Development and evaluation of self-nanoemulsifying drug delivery systems for oral delivery of indomethacin

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Declaration

This thesis describes research conducted in King Saud University in collaboration with UCL School of Pharmacy under the guidance of Dr. Susan Barker, Prof. Duncan Craig and Prof. Hanaa Elsaghir. I declare that the work presented in this thesis is my own and has not previously been submitted for any degree other than that of the degree of Doctor of Philosophy at the University College London. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature:

Date:

Abstract

In this study, indomethacin-loaded self-nanoemulsifying drug delivery systems (SNEDDS) were developed in liquid, solid and carrier-mediated formulations in order to improve the solubility of this model poorly water soluble drug.

Liquid SNEDDS based on Capryol™ 90 (oil phase), Cremophor® RH 40 (surfactant) and Transcutol® HP (co-surfactant) were thermodynamically stable and produced clear nanoemulsions upon dilution. Optimized liquid formulations were transformed into solid SNEDDS by adsorption onto the inert carriers Syloid® XDP 3150, Neusilin® US2 and Florite® PS-200. Ratios of adsorbent: liquid SNEDDS of 1:1.5 and 1:2 resulted in solid SNEDDS formulations that exhibited fair to passable powder flow properties. Carrier-based solid SNEDDS formulations were developed using the solid self-emulsifying carriers Gelucire® 44/14 and Gelucire® 48/16 and prepared by hot melt extrusion.

The absorbent-based solid SNEDDS maintained the self-nanoemulsification properties of the original liquid SNEDDS formulations, with solid state analysis suggesting that the drug had remained in a dissolved state within these formulations. Similarly, physical characterization of the carrier-based solid SNEDDS formulations indicated that the drug was molecularly dispersed within the system and that the self-nanoemulsifying properties of the carrier were unchanged. The only exception was those formulations prepared at the highest drug: carrier ratio (3: 10). For both absorbent-based and carrier-based solid SNEDDS, the in vitro dissolution efficiency was significantly higher than that obtained for the pure drug. However, incorporation of adsorbents into Gelucire®-based solid SNEDDS formulations resulted in reduced dissolution of the drug. Gelucire®48/16-based solid SNEDDS prepared at 50^{o}C were more physically stable to storage at 30^{o}C/75% RH for 6 months than formulations processed at 40^{o}C, suggesting that complete melting of the carrier during manufacture is essential for production of physically stable formulations.

Overall, a range of liquid, solid and carrier-based SNEDDS formulations were successfully developed and offer useful alternatives to improving the solubility of poorly water-soluble drugs.

Impact statement

The oral route is the most commonly used method for administration of drugs, with nearly 80% of the marketed dosage forms being delivered orally. This route of drug administration is the most convenient and non-invasive, leading to better patient compliance. Aqueous solubility of drugs is one of the most important factors that determine their dissolution performance and hence oral absorption and bioavailability. About 70% of new drug substances are poorly water soluble and exhibit slow dissolution rates and often incomplete oral bioavailability. Therefore, different formulation strategies have been investigated for enhancing the solubility of poorly soluble drugs with the aim of improving oral bioavailability.

This study investigated the utility of self-nanoemulsifying drug delivery systems (SNEDDS) as a lipid-based formulation approach to improve solubility and in vitro dissolution performance of the poorly soluble model drug, indomethacin. For this purpose, different drug-loaded SNEDDS were prepared in liquid, solid and carrier-based formulations. Liquid indomethacin-loaded SNEDDS formulations prepared with Capryol™ 90 (oil phase), Cremophor® RH 40 (surfactant) and Transcutol® HP (co-surfactant) showed maximum self-nanoemulsification efficiency and the smallest droplet size compared to other liquid SNEDDS formulations tested. Solid SNEDDS of indomethacin were produced by either adsorbing the optimum liquid SNEDDS on to different adsorbents like Syloid® XDP 3150, Neusilin® US2 and Florite® PS-200 or by directly dissolving the drug in semisolid or solid self-emulsifying carriers such as Gelucire®44/14 and Gelucire®48/16, adopting the hot melt extrusion (HME) technique for manufacture. All solid SNEDDS produced demonstrated self-nanoemulsification properties after dilution with aqueous media and showed significantly higher in vitro dissolution performance compared to the pure drug. Physicochemical characterization of these solid formulations proved that the drug remained in a solubilized state within the formulations.

The results of this study enhance and add to the knowledge in the literature on the improvement of solubility of poorly soluble drugs and the development of industrially-feasible lipid-based oral formulations. Adoption of the novel adsorbents used in this study, such as Florite® PS-200 and Neusilin®US2, to solidify liquid SNEDDS of indomethacin could be applicable on an industrial level to produce homogenous powder formulations, especially when liquid SNEDDS are sprayed on to the adsorbent and mixed with high shear mixers. These powder formulations could be further extended on the industrial scale to formulate self-emulsifying tablets that will contribute to increasing the currently limited number of commercially available solid SNEDDS dosage forms.

Successful development of solid SNEDDS of indomethacin by direct dissolving of the drug in solid self-emulsifying carriers may increase the possibility of using these types of carriers for direct production of highly stable solid SNEDDS formulations. Utilization of hot melt extrusion technology instead of traditional melting methods for processing solid self-emulsifying carriers and developing stable solid SNEDDS formulations will certainly ease the scaling up of this continuous process to the industrial level.

Overall, a range of liquid, solid and carrier-based SNEDDS formulations were successfully developed and provide alternative methods of improving the solubility of poorly water-soluble drugs. These formulations are potentially scalable, offering alternative industrially-feasible formulations of poorly soluble drugs.

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List of abbreviations

ANOVA	Analysis of variance
BCS	Biopharmaceutics classification system
CI	Compressibility index
DE	Dissolution efficiency
DLS	Dynamic light scattering
DSC	Differential scanning calorimetry
FTIR	Fourier transform infrared
GIT	Gastrointestinal tract
GRAS	Generally regarded as safe
HLB	Hydrophile lipophile balance
HME	Hot melt extrusion
HR	Hausner's ratio
ICH	International conference on harmonization
LCT	Long chain triglyceride
LEMS	Liquid encapsulation micro-spray sealing
LFCS	Liquid formulation classification system
MCC	Microcrystalline cellulose
MCT	Medium chain triglyceride
MDT	Mean dissolution time
PCS	Photon correlation spectroscopy
PDI	Polydispersity index
PEG	Polyethylene glycol
SEDDS	Self-emulsifying drug delivery system
SEM	Scanning electron microscopy
SEOF	Self-emulsifying oil formulation
SLN	Solid lipid nanoparticles
SMEDDS	Self-microemulsifying drug delivery system
SNEDDS	Self-nanoemulsifying drug delivery system
S-SEDDS	Solid self-emulsifying drug delivery system
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
TPGS	D-α-tocopheryl polyethylene glycol 1000 succinate
XRD	X-ray diffraction

Chapter 1

Introduction

1.1. Oral route of drug administration

The oral route of drug administration is the most commonly employed method, administering nearly 80% of the commercially available dosage forms (Morishita and Peppas, 2012). Oral drug administration offers the most convenient and non-invasive way of drug delivery that results in better patient compliance. Also, it is the most cost-effective to pharmaceutical industry with a wide range of dosage form designs and the least sterility limitations (Krishnaiah, 2010). However, the bioavailability of drugs administered orally depends on many factors that are related to the aqueous solubility of the drug in gastro-intestinal (GI) fluids, drug intestinal permeability, drug stability in the GI environment, and drug metabolism in the liver (Kalepu et al., 2013, Krishnaiah, 2010, Porter et al., 2008). Aqueous solubility and intestinal permeability of the drug are considered as the key factors that affect oral absorption and bioavailability (Beig et al., 2012, Krishnaiah, 2010, Vieth et al., 2004).

With significant increase in the number of pharmacologically active compounds discovered, it was reported that nearly 70% of new drug candidates exhibit poor aqueous solubility (Ku and Dulin, 2012). Formulation of these compounds into oral dosage forms with improved bioavailability represents a challenge in the area of pharmaceutical research (Buckley et al., 2012, Dahan and Hoffman, 2008, Lipinski et al., 2001).

Aqueous solubility is the primary factor that determines the bioavailability of orally administered drugs by affecting their dissolution properties (Kawabata et al., 2011). Poorly water soluble drugs, with aqueous solubility less than 100 µg/ml and administered in doses more than 100 mg, exhibit a slow dissolution rate and often incomplete bioavailability. For these drugs, the dose/solubility ratio or the volume of gastrointestinal fluids required to dissolve the administered dose may exceed the available volume of fluids and this lead to incomplete bioavailability of oral dosage forms (Horter and Dressman, 2001). Attempts to improve oral efficacy of such drugs by increasing the dose may result in gastrointestinal toxicity and therefore reduced patient compliance. In addition, poor powder properties, and high cost of manufacturing may arise during drug product development (Kawabata et al., 2011). Therefore, different approaches to improve aqueous solubility of poorly soluble drugs have been investigated. For example, modification of the chemical structure or design of prodrugs during the early optimization phase of poorly soluble drugs has been studied as possible options to enhance the aqueous solubility. Also, other approaches that focused on improving the dissolution of poorly soluble drugs have been considered as alternative methods of increasing the solubility of poorly soluble drugs (Kawabata et al., 2011). The following section

describes different formulation approaches that are based on the biopharmaceutical characteristics of drug substances.

1.2. Biopharmaceutics classification system and different formulation approaches

Application of the biopharmaceutics classification system (BCS) helps to understand the physicochemical and biopharmaceutical properties of drugs in order to improve developing pharmaceutical drug products (Kawabata et al., 2011). According to BCS, drugs are grouped into four classes (I – IV) - depending on their aqueous solubility and intestinal permeability (**Figure 1.1**).

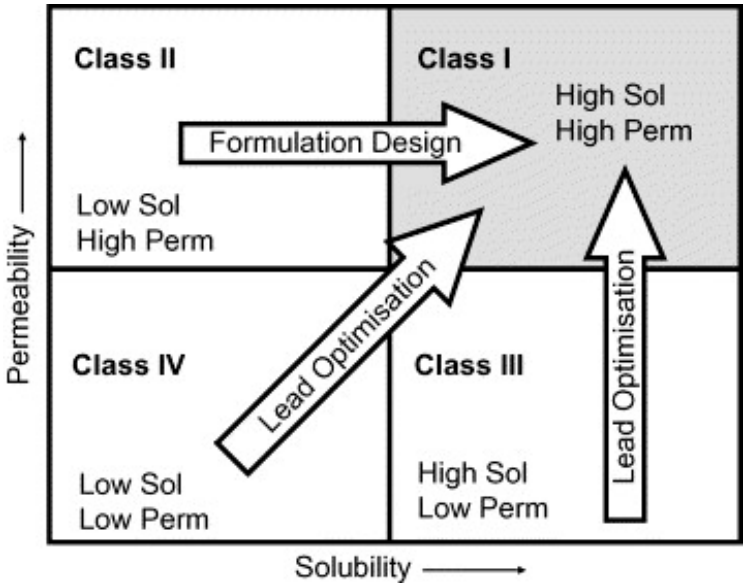


Figure 1.1 Biopharmaceutics classification system (BCS) (Pouton, 2006)

According to FDA (2000), a drug substance is said to be “highly soluble” when the maximum dose is soluble in an amount of 250 ml or less of aqueous medium over the pH range of 1 – 7.5 at 37^{o}C. Also, FDA considers the drug to be “highly permeable” when 90% or more of the administered dose is absorbed in humans.

Generally, oral absorption of class I drugs (high solubility and high permeability) has no rate-limiting step and, therefore, well-designed conventional solid dosage forms will ensure rapid dissolution in gastrointestinal tract (Pouton, 2006). On the other hand, oral bioavailability of BCS class II drugs (low solubility and high permeability) is thought to be dissolution rate limited and enhancement of the dissolution rate of the drug will maximize its oral absorption (Kawabata et al., 2011, Pouton, 2006). Considering class III drugs (high solubility and low permeability), their oral absorption is limited by membrane

permeability. Improved efficacy of such drugs can be achieved upon administration of a high dose (Desai et al., 2012) or upon addition of permeation enhancers, such as fatty acids, bile salts or surfactants, to the drug product (Kawabata et al., 2011). Finally, the bioavailability of class IV drugs is limited by both low solubility and low intestinal permeability. Strategies to enhance aqueous solubility of class II drugs can be applied for class IV drugs, even though low permeability remains as a barrier to overcome (Kawabata et al., 2011, Pouton, 2006).

A schematic presentation of different formulation approaches based on the BCS is depicted in **Figure 1.2**. As can be seen, the solubility, dissolution rate and thus bioavailability of BCS class II drugs can be improved by different formulation methods which can be utilized during both preformulation studies and formulation product development (Kawabata et al., 2011). The formulation methods that can be utilized during the preformulation studies include crystal modifications to utilize the metastable crystalline form of the drug (Blagden et al., 2007), formation of salts of ionizable drugs (Elder et al., 2013, Guzman et al., 2007), and co-crystal formation (Elder et al., 2013, Jung et al., 2010). On the other hand, particle size reduction (Horter and Dressman, 2001, Krishnaiah, 2010, Merisko-Liversidge et al., 2003), pH modification (Riis et al., 2007), amorphization (Kawabata et al., 2011), complexation with cyclodextrins (Brewster and Loftsson, 2007, Hassan et al., 2007) and lipid formulations (Dahan and Hoffman, 2008, Kossena et al., 2007, Pouton, 2006) can be employed during the formulation design phase.

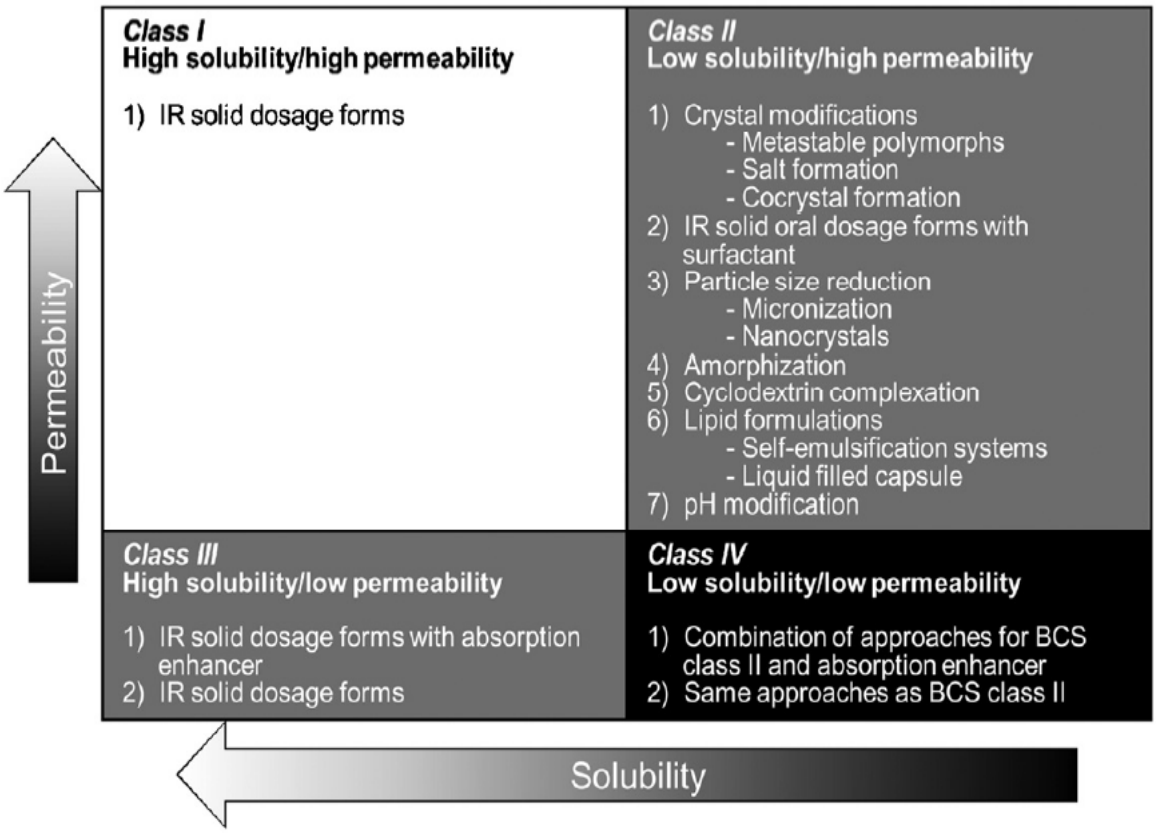


Figure 1.2 Different formulation approaches based on BCS (Kawabata et al., 2011)

All formulation methods assigned for BCS class II drugs aim to enhance their dissolution rate which will then reflect on enhanced oral bioavailability of this class of drugs. Therefore, modification of the factors that affect the dissolution rate will improve the dissolution and hence, the bioavailability. The relation between these factors affecting dissolution rate was described by the Noyes-Whitney equation (Sun et al., 2012) as follows:

$$\frac{dC}{dt} = \frac{DA}{Vh}(C_s - C_x) \qquad \text{(Equation 1.1)}$$

where dC/dt is the dissolution rate, D is the diffusion coefficient, A is the surface area, V is the volume of the dissolution medium, C_s is the saturation solubility, C_x is the drug concentration of bulk solution, and h is the hydrodynamic boundary layer thickness.

From this equation, it can be noticed that reduction of particle size, for instance, will cause an increase in the dissolution rate of drugs due to increased total surface area of drug particles (Horter and Dressman, 2001) and decreased diffusion layer thickness (Mosharraf and Nyström, 1995).

Considering the various technologies available for poorly soluble drugs, lipid formulation approaches appear promising for marked enhancement of solubility, dissolution properties and oral bioavailability. The following section will be focused on description of different lipid-based formulations and their role in improvement of solubility and dissolution properties.

1.3. Lipid formulations

Utilization of lipid formulations to enhance gastrointestinal absorption of poorly water soluble drugs has gained much popularity in the area of pharmaceutical research (Pouton, 2006). Enhanced absorption of lipophilic drugs from lipid-based formulations can be attributed to several different factors.

The presence of lipids in the GI tract promotes biliary secretions, including phospholipids, bile salts and cholesterol. These products, together with gastric movement, form emulsions that enhance the solubilization of poorly soluble drugs (Kossena et al., 2007). Also, lipids present in these formulations may undergo enzymatic degradation in the GIT. Subsequent interaction of the formed hydrolytic products with biliary secretions will lead to the formation of micellar structures that prevent drug precipitation (Dahan and Hoffman, 2008). In addition, surfactants incorporated into these delivery systems may contribute to solubilization of the lipophilic drug (Dahan and Hoffman, 2008).

The ability of lipids to prolong gastric residence time may contribute to increased dissolution of the drug at the absorptive site and subsequently improvement of absorption (Dahan and Hoffman, 2008). However, enhancement of intestinal permeability by lipids (Constantinides and Wasan, 2007) is not considered as a mechanism for the enhancement of oral absorption of poorly water soluble (class II) drugs for which permeability is not a rate-limiting step (Dahan and Hoffman, 2008), but may be important for class III and IV drugs.

Lipid-based formulations consist of a drug dissolved in a mixture of different excipients with a wide variety of physicochemical properties, including triglycerides, mono and diglycerides, lipophilic or hydrophilic surfactants and cosolvents (Pouton, 2006). In order to identify the most appropriate formulations for specific drugs, based on their physicochemical properties, and to help identification of their performance characteristics, the lipid formulation classification system (LFCS) was introduced in the year 2000 (Pouton, 2000) and then updated in the year 2006 (Pouton, 2006).

According to Pouton (2000), three main types of lipid formulation systems were identified: Types I, II, and III, with Type III being sub-divided into IIIA and IIIB. Type I

systems comprise simple and biocompatible formulations containing the drug dissolved in triglycerides and/or mixed glycerides. Type II systems include a lipophilic surfactant (HLB < 12) to improve the solvent capacity of the formulation. This type of formulation is characterized by efficient self-emulsification and by the absence of water soluble components (Pouton, 2000). Type III systems incorporate hydrophilic surfactants (HLB > 12) and/or water-soluble co-solvents (Type IIIA) with oils. Type IIIB is characterized by containing greater proportions of water-soluble components which may reach up to 50% of the formulation (Pouton, 2000).

The LFCS **(Table 1.1)** was updated in 2006 (Pouton, 2006) and included an additional category (Type IV) of lipid formulations comprising hydrophilic surfactants and co-solvents with no oils. According to Pouton (2006), mixing a surfactant with a co-solvent increases solvent capacity on dilution, facilitates dispersion of the surfactant and reduces variability and irritancy caused by high concentrations of surfactant. Although Type IV formulations can be useful for hydrophobic drugs, the safety of these systems has to be evaluated especially for chronic use (Pouton, 2006).

General properties, advantages and disadvantages of each type of lipid formulation (Pouton, 2006) are shown in **Table 1.2**.

Table 1.1 The proposed lipid formulation classification system (LFCS) showing typical composition of various types of lipid formulations (Pouton, 2006)

Excipients in formulation	Content of formulation (% w/w)				
	Type I	**Type II**	**Type IIIA**	**Type IIIB**	**Type IV**
Oils: triglyceride or mixed mono and diglycerides	100	40 – 80	40 – 80	< 20	-
Water insoluble surfactant (HLB < 12)	-	20 – 60	-	-	0-20
Water soluble surfactant (HLB > 12)	-	-	20 – 40	20 – 50	30 – 80
Hydrophilic cosolvents (e.g., PEG, propylene glycol, Transcutol)	-	-	0 – 40	20 – 50	0 – 50

Table 1.2 Characteristic features, advantages and disadvantages of the various types of lipid formulations (Pouton, 2006).

LFCS type	Characteristics	Advantages	Disadvantages
Type I	Non-dispersing; requires digestion	GRAS[1] status, simple; excellent capsule compatibility	Formulation has poor solvent capacity unless drug is highly lipophilic
Type II	SEDDS[2] without water soluble component	Unlikely to lose solvent capacity on dispersion	Turbid o/w dispersion (particle size 0.25 to 2 µm)
Type IIIA	SEDDS/SMEDDS[3] with water soluble components	Clear or almost clear dispersion; drug absorption without digestion	Possible loss of solvent capacity on dispersion; less easily digested
Type IIIB	SMEDDS with water soluble component	Clear dispersion; drug absorption without digestion	Likely loss of solvent capacity on dispersion
Type IV	Oil-free formulation based on surfactant and co-solvents	Good solvent capacity for many drugs; disperses to micellar solution	Loss of solvent capacity on dispersion; may not be digestable

[1] Generally Regarded As Safe
[2] Self-Emulsifying Drug Delivery Systems
[3] Self-Micro Emulsifying Drug Delivery Systems

1.3.1. Selection of a suitable lipid-based formulation

Improvement of low GI absorption of poorly soluble drugs requires a careful consideration of the physicochemical properties of the drug candidate in addition to the interaction of the formulation with the GIT (Hauss, 2007).

1.3.1.1. Physicochemical considerations

Selection of the most suitable lipid-based formulation for specific drugs is largely determined by their physicochemical properties. For example, some poorly water soluble drugs that have poor solubility in glycerides as well as mixed micelles of bile salts and lecithin are not capable of being formulated in Type I, Type II or Type IIIA systems (Pouton and Porter, 2008). Also, drugs with limited solubility in both water and lipid (log

P value approximately 2) are unlikely to have improved absorption from lipid formulations (Pouton and Porter, 2008, Pouton, 2000). In addition, the bioavailability of lipophilic drugs (high log *P* values > 5) may be greater from lipid formulations due to incorporation into mixed micelles (Pouton and Porter, 2008) and enhancement of their dissolution in the gut lumen for more efficient absorption (Pouton, 2000).

Chemical and physical stability of the drug must be essentially considered before selection of a suitable lipid formulation (Hauss, 2007).

1.3.1.2. Biopharmaceutical considerations

Close consideration of the biopharmaceutical properties of the drug is important for selection of the most suitable lipid formulation for oral use. Physiological factors that may affect the rate of drug absorption from the GIT and its transport to the systemic circulation require essential determination in order to formulate the optimum lipid-based formulation (O'Driscoll and Griffin, 2008).

The solvent capacity of the lipid or its ability to solubilize the entire dose of the drug to allow its GI absorption is a prime consideration for lipid formulations (Pouton, 2000). The lipid excipient should solubilize the drug and maintain it in a solubilized state until GI absorption takes place (Hauss, 2007, Pouton, 2006). Lipid systems containing hydrophilic excipients, such as Type III formulations, are more prone to drug precipitation upon dilution and dispersion (Pouton, 2006). The possibility of precipitation can be anticipated by investigating the equilibrium solubility of the drug in different components of the formulation after dilution, dynamic dispersion / precipitation performance of the formulation, and then assessing the correlation between the two experiments (Pouton, 2006).

Inclusion of surfactants and co-solvents into lipid formulations must be assessed carefully to avoid drug precipitation on dilution (Pouton and Porter, 2008). It was reported that loss of solvent capacity of lipid formulations upon dilution may be more prominent if a co-solvent is incorporated rather than those containing non-ionic surfactants (Pouton and Porter, 2008). This is due to the fact that the drug solubility in a micellar solution of surfactant is directly proportional to the surfactant concentration or the number of micelles present, whereas the drug solubility in aqueous solutions of co-solvents will be lost upon dilution (Pouton and Porter, 2008).

Type III and Type IV formulations may lose their solvent capacity on dilution due to partitioning of water-soluble surfactant components into the continuous phase. Therefore, these types of formulations must be tested for in vitro dispersion to predict precipitation which may take place in the intestinal lumen (Pouton and Porter, 2008).

In general, the proportion of the components of lipid formulations should be monitored closely to avoid the likelihood drug precipitation that may occur with slight modifications of the components (Pouton, 2006).

Moreover, the susceptibility of lipid formulations to digestion in the small intestine should be considered for selection of the type of formulation. Lipids undergo digestion in the GIT by the effect of gastric lipases (lipolysis) and subsequently, their digestion products may interact with biliary secretion of bile salts, phospholipids and cholesterol present in the intestinal lumen to form micellar structures to solubilize drugs (Dai, 2010). It has been reported that digestion of lipid components of the formulation may lead to loss of solvent capacity, and subsequent reduction of solubility of the drug in the gut lumen resulting in precipitation of the drug and reduction of the absorption rate (Pouton, 2000, Pouton and Porter, 2008). The presence of surfactants in Types II, III, and IV formulations may inhibit the digestion of the oil within these formulations (Pouton, 2000).

In vitro tests for lipid digestion, using a lipolysis model, may give a prediction of the possibility of in vivo precipitation of lipid-based formulations (Dai, 2010, Dahan and Hoffman, 2008). In vitro lipolysis test is necessary for evaluation of different types of lipid-based formulations including Type IV formulations because surfactants, also, may undergo digestion (Pouton and Porter, 2008). Correlation between the in vitro lipolysis assay and the oral bioavailability has been reported for some lipid based formulations of drugs such as halofantrine (Porter et al., 2004), dexamethasone and griseofulvin (Dahan and Hoffman, 2007).

1.3.2. Formulation approaches of lipid-based drug delivery systems

Lipid-based formulations can be developed in different ways to achieve the desired formulation objectives. The process of development should start from selection of the most appropriate lipid excipients considering their stability, compatibility, fatty acid content, HLB value, and digestibility. The selected lipid excipients may affect the solubility and dissolution / dispersion properties of the formed system. Formulation techniques suitable for the desired dosage form in addition to drug loading are then to be identified (Kalepu et al., 2013).

Different lipid-based formulation approaches have been designed and investigated. These include: lipid solutions (Grove et al., 2005, Mu et al., 2013), lipid suspensions (Larsen et al., 2008, Mu et al., 2013), liposomes (Chen et al., 2013, Chen et al., 2009, Hu et al., 2013), solid lipid nanoparticles (Mehnert and Mäder, 2012, Muchow et al., 2008) and self-emulsifying drug delivery systems (SEDDSs) (Porter et al., 2008, Pouton and Porter, 2008). A brief description of these different lipid-based

formulation approaches and their benefit in improving the dissolution properties and/or bioavailability of many poorly soluble drugs is given below.

1.3.2.1. Lipid solutions

Dissolving a poorly soluble drug in lipids is the simplest method to improve bioavailability (Mu et al., 2013). Careful selection of the appropriate lipid excipient is important for this type of formulation due to the wide variability in physicochemical properties and digestibility of lipids which may affect solubilization of the drug (Mu et al., 2013). For example, the solubility of seocalcitol, a poorly soluble vitamin D analogue with log P value 4.8, was significantly increased upon dissolving in medium chain triglycerides (5.3 mg/g) and long chain triglycerides (1.7 mg/g) compared to the aqueous solubility (20 ng/g) of the drug (Grove et al., 2005). In addition, oral administration of both lipid solutions of seocalcitol in rats resulted in two-fold increase in the bioavailability of the drug, compared to only 10 ± 5% bioavailability obtained from a reference propylene glycol solution. This enhancement of solubility was attributed to the ability of lipid formulations to keep the drug solubilized in the GIT until absorption is complete. No significant differences were detected between the medium and long chain triglycerides (Grove et al., 2005).

Solubilization of the drug during in vitro digestion was reported to be affected by different factors that are related to type of lipid solutions (Mohsin, 2012), type of drugs investigated (Dahan and Hoffman, 2007) and amount of lipid used in the formulation (Porter et al., 2004).

Formulation of the lipophilic drugs dexamethasone and griseofulvin in different lipid solutions comprising either small chain triglyceride (SCT), medium chain triglyceride (MCT) or long chain triglyceride (LCT) affected their in vitro performance as well as the in vivo bioavailability. The effect of different lipids on the in vitro and in vivo performance of dexamethasone formulations was comparable, whereas griseofulvin formulations showed better in vivo and in vitro performance from the MCT formulations compared to LCT and SCT (Dahan and Hoffman, 2007). The solubilization of the drug in the GIT was also affected by the type of the lipid used in the formulation (Mohsin, 2012). Formulation of fenofibrate lipid solutions in MCT resulted in about 5 – 7 % of the drug dissolved in the aqueous phase after in vitro digestion, whilst 21 – 36 % of fenofibrate was dissolved following digestion of a LCT (soybean oil) formulation (Mohsin, 2012). Furthermore, the amount of the lipid in the formulation may affect the in vitro solubilization of the drug as well as its bioavailability. For example, Porter et al. (2004) reported that more halofantrine was solubilized in the aqueous phase when

formulated in MCT formulations containing high lipid amount (≈25 mg triglyceride / ml digestion medium), whereas improved solubilization capacity of the drug was observed from the LCT formulations prepared with low lipid content (≈5 mg triglyceride / ml digestion medium). The oral bioavailability of halofantrine after oral administration of LCT formulations at low lipid content was higher than that obtained from MCT formulations, indicating good correlation between in vitro solubilization data and in vivo oral bioavailability in dogs (Porter et al., 2004).

1.3.2.2. Lipid suspensions

Lipid based suspensions may be useful when solubility in lipids is very limited (Mu et al., 2013). A study has shown that the oral bioavailability of danazol from different lipid based suspensions did not differ from that obtained when a lipid solution was orally administered in rats, indicating that a lipid-based suspension may perform as a lipid solution (Larsen et al., 2008). However, formulation of lipid suspensions may be limited by their physical stability and the crucial need for sedimentation control (Mu et al., 2013).

1.3.2.3. Liposomes

Liposomes are enclosed vesicles composed of one or two phospholipid bilayers surrounding a central aqueous cavity. Because of their biphasic property, liposomes have the ability to incorporate both hydrophilic and lipophilic drugs (Krishnaiah, 2010). These lipid-based formulations have been shown to improve oral absorption of different types of drugs such as insulin (Hu et al., 2013, Zhang et al., 2014, Niu et al., 2014), fenofibrate (Chen et al., 2009), and cyclosporine A (Chen et al., 2013). Enhancement of oral absorption by liposomal formulations has been attributed to possible increase in solubility of the drug, protection against digestive degradation, and enhanced permeation through the intestine (Hu et al., 2013). Also, liposomal phospholipids may interact with bile salts in the gastrointestinal tract to form mixed micelles that will enhance oral absorption of poorly water soluble drugs (Chen et al., 2009).

Application of liposomes for oral administration is limited by the high susceptibility of phospholipids and cholesterol to possible hydrolysis due to the effect of gastric acid, pancreatic lipases, and micellization by bile salts (Parmentier et al., 2012). Different liposomal modifications such as polymer coating of the vesicles (Chen et al., 2013) and interaction of bile salts with phospholipids (Hu et al., 2013, Niu et al., 2014, Chen et al., 2009) have been investigated to evaluate stability of liposomes. However, accurate prediction of the stability of liposomes in human GIT from in vitro stability assays is

dependent on the degree of simulation of the physiological conditions in addition to the type of the animal model selected for the assays (Parmentier et al., 2012).

1.3.2.4. Solid lipid nanoparticles

Solid lipid nanoparticles (SLN) are highly stable matrix systems that utilize non-toxic solid lipids for drug delivery (Mehnert and Mäder, 2012, Müller et al., 2000). Because these systems incorporate solid lipids, they have been used for controlling drug release after oral administration (Mehnert and Mäder, 2012, Müller et al., 2008). Solid lipid nanoparticles can be produced by different technologies such as high pressure homogenization and microemulsion techniques (Muchow et al., 2008). Those produced by high pressure homogenization methods may reach an average particle size below 500 nm (Mehnert and Mäder, 2012).

Enhanced oral bioavailability of several drugs such as ferrous sulfate (Zariwala et al., 2013), risperidone (Silva et al., 2012), lovastatin (Suresh et al., 2007), and nitrendipine (Kumar et al., 2007) has been reported when these drugs were loaded into solid lipid nanoparticles. Improved oral absorption from SLN was attributed to solubilization of the drug in the micelles that form upon degradation of lipids in the gut wall (Muchow et al., 2008).

Although solid lipid nanoparticles are safe and tolerable, they have a relatively low drug loading capacity and potential displacement of the incorporated drug from the formed SLN may occur during storage. Expulsion of the drug from SLN may be due to possible crystallization of the lipids during storage and subsequent formation of a more densely packed crystalline structure that has low drug affinity (Muchow et al., 2008).

1.3.2.5. Self-emulsifying drug delivery systems

The following sections of this review will focus on self-emulsifying drug delivery systems as a part of the proposed study.

1.4. Self-emulsifying drug delivery systems as a tool for improving in vitro dissolution and oral absorption of poorly soluble drugs

1.4.1. Definition and general properties

Self-emulsifying drug delivery systems (SEDDS) or self-emulsifying oil formulations (SEOF) are isotropic mixtures of natural or synthetic oils with lipophilic or hydrophilic surfactant, co-solvents, and the solubilized drug substance (Porter et al., 2008, Neslihan Gursoy and Benita, 2004, Hauss, 2007). These systems are Type II and Type III formulations in the LFCS (Mu et al., 2013) and can rapidly form fine oil in water emulsions, microemulsions or nanoemulsions upon dispersion in the gastrointestinal fluids under mild agitation produced by the digestive motility of the stomach and the intestines (Porter et al., 2008, Neslihan Gursoy and Benita, 2004, Hauss, 2007). Based on the size range of their oil droplets, SEDDS can be classified into self-microemulsifying systems (SMEDDS) with oil droplet size from 100 to 250 nm and self-nanoemulsifying systems (SNEDDS) with oil droplet size less than 100 nm (Kawabata et al., 2011, Kohli et al., 2010).

Compared to lipid solutions, SEDDS have the potential of increased drug loading capacity because of increased solubility of poorly soluble drugs with intermediate partition coefficient ($2 < \log P < 4$) in the amphiphilic surfactants, co-surfactants and co-solvents components of the formulation (Pouton, 2000). In addition, formation of submicron droplets will provide a large interfacial surface area for transfer of the drug resulting in increased rate and extent of absorption and hence, improved bioavailability (Chakraborty et al., 2009). Also, SEDDS may enhance the bioavailability of poorly soluble drugs, for which absorption is dissolution rate limited, because these formulations maintain the drug in a dissolved state throughout the GI tract (Chakraborty et al., 2009, Tang et al., 2008). Moreover, anhydrous SEDDS may provide protection for drugs from the enzymatic and chemical hydrolysis within the aqueous environment of the GIT (Gupta et al., 2013).

1.4.2. Selection of suitable drug candidate for SEDDS

Self-emulsifying drug delivery systems can be used as a formulation approach for drugs that have poor water solubility and slow dissolution rates (Kohli et al., 2010). Therefore, determination of the drug's lipophilicity (or its octanol : water log *P*) and its solubility in the selected lipid excipients and in mixtures of these lipids may be the most important parameters to determine the feasibility of the SEDDS technology for the drug (Mu et al., 2013, Hauss, 2007). Interaction between different formulation components

may influence the final properties of a formulated SEDDS or SMEDDS, such as dispersion behaviour of the formulation upon dilution and solubility of the drug in the excipients mixture, and in this case, a compromise of the formulation characteristics is required in order to predict the optimal formulation (Holm et al., 2006). In addition, because determination of the aqueous solubility and/or log P do not reflect the in vivo behaviour of the SEDDS formulation, each drug compound must be assessed individually to predict the usefulness of SEDDS formulation (Kohli et al., 2010) .

Further to the requirement of low dose of the drug (Kohli et al., 2010), the drug compound must exhibit high chemical stability in the selected lipid excipients (Kawabata et al., 2011).

1.4.3. Mechanisms of self-emulsification

Reiss (1975) has suggested that self-emulsification takes place when the entropy change that favours dispersion is greater than the energy required to increase the surface area of the dispersion. The free energy of formation of a conventional emulsion (G) is a direct function of the interfacial energy required to create a new interface between the oil and water phases, and can be represented by the equation:

$$\Delta G = \sum N\pi r^2 \sigma \qquad \text{(Equation 1.2)}$$

where, G is the free energy associated with emulsification process, N is the number of droplets with radius r, and σ is the interfacial energy (Gupta et al., 2013).

An emulsion is an unstable system because oil and water phases tend to separate in order to reduce the high free energy of the system at the interfacial area. Addition of emulsifying agents tends to reduce the interfacial energy between the two phases due to formation of a mechanical barrier around the emulsion droplets preventing coalescence (Gupta et al., 2013). Thus, formation of a stable emulsion with negative free energy takes place. In contrast, the free energy required to form the emulsion in self-emulsifying formulations is low and therefore, emulsification takes place spontaneously (Neslihan Gursoy and Benita, 2004). Therefore, formulation of a stable and spontaneous dispersion for oral administration may require the incorporation of a surfactant which is able to reduce the interfacial energy between the two phases and then lower the free energy of the system to the minimum (Shafiq et al., 2007).

In addition, dielectric spectroscopy studies have revealed that emulsion formation may be related to the formation of a liquid crystalline phase (liquid crystal) between the oil/surfactant and the water phases (Craig et al., 1995). Penetration of water into these liquid crystals will lead to continuous solubilization of water within the oil droplets until

the formation of dispersed liquid crystal phase, for which the actual amount depends on concentration of the surfactant in the binary mixture. Formation of liquid crystal interface around the oil droplets upon dispersion in water will lead to stabilization of SEDDS formulation and prevention of coalescence (Gupta et al., 2013, Neslihan Gursoy and Benita, 2004).

1.4.4. Improvement of oral absorption by SEDDS

Different studies have shown that the bioavailability of poorly water soluble drugs can be improved upon oral administration of different SEDDS formulations (Seo et al., 2013, Holm et al., 2012, Wang et al., 2010, Atef and Belmonte, 2008). A study conducted in dogs compared the oral bioavailability of a lipophilic model drug after administration of SEDDS, polyethylene glycol (PEG) 400 solution, capsule and tablet dosage forms. The results showed higher values of maximum plasma concentration (C_{max}) and area under the curve (AUC) from the SEDDS formulation compared to other dosage forms tested (Shah et al., 1994). Also, the absolute bioavailability and C_{max} of halofantrine were significantly increased when the drug was administered orally as SNEDDS in rats compared to an oily solution prepared in soy bean oil (Holm et al., 2012). In addition, oral administration of docetaxel loaded SNEDDS (Seo et al., 2013) in mice resulted in 6.5 fold higher absolute bioavailability (17%) and significantly higher AUC and C_{max} compared to docetaxel solution. Another study conducted in rats showed more than two-fold increase in oral bioavailability of the antiepileptic drug, phenytoin, when this poorly water soluble drug was administered orally in stable SEDDS formulation in comparison to the commercially available suspension (Atef and Belmonte, 2008).

Improved bioavailability of poorly soluble drugs may be explained on the basis of rapid drug release and increased drug solubilization in the GIT (Shah et al., 1994). During transport of the SEDDS formulation throughout the GI tract, the drug may partition from the droplets for delivery into the aqueous intestinal fluids. A faster rate of drug release from the droplets into the aqueous media may depend on the droplet/particle size and the polarity of the oil droplets (Shah et al., 1994). The presence of smaller and uniform droplets in SEDDS formulation will provide a short diffusion path for the drug to be released from the formulation (Shah et al., 1994). The polarity of SEDDS formulations is determined by the properties of oils and surfactant components, i.e., the hydrophile – lipophile balance (HLB), degree of unsaturation and chain length of the fatty acids, the molecular weight of the hydrophilic portion and concentration of the surfactant. Determination of oil/water partition coefficient of the lipophilic drug may give

an indication of the polarity of the droplets (Shah et al., 1994, Gershanik and Benita, 2000).

It was reported that the ability of self-emulsifying formulations to be digested and/or solubilized in the mixed micelles of bile salts and phospholipids in the small intestine may be an important factor in determining the absorption rate of the drug from SEDDS rather than the particle size of the formulation (Pouton, 2000). For example, investigation of the bioavailability of probucol in minipigs from SNEDDS and SEDDS differing in their mean particle diameter revealed a slight and non-significant improvement of absorption rate and bioavailability of the drug from SNEDDS compared to SEDDS (Nielsen et al., 2008).

Lipids contribute to improvement of the oral bioavailability through different mechanisms that result in modification of the biopharmaceutical properties of the drug (Neslihan Gursoy and Benita, 2004, Gershanik and Benita, 2000). Lipids can increase solubility and dissolution rate of the drug in intestinal fluids, protect the drug from chemical and enzymatic hydrolysis in oil droplets (Kawakami et al., 2002), in addition to enhancement of lymphatic transport through formation of lipoproteins (Sachs-Barrable et al., 2008, Porter and Charman, 2001, Hauss et al., 1998).

The degree of saturation and the fatty acid chain length of the triglycerides in addition to the volume of lipid administered are important factors that affect absorption and distribution of the drug into the blood and lymphatic circulations (Neslihan Gursoy and Benita, 2004). Transport of the drug into the intestinal lymph may contribute to its overall oral bioavailability (Caliph et al., 2000). The drug, first, must associate with the intestinal lipoproteins (chylomicrons) so that it can be transported to the lymphatic circulation. Therefore, administration of highly lipophilic drugs (triglyceride solubility > 50 mg/ml and log *P* > 5) in lipid-based formulations may enhance the lymphatic transport of these drugs as well as their systemic absorption (Caliph et al., 2000). The extent of lymphatic transport of lipophilic drugs depends on the composition of the lipid vehicle in the formulation (Gershanik and Benita, 2000). For instance, a significantly higher lymphatic transport of ontazolast was obtained from a soy bean-based emulsion formulation in comparison to a SEDDS formulation prepared from Peceol/Tween 80 system (Hauss et al., 1998).

The effect of varying fatty acid chain length of triglycerides on the extent of lymphatic and portal absorption of the drugs from different lipid-based formulations has been studied (Caliph et al., 2000, Holm et al., 2003). A study has been conducted to investigate the effect of varying the chain length of structured triglycerides (the glycerol backbone of triglyceride is esterified with different fatty acid chains) on the lymphatic

transport and oral absorption of halofantrine from different SMEDDS formulations (Holm et al., 2003). The results showed an increase in the lymphatic transport of halofantrine (estimated as cumulative % of the administered dose) from SMEDDS formulated with long chain structured triglycerides (27.4 % ± 1.3) compared to that obtained from SMEDDS formulated using medium chain structured triglycerides (17.9 % ± 1.3). However, SMEDDS formulations prepared with medium chain lipids showed higher total bioavailability (56.9 %) than those incorporating long chain lipids (37.2 %). It was assumed that the short and medium chain fatty acids cannot be incorporated well with intestinal lipoproteins for lymphatic transport process, but they can transported via the portal blood to the systemic circulation with a result of enhanced oral bioavailability (Neslihan Gursoy and Benita, 2004, Caliph et al., 2000). Conversely, the long chain fatty acids and monoglycerides enhance the lymphatic transport because they may undergo re-esterification within the intestinal cells to form triglycerides which can be associated with intestinal lipoproteins and then transported to the intestinal lymph (Neslihan Gursoy and Benita, 2004, Caliph et al., 2000).

Therefore, alteration of the components in SEDDS formulations can be taken as a measure to optimize formulations and improve oral bioavailability of poorly soluble drugs. A study has reported that oral bioavailability of cyclosporine A formulated as a SEDDS changed with changing the type of lipid excipient and their ratio (Odeberg et al., 2003). A SEDDS formulation prepared from fractionated oat oil and medium chain monoglyceride showed an absorption profile of cyclosporine A that is comparable to a reference commercial product (Sandimmun Neoral®), whereas formulations comprising sphingolipids, cholesterol and medium chain triglyceride showed almost no absorption of the drug (Odeberg et al., 2003).

Incorporation of surfactants into SEDDS may also influence the dissolution characteristics of the formulation (Kim et al., 2000) and consequently its oral absorption. It has been postulated that surfactants may partition into the epithelial cell membrane and change the structural arrangements of its lipid bilayer leading to improved intestinal permeability (Neslihan Gursoy and Benita, 2004, Kommuru et al., 2001). Excess surfactant that may come in contact with the membrane may cause extensive damage of the intestinal lipid bilayer to the degree of dissolving it and forming mixed micelles from the surfactant and the membrane (Kommuru et al., 2001, Scott Swenson and Curatolo, 1992). Therefore, hydrophobic surfactants, which cannot dissolve in the aqueous intestinal fluids, will be considered as poor absorption enhancers while surfactants that are too hydrophilic will not partition into the lipophilic membrane (Scott Swenson and Curatolo, 1992). In this instance, the HLB value of surfactants may be considered

although no general correlation has been identified between the HLB of the surfactant and its ability to enhance absorption (Kommuru et al., 2001).

Inclusion of surfactant into SEDDS formulation of coenzyme Q_{10} has resulted in a two-fold enhancement of oral bioavailability of the drug in comparison to a formulation of powder filled into capsule. This bioavailability enhancement was partly attributed to the ability of the surfactant to increase mucosal permeability (Kommuru et al., 2001).

