potential for modifying and improving the ME process by enabling proper short-term stabilization of ME-based suspensions. Although ultrasonics has been used for years in research and diagnostics, its use in chemical processes has only developed in recent years. This is because high-intensity systems have become available that can deliver ultrasonic power on industrially relevant scales (McCausland et al. 2001). The chemical effects of ultrasound derive primarily from acoustic cavitation. Ultrasound is already known to influence the crystallization systems in several ways: (1) reduction in induction time for crystallization; (2) narrowing down crystal size distribution (CSD) with simultaneous reduction in dominant crystal size; and (3) change in crystal geometry (in polymorphic systems) (Nalajala and Moholkar, 2011). Studies on organic melts by Kapustin have shown that ultrasound reduces the time needed for complete crystallization by a large factor; his results suggest that the mechanism is often simple: the field produced by ultrasonic cavitation breaks off microcrystals from the growth surface on the melt, which act as fresh nuclei (Kapustin, 1950; Kapustin, 1952). More recently, studies

of the so-called sonocrystallization of melts suggest that these effects may be due to small bubbles produced by high-intensity ultrasonic waves acting as nuclei themselves, or because fluctuations in the pressure and temperature associated with the ultrasonic wave disturb the equilibrium between solid and liquid phases (McClements, 1995). Cavitation-induced sonochemistry provides a unique interaction between energy and matter, with hot spots inside the bubbles of ~5000 K, pressures of ~1000 bar, and heating and cooling rates of >1010 K s^{-1} (Bang and Suslick, 2010). These extraordinary conditions permit access to a range of transport phenomena normally not accessible. It is suggested that the use of ultrasound during the solidification or cooling step may enhance both the rate of crystallization from the melt emulsion and the rate at which stabilizer is adsorbed onto newly-formed particle surfaces.

While the incorporation of particles produced by size-reduction methods into common solid dosage forms has been investigated appreciably, the integration of those produced by ME into polymer films remains to be well-studied. Polymer films have great potential over other dosage forms for delivery of poorly water-soluble drugs. A distinct advantage is a larger surface area, which leads to rapid disintegration and dissolution in the oral cavity, resulting in increased bioavailability (Sievens-Figueroa et al. 2012; Beck et al. 2013). Polymer films are an easily administered dosage form, a fact which has led to improved acceptability and compliance among geriatric and pediatric patients. This dosage form also exhibits manufacturing advantages, including continuous manufacturing capabilities that enable full product characterization with efficient in-line monitoring of quality (Dixit and Puthli, 2009). The interest in pharmaceutical films for oral application has increased based on recent publications and patents (Perumal et al.

2008; Garsuch and Breitkreutz, 2010; El-Setouhy and Abd El-Malak, 2010; Arya et al. 2010; Bess et al. 2010; R. K. Yang et al. 2010; and Yang et al. 2011). In this thesis, a variant of the film preparation process outlined in Sievens-Figueroa et al. (2012) is used to produce polymer films containing dispersed drug particles as the final dosage form for drug delivery applications. Melt emulsification is used to produce stable fenofibrate and ibuprofen suspensions; produced fenofibrate suspensions are then transformed into polymer films.

1.4 Scope and Organization of Thesis

The following chapters outline a study of the melt emulsification process and the feasibility of its use to produce suspensions of BCS Class II drug particles with enhanced short-term (i.e., up to one week) stability. Chapter 2 describes the experimental details of the study, including methods and materials for suspension and film production, as well as methods for product characterization. Results of the experiments and discussion of these results are presented in Chapter 3. Chapter 4 is a competitive assessment of the use of nanoparticle and amorphous particle technologies for solubility enhancement, focusing on common methods to produce the particles and limitations of the respective technologies. Finally, Appendix A introduces proof-of-concept experiments for the investigation of producing amorphous particles in the melt emulsification and polymer film formation processes.

CHAPTER 2

EXPERIMENTAL SECTION

This chapter provides an outline of the experimental details of the study presented in this thesis. It focuses on the methods and materials used for the production of stable BCS Class II drug suspensions by melt emulsification, the incorporation of suspensions into polymer strip films, and the characterization of both suspensions and films.

2.1 Melt Emulsification

Materials

Melt emulsification experiments were carried out with the poorly water-soluble drugs fenofibrate (FNB, Jai Radhe Sales, Ahmedabad, India) and ibuprofen (IBU, Alfa Chem, NY, USA). The chemical structure of the two drug molecules are shown in Figure 2.1. FNB has a melting temperature between 78–81 °C; IBU, between 75–78 °C. As-received

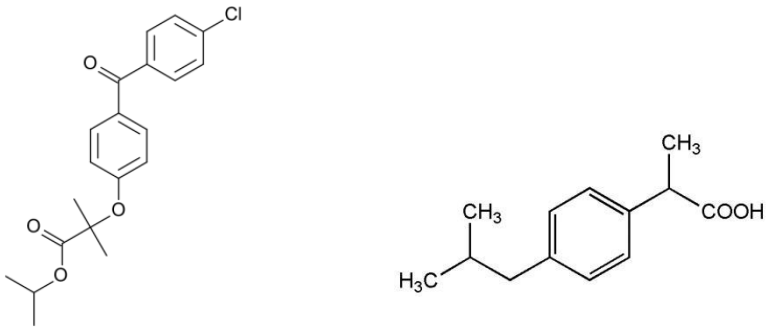

Figure 2.1 Chemical structures of fenofibrate (left) and ibuprofen (right).

drug particles were used in all experiments. To stabilize the droplets in the emulsion, as well as the particles after solidification, surfactants and polymers were used. All additives are summarized with their median molecular weight in Table 2.1. In addition, for the

non-ionic surfactants (Pluronic F68, Pluronic F127, and Tween 80) and the anionic surfactant sodium dodecyl sulfate, an HLB (hydrophilic-lipophilic-balance) value and CMC (critical micellar concentration) are listed in the table. The critical micelle concentration (CMC) is the concentration at which the surfactant molecules saturate the solution and form micelles upon further addition of surfactant (Feng et al. 2006). It stands to mention that the CMC values for Pluronics are not well-defined. All stabilizers were purchased from Sigma Aldrich, with the exception of HPMC E3 (Methocel©, Dow Chemicals, Midland, MI, USA), and were used as-received. Unless otherwise specified, drug concentrations are weight concentrations with respect to (wrt) deionized water, whereas the stabilizer concentrations are expressed wrt the drug loading.

Table 2.1 Overview of Stabilizers Used in Melt Emulsification

Stabilizer	Abbreviation	HLB value	Median Molecular Weight (kDa)	CMC (mM)
Pluronic F68	PF 68	29	8,400	[a]0.04; [b]0.9; [c]~17.9
Pluronic F127	PF 127	22	12,600	[b]0.5; [c]3.97
Tween 80	T 80	15	~1,310	[a]0.012
Polyvinylpyrrolidone K30	PVP K30	-	40,000	-
Hydroxypropyl Methylcellulose E3	HPMC E3	-	20,300	-
Sodium Dodecyl Sulfate	SDS	[d]40	288.4	[e]8.0

Sources: Unless otherwise noted: (Knieke et al. 2013b); [a]Sigma-Aldrich; [b](Georgieva et al. 2009); [c](Alexandridis et al. 1994); [d](Kawakatsu et al. 1997); [e](Dominguez et al. 1997)

Methods

The melt emulsification process is depicted in Figure 2.2 and contains the following steps: 1) the stabilizer was completely dissolved in deionized water; 2) the as-received FNB or IBU particles were dispersed in the aqueous solution and the system was heated to temperatures above the melting point of the drug. To ensure a complete melting and to

reduce the viscosity of the melt, temperatures at least 10 °C above the melting point of the drug were attained. Gentle magnetic stirring was applied during the heating process; 3) emulsification was carried out with high-intensity ultrasound by an ultrasonic probe (Vibra Cell VCX 750, Sonics & Materials, Inc., Newtown, CT) for 5 min at 100% intensity with a 10s:1s pulse ratio (i.e., a 10 s pulse on, followed by a 1 s pulse off). During the emulsification step, the system was held at an elevated temperature (10–15°C above the melting point temperature) to avoid solidification of the melt; 4) Rapid cooling of the hot emulsion in a thermostatic saline bath at –3.0 °C (NESLAB TRE 10 Digital One, Thermo Fisher Scientific, Newington, NH) was employed for 10 min, both in the presence and absence of HIU. Sonocrystallization was carried out with another ultrasonic probe (Omni Sonic Ruptor 250, Omni, Int'l, Kennesaw, GA) at 50% intensity and a 1.8s:0.2s pulse ratio. In all experiments, the volume of deionized water was 50 ml, and, unless otherwise noted, a 1% (w/w, wrt DI water) drug suspension was realized.

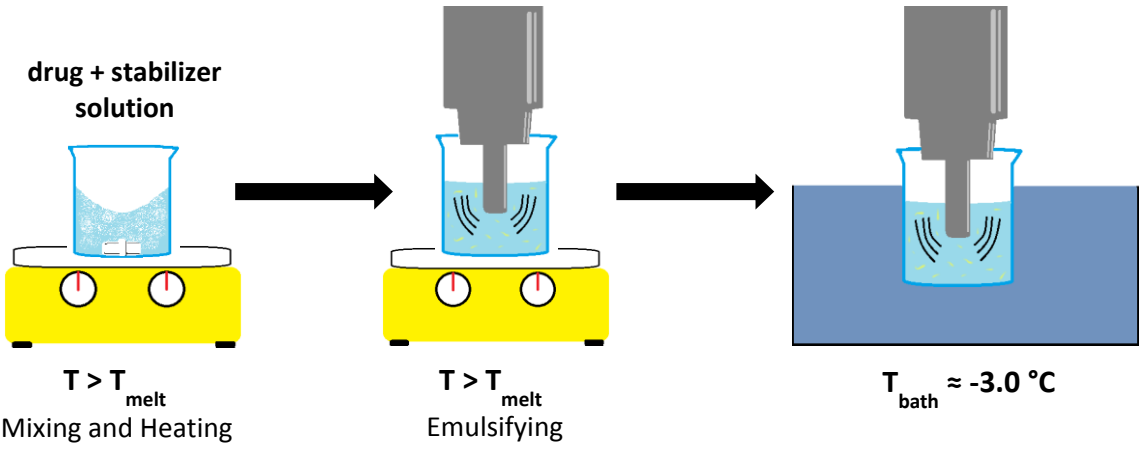

Figure 2.2 Experimental setup for the melt emulsification process.

Sample Characterization

Particle size analysis and morphology

Particle size distributions of the FNB suspensions were measured with the laser diffraction device LS 13 320 (Coulter Beckman, Inc.; Brea, CA, USA) after the emulsification step, at several time points after the cooling period, during film production, and upon film redispersion. Samples were added drop-wise until the polarization intensity differential scattering (PIDS) reached above 40% for all the samples. Particle size distribution was computed by the software using Mie scattering theory with a dispersant refractive index of 1.33 and the particle refractive index of 1.546 for FNB and 1.436 for IBU. All particle size distributions presented here are volume-weighted distributions of particle size. Morphological evaluation of the produced nanoparticles was conducted through scanning electron microscopy (LEO 1530 SVMP, Carl Zeiss; Peabody, MA, USA). Digital images were obtained at an accelerating voltage of 2 kV using an InLens detector. The SEM samples were prepared by diluting the suspensions in de-ionized water and subsequently spreading the dilution onto cleaned Si-wafer. The wafers were dried in dust-free conditions in a desiccator and coated with carbon in the system BAL-TEC MED 020 (BAL-TEC AG; Balzers, Switzerland) to reduce charging effect during imaging.