Also, presence of surfactants in lipid formulations may prevent precipitation of the drug in the intestinal lumen by solubilization process. The solubilized drug will disperse effectively with intestinal fluids and then the drug is rapidly absorbed independently on lipid digestion (Gershanik and Benita, 2000). A study showed that the oral absorption of an oily and poorly water soluble drug increased with increasing the concentration of the surfactant (Tween 80) in the tested emulsion formulation (Toguchi et al., 1990). However, the increased absorption of the drug was proved to be due to the smaller droplet size of the formulation and not due to the increased amount of solubilized drug obtained with increasing surfactant content of the systems (Toguchi et al., 1990). Also, it has been shown that micellar solubilization of the drug may affect its transport through the intestinal membrane and, hence, the extent of oral absorption (Chiu et al., 2003). In vitro permeability studies of cyclosporine reported a reduction in the transport of the drug through a Caco-2 cell line when surfactants were incorporated into the PEG solutions above their critical micelle concentrations. Decreased transport of cyclosporine was attributed to entrapment of the drug within micelles (Chiu et al., 2003).

Therefore, the impact of excess surfactants on the behavior of lipid formulation has to be determined prior to the design of formulation in order to optimize the product for specific performance (Yoon and Burgess, 1996).

1.4.5. Excipients for self-emulsifying drug delivery systems

Self-emulsifying drug delivery systems comprise a wide variety of excipients that differ in their physicochemical and biological properties, which therefore require careful consideration to obtain the most appropriate combinations in order to facilitate more efficient design of SEDDS (Kohli et al., 2010). Various components of self-emulsifying systems including oils, surfactants, and co-surfactants should be selected carefully in order to formulate liquids that are clear when mixed with aqueous phase at room temperature (Balakrishnan et al., 2009a, Balakrishnan et al., 2009b). Formulation of SEDDS requires a crucial consideration of the appropriate mixture of oils, surfactants and co-solvents that will solubilize the entire dose of the drug in a sufficient volume suitable for oral administration (Gupta et al., 2013, Kohli et al., 2010). Study of drug

solubility in various components of SEDDS is important to identify the stability of formulations because precipitation of drug in some formulations may take place before in situ solubilization (Parmar et al., 2011). However, total solubility of the drug in the whole lipid mixture should be considered more than its solubility in individual components of the lipid mixture (Rahman et al., 2013). Different factors may affect the efficiency of self-emulsification process. These include the type and ratio of the oil and surfactant, the concentration of the surfactant used and the temperature at which self-emulsification process takes place (Rahman et al., 2013, Gupta et al., 2013, Shah et al., 1994). Determination of the drug's compatibility, solubility and stability in the selected excipients should take place in order to define the best combinations for the drug (Rahman et al., 2013).

In addition to the crucial requirement of chemical and physical stability of the drug in the formulation, controlling the chemical and physical stability of the chosen excipients during formulation development is necessary for constant dissolution properties of the drug throughout the formulation shelf life (Gupta et al., 2013).

In general, more efficient SEDDS may be prepared with lipid components that are selected to attain maximum drug loading capacity, minimum self-emulsification time, reduced droplet size variation due to pH and composition of the aqueous medium and minimum or prevented drug hydrolysis or metabolism in the gastric fluids (Rahman et al., 2013). Determination of solubilization capacity or drug loading of a self-emulsifying formulation is necessary to avoid drug precipitation that may take place in vivo. Self-emulsifying systems that contain drug concentrations exceeding its solubilization capacity are expected to show drug precipitation upon dilution. Therefore, much attention should be paid to avoid drug precipitation in diluted systems (Narang et al., 2007). In addition, good solubilizing capacity of self-emulsifying formulation is required for stabilization of the drug in solution (Wang et al., 2009).

Particle size of an emulsion is an important factor that influences the rate and extent of drug release as well as in vivo absorption and consequently affects self-emulsification performance of the formulation (Constantinides et al., 1994). It was suggested that the faster release rate of simvastatin from SMEDDSs, compared to conventional tablets, was due to smaller droplet size obtained by dissolving the drug in SMEDDS formulation (Kang et al., 2004). Also, smaller droplet size may contribute to enhanced in vivo drug absorption due to larger interfacial surface area obtained by smaller particles (Liu et al., 2009).

1.4.5.1. Lipids / Oils

Lipids are considered as important excipients in the formulation of SEDDS. They are amphiphilic in nature comprising both lipophilic and hydrophilic portions in their molecular structure. Lipids differ in their fatty acid composition, melting point, HLB, and solubility in non-polar organic solvents (Jannin et al., 2008). Lipids participate in the improvement of absorption from the GI tract due to their ability to solubilize part of the lipophilic drug and to increase the fraction of the drug transported by the intestinal lymphatic circulation (Neslihan Gursoy and Benita, 2004). Therefore, solubilization capacity by an oily phase is an important factor that should be considered in selecting the type of the oil phase (Pouton and Porter, 2008).

1.4.5.1.1. Natural lipids (Triglycerides)

Natural oils and fats consist of mixtures of triglycerides which contain fatty acids of different chain length; namely: short (< 5 carbons), medium (6 to 12 carbons) and long chains (> 12 carbons); with different degrees of unsaturation. The melting point of the oil increases with increasing the fatty acid chain length and decreases with increasing the degree of unsaturation of the triglyceride (Rahman et al., 2013). Triglyceride vegetable oils are commonly present in food and are generally regarded as safe (GRAS) excipients in lipid-based formulations because of their ability to be completely digested and absorbed from the GI tract. The solvent capacity of triglycerides depends on the effective concentration of the ester groups, and therefore, medium chain triglyceride (MCT) possesses higher solvent capacity compared to long chain triglycerides (LCT) (Cao et al., 2004). In addition, MCTs have higher fluidity, lower susceptibility to oxidation, and better self-emulsification properties.

1.4.5.1.2. Semi-synthetic and synthetic lipids (mixed glycerides and polar oils)

Chemical interaction of the MCTs, or glycerides derived from natural oils, with a single or different hydrophilic chemical entities has led to the production of wide varieties of liquid or semisolid (thermo softening) excipients that find wide application in lipid-based formulations due to their solubilizing, surfactant, and wetting properties. These vehicles can be used in SEDDS and SMEDDS as emulsifiers and co-emulsifiers, in addition to their ability to be filled into soft and hard gelatin capsules while in the molten state (Gibson, 2007). However, semi-solid or thermo softening excipients with a melting range of 26 to 70 ^{o}C can be filled only into hard gelatin capsules because of their waxy state at ambient room temperature (Rahman et al., 2013).

Mixed glyceride excipients, with different contents of mono-, di- and triglycerides, can be obtained by partial hydrolysis of triglycerides or glycerolysis (Gibson, 2007). Different properties of mixed glycerides including their chemical composition, physical appearance, melting behavior and HLB values are affected by the original source of triglycerides and the degree of hydrolysis induced during production process (Gibson, 2007). Common excipients of this type include: glyceryl monocaprylocaprate (Capmul® MCM); glyceryl monostearate (Geleol®, Imwitor® 191); glyceryl monooleate (Peceol™) and glyceryl monolinoleate (Maisine™ 35-1) (Rahman et al., 2013). Another example of pharmaceutical excipients, polyoxylglycerides or macrogolglycerides, are produced by polyglycolysis of vegetable oils with polyoxyethyleneglycols (PEG) of certain molecular weight. The physical appearance of these excipients may range from viscous liquids to solids at room temperature, depending on their composition of mono-, di- and triglycerides and mono- and diesters of PEG which aid their ready dispersion in water. Examples of this type of excipients may be composed of unsaturated long chain fatty acids which include oleyl polyoxylglycerides (Labrafil®M1944CS) and linoleyl polyoxylglycerides (labrafil®M2125CS), saturated medium chain fatty acid esters such as lauroyl polyoxylglycerides (Gelucire®44/14) or saturated long chain fatty acid esters such as steroyl polyoxylglycerides (Gelucire®50/13) (Rahman et al., 2013). Different examples of polyoxylglycerides were shown to improve solubility and bioavailability (Chambin and Jannin, 2005, Sha et al., 2005).

Mixed mono- and diglycerides of long chain fatty acids are suitable for liquid formulations when waxy materials present technical problems. Compared to triglycerides, mixed long chain glycerides are more useful to solubilize drugs, with exception of highly lipophilic drugs, especially in Type II and Type III self-emulsifying formulations (Pouton and Porter, 2008). Mixed glycerides of medium chain fatty acids have gained much attention, compared to conventional MCTs, because of their higher solvent capacity, lower susceptibility to oxidation and additional surfactant properties that enhance their emulsification power (Porter et al., 2008). However, digestion of medium chain glycerides should be evaluated prior to their selection for lipid-based formulations (Porter et al., 2008).

More polar oily excipients such as sorbitan fatty acid esters (Spans) resemble mixed glycerides in their physical properties and their ability to improve solvent capacity and dispersion of the formulation. Examples of polar oils include the more lipophilic sorbitan trioleate (Span 85), sorbitan monooleate (Span 80) and free fatty acids such as oleic acid (Strickley, 2007, Gibson, 2007).

1.4.5.2. Surfactants

Incorporation of surfactants in SEDDS is essential for the self-emulsifying properties. Surfactants can dissolve high proportions of hydrophobic drug compounds in the formulation due to their amphiphilic nature (Kohli et al., 2010, Neslihan Gursoy and Benita, 2004). Also, they facilitate the dispersion process by reducing the interfacial tension between oil and water phases with subsequent formation of a flexible film around the droplets (Rahman et al., 2013). In order for predictable self-emulsifying properties to take place in SEDDS formulations, surfactants are required to be incorporated in high concentrations ranging from 30 to 60% w/w of the formulation (Rahman et al., 2013, Neslihan Gursoy and Benita, 2004). However, the high risk of GI irritation associated with incorporation of higher concentrations of surfactants may require essential consideration of the safety of the used concentration of each surfactant (Gupta et al., 2013, Rahman et al., 2013). SMEDDS and SNEDDS formulations may have reduced irritation potential with high surfactant concentrations. This is due to rapid gastric emptying and rapid dispersion of the extremely small droplets of these SMEDDS and SNEDDS formulations throughout the GI fluids (Gupta et al., 2013). Generally, natural emulsifiers such as lecithin and medium chain monoglycerides (MCM) are safer than synthetic surfactants, but with less self-emulsification properties (Constantinides, 1995). In addition, nonionic surfactants are less toxic than the ionic surfactants and may produce highly stable emulsions with a high safety profile (Gershanik and Benita, 2000).

The surfactants used in SEDDS formulations are required to have relatively high HLB values and hydrophilicity in order to promote rapid dispersion in the aqueous GI fluids and immediate formation of fine o/w droplets (Rahman et al., 2013). In addition, better self-emulsifying properties can be obtained by the use of a mixture of surfactants rather than the use of a single surfactant (Li et al., 2005). It was proposed that an increase in the microemulsion area in the phase diagram and possible higher drug loading could be obtained if mixtures of surfactants were combined. In addition, the ability of the surfactant to partition into the oil-water interface may increase resulting in a decreased interfacial energy and a more stable formulation (Li et al., 2005).

Furthermore, the concentration of the surfactant added to SEDDS may affect the droplet size. In general, a smaller mean droplet size is produced with increasing the concentration of the surfactant used. The surfactant molecules added will be adsorbed at the interface between oil and water phases resulting in stabilization of oil droplets. However, increasing the concentration of the surfactant may sometimes lead to increased water penetration into oil droplets and subsequent disruption of the interface

between oil and water phases resulting in removal of oil droplets into the aqueous phase (Kommuru et al., 2001, Pouton, 1997).

Water-soluble surfactants are the most widely employed surfactants for SEDDS or SMEDDS formulations (Pouton, 2006). Having HLB values ≥ 12, micellar solutions of water soluble surfactants can be formed above their critical micelle concentration where they dissolve in water at low concentrations. Depending on the method of production, the lipophilic part of water-soluble surfactants may contain saturated or unsaturated fatty acids. For example, Cremophor® RH40 and Cremophor® EL are relative derivatives of castor oil but with more saturated alkyl chains in Cremophor RH40 and unsaturated alkyl chains in Cremophor EL, according to the degree of hydrogenation of castor oil. The use of water-soluble ester surfactants in pharmaceutical products is limited by their safety profile rather than by their advantageous effect on the physicochemical properties of the products (Rahman et al., 2013, Pouton and Porter, 2008).

Other water-soluble ester surfactants include the polyalcohol esters of edible fatty acids which are produced by esterification of vegetable oils. These amphiphilic esters possess medium to high HLB, based on the process of synthesis, and can be used in SEDDS to enhance solubility and bioavailability (Rajebahadur et al., 2006). Examples of this group include polyglyceryl oleate (Plurol™Oleique CC497), propylene glycol monocaprylate (Capryol™90), propylene glycol monolaurate (Lauroglycol™90), polyoxyethylene–8 stearate (Mirj®45), polyoxyethylene–40 stearate (Mirj®52), polyoxyethylene–15 hydroxystearate (Solutol®HS15), and polyoxyethylene–20 sorbitan monooleate or polysorbate 80 (Tween®80) (Rahman et al., 2013).

Water-insoluble surfactants possess intermediate HLB values from 8 to 12 and can adsorb strongly at the interface between oil and water phases. These surfactants can form micelles but their hydrophilicity may not be sufficient to affect the self-emulsification process. The solubility of these surfactants in water is generally low, but they may disperse in water when shear is applied to form an emulsion (Kalepu et al., 2013). Examples of this type of surfactant are mainly oleate esters such as polyoxyethylene–20 sorbitan trioleate (polysorbate 85 or Tween®85) with HLB value equal to 11; and polyoxyethylene–25 glyceryl trioleate (Tagat TO®) with HLB value equal to 11.5 (Pouton and Porter, 2008). Mixing surfactants with different HLB values may produce systems with different properties if compared to systems prepared from a single surfactant. Differences in behaviour between the two systems can be explained on the basis that the mixture system may contain both water soluble and water insoluble molecules while the single surfactant system will contain only water insoluble molecules. For example, a mixture of Tween 80 and Span 80 with an average HLB value of 11 will

possess different emulsifying properties compared to a system containing only Tween 85 with an HLB value of 11. Small fractions of water soluble components present in Tween 85 may not be enough to affect the properties of the formulation upon dispersion, while the properties of systems prepared from mixtures of Tween 80 and Span 80 may be influenced by the more dominant water soluble portions present in the surfactant mixture (Pouton and Porter, 2008).

1.4.5.3. Co-solvents or co-surfactants

Formulation of more effective SEDDS may require incorporation of some organic solvents to aid in the dissolving of large quantities of either the drug or the hydrophilic surfactant in the lipid base. The most commonly used materials include ethanol, glycerol, propylene glycol and polyethylene glycol (PEG) 400. These solvents may act as co-surfactants in the microemulsion systems (Gershanik and Benita, 2000). Also, incorporation of high concentrations of co-solvents may lead to improved solvent capacity of the formulation for drugs that dissolve freely in co-solvents. However, high concentrations of co-solvents may cause precipitation of the drug upon dilution and dispersion in water. Therefore, careful consideration of the concentration of co-solvents to be used in the formulation is important to avoid problems of drug precipitation and immiscibility of some co-solvents with lipid components (Pouton and Porter, 2008). In addition, incorporation of co-surfactants in lipid-based formulations may allow the formation of fluid and sufficiently flexible interfacial film which is capable to take different curvatures necessary for the development of microemulsions over a wide range of composition (Lawrence and Rees, 2000). Compared to alcohol-free formulations, conventional SEDDS containing organic solvents may develop drug precipitation due to evaporation of the used organic solvents into the shells of soft or hard gelatin capsules. However, alcohol-free lipid formulations may have limited dissolution of the drug (Constantinides, 1995).

Construction of ternary phase diagram is a useful tool to optimize the amounts of components in SEDDS and to determine the system with the most suitable concentration of surfactants or surfactant/co-surfactant blends that can reduce the free energy of the system and increase the dispersion entropy resulting in spontaneous, and thermodynamically stable dispersions (Shafiq et al., 2007). Identification of minimum concentrations of surfactants that yield stable nanoemulsion formulations is necessary to avoid GI irritation that may be caused by large amounts of surfactants (Duc Hanh et al., 2015, Shafiq et al., 2007). Also, the effect of different components on the phase behavior

of different systems can be determined from phase diagrams (Lawrence and Rees, 2000).

1.4.5.4. Other additives

Protection of lipid-based formulations against oxidation may require the addition of different lipid soluble antioxidants. These may include α-tocopherol, β-carotene, butylated hydroxytoluene (BHT), butylated hydroxyanisol (BHA) or propyl gallate (Gibson, 2007).

1.4.6. Approaches for oral delivery of SEDDSs

Formulation of self-emulsifying drug delivery systems in an acceptable dosage form for oral administration represents a challenge that has to be considered. Generally, SEDDSs are prepared in liquid form. However, some limitations associated with these liquid formulations have led to the production of solid self-emulsifying drug delivery systems (S-SEDDSs) as alternative option for oral delivery (Gupta et al., 2013).

1.4.6.1. Capsule filling with liquid self-emulsifying drug delivery systems

Capsule filling offers a simple method of manufacturing for oral delivery of liquid SEDDSs. This method provides high drug loading capacity (up to 50% w/w), especially for low dose and highly potent drugs (Jannin et al., 2008, Tang et al., 2008). It involves filling the formulation into the capsule body which will then be sealed with the capsule cap either by banding with a gelatin band or by using liquid encapsulation micro-spray sealing (LEMS) technology (Jannin et al., 2008). Capsule filling technology has been utilized for development of Liquid Oros technology (Alza Corporation) based on the osmotic principle for controlled delivery of insoluble drug substances or peptides formulated in a liquid self-emulsifying formulation. This system expands when it comes into contact with water and then expels the drug formulation through an orifice in the capsule (Tang et al., 2008).

Some factors may limit the application of capsule filling as a method of manufacturing of conventional liquid SEDDSs. Compatibility of the excipients with the capsule shell is an important factor that must be considered (Balakrishnan et al., 2009a, Jannin et al., 2008, Tang et al., 2008). Also, storage temperature may affect the solubility of different ingredients in the final product because precipitation of the drug and/or excipients may take place at lower storage temperatures. These precipitated materials may prevent existence of the drug in solution or within fine emulsion droplets, if could not be dissolved again by warming the filled capsules to room temperature (Balakrishnan et

al., 2009a). In addition to possible irreversible precipitation, production of liquid SEDDSs may be restricted by the high cost, reduced stability of the liquid preparations, the need for large volumes of the dose for administration, difficult handling and transportation, in addition to limited choices of dosage forms (Gupta et al., 2013, Tang et al., 2008) .

1.4.6.2. Production of solid self-emulsifying drug delivery systems (S-SEDDSs)

Solid self-emulsifying drug delivery systems (S-SEDDS) have been employed as an alternative approach for SEDDSs. These systems are produced by solidification of liquid SEDDSs into powders which can be formulated into different solid dosage forms such as tablets, capsules, pellets and beads. Transformation of liquid SEDDSs into solid dosage forms should not change the release characteristics of the drug or the self-emulsification process that will take place in the GI tract. Therefore, S-SEDDSs possess the advantages of both SEDDS formulations and solid dosage forms. These systems are characterized by high stability and reproducibility of the formulation, improved drug solubility and bioavailability, ease of handling and transportation in addition to better patient compliance (Gupta et al., 2013).

Different solidification technologies that have been utilized to produce S-SEDDSs from the liquid SEDDSs are discussed below.

1.4.6.2.1. Solidification techniques to produce S-SEDDS

i. Spray drying

In the spray drying technique, the liquid formulation is first prepared by mixing the drug, lipids, surfactants and solid carriers (like silicon dioxide) with an organic or aqueous solvent. The prepared formulation is then sprayed or atomized into a hot air chamber where the organic solvent or water contained in the formulation evaporates, and consequently, solid microparticles are produced under controlled conditions of temperature and airflow pattern. The resulting microparticles can be compressed into tablets or alternatively filled into hard shell capsules (Jannin et al., 2008, Tang et al., 2008).

Solid SMEDDS of nimodipine (Yi et al., 2008) prepared by a spray drying technique, using dextran as a solid carrier, showed faster release rate of the drug, compared to conventional tablets, and improved oral bioavailability in rabbits. Also, enhanced anti-tumour activity and reduced toxicity of docetaxel (Seo et al., 2013) was observed when self-nanoemulsifying formulation was spray dried into solid SNEDDS using colloidal silica as an inert porous carrier.

Furthermore, application of spray drying to formulate solid SEDDS of dexibuprofen using Aerosil® 200 as a solid carrier resulted in two fold higher bioavailability of the drug following oral administration to rats when compared to dexibuprofen powder. Such results indicated that the self-emulsification properties of the liquid SEDDS were preserved after solidification (Balakrishnan et al., 2009a).

ii. Adsorption onto solid carriers

A simple adsorption process involves the addition of a liquid self-emulsifying formulation onto a solid carrier by mixing in a blender to obtain a free flowing powder which can be directly filled into capsules or compressed into tablets after mixing with suitable excipients (Gupta et al., 2013, Jannin et al., 2008). In this adsorption technique, good content uniformity can be obtained and up to 70% of lipid formulation may be adsorbed onto suitably selected carriers (high lipid exposure). However, decreased drug loading capacity may be encountered with this method due to subsequent dilutions of the lipid formulation during mixing with solid carriers and then during mixing with excipients required for compression into tablets (Gupta et al., 2013, Jannin et al., 2008).

Solid carriers that are able to adsorb large quantities of the liquid lipid formulation should be selected to ensure increased drug loading capacity as well as lipid exposure. For example, different grades of Neusilin® (magnesium aluminometasilicate), Florite® (calcium silicate), Syloid® (porous silicon dioxide), Aerosil® (colloidal silicon dioxide) and Avicel® (microcrystalline cellulose (MCC)) were used as adsorbents to transform liquid SEDDS into solid SEDDS.

Different studies have explored the use of Neusilin® (magnesium aluminometasilicate) as an adsorbent to transform liquid SEDDS into solid SEDDS and to improve the solubility, dissolution properties and bioavailability of poorly soluble drugs (Agarwal et al., 2009, Beg et al., 2016, Kallakunta et al., 2012, Parmar et al., 2015). For example, utilization of Neusilin® US2 as a solid inert carrier to formulate solid SNEDDS of olmesartan medoxomil (Beg et al., 2016) from optimized liquid SNEDDS, resulted in improvement of drug release rate compared to other adsorbents tested like Aerosil® 200. In addition, a 2.32 – 3.27 fold enhancement in the bioavailabilty parameters of the drug from solid SNEDDS formulated with Neusilin®US2 was observed compared to pure drug solution.

Another study, aimed to improve the solubility of the poorly soluble drug, lercanidipine hydrochloride, employed Neusilin®US2 as an inert carrier to produce a self-emulsifying powder of the drug (Kallakunta et al., 2012). The self-emulsifying powder was produced by simple adsorption of the optimized liquid SEDDS onto Neusilin®US2

using a mortar and a pestle. The formulated self-emulsifying powder showed good flowability, preserved the self-emulsification properties of the liquid SEDDS and exhibited high dissolution efficiency value, compared to the pure drug. Further investigation of the produced self-emulsifying powder by DSC and XRD revealed that the drug was molecularly dispersed in the liquid SEDDS.

Different grades of Florite® (calcium silicate) have been used to formulate solid SEDDS of gentamicin (Ito et al., 2005), nifidipine (Weerapol et al., 2015a) and griseofulvin (Agarwal et al., 2009). The use of calcium silicate in these formulations resulted in positive effects on both dissolution and bioavailability of the drug.

The effectiveness of various grades of Syloid® (porous silicon dioxide) as carriers or adsorbents for different lipid-based formulations has been investigated in different studies (Agrawal et al., 2015, Wang et al., 2010). For example, Syloid® 244FP was utilized to convert liquid SEDDS of glipizide into solid SEDDS. The developed solid SEDDS showed a significant improvement in the dissolution rate of the drug indicating that the drug in the solid formulations could dissolve rapidly and completely compared to the pure drug. Also, in vivo evaluation of the formulated solid SEDDS proved increased therapeutic efficiency of the drug in reduction of blood glucose level compared to the pure drug. This improvement in bioavailability of glipizide was attributed to improved solubility and dissolution rate of the drug from the solid SEDDS (Agrawal et al., 2015). Similar findings were also reported for the production of self-emulsifying pellets of nitrendipine from liquid SEDDS formulation (Wang et al., 2010). The self-emulsifying pellets were produced by adsorption of the liquid SEDDS of nitendipine onto Syloid® 244 FP, followed by extrusion/spheronization process to formulate pellets. The developed self-emulsifying pellets preserved the self-emulsification properties of the liquid SEDDS. The results of the in vitro dissolution and the oral bioavailability of the formulated pellets were significantly higher than that obtained for conventional tablets and were not significantly different than the results obtained for liquid SEDDS formulation.

Syloid® XDP grades were specifically engineered to improve oil adsorption capacity of lipid-based formulations and conversion into free flowing powder from which tablets and capsules can be produced.

Investigation of the utilization of microcrystalline cellulose (MCC) as an adsorbent to transform liquid SEDDS into solid formulations has been conducted (Abdalla et al., 2008, Li et al., 2013). The developed solid SEDDS maintained the self-emulsification properties of the liquid formulations. It also showed an enhancement of the in vitro drug release in comparison to the pure drug.

Several studies have described the use of colloidal silicon dioxide as an adsorbent to formulate solid self-emulsifying drug delivery systems (Balakrishnan et al., 2009a, Beg et al., 2016, Beg et al., 2012, Parmar et al., 2015). Enhancement of the in vitro drug dissolution and bioavailability of the tested drugs were obtained from the solid SEDDS formulated with Aerosil® 200, compared to the pure drug or the commercially available products.

iii. Melt granulation

In melt granulation or pelletization, a powder mix is converted into granules or spheronized pellets by blending a meltable binder with the powder mix using high shear mixing. The heat generated during mixing, due to friction, will cause melting of the solid or semi solid binder "melt-in process" and therefore, liquid bridges are formed with the powder particles leading to production of small granules. These granules can be transformed further into spheronized pellets under controlled conditions if high shear mixing is continued. Similar to conventional wet granulation, the meltable binder can be also sprayed, while in molten state, onto the powder particles (Jannin et al., 2008, Kalepu et al., 2013). The simple process of melt granulation does not require the addition of solvents and may provide up to 85% drug loading capacity (Jannin et al., 2008).

Melt granulation process can be controlled by controlling different parameters that are related to the binder such as particle size, concentration and viscosity on melting; or those related to the process such as mixing time and impeller speed (Jannin et al., 2008, Kalepu et al., 2013).

Polyoxylglycerides (Gelucires®), and partial glycerides or polysorbates are among the different meltable binders that can be employed for melt granulation process to produce solid SEDDS (Tang et al., 2008).

iv. Extrusion spheronization

Melt extrusion is a solvent-free method that involves forcing a material which possesses plastic properties through a die; under controlled conditions of temperature, pressure and flow rate, to produce cylindrical extrudates. High drug loading (60%) and good content uniformity can be obtained from this method of solidification especially if applied to low dose, highly potent drugs (Gupta et al., 2013, Jannin et al., 2008, Tang et al., 2008). The process of extrusion spheronization requires wet massing of a mixture of the drug and different excipients with the binder. The mixture is then extruded into rope-like (rods) extrudates which can be cut (spheronized) into uniform spheroids. The produced spheroids are dried and sifted to obtain the required size distribution (Gupta et

al., 2013, Tang et al., 2008). The melt extrusion method may result in dispersion of drugs at a molecular level in the used matrix. Also, amorphous solids or different polymorphic forms can be processed by this method (Breitenbach, 2002).

Different formulation variables may affect the properties of pellets produced by extrusion/spheronization method (Newton et al., 2001). For example, the relative amounts of self-emulsifying system and water may significantly affect the extrusion force, size spread and roughness of produced pellets. Therefore, it has been suggested that the maximum quantity of the liquid lipid formulation that can be solidified by extrusion spheronization may form 42% of the dry pellet weight (Newton et al., 2001).

Lipid based excipients such as Gelucire® 44/14 can be added to extrusion formulations to improve the dissolution rate and bioavailability of poorly soluble drugs or to provide controlled release characteristics (Mehuys et al., 2005, Serratoni et al., 2007, Hülsmann et al., 2000). For example, an enhancement of the dissolution rate of two model drugs (methyl and propyl paraben) was obtained from pellets prepared by extrusion spheronization of a mixture containing a self-emulsifying mixture consisting of equal parts of mono-diglycerides (Imwitor® 742), polysorbate 80 and microcrystalline cellulose. In addition, application of a coat of ethylcellulose, talc and glycerol onto the pellets resulted in a reduced rate of release of the drug depending on the amount of the coat added to the pellets (Serratoni et al., 2007). Moreover, a significantly higher dissolution rate was observed for 17β-estradiol hemihydrate solid dispersions prepared by melt extrusion of a mixture containing 10% 17β-estradiol hemihydrate, 40% Gelucire® 44/14 and 50% polyvinylpyrrolidone (PVP). About 57% of the drug was released in 60 minutes from the solid dispersions compared to less than 2% of the drug dissolved from the pure non-micronized powder, which indicates more than 30 fold improvement in the release rate of the drug (Hülsmann et al., 2000).

Utility of extrusion spheronization as a technique of solidification was demonstrated in the preparation of diazepam (Abdalla and Mäder, 2007), progesterone (Tuleu et al., 2004) and nitrendipine (Wang et al., 2010) self-emulsifying pellets. Good physical properties in terms of friability, shape and self-emulsifying characteristics were obtained from self-emulsifying pellets of diazepam produced by extrusion spheronization. A faster release rate of diazepam without crystallization was also observed from these pellets during the time of dissolution study (Abdalla and Mäder, 2007). In their study, Tuleu et al. (2004) found that the formulated pellets of progesterone showed nearly 100% release of the drug within 30 minutes. In addition, oral administration of these pellets to dogs showed an AUC that was seven times greater than that obtained when progesterone aqueous suspension was administered (Tuleu et al., 2004). Furthermore,

significantly higher in vitro release performance of nitrendipine (more than 80% within 30 minutes) was observed from self-emulsifying pellets prepared by extrusion spheronization (Wang et al., 2010) compared to conventional tablets. Significant improvement in nitrendipine absorption with a relative bioavailability of 159.87% was also noted following oral administration of self-emulsifying pellets to dogs in comparison to conventional nitrendipine tablets.

Improvements in the release rate and bioavailability of these drugs from pellets prepared via extrusion spheronization indicate the suitability of this method to produce solid self-emulsifying dosage forms from liquid SEDDS formulations.

v. Lyophilization

The technique of lyophilization or freeze drying involves dissolving the drug and the carrier in a common solvent followed by freezing and then sublimation to obtain a lyophilized molecular dispersion (Gupta et al., 2013). This technique of solidification has been employed in the preparation of solid self-nanoemulsifying systems for oral delivery of a therapeutic combination of tamoxifen and quercetin (Jain et al., 2014a, Jain et al., 2014b). The developed formulation showed improved therapeutic efficacy and reduced toxicity of tamoxifen citrate in comparison to free tamoxifen citrate and its combination with quercetin (Jain et al., 2014a, Jain et al., 2014b). Also, a stable solid self-nanoemulsifying formulation of an anti-viral drug (adefovir dipivoxil) that was prepared with the lyophilization technique showed improved in vitro release performance of the drug in addition to enhanced ex vivo absorption from the intestine in comparison to that obtained from plain drug suspension (Gupta et al., 2011).

1.4.6.2.2. Solid self-emulsifying dosage forms

Extensive investigation of the development of S-SEDDSs has been conducted to study the applicability of these systems and to determine their impact on different physical and biopharmaceutical properties of the incorporated drugs. Application of different solidification technologies facilitated the transformation of liquid lipid formulations into different solid dosage forms and therefore become no longer limited to capsule filling (Jannin et al., 2008). Different self-emulsifying solid dosage forms such as tablets, capsules, pellets, beads, suppositories, implants and solid dispersions were produced from the initially formulated liquid SEDDSs. **Table 1.3** summarizes some examples of self-emulsifying solid dosage forms which have been developed using different solidification techniques.

Table 1.3 Some examples of S-SEDDS dosage forms developed by different solidification techniques.

No.	S-SEDDS dosage form	Model drug	Solidification technique	Reference
1.	Tablets	Co-enzyme Q10	Adsorption onto carrier	(Nazzal et al., 2002)
		Ketoprofen	Gelling with Aerosil® 200	(Patil et al., 2004)
		Isradipine	Formulation of matrix with HPMC	(Tran et al., 2013)
		Carvedilol	Osmotic pump tablets	(Wei et al., 2007)
		Cyclosporine A	Osmotic pump tablets	(Zhang et al., 2013)
2.	Pellets	Diazepam	Extrusion / Spheronization	(Abdalla and Mäder, 2007)
		Piroxicam	Wet granulation	(Franceschinis et al., 2011)
		Nitrendipine	Extrusion / Spheronization	(Wang et al., 2010)
3.	Beads	Laoratadine	Loading to porous polystyrene beads	(Patil and Paradkar, 2006)
4.	Microspheres	Zedoary turmeric oil	Quasi-emulsion solvent diffusion method	(You et al., 2005)
5.	Nanoparticles	Paclitaxel	Multiple emulsion (w/o/w) solvent evaporation method	(Trickler et al., 2008)
6.	Phospholipid suspensions	Leutin	Dispersion into a matrix of Phasol® 53 MCT	(Shanmugam et al., 2011)
7.	Suppositories	Indomethacin	Filling into hollow gelatin suppositories	(Kim and Ku, 2000)
8.	Implants	Carmustine	Incorporation into PLGA, poly(d,l-lactide-co-glycolide) wafer by compression molding	(Chae et al., 2005)
9.	Solid dispersions	Piroxicam	Melting method	(Karataş et al., 2005)
		Nifidipine	Melting method	(Vippagunta et al., 2002)
		Diazepam	Melt agglomeration	(Seo et al., 2003)

Among the above tabulated self-emulsifying solid dosage forms, formulation of self-emulsifying solid dispersions appears as a prominent approach to develop solid self-emulsifying drug delivery systems that would enhance the solubility, dissolution and bioavailability of poorly soluble drugs. The following section will highlight the concept of self-emulsifying solid dispersions and their applicability in the manufacture of S-SEDDSs as a part of this study.

1.5. Self-emulsifying solid dispersions

1.5.1. Definition, advantages and limitations

Solid dispersions are formulations comprising the dispersion of one or more drugs into an inert hydrophilic carrier or matrix at the solid state (Vo et al., 2013, Vasconcelos et al., 2007). The dispersed drug may exist as dissolved molecules or amorphous or crystalline particles while the carrier may exist in amorphous or crystalline states (Vo et al., 2013). Formulation of solid dispersions has been reported as an approach to improve the solubility and dissolution rates of poorly soluble drugs (Leuner and Dressman, 2000), possibly by reducing the particle size of the drug to the molecular level, enhancing the wettability and porosity, and changing the drug crystallinity into an amorphous state (Vo et al., 2013, Vasconcelos et al., 2007).

Solid dispersion technology, as an approach to improve the bioavailability of poorly soluble drugs, has many advantages over other techniques that can be used for the same purpose such as salt formation, micronization, nanosizing, solubilization by co-solvents and others (Vo et al., 2013). Solid dispersions may result in reduction of particle size to possibly molecular level while the conventional size reduction methods produce particles in the range of 2–5 μm which can easily agglomerate in the formulation, during dissolution studies or during storage (Pouton, 2006, Vo et al., 2013). Application of more complicated nanosizing methods to produce drug nanocrystals may require special stabilizers and equipments (Möschwitzer, 2013). In addition, drug particles in solid dispersions will not form agglomerates due to their interaction with the carrier. Therefore, the drug is released or dissolved rapidly upon contact with GI fluids to form a supersaturation state which may facilitate rapid drug absorption (Vasanthavada and Serajuddin, 2007). Moreover, if the drug particles precipitated from solid dispersions, the precipitated particles will exhibit higher in vitro dissolution because of their preserved submicron size (< 1 μm) and also their higher energy state may lead to faster dissolution for in vivo conditions (Serajuddin, 1999).

Production techniques of solid dispersions are simpler and more widely applicable compared to other approaches applied for enhancing the bioavailability of poorly soluble drugs. Also, solid dispersions can be manufactured in oral solid dosage forms for much better patient compliance (Serajuddin, 1999).

Solid dispersions also enhance the wettability and porosity of the drug especially when surfactants are added. Improved wettability of the drug is achieved due to absorption of water by the carrier surrounding drug particles leading to improved dissolution of the drug. The polymeric carriers incorporated into solid dispersions may also enhance the solubility of the drug. Solid dispersions prepared by the solvent method are characterized by their high porous structure. Some channels can be produced in solid dispersions structure upon solvent removal resulting in increased porosity and specific surface area and consequently increased dissolution rate of the drug (Vo et al., 2013).

Difficulties in controlling the physical and chemical stability problems of the drug or the solid dispersion formulation have led to limited number of commercially available solid dispersions (Serajuddin, 1999). The most important problem with solid dispersions is the recrystallization of the drug from the amorphous state, either during manufacturing process or subsequent storage. Recrystallization may result in reduced bioavailability of the drug from solid dispersions (Vo et al., 2013). Stability of amorphous drugs during storage is highly influenced by the moisture and humidity levels as well as by the increased temperature which may accelerate the mobility of the drug and hence, drug crystallization. Also, the carriers or the polymers used in solid dispersions can absorb moisture leading to phase separation, crystal growth and conversion into the crystalline state. Therefore, control of the storage temperature and moisture is crucial for the physical stability of the drug. (Vasconcelos et al., 2007).

Different strategies were proposed to overcome the recrystallization tendency, which is the main disadvantage of solid dispersions, and then to maximize the stability of these formulations (Vasconcelos et al., 2007, Konno and Taylor, 2006, Bhugra et al., 2007). In order to prevent drug recrystallization in solid dispersions, a suitable polymer that is characterized by low hygroscopicity and strong molecular interaction with the drug must be selected (Vo et al., 2013). Molecular interaction between the drug and the polymer through hydrogen bonding is required to increase the solid miscibility and thus prevent phase separation and drug recrystallization (Karavas et al., 2006). Low hygroscopicity of the polymer may reduce water absorption by the polymer and thus decrease drug mobility (Vo et al., 2013).

Furthermore, Serajuddin (1999) has proposed that the stability problems of solid dispersions can be reduced when self-emulsifying or surface active excipients are employed as carriers in production of solid dispersions. Solid dispersions incorporating surface active or self-emulsifying carriers are categorized as lipids or lipid-based formulations since these carriers are either glycerides or chemically related to glycerides. These formulations can be prepared by simple dispersions of drugs in dietary glycerides (oils) or in complex mixtures of triglycerides, partial glycerides, surfactants, co-surfactants and co-solvents. According to formulation composition, lipid-based solid dispersions can be classified as non-emulsifying, self-emulsifying, or self-microemulsifying drug delivery systems (Vasanthavada and Serajuddin, 2007). Alternatively, these formulations can be classified depending on the size of oil droplets produced when lipid-based formulations are mixed with aqueous medium. Formulations that produce oil droplets greater than 100 µm in size are classified as Type I lipid formulations, while those which produce oil droplets in the size range from 50 to 100 µm are classified as Type IIIB lipid formulations (Pouton, 2000).

1.5.2. Carriers for solid dispersions

Selection of an appropriate carrier is an important consideration in preparation of amorphous solid dispersions. Utilization of hydrophilic polymeric compounds in solid dispersions may decrease molecular mobility of the amorphous drug leading to inhibition of crystallization and therefore, stabilization of the system (Paudel et al., 2013). Solubility of the drug in the selected polymer with possible formation of strong intermolecular interactions such as intermolecular H-bonding may increase the resistance of the solid dispersion systems to recrystallization. Also, the structure of carriers has to be considered for poorly soluble drugs of known chemical structure (Van Eerdenbrugh and Taylor, 2011). Production of homogenous dispersions may require a difference between the solubility parameters of the drug and the carrier to be less than 7.5 (Greenhalgh et al., 1999).

A wide variety of carriers (either self-emulsifying or not self-emulsifying) have been utilized for production of solid dispersions. Carriers that are not self-emulsifying include polyethylene glycols (PEGs), polyvinylpyrrolidone (PVP), cellulose derivatives, polymethacrylate polymers and sugars (Leuner and Dressman, 2000). On the other hand, self-emulsifying and surface active carriers comprise Gelucires® (Chauhan et al., 2005b, Paudel et al., 2013), Poloxamers or Pluronics (Piao et al., 2014, Tran et al., 2013, Nepal et al., 2010a, Shah and Serajuddin, 2012, Passerini et al., 2002), D-α-tocopheryl

polyethylene glycol 1000 succinate (TPGS) (Shin and Kim, 2003, Khoo et al., 2000, Goddeeris et al., 2008) and phospholipids (Hussain et al., 2012, Sosada et al., 2006).

In solid dispersions prepared with water soluble carriers that are not self-emulsifying (such as polyethylene glycols, PEGs), rapid dissolution of the water soluble carrier matrix causes the formation of a highly concentrated solution of the drug that precipitates on the surface of the dissolving carrier matrix preventing further dissolution of the dispersed drug. Therefore, sifting and pulverization of these solid dispersions are required to enhance drug release through increasing the surface area of dispersed particles. In addition employment of non-surface active carriers such as soft and tacky PEGs may hinder pulverization and milling of the produced solid dispersions. Moreover, capsule filling or tableting of these conventional solid dispersions may become difficult due to poor flow and mixing properties of the produced powders. On the other hand, incorporating self-emulsifying or surface active lipids in solid dispersions prevents the formation of an undissolved drug surface layer on the excipient during dissolution process (Vasanthavada and Serajuddin, 2007). Even though the released drug particles may remain undissolved when their concentration exceeds their saturation solubility, they will usually be dissolved, emulsified or dispersed by the surface active properties of the dissolving carrier. The subsequent increase in surface area of these finely divided drug particles will promote their dissolution in GI fluids (Serajuddin, 1999).

Self-emulsifying solid dispersions are relatively easily manufactured and can be filled directly into hard gelatin capsules while in the molten state to solidify upon cooling at ambient room temperature. Therefore, sifting, milling and mixing procedures are not required (Vasanthavada and Serajuddin, 2007).

An overview of Gelucires® as self-emulsifying carriers that will be evaluated in this study to formulate Gelucire®-based self-nanoemulsifying formulations is given below.

1.5.3. Gelucires® as surface active and self-emulsifying carriers

Gelucires® are polyglycolized glycerides, waxy semi-solid, surfactants composed of a mixture of glyceryl and PEG 1500 esters of long chain fatty acids. Different grades are available for these carriers based on their HLB values (range of 1-18) and melting points (33-70^{o}C). Gelucire®44/14, for which the melting point is 44^{o}C and the HLB value equals 14, and Gelucire®50/13 are the most commonly employed grades in solid dispersions (Paudel et al., 2013). The mechanism of drug release from these surfactants is determined by their HLB values. High HLB Gelucires® release the drug by diffusion and erosion mechanism while low HLB grades release the drug by either simple diffusion or complex release kinetics (Chauhan et al., 2005a).

Employment of Gelucires® as self-emulsifying carriers to improve the limited solubilizing capacity of solid dispersions may decrease recrystallization and precipitation of the drug upon exposure to liquid medium leading to improved dissolution and bioavailability of the drug (Tran et al., 2009, Barker et al., 2003, Faisal et al., 2013). For example, utilization of the self-emulsifying carrier, Gelucire® 44/14, in the preparation of solid dispersions of a poorly soluble drug, aceclofenac, by the melting method improved the dissolution rate of the drug due to modification of drug crystallinity into the amorphous form by the carrier upon exposure to aqueous media (Tran et al., 2009). Also, preparation of α-tocopherol solid dispersions in Gelucire® 44/14 by the melting method resulted in a 2-folds increase in oral bioavailability of α-tocopherol in human volunteers compared to a commercial product containing an equivalent amount of the drug. This increase in oral bioavailability of α-tocopherol was due to formation of fine emulsion droplets of the vitamin upon dispersion in GI fluids (Barker et al., 2003). Further, Faisal et al. (2013) prepared self-emulsifying solid dispersions of lycopene by the conventional solvent evaporation method using Gelucire® 44/14 as the dispersion carrier, in order to improve the oral bioavailability of the antioxidant drug. An increase in the oral bioavailability of lycopene by 2.4-fold was observed in fasted pigs, relative to Lycovit® formulation. However, these in vivo results did not correlate with the lower in vitro dissolution profile obtained from the prepared self-emulsifying solid dispersions compared to Lycovit® formulation. The absence of in vitro/in vivo correlation was attributed to possible contribution of the chosen lipid excipients to stimulation of the intestinal lymphatic uptake of the drug and hence, to improvement of the overall absorption (Faisal et al., 2013). Further, improved oral bioavailability of some drugs dispersed in Gelucire® 44/14, alone or in combination with other excipients, such as halofantrine (Khoo et al., 2000), ontazolast (Hauss et al., 1998) and piroxicam (Yüksel et al., 2003) was explained on the basis of enhanced lymphatic transport and intestinal permeability of drug particles (Hauss et al., 1998).

Gelucire® 44/14 was also employed as a solubilizer, either alone or in combination with other ingredients, to enhance the solubility and dissolution properties of some poorly water soluble drugs such as piroxicam (Karataş et al., 2005), carbamazepine (da Fonseca Antunes et al., 2013), phenytoin (Kawakami et al., 2004), glimepiride (Makar et al., 2013), and naproxen (Nagabandi et al., 2014). Improvement in solubility and dissolution rate of poorly soluble drugs dispersed in Gelucire® 44/14 was attributed to the solubilizing effect of this carrier, improved wetting of drug particles in the dissolution medium, and emulsifying properties of the carrier (Gattefossé, 2012, Karataş et al., 2005).

Gelucire® 50/13 has been employed in several studies to improve the solubility and dissolution rate of poorly soluble compounds. For example, the solubility of indomethacin increased by 3.5 fold when the drug was formulated as solid dispersions in Gelucire® 50/13 (El-Badry et al., 2009). These solid dispersion exhibited higher drug release in phosphate buffer pH 7.4 compared to both the physical mixture and the pure drug. Enhanced solubility and dissolution rate of indomethacin from solid dispersions prepared in Gelucire® 50/13, at 1:4 ratio, was attributed to decreased particle size of the drug, improved wettability of drug particles because of hydrophilicity of the carrier and decreased crystallinity of the drug which was shown by DSC.

The potential of use of Gelucire® 50/13 to enhance the solubility and dissolution profile of a poorly soluble drug, carvedilol (Potluri et al., 2011) was also investigated. It was reported that the formulated solid dispersions of carvedilol in Gelucire® 50/13 showed significant enhancement in the solubility (60 fold) and dissolution profile of carvedilol compared to the pure drug. The enhanced solubility and dissolution characteristics of carvedilol were attributed to enhanced drug wettability and absence of crystalline structure of drug particles in Gelucire® 50/13 solid dispersions.

Similar findings were also reported for solid dispersions of loratadine in Gelucire® 50/13 (Bandari et al., 2014), where a 100 fold increase in the solubility of loratadine was observed, compared to the solubility of pure drug, in addition to enhanced dissolution performance from the solid dispersions.

Gelucires® can also be used with other surfactants to improve properties of solid dispersions. For example combination of Gelucire® 50/13 with Pluronic® F68 in solid dispersions of nifidipine resulted in a higher dissolution rate of the drug from solid dispersions, compared to the crystalline drug of the same particle size, due to increase in wettability and solubility of the drug in the presence of the two surfactants (Vippagunta et al., 2002). Also, Karataş et al. (2005) have used a combination of Gelucire® 44/14 with Labrasol® in order to improve solubility and dissolution rate of piroxicam from solid dispersions. The enhanced dissolution rate of the drug from these solid dispersions was attributed to the solubilizing effects of Gelucire® 44/14 and possibly Labrasol®, and also to improved wettability of piroxicam in the dissolution medium due to the amphiphilic properties of the used surfactants. In addition, combining Gelucire® 44/14 with labrasol® in solid dispersions of piroxicam showed improved relative bioavailability of the drug in human volunteers as compared to pure piroxicam filled in hard gelatin capsules (Yüksel et al., 2003).

Recently, Gelucire® 48/16 was introduced into the field of pharmaceutical research. This Gelucire® grade possesses a melting point of 48^{o}C and HLB value of 16.

Two case studies were conducted by Gattefossé (2015) on two poorly soluble, class II compounds (piroxicam and curcumin) to demonstrate the solubilizing properties of Gelucire®48/16. A significant increase in equilibrium solubility of both compounds was produced by Gelucire®48/16, with drug saturation levels kept below 80% to avoid the risk of precipitation in aqueous fluids. Evaluation of re-dispersibility of the produced formulations showed that micellar solutions obtained in purified water were homogenous and no precipitation of either drug was noticed. Also, encapsulation of both drugs within micelles did not change the size of the dispersion from the nanoscale range (Gattefossé, 2015). An in vitro lipolysis test for solid dispersions of Gelucire®48/16 with piroxicam or curcumin confirmed that Gelucire® 48/16 preserved its solubilizing properties during digestion by pancreatic enzymes. Therefore, it was proposed that Gelucire® 48/16 may contribute to enhanced in vivo absorption and bioavailability of both drugs as it maintains high drug concentration in solution during in vitro digestion for 60 minutes.

In a statistical experimental design conducted by Ganesh (2016), it was shown that an optimum solid dispersion formulation of rivaroxaban (class II drug used to treat deep vein thrombosis and myocardial infarction) with a mixture comprising Gelucire®48/16, Compritol®HD5 ATO and Labrasol® produced the maximum dissolution profile of the drug (rivaroxaban) with 64.7% released within 5 minutes. These results were attributed to the emulsifying effect of Gelucire®48/16. However, another study that formulated solid dispersions of glimepiride utilizing different Gelucires® showed that the dissolution profile obtained for solid dispersions prepared with Gelucire®55/18 was higher than those obtained from formulations prepared with Gelucire®48/16 or Gelucire®44/14 (Sambasivarao, 2016).

In addition, the in vitro digestion testing of formulations prepared by dispersion of model class II drugs (piroxicam, curcumin and nifidipine) in Gelucire® 48/16 was evaluated in comparison to other water soluble and water dispersible surfactants such as Tween® 80, Cremophor® RH40 and Labrasol® ALF (Jannin et al., 2015). It was observed that dispersions of piroxicam and curcumin prepared with Gelucire® 48/16 showed the best performance during in vitro lipolysis, compared to other formulations prepared with different water soluble or water dispersible surfactants. The enhanced in vitro digestion of formulations prepared with Gelucire® 48/16 was explained on the basis that Gelucire® 48/16 maintained both drugs in a dissolved state in the aqueous phase during the 1-h in vitro digestion test. The study concluded that enhanced drug solubilization affected by excipients like Gelucire® 48/16 is crucially required to maintain the drug in solution after dispersion and digestion (Jannin et al., 2015).

1.5.4. Manufacture of self-emulsifying solid dispersions

Solid dispersions can be produced by three different methods: melting (fusion) method, solvent method and melting–solvent method. The choice among solid dispersion preparation methods is controlled by the physicochemical properties of drugs and carriers (Vo et al., 2013, Paudel et al., 2013). For example, limited application of melting process may arise from the crucial requirement of compatibility and miscibility of the drug and the carrier at the heating temperature. Phase separation may occur due to incompatibility and incomplete miscibility of the drug in the melted and highly viscous polymeric carrier (Vasconcelos et al., 2007, Vo et al., 2013). Addition of surfactants can maintain miscibility of the drug in the melted carrier. Also, slow cooling of the melted drug-carrier mixture can lead to phase separation and therefore, fast cooling can maintain the drug in an amorphous miscible state (Vo et al., 2013). In addition, the use of high temperatures in the melting method may cause degradation of thermo-labile drugs or carriers that are melted together at the same time. However, suspending the drug in a previously melted carrier may reduce the process temperature and the time of heating (Mehanna et al., 2010).

Modifications in melting methods to avoid different limitations such as reduction of the process temperature and the time of drug heating have led to other techniques for production of solid dispersions. Among these new modified methods, the technique of hot melt extrusion (HME) appears as an alternative and promising tool for production of different solid dispersions.

The following section gives a brief description of the HME technology which was adopted in this study to produce Gelucire®-based SNEDDS formulations.

1.5.5. Hot Melt Extrusion (HME) technique for production of Gelucire®-based SNEDDS of indomethacin

Hot melt extrusion (HME) is a novel processing technique that has been used in the plastics industry since 1930. Application of the HME technique in the pharmaceutical industry has steadily grown worldwide since the early 1980s. HME involves conveying raw materials through a hopper to rotating screws under controlled heating temperatures, pressure and screw speed; and then forcing the melted mixture through an orifice or a die to produce extrudates of various shapes and sizes. This technique has been applied very widely in the manufacture of different pharmaceutical dosage forms (Crowley et al., 2007, Maniruzzaman et al., 2012).

Generally, a hot melt extruder consists of single or twin screws placed inside a cylindrical stationary barrel. The twin screws rotate in the same (co-rotating) or opposite (counter-rotating) direction. The stationary barrel is manufactured in segments that can be bolted or sealed together. A die, which can determine the size and the shape of the extrudate, is connected at the end of the barrel. Single screw extruders are simple but do not possess the capability of efficient mixing compared to twin screw extruders. Therefore, twin screw extruders **(Figure 1.3)** are preferred for manufacturing of most pharmaceutical formulations where homogenous mixing of formulation ingredients is essential. Also, twin screw extruders provide the possibility to manipulate and optimize process conditions to accommodate the wide range of pharmaceutical formulations (Maniruzzaman et al., 2012).

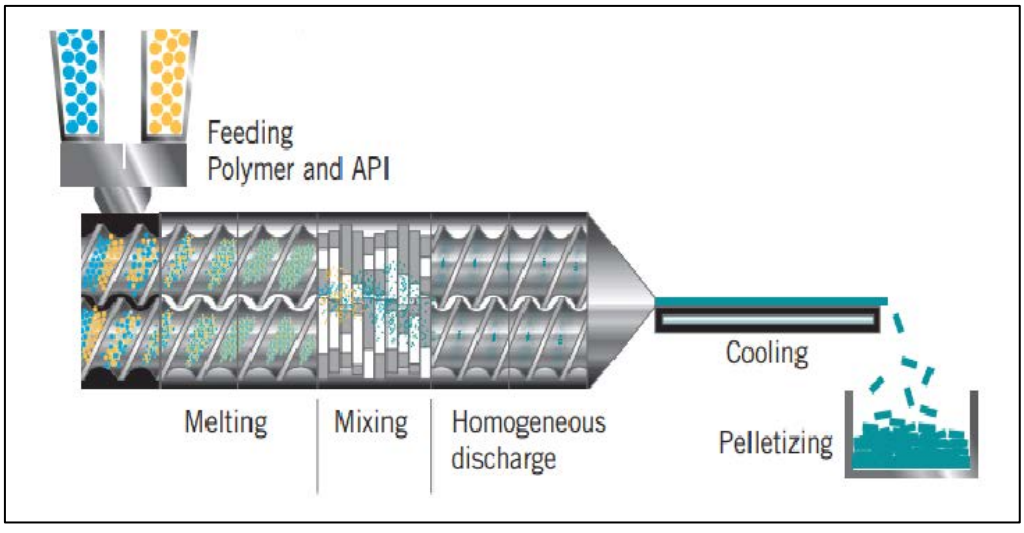


Figure 1.3 Schematic diagram of hot melt extrusion process

Although only a limited number of polymers and excipients can be processed by HME technology, HME offers several advantages over traditional manufacturing processes. This solvent-free technique is considered as an economic continuous process with fewer production steps, reduced processing time and ease of control of processing parameters, in addition to efficient mixing that is reflected by good content uniformity of the produced extrudates (Maniruzzaman et al., 2012). Also, the risk of decomposition of thermolabile drugs when processed by HME can be reduced by limiting and controlling the residence time of the heat sensitive materials within the extruder (Vo et al., 2013).

In pharmaceutical practice, HME is mainly used to enhance the dissolution rate and hence the bioavailability of poorly soluble drugs by formation of solid solutions or solid dispersions. Improvement of dissolution rate and bioavailability by HME manufacturing processes could be due to the fact that crystalline drugs can be converted into the amorphous state that would be more water soluble. Also in HME, poorly water soluble compounds are dispersed at the molecular level especially with hydrophilic polymers to produce solid solutions or solid dispersions (Maniruzzaman et al., 2012, Maniruzzaman et al., 2013b). For example, the dissolution rates of two poorly soluble drugs, famotidine and indomethacin, from solid dispersion prepared with different hydrophilic polymers using HME were higher compared to the pure drug alone (Maniruzzaman et al., 2013b). HME can be also used for the production of modified release formulations and for taste masking of bitter drugs (Maniruzzaman et al., 2012, Maniruzzaman et al., 2013b).

Pharmaceutical materials that can be processed by HME should possess thermoplastic behavior which is the ability to deform easily upon heating in the extruder and then solidify upon exiting the die part of the instrument. Thermal stability at the processing temperature is an essential requirement for each material to be processed by HME, although the reduced processing time in this technology may not exclude all thermolabile compounds. In addition, pharmaceutical materials that can be used in HME should be pure and safe, similarly to the materials used for traditional manufacturing methods (Crowley et al., 2007).

Selection of different excipients for HME processing may affect the properties of the final HME formulations, similarly to those used in conventional dosage forms. These excipients may include matrix carrier, release modifiers, bulking agents, thermal lubricants and antioxidants. For example, release modifiers can be incorporated in HME to decrease or increase the release rate of the final HME formulations. Plasticizers also can be added in HME to reduce the processing temperatures and hence, decrease the

drug and carrier decomposition. Low molecular weight polyethylene glycol and surfactants are examples of the materials that can be used as plasticizers during manufacturing by HME (Crowley et al., 2007). Therefore, surfactants can be used as plasticizers in HME to produce solid dispersions in addition to their effect as solubilizing agents (Ghebremeskel et al., 2006).

1.6. Aims of the study

The aim of this study is to investigate different self-nanoemulsifying drug delivery systems (SNEDDSs) prepared in liquid, solid and carrier-based formulations and to assess the effect of these types of formulations on the solubility and dissolution properties of a poorly water soluble drug.

Indomethacin was selected as a model drug for this study. Therapeutically, it is a potent non-steroidal anti-inflammatory and analgesic drug which is indicated for treatment of pain and swelling associated with rheumatoid arthritis, osteoarthritis, gout and other acute musculoskeletal disorders (El-Badry et al., 2009, Shakeel et al., 2013a, Taha, 2009). It is available commercially in the form of capsules, suspensions, suppositories and injections.

According to the biopharmaceutical classification system (BCS), indomethacin is classified as a Class II compound because of its poor aqueous solubility and high permeability. The dissolution rate of Class II drugs is a limiting factor in their GI absorption because it affects the concentration of drugs at the absorption membrane surface over time (Löbenberg and Amidon, 2000). Therefore, improving the solubility of Class II drugs in order to improve their dissolution profile is a crucial requirement in enhancing their oral absorption and bioavailability.

Many investigations have been conducted to evaluate indomethacin performance from self-emulsifying formulations (Taha, 2009, Kim and Ku, 2000, Stillhart and Kuentz, 2012, Shakeel et al., 2013b, Prasad et al., 2013, Shakeel et al., 2013c). Incorporation of indomethacin into SEDDS was advantageous in enhancing solubility, dissolution profile and bioavailability of the drug. Most of these SEDDS were evaluated based on the solubility of the drug in lipid excipients in addition to spontaneous dispersion after addition to aqueous fluids. However, inhibition of drug precipitation upon dilution with GI fluids and sustaining supersaturation levels of the drug may remain as the main challenges encountered with SEDDS formulations (Prasad et al., 2013). Selection of the most appropriate excipients may reduce their physical and chemical interaction with the drug and therefore, may inhibit drug precipitation. Consequently, a well-designed

SEDDS formulation of indomethacin using the proper excipients may further lead to substantial enhancement of oral bioavailability of the drug.

In addition, different formulations of indomethacin in solid dispersions using various hydrophilic carriers showed remarkable improvements in the dissolution rate of the drug that were attributed to reduction in drug crystallinity, solubilization effect of the carrier, enhancement of drug wettability, reduction of drug particle size and prevention of agglomeration of drug particles (El-Badry et al., 2009, Valizadeh et al., 2004, Wang et al., 2007). However, physical instability of these dispersions and possible conversion into the less soluble crystalline states may be encountered during processing, dissolution or under different storage conditions.

Therefore, considering the type and the amount of the carrier employed in solid dispersion formulations may assist in inhibiting drug recrystallization from these preparations. Surface active carriers such as Gelucires® may be promising in solid dispersions to improve solubility and dissolution of poorly soluble drugs and minimize tendency of recrystallization. Also, conducting of stability studies on prepared formulations may contribute to identification of the most suitable storage conditions that will maintain stability for extended periods of time.

With the introduction of some novel solid adsorbents and self-emulsifying carriers, this study aims to formulate different SNEDDSs with a view to enhance solubility, dissolution profiles and/or bioavailability of poorly water soluble drugs taking the weakly acidic indomethacin as a model drug. Approaching the most suitable formulation may achieve further goal of the study to aim at large scale production.

<u>The objectives of this study can be summarized as follows:</u>

1. To design and formulate suitable liquid SNEDDS of indomethacin using different combinations of oils, surfactant and co-surfactants, with a view to enhancing the solubility of the drug. Different combinations of components will be optimized for the best formulation by construction of ternary phase diagrams. The formulated systems will be evaluated for drug solubilizing potential, thermodynamic stability, self-emulsification efficiency, droplet size, polydispersity index and zeta potential for the purpose of optimization of drug-loaded liquid SNEDDS. This objective will be presented in **Chapter 3** of this thesis.

2. To investigate the feasibility of using adsorption onto solid carriers as a solidification technique to convert the optimum liquid SNEDDS (developed in **Chapter 3**) into solid SNEDDS. Different categories of inert carriers or adsorbents will be utilized for this

purpose. The formulated solid SNEDDS will be assessed for their powder properties and optimum formulations will be evaluated for their drug content, redispersibility and dissolution properties. Different physical characterization techniques such as Fourier Transform Infrared (FTIR), differential scanning calorimetry (DSC), X-ray diffraction (XRD), and scanning electron microscopy (SEM) will be applied on optimum solid SNEDDS formulations to confirm amorphous or crystalline states of the drug. This part of the work will be presented in **Chapter 4** of this thesis.

3. To investigate the utility of Gelucire®44/14 and Gelucire®48/16 as lipidic carriers in formulation of Gelucire-based SNEDDS adopting the hot melt extrusion technique, with a view to enhance the solubility and dissolution properties of indomethacin. The formulated Gelucire®-based SNEDDS will be evaluated for their drug content, dissolution properties, redispersibility and droplet size. Also, Fourier Transform Infrared (FTIR), differential scanning calorimetry (DSC), X-ray diffraction (XRD), and scanning electron microscopy (SEM) will be conducted for physical characterization of the formulated products to assess crystalline and amorphous states of the drug and to identify optimum Gelucire-based formulations. This part of work will be presented in **Chapter 5** of this thesis.

4. To conduct stability studies on optimum Gelucire®-based SNEDDSs (defined from **Chapter 5**) in order to assess the tendency of these formulations for recrystallization and also to determine the most suitable storage conditions. Investigation of drug dissolution properties, differential scanning calorimetry (DSC), X-ray diffraction (XRD), and scanning electron microscopy (SEM) will be carried out in order to determine the effect of aging on the physicochemical properties of the drug. This part of the study will be presented in **Chapter 6** of this thesis.

Chapter 2

Materials & Methods

2.1. Introduction

In this study, different self-nanoemulsifying drug delivery systems (SNEDDSs) of indomethacin were prepared as liquid, solid, and Gelucire®-based formulations. The liquid SNEDDSs of indomethacin were first formulated using different mixtures of oils, surfactants and co-surfactants. Studies of solubility, ternary phase diagram, self-nanoemulsification and system stability were performed to optimize the formulation components. In order to characterize different liquid formulations, dynamic light scattering (DLS) was employed for measurement of droplet size, polydispersity index (PDI) and zeta potential.

Solidification of the optimum liquid SNEDDSs of indomethacin was performed by adsorption onto different solid adsorbents. The obtained solid SNEDDS formulations were evaluated for flow properties using angle of repose method in addition to calculation of Hausner's ratio and Carr's index from tapped density experiments. Optimum solid SNEDDS formulations were then evaluated for redispersibilty, drug content and dissolution properties. Calculation of the dissolution parameters (dissolution efficiency after 15 min (DE_{15min}) and % drug released after 15 min (Q_{15min})) of solid SNEDDSs of indomethacin in addition to differential scanning calorimetry (DSC), X-ray diffraction (XRD) and Fourier Transform InfraRed (FTIR) were also conducted for characterization.

Different formulations of Gelucire®-based SNEDDS of indomethacin were prepared by mixing the drug with Gelucire® 48/16 at both 40^{o} and 50^{o}C using a hot melt (twin-screw) extruder. All formulations were tested for self-emulsification efficiency and redispersibilty. The physical properties of these formulations were characterized by DSC, XRD and FTIR. In addition, the dissolution parameters (mentioned above) of Gelucire®-based SNEDDS formulations were determined and calculated.

This chapter provides an outline of the materials used in the study in addition to brief description of the techniques and methods used in evaluation and physical characterization of different SNEDDSs of indomethacin.

2.2. Materials

2.2.1. The model drug (Indomethacin)

In this study, indomethacin (IND) was chosen as a model drug. It is a non-steroidal anti-inflammatory agent that is used as antirheumatic agent to reduce pain, fever and swelling. Upon oral administration, indomethacin acts to reduce inflammation and pain mainly by non-selective inhibition of cyclooxygenase enzymes thus reducing prostaglandin production, which may lead to irritation and ulceration of gastric mucosa upon frequent and chronic ingestion of the drug (Odabasoglu et al., 2008, Taha, 2009).

Indomethacin is a weak acid with a pK_a value of 4.5 (Beetge et al., 2000) and therefore, it may dissolve in the intestinal alkaline pH and precipitate in the acidic pH of the stomach (Shakeel et al., 2013c). Its melting point falls in the range of 158 to 162^oC depending on its polymorphic form (Wang et al., 2007). Indomethacin was reported to exist in different polymorphic forms (Lin, 1992, Slavin et al., 2002). According to Slavin et al. (2002), the most commonly obtainable forms are the crystalline γ-form and α-form, while the β-form is considered as a solvate that can be obtained from different solvents under supersaturation conditions. Amorphous indomethacin may undergo crystallization during storage to form either the more thermodynamically stable γ-form; if stored below its glass transition temperature (T_g) (Beetge et al.), or the less stable α-form; if stored above T_g (Yoshioka et al., 1994).

The aqueous solubility of indomethacin measured in distilled water (pH 5.5) was reported to be 0.937 µg/ml at 298.15^oK, (Shakeel et al., 2013a, Löbenberg and Amidon, 2000) which may account only for 0.004% of the administered dose. In addition, indomethacin possesses high permeability because, according to FDA (2000), 90% or more of the administered dose of the drug is absorbed. A value of permeability of 4.167 x 10^{-4} cm/sec was reported for highly permeable drugs (Levitt, 2013).

The molecular structure of indomethacin is presented in **Figure 2.1,** while its physicochemical properties and toxicity are shown in **Table 2.1**.

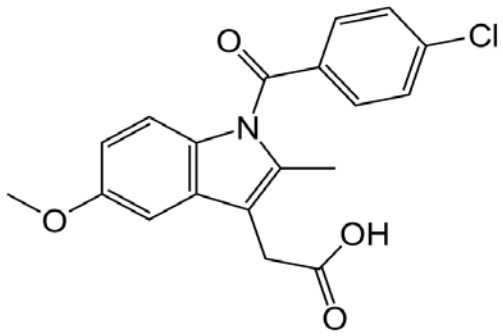


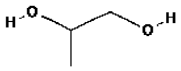
Figure 2.1 Chemical Structure of indomethacin

Table 2.1 Physicochemical properties and toxicity of indomethacin (Pubchem, 2017)

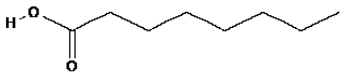
Properties	Description
Manufacturer	Sigma-Aldrich Chemie GmbH, Germany
IUPAC name	1-(4-chlorobenzoyl)-5-methoxy-2-methyl-3-indoleacetic acid
Empirical formula	$C_{19}H_{16}ClNO_4$
Physical appearance	Pale-yellow to yellow-tan, crystalline powder
Melting point	158 to 162^{o}C
Molecular weight	357.79 g/mol
Solubility	Soluble in ethanol, ether, acetone, castor oil; practically insoluble in water (0.937 mg/L at 25^{o}C)
Toxicity	Increased risk of bleeding, ulceration, and perforation of the gastrointestinal tract is associated with overdosage

2.2.2. Capryol™ 90 (propylene glycol monocaprylate)

Capryol 90 is a nonionic water insoluble surfactant which can be used in oral lipid-based formulations such as SEDDS. Also, it can be used as a co-surfactant and solubilizer in topical dosage forms (Gattefossé, 2012). The chemical structure of this compound is given in **Figure 2.2** and the physicochemical properties and toxicity are presented in **Table 2.2**.



Propylene glycol



Caprylic acid

Figure 2.2 Chemical structure of Capryol™ 90 (propylene glycol monocaprylate) (Gattefossé, 2012, Pubchem, 2017)

Table 2.2 Physicochemical properties and toxicity of Capryol™ 90 (Gattefossé, 2012, Pubchem, 2017)

Properties	Description
Manufacturer	Gattefossé, Lyon, France
USP NF name	Propylene glycol monocaprylate
Chemical description	Propylene glycol esters of caprylic acid (C8), mainly composed of monoesters and a small fraction of diesters.
Empirical formula	$C_{11}H_{24}O_4$
Physical appearance	Light yellow oily liquid with characteristic odour
HLB	5
Molecular weight	220.309 g/mol
Solubility	Insoluble in water
Toxicity	Safety of use is inferred by precedence of use in approved pharmaceutical products

2.2.3. Labrafac^{TM} lipophile WL 1349 (medium chain triglyceride)

Labrafac^{TM} lipophile WL 1349 is an oily vehicle and solubilizer that can be used for oral, topical, and parenteral lipid-based formulations (Gattefossé, 2012). The chemical structure, physicochemical properties and toxicity of this compund are presented in **Figure 2.3** and **Table 2.3**, respectively.

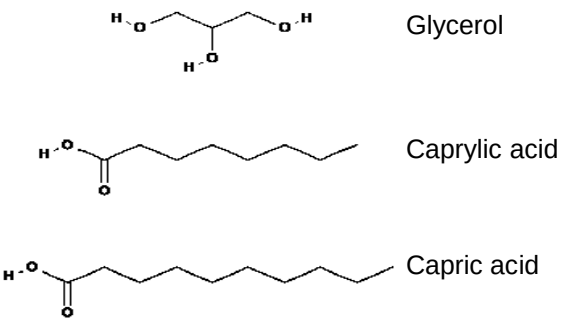


Figure 2.3 Chemical structure of Labrafac^{TM} lipophile WL 1349 (medium chain triglyceride) (Pubchem, 2017)

Table 2.3 Physicochemical properties and toxicity of Labrafac^{TM} lipophile WL 1349 (medium chain triglyceride) (Gattefossé, 2012, Pubchem, 2017)

Properties	Description
Manufacturer	Gattefossé, Lyon, France
USP NF name	Medium chain triglycerides or (glycerides, mixed decanoyl and octanoyl)
Chemical description	Consists of medium-chain triglycerides of caprylic (C_8) and capric (C_{10}) acids
Empirical formula	$C_{21}H_{44}O_7$
Physical appearance	Yellow liquid with characteristic odour
HLB	1
Molecular weight	408.576 g/mol
Solubility	Insoluble in water
Toxicity	Safety of use is inferred by food additive status and precedence of use in approved pharmaceutical products

2.2.4. Labrafil® M 2125 CS (polyoxylglyceride)

Labrafil® M 2125 CS is a nonionic water-dispersible surfactant that can be used for lipid-based formulations to solubilize and enhance oral bioavailability of poorly water-soluble drugs. The physicochemical properties and toxicity of this compound are presented in **Table 2.4**.

Table 2.4 Physicochemical properties and toxicity of Labrafil® M 2125 CS (polyoxylglyceride) (Gattefossé, 2012, Pubchem, 2017, Rowe et al., 2009)

Properties	Description
Manufacturer	Gattefossé, Lyon, France
USP NF name	Linoleoyl polyoxyl-6 glycerides (or Linoleoyl macrogol-6 glycerides)
Chemical description	Consists of mono-, di- and triglycerides and PEG-6 (MW 300) mono- and diesters of linoleic ($C_{18:2}$) acid
Physical appearance	Yellow liquid with light odour
HLB	9
Solubility	Dispersible in water, soluble in many organic solvents
Toxicity	Generally regarded as relatively nonirritant and nontoxic

2.2.5. Transcutol® HP (diethylene glycol monoethyl ether)

Transcutol® HP is a highly pure solvent and solubilizer that improve solubility and bioavailability of poorly soluble drugs. The chemical structure, physicochemical properties and toxicity of this compound are presented in **Figure 2.4** and **Table 2.5**, respectively.

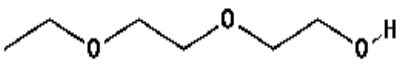


Figure 2.4 Chemical structure of Transcutol® HP (diethylene glycol monoethyl ether) (Pubchem, 2017)

Table 2.5 Physicochemical properties and toxicity of Transcutol® HP (diethylene glycol monoethyl ether) (Gattefossé, 2012, Pubchem, 2017)

Properties	Description
Manufacturer	Gattefossé, Lyon, France
USP NF name	Diethylene glycol monoethyl ether
Chemical description	Consists of highly purified diethylene glycol monoethyl ether
Empirical formula	$C_6H_{14}O_3$
Physical appearance	Colourless liquid with light odour
Molecular weight	134.175 g/mol
Solubility	Miscible in water and common organic solvents
Toxicity	Safety of use and low irritancy inferred by numerous toxicological studies and precedence of use in approved pharmaceutical products

2.2.6. Cremophor® RH 40 (polyoxyl 40 hydrogenated castor oil)

Cremophor® RH 40 (or Kolliphor RH40) is a polyoxyl 40 hydrogenated castor oil that can be used as emulsifying, solubilizing and wetting agent in lipid-based formulations. The physicochemical properties and toxicity of this compound are presented in **Table 2.6**.

Table 2.6 Physicochemical properties and toxicity of Cremophor RH40 (polyoxyl 40 hydrogenated castor oil) (BASF, 2014, Rowe et al., 2009)

Properties	Description
Manufacturer	BASF – Germany
USP NF name	Polyoxyl 40 hydrogenated castor oil
Chemical description	Nonionic solubilizer and emulsifying agent obtained by reacting 45 moles of ethylene oxide with 1 mole of hydrogenated castor oil. The main constituent of Cremophor®RH 40 is glycerol polyethylene glycol oxystearate, which, together with fatty acid glycerol polyglycol esters, forms the hydrophobic part of the product. The hydrophilic part consists of polyethylene glycols and glycerol ethoxylate.
Empirical formula	$C_6H_{14}O_3$
Physical appearance	White to yellowish, thin paste at 20°C that liquefies at 30°C. It has a very faint characteristic odour.
HLB	14 – 16
Solubility	Soluble in water, ethanol, chloroform and oils
Toxicity	Essentially non-toxic and non-irritant material as shown by acute and chronic toxicity tests in animals

2.2.7. Tween® 20 (Polysorbate 20)

Polysorbate 20 (Tween 20) is a hydrophilic non-ionic surfactant. Polysorbate 20 is produced by ethoxylation of sorbitan before the addition of lauric acid (Polyoxyethlene (20) sorbitan monolaurate). The ethoxylation process leaves the molecule with 20 units of polyethylene glycol. Polysorbates are widely used as emulsifying agents in preparation of stable oil in water pharmaceutical emulsions. They are also utilized as solubilizing agents for poorly soluble drugs as well as wetting agents (Rowe et al., 2009). The number 20 following the 'Polysorbate' part indicates the type of the fatty acid (lauric acid) linked to the polyoxyethlene sorbitan part of the molecule. The chemical structure of Tween 20 is given in **Figure 2.5**, and the physicochemical properties and toxicity are summarized in **Table 2.7.**

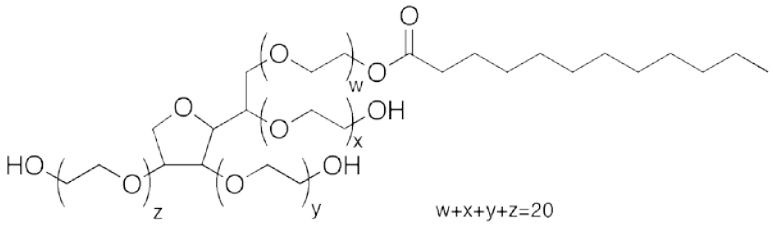


Figure 2.5 Chemical structure of Tween® 20 (Polysorbate 20) (Pubchem, 2017, Rowe et al., 2009)

Table 2.7 Physicochemical properties and toxicity of Tween® 20 (Polysorbate 20) (Pubchem, 2017, Rowe et al., 2009)

Properties	Description
Manufacturer	Sigma-Aldrich Chemie GmbH, Germany
Chemical name	Polyoxyethylene 20 sorbitan monolaurate
Chemical description	A series of partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20 moles of ethylene oxide for each mole of sorbitol and its anhydrides.
Empirical formula	$C_{58}H_{114}O_{26}$
Molecular weight	1128
Physical appearance	Yellow oily viscous liquid with characteristic odour
HLB	16.7
CMC	60 mg/L or ~ $6x10^{-3}$ mole/L
Solubility	Soluble in water and ethanol
Toxicity	Generally regarded as non-toxic and non-irritant material

2.2.8. Tween® 80 (polysorbate 80)

Polysorbate 80 is a non-ionic surfactant that is often used in foods and cosmetics. It is widely used as emulsifying agent in oil in water pharmaceutical emulsions, a solubilizing agent for poorly soluble drugs, as well as a wetting agent. Polysorbate 80 is derived from polyethoxylated sorbitan and oleic acid. The hydrophilic group in this compound is the polyether (or polyoxyethylene) group, while the lipophilic group is the oleic acid. The number 20 following the 'polyoxyethelene' part indicates the total number of oxyethlene –(CH_2-CH_2-O)- groups in the molecule, while the number that follows the 'Polysorbate' part refers to the type of fatty acid (oleic acid) associated with polyoxythelene sorbitan part of the molecule (Rowe et al., 2009).

The chemical structure of Tween 80 is given in **Figure 2.6**, and the physicochemical properties and toxicity are summarized in **Table 2.8.**

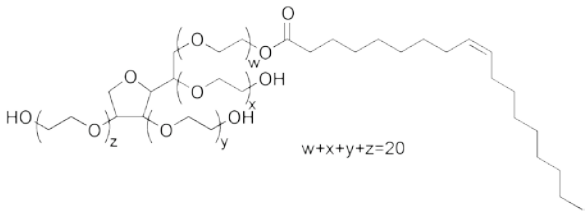


Figure 2.6 Chemical structure of Tween® 80 (Polysorbate 80) (Pubchem, 2017, Rowe et al., 2009)

Table 2.8 Physicochemical properties and toxicity of Tween® 80 (Polysorbate 80) (Pubchem, 2017, Rowe et al., 2009)

Properties	Description
Manufacturer	Sigma-Aldrich Chemie GmbH, Germany
Chemical name	Polyoxyethylene 20 sorbitan mono oleate
Chemical description	A series of partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20 moles of ethylene oxide for each mole of sorbitol and its anhydrides.
Empirical formula	$C_{64}H_{124}O_{26}$
Molecular weight	1310
Physical appearance	Yellow oily viscous liquid with characteristic odor
HLB	15
CMC	13 – 15 mg/liter
Solubility	Soluble in water and ethanol
Toxicity	Generally regarded as non-toxic and no-irritant materials.

2.2.9. Polyethylene glycol 400 (PEG 400)

Polyethylene glycols (PEGs) are widely used in formulation of pharmaceutical preparations such as topical, oral, parenteral, ophthalmic and rectal dosage forms. Also, these excipients have been used in controlled release systems as biodegradable polymeric matrices. Polyethylene glycols occur in different molecular weights (grades). Grades 200 – 600 appear as clear, colorless viscous liquids while grades 1000 and above are solids at room temperature. In addition to their use in different pharmaceutical formulations, polyethylene glycols have been also used to improve the solubility and dissolution properties of poorly water soluble drugs (Rowe et al., 2009). In this study, PEG 400, which is a low molecular weight polyethylene glycol, has been employed as an excipient to improve the solubility of the model drug. The chemical structure of polyethylene glycol is presented in **Figure 2.7**, while the physicochemical properties and toxicity of PEG 400 are summarized in **Table 2.9**.

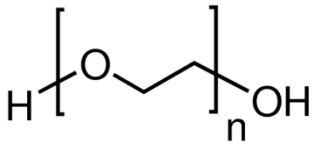


Figure 2.7 Chemical structure of polyethylene glycol

Table 2.9 Physicochemical properties and toxicity of polyethylene glycol 400 (Rowe et al., 2009)

Properties	Description
Manufacturer	Sigma-Aldrich Chemie GmbH, Germany
Chemical name	α-Hydro-ω-hydroxypoly(oxy-1,2-ethanediyl)
Empirical formula	$C_{2n}H_{4n+2}O_{n+1}$, n = 8.7
Molecular weight	380–420 g/mole
Physical appearance	Clear, colorless viscous liquid
Solubility	Soluble in water, alcohol, acetone, and glycerin; insoluble in fats, fixed oils and mineral oil.
Toxicity	Generally regarded as non-toxic and non-irritant material

2.2.10. Propylene glycol

Propylene glycol is a commonly used solvent or co-solvent in different pharmaceutical formulations, cosmetics and foods. It can act to dissolve many poorly soluble drugs in oral solutions and parenteral formulations. It can also be used as a humectant in topical formulations and as a preservative in solution and semisolid formulations (Rowe et al., 2009). **Figure 2.8** and **Table 2.10** show the chemical structure, physicochemical properties and toxicity of propylene glycol, respectively.

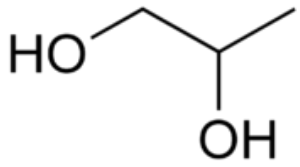


Figure 2.8 Chemical structure of propylene glycol

Table 2.10 Physicochemical properties and toxicity of propylene glycol (Rowe et al., 2009)

Properties	Description
Manufacturer	Sigma-Aldrich Chemie GmbH, Germany
Chemical name	1,2-Propanediol
Empirical formula	$C_3H_8O_2$
Molecular weight	76.09 g/mole
Physical appearance	Clear, colourless, viscous, and practically odourless liquid
Solubility	Miscible in water, ethanol, chloroform, diethyl ether and acetone
Toxicity	Generally regarded as non-toxic and non-irritant material. It is also used extensively in foods and cosmetics.

2.2.11. Neusilin® US2 (Magnesium aluminometasilicate)

Neusilin® is a multifunctional excipient that occurs as fine powder or granules of magnesium aluminometasilicate (MAS). This excipient is widely used in wet granulation and direct compression processes to improve manufacturing of tablets, capsules, powders and granules. Neusilin® is available in various grades that possess different bulk density, water content, pH and particle size. It is amorphous and characterized by very large specific surface area and high oil and water adsorption capacity. The general chemical structure of Neusilin® is presented in **Figure 2.9** and its physicochemical properties and toxicity are presented in **Table 2.11**.

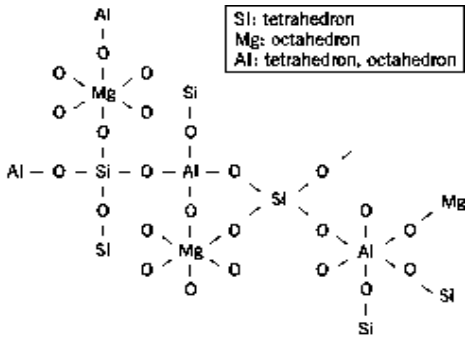


Figure 2.9 Chemical structure of Neusilin® US2 (MAS) (Fuji Chemical Industry, 2014, Tan et al., 2013)

Table 2.11 Physicochemical properties and toxicity of Neusilin®US2 (MAS) (Fuji Chemical Industry, 2014, Tan et al., 2013)

Properties	Description
Manufacturer	Fuji Chemical Industry Co. Ltd, Tokyo, Japan
Chemical name	Magnesium aluminometasilicate
Empirical formula	$Al_2O_3 \cdot MgO \cdot 1.7SiO_2 \cdot xH_2O$
Oil adsorbing capacity (ml/100g)	270 – 340
Water adsorbing capacity (ml/100g)	240 – 310
Particle size distribution (µm)	44 – 177
Specific surface area (m^2/g)	300
Average pore diameter (nm)	5 – 6
Loose bulk density (g/ml)	0.13 – 0.18
Tapped bulk density (g/ml)	0.16 – 0.22
Angle of repose (deg.)	30
Physical appearance	White granules
Solubility	Practically insoluble in water and ethanol
Toxicity	An accepted ingredient by the US Pharmacopoeia / National Formulary with no reports of adverse reactions. The US DMF (type IV) was filed in 1998.

2.2.12. Florite® PS-200 (Calcium silicate)

Florite® is a new multifunctional excipient composed of synthetic calcium silicate. This excipient offers many advantages for cosmetics and pharmaceutical formulations (Tomita Pharmaceutical Co., 2015). It is available commercially in different grades that possess different oil or water absorption capacity. Florite® products are characterized by their high water/oil absorption ability compared to other inorganic materials such as silicone dioxide. Florite® could absorb up to 5 times its weight of liquid and remain a free flowing powder. This exceptional liquid absorbency for both water and oil is due to the unique petaloid crystal structure **(Figure 2.10)** as compared to other calcium silicate.

Due to the petaloid crystal structure, Florite® products possess deep and large macropores which provide the highest liquid absorption capacity. Drug molecules loaded into these macropores are protected from light and oxygen which may result in enhanced stability of the drug. The PS grades of Florite® are suitable for tablet manufacturing and are characterized by low angles of repose. Moreover, Florite® can maintain the pore structure and therefore, preserve the liquid absorbency after compression so that no leaking of liquid during compression process can be observed (Tomita Pharmaceutical Co., 2015).

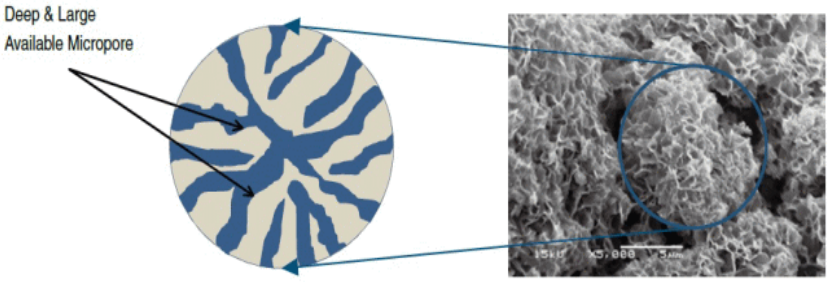


Figure 2.10 Petaloid crystal structure of Florite® products (Tomita Pharmaceutical Co., 2015)

The physicochemical properties and toxicity of calcium silicate (Florite® PS-200) are summarized in **Table 2.12.**

Table 2.12 Physicochemical properties and toxicity of calcium silicate (Florite® PS-200) (Rowe et al., 2009, Tan et al., 2013) (Tomita Pharmaceutical Co., 2015)

Properties	Description
Manufacturer	Tomita Pharmaceutical Co. Ltd, Japan
Chemical name	Calcium silicate
Empirical formula	$CaSiO_3$
Molecular weight	116.2 g/mole
Oil absorption capacity (ml/100g)	370
Particle size distribution (µm)	150
Loose bulk density (g/ml)	0.07
Tapped bulk density (g/ml)	0.09
Angle of repose (deg.)	36
Physical appearance	White or off-white granules
Solubility	Practically insoluble in water.
Toxicity	Calcium silicate is included in the FDA Inactive Ingredients Database (oral dosage forms). Practically nontoxic in oral formulations. The DMF submitted for the NF calcium silicate (as Florite® R) is 28644 (type IV).

2.2.13. Syloid® XDP 3150 (Amorphous silicon dioxide)

Syloid® is a chemically prepared amorphous silicon dioxide that is used in many pharmaceutical formulations due its unique features. It is characterized by its morphology, adsorption capacity, particle size, density and internal surface area. High density of Syloid® may aid to create less dust processes during manufacturing of pharmaceuticals. Also, Syloid® could improve the flow properties of pharmaceutical mixtures during manufacturing. In addition, the unique morphology of highly developed network of meso-pores allow Syloid® particles **(Figure 2.11)** to adsorb maximum amounts of liquids and eases the transformation of liquid or lipid-based formulations into free flowing powder. Syloid® is available in different grades that differ in their porosity, internal surface area and oil adsorption capacity. The XDP grades of Syloid® were developed with specific particle size and adsorption capacity to allow higher loading of lipid-based formulations and the formation of free flowing powder that can be easily converted into tablets or capsules.

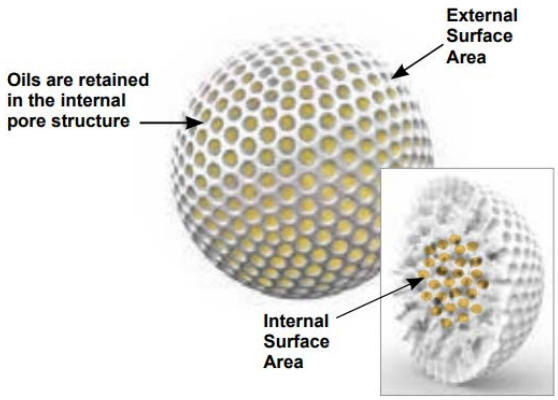


Figure 2.11 Spherical meso-pores of Syloid® particles. (Grace GmbH, 2012)

The physicochemical properties and toxicity of Syloid® XDP 3150 are presented in **Table 2.13**.

Table 2.13 Physicochemical properties and toxicity of amorphous silicon dioxide (Syloid® XDP 3150) (Grace GmbH, 2012)

Properties	Description
Manufacturer	Grace GmbH & Co. KG, Germany
Chemical name	Amorphous silicon dioxide
Pore volume (ml/100g)	174
Mean particle size distribution (µm)	120 – 170
Density at 20^{o}C (g/ml)	2.17 – 2.20
Bulk density at 20^{o}C (Kg/m^{3})	200 – 600
Angle of repose (deg.)	≈32
Physical appearance	White odourless powder
Solubility	Insoluble in water.
Toxicity	Non-toxic and non-irritant

2.2.14. Microcrystalline cellulose (Avicel® PH 102)

Microcrystalline cellulose (MCC) is a widely used pharmaceutical excipient especially in tablet manufacturing. This compound is used as a diluent or a binder in formulation of tablets by wet granulation or direct compression methods. It also possesses some disintegrant and lubricant effects which make it useful in tabletting processes (Rowe et al., 2009). Additionally, this material is used in cosmetic and food industries.

The chemical structure, physicochemical properties and toxicity of MCC are shown in **Figure 2.12** and **Table 2.14**, respectively.

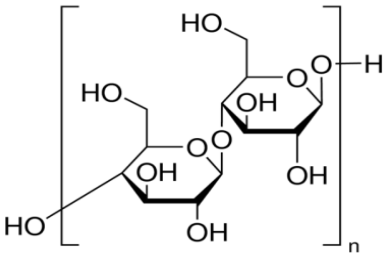


Figure 2.12 Chemical structure of microcrystalline cellulose (MCC)

Table 2.14 Physicochemical properties and toxicity of microcrystalline cellulose (Rowe et al., 2009)

Properties	Description
Manufacturer	Sigma-Aldrich Chemie GmbH, Germany
Chemical name	Cellulose
Empirical formula	$(C_6H_{10}O_5)_n$ where n ≈ 220.
Molecular weight	≈ 36 000
Mean particle size (µm)	100 (Avicel® PH-102)
Specific surface area (m^2/g)	1.21–1.30 m^2/g (Avicel® PH-102)
Physical appearance	White, odourless, tasteless, crystalline powder composed of porous particles
Solubility	Practically insoluble in water and most organic solvents, slightly soluble in 5% w/v sodium hydroxide solution.
Toxicity	Widely used in oral pharmaceutical formulations and food products and is generally regarded as a relatively nontoxic and nonirritant material.

2.2.15. Aerosil® 200 (colloidal silicon dioxide)

Aerosil® (colloidal silicon dioxide) is a commonly used excipent in pharmaceutical formulations and cosmetics. Commercially, Aerosil® is available in different grades that are obtained by modifications in its manufacturing processes. Aerosil® is characterized by its high specific surface area and small particle size which may improve the flow properties of powders especially in tablet manufacturing and capsule filling (Rowe et al., 2009).

The physicochemical properties and toxicity of colloidal silicon dioxide used in this study (Aerosil® 200) are shown in **Table 2.15**.

Table 2.15 Physicochemical properties and toxicity of colloidal silicon dioxide (Aerosil® 200) (Rowe et al., 2009)

Properties	Description
Manufacturer	Evonik Industries AG, Essen, Germany
Chemical name	Silica
Empirical formula	SiO_2
Molecular weight	60.08
Mean particle size (µm)	Primary particle size is 7–16 nm. Aerosil forms loose agglomerates of 10–200 µm
Specific surface area (m^2/g)	200 ± 25
Physical appearance	Light, loose, bluish-white-colored, odourless amorphous powder.
Solubility	Practically insoluble in organic solvents and water; soluble in hot solutions of alkali hydroxide
Toxicity	Generally regarded as an essentially nontoxic and nonirritant excipient and widely used in oral and topical pharmaceutical products

2.2.16. Gelucire® 44/14

Gelucires® are lipid-based, amphiphilic excipients that possess hydrophilic and hydrophobic properties due to their composition of fatty acid esters of polyethylene glycol (PEG) and glycerides (Svensson et al., 2004). Gelucires® are available in several grades with a wide range of properties due to differences in their melting point and hydrophile-lipophile balance (HLB). Each grade of Gelucires® is denoted by two numbers at the end of 'Gelucire' indicating the values of melting point and HLB (Gattefossé, 2012). Grades with low HLB values are mainly composed of partial glycerides, while grades with high HLB values (above 10) are composed of mixtures of partial saturated glycerides and PEG esters (Bandari et al., 2014).