2.2 Polymer Film Production

Materials and Methods

Preparation of films containing nanoparticles

Figure 2.3 shows a schematic of the process used for film formation. A solution containing 15% HPMC E15 LV (wrt to solution weight) (15 g) and 5% glycerin (wrt to solution weight) (5 g) was prepared by adding the glycerin to water (80 g) and heating to 80 °C. This composition was chosen in order to ensure a final suspension viscosity and film quality acceptable for characterization. The polymer was then added until well dispersed and the temperature was decreased to room temperature to dissolve the polymer completely. The components were mixed until a transparent, yellowish solution was obtained. The resulting solution was then let to rest until no bubbles were seen. The polymer solution (30 g) was then added to the suspension produced from ME (30 g), and then mixed for 5 h at ~300 rpm using a dual-impeller mixer (McMaster, Catalog no. 3471K5, Los Angeles, CA, USA) attached to a motor (IKA RW16 Basic Overhead Stirrer) and left to rest until no bubbles were observed. When PF68 was present in the original drug suspensions, the final FNB film suspensions had 7.5% HPMC, 2.5% glycerin, 0.493% FNB, and 0.246% PF68. When PF68 was absent from the original drug suspensions, the final FNB film suspensions had 7.5% HPMC, 2.5% glycerin, and 0.495% FNB. The final viscous suspensions were then casted onto a stainless steel plate using an Elcometer 3700 Doctor Blade (Elcometer, USA). The wet film thickness was set to 1000 μm by adjusting the Doctor Blade aperture accordingly. The films were then dried overnight in an oven (Gallenkamp 300 Plus Series, UK) at 40 °C. The theoretical amount of drug in the resulting films accounted for either 4.65% (w/w) of the film

composition (PF68 present) or 4.76% (w/w) of the film composition (PF68 absent), assuming all of the water had been removed during the drying step.

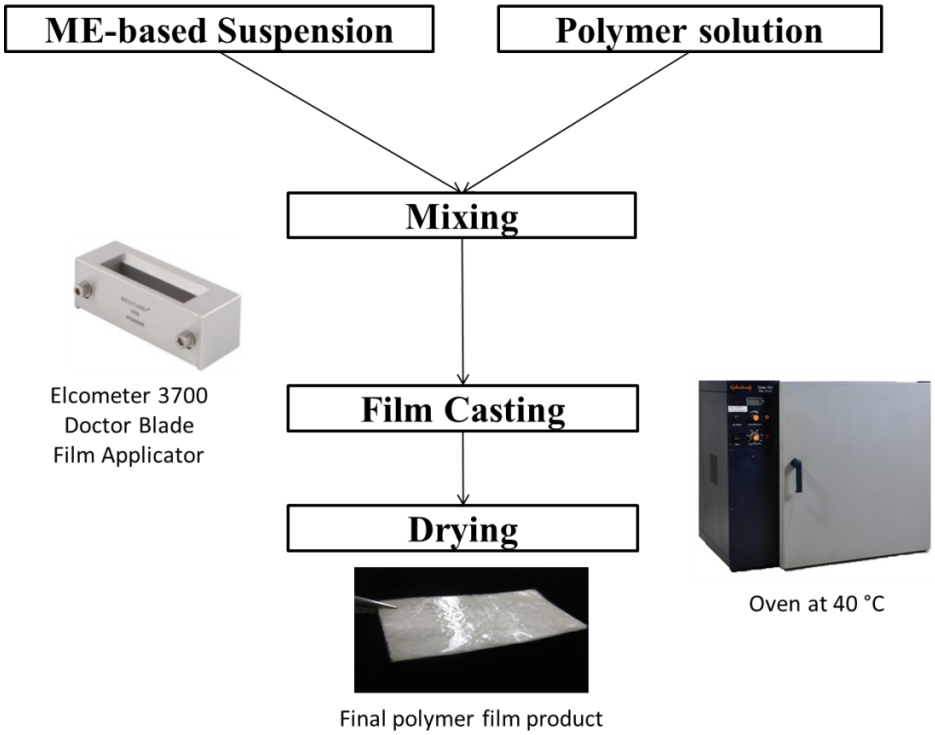

Figure 2.3 Experimental setup for the film formation process.

Characterization of the ME-based films

The redispersion and dissolution behaviors of four films containing different FNB suspensions were studied. The suspensions were as follows:

a) As-received, non-processed FNB with no stabilizers

b) FNB with no stabilizers processed in the presence of HIU during cooling

c) FNB stabilized with PF68 in the presence of HIU during cooling

d) FNB stabilized with PF68 in the absence of HIU during cooling

Assay of Films

Six individual 5.07 cm^2 (2.54 cm diameter) circular discs from each film produced were dissolved in separate 100 ml aqueous solutions of 18.7 mM SDS. The absorbances of 2 ml aliquots from the prepared samples of dissolved FNB were measured at a wavelength of 290 nm by Ultraviolet (UV) spectroscopy in a UV Spectrophotometer (Evolution 300, Thermo Scientific; Santa Clara, CA, USA). These six replicates from each film were used to find the mean drug content in the films. This information, along with the relative standard deviation, is presented in Table 3.1 of the results and discussion chapter.

Redispersion of Films

Circular samples, 5.07 cm^2 (2.54 cm diameter) of each film produced were dispersed in a 50 ml beaker containing 10 ml de-ionized water and stirred magnetically at a speed of ~475 rpm for 5 min. An aliquot of the sample was pulled from the beaker while the suspension was being stirred and particle size was measured in the LS13320.

Dissolution of Films

Dissolution tests of the produced polymer strip films containing fenofibrate suspensions were carried out and the results were compared with those of films containing as-received and non-stabilized drug particles. The dissolution experiments were performed with a USP type IV apparatus (CE7*smart*, Sotax; Allschwil, Switzerland) with cells of an internal diameter of 22.6 mm (Kakhi, 2009; Heng et al. 2008). Films were horizontally positioned in the cells with 5 g of glass beads (1 mm in diameter) filling up the conical part at the bottom of each cell. Glass microfiber filters (0.2 μm, GF/D grade) were used

in the filter-head for each experiment. As a dissolution medium, 600 mL of an 18.7 mM SDS (above the CMC) solution were used at 37 ± 0.5 °C. Each cell flow rate was set to 16 ml/min. A Thermo Evolution UV spectrophotometer was used to automatically measure the FNB concentration using a previously made calibration curve. Six samples were used and the average drug release and standard deviation were plotted as a function of time. Circular film samples with an area of 1.98 cm^2 (1.59 cm diameter) and an average thickness of 65 μm were used for the dissolution test.

Differential Scanning Calorimetry

Curves of heat flux versus temperature were constructed using DSC (DSC30 using STARe software, Mettler Instrument, Hightstown, NJ). Samples of filtered suspensions and final film product (ca. 10 mg) were placed in 40-μL aluminum pans (Mettler Instrument). An identical empty pan was used as a reference. The samples were scanned at 10 °C/min with a nitrogen purge. The temperature, time constant, and the heat flow calibrations for the instrument were performed using indium and zinc.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Melt Emulsification

The size of emulsified drug droplets and, hence, drug particles produced by melt emulsification (ME) depends on the agitation conditions during the emulsification and cooling steps and the stabilizer type and concentration. Agitation conditions during the emulsification step were optimized in a prior study (Knieke et al. 2013b), and these optimum conditions were used throughout the work presented in this thesis. In this way, the process of preparing fine particles by ME was optimized by variations in the remaining parameters to achieve stable suspensions.

3.1.1 Effects of Agitation Methods during Cooling

The treatment of emulsions during the cooling step for particle formation was varied to examine effects on the particle size measured immediately after cooling as well as the short-term physical stability (i.e., up to one week) of the suspensions. Each formulation in this section consists of 1 % FNB (w/w) and 0.5 % PF127 (w/w) wrt deionized water. This formulation was chosen based on optimum low-drug-loading results from Knieke et al. (2013b). Size distributions are described in terms of D10, D50, and D90, also known as passing sizes. The D10 value represents the particle size which 10% of the tested population is smaller than. Similarly, D50 size is larger than the value of 50% of the tested population, and D90 size is larger than the value of 90% of the tested population. Figure 3.1 displays the effect of gentle stirring by a metal rod during cooling on the initial particle size and subsequent particle stability. Immediately after the cooling step, it is

possible that the drug is still in an amorphous state due to rapid cooling and the observation of FNB exhibiting high relative glass-forming ability when supercooled from the molten state (Baird et al. 2010). It has been observed that this so-called supercooled liquid is very sticky and tends to adhere to other particles and the beaker walls (Knieke et al. 2013b). As time goes on, the amorphous particles in water may recrystallize. In the absence of detailed microstructural characterization of the particle samples taken at different time points, it is hard to suggest when and how such amorphous–crystalline transition occurs. It is speculated that both supercooled droplets and recrystallized particles experience coalescence and subsequent particle growth as well as aggregation as a result of Brownian collisions within an hour while stored at ambient conditions. Indeed, Figures 3.1 and 3.2 suggest the presence of both large particle aggregates and dendritic crystal formations (typical of crystal growth).

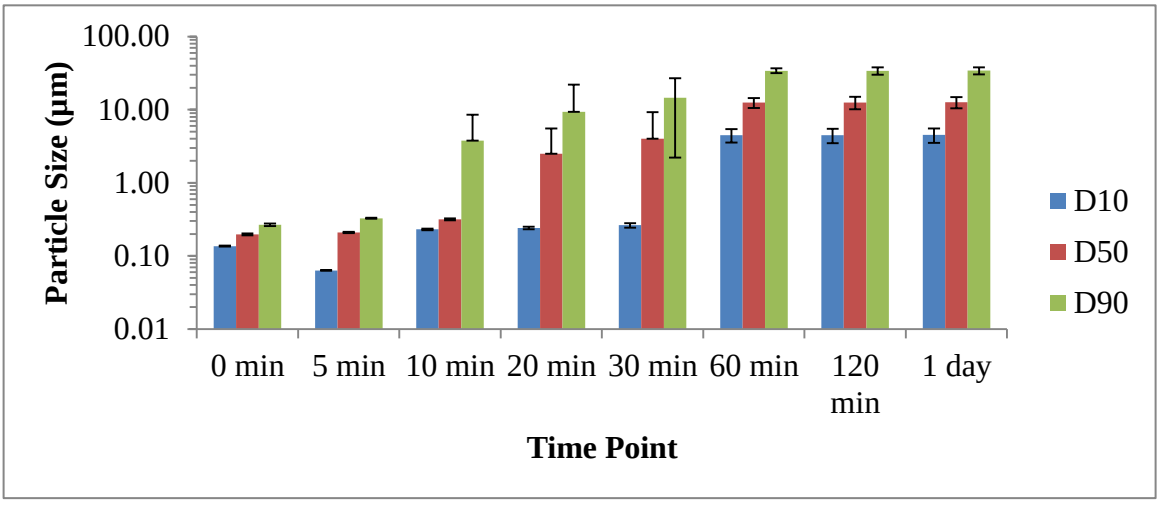

Figure 3.1 Effect of using gentle stirring during cooling on particle size and stability.