Gelucire® 44/14 is a non-ionic surfactant produced by the reaction of hydrogenated palm kernel oil and PEG 1500. The final composition for Gelucire® 44/14 comprises 72% PEG esters, 20% glycerides, and 8% pure PEG. The amount of free glycerol is in the range 0 – 3% (Svensson et al., 2004). Gelucire® 44/14 is characterized by its surface active properties and ability to self-emulsify when in contact with aqueous fluids to produce fine dispersion or emulsion (da Fonseca Antunes et al., 2013, Gattefossé, 2012).

The physicochemical properties and toxicity of this carrier are presented in **Table 2.16**.

Table 2.16 Physicochemical properties and toxicity of Gelucire® 44/14 (Gattefossé, 2012)

Properties	Description
Manufacturer	Gattefossé, Lyon, France
USP NF name	Lauroyl polyoxyl-32 glycerides
Chemical description	Consists of a small fraction of mono, di- and triglycerides and mainly PEG-32 (MW 1500) mono- and diesters of lauric acid (C_{12})
Physical appearance	White semi-solid waxy material, with light odour
Melting range (^{o}C)	42.5 – 47.5
Calculated/Practical HLB	14/11
CMC (mg/L, 25 ^{o}C)	72 ± 53
Solubility	Water dispersible
Toxicity	Safety of use is inferred by precedence of use in approved pharmaceutical products and extensive toxicological evaluations.

2.2.17. Gelucire® 48/16

Gelucire® 48/16 is a pure non-ionic surfactant with no glyceride fraction. It is a PEG 32 ester lipid excipient comprising a mixture of stearic and palmitic acid. According to the manufacturer, the final composition of Gelucire® 48/16 comprises 40 – 60% of stearic acid (C_{18}) and the sum of palmitic (C_{16}) and stearic acid (C_{18}) is ≥90% (Gattefossé, 2015).

Gelucire® 48/16 self-emulsifies in aqueous media into micellar solutions when used alone or in combination with low concentrations of other water miscible solvents (such as Transcutol® HP). The micellar solutions produced may encapsulate the active ingredient within micelles. Therefore, Gelucire® 48/16 can be employed as a solubilizer for poorly soluble drugs with low log P values (Gattefossé, 2015).

The physicochemical properties and toxicity of Gelucire® 48/16 are given in **Table 2.17**.

Table 2.17 Physicochemical properties and toxicity of Gelucire® 48/16 (Gattefossé, 2015)

Properties	Description
Manufacturer	Gattefossé, Lyon, France
USP NF name	Polyethylene glycol monostearate
Chemical description	Consists of PEG-32 (MW 1500) esters of palmitic (C_{16}) and stearic (C_{18}) acids
Physical appearance	White waxy pellets with faint odour
Melting range (^oC)	46 – 50
Calculated/Practical HLB	16/12
CMC (mg/L, 25 ^oC)	153 ± 31
Particle size (37°C, 1g/200 ml water)	7 ± 1nm
Solubility	Water dispersible
Toxicity	Gelucire 48/16 meets the requirement of USP/NF for polyoxyl stearate. Polyoxyl stearates are listed in US FDA inactive ingredient database for different dosage forms and administration routes. They have been used in oral, topical, vaginal, and rectal pharmaceutical products. A type IV drug master file (DMF) is registered with the US FDA.

2.2.18. Gelucire® 50/13

Gelucire® 50/13 is a non-ionic water dispersible surfactant. It is a stearoyl polyoxyl-32 glycerides lipid excipient comprising a mixture of short, medium and long chain fatty acids. According to the manufacturer, the final composition of Gelucire® 50/13 comprises ≤ 3% caprylic acid (C_8), ≤ 3% capric acid (C_{10}), ≤ 5% lauric acid (C_{12}), ≤ 5% myristic acid (C_{14}), 40 – 50% palmitic acid (C_{16}), and 48 – 58% stearic acid (C_{18}). The sum of palmitic and stearic acid is ≥90% (Gattefossé, 2012).

Because Gelucire® 50/13 is water dispersible, it self-emulsifies in aqueous media into a coarse emulsion (Gattefossé, 2012). Therefore, this excipient has been employed in several studies to improve the solubility and dissolution rate of poorly soluble compounds (Bandari et al., 2014, El-Badry et al., 2009, Potluri et al., 2011).

The physicochemical properties and toxicity of this carrier are presented in **Table 2.18**.

Table 2.18 Physicochemical properties and toxicity of Gelucire® 50/13 (Gattefossé, 2012)

Properties	Description
Manufacturer	Gattefossé, Lyon, France
USP NF name	Stearoyl polyoxyl-32 glycerides
Chemical description	Consists of mono, di- and triglycerides and PEG-32 (MW 1500) mono- and diesters of palmitic (C_{16}) and stearic (C_{18}) acids
Physical appearance	White waxy pellets with light odor
Melting range (^oC)	46 – 51
Calculated/Practical HLB	13/11
CMC (mg/L, 25 ^oC)	100
Solubility	Water dispersible
Toxicity	Safety of use is inferred by toxicological data and precedence of use in approved pharmaceutical products.

2.3. Formulation methods

2.3.1. Formulation of liquid self-nanoemulsifying drug delivery systems (SNEDDSs)

The type and the concentration of the components of SNEDDSs greatly influence the properties of the produced nanoemulsions such as the self-nanoemulsification efficiency, the droplet size and the polydispersity index. Therefore, initial selection of the components of SNEDDSs should be followed by optimization of the amounts of these components. The primary factors that should be considered to select the appropriate components are their ability to dissolve the drug and their ability to form spontaneous nanoemulsions upon dispersion in liquid medium. After choosing the potential components of SNEDDSs, their phase behaviour should be evaluated to identify different phases in addition to phase transitions. After that, ternary phase diagrams can be plotted to determine the self-nanoemulsification areas (Date et al., 2010). Components that produce larger self-nanoemulsifying areas possess greater self-nanoemulsification efficiency.

The effect of the drug on the size of self-nanoemulsification regions should be evaluated as well because some drugs may reduce the size of the self-nanoemulsification areas in ternary phase diagrams. Identification of the self-nanoemulsification regions may help to optimize the composition of SNEDDSs. In addition, different properties of the final SNEDDS should be evaluated including: self-nanoemulsification time and efficiency, droplet size, polydispersity index and zeta potential. Determination of zeta potential of SNEDDSs may give information on formulation stability. The morphological properties of the droplets of the final SNEDDS can be studied using transmission electron microscopy (TEM), while the in vitro drug release of the formulated SNEDDS can be evaluated in different dissolution media (Date et al., 2010).

Self-nanoemulsifying formulations can be also optimized before in vivo studies by subjecting it to in vitro lipolysis studies. These studies can be conducted using an in vitro lipolysis model that simulates digestion process in the intestine and also determines the possibility of drug precipitation as well as the in vitro / in vivo correlation (Date et al., 2010).

The above general rules for formulation of liquid SNEDDSs were applied in this study to prepare liquid SNEDDSs of indomethacin as follows:

2.3.1.1. Determination of indomethacin solubility in various components

The solubility of indomethacin in different oils, surfactants and co-surfactants was determined according to the method of Date and Nagarsenker (2007). In this method, an excess amount of the drug was mixed with fixed amounts of the excipient and the mixtures were shaken for 48 hours at 25^{o}C to attain equilibrium. Then, samples were centrifuged to remove undissolved drug, filtered through a 0.45 µm membrane filter, and the supernatant was suitably diluted before spectrophotometric analysis to determine the amount of the drug dissolved in each excipient.

2.3.1.2. Screening of surfactants for emulsifying ability

The emulsification ability of different surfactants was evaluated by mixing the surfactant with the selected oily phase in a 1:1 weight ratio following the method of Date and Nagarsenker (2007). The mixtures were vortex mixed and diluted up to 200 fold dilution. The ease of formation of an emulsion was assessed by observing the number of inversion of the volumetric flask required to obtain a uniform emulsion. The resulting emulsion was also examined visually for relative turbidity according to different grading systems (Grades A – E) described by Khoo et al. (1998) that depict the spontaneity and appearance of the nanoemulsion formed upon dilution. Mixtures that showed grades A and B upon dilution were assigned for further evaluation.

2.3.1.3. Screening of co-surfactants for emulsifying ability

The ability of co-surfactants (or co-solvents) to improve the emulsification ability of surfactants was also evaluated according to the method of Date and Nagarsenker (2007). Mixtures of the selected oily phase, surfactants and co-surfactants (or co-solvents) were mixed at a ratio of 3:2:1, respectively, and then diluted with distilled water for 200 fold dilution. The appearance and the ease of formation of nanoemulsion were assessed as described above for screening of surfactants.

2.3.1.4. Construction of ternary phase diagrams

According to the method of Shafiq et al. (2007) and Shafiq-un-Nabi et al. (2007), ternary phase diagrams were constructed in the absence of indomethacin to identify the self-emulsifying regions and also to determine the optimum amounts of different components of SNEDDS. Distilled water was used as the aqueous phase for development of these phase diagrams. Different combinations of the oil phase (Capryol™ 90), surfactants (Tween®80, Tween®20 and Cremophor®RH 40) and co-surfactant (Transcutol®HP) were prepared and grouped into 3 groups. In each group,

surfactants and co-surfactants mixtures (Smix) were mixed in different weight ratios so that the concentration of surfactant increases with respect to co-surfactant and the concentration of co-surfactant increases with respect to surfactant.

For each phase diagram, oil (Capryol™ 90) and specific Smix ratio were prepared and mixed thoroughly in different weight ratios. Phase diagrams were constructed using the water titration method, in which each combination of oil and Smix was slowly titrated with successive and fixed portions of water to produce water concentration ranging from 9% to 95% w/w. After each water addition, the mixtures were mixed and visual observations of different physical states were marked on a three component phase diagram. In plotting ternary phase diagram, one axis represents the oil phase, the second represents the Smix and the third represents the aqueous phase.

2.3.1.5. Selection of the best combinations from ternary phase diagrams

After construction of the ternary phase diagrams, the combinations of oil and surfactant / co-surfactant mixtures (Smix) that produce the maximum nanoemulsion region were selected to prepare indomethacin-loaded liquid SNEDDSs. The nanoemulsion region is characterized by formation of a transparent and easily flowable nanoemulsion. In order to cover the entire range of occurrence of self-nanoemulsification in each phase diagram, different oil compositions with fixed Smix percentages were adopted to formulate different liquid SNEDDS of indomethacin.

Prior to formulation of indomethacin-loaded liquid SNEDDSs, the effect of addition of indomethacin on phase behavior in the selected combinations was investigated. Observation of any phase behaviour changes in each formulation was recorded.

2.3.1.6. Preparation of drug-loaded self-nanoemulsifying formulations

Indomethacin was added to optimized blank ternary systems at a drug loading concentration of 2.5% w/w, which reflects the indomethacin therapeutic dose (25 mg). Final mixtures were mixed and shaken for 24 hours at 25^{o}C in an isothermal shaking water bath to ensure complete solubilization.

2.3.1.7. Thermodynamic stability tests

According to the methods of Shakeel et al. (2009) and Shafiq et al. (2007), prepared formulations were subjected to thermodynamic stress tests (including centrifugation, heating cooling cycle, and freeze thaw cycle) to eliminate metastable, unstable and biphasic formulations. Formulations that passed the thermodynamic stress tests were subjected to self-nanoemulsification tests.

2.3.1.8. Self-nanoemulsification efficiency tests

Self-nanoemulsification tests were carried out to investigate any drug precipitation that may take place upon dilution of formulations with different diluents. Each drug-loaded self-emulsifying formulation was diluted 200 fold with deionized water or 0.1 N HCl and the self-nanoemulsification performance was evaluated visually according to different grading systems described by Khoo et al. (1998). Formulations that showed grades A and B upon dilution were subjected for further evaluation.

2.3.1.9. Transmission electron microscopy (TEM) studies

Studies using transmission electron microscopy (TEM) were conducted to obtain information on the morphological structure of the selected indomethacin-loaded SNEDDS. Samples were diluted, directly deposited on coated copper grids and stained with 1% uranyl acetate. The coated grid was left to dry and then observed for TEM.

2.3.2. Formulation of solid self-nanoemulsifying drug delivery systems (SNEDDSs)

Generally, development of solid SNEDDSs from liquid SNEDDSs requires optimization of the liquid formulation and selection of the most suitable excipients with the most appropriate solidification technique in order to achieve optimum product performance. For example, utilization of carriers with high specific surface area and/or high porosity would be beneficial to load the dosage in an acceptable amount of the final solid SNEDDS. Carriers with good flowability would be suitable for solidification by adsorption and for further processing steps such as direct compression or capsule filling. Also, selection of porous carriers will decrease the possibility of leaking of SEDDSs formulation during compression. Therefore, in formulation of solid SNEDDS, a compromise must be established between the highest amount of drug that can be incorporated and the best physical properties of the solid formulation (Mandić et al., 2017).

Adsorption of liquid SNEDDSs onto solid carriers appears as a favourable solidification technique due to fewer processing steps, compared to other solidification methods such as spray drying or wet granulation. This method of solidification can be implemented on an industrial level where homogenous products can be obtained by spraying the liquid formulation onto the carrier or by using high shear mixing technique (Mandić et al., 2017).

The method of adsorption of liquid SNEDDSs onto solid carriers is simple and involves blending the liquid formulation with the solid carrier or adsorbent in a blender or

by using a mortar with pestle. The obtained powder can be directly filled into capsules or mixed with appropriate excipients and then compressed into tablets (Gupta et al., 2013).

In this study, different inert carriers including Avicel® PH102 (microcrystalline cellulose), Aerosil® 200 (colloidal silicone dioxide), Syloid® XDP 3150 (Porous silicone dioxide), Florite® PS-200 (Calcium silicate) and Neusilin® US2 (Magnesium aluminometa silicate) which possess different physical properties were utilized to load the optimized liquid indomethacin SNEDDS.

The methods adopted in this work to formulate solid SNEDDSs of indomethacin were as follows:

2.3.2.1. Preparation of solid SNEDDSs of indomethacin by adsorption technique

The solid SNEDDS of indomethacin were prepared by simple mixing of the liquid SNEDDS formulations with different adsorbents at different adsorbent: liquid formulation ratios (by weight). Mixing was performed in a mortar using a pestle. The resulting granular mass was passed through a 250 µm sieve for uniformity in particle size. The powder samples were stored over anhydrous calcium chloride in a desiccator until further evaluation.

2.3.2.2. Determination of flow properties of indomethacin-loaded solid SNEDDS by angle of repose method

Determination of powder flow is an important requirement prior to some pharmaceutical manufacturing processes such as tableting and capsule filling. Angle of repose is one of the most commonly applied methods to determine flow properties of powder which are related to the inter-particulate friction between particles.

In this study, the fixed funnel method was used to determine the flow properties of different solid self-emulsifying formulations and to calculate the angle of repose (θ). A funnel was secured to a stand with its tip adjusted at a fixed height (H) above the horizontal surface. Powder formulation was passed through the funnel until the apex of pile touched the tip of the funnel. The angle of repose was calculated using the equation:

$$tan\theta = \frac{H}{r} \qquad \text{(Equation 2.1)}$$

where (r) is the radius of the pile of powder.

The obtained values of angle of repose for different solid SNEDDS formulations were compared to the general scale of flowability for angle of repose depicted in British Pharmacopoeia (2015).

2.3.2.3. Determination of packing properties of indomethacin-loaded solid SNEDDS by measuring Carr's compressibility index (CI) and Hausner's ratio (HR)

Measurement of compressibility index (CI) and Hausner's ratio (HR) can be used as an alternative method to predict the flow properties of powder. The value of compressibility index (CI) can be influenced by the surface area, size and shape, moisture content, bulk density and cohesiveness of particles. Therefore, it represents an indirect measure of all of these properties. Measurement of the values of compressibility index (CI) and Hausner's ratio (HR) is basically performed by measuring the initial apparent volume, (V_o), and the final tapped volume, (V_f), of the powder which is obtained after tapping the powder until no additional changes in the volume can be observed. The compressibility index (CI) and the Hausner's ratio (HR) can be calculated from the following equations (British Pharmacopoeia, 2015):

$$\text{Compressibility index} = \frac{V_o - V_t}{V_o} \times 100 \qquad \text{(Equation 2.2)}$$

$$\text{Hausner's ratio} = \frac{V_o}{V_f} \qquad \text{(Equation 2.3)}$$

Also, the compressibility (CI) index and Hausner ratio (HR) can be calculated using the measured values of bulk density (ρ_{bulk}) and tapped density (ρ_{tapped}) as follows:

$$\text{Compressibility index} = \frac{\rho_{tapped} - \rho_{bulk}}{\rho_{tapped}} \times 100 \qquad \text{(Equation 2.4)}$$

$$\text{Hausner's ratio} = \frac{\rho_{tapped}}{\rho_{bulk}} \qquad \text{(Equation 2.5)}$$

In order to study the flow and packing properties of different indomethacin-loaded solid SNEDDS formulations, Carr's compressibility index (CI) and Hausner's ratio (HR) were assessed by introducing a known weight of the powder into a graduated cylinder through a funnel. The cylinder was dropped onto a wooden surface three times from a height of 1 inch at 2 seconds intervals. The bulk density (ρ_{bulk}) in g/cm^3 was calculated by dividing the weight of the sample by the obtained initial apparent volume (V_o) of the sample. Then, the graduated cylinder was tapped until a constant volume is obtained and the final tapped volume (V_f) of the powder was recorded. The tapped density (ρ_{tapped}) in g/cm^3, was calculated by dividing the weight of the powder by the final tapped volume (V_f). Finally, Carr's compressibility index and Hausner's ratio were calculated using the above mathematical relations.

The obtained values of Carr's compressibility index (CI) and Hausner's ratio (HR) were compared to the scale of flowability for these parameters presented in the British Pharmacopoeia (2015).

2.3.2.4. Determination of drug content of indomethacin-loaded solid SNEDDS

In this study, the drug content was determined for drug-loaded solid SNEDDSs in order to calculate the amount of the drug loaded upon adsorption of the liquid SNEDD formulations on to different carriers.

An accurately weighed amount of the resulting drug-loaded solid SNEDDS formulation was dispersed in a suitable quantity of methanol and shaken thoroughly to ensure release and dissolution of the drug in methanol. The samples were centrifuged at 3000 rpm for 15 minutes to separate undissolved excipients. The supernatant was filtered through a 0.45 µm membrane filter and the filtrate was assayed spectrophotometrically for the drug at a wavelength of 320 nm. The drug content in each sample was calculated as milligrams of the drug per gram of the product using the following equation:

$$drug\ content = \frac{drug\ content\ in\ the\ weight\ taken\ from\ solid\ \ SNEDDS}{weight\ of\ the\ solid\ SNEDDS\ taken} \qquad \text{(Equation 2.6)}$$

Also, the drug content in each formulation was estimated as % of the theoretical amount added using the following equation:

$$\%\ of\ theoretical = \frac{actual\ drug\ content}{theoretical\ drug\ content} \times 100 \qquad \text{(Equation 2.7)}$$

Calculation of theoretical drug content was based on assuming that the entire amount of drug present in liquid SNEDDS formulation gets adsorbed onto the carrier with no loss at any stage of preparation of the solid SNEDDS.

The experiments were repeated in triplicate for each produced batch of powder and then the results were averaged ± standard deviation.

2.3.3. Formulation of carrier-based self-nanoemulsifying drug delivery systems (SNEDDSs) by hot melt extrusion (HME)

Hot melt extrusion (HME) is considered as an advanced pharmaceutical manufacturing technique that can be applied to overcome the poor water solubility of some compounds and therefore to enhance their oral delivery. This continuous process is solvent free, cost-effective and can be easily scaled up. It is based on mixing the drug with a polymeric or lipidic carrier to form molecularly dispersed or amorphous solid dispersions. The obtained extrudate can be milled and formulated into different dosage forms like tablets, capsules, pellets and implants (Repka et al., 2007).

In this study, a twin screw extruder (micro-compounder, MC 15, Xplore Instruments BV, Sittard, The Netherlands) was used to formulate Gelucire®-based SNEDDSs of indomethacin. This instrument is depicted in **Figure 2.13**.

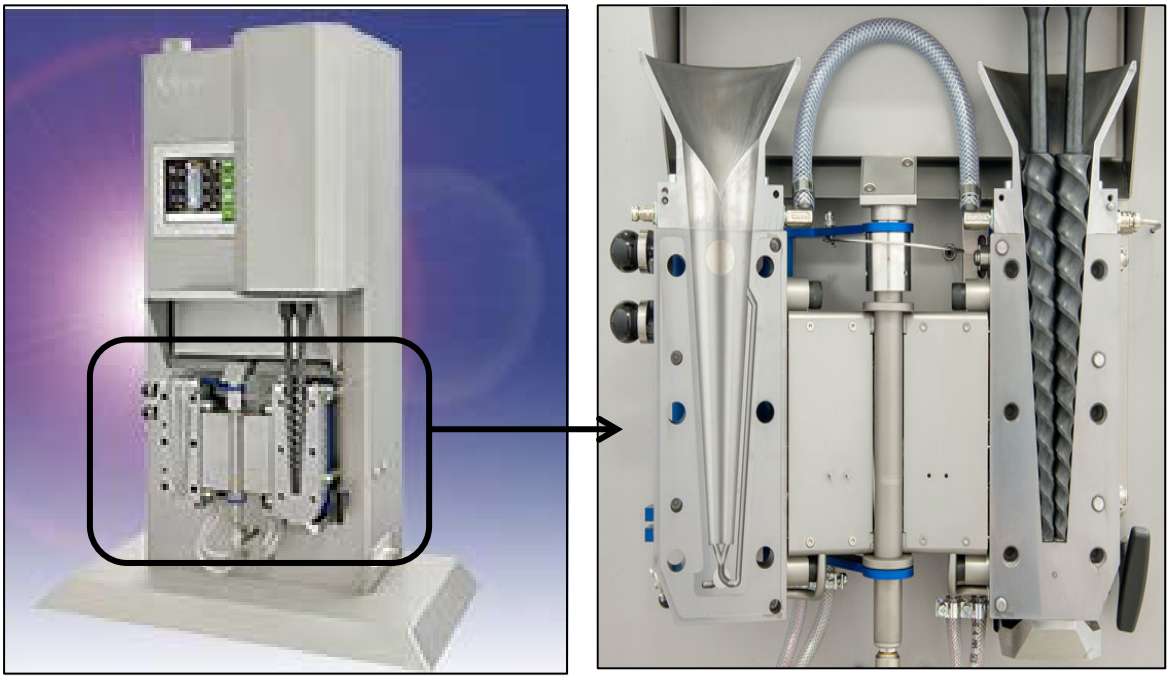


Figure 2.13 Left side: Twin screw extruder (micro-compounder, MC 15, Xplore Instruments). Right side: The vertical co-rotating twin screws.

The extruder is equipped with vertical co-rotating twin screws which possess a length to diameter (L/D) ratio of 150: 18 and a volume of 15 cm^3.

In order to formulate Gelucire®-based SNEDDSs of indomethacin, physical mixtures of the drug with Gelucire® 44/14 or Gelucire® 48/16 were introduced into the twin screw extruder through the hopper. The temperature of the extruder barrel was adjusted at 40°C (below the melting point of both Gelucires®) or at 50°C (the melting point of Gelucire® 48/16). A constant screw rotational speed of 30 rpm was maintained. The mass was mixed for 5 minutes inside the barrel before extrusion through a die with 1 mm diameter. After cooling the extruded products at room temperature, these were cut or crushed into small pieces and then sieved through 500 µm sieve to obtain a granular product that was used for further experiments.

2.3.3.1. Determination of drug content of Gelucire®-based SNEDDS of indomethacin

The drug content of Gelucire®-based indomethacin-loaded SNEDDS was determined similarly as described previously under **section 2.3.2.4** for determination of drug content of indomethacin-loaded solid SNEDDS.

Determination of drug content was repeated in triplicates for each formulation and the results were averaged ± standard deviation.

2.4. Evaluation methods

2.4.1. Dynamic light scattering (DLS)

Dynamic light scattering (DLS) is a technique applied for measuring the size and size distribution of nanoparticles especially in liquid form. Compared to imaging techniques such as electron microscopy that can be used for measuring the size of nanoparticles, DLS does not require drying of the sample and therefore, the properties of the particles in the liquid dispersion remain unaffected (Pecora, 2000). In DLS, the size of the particles is determined by measuring the changes in the intensity of light scattered from a liquid dispersion. This technique is also referred to as photon correlation spectroscopy (PCS). According to Pecora (2000), particles are illuminated with a source of light (laser) in DLS and the intensity of fluctuation of scattered light is analyzed by the instrument to calculate a correlation function that can be used to obtain the size distribution of the sample.

In this study, a Malvern Zetasizer Nano-ZS **(Figure 2.14),** which is based on DLS, was used to measure the droplet size, polydispersity index (PDI) and zeta potential of SNEDDSs of indomethacin.

Dynamic light scattering measures the Brownian motion of the particles and relates it to the size of the particles. Brownian motion of the particles takes place due to random collision with the molecules of the dispersion liquid. As smaller particles move faster and larger particles move slower, the Zetasizer uses this information to relate the speed of Brownian motion of the particles to their size. The relationship between the size of the spherical particle and its speed is defined in the Stokes-Einstein equation:

$$D_h = \frac{k_B T}{3\pi\eta D_t} \qquad \text{(Equation 2.8)}$$

where D_h is the hydrodynamic diameter, D_t is the translational diffusion coefficient, k_B is Boltzmann's constant, T is the absolute temperature, and η is the dynamic viscosity.

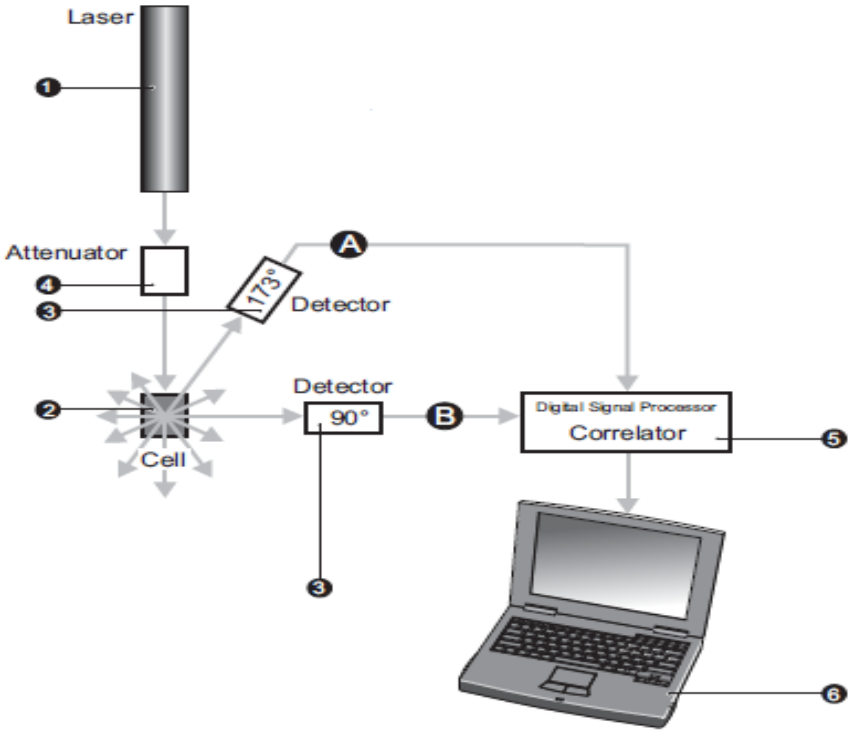


Figure 2.14 Components of a typical DLS system. The laser (1), measurement cell (2), detector (3), attenuator (4), correlator (5) and data handling PC (6). Detectors can be placed at either 90^{o} or at a wider angle at 173^{o}. Adapted from (Malvern Instruments, 2004).

The polydispersity index (PDI) is another important parameter that can be determined by Zetasizer. This parameter is dimensionless and has to be evaluated especially in a multimodal distribution to describe the diameter distribution in a sample of SNEDDS dispersed in aqueous media. The value of polydispersity index indicates uniformity in droplet size distribution within the formulation. Values less than 0.05 are rarely seen other than with highly monodisperse standards, while values greater than 0.7 indicate that the sample has a very broad size distribution of particles and may not be suitable for analysis by DLS.

Malvern Zetasizer can be used also for measurement of the zeta potential of nanoparticles in liquid dispersions. Measurement of the value of the zeta potential gives an indication of stability of self-emulsifying formulations, which is directly related to the magnitude of surface charges on emulsion droplets (Balakumar et al., 2013). The magnitude of zeta potential of a liquid dispersion indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in that dispersion. Formulations

with high values of zeta potential (in either negative or positive charge) are more stable with less tendency of particle aggregation than those with low values of zeta potential. In general, colloidal dispersions are considered as stable formulations when their zeta potential values range between 25 and 30 mV in either charge (Bali et al., 2011, Shakeel et al., 2013b).

In practice, zeta potential can be measured using the Malvern zetasizer. The sample of liquid dispersion is placed in a capillary cell with electrodes at either end to which an electric field is applied **(Figure 2.15).** Then charged particles in the dispersion will migrate towards the electrode of opposite charge with a certain velocity that is proportional to the magnitude of the zeta potential (Malvern Instruments, 2004).

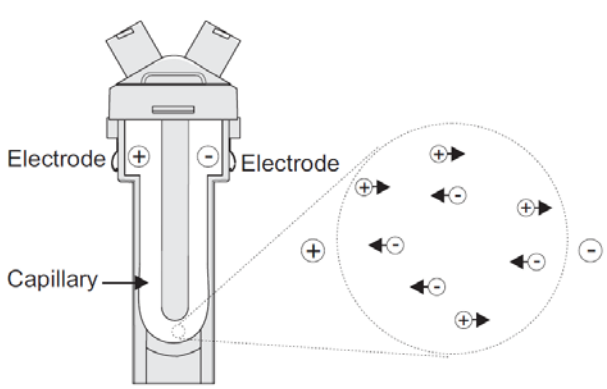


Figure 2.15 Illustration of a folded capillary cell for measurement of zeta potential in DLS system. Adapted from (Malvern Instruments, 2004)

The velocity of the particles moving in the electric field (known as the electrophoretic mobility) is dependent on the zeta potential, strength of the electric field, viscosity and dielectric constant of the medium. Zetasizer can obtain the zeta potential of a liquid dispersion by applying Henry's equation as follows:

$$U_E = \frac{2\varepsilon\, z\, f(K_a)}{3\eta} \qquad \text{(Equation 2.9)}$$

where U_E is the electrophoretic mobility, ε is the dielectric constant, z is the zeta potential, $f(K_a)$ is the Henry function, and η is the viscosity.

The Henry's function, $f(K_a)$, has two values, either 1.5 or 1.0 which can be used as approximations in measurement of zeta potential. When zeta potential is determined in aqueous media with moderate concentration, the Henry's function ($f(K_a)$) is considered as 1.5 and can be referred to as Smoluchowski approximation. On the other hand, upon measuring zeta potential in non-aqueous media with low dielectric constant, the Henry's function ($f(K_a)$) takes the value of 1.0 and can be referred to as Huckel approximation.

In this project, Malvern Zetasizer nano-ZS was used to measure the droplet size, polydispersity index and zeta potential of the liquid, solid and Gelucire®-based SNEDDS of indomethacin. An amount of each formulation was dispersed in purified water to obtain a fixed final drug concentration. Samples were filled into acrylic cuvettes for measurement of droplet size. The light scattering was measured at a scattering angle of 90° and a temperature of 25°C. In the case of measurement of zeta potential, the samples were prepared similarly and filled into folded capillary cells. All measurements were repeated in triplicates and the results were averaged ± standard deviation.

2.4.2. In vitro dissolution studies

Dissolution is the process of transforming drug molecules from the solid state into solution. Different stages (or processes) are involved in the dissolution of a drug from solid dosage forms. When a solid dosage form is placed in contact with an aqueous medium, it starts to pass into solution from the intact dosage form. Also, the solid dosage form may disintegrate into granules which may in turn deaggregate into fine particles. Therefore, disintegration, deaggregation and dissolution occur simultaneously during the release of the drug from the solid dosage form. These processes of dissolution from solid dosage forms are illustrated in **Figure 2.16.**

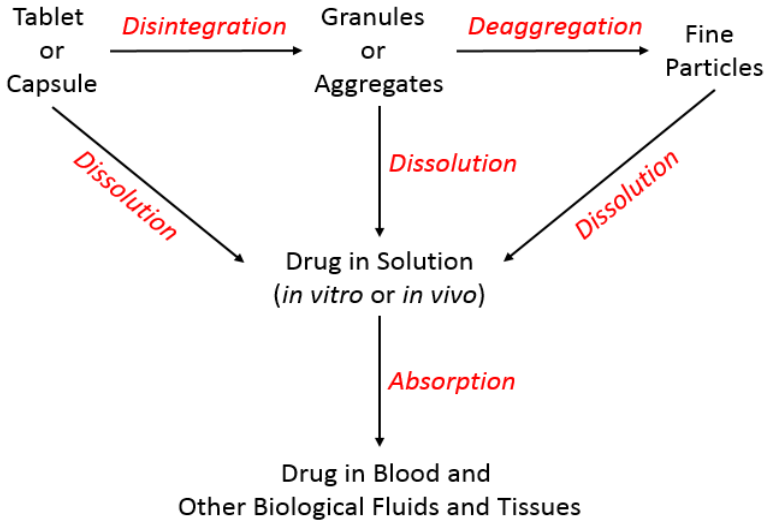


Figure 2.16 Different processes involved in the dissolution of solid dosage forms (Abdou, 1989)

In pharmaceutical practice, dissolution rate can be defined as "the amount of drug substance that goes in solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition" (Abdou, 1989). The

correlation between the dissolution rate and the solubility gradient of the solid particles has been described by Noyes-Whitney equation which can be written as follows (Sun et al., 2012) :

$$\frac{dC}{dt} = \frac{DA}{Vh}(C_s - C_x) \qquad \text{(Equation 2.9)}$$

where dC/dt is the dissolution rate, D is the diffusion coefficient of the solute in solution, A is the surface area of the solid, V is the volume of the dissolution medium, C_s is the saturation solubility of the solid, C_x is the drug concentration in the bulk solution, and h is the thickness of the diffusion layer.

The in vitro dissolution test is routinely applied in pharmaceutical drug development to obtain information on drug release for quality control purposes in order to assess consistency of batch manufacturing. Also, this test can be applied to predict the in vivo performance of the drug formulation, and then to establish in vivo – in vitro relationship between drug absorption and its release from the dosage form (Qiu et al., 2014). In addition, dissolution testing can be employed to aid in choosing among different formulations manufactured using various excipients, with the aim to select the optimum formulation that exhibits the most desirable and reproducible dissolution profile (Dressman et al., 1998, Qiu et al., 2014).

Four different assemblies of dissolution apparatus were defined by the British Pharmacopoeia (2015) including: apparatus I (basket apparatus), apparatus II (paddle apparatus), apparatus III (reciprocating cylinder) and apparatus IV (flow-through cell). The choice of the apparatus to be used for in vitro dissolution studies is governed by the physicochemical properties of the dosage form. In this study, Pharma Test Dissolution apparatus (Paddle apparatus) was used to assess the release profiles of indomethacin from different solid and carrier-based SNEDDS formulations.

Different dissolution media can be used to conduct the in vitro dissolution studies. Buffers, acidic solutions, surfactants and buffers or acidic solutions combined with surfactants are among the various dissolution media that can be used. Also, biorelevant media prepared with bile salts or other physiological ingredients can be used as dissolution media especially when in vivo – in vitro correlation studies are to be conducted (Fotaki et al., 2013). Selection of the most appropriate dissolution medium for a dissolution study is based on the dissolution properties expected for a drug and/or product. Before conducting a dissolution test, the solubility of the drug in the dissolution medium has to be evaluated. The pH of the dissolution medium is another important aspect that must be considered so that the in vitro dissolution environment would be relevant to the physiological conditions. Generally, pharmacopeial monographs of drugs

specify the type, composition and pH of the dissolution medium required for dissolution testing of specific drugs.

In general, selection of the dissolution medium for oral dosage forms should be based on drug properties such as drug solubility and stability, in addition to formulation (product) components (excipients) used, and interaction between the components (Fotaki et al., 2013).

In this research, the in vitro dissolution studies of different indomethacin solid and carrier-based SNEDDS formulations were carried out in dissolution apparatus II (Paddle method) according to the requirements specified in the British Pharmacopoeia (2015) for indomethacin capsules. The dissolution medium composed of 900 ml phosphate buffer pH 7.2 maintained at 37 ± 0.5^{o}C and the rotational speed was adjusted at 50 rpm. Phosphate buffer pH 7.2 was prepared according to the British Pharmacopoeia (2015) by mixing 50 ml of 0.2M potassium dihydrogen orthophosphate with 35 ml of 0.2M sodium hydroxide and diluting to 200 ml with water. Volumes of these solutions were corrected accordingly to prepare the total volumes required for dissolution studies. An amount of solid SNEDDS formulation equivalent to 25 mg of indomethacin was filled in suitable number of hard gelatin capsules (size 000) and used for dissolution studies. For Gelucire-based SNEDDSs, an amount of the formulation equivalent to 25 mg of the drug was directly dropped into the dissolution medium. Samples were withdrawn at predetermined time intervals. An equal volume of fresh dissolution medium maintained at the same temperature was added to keep constant volume during dissolution study. The collected samples were filtered through 0.45 µm syringe filter, suitably diluted and then assayed for the content of indomethacin by UV spectrophotometry at 320 nm. Experiments were performed in triplicates and the results are averaged ± standard deviation (SD).

The dissolution profile of indomethacin released from the tested formulations was compared to that obtained when the same quantity of pure indomethacin was filled in capsules.

In order to compare the resulting drug dissolution profiles, different approaches can be applied for this purpose. Dissolution profiles can be compared according to the model of drug release, i.e., zero order, first order, Higuchi, and Hixson-Crowell. Also, the ANOVA approach, the difference (f_1) and similarity (f_2) factors (fit factors) and the dissolution efficiency (DE) can be used to compare different dissolution profiles. While the ANOVA approach identifies the statistical equivalence of formulations, the pharmaceutical equivalence of two formulations can be identified by the difference (f_1) and similarity (f_2) factors. However, the fit factors do not provide information on the

consistency of individual batches. Therefore, the dissolution efficiency (DE) can be used as a parameter to analyze dissolution profiles and also to compare pairs of formulations. The use of DE (which considers the whole dissolution process) for comparison of dissolution profiles is preferred over comparison that is based on a single time point (mean % dissolved at a selected time) (Anderson et al., 1998).

The concept of dissolution efficiency (DE) was introduced by Khan and Rhodes (1972). The dissolution efficiency can be defined as "the area under the dissolution curve up to a certain time, t, expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time" (Anderson et al., 1998).

The value of DE can be calculated as follows:

$$DE = \frac{\int_{t1}^{t2} y.dt}{y_{100}.(t_2 - t_1)} \times 100\% \qquad \text{(Equation 2.10)}$$

where y is the % of dissolved product, t_1 and t_2 are the time points.

For calculation of DE, the value of $t_1 = 0$ is generally selected while the value for t_2 can be set at the time that corresponds to 70 – 90% dissolution. However, early portions of the dissolution curve can be analyzed (Anderson et al., 1998).

The mean dissolution time (MDT) is a parameter that represents an arithmetic mean value for any dissolution profile. Estimation of MDT can provide information on in vitro / in vivo correlation and can be used to compare different dissolution profiles statistically. Different methods based on model dependent and model independent approaches can be used to calculate MDT. The model independent method uses the amount of drug dissolved in dissolution medium after specifically defined time intervals. Generally, the calculation method is based on using the trapezoidal rule to calculate the area under the dissolution curve (Podczeck, 1993). The following equation can be used to calculate the MDT of a given dissolution profile (Rinaki et al., 2003):

$$MDT = \frac{\int_0^{W\infty} t.dW(t)}{\int_0^{W\infty} dW(t)} \qquad \text{(Equation 2.11)}$$

where W(t) is the cumulative amount of drug dissolved at time (t).

In this study, the obtained dissolution profiles of different SNEDDS formulations were compared using the DE and mean % drug released after specific times. The DE after 15 minutes (DE_{15min}) was calculated using DDSolver as Excel add inn. This was chosen because of good separation of dissolution curves observed at this time interval for the tested formulations. Also, the mean % drug released after 15 minutes (Q_{15min}) and the MDT were used to compare different dissolution profiles.

2.4.3. Ultraviolet/Visible spectrophotometry

Ultraviolet/visible (UV/Vis) spectroscopy is an analytical technique that can be used for quantitative determination of compounds that absorb UV/Vis radiation. UV/Vis spectroscopy measures absorbance or reflectance in the ultraviolet (wavelength 200 – 400 nm) and visible (400 – 800 nm) spectral regions. This technique is based on the fact that when organic molecules are exposed to electromagnetic radiation in the UV/Vis regions of the spectrum, they undergo electronic transitions in the outer orbitals (Martin et al., 1993).

By application of the Beer – Lambert law, UV/Vis spectroscopy can be used to determine the concentration of organic molecules in a solution at a fixed path length. The Beer – Lambert law relates the absorbance of a solution to the concentration of the absorbing molecules as in the following equation (Martin et al., 1993):

$$A = \varepsilon bc \qquad \text{(Equation 2.11)}$$

where *A* is the measured absorbance (the amount of light absorbed by the sample), *ε* is a constant known as the molar absorbitivity (or molar extinction coefficient), *b* is the path length of the radiation passing through the sample (or the path length of the cell which usually equals to 1 cm), and *c* is the concentration of the absorbing substance.

In this study, UV spectroscopy was used as the analyzing technique to determine the concentration of indomethacin in different formulated SNEDDSs. For this purpose, standard calibration curves of indomethacin were constructed in both methanol and phosphate buffer. Different concentrations of indomethacin ranging from 2.5 to 45 µg/ml were prepared in the assigned medium and the absorbance of these solutions was determined spectrophotometrically at the maximum wavelength (λ_{max} = 320 nm) corresponding to indomethacin (British Pharmacopoeia, 2015, Inada et al., 2013, Yadav and Yadav, 2009) using the corresponding medium as a reference. To obtain the standard calibration curve, the measured absorbance was plotted against the corresponding concentrations and the equation that describes the relationship between the concentration and the absorbance was estimated.

In order to determine the concentration of indomethacin in different formulated SNEDDSs, scanning of each sample was carried out at a wavelength of 320 nm using a Libra S22 UV/Vis spectrophotometer (Biochrom Ltd., Cambridge, UK). Readings of absorbance were fitted into the equation of the corresponding calibration curve and the concentration of the drug was calculated.

2.5. Solid state characterization methods

2.5.1. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is the most widely applied thermal analysis technique. In this technique, samples are subjected to linear heating and cooling cycles to obtain information on their melting, decomposition, re-crystallization and possible glass transition. In DSC analysis, the energy changes that occur in the sample during these thermal processes are measured together with the time or the temperature at which these changes take place. This thermal analysis technique is simple, rapid and requires only a small sample size that can be heated over a wide temperature range (from – 120 to 600^{o}C) according to the instrument used (Gabbott, 2008, Reading and Craig, 2007).

Two different types of DSC instruments have been identified: the heat flux and the power compensation. Heat flux DSC (**Figure 2.17)** is made of a single furnace that incubates the sample and empty reference pans. The sample and the reference pans are heated equally in the furnace and the temperature difference between the two pans is measured by a pair of thermocouples located beneath the pans. The amount of heat flow from the furnace to the cells can be estimated from the following equation:

$$\frac{dQ}{dt} = \frac{\Delta T}{R} \qquad \text{(Equation 2.12)}$$

where dQ/dt is the heat flow, ΔT is the temperature difference between the furnace and the pans, and R is the thermal resistance of the heat flow between the furnace and the pans (Reading and Craig, 2007).

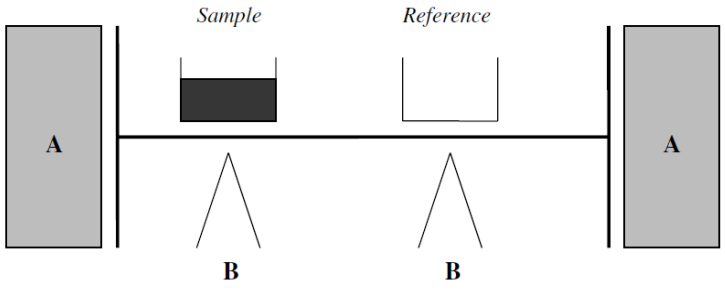


Figure 2.17 Schematic representation of heat flux DSC. A =furnace, B = thermocouple (Reading and Craig, 2007)

The other type of DSC instrument is the power compensation DSC (**Figure 2.18**). This system utilizes two furnaces, one for the sample pan and the other for the reference

pan. Both furnaces have the same temperature program. In this system, the difference in the power supplied for both furnaces in order to keep both pans at the same temperature is measured and recorded. Therefore, in power compensation type, the sample is subjected to programmed heating, in contrast to the heat flux approach where the furnace is the part subjected to programmed temperature (Reading and Craig, 2007).

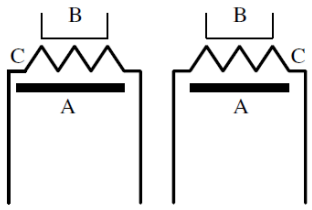


Figure 2.18 Schematic representation of power compansation DSC. A = furnaces, B = sample and reference pans, C = sample and reference platinum resistance thermometers (Reading and Craig, 2007).

Both types of DSC analysis are sensitive and accurate. However, higher heating and cooling rates can be achieved with the double furnaces in power compensation DSC, while better baseline stability can be obtained with the single furnace in heat flux DSC.

The heat capacity (C_p) represents the amount of energy required to raise the temperature of the sample by 1K. This parameter can be expressed as:

$$\frac{dQ}{dt} = C_P \times \frac{dT}{dt} \qquad \text{(Equation 2.13)}$$

where dQ/dt is the heat flow and dT/dt is the heating rate (Reading and Craig, 2007).