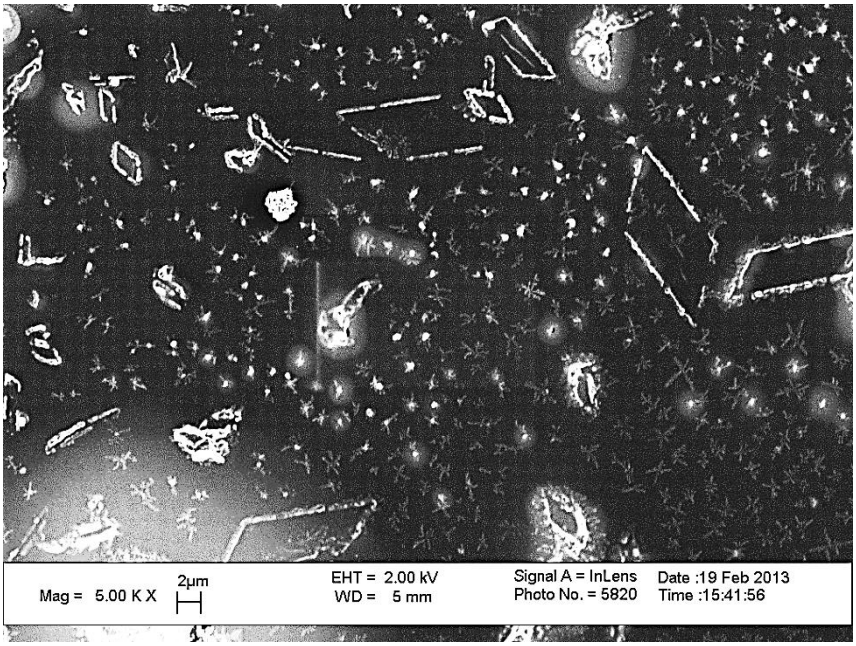

Figure 3.2 SEM micrograph of FNB particles produced with gentle stirring during cooling (30 min after the cooling step).

. High-intensity ultrasound (HIU), also known as sonication, was employed as a treatment during the cooling step. Results indicate that particles produced in the presence of HIU during cooling exhibit enhanced physical stability (i.e., no size increase), even over the course of 7 days (Figure 3.3). Moreover, after 7 days, the HIU treated sample showed much smaller particles (Figure 3.3.) than the gentle-stirred sample (Figure 3.1). The SEM micrograph in Figure 3.4 (Mag. = 30KX) depicts<600 nm primary particles produced in the presence of HIU during cooling. A comparison of the particle sizes shown in Figure 3.3 and Figure 3.4 suggests that the particles are somewhat aggregated even in the HIU treated samples. The larger *initial* (0 min) particle size distribution, in comparison with suspensions produced with gentle stirring during cooling, is the result of more intense agitation during HIU treatment. When HIU is applied to a cooled melt-emulsion system, recrystallization is not instantaneous; therefore, there is a greater

likelihood that soft particles initially collide and aggregate during cooling, before crystallization and stabilizer adsorption can take place. With the use of HIU, preparation of ME-based drug suspensions with up to 1-week stability has been successfully demonstrated.

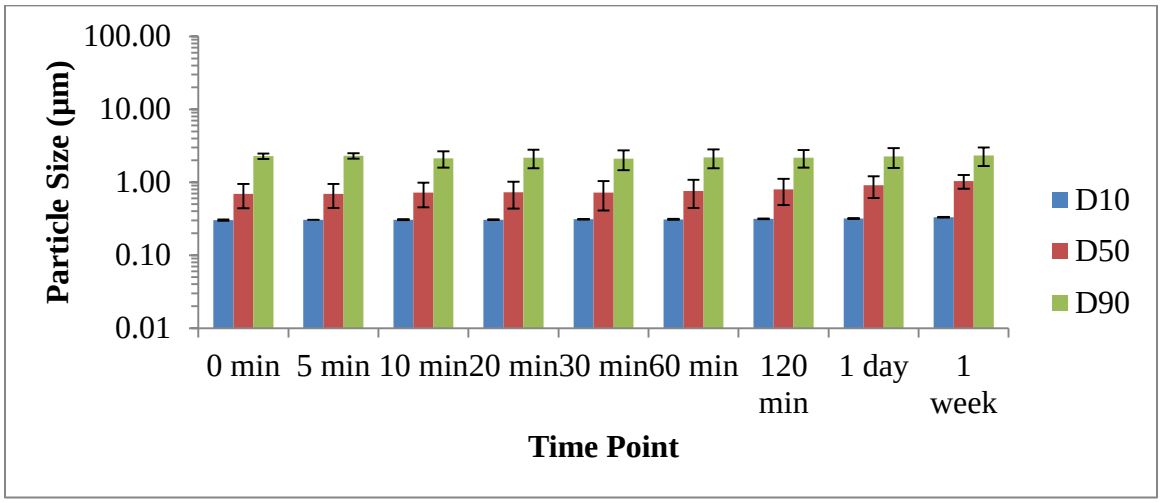

Figure 3.3 Effect of using HIU during cooling on particle size and stability.

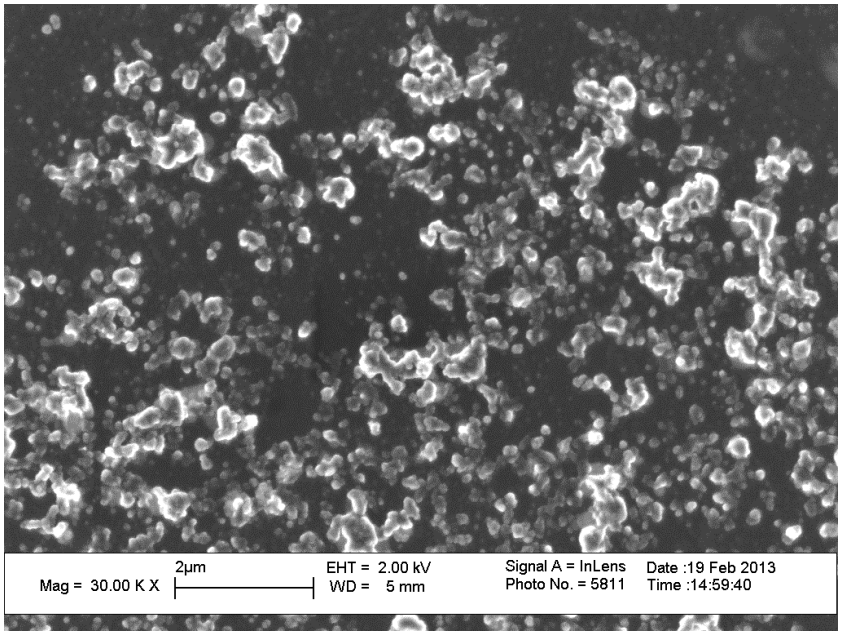

Figure 3.4 SEM micrograph of FNB particles produced with HIU during cooling (30 min after the cooling step).

During sonication, due to the propagation of ultrasound through water, high and low pressure cycles are created. Small cavities are generated during the low pressure cycle that grows in size during further cycles. During the high pressure cycle cavities collapse creating shock waves and liquid jet streams. This phenomenon, termed as cavitation, produces transient high local pressures and high temperatures (Sangawar and Gupta, 2009). Sonication has been used in literature to deagglomerate the agglomerates (Kusters et al. 1993; Faure et al. 2010) and aggregates (Ding and Pacek, 2008) of nanoparticles. As the clusters break down and fresh surfaces are exposed, the stabilizers or additives like surfactants can adsorb to the fresh surfaces and prevent the reaggregation of the primary particles. If there is no stabilizers or if the stabilizers do not adsorb to the fresh surfaces fast, the particles can aggregate. It is also observed that when HIU is applied to an emulsion system undergoing cooling, the temperature was higher due to higher energy dissipation (to be illustrated in Figure 3.7 below). The presence of higher temperatures in the emulsified system during cooling may result in (a) faster and more effective mass transfer of the stabilizer (due to lower suspension viscosity and higher stabilizer diffusivity) to recrystallized particle surfaces, thus possibly allowing faster adsorption and (b) preventing the formation of amorphous particles.

Besides HIU, two agitation methods of providing mechanical disturbance were explored in the hopes of producing stable FNB suspensions:

1. Mixing with a double impeller blade (described in section 2.2) at 1200 RPM
2. Same as (1), with the inclusion of 25 ml of 100 micron yttrium-stabilized zirconia beads.

Mixing alone with an impeller blade did not impart physical stability to the produced suspensions (Figure 3.5). However, with the addition of 100 µm yttrium-stabilized zirconia beads, which are used as media in the wet stirred media milling process, particles remained relatively stable, though particle size distributions achieved were unfavorably larger than those observed when HIU is used as the agitation method (Figure 3.6).

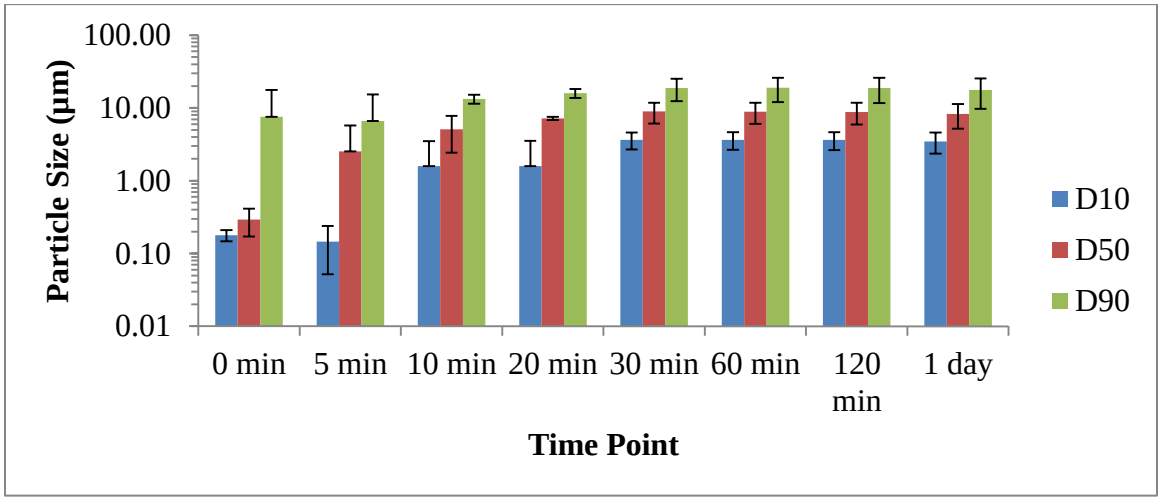

Figure 3.5 Effect of using impeller blade mixing on particle size and stability.

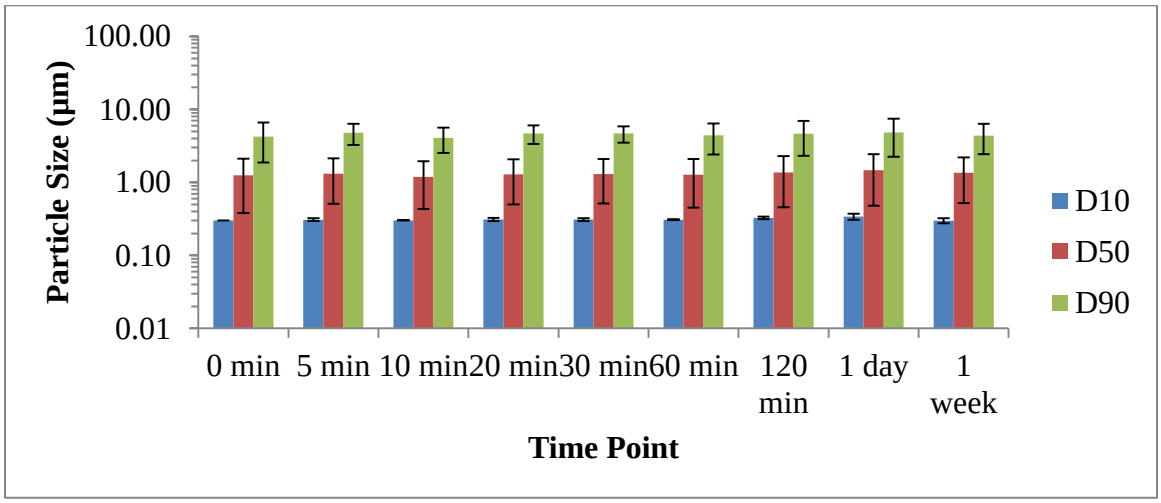

Figure 3.6 Effect of using impeller blade mixing with zirconia beads on particle size and stability.

Figure 3.7 depicts cooling rates for each agitation method used during the cooling step. Higher temperatures when HIU is employed can be explained as follows. During HIU, cavitation provides a unique interaction between energy and matter, with hot spots inside the bubbles of ~5000 K, pressures of ~1000 bar, and heating and cooling rates of >1000 K s^{-1} (Bang, J. H. and Suslick, K. S., 2010). Even though these bubbles are very small, this can have dramatic effect on the rate of heat generated in the emulsion system. Also, since such intense heat generation is most likely not taking place in systems exposed to agitation other than HIU, cooling provided by the thermostatic saline bath will lead to slower decrease in temperature in the HIU treatment as compared with the other agitation cases.

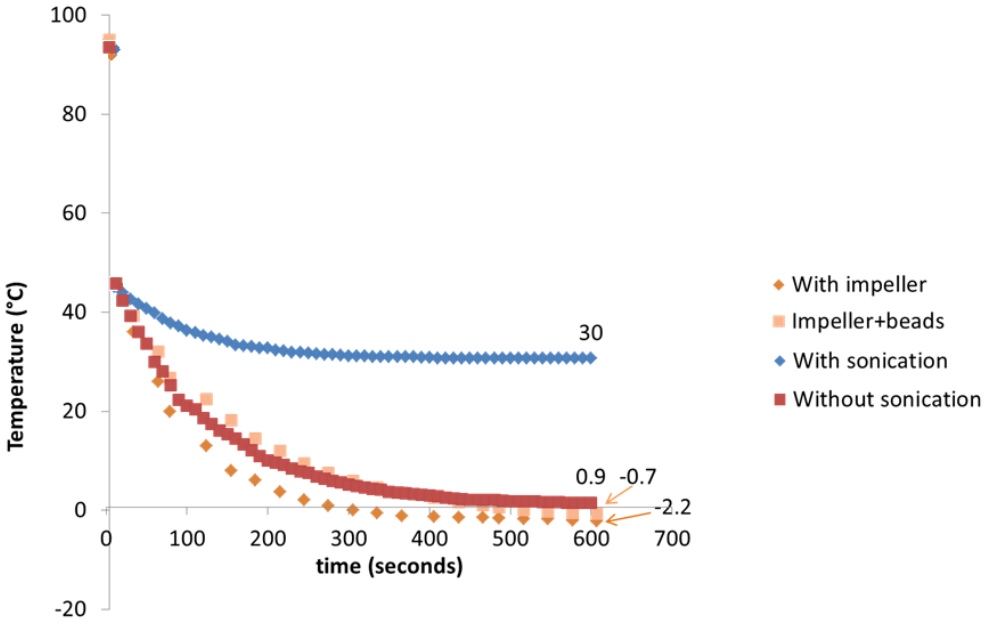

Figure 3.7 Effect of agitation methods on the cooling rate of emulsions.

The application of HIU during the cooling step was further optimized. A brief study (Figure 3.8) was carried out to find an optimum intensity specific to the ultrasonic

probe used in this study. The duration of the ultrasonic treatment was maintained at 10 min (the complete duration of the cooling step).

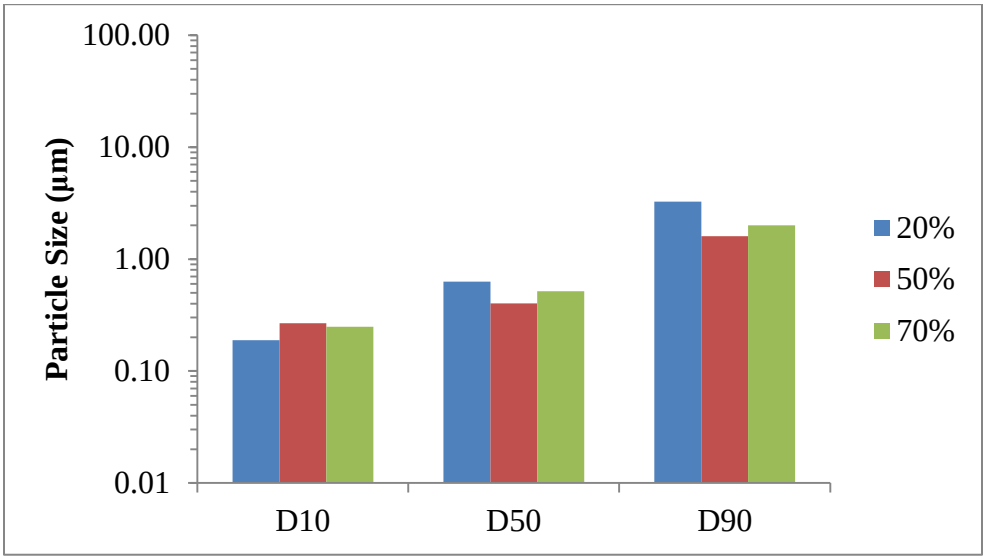

Figure 3.8 Effect of ultrasonic intensity on the initial suspension particle size distribution.

It can be argued that the difference between intensities is not very distinct; nevertheless, given more favorable results in the initial 50% and 90% passing particle sizes, the 50%-intensity setting was used for the remainder of the study.

In order to confirm the nature of the particles formed during the ME process, DSC characterization was performed on FNB suspensions stabilized with PF68 and made either in the presence or absence of HIU during cooling (Figure 3.9). After thirty minutes of being stored at ambient conditions after the cooling step, the suspensions were syringe filtered through 0.2 µm filters, and the filtrates were dried at room temperature and characterized. The presence of endothermic peaks near 80 °C, along with the knowledge that the only solids in the suspension are the drug particles, confirms the presence of crystalline FNB particles in both cases. Even if amorphous particles had been produced in the case where HIU was absent from the cooling step, it is shown that they recrystallized

within a half hour of the cooling step, due to thermodynamically metastable state of the amorphous particles. This may not be considered entirely conclusive, however, without further confirmation with x-ray powder diffraction analysis.

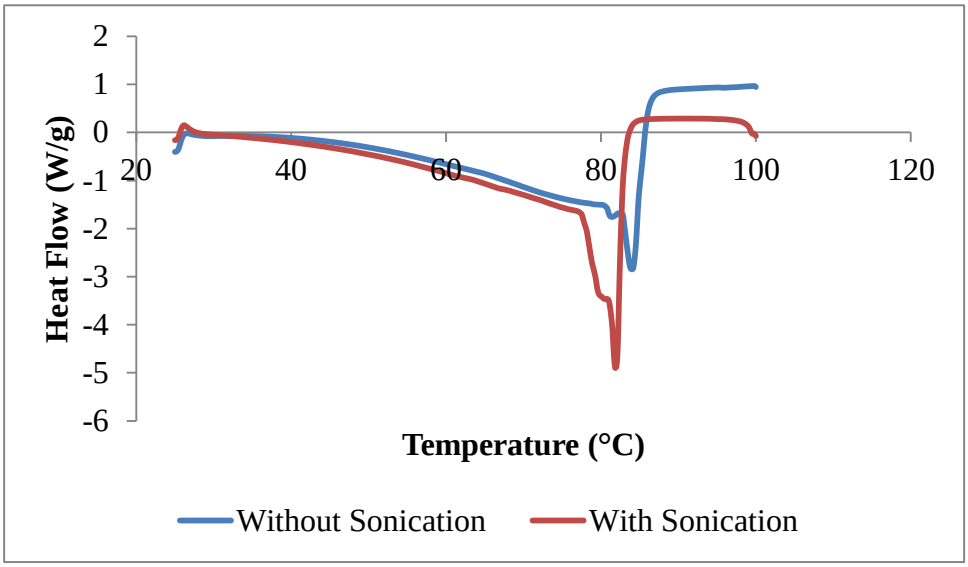

Figure 3.9 DSC thermograms of FNB particles made with and without sonication during cooling.

3.1.2 Effects of Additives on Physical Stability

Given the optimization of treatment during the cooling step with the addition of HIU, it was necessary to perform a stabilizer screening study in order to potentially enhance the stability of FNB suspensions produced by ME. Figures 3.10 and 3.11 depict the effect of stabilizer type and concentration on the median size of emulsified droplets and initial particles, respectively. HPMC E3 and PVP K30 are steric polymers. The former is a cellulosic semi-synthetic polymer; the latter, a synthetic polymer. PF68, PF127, and T80 are non-ionic surfactants, and SDS is an anionic surfactant. At all concentrations, it can be seen that surfactants as a whole do a better job of maintaining small emulsion droplet sizes, with T80 performing the best (Figure 3.10). In general, similar behavior was

observed for the initial median size (Figure 3.11), with PF68 being the best surfactant. However, at 100% polymer concentration wrt FNB, steric stabilization provided by HPMC E3 trumps the effects of some surfactants. This is most likely due to solubilization and subsequent Ostwald ripening of drug particles at concentrations well above these surfactants' critical micellar concentrations (CMCs). In some experiments conducted at high concentrations of the surfactants, including those eventually considered optimized cases, the surfactants' CMCs were exceeded.