Another DSC technique, modulated temperature differential scanning calorimetry (MTDSC), was introduced as an extension to the conventional DSC techniques. This technique involves the application of a sinusoidal heating wave to the standard linear temperature program. The heating rate in this technique also modulates due to modification of the temperature. The constant heating rate utilized in conventional DSC methods is not capable of separating overlapping thermal processes (Reading et al., 2007). For example, determination of the glass transition temperature (T_g) of polymers requires separation of heat flow due to heat capacity from the heat flow mediated by other overlapping thermal events like desolvation, dehydration or other enthalpic relaxations that possess stronger thermal signals in comparison to the weak T_g (Knopp et al., 2016). Therefore, modulation of the heating rate in MTDSC allows the separation of overlapped reversible (heat flow due to heat capacity differences such as melting and

glass transition) and non-reversible (heat flow due to a chemical or physical event such as crystallization and decomposition) thermal events taking place in the sample during heating and cooling conditions. MTDSC is more sensitive and gives higher resolution than the conventional DSC (Knopp et al., 2016, Reading et al., 2007).

In this study, conventional DSC analysis was performed using a single furnace, heat flux DSC (DSC 4000, Perkin Elmer, US). Accurately weighed samples (approximately 5 – 10 mg) were placed in a standard aluminum pan and scanned at a controlled heating rate of 10^{o}C/min from 25 – 300^{o}C under nitrogen gas flow of 20 ml/minute. The temperature and the energy changes, such as melting or crystallization that took place during heating were recorded.

2.5.2. Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) is a thermal analysis technique that measures the changes in weight of a sample while it is heated at a constant heating rate. This technique is used to determine different characteristics of materials that exhibit weight loss or gain (such as oxidation, evaporation or decomposition) upon heating or cooling. It is also applied to evaluate thermal stability of pharmaceutical compounds prior to their use in formulation methods that involve heating. Samples are considered to be thermally stable if no or negligible loss in weight is observed over the temperature range selected. This stability can be verified in the form of no or negligible slope of the TGA curve.

The basic TGA system consists of a precision balance and a pan to load the sample in addition to a furnace which can be programmed for a constant heating rate. The sample (about 3 – 5 mg) is placed in the pan which is hung by a wire beneath the sensitive balance. An inert purge gas (like nitrogen) flows over the sample to prevent oxidation and other undesirable reactions. The sample in the heating pan is heated at a constant heating rate to temperatures that may reach up to 1000^{o}C. The instrument weighs the sample continuously during heating process and the results are recorded and presented as plots of the % weight loss against the temperature (Saunders and Gabbott, 2011).

TGA was employed in this study to evaluate the thermal stability of indomethacin, Gelucire®44/14, Gelucire®48/16 and other adsorbents before employment in formulation of Gelucire-based SNEDDS adopting hot melt extrusion technique. Thermal stability was assessed using Pyris 1 TGA (Perkin Elmer, Waltham, Massachusetts, USA). The analysis was performed on samples of approximately 3 – 5 mg in the temperature range between 30^{o} and 250^{o}C and at a heating rate of 10^{o}C / minute.

2.5.3. X-Ray Diffraction (XRD) analysis

X-ray diffraction is a common analytical technique that is used for characterization of crystalline materials. It has been also employed for quantitative analysis and qualitative identification of unknown compounds.

This technique is based on the fact that atoms in a crystal diffract X-ray beams in a way similar to diffraction of ordinary light by the plane of a diffraction grating. Each substance reflects (or diffracts) the X-ray beam and hence, a particular diffraction pattern can be produced and can be used as a 'fingerprint' for each compound and crystal form. Therefore, an unknown powder can be identified by comparing its diffraction pattern to those of known substances or by comparing its distances of various planes (interplanar spacing or d-spacing) obtained from the diffraction pattern to those values recorded for known compounds (Abdou, 1985).

The unique diffraction pattern obtained from crystalline materials is a result of the arrangement of atoms or molecules in those crystalline materials in addition to the interatomic distance between them. In a diffraction pattern, the position of the X-ray diffraction peaks (expressed as their incidence angle, θ) can be obtained from Bragg's equation, which relates the wavelength of the X-ray beam (λ) to the incidence angle of X-ray beam (θ) and the interplanar spacing of a set of diffracting planes (d) as follows:

$$n\lambda = 2d\ sin\theta \qquad \text{(Equation 2.14)}$$

where n is the order of the diffractions.

Therefore, any changes in the d-spacing (due to crystal deformation during different processes) will be inversely proportional to the incident angle of X-ray beam (θ). These changes can be observed in the form of shifting of the position of X-ray diffraction peaks to higher or lower θ values (Bandyopadhyay et al., 2005).

Particles of an amorphous material usually do not possess ordered structure like crystalline materials and their atoms will diffract the X-rays in many directions. Therefore, the resulting X-ray diffraction pattern of amorphous materials contains no sharp crystalline (Braggs) diffraction peaks like crystalline materials but show a broad halo. This halo pattern is needed for characterization because it confirms that the material is amorphous (Gilmore, 2011).

X-ray diffractometers are composed of three main parts including: the X-ray tube, the sample holder and the X-ray detector (**Figure 2.19**). The incident beam optics conditions the X-ray beam before hitting the sample, while the goniometer represents a platform that holds and moves the sample, optics, detector and/or the tube.

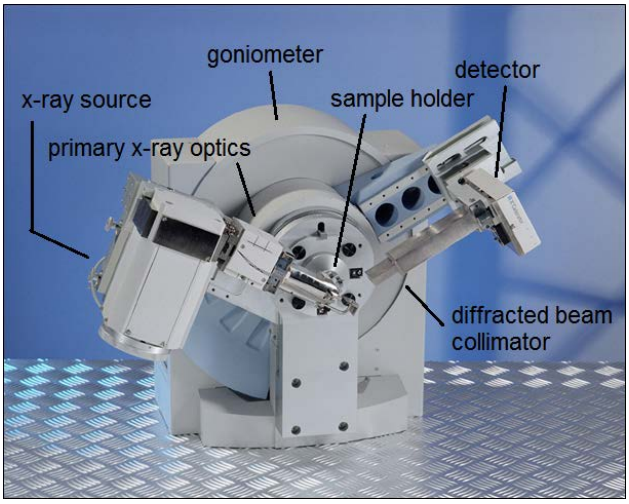


Figure 2.19 Basic components of X-ray diffractometer with the goniometer

Diffraction methods are based on generation of X-rays by a cathode ray tube by heating a filament to produce electrons. The X-rays produced are then filtered to produce monochromatic radiation, collimated to concentrate and directed to hit the sample by applying a high voltage for acceleration. The sample holder usually rotates in the path of the X-ray beam at an angle θ, while the X-ray detector collects the diffracted X-rays and rotates at an angle of 2θ. As the sample and the detector rotate through their respective angles, the characteristic X-ray spectra are obtained by recording the intensities of the X-rays reflected by the sample at different angles (the beam incident angle and the beam reflected angle). For a typical X-ray diffraction pattern, the data are collected at 2θ from about 5^o to 70^o which represent the angles preset in the X-ray scanning. The obtained X-ray spectrum is commonly presented as an X – Y plot (or as a table) of peak positions at 2θ against X-ray counts or intensity (Abdou, 1985).

XRD analysis is accurate, rapid, reliable, non-destructive and requires minimal sample preparation. It includes two types: the X-ray powder diffraction (XRPD) and the single-crystal XRD. The XRPD analysis is a commonly used technique with wide applications in the pharmaceutical field. This technique can be utilized during drug development, manufacturing and quality control of manufactured pharmaceutical formulations. XRPD can provide information on the degree of crystallinity and amorphous content of pharmaceutical mixtures. Also, this technique is useful for quantitative analysis of pharmaceutical mixtures and can be used to obtain accurate percentage of components of mixtures. This is based on the fact that the intensity of a diffraction peak of one component of the mixture is directly proportional to the concentration of that component in the mixture. In order to determine the concentration

of one component in a mixture by XRD, a known amount of a well characterized standard is added to the mixture containing the component to be quantified and the amount of the component is determined relative to a certain % content of the added standard. In addition, XRPD technique can be applied for qualitative analysis of compounds, based on the fact that even chemically related compounds possess different and unique 'fingerprint' diffraction patterns that can be used for their identification (Gilmore, 2011).

In this work, XRPD was employed as one of the characterization techniques adopted for evaluation of the produced solid and carrier-based SNEDDSs of indomethacin. For the purpose of comparison, the XRPD analysis was also conducted for indomethacin and different excipients used for different formulations. Ultima IV diffractometer (Rigaku Co., Ltd., Tokyo, Japan) was used for this purpose. A copper X-ray source was used and maintained at 40 kV of tube voltage and 40 mA of tube current to produce emissions of 0.15406 nm. The samples were scanned at 3−60° 2θ range at a scanning speed of 0.5 deg./min. Data were collected using a step scan mode with step size of 0.02^{o} and counting time of 1 second per step.

2.5.4. Fourier-Transform Infra-Red (FTIR) analysis

Infrared (IR) radiation is a type of electromagnetic radiation that starts after the visible region at 700 nm. The IR spectral region comprises three subdivisions that lie between different wavelength ranges including: the near IR, mid IR and far IR. In pharmaceutical analysis, the most commonly used region of the IR spectrum is the mid IR region because it is the region where fundamental vibrations of polyatomic molecules take place. This region falls in the range between 2.6 – 26 µm (wavelength) or 4000 – 400 cm^{-1} (wavenumber). The wavenumber is usually used in IR spectrum rather than the wavelength because the wavenumber is directly related to the energy and the frequency of the radiation which in turn can be directly related to the molecular vibrational changes. Also, the low energies of IR radiation are not adequate to cause electron transitions but they are adequate to induce vibrational changes within molecules. Therefore, when IR radiation hits a sample at particular wavelength, their low energy causes vibrational changes within the sample molecules, rather than causing electron transitions as seen with UV radiation (Bunaciu et al., 2010).

IR spectroscopy is based on passing IR radiation through a sample and measuring the amount of the IR radiation absorbed by the sample molecules at particular wavelength or frequency. Absorption of IR radiation by atoms of a sample causes atomic vibrational changes according to bond strength, atomic masses, and inter- and

intramolecular interactions. IR spectra usually provide information on these vibrational changes by displaying the percent transmittance to the wavenumber of the incident radiation.

Although IR spectra are complex, it provide information about the presence or absence of certain functional groups and therefore it can be used as fingerprints to compare or identify samples or compounds. For example, the IR spectra of known organic compounds can be produced and used as a fingerprint library for identification of unknown compounds via spectral comparison. Also, the molecular structure of unknown compounds can be elucidated by detection of the presence of specific functional groups of these compounds in their IR spectra. However, IR spectra do not provide detailed information on the molecular formula or the structure of a compound.

For most molecules, IR absorption peaks appear in the mid IR region between 4000 and 400 cm^{-1}. The position and intensity of these vibrational bands vary according to the type of atomic chemical bond, conformation of the chemical bonds and their adjacent chemical groups. Therefore, each functional group (such as C=O, C-H, N-H, O-H) produces vibrational peaks, at specific spectral region, which can be used for interpretation of vibrational spectra (Bunaciu et al., 2010).

Although the original IR spectroscopic analysis methods are rapid and non-destructive, it had only limited applications in quantitative analysis. However, the use of IR spectroscopy for quantitative analysis has grown widely with the introduction of the concept of Fourier transform infrared (FTIR). The FTIR spectrometers combine interferometer or Michelson interferometer (**Figure 2.20**) with sensitive IR detector. The interferometer is placed between the source of the radiation and the sample. An IR beam emitted by the source is split by a beam splitter into two beams. The two reflected beams are then combined at the beam splitter according to the position of a movable mirror. The non-absorbed beam leaves the interferometer and becomes focused on the detector which performs complex mathematical calculations to produce the interferogram by relating the intensity of the combined beams to the position of the moving mirror. In FTIR, highly developed computer programs and software are employed to convert the interferograms into spectrum (Abdou, 1985, Bunaciu et al., 2010, Markovich and Pidgeon, 1991).

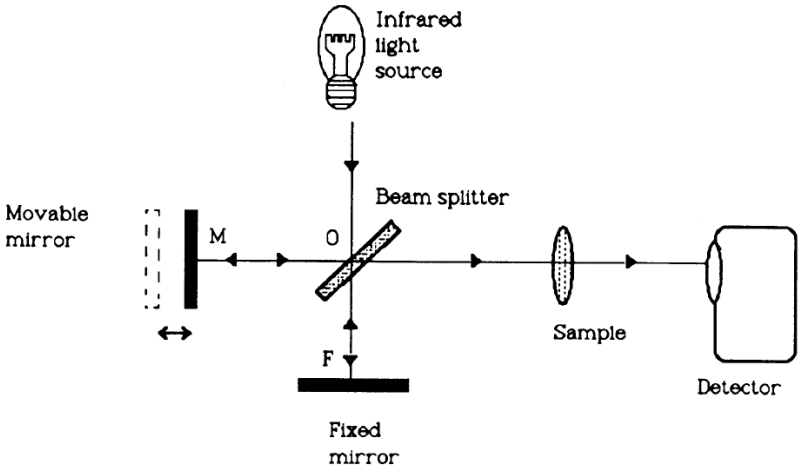


Figure 2.20 Essential components of Michelson interferometer (Markovich and Pidgeon, 1991)

FTIR spectra usually display the % transmittance of radiation at respective wavenumbers. For transmission mode measurement, the detector is usually situated behind the sample so that the fraction of transmitted radiation is collected. Also, solid samples are dispersed in potassium bromide (KBr) disc so that their particle size is smaller than the wavelength of the radiation to avoid scattering effect (Bunaciu et al., 2010).

In this study, FTIR analysis was adopted for characterization and evaluation of the produced solid and carrier-based SNEDDSs of indomethacin. For the purpose of comparison, the FTIR spectra was also obtained for indomethacin and different excipients used for different formulations. All FTIR spectra were recorded in the scanning range of 4000 – 400 cm^{-1} using a Fourier transform infrared (FTIR) spectrophotometer (Nicolet 6700, Thermo Fisher Scientific, Massachusetts, USA) to identify possible interactions between the drug and different excipients used in the formulations. A mixture of formulation sample (4 mg) and dry potassium bromide (IR grade, 200 mg) was lightly ground and compacted to form a disc using a hydraulic press and scanned at a speed of four scans/second.

2.5.5. Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) is an imaging technique that is used widely for characterization of the size and the surface properties of solid samples. Images of the sample are produced by focusing a beam of electrons on the surface of the sample.

Interaction of these electrons with the sample atoms will produce different signals that provide information about topographical features of the sample such as cracks, crystal faces and surface roughness.

SEM is widely used in the pharmaceutical industry during development and optimization of manufactured dosage forms, especially the solid forms. This technique is applied for investigation of the surface properties of active pharmaceutical ingredients and the excipients used in different pharmaceutical formulations. In SEM, the beam of electrons is generated by a cathode source, accelerated by application of high voltage and focused by the objective lens before it enters the sample chamber. In the SEM, different magnifications can be obtained for the surface of the sample. In addition, the linear dimensions of sample particles can be measured with a scale bar superimposed upon the image. The sample is mounted onto a sample holder or stub by putting an adhesive pad on the stub and gently dipping it into the sample to form a thin layer that sticks to the pad. In order to make the pharmaceutical sample electrically conductive, the sample is coated with a thin layer of gold or platinum using a sputter coating unit. This coating aids to give brighter images at low electron voltage and also improves the thermal stability of the sample by conducting heat from the sample upon hitting by the beam of electrons (Nichols et al., 2011).

The morphological properties of indomethacin, different excipients and the formulated solid and carrier-based SNEDDSs were investigated using JSM-6060LV scanning electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 10 kV. Samples were lightly sprinkled on double-sided sticky tape, affixed to an aluminum stub, and made electrically conductive with a gold coating (13 – 14 nm/min; 45 s; 20 mA) under vacuum using JFC-1600 Auto Fine Coater (Jeol, Tokyo, Japan). Micrographs were recorded at different magnifications to study the surface and morphological characteristics.

2.5.6. Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) is a commonly used technique that provides information on the surface morphology, size, shape and structure of biological samples as well as inorganic materials. This technology uses a beam of electrons that can pass through the sample to produce information on structural features, size and shape (Nichols et al., 2011).

Compared to conventional light microscopy, TEM can produce images or micrographs of higher resolution and magnification due to shorter wavelength of electrons compared to photons. This instrument can be operated to produce images with

magnification of 1000 – 250,000x on the screen. Also, TEM can provide images of higher resolution than scanning electron microscope that only views and scans the surface of a sample. Therefore, samples for TEM are required to be of a thickness of less than 100 nm to allow the slow electrons to penetrate the sample, while samples for scanning electron microscopy (SEM) can be of a thickness greater than 10 µm to obtain surface features (Nichols et al., 2011). In addition, TEM analysis can produce micrographs of particles of less than 1 nm in size.

The TEM is composed of three main parts. The first part comprises the electron gun which produces the beam of electrons, and the condenser system that focuses the electron beam onto the sample. The second part is the image producing part that consists of the objective lens, the movable sample stage, and the projector lenses. In this part, the electrons are focused by the objective lens to pass through the sample, where part of the electron beam is transmitted through the sample and the other part is emitted and focused as an image with high magnification on the projector lens. The third part of the TEM is the image recording part which consists of a fluorescent screen to view the images and a digital camera that record images. A vacuum system is another essential component in TEM to ensure that electrons will not interfere with gas atoms (Dykstra and Reuss, 2011).

High resolution, black and white images are produced by TEM due to the interaction between the prepared sample and the beam of electrons in the vacuum chamber. Different parts of the image are displayed in different degrees of darkness due to differences in absorption of electrons caused by different thickness or different composition of the sample. The lighter areas reflect the places where more electrons were transmitted through the sample while the darker regions represent the dense areas of the sample. These differences may provide information on the structure, size and shape of the sample.

Generally, TEM samples are prepared according to the type of the sample and to the type of information required from the test. TEM samples should be prepared to be of less than 100 nm thick so that they can be penetrated by electrons. Usually, the diluted sample is deposited onto films of supporting grids. A standard TEM grid is made of copper, molybdenum, platinum or gold and has a size of 3.05 mm in diameter and a mesh size ranging between few and 100 µm. To enhance the contrast of the produced image, samples are stained with negative staining material such as uranyl acetate or with heavy metals. The staining material will absorb part of the beam of electrons or scatter a part of the electron beam which will be projected on the imaging system. The grid is then placed in the sample holder that is paired with the sample stage. After

insertion into the TEM, the movable sample stage may allow adjustment of the position of the sample to the region where electron beam can be directed (Cheville and Stasko, 2014).

In this study, morphological structures of optimized liquid SNEDDS of indomethacin were investigated using TEM (Philips CM 120 BioTwin, USA). Samples were diluted, deposited on copper grids, stained with 1% uranyl acetate and left to dry before observation under TEM.

2.6. Statistical evaluation methods

In this project, the data of interest were statistically evaluated using one way analysis of variance (ANOVA). Also, significance of difference between the mean of two independent samples was ddetermined using the two samples t-test. Significant differences were determined at a 5% significance level, unless otherwise stated elsewhere. Statistical differences yielding $p < 0.05$ were considered significant. The calculations were performed using MS Excel (2013).

Chapter 3

Development & evaluation of indomethacin-loaded liquid SNEDDS formulations

3.1. Introduction

Self-nanoemulsifying drug delivery systems (SNEDDSs) are isotropic mixtures of oil(s) with surfactant(s), co-surfactant(s) and the solubilized drug (Hauss, 2007, Neslihan Gursoy and Benita, 2004, Porter et al., 2008). These mixtures, when diluted with aqueous fluids and under mild agitation, form quick and spontaneous oil in water nanoemulsions with droplet size ranging from few nanometers to less than 100 nm (Kawabata et al., 2011, Kohli et al., 2010). According to Rehman et al. (2017), these anhydrous formulations can be considered as preconcentrates of nanoemulsions. However, SNEDDSs can offer several advantages compared to nanoemulsions. SNEDDSs can be filled into soft or hard gelatin capsules (due to lack of water content) and this may lead to improved palatability as well as patient compliance. Also, improved physical and chemical stability of the formulation can be obtained upon long term storage of SNEDDSs (Date et al., 2010).

Utilization of SNEDDS formulations is considered as one of the most important approaches to improve solubility and dissolution rate of poorly water soluble (Class II) drugs. Dispersion of SNEDDSs in aqueous fluids leads to formation of fine droplets that contain already dissolved drug in the oil phase and provide large interfacial surface area for transfer of the drug resulting in enhanced rate and extent of absorption and therefore, improved bioavailability (Chakraborty et al., 2009). Also, fine nano-sized droplets may exhibit quick digestion and therefore, faster drug absorption in the gastrointestinal tract (Grove et al.). These lipid formulations can enhance the lymphatic uptake of highly lipophilic compounds (log P > 5 and lipid solubility > 50 mg/g) (Rehman et al., 2017). Compared to lipid solutions, SNEDDSs possess the advantage of increased drug loading capacity due to high content of surfactants and co-surfactants that contribute to enhanced solubility of poorly soluble drugs with intermediate partition coefficient (2 < log P < 4) (Pouton, 2000). In addition, SNEDDSs may provide protection for drugs against the enzymatic and chemical hydrolysis that take place within the aqueous environment of the GIT (Date et al., 2010, Gupta et al., 2013). Furthermore, inhibition of P-glycoprotein mediated drug efflux and improved lymphatic transport by SNEDDS formulations may contribute to enhanced bioavailability of poorly soluble drugs (Date et al., 2010).

The type and the concentration of the components involved in formulation of SNEDDSs have a prominent effect on the final properties of nanoemulsion produced such as the droplet size, polydispersity index and self-nanoemulsification efficiency. Therefore, optimization of the amounts of these components is important for SNEDDS development. Initial selection of SNEDDS components must be based on their ability to

dissolve the model drug as well as their ability to form spontaneous nanoemulsion upon contact with aqueous medium. Also, the phase behavior of the constituents should be evaluated to determine different phases and phase transitions. Plotting of ternary phase diagrams is important to identify the self-nanoemulsification areas where spontaneous nanoemulsions with droplet size of less than 100 nm can be produced. In addition, the effect of the presence of the drug on the size of self-nanoemulsification zones in ternary phase diagrams should be evaluated, because some drugs may reduce the size of these regions. Therefore, optimization of SNEDDS and finalizing its composition are based on determination of the self-nanoemulsification areas as well as evaluation of phase behavior (Date et al., 2010). Other optimization techniques of SNEDDS composition, such as statistical experimental design and response surface methodology, can be applied with lesser number of experiments (Date et al., 2010).

Characterization of the final SNEDDS formulation for different properties must be also considered. Parameters such as the droplet size, polydispersity index, zeta potential, thermodynamic stability, self-nanoemulsification test and in vitro drug dissolution should be carefully evaluated. Also, the in vitro lipolysis model, which simulates digestion in the small intestine, can be used to study the digestion of SNEDDS formulations in addition to their tendency to precipitation. Application of in vitro lipolysis model is useful for optimization of SNEDDS formulations before in vivo studies and also to establish in vivo / in vitro correlation (Date et al., 2010).

In this part of the study, development of indomethacin-loaded SNEDDS formulations will be carried out utilizing different combinations of oils, surfactants and co-surfactants aiming to enhance the solubility of the poorly soluble model drug, indomethacin. Composition of different formulations will be optimized using drug solubility, ternary phase diagram, self-nanoemulsification test and system stability. The formulated systems will be characterized for droplet size, polydispersity index and zeta potential.

3.2. Materials

- Indomethacin was obtained from Sigma-Aldrich Chemie GmbH, Germany.
- Capryol™ 90 (propylene glycol monocaprylate), Labrafac™ lipophile WL 1349 (medium chain triglyceride), Labrafil® M 2125 CS (polyoxylglyceride), and Transcutol® HP (diethylene glycol monoethyl ether) were kindly provided by Gattefosse Co., France.
- Cremophor® RH 40 (polyoxyl 40 hydrogenated castor oil) was kindly provided by BASF Co. (Germany).

- Tween® 20 (polysorbate 20) was obtained from BDH Laboratory Supplies, Poole, England.
- Tween® 80 (polysorbate 80) was obtained from Sigma-Aldrich Chemie GmbH, Germany.
- PEG 400 (polyethylene glycol 400) was obtained from BDH Laboratory Supplies, Poole, England.
- Propylene glycol was obtained from Riedel-de Haen AG, Sleeze – Hannover, Germany.
- Hydrochloric acid was obtained from Fisher Scientific UK Limited, Leicestershire, UK.
- Methanol was obtained from Fisher Scientific UK Limited, Leicestershire, UK.

3.3. Methods

3.3.1. Construction of standard calibration curve of indomethacin in methanol

A stock solution of indomethacin (100 mg / 100 ml) was prepared in methanol. Diluted indomethacin solution (10 mg / 100 ml) in methanol was prepared from the stock solution. Then, serial dilutions were prepared from that diluted indomethacin solution in methanol to obtain different concentrations ranging from 2.5 to 45 µg/ml. The absorbance of these serial dilutions was determined spectrophotometrically at λ_{max} 320 nm, using methanol as a reference. Each sample was analyzed in triplicate and the results are presented as mean ± SD. The measured absorbance was plotted against the corresponding concentrations to obtain the standard calibration curve.

The inter-day accuracy of the assay method for determination of indomethacin concentrations was assessed by calculating the % recovery of three different concentrations (10, 20, and 30 µg/ml) of the drug solution on different days. Three readings were recorded for each sample in methanol at λ_{max} 320 nm. The readings were fitted into the regression equation and the % recovery was calculated according to following equation:

$$\%\, recovery = \frac{recovered\ or\ found\ concentration}{added\ concentration} \times 100 \qquad \text{(Equation 3.1)}$$

Following the method of Sawant et al. (2010), the intra-day and inter-day precision (reproducibility) of the assay method was evaluated by calculating the % relative standard deviation (%RSD) obtained on measuring the absorbance of three

different concentrations (10, 20, and 30 µg/ml) of the drug solution prepared and analyzed on the same day (three sets) or on different days (five sets).

3.3.2. Determination of indomethacin solubility in various components

The solubility of indomethacin in different oils, surfactants, co-surfactants, or mixtures of oils and surfactants was determined according to the method of Date and Nagarsenker (2007). An excess amount of the drug was added to 2 g of each of the selected excipients (or blends of excipients) in screw capped glass vials. The samples were mixed by vortexing to enable proper mixing of the drug with the vehicles. The vials were shaken for 48 hours in an isothermal shaking water bath adjusted at 25 ^{o}C. Equilibrated samples were then centrifuged at 5000 rpm for 15 minutes to remove undissolved drug. The supernatant was aspirated and filtered through a 0.45 µm membrane filter. Aliquots of the supernatant were diluted properly with methanol and the concentration of indomethacin was determined spectrophotometrically at 320 nm against a blank prepared from each excipient in methanol. Tests were repeated in triplicate and the results are presented as mean ± SD.

3.3.3. Screening of surfactants for emulsifying ability

Different surfactants were evaluated for their emulsification ability by mixing the surfactant with the selected oily phase in a 1:1 weight ratio following the method of Date and Nagarsenker (2007). The mixtures were homogenized by vortexing, and 100 mg of each mixture was accurately weighed and added to 20 ml distilled water (200 fold dilution) in a volumetric flask. The ease of formation of emulsion was assessed by observing the number of inversions of the volumetric flask required to obtain a uniform emulsion. The resulting emulsion was also examined visually for relative turbidity according to different grading systems described by Khoo et al. (1998) which can be summarized as follows:

- Grade A: indicates rapid formation of clear or slightly bluish nanoemulsion that emulsifies within 1 minute.
- Grade B: denotes rapid formation of less clear or bluish white nanoemulsion that emulsifies within 2 minutes.
- Grade C: reveals formation of bright white, milky emulsion that emulsifies within 2 minutes.
- Grade D: indicates formation of dull, greyish emulsion that emulsifies slowly (more than 2 minutes).

- Grade E: represents formulations with poor emulsification and large oil globules present on the surface.

Mixtures that showed grades A and B upon dilution were assigned for further evaluation.

3.3.4. Screening of co-surfactants for emulsifying ability

The relative efficacy of co-surfactants (or co-solvents) to improve emulsification ability of surfactants was also evaluated according to the method of Date and Nagarsenker (2007). Mixtures of the selected oily phase, surfactants and co-surfactants (or co-solvents) were mixed and homogenized at a ratio of 3:2:1, respectively. Hundred milligrams of each mixture was accurately weighed and added to 20 ml distilled water (200 fold dilution). The relative turbidity and the ease of formation of the resulting emulsion were assessed as described above for screening of surfactants.

3.3.5. Construction of ternary phase diagrams

Ternary phase diagrams were constructed to identify the regions in which the formulations could self-emulsify upon dilution and gentle agitation. Also, construction of ternary phase diagrams may be beneficial to determine the relative amounts of different components of SEDDS; oil phase, surfactant and co-surfactant (Balakrishnan et al., 2009a, Kommuru et al., 2001).

Based on the solubility study of indomethacin, Capryol™ 90 was chosen as the oil phase. Tween® 80, Tween® 20 and Cremophor® RH 40 were used as surfactants whereas Transcutol® HP was employed as a co-surfactant. Distilled water was used as the aqueous phase for development of these phase diagrams. According to the method of Shafiq et al. (2007) and Shafiq-un-Nabi et al. (2007), ternary phase diagrams were constructed in the absence of indomethacin to identify the self-emulsifying regions. Different combinations of the oil phase, surfactants and co-surfactants were prepared and could be grouped into 3 groups (**Table 3.1**). Surfactants and co-surfactants (Smix) in each group were mixed in different weight ratios (1:0, 1:1, 1:2, 1:3, 2:1, 3:1, and 4:1) so that the concentration of surfactant increases with respect to co-surfactant and the concentration of co-surfactant increases with respect to surfactant.

Table 3.1 Different combinations of oil, surfactants and co-surfactant used in construction of phase diagrams

Group	Oil	Surfactant	Co-surfactant
I	Capryol™ 90	Tween® 80	Transcutol® HP
II	Capryol™ 90	Tween® 20	Transcutol® HP
III	Capryol™ 90	Cremophor® RH 40	Transcutol® HP

For each phase diagram, oil (Capryol™ 90) and specific Smix ratio were prepared and mixed thoroughly in different weight ratios of 0.9:0.1, 0.8:0.2, 0.7:0.3, 0.6:0.4, 0.5:0.5, 0.4:0.6, 0.3:0.7, 0.2:0.8, and 0.1:0.9 in suitable glass vials. Phase diagrams were constructed using the water titration method, in which each combination of oil and Smix was slowly titrated with water. Hundred microliter portions of distilled water were added, at room temperature (25 ± 2^{o}C), to the oil and surfactant mixture to produce a water concentration ranging from 9% to 95% w/w. After each water addition, the mixtures were vortexed for 10 to 20 seconds and visual observations were recorded for the following physical states (**Figure 3.1**):

1. Transparent, easily flowable nanoemulsion (NE).
2. Transparent, non-flowable gel or nanogel (NG).
3. Milky (cloudy), easily flowable emulsion (E).
4. Milky non-flowable emulsion or emulgel (EG).

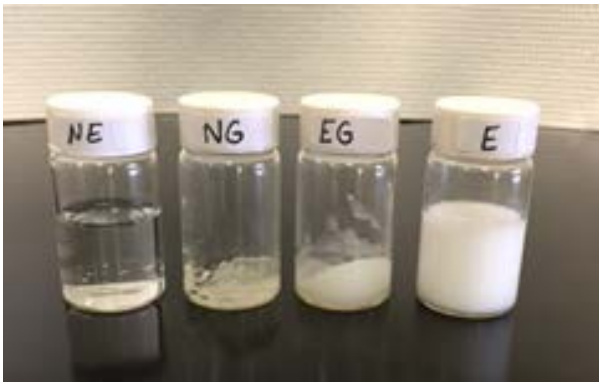


Figure 3.1 Different physical states recorded from ternary phase diagram (NE = Nanoemulsion, NG = Nanogel, EG = Emulgel, and E = Emulsion).

For each Smix ratio, the different physical states were marked on a three component phase diagram, with one axis representing the oil phase, the second representing the mixture of surfactant and co-surfactant (Smix) and the third

representing the aqueous phase. All experiments were repeated in duplicate and similar observations were recorded between repeats.

Ternary phase diagrams were plotted using Ternary 1.37 software (ZetaWare, USA).

3.3.6. Selection of formulations from phase diagrams

Selection of different formulations from the nanoemulsion region, in specific phase diagrams, was based on different oil compositions. In order to cover the entire range of the nanoemulsion region in each specific phase diagram, different oil compositions of 30 % and 50 % were selected and the concentration of the surfactant/co-surfactant (Smix) was calculated according to their ratios.

3.3.7. Effect of the drug on phase behaviour of formulations selected from phase diagrams

The effect of the presence of the drug on phase behaviour was investigated by dissolving 25 mg of indomethacin in selected formulations from each phase diagram. For this purpose, water was added in increasing increments and the new percentages of oil, Smix and water were re-calculated and then re-fitted in the specific phase diagram. Observation of any changes in phase behavior was recorded for each formulation.

3.3.8. Determination of indomethacin solubility in selected formulations

An excess quantity of indomethacin was added to ternary formulations selected from the nanoemulsion regions in phase diagrams. Blank ternary systems were first prepared by mixing the oil phase with the surfactant/co-surfactant mixture. The excess amount of the drug was added to prepared blank systems and mixed by vortexing. The formed suspensions were shaken for 48 hours at 25 ^{o}C in an isothermal shaking water bath. Equilibrated mixtures were then centrifuged at 5000 rpm for 15 minutes to remove undissolved drug. The supernatant was aspirated, filtered through a 0.45 µm membrane filter, suitably diluted with methanol and the concentration of indomethacin was determined spectrophotometrically at 320 nm, against a blank prepared from each mixture in methanol. Triplicate samples were analyzed and the results presented as mean ± SD.

3.3.9. Preparation of drug-loaded self-nanoemulsifying formulations

Indomethacin at a drug loading concentration of 2.5% w/w was added to selected blank ternary formulations. The 2.5% w/w drug loading in SNEDDS reflects the

indomethacin therapeutic dose (25 mg) in a reasonably small volume (approximately 1 ml). Final mixtures were mixed by vortexing until clear systems were obtained. Systems containing indomethacin were shaken for 24 hours at 25 ^{o}C in an isothermal shaking water bath to ensure complete solubilization.

3.3.10. Thermodynamic stability tests

According to the methods of Shakeel et al. (2009) and Shafiq et al. (2007), prepared formulations were subjected to thermodynamic stress tests to eliminate metastable, unstable and biphasic formulations.

3.3.10.1. Centrifugation

Prepared self-emulsifying formulations were centrifuged at 5000 rpm for 15 minutes. Formulations that did not show phase separation were subjected to the heating – cooling test.

3.3.10.2. Heating cooling cycle

Formulations that passed the centrifugation test were subjected to six cycles between cooling (4^{o}C) and heating (45^{o}C) with storage at each temperature for not less than 48 hours. Formulations which were stable at these temperatures and did not show any phase separation were evaluated for freeze – thawing test.

3.3.10.3. Freeze-thaw cycle

In this stress test, formulations were exposed to three cycles of freezing and thawing between –21 ^{o}C and +25 ^{o}C, with storage for periods of not less than 48 hours at each temperature.

Formulations that passed thermodynamic stress tests were taken for self-nanoemulsification tests.

3.3.11. Self-nanoemulsification efficiency tests

Evaluation of the efficiency of self-nanoemulsification of different drug-loaded liquid SNEDDS was carried out to investigate any drug precipitation that may take place upon dilution of formulations with different diluents such as deionized water and 0.1 N HCl. This test was performed as similarly described for screening of surfactants and co-surfactants emulsifying ability. One milliliter of each drug-loaded self-emulsifying formulation was diluted with deionized water or 0.1 N HCl at a dilution ratio of 1:200. The self-nanoemulsification performance of each formulation was evaluated visually

according to different grading systems described by Khoo et al. (1998) and summarized earlier.

Formulations that showed grades A and B upon dilution were subjected for further evaluation.

3.3.12. Characterization of indomethacin-loaded SNEDDSs

3.3.12.1. Measurement of particle size

Mean particle size and polydispersity index (PDI) of selected formulations were determined by photon correlation spectroscopy using Malvern Zetasizer Nano-ZS. Photon correlation spectroscopy utilizes the Brownian motion of the particles to analyze the fluctuations in light scattering that take place due to this motion. Light scattering was measured at a scattering angle of 90^{o} and a temperature of 25^{o}C. Samples of indomethacin-loaded SNEDDS were suitably diluted with deionized water (1:200), filtered through a 0.45 µm membrane filter, and then filled into an acrylic cuvette for measurement. All experiments were repeated thrice and the values of z-average diameter were used.

In this study, Malvern Zetasizer Nano-ZS, which is based on dynamic light scattering (DLS) technique, was used to measure the droplet size of diluted indomethacin-loaded SNEDDS. Dynamic light scattering measures the Brownian motion of the particles and relates it to size of the particles. As smaller particles move faster and larger particles move slower, the Zetasizer uses thise information to relate the speed of Brownian motion of the particles to their size. According to Pecora (2000), particles are illuminated with a source of light (laser) in DLS technique and the intensity of fluctuation of scattered light is analyzed by the instrument to calculate a correlation function that can be used to obtain the size distribution of the sample.

The polydispersity index (PDI) is another important parameter that can be determined by Zetasizer. According to Liu et al. (2009), this parameter has to be evaluated especially in a multimodal distribution to describe the diameter distribution in a sample of SNEDDS dispersed in aqueous media. The value of polydispersity index indicates uniformity in droplet size distribution within the formulation.

3.3.12.2. Measurement of zeta potential

Measurement of the value of zeta potential gives an indication of stability of self-emulsifying formulations, which is directly related to the magnitude of surface charges on emulsion droplets (Balakumar et al., 2013). The zeta potential of selected formulation

was determined using Malvern Zetasizer Nano-ZS. Samples were properly diluted with deionized water (1:200) and filtered through a 0.45 µm membrane filter before measurement.

3.3.12.3. Transmission electron microscopy (TEM) studies

Studies using transmission electron microscopy (TEM) were conducted to reveal morphology and structure of the nanoemulsions produced from selected self-emulsifying formulations. To perform TEM experiments, samples of indomethacin-loaded SNEDDS were diluted with deionized water to a ratio of 1:200 and filtered through a 0.45 µm membrane filter. A drop of the diluted formulation was directly deposited on coated copper grids with a mesh size of 300 and stained with one drop of 1% uranyl acetate. The coated grid was left to dry for 30 seconds and then observed for TEM (Philips CM 120 BioTwin, USA) equipped with AMT software.

3.3.13. Statistical analysis

Solubility data and characterization parameters were statistically evaluated using one way analysis of variance (ANOVA) with t-test. Significant differences were determined at a 5% significance level, unless otherwise stated elsewhere. Statistical differences yielding ($p < 0.05$) were considered significant.

3.4. Results and Discussion

3.4.1. Standard calibration curve of indomethacin in methanol

The standard calibration curve of indomethacin was constructed in methanol to obtain different concentrations ranging from 2.5 to 45 µg/ml, for which the absorbance readings were determined spectrophotometrically at λ_{max} 320 nm (**Table 3.2 and Figure 3.2**). The standard calibration curve was linear over the concentration range studied and obeys Beer-Lambert's law with a correlation coefficient (r^2) 0.999. The corresponding regression equation was found to be Y = 0.0179X – 0.0043.

Table 3.2 Data of the standard calibration curve of indomethacin in methanol assayed spectrophotometrically at λ_{max} 320 nm

Concentration (µg/ml)	Mean absorbance ±SD (n = 3)
2.5	0.051 ± 0.003
5	0.089 ± 0.002
10	0.172 ± 0.001
15	0.263 ± 0.003
20	0.335 ± 0.002
25	0.433 ± 0.002
30	0.53 ± 0.005
35	0.619 ± 0.004
40	0.719 ± 0.003
45	0.811 ± 0.002

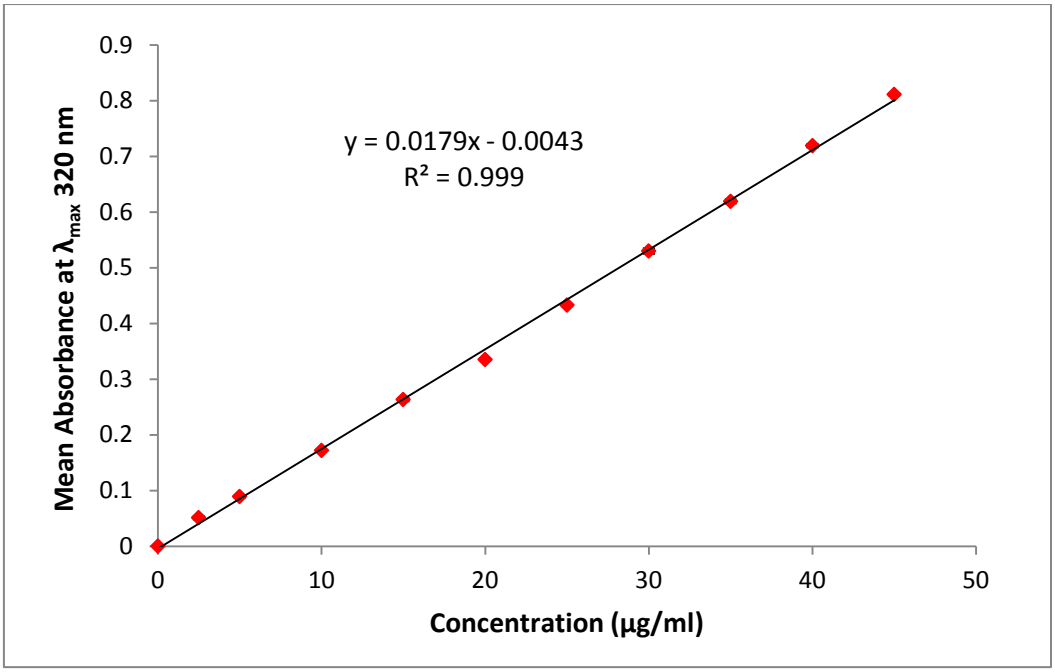


Figure 3.2 Standard calibration curve of indomethacin in methanol assayed spectrophotometrically at λ_{max} 320 nm. (Small standard deviation bars are added but can't be visualized relative to the marker size).

Determination of the accuracy of the spectrophotometric measurement indicated that the % recoveries of indomethacin for the three concentrations (97.48% – 101.47%) with small %RSD values (0.44 – 1.38) were satisfactory for this analytical method. In addition, the %RSD values for the inter-day and intra-day precision varied from 1.07 – 2.13, and 0.17 – 1.17, respectively. The values of %RSD obtained for accuracy and precision of the assay remained within 5% which is acceptable according to ICH guidelines on validation of analytical procedures (ICH, 2005, Nováková et al., 2005).

3.4.2. Solubility studies

Results of the solubility testing of indomethacin in different oils, surfactants, co-surfactants and water are shown in **Figure 3.3**. It is apparent that indomethacin was more soluble in various vehicles used compared to its resulting solubility in water (0.02 ± 0.01 mg/g).

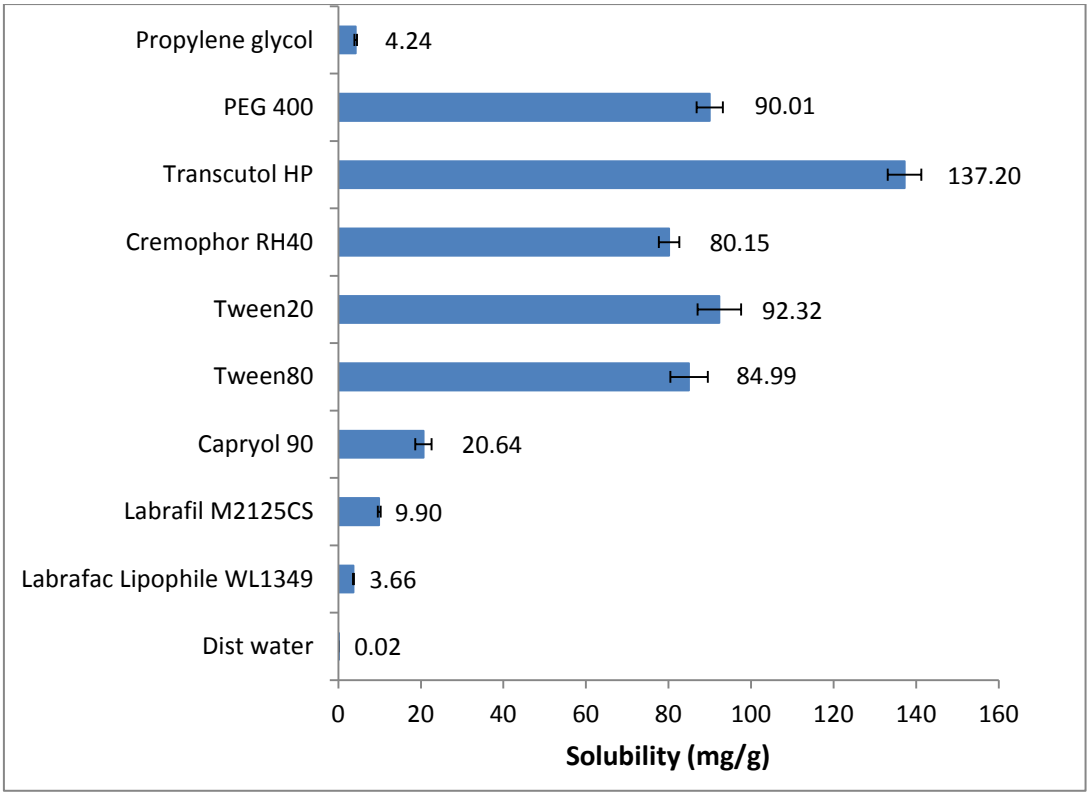


Figure 3.3 Solubility of indomethacin in different oils, surfactants, and co-surfactants at 25°C. (Mean ± SD, n=3).

Figure 3.3 shows the solubility of indomethacin in three different oily phases with varying hydrocarbon chain lengths (C_8 to C_{18}) namely: Capryol™ 90 (C_8 propylene glycol monocaprylate, HLB = 5), Labrafac™ lipophile WL 1349 (medium chain triglyceride, HLB = 1), and Labrafil® M 2125 CS (C_{18} polyoxylglyceride, HLB = 9).

Among the three tested oils, indomethacin exhibited a significantly higher solubility ($p < 0.05$) in Capryol™ 90 (20.64 ± 1.97 mg/g) compared to Labrafac™ lipophile WL 1349 (3.66 ± 0.07 mg/g) or Labrafil® M 2125 CS (9.90 ± 0.35 mg/g). The higher drug solubility in Capryol™ 90 could be attributed to its HLB value and carbon chain length. Similar findings were reported for improved solubility of tamoxifen citrate in Capryol™ 90 compared to that obtained in Labrafac™ lipophile WL 1349 (Elnaggar et al., 2009). Capryol™ 90 has a higher HLB value (HLB = 5) compared to the highly lipophilic Labrafac™ lipophile WL 1349 (HLB = 1) and also possesses medium carbon chain length compared to Labrafil® M 2125 CS (C_{18} polyoxylglyceride). It was reported that highly polar lipids are required for nanoemulsion formation (Kawakami et al., 2002) and that the polarity of lipids will decrease with increasing the length of the alkyl chain (Shen and Zhong, 2006).

Because drug loading capacity is the main factor that should be considered for choosing the oily phase (Duc Hanh et al., 2015, Pouton and Porter, 2008), Capryol™ 90 was selected as the oil component to formulate indomethacin-loaded SNEDDS and consequently was subjected for further evaluation in presence of surfactants and co-surfactants.

Three non-ionic surfactants; Cremophor® RH 40 (polyoxyl 40 hydrogenated castor oil, HLB = 14), Tween®20 (polysorbate 20, HLB = 16.7), and Tween® 80 (polysorbate 80, HLB = 15) were used in this study. All surfactants tested exhibited high solubilizing potential for indomethacin (**Figure 3.3**), with 92.32 ± 5.30 mg/g solubilized by Tween®20 , followed by 84.99 ± 4.52 mg/g solubilized by Tween® 80, and 80.15 ± 2.49 mg/g solubilized by Cremophor® RH 40. A significant difference in solubility potential of the drug due to surfactants can be observed only between Tween®20 and Cremophor® RH 40.

Selection of surfactants should be based on its emulsification efficiency for the selected oil more than its solubilizing potential for the drug (Duc Hanh et al., 2015). Therefore, the miscibility of the above surfactants with the selected oil (Capryol™ 90) at a 1:1 weight ratio was investigated according to the method reported by Balakrishnan et al. (2009a) and Date and Nagarsenker (2007). Emulsification studies showed that all tested surfactants (Cremophor® RH 40; HLB = 14, Tween®20; HLB = 16.7, and Tween® 80 ; HLB = 15) were able to produce clear nanoemulsions with Capryol™ 90 upon dilution, and hence, these surfactants were employed in further studies.

Although surfactants provide a mechanical barrier against coalescence of emulsion droplets, by reducing the interfacial energy of these droplets, the system is still considered thermodynamically unstable and the separation of the two phases is only delayed (Craig et al., 1995). The use of a single surfactant may not be enough to achieve a transient negative interfacial energy or a fluid interfacial film. Hence, addition of a co-surfactant may provide sufficient flexibility to the interfacial film so that various curvatures can be available to form nanoemulsions over a wide range of composition (Rahman et al., 2013, Shafiq et al., 2007). Co-solvents can be incorporated into lipid-based systems to act as co-surfactants, due to their ability to dissolve large quantities of either the drug or the hydrophilic surfactant in the lipid base (Neslihan Gursoy and Benita, 2004).

Therefore, a co-surfactant; Transcutol® HP (diethylene glycol monoethyl ether, HLB = 4.2) and two co-solvents; polyethylene glycol 400 (PEG 400) and propylene glycol were evaluated in this study. From the results presented in **Figure 3.3**, it is obvious that

among all examined vehicles, Transcutol® HP showed the highest capacity to dissolve indomethacin (137.20 ± 4.06 mg/g).

Results of the study of the ability of co-surfactants (or co-solvents) to improve the emulsification ability of surfactants are presented in **Table 3.3**. The co-surfactant and co-solvents used were equivalent in improving emulsification ability of surfactants as demonstrated by grades A and B produced upon dilution with distilled water.

The relatively high hydrophilicity of co-solvents (PEG 400 and propylene glycol) may lead to an increased risk of destroying the emulsion in comparison to when Transcutol® HP is used (Zhang et al., 2008). This is because the high water solubility of these co-solvents may cause them to redistribute between the aqueous phase and the emulsion–water interface upon dilution, leading to loss of solvent capacity of the vehicle (Patel and Vavia, 2007). Therefore, Transcutol® HP was selected as the co-surfactant to conduct further studies aiming to improve the drug loading capabilities and form spontaneous fine nanoemulsions. Transcutol® HP was reported by Sullivan et al. (2014) to be well tolerated orally by different animals in addition to its well-known safe use in food and cosmetics industry.

Table 3.3 Classification of systems composed of oil (Capryol™90): surfactant: co-surfactant (or co-solvent) at a ratio of 3:2:1 as grade A and B upon dilution (200 fold) with distilled water.

Surfactant	**Co-surfactant / co-solvent**	**Visual grade**
Cremophor® RH 40	Transcutol® HP	A*
	PEG 400	A
	Propylene glycol	A
Tween®20	Transcutol® HP	A
	PEG 400	A
	Propylene glycol	A
Tween® 80	Transcutol® HP	A
	PEG 400	B*
	Propylene glycol	B

*A = clear or slightly bluish nanoemulsion that emulsifies within 1 minute, *B = less clear or bluish white nanoemulsion that emulsifies within 2 minutes.

Based on the results of excipient screening, Capryol™ 90 (the oily phase), Cremophor® RH 40, Tween®20, and Tween® 80 (surfactants), in addition to Transcutol® HP (co-surfactant) were selected to formulate indomethacin-loaded SNEDDS. The appropriate amounts of the selected oil, surfactants and co-surfactant were determined by constructing phase diagrams.

3.4.3. Construction of ternary phase diagrams

In order to identify the self-emulsifying regions and to optimize the percentages of different liquid SNEDDS components, a ternary phase diagram was constructed in the absence of indomethacin.

Based on the data obtained from solubility studies, Capryol™ 90 was used as the oil phase. Cremophor® RH 40, Tween®20, and Tween® 80 were used as surfactants; and Transcutol® HP was used as a co-surfactant to construct ternary phase diagram. For more detailed study of phase diagrams, these components were divided in different group combinations; Groups I to III; (**Table 3.1**). Different ratios of surfactant/co-surfactant from each group were used to prepare different ternary systems. Ternary phase diagrams were constructed separately for each group (**Figures 3.4 to 3.6**) and the sizes of self-nanoemulsion regions were compared. Identification of optimal composition of formulation is based on comparison of the size of the self-nanoemusifying zones in

different phase diagrams. Larger self-nanoemusifying areas indicate greater self-nanoemusification efficiency of the tested ternary formula (Duc Hanh et al., 2015).

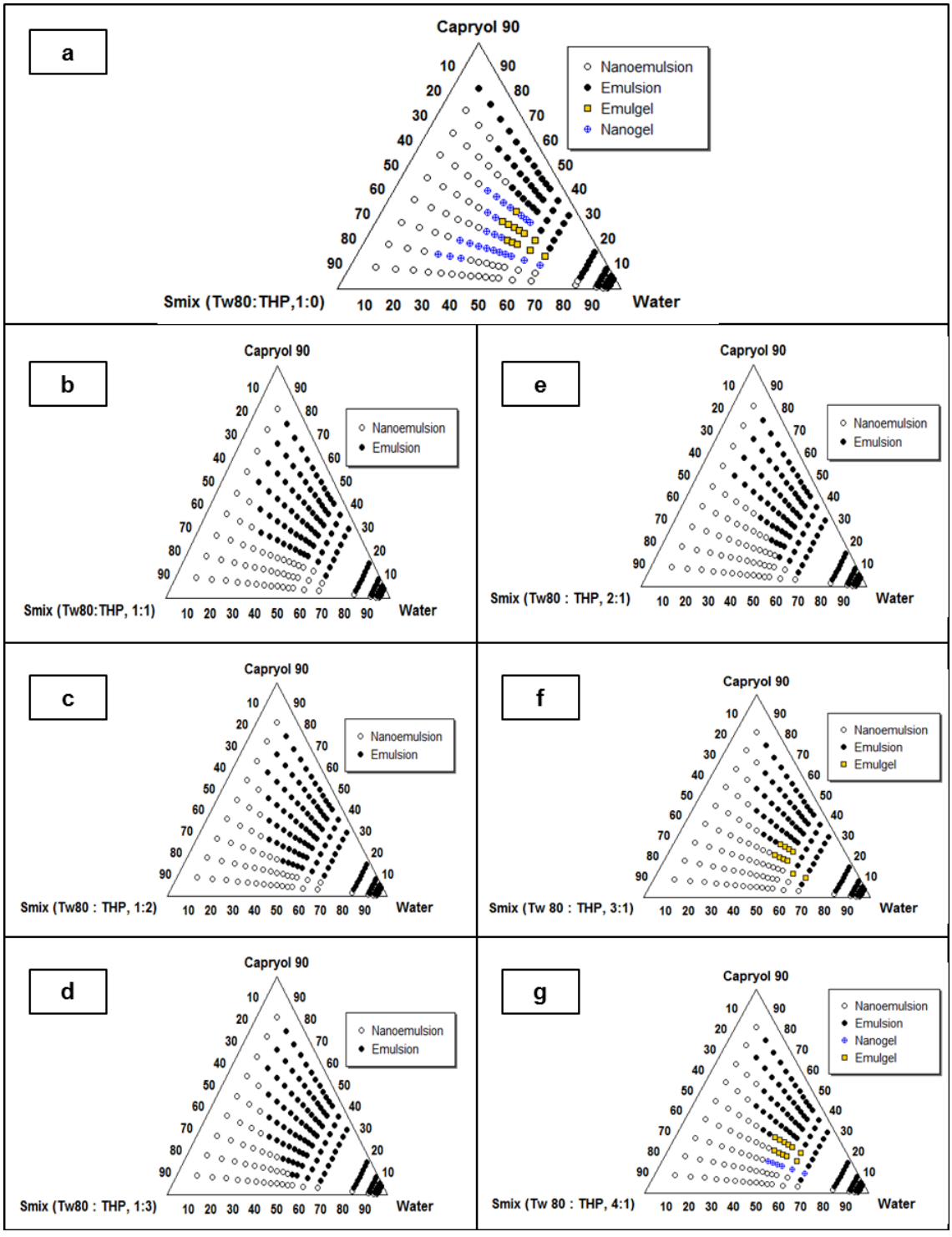


Figure 3.4 Ternary phase diagram of group I at different Smix (Tween®80; Tw80; & Transcutol® HP; THP) ratios.

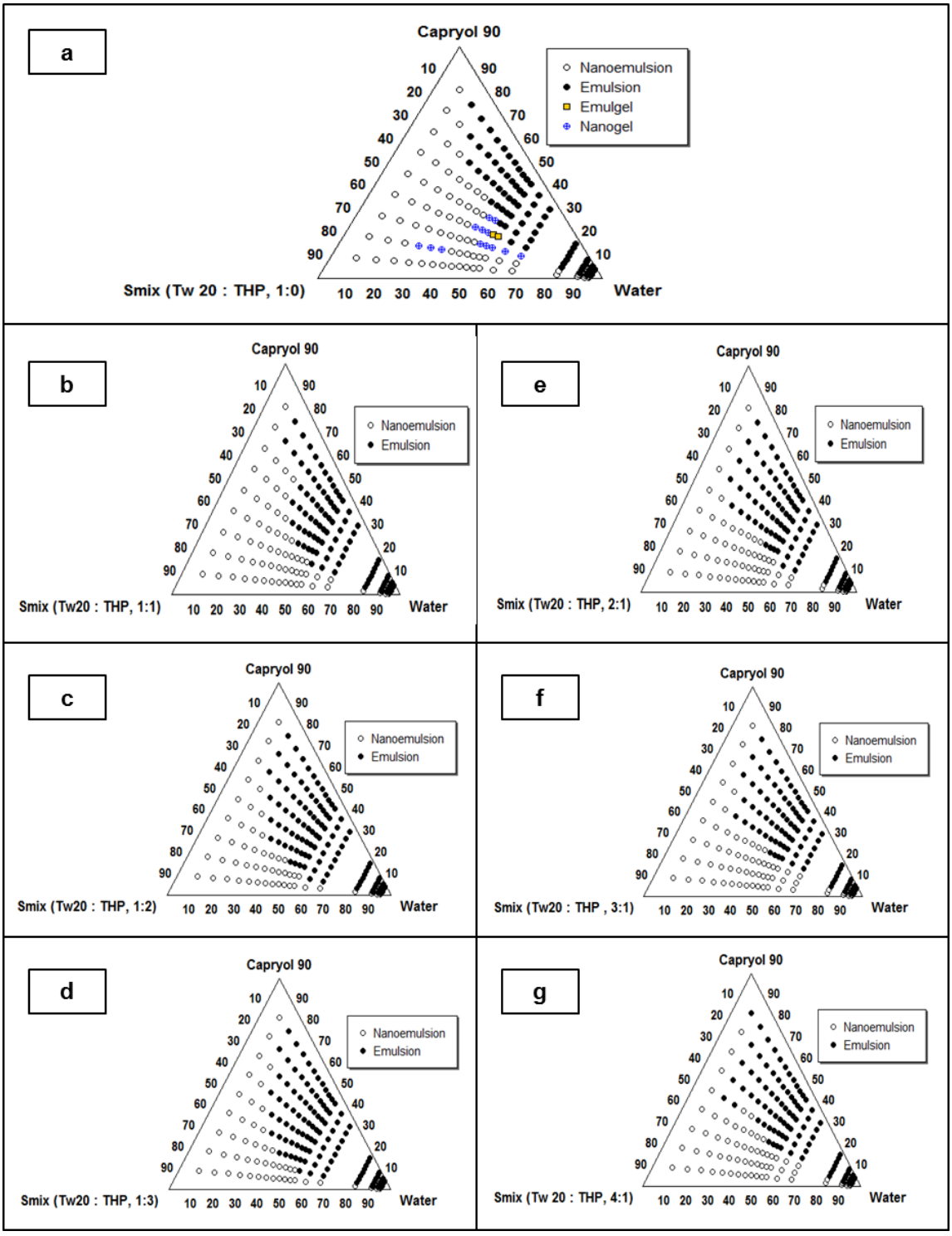


Figure 3.5 Ternary phase diagram of group II at different Smix (Tween®20; Tw20; & Transcutol® HP; THP) ratios.

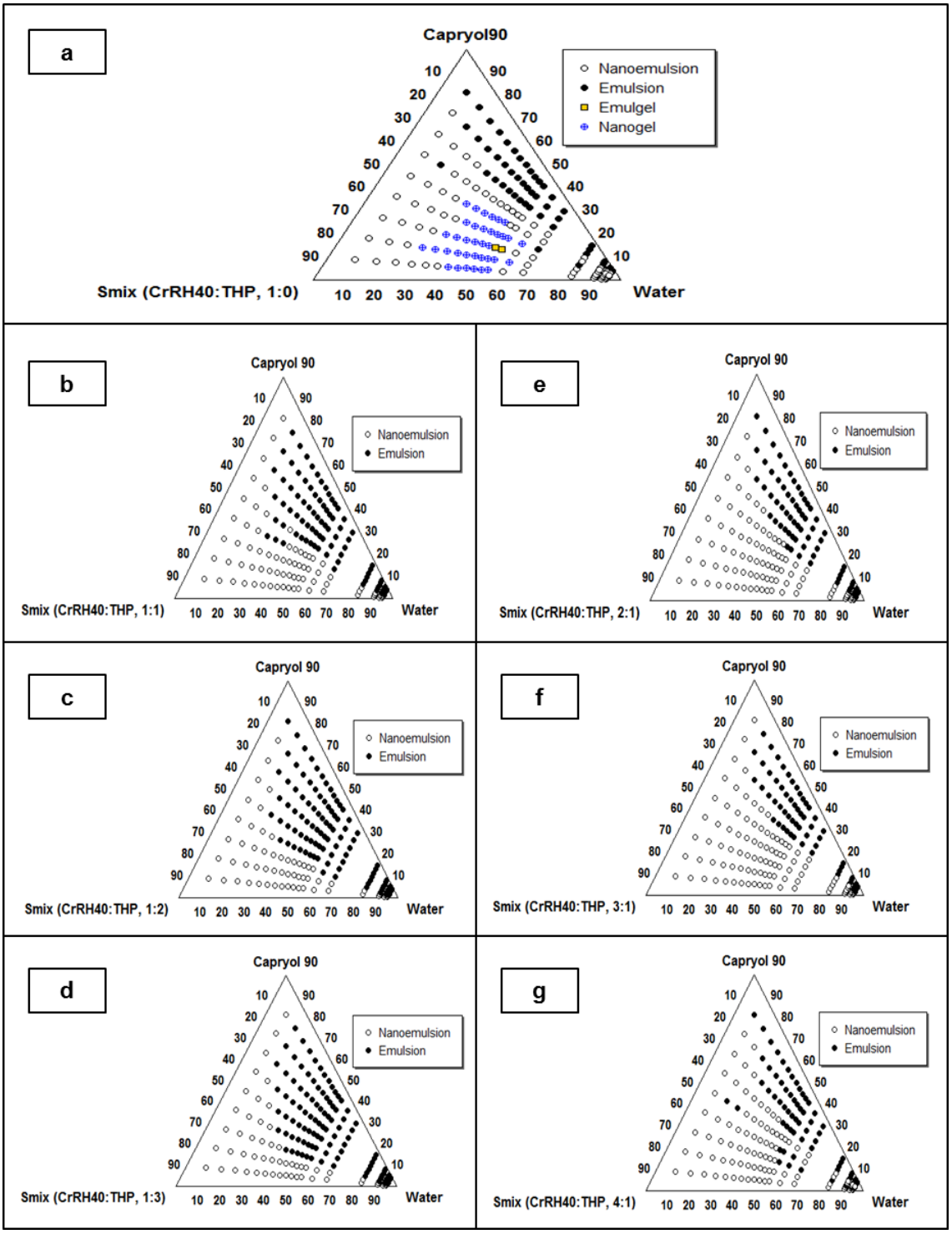


Figure 3.6 Ternary phase diagram of group III at different Smix (Cremophor®RH40; CrRH40; & Transcutol®HP; THP) ratios.

In **Figure 3.4 (a)** it can be seen that when Tween® 80 was used alone, a good size of nanoemulsion region along with small nanogel and emulgel areas were observed. Addition of a co-surfactant (Transcutol HP) to Tween® 80 in equal amounts (Smix ratio 1:1) produced a reduction in the nanoemulsion region and a disappearance of the nanogel and emulgel areas (**Figure 3.4 (b)**). The nanoemulsion region was further decreased upon increasing the co-surfactant concentration to Smix ratio of 1:2 (**Figure 3.4 (c)**) or 1:3 (**Figure 3.4 (d)**), in comparison to the ratio of 1:1. This can be explained on the basis of reduction of surfactant content with increasing co-surfactant concentration (Duc Hanh et al., 2015). On the other hand, increasing the concentration of the surfactant with respect to co-surfactant (Smix ratio 2:1) showed an improvement of the nanoemulsion area (**Figure 3.4 (e)**) compared to the 1:1 ratio. Further increases in the area of nanoemulsification were observed upon increasing the concentration of the surfactant in the Smix to the ratio of 3:1 (**Figure 3.4 (f))** or 4:1 (**Figure 3.4 (g)**). In addition, a small emulgel area was observed with the Smix ratio of 3:1 (**Figure 3.4 (f))** while small nanogel and emulgel areas were apparent with the Smix ratio of 4:1 (**Figure 3.4 (g)).** Hence, surfactant/co-surfactant (Smix) ratios of 1:0, 2:1, 3:1, and 4:1 were selected from Group I for further evaluation, because of good size nanoemulsion areas.

In the case of Group II illustrated in **Figure 3.5 (a – g)**, smaller nanogel and emulgel areas and a reasonable nanoemulsion region were noticed when Tween® 20 was used alone (**Figure 3.5 (a)**). Inclusion of co-surfactant with the surfactant at a ratio of 1:1 resulted in removal of nanogel and emulgel areas with slight reduction in the nanoemulsification area (**Figure 3.5 (b)**). Further reduction in the nanoemulsion area was observed upon increasing co-surfactant concentration to the ratios of 1:2 (**Figure 3.6 (c)**) and 1:3 (**Figure 3.5 (d)**). Also, increasing the concentration of surfactant in Smix with respect to co-surfactant to 2:1 ratio (**Figure 3.5 (e)**) decreased the nanoemulsion area compared to 1:1 ratio. Additional increase in surfactant concentration (**Figure 3.5 (f and g)**) showed smaller extent of improvement in the nanoemulsion region. Therefore, the surfactant/co-surfactant (Smix) ratios 1:0 and 1:1 were chosen for further studies, since the size of the nanoemulsion region was optimum with these ratios.

Phase diagrams of Group III are shown in **Figure 3.6 (a – g)**. The combination of Cremophor® RH 40 (surfactant) with Capryol™ 90 (oil phase) produced large nanogel and emulgel areas (**Figure 3.6 (a)**) and hence, small nanoemulsion region. When the co-surfactant was included with the surfactant in equal amounts (**Figure 3.6 (b)**), the nanogel and emulgel areas changed to easily flowable nanoemulsion region. This could be explained on the basis that the co-surfactant may cause the oil phase to penetrate into the hydrophobic portion of the surfactant. As a result, further reduction of the

interfacial tension is attained and consequently the fluidity of the system increases (Shafiq et al., 2007). Doubling the concentration of the co-surfactant (Smix 1:2 ratio) led to reduction in the area of nanoemulsion region (**Figure 3.6 (c)**) compared to 1:1 ratio. This could be due to reduction of the surfactant concentration brought by increasing the content of co-surfactant. (Duc Hanh et al., 2015). Further reduction in the area of nanoemulsification was observed with further increase in co-surfactant concentration to Smix ratio 1:3 (**Figure 3.6 (d)**). In contrast, increasing surfactant concentration with respect to co-surfactant (Smix ratio 2:1) cause a notable increase in the area of nanoemulsification, compared to ratio 1:1 (**Figure 3.6 (e)**). Additional increase in surfactant amount to Smix ratio 3:1 (**Figure 3.6 (f)**) also increased the area of nanoemulsion region. However, increasing the Smix ratio to 4:1 resulted in small reduction in nanoemulsion region (**Figure 3.6 (g)**) which indicates that optimum emulsification was attained.

Therefore, surfactant/co-surfactant (Smix) ratios of 2:1 and 3:1 were selected as optimal ratios from Group III because of maximum nanoemulsification produced by these Smix ratios.

Comparing the size of the nanoemulsion regions, it appears that the self-nanoemulsifying properties of ternary formulations containing Cremophor® RH 40 are better than those obtained when Tween® 80 or Tween® 20 were used. Although these surfactants possess high HLB values, the difference in their self-nanoemulsifying properties could be due to differences in their structure and chain length (Date and Nagarsenker, 2007). Also, differences in lipid – surfactant affinity may contribute to different self-emulsifying abilities (Sánchez et al., 2001).

In addition, it is obvious from phase diagrams plotted for Tween® 80 or Cremophor® RH 40 (Groups I and III, **Figures 3.4 and 3.6**) that increasing the content of the surfactant led to increased clarity of the produced emulsions which was observed visually. This is because increasing the concentration of surfactant at the interface may reduce the oil content and hence the size of generated emulsion particles which may reflect on more clarity of the formulation (Levy and Benita, 1990). However, reduced clarity of ternary formulations prepared using Tween 20 (Group II, **Figure 3.5**) was observed upon increasing their content of surfactant. This may be due to the fact that increasing surfactant concentration may facilitate water penetration into oil droplets leading to their disruption and ejection of oil droplets into the aqueous phase (Pouton, 1997). Similar observations were reported in different studies of self-emulsifying systems (Kommuru et al., 2001, Parmar et al., 2011, Wang et al., 2009).

The high HLB surfactants employed in this study (Tween® 80; HLB = 15, Tween®20; HLB = 16.7 and Cremophor® RH 40; HLB = 14) were mixed with low HLB co-surfactant (Transcutol®HP, HLB = 4.2) in order to formulate more stable self-emulsifying formulations. The co-surfactant is reported to penetrate into the surfactant film and create void spaces among the molecules which may lead to increased interfacial fluidity of surfactant boundaries (Constantinides and Scalart, 1997).

3.4.4. Selection of formulations from ternary phase diagram

Because no clear basis has been reported in the literature for the selection of nanoemulsion formulations from the phase diagram, it is possible to prepare many different formulations from the nanoemulsion region of the diagram (Shafiq et al., 2007). Based on the results obtained from constructing ternary phase diagram, it is obvious that optimal nanoemulsion zones can be obtained by different Smix ratios prepared from each group of components (Groups I to III). The Smix ratios that produced maximum area of nanoemulsion region were selected for preparation of indomethacin-loaded SNEDDS using different groups of components.

Based on the results obtained from the constructed phase diagrams, it can be seen that Smix ratios of 1:0, 2:1, 3:1, and 4:1 from Group I (containing Tween® 80), Smix ratios of 1:0 and 1:1 from Group II (containing Tween® 20), and Smix ratios of 2:1 and 3:1 from Group III (containing Cremophor® RH 40) resulted in optimum emulsification. Therefore, these eight mixtures of different combinations of surfactants and co-surfactants were employed for this study.

In order to cover the entire range of self-nanoemulsification occurrence in each phase diagram, different oil compositions of 30% w/w and 50% w/w were selected. The composition of selected formulations is illustrated in **Table 3.4**.

Prior to formulation of drug-loaded SNEDDS, the effect of incorporation of indomethacin on phase behavior of the selected formulations was investigated. It was found that the addition of indomethacin did not affect phase behavior of selected formulations or the nanoemulsion area of phase diagrams. Similar findings were reported by Shafiq et al. (2007) upon construction of phase diagrams of ramipril nanoemulsions.

Table 3.4 Composition of selected self-nanoemulsifying formulations

Group	Code	Oil %	Surfactant %	Co-surfactant %	Smix ratio
Group I (Capryol™ 90, Tween® 80, Transcutol® HP)	F1	50	50	--	1:0
	F2	30	70	--	1:0
	F3	50	33.4	16.6	2:1
	F4	30	46.7	23.3	2:1
	F5	50	37.5	12.5	3:1
	F6	30	52.5	17.5	3:1
	F7	50	40	10	4:1
	F8	30	56	14	4:1
Group II (Capryol™ 90, Tween® 20, Transcutol® HP)	F9	50	50	--	1:0
	F10	30	70	--	1:0
	F11	50	25	25	1:1
	F12	30	35	35	1:1
Group III (Capryol™ 90, Cremophor® RH40, Transcutol® HP)	F13	50	33.4	16.6	2:1
	F14	30	46.7	23.3	2:1
	F15	50	37.5	12.5	3:1
	F16	30	52.5	17.5	3:1

3.4.5. Determination of indomethacin solubility in selected formulations

The saturated solubility of indomethacin in different self-emulsifying formulations (F1 to F16) was determined as described earlier in experimental section, and the results are presented in **Table 3.5**.

From the results presented in **Table 3.5**, it is clear that the solubility of indomethacin in selected SNEDDS increased by about 1500 to 2000 fold compared to its solubility in water. Therefore, using SNEDDS proved to enhance the solubilization capacity of indomethacin. However, no significant difference was detected ($p > 0.05$) in maximum drug loaded into different SNEDDS formulations studied here.

Upon visual examination of the results, it was also noticed that the solubility of indomethacin in different self-emulsifying formulations increased with increasing the content of both oil and surfactant. This observation was more obvious in formulations F15 & F16. It appeared that formulations containing 50% of oil showed higher indomethacin solubility than those containing 30% of oil. This may be due to high affinity of the drug to the oil. Also, self-emulsifying systems prepared with high content of

surfactant showed better indomethacin solubilizing capacity. These findings are in disagreement with those obtained for formulating SMEDDS of antitumor agent, oridonin (Liu et al., 2009).

It is obvious that the minimal indomethacin saturated solubility observed for the selected formulations was 30.78 ± 2.04 mg/g. However, a fixed indomethacin concentration of 2.5% w/w (25 mg/g) was selected to be loaded in all self-emulsifying formulations. This concentration reflects the indomethacin therapeutic dose (25 mg) and is lower than the minimal saturated solubility observed here. Accordingly, it was expected to provide spontaneous emulsification of SNEDDS with a low tendency of drug precipitation upon aqueous dilution. Also, using fixed concentration of indomethacin in all formulations was proposed to exclude the effect of varying the drug concentration on the self-emulsifying efficiency of the systems.

It was reported that increased drug loading in SNEDDS above the saturated solubility of the drug may result in increased droplet size (Wang et al., 2009). This was attributed to the fact that high drug concentration may cause precipitation and deposition of drug particles in the oil-water interface causing rigid and less flexible films that could hinder spontaneous emulsification of the SNEDDS (Wang et al., 2009, Park and Kim, 1999).

3.4.6. Formulation and thermodynamic stability studies of indomethacin-loaded self-emulsifying formulations

Indomethacin was loaded in a 2.5% w/w concentration to all selected self-emulsifying systems (F1 to F16). The prepared formulations were kept in closed containers and tested for thermodynamic stability.

Thermodynamic stability studies were carried out to determine the effects of temperature variation and centrifugation on precipitation or phase separation of the formulated SNEDDSs (Duc Hanh et al., 2015).

It was found that all tested self-emulsifying formulations (F1 to F16) were stable and did not show evidence of phase separation in the centrifugation stress test. Also, no precipitation of indomethacin or phase separation was observed following cooling – heating or freezing – thawing cycles (**Table 3.5**). Therefore, all tested formulations (F1 to F16) were taken for self-nanoemulsification efficiency testing.

3.4.7. Self-nanoemulsification efficiency tests

In this study, self-nanoemulsification efficiency tests (or dispersibility tests) were carried out to evaluate any drug precipitation or phase separation upon dilution with

deionized water or 0.1N HCl. The results of these tests are shown in **Table 3.5**. No significant difference was observed in self-nanoemulsification performance between formulated SNEDDSs dispersed in either deionized water or 0.1 N HCl. Similar findings were reported for glibenclamide-loaded nanoemulsions (Shakeel et al., 2013), rosuvastatin calcium–loaded SNEDDS (Balakumar et al., 2013), and phyllanthin-loaded SMEDDS (Duc Hanh et al., 2015).

Formulated SNEDDSs that passed this test in grades A and B were chosen for further evaluation. Formulations with grades A and B are expected to remain as SNEDDSs upon dispersion in GI fluids (Shakeel et al., 2013b).

Table 3.5 Saturated solubility, thermodynamic stability and dispersibility of indomethacin in selected self-emulsifying formulations

Group	Code	Saturated solubility (mg/g) (Mean±SD)	Thermodynamic stability			Dispersibility	
			Centrifugation	Cooling /Heating	Freeze / Thawing	De-ionized water	0.1N HCl
Group I	F1	34.78 ± 1.84	✓	✓	✓	B	B
	F2	30.78 ± 2.04	✓	✓	✓	B	B
	F3	36.63 ± 2.28	✓	✓	✓	B	B
	F4	34.50 ± 0.79	✓	✓	✓	B	B
	F5	40.65 ± 1.57	✓	✓	✓	B	B
	F6	38.53 ± 1.44	✓	✓	✓	B	B
	F7	42.18 ± 1.29	✓	✓	✓	B	B
	F8	40.23 ± 0.65	✓	✓	✓	B	B
Group II	F9	41.42 ± 1.48	✓	✓	✓	B	B
	F10	36.35 ± 0.94	✓	✓	✓	B	B
	F11	43.16 ± 0.89	✓	✓	✓	B	B
	F12	36.78 ± 0.44	✓	✓	✓	B	B
Group III	F13	38.92 ± 0.89	✓	✓	✓	A	A
	F14	34.88 ± 0.83	✓	✓	✓	A	A
	F15	45.45 ± 1.23	✓	✓	✓	A	A
	F16	34.21 ± 0.59	✓	✓	✓	A	A

SD = Standard deviation.

3.4.8. Characterization of indomethacin-loaded SNEDDS

3.4.8.1. Measurement of particle size

The mean droplet size and polydispersity index (PDI) determined for different indomethacin-loaded SNEDDS (F1 to F16) are shown in **Table 3.6**.

Incorporation of different surfactants into indomethacin-loaded SNEDDS formulations resulted in significantly different droplet size ($p < 0.05$); as revealed by one-way ANOVA with t-test. Among the tested formulations, SNEDDS formulations prepared with Cremophor® RH 40 and Transcutol® HP as surfactant and co-surfactant,

respectively, showed a significantly smaller droplet size ($p < 0.05$) compared to other formulations prepared with Tween® 80 and Tween® 20 as surfactants.

Table 3.6 Mean droplet size, polydispersity index (PDI) and zeta potential of indomethacin-loaded liquid SNEDDS formulations (n=3)

Group	Code	Mean droplet size (nm) ± SD	Mean PDI ± SD	Mean Zeta potential (mV)	Zeta deviation (mV)
Group I	F1	102.05 ± 1.20	0.531 ± 0.008	-16.4	4.5
	F2	150.05 ± 0.64	0.449 ± 0.005	-17.2	4.0
	F3	88.06 ± 0.28	0.464 ± 0.004	-25.5	5.2
	F4	133.95 ± 0.21	0.489 ± 0.008	-21.6	4.8
	F5	98.465 ± 0.91	0.492 ± 0.004	-22.4	4.5
	F6	130.90 ± 1.98	0.453 ± 0.006	-19.8	4.4
	F7	109.65 ± 0.92	0.448 ± 0.001	-21.7	4.1
	F8	136.55 ± 0.49	0.518 ± 0.002	-19.7	4.4
Group II	F9	213.85 ± 1.63	0.391 ± 0.003	-26.8	4.9
	F10	176.75 ± 9.69	0.651 ± 0.039	-33.9	6.5
	F11	234.90 ± 5.52	0.353 ± 0.038	-29.4	4.5
	F12	323.60 ± 0.14	0.296 ± 0.001	-36.0	4.8
Group III	F13	71.44 ± 0.92	0.238 ± 0.001	-22.8	8.1
	F14	20.68 ± 0.03	0.110 ± 0.020	-25.2	8.3
	F15	70.34 ± 0.98	0.265 ± 0.001	-31.5	8.0
	F16	22.69 ± 0.06	0.130 ± 0.004	-16.2	9.9

It can be noted that formulations prepared with a high percentage (50%) of oil phase (Capryol™ 90) showed smaller droplet diameters compared to formulations prepared with lower oil content (30%). Larger droplets obtained with low oil concentrations may be because formulations prepared with lower oil content may consequently contain higher proportions of the surfactant/co-surfactant mixture. Higher surfactant concentrations relative to the oil phase may in turn give multiple micelles or closely packed surfactant molecules around the particles which may lead to formation of larger dense particles.

For SNEDDS formulations (F1 to F8) prepared with different ratios of Tween® 80 and Transcutol® HP as surfactant/co-surfactant mixture (Smix), it was noted that

increasing the surfactant concentration in Smix resulted in significantly smaller droplet sizes, until the Smix ratio 3:1 where only a slight increase in droplet size was observed with further increasing concentration of surfactant. This can be explained on the basis of adsorption of the surfactant around the oil-water interface of a droplet with subsequent reduction of interfacial tension of the system (Wang et al., 2009). Also, high concentrations of surfactant may lead to formation of closely-packed films at the oil-water interface which may stabilize the oil droplets (Kommuru et al., 2001, Parmar et al., 2011). When the concentration of the surfactant was increased beyond that in Smix ratio of 2:1, an increase in droplet size was observed for formulations F5 to F8. Similar findings were observed with SEDDS of coenzyme Q10 (Kommuru et al., 2001), SNEDDS of ibuprofen (Wang et al., 2009) and lercanidipine-loaded SNEDDS (Parmar et al., 2011). The increase in droplet size with increased surfactant concentration can be explained based on the fact that high concentrations of surfactants may cause breakage of the oil-water interface due to increased water penetration with resultant ejection of oil droplets into the aqueous phase (Parmar et al., 2011). Also, increased viscosity of the system by addition of higher amounts of surfactant may lead to high rigidity of the interface which may require more energy to produce fine dispersions (Wang et al., 2009, Pouton, 1997). Furthermore, increasing the concentration of surfactant will subsequently decrease the content of the co-surfactant in the Smix leading to a decrease in the flexibility of the interfacial film with consequent increased bending stress and rigidity of the interface (Kawakami et al., 2002).

Incorporation of co-surfactant (Transcutol® HP) in formulations F3 and F4 led to a reduction in their droplet size compared to formulations F1 and F2 prepared without co-surfactant (**Table 3.6**). Reduction in the mean droplet size upon addition of co-surfactant could be due to the fact that co-surfactants may lower the bending stress of the interface by decreasing the interfacial tension and thus increasing fluidity of that film (Kommuru et al., 2001). Also, co-surfactants participate to form a mechanical barrier against coalescence or aggregation of dispersed particles. Moreover, depending on their structure and chain length, co-surfactants possess the ability to penetrate the interfacial surfactant monolayer which may lead to improvement of emulsification of surfactants (Parmar et al., 2011).

Indomethacin self-emulsifying formulations (F9 to F12) prepared with Tween® 20 and Transcutol® HP as the surfactant/co-surfactant mixture (Smix) exhibited larger droplet size compared to formulations prepared with Tween® 80 (F1 to F8) or Cremophor® RH 40 (F13 to F16). It is apparent that the use of different surfactants with different HLB values produced different droplet size of the microemulsion upon dilution.

In this study, the droplet size was smaller in formulations prepared with of Cremophor® RH 40, followed by those containing Tween® 80, and the largest droplet size was observed for systems prepared with Tween® 20.

Similarly, Vitamin D (Guttoff et al., 2015) and Vitamin E (Saberi et al., 2013) loaded nanoemulsion formulations prepared with Tween® 80 showed smaller droplet size than nanoemulsion systems prepared with Tween® 20.

Larger droplet size observed for self-emulsifying formulations prepared with Tween® 20, compared to those observed with formulations containing Tween® 80 could be attributed to differences in the molecular geometry of both surfactants (Saberi et al., 2013, Guttoff et al., 2015). Molecular geometry of a surfactant can be described by a packing parameter, which relates the cross sectional area of the tail group to that of the head group of the surfactant. The packing parameter is believed to affect packing of the surfactant molecules at the oil-water interface, which in turn may reflect differences in interfacial properties such as surface energy and dynamics which affect the formation of ultrafine oil droplets (Guttoff et al., 2015, Hashem et al., 2011, Saberi et al., 2013).

The differences in chemical structure between Tween® 20 and Tween® 80 might be also considered. Tween® 80 possesses a large cross sectional area of its 18 carbon atoms non-polar tail groups, therefore, it has a higher packing parameter than Tween® 20 (12 carbon atoms). Also, Tween® 80 has unsaturated and curved (kinked) non-polar tail groups of long chain oleic acid, while Tween® 20 has saturated and linear non-polar tail groups of medium chain lauric acid. These differences will affect packing of the surfactant molecules at the oil-water boundaries which in turn affect the tendency of spontaneous formation of ultrafine oil droplets upon dilution of the formulation (Saberi et al., 2013, Guttoff et al., 2015, Marasini et al., 2012).

An increased droplet size of F11 and F12 upon incorporation of the co-surfactant (Transcutol® HP), compared to F9 and F10 prepared without co-surfactant, was observed. Increased droplet size by increasing the concentration of co-surfactant could be due to expansion of the interfacial film produced by the co-surfactant (Gao et al., 1998). Similar findings were reported for microemulsion systems of cyclosporine A prepared with Cremophor® EL as surfactant and Transcutol® HP as co-surfactant (Gao et al., 1998).

Indomethacin-loaded SNEDDS formulations (F13 to F16) prepared with Cremophor® RH 40 as a surfactant, and Transcutol® HP as a co-surfactant, showed the lowest droplet size ($p < 0.05$) ranging between 20.68 ± 0.03 nm and 71.44 ± 0.92 nm, compared to all other formulations. The droplet size distribution of nanoemulsions generated from these indomethacin-loaded SNEDDS formulations is presented in **Figure**

3.7. It was observed that decreasing the oil content of the formulations resulted in a decrease in the size of formulation droplets. Similar results were reported for lercanidipine-loaded SNEDDS (Parmar et al., 2011). However, formula F16 showed larger droplet size (22.69 ± 0.06 nm) compared to that exhibited by formula F14 (20.68 ± 0.03 nm) although both formulations contain the same oil content. Such finding may be attributed to the high surfactant content used in formula F16 (Smix ratio = 3:1) compared to that used in formula F14. Higher surfactant concentrations may enhance water penetration into the oil droplets leading to interfacial disruption and subsequent expulsion of oil droplets into the aqueous phase (Parmar et al., 2011).

Addition of Transcutol® HP to Cremophor® RH 40 in SNEDDS formulations of indomethacin (F13 to F16) could also lead to the smaller droplet size of these formulations. Again, Transcutol® HP may play a role in further reduction of interfacial tension and may provide more flexibility for the interfacial film leading to reduced droplet size (Shakeel et al., 2013, Bali et al., 2011).

Overall, it appears that the smallest droplet size was obtained from systems prepared with Cremophor® RH 40, while those systems containing Tween® 80 or Tween® 20 produced larger droplet sizes. The small droplet size obtained from systems containing Cremophor® RH 40 could be attributed to the molecular structure of the surfactant and co-surfactant. As reported by Nepal et al. (2010b), effective arrangement of the co-surfactant (Transcutol® HP) with Cremophor® RH 40 in mixed films at the oil-water interface appears to be the reason for marked reduction of droplet size as a consequence of great reduction in interfacial tension.

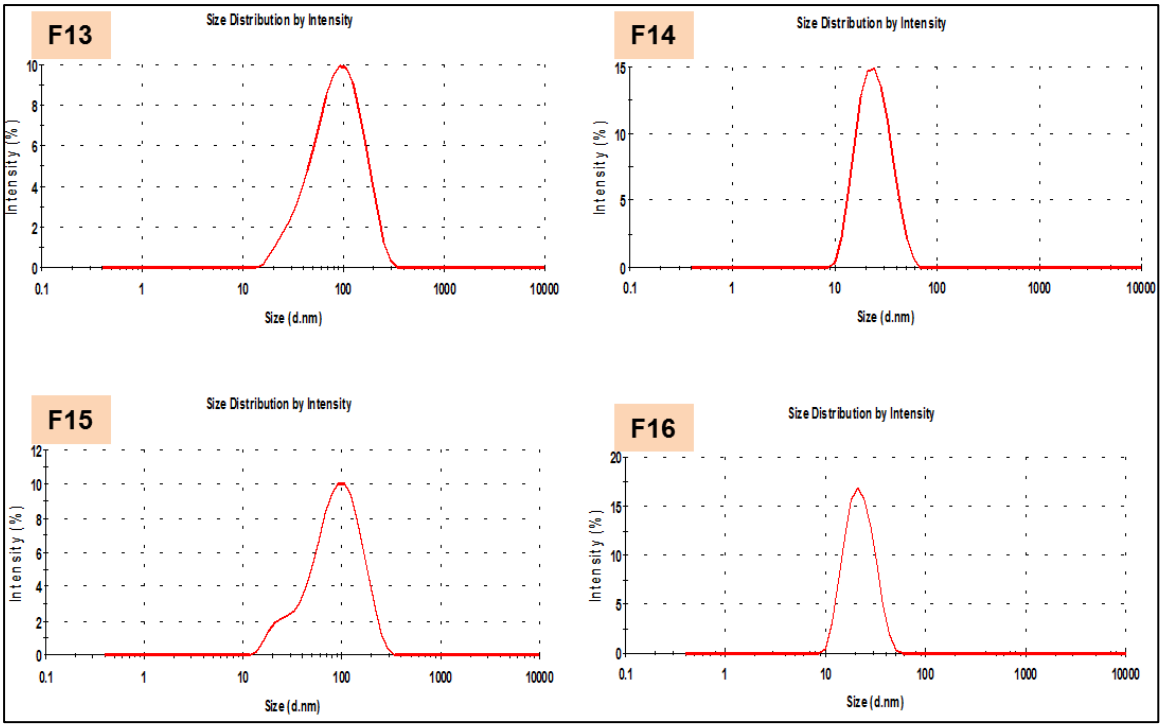


Figure 3.7 Droplet size distribution of nanoemulsions generated from indomethacin-loaded liquid SNEDDS formulations (F13 – F16).

3.4.8.2. Zeta potential measurements

Another property of SEDDS to be evaluated is the zeta potential or the magnitude of surface charges on the surface of particles. It was reported that the surface charge carried by oil droplets may affect in vivo absorption of the drug from the lipid-based formulation (Gershanik et al., 1998).

The magnitude of zeta potential indicates the degree of repulsion between adjacent, similarly charged particles and can be related to stability of the SEDDS formulation (Bali et al., 2011, Parmar et al., 2011). High zeta potential values indicate stable formulations that can resist coalescence of particles, whilst formulations with low values of zeta potential may exhibit flocculation of particles when attraction exceeds repulsion (Parmar et al., 2011). In general, formulations with zeta potential values between 25 and 30 mV in either charge are regarded as stable formulations (Bali et al., 2011, Shakeel et al., 2013b). Self-emulsifying formulations possess a negative charge on the oil droplets due to the presence of anionic groups of free fatty acids contained in their composition; the oil, surfactant and co-surfactant (Balakrishnan et al., 2009b).

The values of zeta potential of the formulated indomethacin-loaded SNEDDS (F1 to F16) are presented in **Table 3.6**. The obtained high negative values of zeta potential

indicate that the tested formulations are less likely to flocculate or aggregate during storage or in biological environment (Bali et al., 2011).

Therefore, SNEDDS formulations of indomethacin comprising Cremophor® RH 40 as a surfactant and Transcutol® HP as a co-surfactant (F13 – F16) were selected among all formulations to be subjected for further evaluation. Formulations with Cremophor® RH 40 exhibited the highest self-emulsification efficiency and the smallest droplet size. Generated nanoemulsions from diluted indomethacin-loaded SNEDDS (F13 to F16) possessed a mean droplet diameter in the range between 20.68 ± 0.03 and 71.44 ± 0.92 nm with polydispersity index (PDI) between 0.11 ± 0.02 and 0.265 ± 0.001. As indicated by Pouton and Porter (2008), smaller PDI values indicate that systems possess narrow size distribution and can be considered as adequate formulations. Additionally formulations F13 to F16 showed zeta potential values which suggested that the nanoemulsions would be stable.

The differences in the performance of Tween 20®, Tween 80® and Cremophor® RH 40 as surfactants in this study are somewhat surprising. The HLB values of the three surfactants quoted by their manufacturers are 16.7, 15, and 14-16 for Tween 20®, Tween 80® and Cremophor® RH 40, respectively. Hence, it may be reasonable to expect that Tween 20®-based formulations would behave differently to those based on the other two surfactants, if HLB is the main determinant of performance, and this is in fact seen in this study in both the particle size and zeta potential data. As the HLB values for Tween 80® and Cremophor® RH 40 overlap, it would be expected that their formulations would be similar. However, this was not observed in this study, with the mean droplet size being significantly smaller for the Cremophor® RH 40-based formulations than the Tween 80®-based formulations, although the zeta potential values were more similar between the two sets of samples.