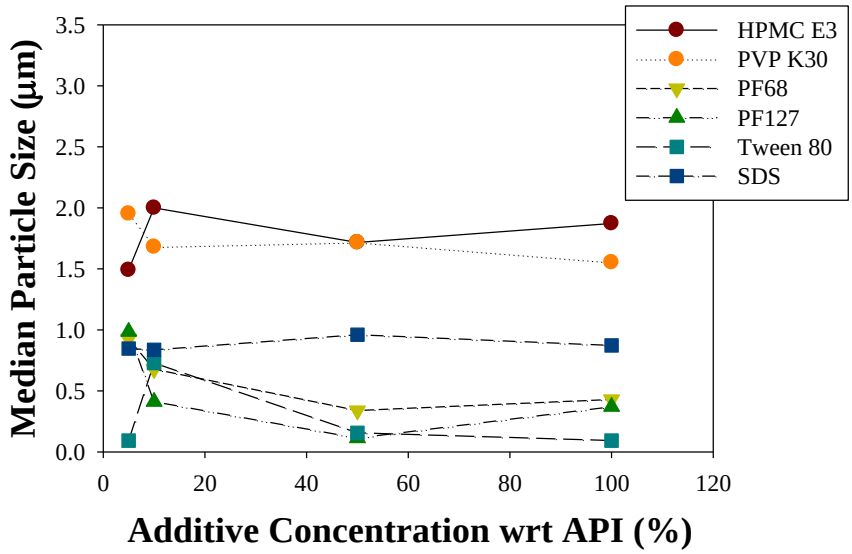

Figure 3.10 Effect of stabilizer and its concentration (wrt drug) on median droplet size.

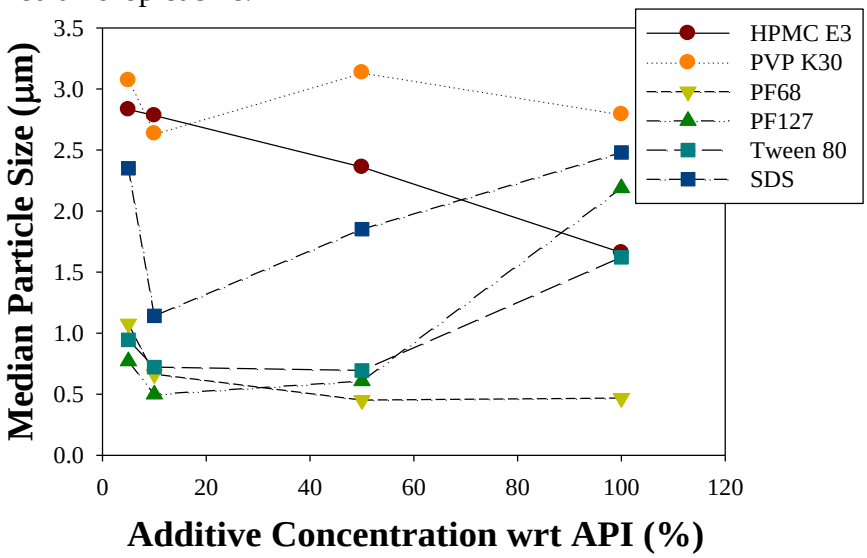

Figure 3.11 Effect of additive and its concentration (wrt drug) on initial median particle size.

Another way to distinguish between surfactant stabilizers is by the use of the hydrophilic-lipophilic-balance (HLB) concept. The HLB value, first introduced by Griffin in 1949, is a quantitative measure of the hydrophilic portion in a non-ionic surfactant. A low HLB number indicates a more lipophilic molecule; a high number, a

hydrophilic molecule (Davies, 1994). As a consequence, for different emulsion systems different optimal HLB values exist which are most suitable in stabilizing the produced droplets (Boyd et al. 1972; Fu et al. 2010). In the case of anionic surfactants, such as SDS, comparative values have been calculated.

Figures 3.12 (a) and (b) respectively depict the median size of emulsion droplets and initial particles against the HLB values and concentrations of surfactants used in the study. The additive concentrations shown on the graphs are wrt drug loading. Figure 3.12a illustrates a general tendency toward an increase in emulsion droplet size for surfactants with higher HLB. T80, with an HLB value of 15, provides the best emulsion stability across all concentrations. Studies done following Griffin's method (Griffin, 1954) suggest that surfactants with HLB values from 12 to 16 are most suitable for stabilizing o/w emulsions. Furthermore, taking into account relative stability in the transition from emulsion to suspension, PF68 was the top performing surfactant. It was observed that suspensions made in the presence of 50% PF68 wrt FNB along with HIU during cooling exhibited 7-day physical stability (stability result not shown). This was thus regarded as the optimum case for the incorporation of ME FNB suspensions into polymer film formation.

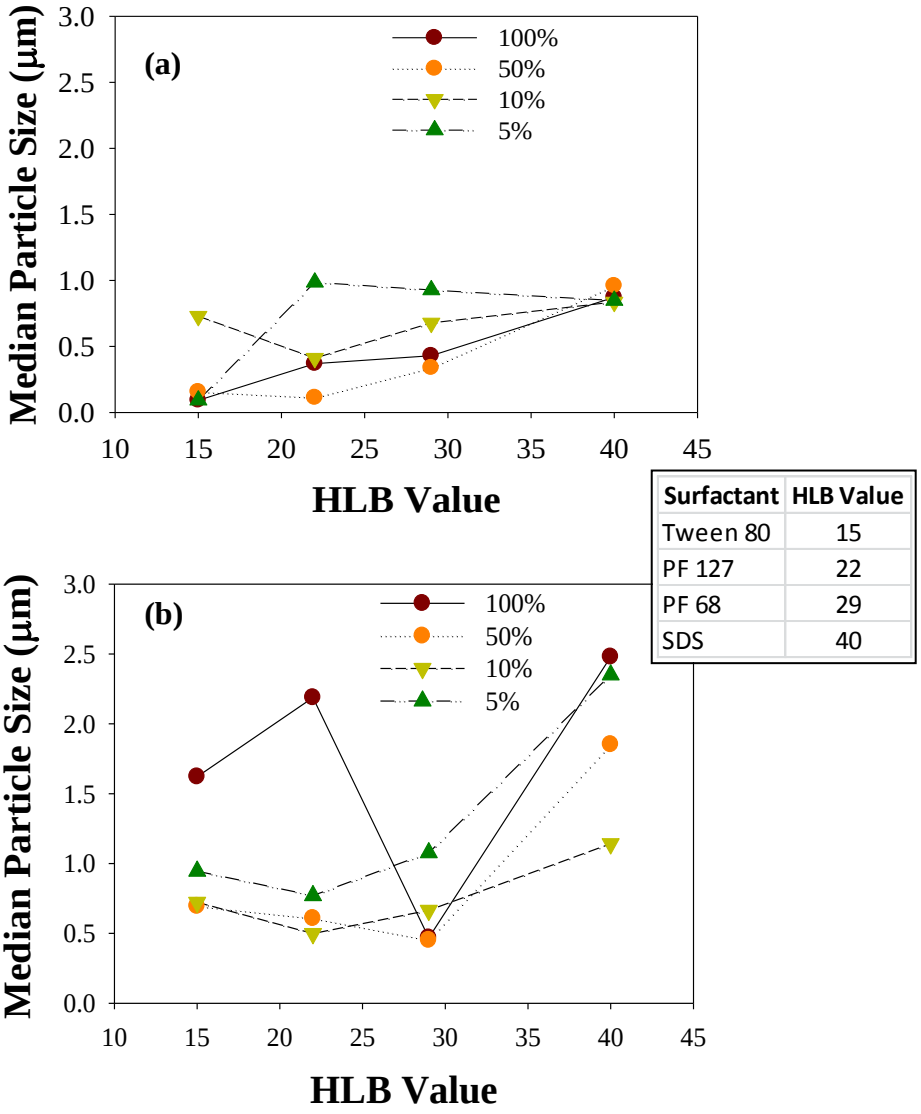

Figure 3.12a and 3.12b Effect of HLB value on droplet (a) and initial (b) median particle sizes. HLB values for each surfactant are provided in the adjoining table.

3.1.3 Effect of Drug

The optimization of the agitation method employed during the cooling step of the ME has been shown. This optimization, along with the knowledge that a screen of available additives will allow for further optimization of particle size distribution and physical

stability, was used to investigate the effects of using another candidate drug for the ME process: ibuprofen.

Figure 3.13 shows a similar trend to the one observed for the stabilizer screen for the ME of FNB: the use of amphiphilic polymeric surfactants PF68, PF127, and T80 allows for smaller initial particle size in comparison to the pure polymers PVP K30 and HPMC E3. The nature of these surfactants allows them to not only adsorb on liquid–liquid interfaces in melt emulsions, but also on particle surfaces upon recrystallization, providing steric stabilization in both cases (Knieke et al. 2013b). At most additive

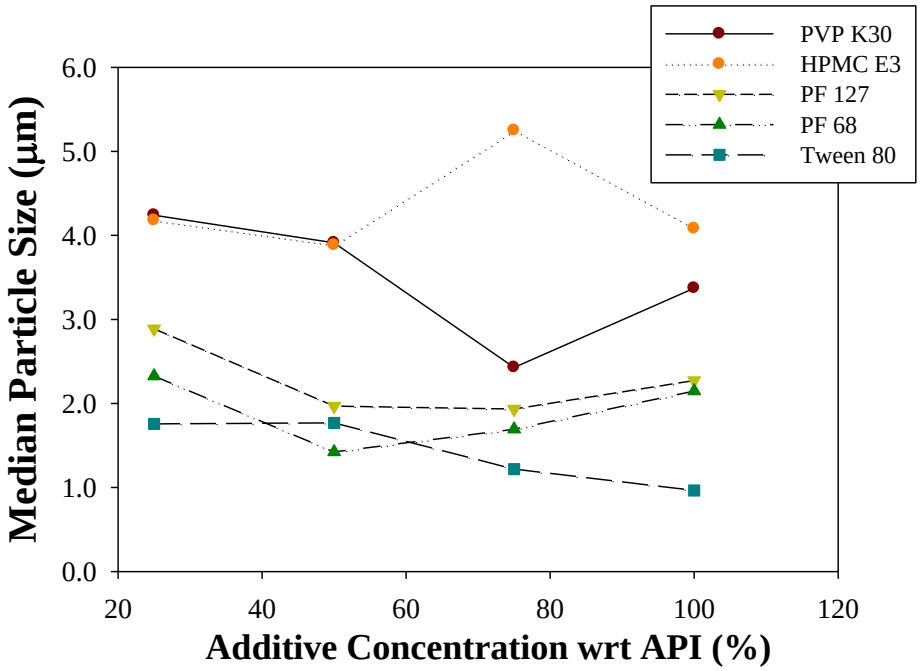

Figure 3.13 Effect of additive and concentration (wrt drug) on the initial median particle size for ibuprofen.

concentrations wrt drug loading (i.e., 25%, 75%, and 100%), a smaller initial median particle size was realized with the use of T80. However, at the concentration optimized for the ME of FNB (50%), PF 68 is once again shown to produce the smallest particles. Again, it was observed that suspensions made in the presence of PF68 and HIU during

cooling exhibited 7-day physical stability (results not shown). This leads to the conclusion that this combination of additive stabilization and agitation during cooling can be considered an optimum for the ME process presented here, and not just for one particular drug system.

3.2 Polymer Films Containing Drug Particles

The redispersion and dissolution behaviors of four polymeric films containing FNB particles processed differently were studied. Processing below refers to the ME process. These four different films were prepared with

a) As-received, non-processed FNB with no stabilizers (control sample)

b) FNB with no stabilizers processed in the presence of high-intensity ultrasound (HIU) during cooling

c) FNB stabilized with PF68 in the presence of HIU during cooling

d) FNB stabilized with PF68 in the absence of HIU during cooling

For the remainder of section 3.2, (a), (b), (c), and (d) notation refer to the cases above. All films had a similar average thickness (~65-70 μm).

3.2.1 Redispersion

In the following figures depicting the redispersion results (Figures 3.14 a, b, c, and d), the "0 min" size refers to the particle size immediately after the cooling step. In the case of the as-received particles, this size refers to that measured after mixing 0.5 g of as-received FNB with 50 ml of DI water for 30 min at ~600 rpm with a magnetic stir bar. In all cases, the film precursor size was measured after the original suspensions were mixed with film solution for 5 h to form a so-called film precursor suspension. The redispersion

particle size was measured after a 5.07 cm^2 (2.54 cm diameter) disc of each film produced was dispersed in a 50 ml beaker containing 10 ml de-ionized water and stirred magnetically at a speed of ~475 rpm for 5 min. For each size measurement, an aliquot of the sample was pulled from the container while the suspension was being stirred and particle size was measured in the LS13320.

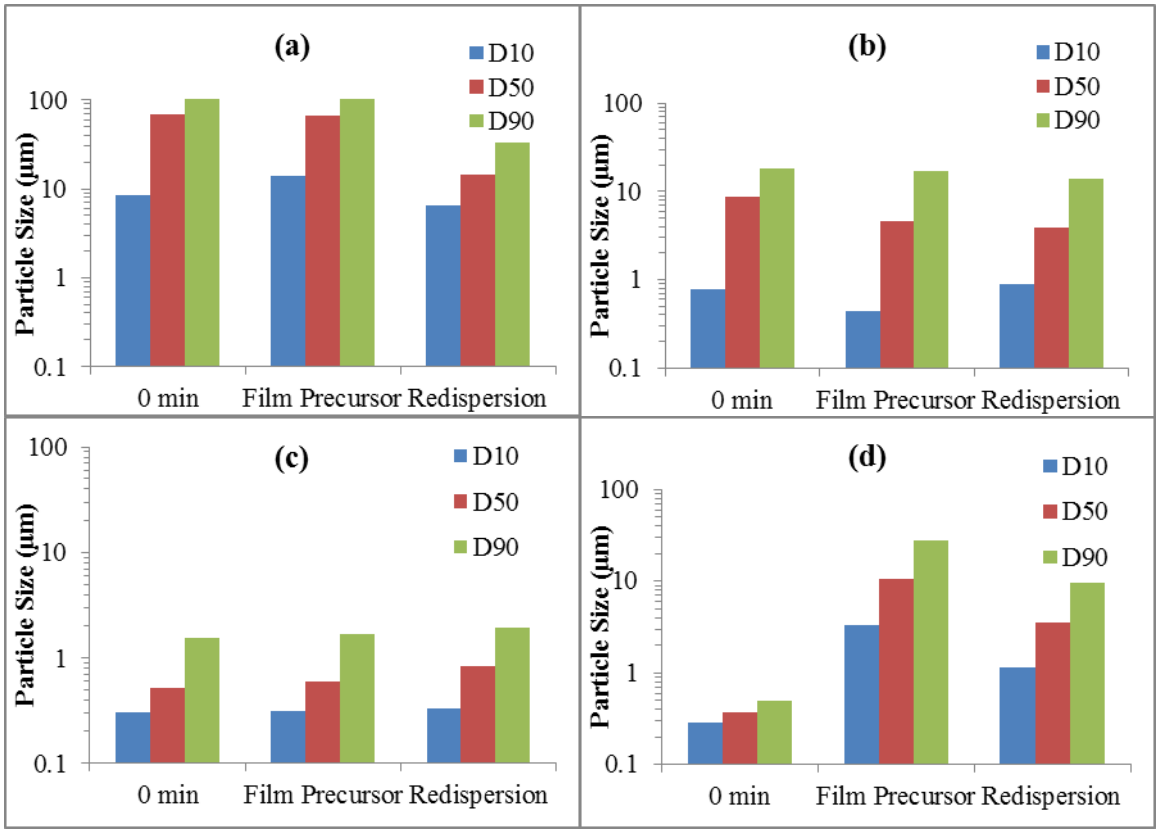

Figure 3.14 (a)-(d) Particle size as a function of time during film formation process. Film (a) contains as-received, non-processed particles; (b) contains ME FNB particles produced without stabilizers and with HIU during cooling; (c) contains ME FNB particles produced with both PF68 and HIU during cooling; (d) contains ME FNB particles produced with PF68 and without HIU during cooling. "0 min" refers to particle size immediately after mixing (a) or cooling (b-d). "Film Precursor" refers to suspension after 5 h mixing with polymer film solution. "Redispersion" refers to particles redispersed from films in deionized water.

As expected, the large as-received FNB particles (a) remained large throughout the entire film formation process, as large particles were observed upon film redispersion.

Some size reduction occurred, most likely as a result of the mixing step. Particle disaggregation is not a typical phenomenon observed during the drying of films; it is known that colloidal particles confined in liquid films experience attractive capillary forces, which can cause particle aggregation (Danov et al. 2001). Aggregated particles produced without additives in the presence of HIU during cooling were recovered in the redispersion of film (b). The film containing FNB particles produced in the presence of HIU during cooling and PF68 (c) exhibited smaller sizes due to the prevention of particle aggregation via steric stabilization. Film containing those particles produced with PF68 and in the absence of HIU during cooling exhibited dramatic particle growth during the mixing step of the film formation process. Without the enhanced stabilizer adsorption and crystallization dynamics assumed to exist in the presence of HIU, this lack of physical stability was to be expected.

3.2.2 Dissolution

The dissolution profiles of the four discussed films, whose redispersion behaviors were illustrated in Figures 3.14a–d, are depicted in Figure 3.15. The first things to notice are the considerable error bars associated with the dissolution profiles of films (a) and (b). These are representations of the poor content uniformity of these films, as made evident by their respective assays (Table 3.1). The absence of proper stabilization of drug suspensions and the presence of large particles/aggregates in films (a) and (b) may have resulted in sedimentation and poor dispersion in the polymer films during drying. The aggregates have smaller surface area than the constituent fine primary particles, which explains the slower dissolution of FNB from the films produced in cases (a) and (b). The smaller variability of the dissolution and faster FNB dissolution associated with the

profiles of films (c) and (d) – those made with PF68 – support the fact that proper additive stabilization in drug suspensions is necessary for reducing the extent of drug particle aggregation and this producing polymer films homogeneously laden with drug particles.

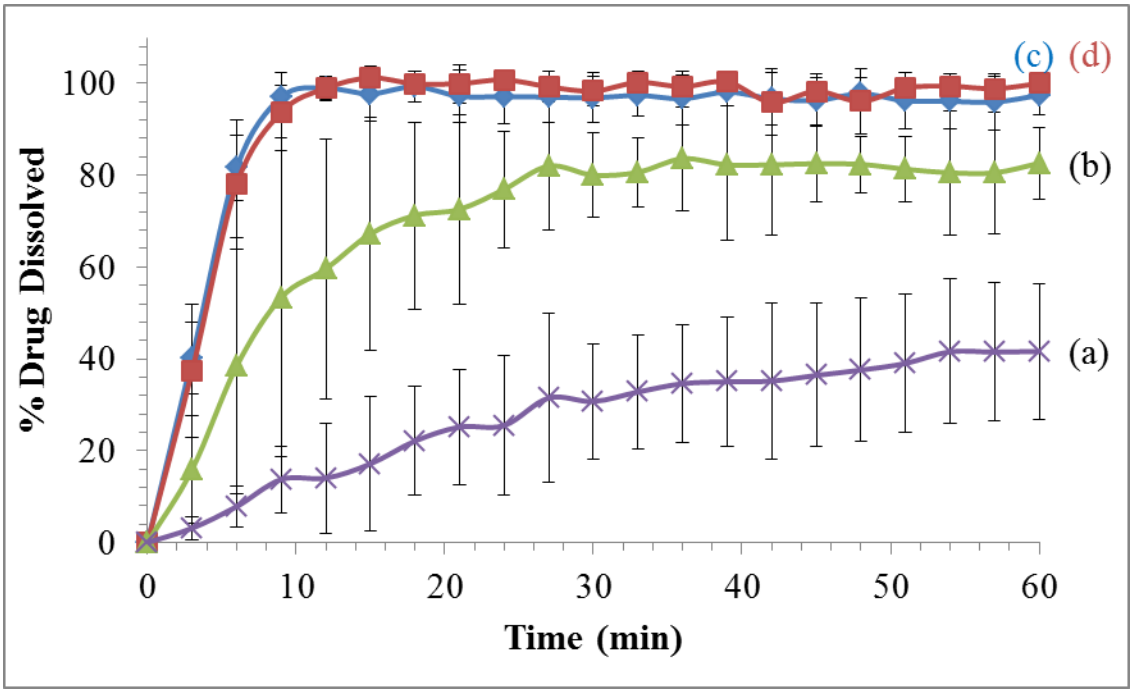

Figure 3.15 Effect of stabilization and process conditions for producing FNB suspensions on the dissolution behavior of polymer strip films.

Table 3.1 FNB Content in Polymer Films

Film	Loading of drug (% w/w)[a]	Drug content (% w/w)
a	4.76	1.64±38.14
b	4.76	2.30±21.56
c	4.65	4.56±2.40
d	4.65	4.07±2.27

[a] % w/w is with respect to weight of films after drying.

The next thing to notice is the fact that films (c) and (d) exhibited almost the same dissolution behavior. It stands to mention that this has occurred in spite of the fact that redispersion results show larger particles being released from film (d) than from film (c). Firstly, as dissolution medium, 600 mL of an 18.7 mM SDS solution were used at 37 ± 0.5 °C; the SDS concentration was higher than the CMC value, which caused an increase in the solubility of FNB in water. It is possible that the dissolution method has poor discriminatory power upon use of an SDS solution of this concentration, which was somewhat required to get detectable assays during the dissolution test. By this reasoning, however, and the fact that all films studied were of similar thicknesses, films (b) and (d) should also have similar dissolution profiles, since the redispersion results of these two films reveal the presence of similarly-sized particles/aggregates. This is not the case, however. A possible secondary explanation for the indistinguishable nature of the dissolution profiles of films (c) and (d) may lie in the use of PF68. This polymeric surfactant, unlike other surfactants, does not have a distinct CMC (Ozturk and Hu, 2005; Chalmers et al. 2009). In this way it is speculated that PF68 in films (c) and (d) may form micelles which aid in the solubilization of FNB particles. In conjunction with excess of surfactant SDS present in the dissolution medium, this may be sufficient to ensure fast dissolution of FNB particles from polymer strip films.