The observed difference in performance may be attributed to the different chemical structures of Tween 80® and Cremophor® RH 40. Tween 80® is a bulkier molecule, whereas Cremophor® RH 40 is a more linear molecule, hence it would be expected that the packing of the Cremophor® RH 40 components into the emulsion droplet would be more regular and consistent, leading to smaller droplets. Additionally, Nepal et (2010b) have suggested that Cremophor® RH 40 has greater affinity for the oil phase than the Tweens® and would thus have greater contact with the Capryol™ 90 in these formulations, leading to reduced interfacial tension and smaller droplets after dilution. Finally, the larger polyethylene oxide content of Cremophor® RH 40 compared to the other surfactants may help to increase the solubility of the drug and prevent drug precipitation as suggested by (Elnaggar et al., 2009).

3.4.8.3. Transmission electron microscopy (TEM) studies

The structure and morphology of selected indomethacin-loaded SNEDDS (F13 – F16) were evaluated using TEM. The photographs obtained are presented in **Figure 3.8**. It is evident that all particles possessed spherical shape after dilution. The particles in F14 formulation showed the smallest size while those in formulations F13 and F15 exhibited larger size. These results are consistent with that obtained in droplet size analysis.

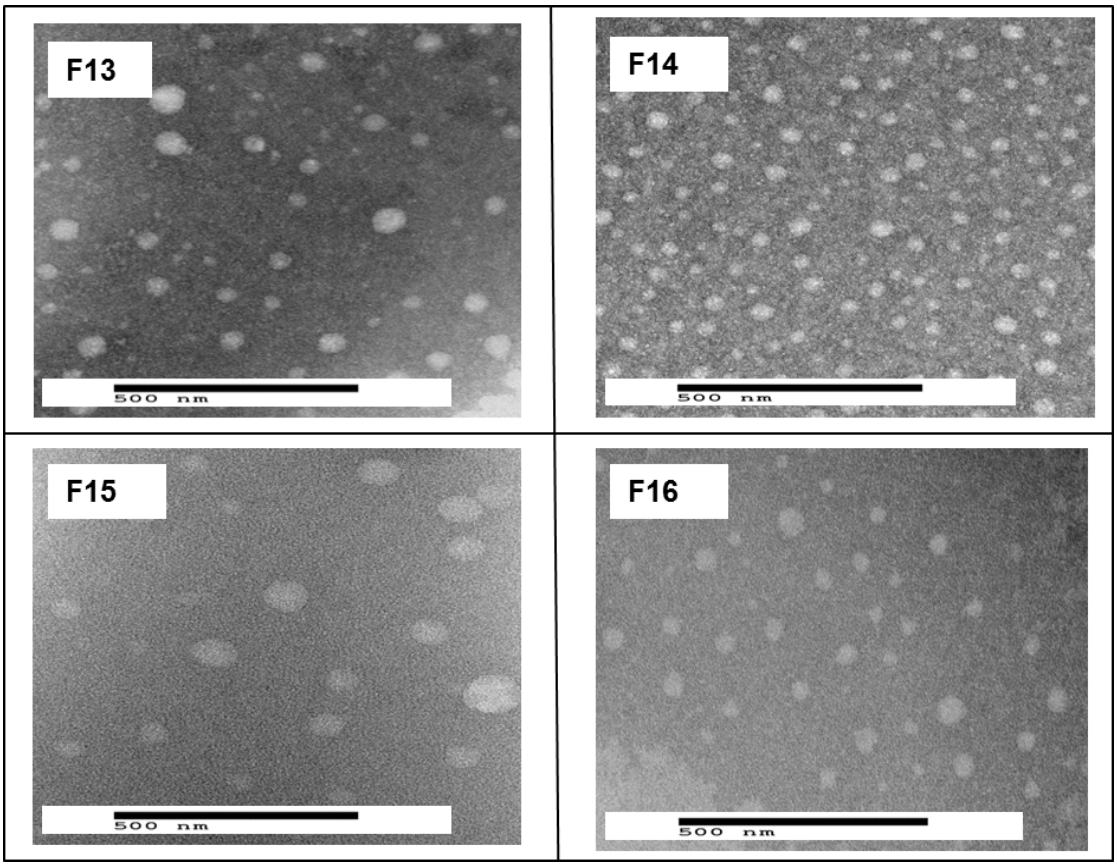


Figure 3.8 TEM photographs of selected indomethacin-loaded liquid SNEDDS.

3.5. Conclusion

In view of the above results, it can be seen that SNEDDS formulations of indomethacin (F13 to F16) prepared with Cremophor® RH 40 and Transcutol®HP as surfactant/co-surfactant mixture were thermodynamically stable over a relatively short time period and exhibited the highest self-emulsification efficiency. These systems utilizing Cremophor® RH 40 showed the smallest droplet size (less than 100 nm) with good polydispersity index (0.11 ± 0.02 to 0.265 ± 0.001) compared to all other tested

formulations. SNEDDS formulations prepared with Cremophor® RH 40 may possess some advantages due to this particular surfactant. It was reported that the use of Cremophor® RH 40 as a surfactant in systems designed for oral administration could be more suitable and advantageous because it is less readily digested due to the low reactivity of its saturated backbone. Also, the larger polyethylene oxide content of Cremophor® RH 40 may be responsible for its lower susceptibility to hydrolysis by pancreatic enzymes. These preferential properties of Cremophor® RH 40 may contribute to prevent drug precipitation from formulations designed with this surfactant (Porter et al., 2008, Elnaggar et al., 2009). Additionally, Cremophor® RH 40 was reported as a bioavailability enhancer through its action as an inhibitor of cytochrome P450 (CYP) 3A enzyme that can diminish bioavailability of some lipophilic drugs (Elnaggar et al., 2009). Improved bioavailability of some drugs formulated as self-emulsifying formulations comprising Cremophor® RH 40 include atorvastatin (Shen and Zhong, 2006), tamoxifen citrate (Elnaggar et al., 2009), and probucol (Nielsen et al., 2008). As a result of studies of improvement of bioavailability due to Cremophor® RH 40, this surfactant was utilized in one of the commercially available SEDDS products; Neoral® (Elnaggar et al., 2009).

Added to the above characteristics that could be related to Cremophore® RH 40, formulations F13 to F16 were prepared with surfactant concentrations between 33.4% and 52.5% (w/w) which were in the range of 30 to 60% that was reported to be necessary to formulate stable SNEDDS (Neslihan Gursoy and Benita, 2004, Tang et al., 2008). Moreover, these systems contained effective surfactant/co-surfactant ratios of 2:1 and 3:1 which resulted in maximum self-nanoemulsifying regions that are devoid of any gel-like zones as presented in their particular phase diagrams.

Accordingly, these four different indomethacin-loaded SNEDDS were found with all of the above properties and hence, selected for further studies that will be focused on transforming these liquid formulations into solid SNEDDS formulations in order to combine the advantages of both lipid-based formulations and solid dosage forms. Different techniques can be used for this purpose. Adsorption onto solid carriers will be utilized in the next part of this study to formulate solid SNEDDS formulations from the optimized liquid formulations. Use of suitable carriers such as silica or silica compounds can be adopted for solidification purposes. The effect of the solidification process on various physicochemical properties of the drug will be evaluated using different characterization and analysis techniques.

Chapter 4

Development & evaluation of indomethacin-loaded solid SNEDDS formulations

4.1. Introduction

Filling of liquid SNEDDSs into hard or soft gelatin capsules is the simplest way for oral administration of these formulations. Capsule filling is suitable for highly potent drugs and provides high drug loading which is determined by the solubility of the drug as well as the capacity of the capsule. However, encapsulation of liquid SNEDDSs may be associated with various limitations. Possible interaction between the formulation excipients and the capsule shell is likely to occur especially if co-solvents were used in production of the formulation. Also, the components may leach or leak out the capsule shell and the drug may precipitate out the formulation at lower storage temperatures. In addition, the components of the liquid fill may be susceptible for solidification at lower storage temperatures. Higher costs, slow production capacity and difficult handling compared to solid dosage forms may also add to these limitations (Mandić et al., 2017). Therefore, transformation of liquid SNEDDS into solid dosage forms may be beneficial to avoid different disadvantages associated with production or encapsulation of liquid formulations. Solid self-emulsifying systems produced from liquid formulations are usually filled into capsules or alternatively, processed into different solid dosage forms such as tablets or pellets. Hence, solid SNEDDS formulations comprise the advantages of both liquid SNEDDSs (improved solubility and bioavailability) as well as those of solid dosage forms including high stability, ease of handling and storage, low production cost, reproducibility, accurate dosing, and as a result better patient compliance (Date et al., 2010, Mandić et al., 2017, Rehman et al., 2017).

The simplest method to convert liquid SNEDDS formulations into solid ones is by adsorption of the liquid formulation onto the surface of solid carriers that are highly porous and/or possess high specific surface area. Also, solid carriers used for solidification of liquid SNEDDSs should be inert, compatible (Gupta et al., 2013), provide high drug loading and reasonable dissolution profile, in addition to adequate flowability and compressibility that may be necessary for further manufacturing procedures (Mandić et al., 2017).

Solidification of liquid SNEDDS by adsorption onto solid carriers involves the addition of successive increments of liquid formulation onto inert adsorbent followed by mixing to obtain free-flowing powder that can be directly filled into capsules or alternatively, compressed into tablets after addition of suitable excipients (Gupta et al., 2013, Mandić et al., 2017). The method of adsorption onto solid carriers can achieve high levels of drug loading which may reach up to 80% w/w (Ito et al., 2005) although some studies have reported that loading of 50% w/w of lipid formulation did not affect the flow properties of the obtained self-emulsifying powder (Jannin et al., 2008, Weerapol et

al., 2015a). Also, this method of solidification does not utilize organic solvents and can produce formulations with good content uniformity (Mandić et al., 2017).

Different solid carriers can be utilized for adsorption of liquid SNEDDS formulations. These include: (i) microcrystalline cellulose; (ii) silicon dioxide under the trade name Aerosil® (Evonik Industries AG, 2017) with different grades of various specific surface area; (iii) amorphous silicon dioxide under the trade name of Syloid® (Grace GmbH, 2012) or Sylysia® (Fuji Silysia Chemical Ltd., 2011) with different grades of pore volume; (iv) magnesium aluminometasilicate under the trade name of Neusilin® (Fuji Chemical Industry, 2014) with different grades of particle size and surface properties (neutral or alkaline); (v) calcium silicate under the trade name of Florite® (Tomita Pharmaceutical Co., 2015); and (vi) porous dibasic calcium phosphate anhydrous under the trade name of Fujicalin® (Fuji Chemical Industry Co. Ltd., 2007). These adsorbents are generally recognized as safe materials and were effectively employed for production of solid SNEDDS formulations by the adsorption method (Agarwal et al., 2009, Agrawal et al., 2015, Beg et al., 2016, Ito et al., 2005, Weerapol et al., 2015a).

Different physical properties of solid carriers such as specific surface area, particle size and pore size and shape could influence the rate of drug dissolution from formulated solid SNEDDSs (Agarwal et al., 2009, Weerapol et al., 2015a). For example, the release rate of gentamycin sulfate from solid SEDDS formulated by adsorption of liquid formulation to different carriers differed according to the specific surface area of the carrier used. Higher release rate of the drug was observed from solid SNEDDS formulations prepared with low specific surface area carrier (Sylysia® 320) compared to formulations prepared with high specific surface area (Florite® RE) (Ito et al., 2005). On the other hand, Agarwal et al. (2009) have reported that the dissolution rate of griseofulvin-loaded solid SEDDS prepared by adsorption onto different carriers increased with increasing the specific surface area of the carrier. In that study, it was shown that solid SEDDS of griseofulvin prepared with two different particle size grades of Neusilin® US2 having similar specific surface area (≈ 300 m^2/g) showed higher release rate than formulations prepared with different particle size grades of silicon dioxide (specific surface area ≈ 150 – 230 m^2/g) or calcium silicate (specific surface area ≈ 50 m^2/g). These results were explained on the basis of the size and depth of the pores of the carrier which are dependent on the specific surface area and particle size of the adsorbent material. The authors hypothesized that liquid lipid formulation may fill the pores of the adsorbent in different ways which may affect their dissolution. It was suggested that lipid formulation may fill the pores either partially, completely or just

adsorbed on the surface of adsorbents depending on the specific surface area of the adsorbent. For instance, adsorbents with high specific surface area (≥ 300 m^2/g) like Neusilin® may possess large number of narrow and long intraparticular pores that contribute to high surface area. Lipid formulation may partially fill the pores of this type of adsorbents and a decreased release rate may be observed due to increased pore length in which the lipid formulation get entrapped. Limited access of dissolution medium to the formulation retained inside the pores may be responsible for decreased release rate of the drug. In addition, the use of larger particle size adsorbents such as Neusilin® US2 (particle size ≈ 80 µm) resulted in decreased release rate of griseofulvin from solid SNEDDS compared to that obtained when smaller particle size adsorbents such as Neusilin® UFL2 (particle size ≈ 5 µm) were used. Again, this was attributed to greater pore length in adsorbents with larger particle size than in those having small particle size. On the other hand, it was proposed that lipid formulation will completely fill the pores of adsorbents having intermediate specific surface area (≈ 150 – 230 m^2/g) like silica-based adsorbents, so that a greater portion of the lipid formulation will interact with the adsorbent pores through hydrophobic interaction and this may lead to possible precipitation of the drug and subsequently, decreased dissolution rate was observed. In the case of adsorbents with low specific surface area (≤ 50 m^2/g), limited area will be available for adsorption of lipid formulations so these formulations will be adsorbed as thin film through hydrophobic interaction with the surface of the adsorbent and this may lead to precipitation of griseofulvin from the lipid formulation resulting in reduced dissolution rate of the drug. The authors also explained the results based on the affinity of griseofulvin to the surface of the adsorbent used. It was proposed that griseofulvin possess high affinity to interact with the hydrophobic surface of silica-based adsorbents and this may result in precipitation of griseofulvin due to its low solubility in the dissolution medium. Therefore, highest precipitation is likely to occur with adsorbents having low specific surface area (≤ 50 m^2/g), while lowest precipitation rate is expected with highly porous adsorbents possessing high specific surface area (≥ 300 m^2/g) in which lipid formulations are incorporated as droplets in the pores and a minimal contact and interaction between the adsorbent and the lipid formula can be obtained.

Therefore, careful consideration of the specific surface area, particle size, the amount and the type of the adsorbent is important in development of solid SNEDDS formulations using the adsorption method. Also, possible interaction between lipid formulation and the carrier should be estimated carefully because it may lead to incomplete or delayed drug release (Agarwal et al., 2009).

Enhancement of the dissolution rate of the drug obtained from solid SNEDDSs prepared by the adsorption method may be attributed to the fact that the drug is maintained in a solubilized form after preparation of the solid product (Date et al., 2010, Raval et al., 2012). The solubilized form of the drug may contribute to rapid formation of very small droplets and faster drug release upon contact with the dissolution medium.

The final solid SNEDDS should be evaluated for different parameters before formulation into solid dosage forms such as tablets or capsules. For example, the ratio of the liquid SNEDDS: adsorbent should be carefully determined. Also, the flow properties such as angle of repose, compressibility index in addition to particle size distribution must be evaluated prior to final conversion into tablets or capsules. Formation of spontaneous nanoemulsion upon dispersion of the final product in a liquid medium may be the rate-limiting step for both in vitro dissolution and in vivo absorption of the drug. Therefore, determination of the self-nanoemulsifying properties of the final product and droplet size of the formed nanoemulsion should be carefully considered as it would determine the in vivo performance of the formulation. The globule size of the obtained nanoemulsion may be affected by the type of the carrier as well as the components of SNEDDS formulation (Date et al., 2010). Generally, solidification procedures should not change the self-emulsifying properties and the droplet size of liquid SNEDDS formulations. This is important to ensure that improved bioavailability of the drug achieved with liquid formulations will be preserved after solidification (Mandić et al., 2017).

Furthermore, physical characterization of the formulated solid SNEDDSs using DSC, XRD and scanning electron microscopy is important to reveal any precipitation of the drug that may have occurred during preparation. For instance, the absence of drug melting endotherms in DSC traces indicates that the drug has remained in a solubilized form within solid SNEDDS formulations and has not precipitated in a crystalline form. Similarly, an absence of crystalline peaks related to the drug in XRD diffractograms also suggests that the drug remains in a dissolved state in the solid SNEDDSs. Investigation of solid SNEDDSs with scanning electron microscopy may provide information on the surface characteristics of particles, while in vitro dissolution studies may give information on possible performance of solid SNEDDS formulation in the GI tract (Date et al., 2010).

In this part of study, solidification by adsorption onto solid carriers was utilized to formulate solid SNEDDSs of indomethacin. Liquid SNEDDS formulations of indomethacin (F13 – F16) that were optimized in **Chapter 3** were employed for this purpose. Different types of solid carriers or adsorbents were used to transform these liquid SNEDDS formulations into solid ones. The potential of use of different types of

adsorbents was assessed by evaluation of powder flow properties, self-emulsification efficiency and dissolution rate of the resulting solid indomethacin-loaded SNEDDSs. In addition, physical characterization of formulated solid SNEDDSs was carried out to evaluate the usefulness of this technique of solidification in production of solid SNEDDSs.

4.2. Materials

- Indomethacin was obtained from Sigma-Aldrich Chemie GmbH, Germany.
- Capryol™ 90 (propylene glycol monocaprylate), and Transcutol® HP (diethylene glycol monoethyl ether) were kindly provided by Gattefosse Co., France.
- Cremophor® RH 40 (polyoxyl 40 hydrogenated castor oil) was kindly provided by BASF Co. (Germany).
- Microcrystalline cellulose (Avicel PH 102) was obtained from FMC BioPolymer (PA, USA),
- Aerosil® 200 (silicon dioxide) was obtained from Evonik Industries AG (Germany).
- Syloid®XDP3150 (amorphous silicon dioxide) was kindly provided by Grace GmbH, (Germany).
- Neusilin®US2 (magnesium aluminometasilicate) was kindly provided by Fuji Chemical Industry (Japan).
- Florite®PS-200 (calcium silicate) was kindly provided by Tomita Pharmaceutical Co. (Japan).
- Potassium dihydrogen orthophosphate anhydrous was obtained from Loba Chemie Pvt. Ltd. (Mumbai, India).
- Sodium hydroxide was obtained from Fluka Chemie GmbH (Germany).
- Calcium chloride was obtained from Sigma Aldrich (U.K.).
- Methanol was obtained from Fisher Scientific UK Limited (Leicestershire, UK).
- Deionized water was purified using an Ultra-purification Water System, Millipore Co. Ltd. (Bedford, MA, USA).
- Hard gelatin capsules were obtained from pharma tradechem (Mumbai, India).

4.3. Methods:

4.3.1. Construction of standard calibration curve of indomethacin in phosphate buffer pH 7.2

The standard calibration curve of indomethacin was constructed in phosphate buffer similarly to the standard curve constructed in methanol which was described previously in **Chapter 3**. Briefly, a stock solution of indomethacin was prepared in phosphate buffer pH 7.2 and serial dilutions were then prepared from the stock solution to obtain different drug concentration ranging from 2.5 to 45 µg/ml. The absorbance of these serial dilutions was determined in triplicates at 320 nm, using phosphate buffer pH 7.2 as a reference standard. The standard calibration curve was obtained by plotting the mean absorbance of these dilutions against the corresponding concentrations.

The inter-day accuracy of the assay method for determination of indomethacin concentrations in phosphate buffer was determined as explained in **Chapter 3**. Similarly, the intra-day and inter-day precision (reproducibility) of the assay procedure was evaluated according to the method of Sawant et al. (2010) presented previously in **Chapter 3**.

4.3.2. Preparation of liquid SNEDDSs of indomethacin (optimized from Chapter 3)

Liquid SNEDDS formulations of indomethacin (F13 – F16) that were optimized in **Chapter 3** were employed for transformation into solid SNEDDSs. The liquid formulations were prepared as described in **Chapter 3.** Briefly, blank ternary mixtures of oil (Capryol™ 90), surfactant (Cremophor® RH40) and co-surfactant (Transcutol® HP) were first mixed in borosilicate glass vials using a magnetic stirrer for 10 minutes. The composition of theses formulations is summarized in **Table 4.1**. After preparation of blank ternary systems, indomethacin was added at a concentration of 2.5% w/w. Final mixtures were vortex mixed until clear mixtures were obtained. To ensure complete solubilization, the prepared mixtures were shaken for 24 hours at 25 ^{o}C in an isothermal shaking water bath.

Table 4.1 Composition of optimized liquid Indomethacin SNEDDS formulations.

Formulation Code	Oil (Capryol™ 90) (%w/w)	Surfactant (%w/w)	Co-surfactant (%w/w)	Smix ratio
F13	50	33.4	16.6	2:1
F14	30	46.7	23.3	2:1
F15	50	37.5	12.5	3:1
F16	30	52.5	17.5	3:1

Smix : Cremophor® RH40 (surfactant) & Transcutol® HP (co-surfactant)

4.3.3. Formulation of indomethacin-loaded solid SNEDDS

The adsorption method was employed to transform the optimized liquid SNEDDS of indomethacin into solid SNEDDS formulations. Different solid carriers namely: microcrystalline cellulose, Aerosil® 200, Syloid® XDP3150, Neusilin® US2 and Florite® PS-200 were used to load the liquid SNEDDSs of indomethacin. The liquid lipid formulation was added in portions and mixed with different adsorbents at the following fixed adsorbent to liquid SNEDDS ratios by weight: 1:0.25, 1:0.5, 1:1; 1:1.5 and 1:2. A successive constant portion of liquid SNEDDS formulation was added to the adsorbent placed in a mortar and mixed with a pestle. The granular mass obtained was then passed through 250 µm mesh screen for the purpose of uniformity in particle size. The powder samples were stored in a desiccator over anhydrous calcium chloride until further evaluation.

4.3.4. Evaluation of formulated indomethacin-loaded solid SNEDDS

4.3.4.1. Flow properties (angle of repose method)

Flow properties of the used adsorbents alone and different solid SNEDDS formulations were determined by angle of repose (θ) method as previously described in **Chapter 2**. All experiments were performed in triplicates and results were averaged ± SD.

4.3.4.2. Carr's compressibility index (CI%) and Hausner's ratio (HR)

The flow and packing properties of blank adsorbents and different indomethacin-loaded solid SNEDDS powder formulations were also assessed by measuring Carr's compressibility index (CI%) and Hausner's ratio (HR). Measurements and calculations of these values were performed as previously described in **Chapter 2**.

All experiments were done in triplicates, and the resulting mean values of CI% and HR were compared to the scale of flowability for these parameters presented in the British Pharmacopoeia (2015).

Powder formulations that showed adequate flow properties according to the pharmacopoeial scale of flowability were subjected for further evaluation.

4.3.4.3. Determination of drug content

The drug content in each indomethacin-loaded solid SNEDDS formulation was determined as previously stated in Chapter 2. All experiments were performed in triplicates and the results were averaged ± Standard deviation (SD).

4.3.4.4. Redispersibility of solid SNEDDS formulations (Droplet size measurement)

Redispersibility of optimized indomethacin-loaded solid SNEDDS formulations was evaluated by measuring the globule size using a Malvern Zetasizer nano-ZS (Malvern Instruments, Worchestershire, UK). According to the method of Kanaujia et al. (2014), each formulation sample was dispersed in deionized water to obtain a final indomethacin concentration of 100 µg/ml. The mixtures were shaken gently for 5 minutes and then centrifuged at 3000 rpm for 15 minutes and filtered through a 0.45 µm syringe filter. The average globule size, polydispersity index and zeta potential of the nanoemulsion formed from S-SNEDDS were determined by Malvern Zetasizer. All measurements were made in triplicates and the results are presented as mean ± standard deviation (SD).

4.3.4.5. Solid state characterization of indomethacin-loaded solid SNEDDS formulations

4.3.4.5.1. Fourier transform infrared spectroscopy (FTIR)

In order to identify possible interaction between the drug and different excipients used in the formulations, FTIR spectra of optimized drug-loaded solid SNEDDS formulations were recorded in the scanning range of 4000 – 400 cm^{-1} using Fourier transform infrared (FTIR) spectrophotometer (Nicolet 6700, Thermo Fisher Scientific, USA). The FTIR spectra of indomethacin and different carriers used for different formulations were also obtained for the purpose of comparison. To perform the test, an amount of each formulation sample (4 mg) was mixed with dry potassium bromide (IR grade, 200 mg), lightly ground and compacted into a disc using hydraulic press and then scanned at a speed of 4 scans/second.

4.3.4.5.2. Differential scanning calorimetry (DSC)

Thermal behavior of indomethacin, solid carriers and optimized drug-loaded solid SNEDDS formulations was studied using a single furnace, heat flux DSC (DSC 4000, Perkin Elmer, US). An accurately weighed sample (5 – 10mg) was placed in a flat bottomed standard aluminum pan and scanned at a heating rate of $10^{o}C$/ minute from 25 – 300 ^{o}C under a nitrogen gas flow rate of 20 ml/min.

4.3.4.5.3. Powder X-ray diffraction (XRD)

The X-ray diffraction patterns of indomethacin, solid carriers and optimized drug-loaded solid SNEDDS formulations were recorded at ambient temperature using Ultima IV diffractometer (Rigaku Co., Ltd., Tokyo, Japan) equipped with a copper X-ray source maintained at 40 kV of tube voltage and 40 mA of tube current to produce emissions of 0.15406 nm. The samples were scanned at 3−60° 2θ range at a scanning speed of 0.5 deg. /min. Data were collected using a step scan mode with step size of 0.02^{o} and counting time of 1 second per step.

4.3.4.5.4. Scanning electron microscopy (SEM)

The morphological characteristics of indomethacin, solid carriers and optimized drug-loaded solid SNEDDS formulations were investigated using JSM-6060LV scanning electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 10 kV. Prior to observation under SEM, double-sided sticky tape with lightly sprinkled sample was

affixed to aluminum stub and made electrically conductive with a gold coating (13 – 14 nm/min; 45 s; 20 mA) under vacuum using JFC-1600 Auto Fine Coater (Jeol, Tokyo, Japan). Micrographs were recorded at different magnifications and analyzed for the surface and morphological characteristics.

4.3.4.6. In vitro dissolution studies

In vitro dissolution studies of optimized drug-loaded solid SNEDDS formulations were performed according to British Pharmacopoeia (2015) using dissolution type II apparatus (Pharma Test, Hainburg, Germany) with 900 ml phosphate buffer pH 7.2 maintained at 37 ± 0.5 ^{o}C and at a rotation speed of 50 rpm. An amount of drug-loaded solid SNEDDS formulation equivalent to 25 mg of indomethacin was filled in suitable number of hard gelatin capsules (size 000) and used for dissolution studies. Samples (5 ml) were withdrawn at predetermined time intervals. An equal volume of fresh dissolution medium maintained at the same temperature was added to keep constant volume during dissolution study. The collected samples were filtered through 0.45 µm syringe filters and then assayed for the content of indomethacin by UV spectrophotometry at 320 nm. For the purpose of comparison, the experiment was repeated with the same quantity of indomethacin (25 mg) taken from the optimized liquid SNEDDS formulation as well as from pure drug powder and filled in hard gelatin capsules. All experiments were performed in triplicates and results were averaged ± SD. Dissolution efficiency after 15 minutes (DE_{15min}), mean dissolution time (MDT) and % released after 15 minutes ($\%Q_{15min}$) were used to compare the dissolution performance of different drug-loaded solid SNEDDS formulations. The DE_{15min} was determined for the time intervals of dissolution study (from 0 – 15 min) while MDT was calculated for all dissolution time interval (from 0 - 60 min) using DDSolver as Excel add inn.

4.3.4.7. Statistical analysis

One way analysis of variance (ANOVA) was used with t-test to detect differences between the data of interest. Significant differences were determined at a 5% significance level, unless otherwise stated elsewhere. Statistical differences yielding ($p < 0.05$) were considered significant.

4.4. Results and Discussion

4.4.1. Standard calibration curve of indomethacin in phosphate buffer pH 7.2

The standard calibration curve of indomethacin was constructed in phosphate buffer pH 7.2 to obtain different concentrations ranging from 2.5 to 45 µg/ml. The absorbance readings of these concentrations were determined spectrophotometrically at λ_{max} 320 nm and the results were plotted against the corresponding concentrations as presented in **Table 4.2** and **Figure 4.1**. The standard calibration curve was linear over the concentration range studied and obeys Beer-Lambert's law with a correlation coefficient (r^2) 0.999. The corresponding regression equation was found to be Y = 0.0227X + 0.0047.

Table 4.2 Data of the standard calibration curve of indomethacin constructed in phosphate buffer pH 7.2 assayed at 320 nm.

Concentration (µg/ml)	Mean absorbance ±SD (n = 3)
2.5	0.063 ± 0.002
5	0.114 ± 0.001
10	0.228 ± 0.001
15	0.345 ± 0.001
20	0.455 ± 0.004
25	0.579 ± 0.004
30	0.699 ± 0.003
35	0.805 ± 0.003
40	0.923 ± 0.001
45	0.998 ± 0.001

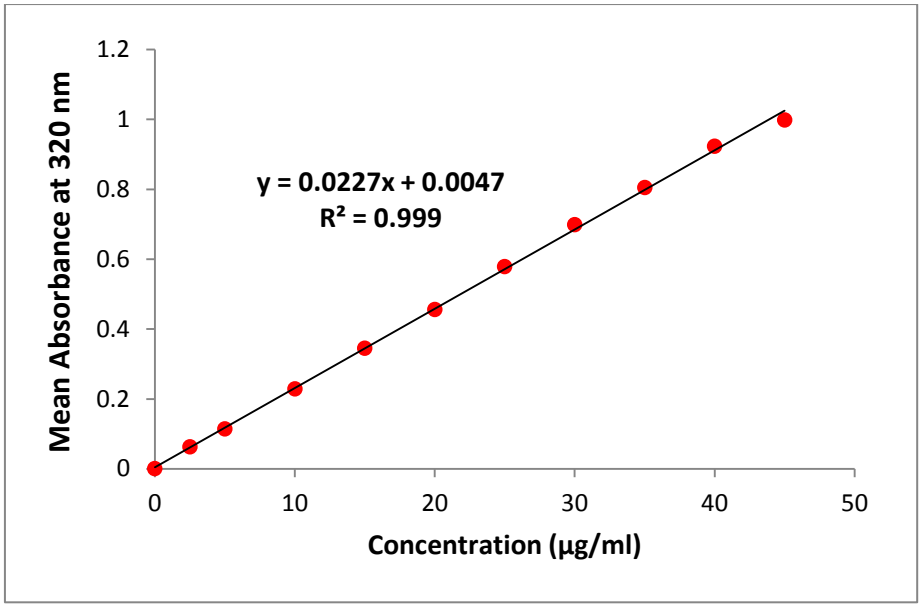


Figure 4.1 Standard calibration curve of indomethacin in phosphate buffer pH 7.2 assayed at 320 nm. (Small standard deviation bars were added but can't be visualized relative to the marker size).

The spectrophotometric assay in phosphate buffer showed accuracy with % recoveries of indomethacin in the range of 97.05 – 101.57% for the three concentrations selected. Also, small %RSD values (0.78 – 1.20) were satisfactory for this analytical method. In addition, determination of inter-day and intra-day precision of the assay method revealed %RSD values of 0.90 – 2.24 and 0.44 – 1.35, respectively. The values of %RSD obtained for accuracy and precision of the assay remained within 5% which is acceptable according to ICH guidelines on validation of analytical procedures (ICH, 2005, Nováková et al., 2005).

4.4.2. Formulation of Indomethacin-loaded solid SNEDDS

Drug-loaded solid SNEDDS formulations were formulated by adsorbing the optimized liquid SNEDDS of indomethacin onto different solid carriers selected for this study. The liquid lipid formulation was added in successive constant portions to the adsorbent at different adsorbent: liquid SNEDDS ratios of 1: 0.25, 1: 0.5, 1: 1; 1: 1.5 and 1: 2. The resulting granular mass varied in their consistency and appearance according to the type and/or the amount of the liquid SNEDDS formulation added to the adsorbent. For example, utilization of MCC as a solid adsorbent resulted in greasy mixtures upon

addition of any of the liquid SNEDDS formulations. The greasiness of these mixtures increased with increasing the amount of the liquid SNEDDS formulation (at the ratios of 1: 1.5 and 1: 2). Also, the addition of any of the liquid SNEDDS to Aerosil® 200 at all the ratios tested, produced solid mixtures that were fluffy and difficult to handle.

On the other hand, the appearance and consistency of solid mixtures produced with Neusilin® US2 or Florite® PS-200 varied according to the type of liquid SNEDDS added. Greasy solid mixtures were obtained upon the addition of F13 or F15 to any of these two carries, while less greasy blends were produced when F14 or F16 were added to these carriers, especially at high adsorbent: liquid SNEDDS ratios (1: 1.5 and 1: 2).

In addition, the solid mixtures obtained by adding any of the liquid SNEDDS formulations (F13 – F16) to Syloid® XDP3150 at different ratios were not too greasy and could be handled easily.

In order to confirm the above differences in the physical appearance of different solid mixtures, the flow properties of different drug-loaded solid SNEDDS were evaluated in the next section.

4.4.3. Flow properties of different indomethacin-loaded solid SNEDDS formulations

Determination of the flow properties of solid SNEDDS powder formulations helps to identify the most appropriate formulation that can be successfully filled into capsules or alternatively, compressed into tablets. The flowability of different indomethacin-loaded solid SNEDDS formulations was determined using angle of repose (θ), Carr's compressibility index (CI%) and Hausner's ratio (HR) and the results are presented in **Table 4.3 until Table 4.7.** According to the scale of flowability presented in British Pharmacopoeia (2015), powder formulations possessing angle of repose in the range of 25^o – 35^o are considered as having acceptable flow properties although powder formulations that show angle of repose in the range of 40^o – 50^o may be adequately manufactured. Also, powder formulations having CI% values below 25 are considered to possess good flow properties. In addition, HR values less than or equal to 1.25 indicate good flow properties although HR values less than 1.34 denote passable flow.

Table 4.3 Flowability parameters of different indomethacin solid SNEDDSs loaded onto Microcrystalline cellulose (Avicel® PH 102).

Liquid SNEDDS	**Ratio of carrier: liq. SNEDDS**	**Angle of repose (θ^o) ±SD**	**Bulk density (ρ_b) (g/cm^3) ±SD**	**Tapped density (ρ_t) (g/cm^3) ±SD**	**Carr's Index (CI%) ±SD**	**Hausner's Ratio (HR) ±SD**
Avicel® PH 102	----	34.15±0.29	0.32±0.01	0.39±0.00	17.95±1.78	1.22±0.02
F13	1:0.25	47.54±0.47	0.31±0.01	0.40±0.01	22.37±2.28	1.29±0.04
	1:0.5	46.02±0.30	0.33±0.00	0.42±0.01	21.43±2.89	1.27±0.04
	1:1	48.02±0.47	0.40±0.01	0.54±0.02	25.79±2.48	1.35±0.05
	1:1.5	N/A	N/A	N/A	N/A	N/A
	1:2	N/A	N/A	N/A	N/A	N/A
F14	1:0.25	48.12±0.64	0.31±0.01	0.41±0.02	24.39±3.28	1.32±0.09
	1:0.5	49.53±0.51	0.34±0.01	0.43±0.01	16.35±2.34	1.26±0.03
	1:1	46.07±0.37	0.42±0.01	0.56±0.02	24.60±2.37	1.33±0.04
	1:1.5	N/A	N/A	N/A	N/A	N/A
	1:2	N/A	N/A	N/A	N/A	N/A
F15	1:0.25	48.07±0.40	0.32±0.01	0.42±0.00	23.82±2.83	1.31±0.04
	1:0.5	49.20±0.41	0.32±0.01	0.43±0.01	25.58±2.41	1.34±0.04
	1:1	47.44±0.50	0.41±0.01	0.54±0.02	24.28±2.26	1.32±0.04
	1:1.5	N/A	N/A	N/A	N/A	N/A
	1:2	N/A	N/A	N/A	N/A	N/A
F16	1:0.25	46.82±0.38	0.30±0.01	0.39±0.01	23.08±2.53	1.30±0.04
	1:0.5	49.03±0.25	0.35±0.01	0.47±0.01	24.60±2.37	1.33±0.04
	1:1	47.35±1.15	0.39±0.02	0.50±0.01	22.10±3.10	1.28±0.07
	1:1.5	N/A	N/A	N/A	N/A	N/A
	1:2	N/A	N/A	N/A	N/A	N/A

N/A: Not Applicable.

Table 4.4 Flowability parameters of different indomethacin solid SNEDDSs loaded onto Aerosil® 200.

Liquid SNEDDS	Ratio of carrier: liq. SNEDDS	Angle of repose (θ^o) ±SD	Bulk density (ρ_b) (g/cm^3) ±SD	Tapped density (ρ_t) (g/cm^3) ±SD	Carr's Index (CI%) ±SD	Hausner's Ratio (HR) ±SD
Aerosil® 200	----	29.67±0.12	0.07±0.00	0.09±0.00	24.89±1.31	1.33±0.02
F13	1:0.25	38.92±0.28	0.36±0.00	0.49±0.01	25.41±1.27	1.34±0.02
	1:0.5	41.72±0.15	0.06±0.00	0.08±0.00	24.23±1.02	1.32±0.02
	1:1	43.74±0.63	0.07±0.00	0.10±0.00	27.06±0.69	1.42±0.01
	1:1.5	44.48±0.16	0.14±0.00	0.22±0.00	36.59±0.31	1.58±0.01
	1:2	46.62±0.55	0.23±0.00	0.34±0.01	32.90±1.84	1.49±0.04
F14	1:0.25	39.19±0.29	0.20±0.00	0.26±0.00	22.28±1.52	1.29±0.03
	1:0.5	44.62±0.36	0.39±0.01	0.50±0.02	21.69±1.50	1.28±0.02
	1:1	45.63±0.59	0.08±0.00	0.11±0.00	27.28±1.03	1.39±0.02
	1:1.5	43.74±0.50	0.12±0.00	0.17±0.00	29.43±0.86	1.42±0.02
	1:2	41.59±0.32	0.07±0.00	0.10±0.00	30.82±1.96	1.43±0.04
F15	1:0.25	39.38±0.24	0.19±0.00	0.25±0.00	25.26±0.80	1.34±0.01
	1:0.5	45.34±0.37	0.34±0.00	0.46±0.01	25.01±1.60	1.33±0.03
	1:1	45.53±0.22	0.06±0.00	0.08±0.00	24.45±0.69	1.32±0.01
	1:1.5	45.77±0.22	0.08±0.01	0.12±0.01	32.33±0.78	1.48±0.02
	1:2	46.33±1.18	0.11±0.00	0.17±0.00	35.68±1.02	1.55±0.02
F16	1:0.25	43.92±0.35	0.12±0.00	0.16±0.00	25.53±1.21	1.34±0.02
	1:0.5	44.62±0.36	0.37±0.01	0.47±0.01	20.85±2.33	1.26±0.04
	1:1	44.16±0.74	0.07±0.00	0.09±0.00	24.89±1.31	1.33±0.02
	1:1.5	41.93±0.32	0.08±0.00	0.11±0.00	27.29±0.64	1.38±0.01
	1:2	46.22±0.74	0.18±0.00	0.26±0.00	30.14±1.29	1.43±0.03

Table 4.5 Flowability parameters of different indomethacin solid SNEDDSs loaded onto Neusilin® US2.

Liquid SNEDDS	Ratio of carrier: liq. SNEDDS	Angle of repose (θ^o) ±SD	Bulk density (ρ_b) (g/cm^3) ±SD	Tapped density (ρ_t) (g/cm^3) ±SD	Carr's Index (CI%) ±SD	Hausner's Ratio (HR) ±SD
Neusilin® US2	----	29.51±0.31	0.18±0.00	0.19±0.00	6.45±1.36	1.07±0.02
F13	1:0.25	41.93±0.32	0.15±0.01	0.21±0.02	28.57±2.48	1.41±0.10
	1:0.5	42.28±0.65	0.20±0.01	0.29±0.01	31.03±1.44	1.45±0.04
	1:1	44.31±1.20	0.14±0.01	0.20±0.01	30.15±2.27	1.43±0.05
	1:1.5	42.11±1.99	0.27±0.01	0.40±0.01	32.82±0.45	1.49±0.01
	1:2	44.07±1.05	0.35±0.02	0.51±0.01	31.37±3.97	1.46±0.06
F14	1:0.25	42.95±1.33	0.19±0.00	0.25±0.01	24.17±1.18	1.32±0.06
	1:0.5	42.43±1.48	0.21±0.00	0.28±0.01	24.75±2.10	1.33±0.05
	1:1	43.65±2.05	0.29±0.00	0.37±0.01	21.63±1.20	1.28±0.03
	1:1.5	40.01±1.33	0.35±0.00	0.46±0.02	23.91±2.79	1.31±0.07
	1:2	39.81±0.59	0.40±0.01	0.51±0.02	23.21±1.41	1.30±0.02
F15	1:0.25	41.86±0.98	0.17±0.01	0.23±0.00	26.63±2.71	1.36±0.05
	1:0.5	45.37±1.61	0.20±0.00	0.28±0.01	28.56±3.17	1.40±0.07
	1:1	44.55±1.45	0.25±0.00	0.36±0.01	30.18±1.76	1.43±0.04
	1:1.5	43.99±1.14	0.33±0.01	0.44±0.02	26.33±3.80	1.36±0.07
	1:2	43.67±0.17	0.36±0.00	0.49±0.03	26.03±3.57	1.35±0.07
F16	1:0.25	42.07±0.75	0.20±0.01	0.26±0.01	23.08±1.75	1.32±0.04
	1:0.5	44.30±0.70	0.18±0.01	0.23±0.01	22.21±1.19	1.29±0.02
	1:1	44.96±1.13	0.26±0.01	0.34±0.02	24.95±3.54	1.33±0.06
	1:1.5	39.27±0.26	0.33±0.01	0.42±0.01	21.43±3.34	1.27±0.04
	1:2	39.80±0.15	0.39±0.00	0.51±0.01	23.87±2.35	1.31±0.04

Table 4.6 Flowability parameters of different indomethacin solid SNEDDSs loaded onto Florite® PS 200.

Liquid SNEDDS	Ratio of carrier: liq. SNEDDS	Angle of repose (θ^o) ±SD	Bulk density (ρ_b) (g/cm^3) ±SD	Tapped density (ρ_t) (g/cm^3) ±SD	Carr's Index (CI%) ±SD	Hausner's Ratio (HR) ±SD
Florite® PS 200	----	33.89±1.78	0.07±0.01	0.07±0.01	7.39±2.29	1.08±0.03
F13	1:0.25	40.44±0.42	0.09±0.00	0.12±0.00	24.05±0.33	1.33±0.00
	1:0.5	45.53±0.51	0.11±0.00	0.13±0.00	20.72±1.95	1.26±0.03
	1:1	49.03±0.34	0.14±0.00	0.18±0.00	20.77±2.04	1.26±0.03
	1:1.5	49.14±0.33	0.17±0.00	0.22±0.01	21.03±1.78	1.27±0.03
	1:2	48.71±0.83	0.21±0.01	0.27±0.01	20.77±2.04	1.26±0.03
F14	1:0.25	43.92±0.35	0.09±0.00	0.12±0.01	24.83±3.07	1.33±0.06
	1:0.5	43.97±0.34	0.09±0.00	0.12±0.00	22.36±2.09	1.29±0.03
	1:1	44.87±0.41	0.13±0.01	0.17±0.01	22.36±2.09	1.29±0.03
	1:1.5	38.35±1.78	0.16±0.00	0.17±0.01	15.54±3.62	1.19±0.05
	1:2	39.65±1.43	0.21±0.01	0.26±0.01	19.44±3.54	1.24±0.05
F15	1:0.25	41.23±0.34	0.10±0.01	0.13±0.02	22.70±7.13	1.30±0.12
	1:0.5	43.98±0.83	0.11±0.00	0.14±0.00	21.43±1.31	1.27±0.02
	1:1	48.87±0.41	0.14±0.00	0.18±0.01	25.57±1.79	1.34±0.03
	1:1.5	48.44±0.40	0.18±0.01	0.23±0.01	23.64±3.48	1.31±0.06
	1:2	48.23±0.67	0.19±0.01	0.24±0.01	19.74±0.44	1.26±0.01
F16	1:0.25	44.58±0.37	0.10±0.01	0.13±0.02	22.36±2.09	1.29±0.03
	1:0.5	43.03±0.49	0.12±0.02	0.15±0.03	23.33±3.47	1.31±0.06
	1:1	44.97±0.34	0.15±0.01	0.20±0.02	23.19±2.40	1.30±0.04
	1:1.5	37.78±1.08	0.18±0.01	0.22±0.00	18.83±3.26	1.23±0.05
	1:2	38.24±0.42	0.19±0.01	0.23±0.01	17.43±1.11	1.21±0.02

Table 4.7 Flowability parameters of different indomethacin solid SNEDDSs loaded onto Syloid® XDP 3150.