CHAPTER 4

COMPETITIVE ASSESSMENT

As expressed in the Introduction, the two classical methods for improving the solubility of poorly water-soluble drugs are reduction of crystalline particle size and the formation of the amorphous form of the drug. This chapter serves as a competitive assessment of these approaches.

4.1 Nanoparticle Technology

Most BCS Class II drugs are characterized by both low equilibrium solubility and a low dissolution rate, due to high stability of these drugs in the crystalline form. It can be understood, therefore, that dissolution will not be a fast process, since the drug molecules are at a preferred energy state. In order to make complete dissolution possible in a time frame which would allow for increased drug efficacy, the dissolution process should be fast. Consequentially, increasing the dissolution rate is an effective way to increase the uptake of a poorly water-soluble drug into the body, enhancing its bioavailability. A modified version of the previously-mentioned Noyes-Whitney equation (1897) describes how drug nanoparticles, exhibiting large surface area with small particle sizes, will enable high dissolution rate and thus bioavailability:

$$\frac{dm}{dt} = k_0 A(C_S - C) \tag{4.1}$$

Here, k_0 is the overall solute transfer coefficient defined by $1/k_0 = 1/k_i + 1/k_c$, where k_i and k_c are the interface rearrangement constant and the external mass transfer coefficient,

respectively, C_S is the saturation solubility, C is the concentration in the bulk solution, A is the total surface area, and m is the amount dissolved at time t. The major contribution to the enhanced dissolution rate of the drug is through the increase of both the specific surface area and the total area A of the particles due to size reduction (Bhakay et al. 2011). Furthermore, if particles are very small (i.e., < 100 nm), solubility can be described as a function of particle size according to the Kelvin equation (Tadros, 2013):

$$S(r) = S_{\infty} exp\left(\frac{2\gamma V_m}{rRT}\right) \tag{4.2}$$

In this equation, $S(r)$ is the solubility as a function of the radius r, S_{∞} is the solubility of a coarse, crystalline particle, γ is the interfacial tension between drug and solvent, V_m is the molar volume of the drug, R is the gas constant, and T is the temperature. This equation clearly shows that solubility increases immensely as the particle size decreases. As a result of the exponential function, this effect becomes drastic at particle sizes < 100 nm.

The effectiveness of nanoparticle and fine particle suspensions of poorly water-soluble drugs in enhancing bioavailability has been proven in animal models and clinical trials with various administration routes, including oral, pulmonary, ocular, and parenteral delivery (Liversidge, G. G., and K. C. Cundy., 1995; W. Yang et al. 2010). The production of suspensions by particle size reduction has been well-studied at NJIT using various techniques, including wet stirred media milling (WSMM) (Bhakay et al. 2011; Bilgili and Afolabi, 2012; Monteiro et al. 2013; Knieke et al. 2013a), liquid antisolvent precipitation (LASP) (Dalvi and Davé, 2009; Beck et al. 2010; Dalvi and Davé, 2010), rapid expansion of supercritical solutions in a liquid solvent (RESOLV)

(Dalvi et al. 2013) and melt emulsification (ME) (Knieke et al. 2013b). In addition, drug suspensions produced by WSMM and LASP have been incorporated into the formation of polymer strip films for solid dosage forms (Sievens-Figueroa et al. 2012; Beck et al. 2013). In all of the above studies, it has been shown that the dissolution rate of nanoparticles (particles $\leq$ 1000 nm in size in prevalent pharmaceutical literature) resulting from the processing is significantly enhanced in comparison to their as-received, unprocessed counterparts. The sizes of as-received drug particles are typically on the order of tens of microns, whereas the resultant suspensions from WSMM, the most robust of the aforementioned techniques, may contain particles as small as 50–250 nanometers while maintaining drug loadings up to 30% (w/w) wrt deionized water (Afolabi et al. 2013, under review). In all cases mentioned, the final drug product was confirmed to be crystalline in nature.

4.2 Amorphous Particles

Several studies have used thermodynamic principles to predict the potential impact of the amorphous form on bioavailability based on *in vitro* dissolution profiles and/or thermal analysis for oral delivery (Hancock and Parks, 2000; Hancock and Zografi, 1997). The amorphous form of a drug has a higher thermodynamic chemical potential than its crystalline counterpart (Hilden and Morris, 2004). The higher thermodynamic activity of the drug can produce supersaturated solutions (Matteucci et al. 2008), thereby providing an opportunity to enhance absorption and bioavailability. In a study by W. Yang et al. (2010), the effect of supersaturation on bioavailability of inhaled nebulized aerosols was compared for amorphous versus crystalline nanoparticulate dispersions. Nanocrystalline formulations of itraconazole (ITZ), another BCS Class II drug, were made by wet

milling, whereas amorphous nanostructured aggregates were made by an ultra-rapid freezing process. Dissolution tests revealed the extent of supersaturation was 4.7-times higher for the amorphous nanostructured aggregates versus nanocrystalline particles, though their dissolution rates were similar. However, the increase in the systemic bioavailability for the amorphous versus crystalline dispersion of about 3.8 times was approximately the same as the increase in supersaturation measured in vitro in simulated lung fluid. The high supersaturation, favored by rapid dissolution of amorphous nanoparticles, prior to particle clearance or crystallization favors high permeability into the bloodstream. It was concluded that pulmonary delivery of amorphous nanoparticle formulations of extremely poorly water-soluble drugs is beneficial for both local and systemic therapy (W. Yang et al. 2010).

Conversely, a study by Meulenaar et al. (2013) reported anomalous dissolution behavior of amorphous capecitabine, a chemotherapeutic drug belonging to BCS Class III. These drugs are characterized by their low permeability and high solubility, which are opposite characteristics from those of Class II drugs. In contrast to what is expected from thermodynamic theory, amorphous capecitabine dissolved significantly slower in comparison to its crystalline counterpart. Experiments revealed a "gelling" phenomenon of amorphous capecitabine in an aqueous environment. The "gel", which was immediately formed upon contact with water, entrapped the capecitabine and significantly slowed down its dissolution. This "gelling" property was hypothesized to be related to the low glass transition temperature (T_g = 19 °C) of amorphous capecitabine, resulting in an instant collapse ("gelling") in an aqueous environment. It was suggested that this dissolution behavior be applied in the development of a sustained release dosage

form (Meulenaar et al. 2013). However, in the interest of increasing the dissolution rate of a drug to prevent its excretion before being therapeutically applicable, it seems that the production of the amorphous form is not always a clear advantage.

Amorphous APIs are susceptible to recrystallization due to the metastability of this high-energy state. Therefore, there is a need to physically stabilize them as solid dispersions in polymeric carriers. Common routes to the production of stable amorphous state drug particles include quenching of melts (Pokharkar et al. 2006; Lakshman et al. 2008), rapid precipitation by antisolvent addition (Chen et al. 2006; Zhang et al. 2006), freeze-drying (Pikal, 1994), and spray-drying (Broadhead et al. 1992; Shen et al. 2010).

Amorphous drugs represent opportunity and, in some cases, necessity in pharmaceutical development. The opportunity arises from the potential to improve bioavailability via use of an amorphous form, rather than a crystalline form. Challenges exist in predicting the solubility enhancement ratio of amorphous solids relative to their crystalline counterparts. These arise due to several factors: (a) a change in thermodynamic activity of amorphous solute as it absorbs water, (b) in the case of ionizable compounds in pure water, the difference in degree of ionization of the solute at different concentrations, and (c) experimentally, onset of crystallization frustrates the attempt to determine the equilibrium solubility of the amorphous solid (Murdande et al. 2010).

It is sometimes the case that no crystalline form is available, in which case it is necessary to deal with the amorphous form; cases where this consideration exists include the use of salts, co-crystals or pharmaceutically-acceptable solvates. The amorphous drug may be isolated by a variety of methods including precipitation or desolvation of a

solvate, and the ease of isolation will be affected by the glass transition temperature (T_g) and the extent to which the T_g is lowered by residual solvent (Hancock and Zografi, 1994). In some cases, amorphous salts have been found to have a higher T_g than the free acid or base (Towler et al. 2008; Tong et al. 2002), and amorphous dispersions may also be used to improve the physical properties.

In addition, a special interest of this thesis was to investigate the feasibility of producing amorphous drug particles by means of melt emulsification and the polymer film formation process. Proof-of-concept experiments are discussed in Appendix A.

CHAPTER 5

CONCLUSIONS

5.1 Melt Emulsification

The process of preparing stable FNB nanosuspensions by ME was optimized. In general, the drug nanoparticles produced right after cooling tend to aggregate and grow significantly during storage. Hence, ensuring the physical stability of the ME-based suspensions appears to be a challenging task. In this thesis, the effects of different agitation methods during the cooling step and stabilizer type/concentration on particle size after cooling and during storage were investigated. The physical stability of the suspensions was improved when proper mechanical disturbance was applied to the emulsions during the cooling step via high-intensity ultrasound (HIU). The median particle size for FNB suspensions produced with HIU during cooling, originally stabilized using 50% PF127 wrt drug loading, was observed to be between 500–600 nm. A stabilizer screening study was performed to ensure the optimized use of additives. Surfactants alone performed more favorably than steric polymers alone in stabilizing both emulsions and subsequent suspensions. The optimum additive was a polymeric surfactant PF68 at a concentration of 50% wrt drug loading. This optimization approach was applied to ibuprofen, another poorly water-soluble drug. The median particle size for suspensions produced with HIU during cooling and stabilized using 50% PF68 wrt drug loading, was observed to be between 400–500 nm for FNB and between 1400–1500 nm for IBU. Combinations of PF68 with pure polymers HPMC E3 or PVP K30 or with T80 (the best emulsifying surfactant) were found to perform less favorably than PF68 alone (results not shown). FNB suspensions produced in the presence of PF68 and HIU during

cooling were incorporated into the polymer film production process. The dissolution and redispersion behaviors of this optimized case were compared to those of films containing non-optimized suspensions.

5.2 Polymer Films Containing Drug Particles

The effects of particle size, ME conditions during cooling, and additive stabilization on the redispersion and dissolution behavior of polymer films containing FNB drug particles were studied. As expected, the film containing non-processed, as-received particles exhibited recovery of large particles upon redispersion and a poor dissolution profile in 18.7 mM SDS medium. The use of only HIU during cooling to process melt emulsions was shown to produce a suspension with particle aggregates due to the absence of proper additive stabilization. The redispersed particle size distribution of this suspension was similar to that of the suspension produced in the presence of PF 68 and the absence of HIU, yet the films containing these respective suspensions exhibited very different dissolution behavior. Furthermore, regardless of a significant difference in redispersed particle size, both of the films containing particles produced in the presence of PF68 expressed the same dissolution profile. This points to the good stabilizing action of PF68, in conjunction with the excess SDS (concentration above the CMC) present in the dissolution medium.

5.3 Future Work

For future work, it is proposed that alternative cooling techniques be investigated for the production of drug nanosuspensions by ME. The use of cryogenic materials such as liquid nitrogen may be employed to instantly quench nano-emulsion droplets. This

technique alone may lead to producing amorphous materials, given the extremely high degree of expected supercooling. In conjunction with proper application of HIU, cryogenic cooling may also lead to stable crystalline nanosuspensions with smaller particle size distributions than the ones observed in this study. In a longer-term study, other candidate drugs for ME may be further investigated, with careful consideration of drug crystallization tendencies (Baird et al. 2010).

APPENDIX A

INVESTIGATING AMORPHOUS PARTICLE FORMATION

Figures A.1 to A.3 depict DSC characterization of particle and film products formed by the following outlined methods.

A.1 Amorphous Particles in Melt Emulsification

The production of amorphous particles is a topic of great interest in pharmaceutical research. It has been discussed that these high-energy particles have been shown to exhibit improved solubility in comparison to their crystalline counterparts. As proof of concept, an experiment was performed to investigate how particles that are initially amorphous behave when processed by the melt emulsification technique presented in this thesis.

A.1.1 Amorphous Particle Production in Solid Dispersion

Amorphous particles of FNB were produced according to the method outlined in Mahapatra et al. (2010). A solid dispersion of FNB with polyethylene glycol (PEG) 3000, a hydrophilic polymer, containing a weight ratio of drug and polymer (1:5) was prepared by melting or fusion method. In this method, an appropriate amount of drug and hydrophilic polymer was melted in a beaker on a hot plate maintained at a temperature slightly above the corresponding melting point of the drug. The mixture was cooled rapidly by placing the beaker in a –3.0 °C bath for about 10 minutes and solidified. The SD was then ground into smaller particles in a mortar and pestle; this powder was

immediately characterized by DSC (Figure A.1), and then stored at room temperature until further study.

The DSC thermograms (without the presence of an endothermic peak at 80 °C characteristic of crystalline FNB) depicted in Figure A.1 confirms both that amorphous FNB particles were indeed produced by the solid dispersion and that the particles remained amorphous after one week. The endothermic peak shown corresponds to the melting point of PEG 3000.

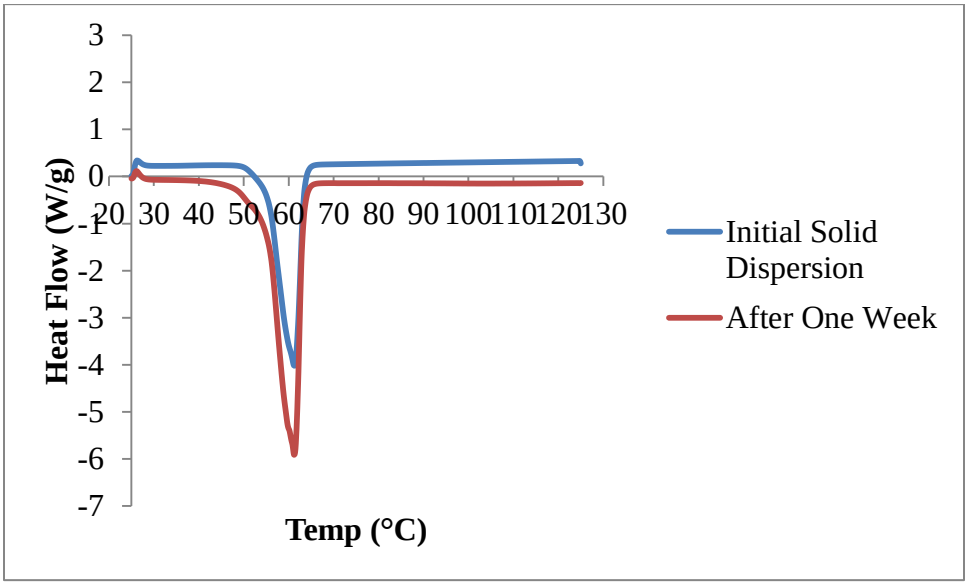

Figure A.1 DSC thermograms of fenofibrate solid dispersion in PEG 3000 after production and one week after production.

A.1.2 Melt Emulsification of Solid Dispersion

A 0.5 g of the solid dispersion powder sample was subjected to the optimized ME process (HIU during cooling and 50% PF68 loading wrt the solid dispersion powder) in a 50 ml batch. In order to investigate the nature of particles, the produced suspension was syringe filtered through a 0.2 µm filter thirty minutes after the cooling step. The filtrate was dried at room temperature and characterized by DSC (Figure A.2). The presence of an

endothermic peak at 80 °C confirms that particles which were initially amorphous were recrystallized during the course of the ME process. Given the fact that the particles were initially amorphous, it is suggested that the ME process presented in this thesis is not suitable for the production of amorphous particles.

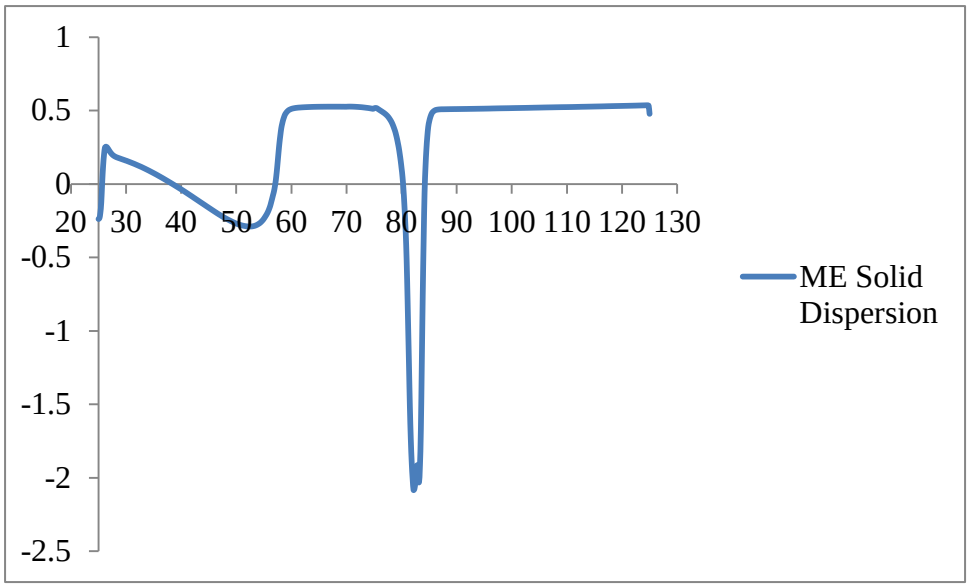

Figure A.2 DSC thermogram of filtrate from suspension of FNB solid dispersion processed by melt emulsification.

A.2 Solid Dispersions by Melt-Quench Method for Film Production

The production of stable amorphous drug particles by solid dispersion in a hydrophilic polymer matrix has been investigated. Polymer strip films have been discussed as an attractive solid dosage delivery system. The combination of the two concepts is an interesting, simple means of producing a solid dosage form infused with drug particles with enhanced solubility. The details of a proof-of-concept experiment and subsequent product characterization are presented here.

A.2.1 Experimental Procedure

0.5 grams of FNB were melted in a beaker with 2.5g of PEG 3000, to maintain congruence with the ratio of drug to polymer in the solid dispersion experiments. When complete melting was observed, 50 g of pre-heated polymer film solution (15% HPMC E15, 5% Glycerin; w/w) was added to the beaker. The system was then subjected to cooling in a –3.0 °C thermostatic saline bath while being mixed by a dual impeller mixer at ~300 rpm for 10 minutes. The final temperature of the system was 8 °C. After allowing for the foam on top of the film suspension to reduce (about five minutes), a film was cast and dried according to previously described techniques. After drying, the film was characterized by DSC (Figure A.3) to observe the crystallinity of the embedded FNB particles.

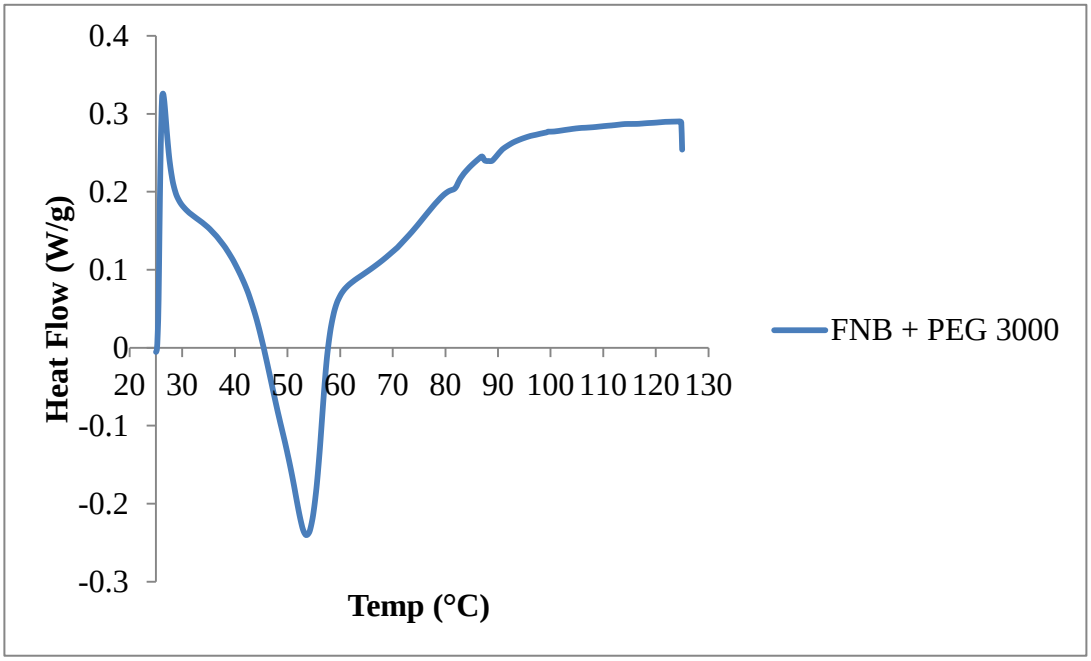

Figure A.3 DSC thermogram of film containing FNB particles produced by melt-quench method.

The lack of a distinct endothermic peak at 80 °C suggests that the FNB particles in the film are at least partially, if not fully, amorphous. Once again, the peak shown corresponds to the melting point of PEG 3000, just as in the previous solid dispersion experiment.

A.2.1 Assessment of Approach

The use of this technique allows for the possibility to combine the production of both amorphous FNB particles and films laden with amorphous particles into a simple melt-quench technique. The experiment required minimal equipment– a heating plate, a cooling bath, and a variable-speed impeller– and resulted in a viscous suspension suitable for film formation. The final film product seemed homogenously laden with particles upon observation. This method does not require the use of surfactants which may be considered unfavorable for consumer dosage formulations. Furthermore, the lack of an aqueous environment accommodates drugs that have melting temperatures greater than 100 °C; this is a major limitation of the melt emulsification process. Given the simplicity of the technique and the possibility of creating amorphous particles in a final dosage form in one step, this concept deserves consideration for future study.

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