Liquid SNEDDS	Ratio of carrier: liq. SNEDDS	Angle of repose (θ^o) ±SD	Bulk density (ρ_b) (g/cm^3) ±SD	Tapped density (ρ_t) (g/cm^3) ±SD	Carr's Index (CI%) ±SD	Hausner's Ratio (HR) ±SD
Syloid® XDP 3150	----	32.70±0.63	0.25±0.01	0.26±0.01	3.80±0.08	1.04±0.00
F13	1:0.25	38.92±0.28	0.30±0.01	0.33±0.01	8.42±2.73	1.09±0.03
	1:0.5	37.47±0.69	0.37±0.01	0.41±0.01	10.00±0.00	1.11±0.00
	1:1	38.01±0.71	0.50±0.01	0.54±0.01	6.84±2.73	1.07±0.03
	1:1.5	40.86±0.87	0.58±0.06	0.69±0.05	16.02±2.89	1.19±0.03
	1:2	42.36±0.53	0.50±0.03	0.61±0.02	18.04±3.03	1.22±0.06
F14	1:0.25	38.71±1.13	0.31±0.00	0.34±0.01	8.33±2.89	1.09±0.03
	1:0.5	39.52±0.82	0.36±0.00	0.40±0.01	8.33±2.89	1.09±0.03
	1:1	37.93±0.32	0.51±0.00	0.54±0.00	5.26±0.00	1.06±0.00
	1:1.5	36.23±0.50	0.63±0.00	0.66±0.02	3.51±3.04	1.04±0.03
	1:2	38.22±0.39	0.45±0.01	0.55±0.02	18.42±4.69	1.23±0.07
F15	1:0.25	38.59±0.34	0.31±0.01	0.34±0.00	8.42±2.73	1.09±0.03
	1:0.5	36.26±0.25	0.36±0.00	0.39±0.01	8.33±2.89	1.09±0.03
	1:1	43.48±0.89	0.49±0.01	0.52±0.00	6.67±2.89	1.07±0.03
	1:1.5	41.51±0.45	0.57±0.03	0.68±0.05	16.18±2.89	1.19±0.03
	1:2	42.00±0.42	0.44±0.03	0.55±0.03	18.92±2.88	1.23±0.04
F16	1:0.25	39.93±0.32	0.30±0.01	0.32±0.02	4.84±0.14	1.05±0.00
	1:0.5	38.92±0.28	0.37±0.00	0.40±0.01	8.33±2.89	1.09±0.03
	1:1	39.08±0.42	0.51±0.01	0.53±0.01	5.18±0.15	1.05±0.00
	1:1.5	37.25±0.31	0.61±0.00	0.65±0.00	5.00±0.00	1.05±0.00
	1:2	39.30±0.31	0.48±0.01	0.58±0.02	17.93±3.58	1.22±0.05

From the results presented in **Table 4.3**, it appears that the addition of microcrystalline cellulose (MCC) or Avicel PH 102 to all liquid indomethacin-loaded SNEDDS formulations (F13 – F16) in the ratios of 1: 0.25, 1: 0.5, and 1: 1 produced mixtures with poor flowability as indicated by the values obtained for angle of repose (46.02 ± 0.30 to 49.53 ± 0.51). The values of CI% (16.35 ± 2.34 to 25.79 ± 2.48) and HR (1.26 ± 0.03 to 1.34 ± 0.04) for these mixtures indicated passable flow which means that these formulation may hang up during filling into capsules or upon further processing into compressed tablets. Further incorporation of higher amounts of the liquid SNEDDS formulations (adsorbent: liquid formulation ratios of 1: 1.5 and 1: 2) resulted in very greasy mixtures for which flowability parameters could not be determined. This may be attributed to reduced porosity of the carrier upon addition of further amounts of the liquid formulation. Lower amounts of drug-loaded liquid SNEDDS formulation may adsorb onto the rough and porous surface of the particles of MCC to produce smoother and less porous surface, while higher amounts of the liquid SNEDDSs may not associate with the solid adsorbent due to reduced porosity of the powder formulation (Abdalla et al., 2008). Similar findings have been reported for incorporation of a self-emulsifying mixture of progesterone onto MCC or Avicel PH 101 to produce pellets (Abdalla et al., 2008), where higher concentrations of the liquid formula incorporated within the cellulose fibers network of MCC as revealed by environmental scanning electron microscopy (ESEM).
Due to the poor flow properties of indomethacin-loaded solid SNEDDS formulated by adsorption of liquid SNEDDS onto MCC and because of difficulty to incorporate higher amounts of the liquid formulation, and hence higher content of the drug, these formulations were excluded from further evaluation studies.

The flowability parameters obtained for drug-loaded solid SNEDDS formulations prepared by adsorption of liquid SNEDDS formulations (F13 – F16) onto Aerosil® 200 are shown in **Table 4.4**. All solid SNEDDS formulations produced by adsorption onto Aerosil® 200 at the adsorption ratios of 1: 0.25, 1: 0.5 and 1: 1 exhibited fair to poor flow properties according to the estimated values of angle of repose (38.92 ± 0.28 to 46.62 ± 0.55). Incorporation of the liquid SNEDDS formulations onto Aerosil® 200 at higher adsorbent: liquid formulation ratios (1: 1.5 and 1: 2) resulted in solid SNEDDS products that were difficult to manipulate and showed higher values of CI% (20.85 ± 2.33 to 36.59 ± 0.31) and HR (1.26 ± 0.04 to 1.58 ± 0.01). Difficult handling of solid formulations prepared using Aerosil® 200 may be attributed to the fluffy nature of this carrier due to the inter-particulate voids present in its structure (Beg et al., 2016). Because of the poor flow behaviour of formulations prepared at low concentrations in addition to difficulty of

handling of solid mixtures prepared at higher ratios, these solid mixtures were excluded from further studies.

The results of determination of flow parameters of solid SNEDDS formulations produced using Neusilin® US2 as an adsorbent for indomethacin liquid SNEDDS formulations (F13 – F16) are summarized in **Table 4.5**. The estimated values of angle of repose (39.27 ± 0.26 to 44.96 ± 1.13) indicated that all indomethacin-loaded solid SNEDDS formulations prepared at different carrier: liquid SNEDDS ratios exhibited fair to passable flow. On the other hand, the determined values of CI% (21.43 ± 3.34 to 32.82 ± 0.45) and HR (1.27 ± 0.04 to 1.49 ± 0.01) for these solid SNEDDS formulation indicated a flow behavior ranging from passable to very poor flow. It was also noticed that addition of Neusilin® US2 to liquid SNEDDS formulations (F13 or F15) resulted in solid mixtures with high values of CI% and HR and hence, poor to very poor flow characteristics. Higher oil content of liquid SNEDDS formulations: F13 and F15 (as presented in **Table 4.1**) may contribute to production of greasy solid mixtures upon their addition to Neusilin® US2. These greasy solid mixtures may not fill properly into capsules and may not be easily processed into tablet dosage forms. On the other hand, solid mixtures produced by adsorption of liquid SNEDDS formulations (F14 or F16) onto Neusilin® US2 exhibited passable flow as indicated by their CI% (21.43 ± 3.34 to 24.95 ± 3.54) and HR (1.27 ± 0.04 to 1.33 ± 0.06) values. Acceptable flow potential of solid mixtures produced using Neusilin® US2 may be due to high adsorption capacity of this carrier (2.7 to 3.4 ml/g) in addition to its larger surface area (≈300 m^2/g) (Tan et al., 2013). Thus, drug-loaded solid SNEDDS formulations prepared using Neusilin® US2 and liquid SNEDDS (F14 & F16) at the ratios of 1: 1.5 and 1: 2 were selected for further investigation because of their free flowing characteristics and higher content of the drug.

Flow parameters of solid SNEDDS formulations prepared using Florite® PS-200 (calcium silicate) as a carrier for loading indomethacin liquid SNEDDS formulations (F13 – F16) are presented in **Table 4.6**. It can be noticed that incorporation of liquid SNEDDS formulations, F13 or F15, onto Florite® PS-200 resulted in solid SNEDDS formulations with flow potential ranging between passable and poor flow characteristics as designated by angle of repose (40.44 ± 0.42 to 49.14 ± 0.33), CI% (19.74 ± 0.44 to 25.57 ± 1.79) and HR (1.26 ± 0.01 to 1.34 ± 0.03). These poor flow properties could be due to higher oil content of liquid SNEDDS formulations F13 & F15 **(Table 4.1)** that may lead to formation of excessively greasy solid mixtures that may be difficult to manipulate during further conversion into capsules or tablets. However, incorporation of liquid SNEDDS formulations F14 or F16 onto Florite® PS-200 produced solid SNEDDS formulation with improved flow properties as shown by the values obtained for angle of repose (37.78 ±

1.08 to 44.97 ± 0.34), CI% (15.54 ± 3.62 to 24.83 ± 3.07) and HR (1.19 ± 0.05 to 1.33 ± 0.06). Addition of higher amounts of liquid formulations F13 and F14 to the carrier (in adsorbent: liquid SNEDDS ratios of 1: 1.5 and 1: 2) resulted in solid mixtures with much improved flow characteristics. This may be due to the deep and large macropores of Florite® PS-200 that may provide high oil adsorption capacity (3.7 ml/g) (Tan et al., 2013). Therefore, indomethacin-loaded solid SNEDDS formulations prepared with Florite® PS-200 and liquid SNEDDS (F14 & F16) at higher ratios (1: 1.5 and 1: 2) were chosen for further evaluation because of their free flowing properties and higher content of the drug.

The flowability parameters obtained for drug-loaded solid SNEDDS formulations prepared by adsorption of liquid SNEDDS formulations (F13 – F16) onto Syloid® XDP 3150 (mesoporous or amorphous silicon dioxide) are shown in **Table 4.7**. It was observed that addition of liquid SNEDDS formulations of indomethacin (F13 – F16) to Syloid® XDP 3150 resulted in solid SNEDDS formulations with acceptable flow properties (angle of repose: 36.23 ± 0.50 to 43.48 ± 0.89; CI%: 3.51 ± 3.04 to 18.92 ± 2.88; HR: 1.05 ± 0.00 to 1.23 ± 0.07). This could be due to the characteristic network of meso-pores of this grade of Syloid® excipient which provides high oil adsorption capacity (≈3.8 ml/g) for this carrier (Grace GmbH, 2012). Also, better flow properties were noticed for solid SNEDDS formulations produced upon incorporation of F14 or F16 onto Syloid® XDP 3150 at adsorbent: liquid SNEDDS ratios of 1: 1.5 and 1: 2. Therefore, these solid formulations were selected for additional investigation.

Overall, different flow properties of various solid SNEDDS formulations produced by adsorption onto different solid carriers could be due to differences in physicochemical properties as well as oil adsorption capacity of these materials. Inadequate flow characteristics observed for indomethacin-loaded solid SNEDDSs developed using MCC or Aerosil® 200; and confirmed by estimated values of angle of repose, CI% or HR; suggested that these groups of formulations may not be suitable for further processing into solid dosage forms like capsules or tablets. Hence, these formulations were excluded from further analysis or evaluation tests.

On the other hand, indomethacin-loaded solid SNEDDSs developed using Neusilin® US2, Florite® PS-200 or Syloid® XDP 3150 as solid carriers showed better flow properties as indicated by the determined values of different flowability parameters. Specifically, the use of these adsorbents for loading liquid formulations F14 & F16 at adsorbent: liquid SNEDDS ratios of 1: 1.5 and 1: 2 resulted in solid mixtures with optimum flow characteristics. These free flowing solid SNEDDS formulations of indomethacin prepared with higher amounts of liquid SNEDDS formulations will

consequently contain higher amounts of the drug and therefore, an amount equivalent to the pharmacological dose of the drug can be weighed from theses formulations and filled into capsules for additional analysis. Thus, these optimum 12 indomethacin-loaded solid SNEDDS formulations prepared from F14 & F16 (highlighted in red in **Tables 4.5 – 4.7**) that showed the smallest droplet size of nanoemulsion among other optimized liquid SNEDDS formulations (F13 & F15) were selected to be subjected for further characterization to investigate the effect of solidification method on different properties of the produced solid SNEDDS formulations such as self-emulsifying properties, droplet size, dissolution characteristics, in addition to identification of crystalline or amorphous state of the drug.

New formulation codes were given for the selected optimum indomethacin-loaded solid SNEDDS formulations. These formulation codes, in addition to formulation composition, are shown in **Table 4.8** and will be used in the following sections of this chapter to designate different solid SNEDDS formulations of indomethacin prepared from the corresponding carrier and liquid indomethacin-loaded SNEDDS formulations.

Table 4.8 Codes and composition of optimum indomethacin-loaded solid SNEDDS formulations

Code	Adsorbent used	Liquid Indomethacin loaded-SNEDDS	Ratio of (adsorbent: liquid SNEDDS)
S1	Syloid® XDP 3150	F14	1:1.5
S2			1:2
S3		F16	1:1.5
S4			1:2
N1	Neusilin® US2	F14	1:1.5
N2			1:2
N3		F16	1:1.5
N4			1:2
R1	Florite® PS-200	F14	1:1.5
R2			1:2
R3		F16	1:1.5
R4			1:2

4.4.4. Drug content of different indomethacin-loaded solid SNEDDS formulations

The results of drug content of different indomethacin-loaded solid SNEDDS formulations are summarized in **Table 4.9**. It was observed that all solid SNEDDS formulations prepared using any of the three solid adsorbents (Syloid® XDP 3150, Neusilin® US2 or Florite® PS-200) revealed values ranging from 94.79% ± 2.23% to 98.84% ± 1.12% calculated as % of theoretical amount added. According to the U.S. FDA (2003) on 'blend uniformity', a given batch of powder may pass drug content uniformity test if the assay of 60 samples or more from that batch showed a relative standard deviation (RSD) ≤ 4%. Although analysis of large number of samples was difficult and not possible with lab scale experiments, results of drug content obtained for various powder formulations (**Table 4.9**) with acceptable values of RSD <3% may indicate that the method adopted in this work is capable of producing solid SNEDDS formulations with reasonable values of content uniformity. Less than 100% drug content values obtained for the tested solid SNEDDS formulations may possibly be attributed to adherence (or sticking) of liquid SNEDDS formulation to the sides of the mortar, and hence loss of drug, during mixing and preparation of different formulations. Similar findings and interpretation were given by Gumaste et al. (2013a) in the evaluation of drug content of various powder formulations prepared by adsorption of liquid SEDDS of probucol onto Neusilin® US2 at 1:1 w/w ratio.

Table 4.9 Results of drug content (Mean ± SD) of different indomethacin-loaded solid SNEDDS formulations

Code	Drug content (Mean ± SD)		RSD (%)
	mg / g	% of theoretical	
S1	14.26 ± 0.39	95.06 ± 2.59	2.73
S2	16.11 ± 0.17	96.65 ± 1.05	1.09
S3	14.32 ± 0.40	95.47 ± 2.65	2.78
S4	15.91 ± 0.32	95.47 ± 1.89	1.98
N1	14.22 ± 0.33	94.79 ± 2.23	2.35
N2	16.10 ± 0.42	96.61 ± 2.53	2.62
N3	14.56 ± 0.18	97.06 ± 1.22	1.26
N4	15.87 ± 0.42	95.20 ± 2.54	2.67
R1	14.83 ± 0.17	98.84 ± 1.12	1.13
R2	16.14 ± 0.43	96.80 ± 2.59	2.68
R3	14.24 ± 0.42	94.93 ± 2.81	2.96
R4	16.07 ± 0.37	96.42 ± 2.23	2.31

4.4.5. Redispersibility of solid SNEDDS formulations (Droplet size measurement)

The redispersibility of the formulated indomethacin-loaded solid SNEDDS was evaluated by reconstituting the powder formulation in deionized water followed by measuring the droplet size, polydispersity index and zeta potential of the resulting nanoemulsions and then comparing the values obtained to those recorded previously for liquid SNEDDS formulations, from which powder formulations were generated. The droplet size is an important factor in immediate self-emulsification performance because it provides information on the rate and extent of in vitro drug dissolution as well as the in vivo absorption. Smaller droplet size allows faster dissolution rate and provides larger interfacial surface area for in vivo drug absorption (Weerapol et al., 2015a).

The results of measurement of droplet size, polydispersity index and zeta potential obtained for solid SNEDDS formulations prepared with Syloid® XDP 3150 (S1 – S4), Neusilin® US2 (N1 – N4) or Florite® PS-200 (R1 – R4) as well as those obtained for optimized liquid SNEDDS formulations (F14 & F16) are presented in **Table 4.10**. Obtained results confirmed insignificant difference ($p > 0.05$) in the mean droplet size of nanoemulsions generated from all solid SNEDDS prepared using the three silicate-

based adsorbents. Also, results showed that the mean droplet size of nanoemulsions produced from dispersed solid SNEDDS formulations (18.49 ± 1.15 to 24.24 ± 0.18 nm) were not significantly different ($p > 0.05$) from the mean droplet size obtained for liquid formulations; F14 (20.68 ± 0.03 nm) and F16 (22.69 ± 0.06 nm).

In addition, low PDI values (0.12 ± 0.004 to 0.31 ± 0.02) were observed for all indomethacin-loaded solid SNEDDS formulations which indicate uniform size distribution of nanoemulsions generated from tested powder formulations. This could be due to the optimized properties of liquid SNEDDS (F14 & F16), shown previously in **Chapter 3,** such as high surfactant content (46.7% – 52.5%), low oil content (30%) in addition to high polarity of their oil component; Capryol™ 90 (C_8, HLB = 6) (Elkadi et al., 2017).

The values of zeta potential of the formulated indomethacin-loaded solid SNEDDS are also presented in **Table 4.10**. The zeta potential values observed for all solid SNEDDS formulations were comparable to the reasonable zeta potential values noticed for the original liquid SNEDDS formulations. High negative values of zeta potential observed for all solid formulations indicate that nanoemulsions produced from tested formulations are highly stable and may less likely flocculate in liquid medium or in GI fluids (Bali et al., 2011).

The results of redispersibility indicate that the self-emulsification ability of liquid SNEDDS formulations was preserved and maintained even after solidification by adsorption onto suitable solid carriers. Similar findings were reported for self-nanoemulsifying powders of isradipine (Ramasahayam et al., 2015), solid self-microemulsifying dispersible tablets of celastrol (Qi et al., 2014) and oral self-emulsifying powder of lercanidipine hydrochloride (Kallakunta et al., 2012).

Table 4.10 Mean droplet size, PDI and zeta potential of formulated indomethacin-loaded solid SNEDDS compared to optimized liquid SNEDDS formulations.

Code	Mean droplet diameter (nm) ± SD	Mean PDI ± SD	Zeta potential (mV)	Zeta deviation (mV)
F14*	20.68 ± 0.03	0.11 ± 0.020	-25.2	8.31
F16*	22.69 ± 0.06	0.13 ± 0.004	-16.2	9.95
S1	23.90 ± 0.06	0.24 ± 0.001	-20.6	7.31
S2	24.24 ± 0.18	0.27 ± 0.003	-16.5	3.78
S3	20.08 ± 0.66	0.26 ± 0.020	-28.0	4.19
S4	21.33 ± 1.35	0.23 ± 0.030	-21.7	5.35
N1	22.06 ± 0.16	0.14 ± 0.010	-22.9	4.27
N2	22.38 ± 0.02	0.30 ± 0.020	-21.0	6.35
N3	20.62 ± 0.12	0.23 ± 0.002	-21.5	5.27
N4	19.23 ± 0.49	0.22 ± 0.020	-18.0	6.02
R1	21.80 ± 0.07	0.12 ± 0.004	-23.1	4.13
R2	20.96 ± 1.44	0.31 ± 0.020	-24.2	5.77
R3	18.49 ± 1.15	0.26 ± 0.050	-24.8	5.60
R4	20.41 ± 0.76	0.31 ± 0.010	-23.4	5.23

* Optimized indomethacin–loaded liquid SNEDDS formulations.

4.4.6. Solid state characterization of indomethacin-loaded solid SNEDDS formulations

4.4.6.1. FTIR spectroscopy

FTIR studies were performed to ascertain if there was any incompatibility and/or interaction between the drug and the adsorbents used in preparation of indomethacin-loaded SNEDDS formulations. FTIR is used for this purpose because each compound absorbs specific radiation frequencies according to its molecular structure. In an FTIR spectrum, the two most important regions to be examined are located in the ranges between 4000 – 1300 and 900 – 650 cm^{-1}. The intermediate region of the spectrum that falls in the range of 1300 – 900 cm^{-1} is known as the 'fingerprint' region (Dupeyrón et al., 2013). Mixing of a drug with an excipient at the molecular level may cause changes in FTIR spectrum of the formulation comprising the drug and the excipient. These changes can be observed in the form of disappearance, shifting or broadening of the characteristic peaks of the drug and the excipient used (Lim et al., 2013).

FTIR spectra of indomethacin, different drug-loaded solid SNEDDS formulations and the corresponding carrier used in various formulations are shown in **Figures 4.2, 4.3 and 4.4**. The resulting FTIR spectrum of pure indomethacin showed specific peaks for the γ-polymorph of indomethacin (Dupeyrón et al., 2013). Two strong peaks were observed in drug FTIR spectrum with the peak appearing at 1716 cm^{-1} related to asymmetric acid C=O of a cyclic dimer, while the peak showing at 1692 cm^{-1} related to the benzoyl C=O (Taylor and Zografi, 1997). Also, other absorption peaks were recorded at 2925 cm^{-1} (C-H stretching vibrations), 1223 cm^{-1} (asymmetric aromatic O-C stretching), and 1068 cm^{-1} (symmetric aromatic O-H stretching). The absorption patterns of indomethacin in the region of 1300 – 650 cm^{-1} are complex because of the aromatic structure of this compound. Therefore, strong or medium intensity bands appearing in this region may be non-specific and less useful for structural characterization, while a weak intensity band in the region above 2000 cm^{-1} may be group-specific (Dupeyrón et al., 2013).

Syloid® XDP 3150 (amorphous silicon dioxide) showed principal peaks at 3346, 1630, 1086, 799 and 458 cm^{-1} (**Figure 4.2**). The FTIR spectrum of all solid SNEDDS formulations prepared with this carrier (S1 – S4) exhibited all characteristic peaks of Syloid® XDP 3150 at the same positions in addition to characteristic drug peaks at 2925, 1716, 1223 and 1068 cm^{-1}. Presence of specific drug peaks in the spectra of different solid SNEDDS formulations indicates that the molecular structure of indomethacin remained intact. Absence of some drug peaks in the spectra of these solid SNEDDS formulations may be because these peaks are more likely to be hidden in the baseline of the corresponding spectra. In addition, no chemical interaction occurred between the drug and the carrier in drug-loaded solid SNEDDS formulations (S1 – S4) because no new extra peaks were observed in their FTIR spectra. Similarly, solid SEDDS of glipizide prepared with the adsorbent; Syloid® 244 FP which is similar to Syloid® XDP 3150 used in this study; did not exhibit chemical interaction upon investigation of the corresponding FTIR spectra (Agrawal et al., 2015).

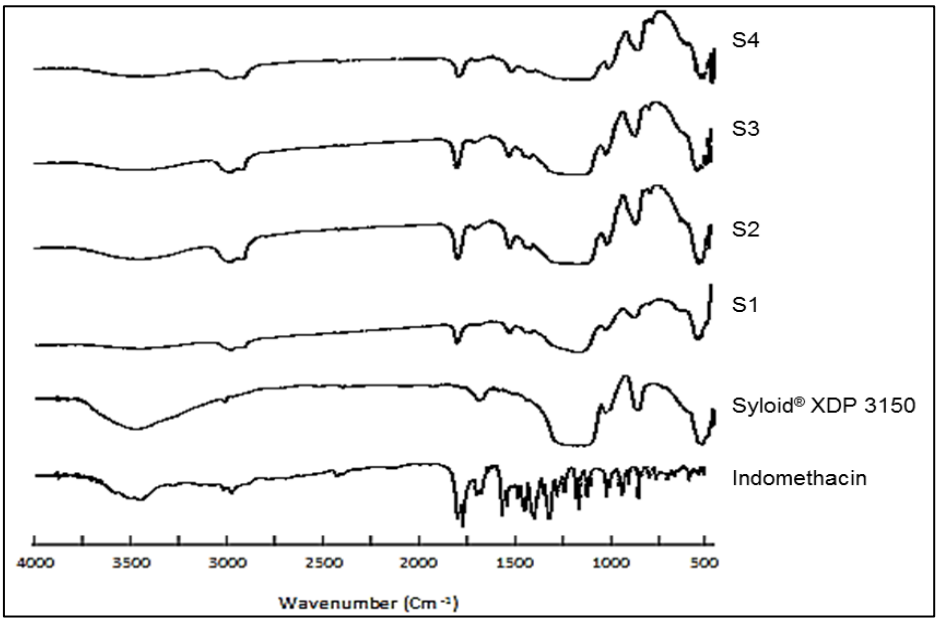


Figure 4.2 FTIR spectra of indomethacin, Syloid® XDP3150 and various drug-loaded solid SNEDDS formulations.

The FTIR spectrum of Neusilin® US2 (magnesium aluminometa silicate) shown in **Figure 4.3** is characterized by the absorption peaks at 3444, 1638, 1380, 1041 and 472 cm^{-1}. All solid SNEDDS formulations prepared using Neusilin® US2 (N1 – N4) showed the absorption peaks of the carrier at the same position as well as the characteristics peaks of the drug mentioned earlier. No chemical interaction was detected within these solid SNEDDS formulations as evidenced by absence of new extra peaks in their corresponding spectra. Similar FTIR findings were reported for solid SNEDDS formulated with Neusilin® US2 such as isradipine-loaded self-nanoemulsifying powders (Ramasahayam et al., 2015) and Valsartan-loaded solid self-nanoemulsifying granules (Beg et al., 2012).

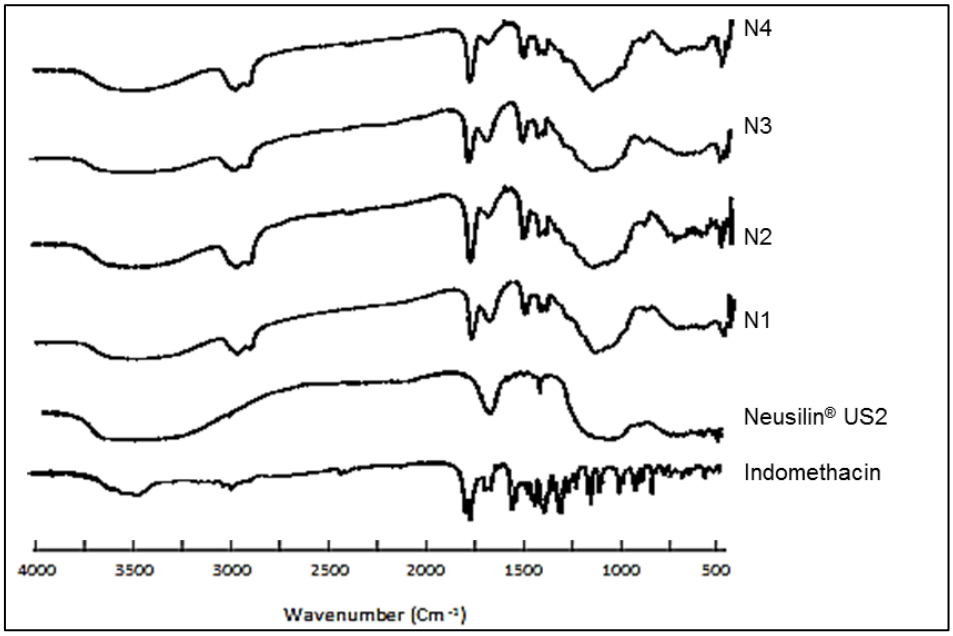


Figure 4.3 FTIR spectra of indomethacin, Neusilin® US2 and various drug-loaded solid SNEDDS formulations.

The FTIR spectrum of Florite® PS-200 (calcium silicate) presented in **Figure 4.4** showed absorption peaks at 3448, 1637, 1348, 1039, 784, 607 and 467 cm^{-1}. All absorption peaks of Florite® PS-200 were observed at the same position in FTIR spectra of solid SNEDDS formulations prepared using this carrier (R1 – R4). Also, the characteristics peaks of the drug mentioned before were present in the spectra of Florite® PS-200-based formulations. In addition, absence of new peaks in these spectra indicates that no chemical interaction occurred between the drug and Florite® PS-200 in the prepared solid SNEDDS formulations.

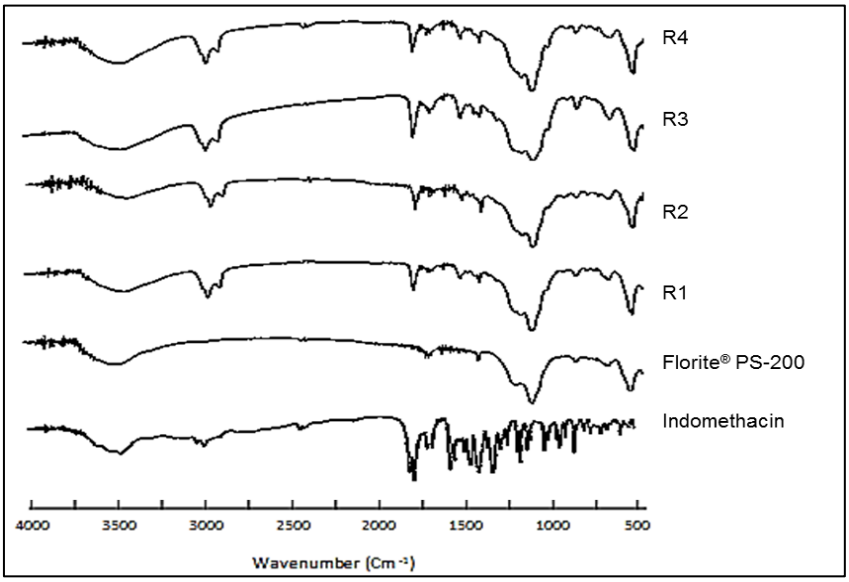


Figure 4.4 FTIR spectra of indomethacin, Florite® PS-200 and various drug-loaded solid SNEDDS formulations.

4.4.6.2. Differential scanning calorimetry (DSC) studies

DSC studies allow determination of physical state, thermotropic phase transition and thermal behaviour of drug within the formulation (Kallakunta et al., 2012, Ramasahayam et al., 2015). DSC traces of different indomethacin-loaded solid SNEDDS powder formulations prepared by adsorption of optimized drug-loaded liquid SNEDSs onto different solid adsorbents are shown in **Figures 4.5, 4.6 and 4.7**.

The DSC trace of pure indomethacin reflects the presence of the drug in the crystalline state with a sharp endothermic peak at 162.31^{o}C which corresponds to its melting point, with ΔH (enthalpy) of 106.2950 J/g. In **Figure 4.5,** it appears that Syloid® XDP 3150 (amorphous silicon dioxide) showed no thermal transitions over the entire range of temperature examined. Also, no representative peaks of indomethacin were observed in the DSC traces of drug-loaded solid SNEDDS prepared with Syloid® XDP 3150 (S1 – S4).

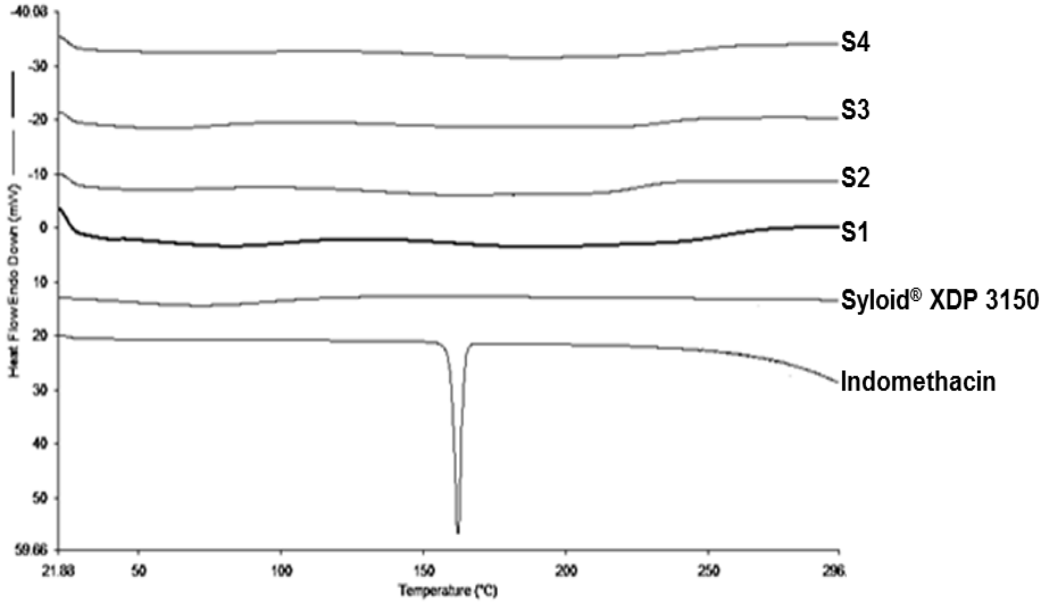


Figure 4.5 DSC traces of indomethacin, Syloid® XDP 3150 and various drug-loaded solid SNEDDS formulations.

The broad endothermic peak of Neusilin® US2 (magnesium aluminometa silicate) observed at 228.61°C (**Figure 4.6)** indicates the amorphous nature of this solid carrier. No representative peaks of crystalline indomethacin were detected for drug-loaded solid SNEDDS prepared with Neusilin® US2 (N1 –N4).

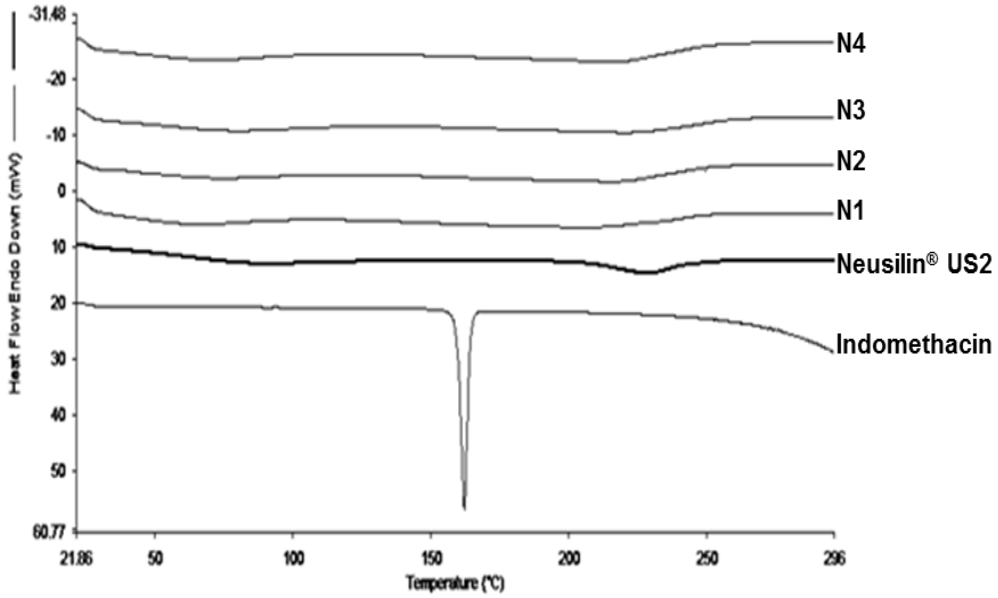


Figure 4.6 DSC traces of indomethacin, Neusilin® US2 and various drug-loaded solid SNEDDS formulations.

In **Figure 4.7**, the DSC trace of the solid carrier; Florite® PS-200 (calcium silicate); did not present any prominent peak over the entire range of the tested temperature. Also, no obvious endothermic peak relating to the melting of crystalline indomethacin was noticed in all drug-loaded solid SNEDDS powder formulations prepared with Florite® PS-200 (R1 – R4).

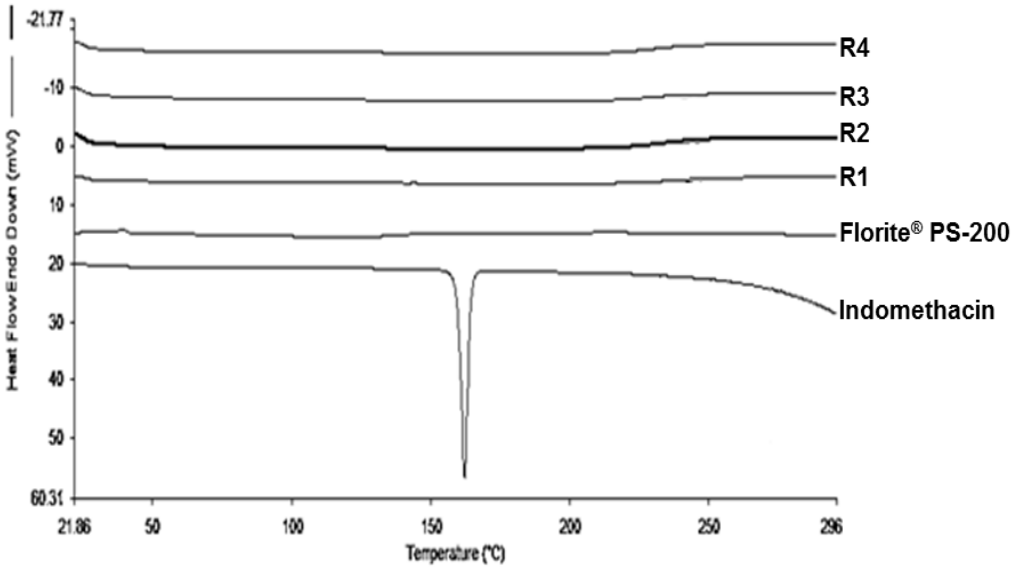


Figure 4.7 DSC traces of indomethacin, Florite® PS-200 and various drug-loaded solid SNEDDS formulations.

Overall, it can be anticipated that absence of a characteristic endothermic peak corresponding to the melting of crystalline indomethacin in all drug-loaded solid SNEDDS formulations prepared with the employed solid carriers; Syloid® XDP 3150, Neusilin® US2 and Florite® PS-200; indicates that the drug remained in a molecularly dissolved state within the self-nanoemulsifying powder formulations. Previous reports suggested that the use of similar grades of the above carriers to formulate different solid SEDDS maintained the drug in a dissolved state within differently formulated solid SEDDS. For example, utilization of Syloid® 244FP (porous silicon dioxide) (Agrawal et al., 2015, Weerapol et al., 2015a), Neusilin® US2 (magnesium aluminometasilicate) (Inugala et al., 2015, Kallakunta et al., 2012, Parmar et al., 2015, Ramasahayam et al., 2015), or Florite® RE (porous calcium silicate) (Weerapol et al., 2015a, Weerapol et al., 2015b) to formulate solid SEDDS produced formulations that did not show any crystalline peak of the corresponding drugs as confirmed by the DSC studies.

4.4.6.3. Powder X-ray diffraction (XRD) studies

XRD analysis is a technique that provides information on the degree of crystallinity and amorphous content of pharmaceutical formulations. It is also useful for quantitative analysis of pharmaceutical mixtures to determine exact percentage of components of a formulation (Gilmore, 2011). In this study, qualitative XRD studies were carried out in order to evaluate the crystallinity of the drug within the formulated products. Different XRD patterns obtained for different indomethacin-loaded solid SNEDDS powder formulations prepared with different solid carriers are depicted in **Figures 4.8, 4.9 and 4.10**. For the purpose of comparison, XRD patterns of indomethacin and carriers employed in different formulations were also obtained.

Indomethacin diffractogram showed characteristics narrow and sharp diffraction peaks at 2θ values of 11.9^{o}, 13^{o}, 17.2^{o}, 19.9^{o}, 20.6^{o}, 21.2^{o}, 22.1^{o}, 23.4^{o}, 24.3^{o}, 26.9^{o}, 29.7^{o} and 31.9^{o} which correspond to the crystalline nature of the drug. The obtained diffractogram of indomethacin is similar to those reported in previous studies (Dupeyrón et al., 2013, Lim et al., 2013).

The XRD pattern of Syloid® XDP 3150 (amorphous silicon dioxide) which is presented in **Figure 4.8**, showed no sharp intrinsic peaks. Instead, a halo peak appears centred on 2θ value of 21° which indicates the amorphous nature of this carrier. Also, it was noticed that the XRD patterns of all solid SNEDDS formulations prepared with Syloid® XDP 3150 (S1 – S4) showed an amorphous halo at 2θ values of 21° as observed for the amorphous carrier. In addition, absence of distinctive crystalline peaks of indomethacin in the diffrctograms of solid SNEDDS (S1 – S4) indicates that the drug remained in a molecularly dissolved state within solid powder formulations prepared with Syloid® XDP 3150. Similar findings were reported for solid-SEDDS of glipizide formulated with porous silicon dioxide (Syloid® 244FP) as an adsorbent. The XRD patterns of glipizide-loaded solid SEDDS revealed the absence of crystalline peak of the drug which confirmed the presence of the drug in a solubilized state within the solid SEDDS formulation (Agrawal et al., 2015).

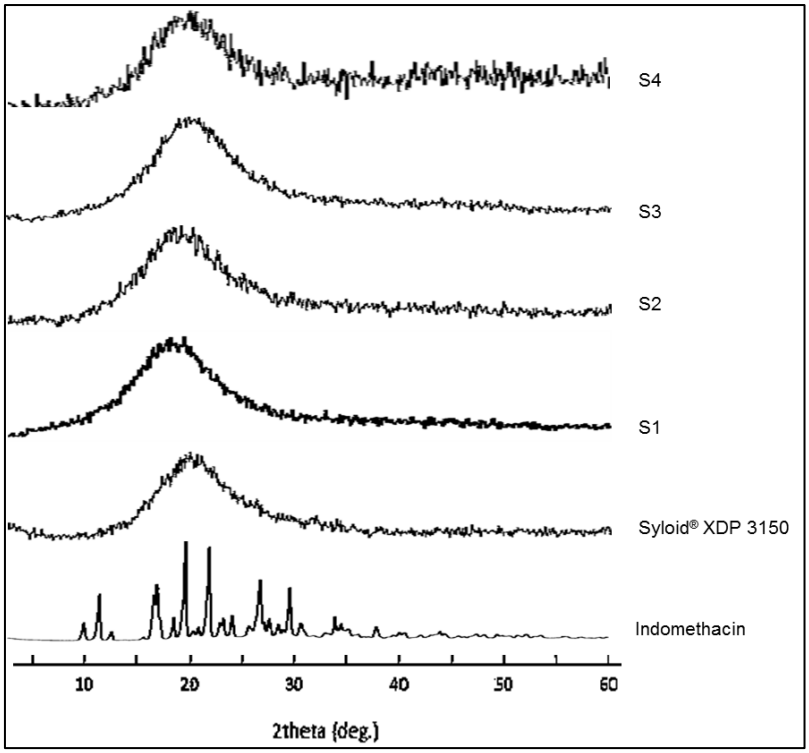


Figure 4.8 XRD diffractograms of indomethacin, Syloid® XDP 3150 and various drug-loaded solid SNEDDS formulations.

The X-ray diffractograms of Neusilin® US2 (magnesium aluminometa silicate) and the solid SNEDDS formulated with this adsorbent are presented in **Figure 4.9**. Neusilin® US2 showed no high intensity peaks but two halo peaks were noticed centred on 2θ values of 20.6° and 35°. No obvious peaks of crystalline indomethacin were observed for all solid SNEDDS formulations prepared with Neusilin® US2 (N1 – N4). This may be because the drug remained in a dissolved state within the self-emulsifying liquid formulation even after adsorption onto Neusilin® US2. Similar observations were reported for self-nanoemulsifying powder of isradipine (Ramasahayam et al., 2015) and self-emulsifying powder of lercanidipine hydrochloride (Kallakunta et al., 2012) prepared using Neusilin® US2 as adsorbent. Investigation of the XRD patterns of these two self-emulsifying powder formulations revealed absence of characteristic drug peaks which was attributed to the fact that the drug remained in a molecularly dissolved state upon adsorption of the liquid self-nanoemulsifying formulations onto Neusilin® US2 (Kallakunta et al., 2012, Ramasahayam et al., 2015).

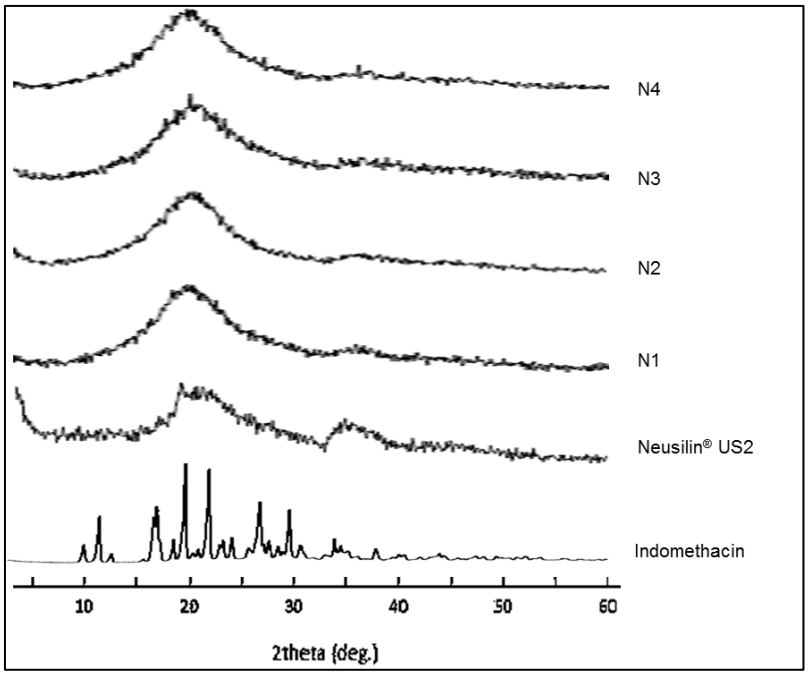


Figure 4.9 XRD diffractograms of indomethacin, Neusilin® US2 and various drug-loaded solid SNEDDS formulations.

Figure 4.10 shows the X-ray diffractograms of Florite® PS-200 (calcium silicate) and indomethacin-loaded solid SNEDDS prepared with this carrier. Florite® PS-200 diffractogram showed sharp diffraction peaks at 2θ values of 5.6°, 10.6°, 21.2°, 28.5°, 38.1°, 44.3° and 50° which correspond to partial crystalline nature of this carrier. Diffraction peaks of Florite® PS-200 were obvious in XRD diffractograms of all drug-loaded solid SNEDDS formulated with this adsorbent (R1 – R4). Again, absence of sharp crystalline peaks of the drug in diffractograms of Florite® - based solid powder formulations indicates that the drug remained molecularly solubilized within the self-emulsifying liquid formulation after solidification process. Similarly, absence of crystalline peaks of the drug was observed in XRD patterns of self-emulsifying powder of nifidipine formulated using a similar grade of porous calcium silicate (Florite® RE) (Weerapol et al., 2015a).

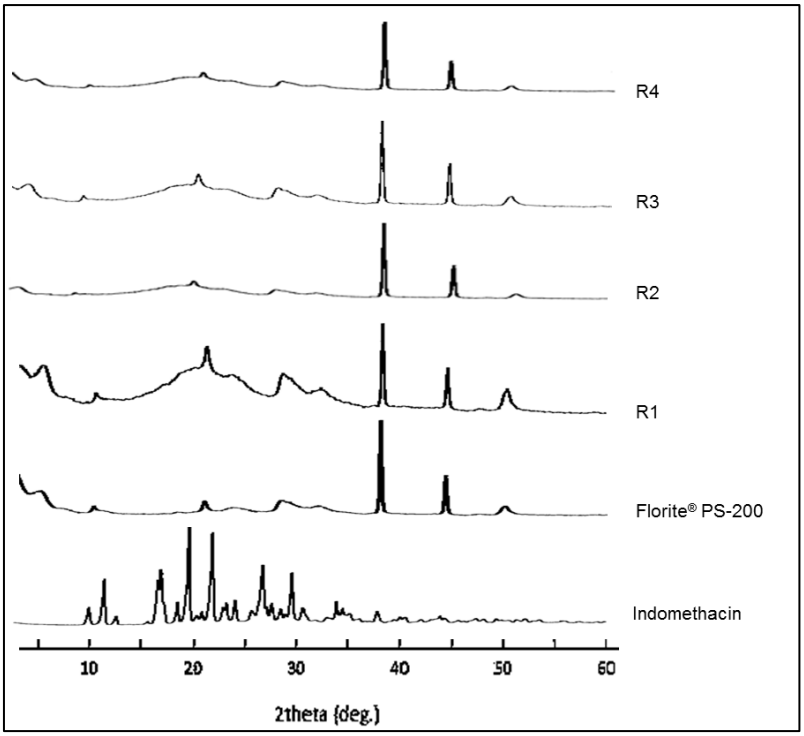


Figure 4.10 XRD diffractograms of indomethacin, Florite® PS-200 and various drug-loaded solid SNEDDS formulations.

4.4.6.4. Scanning electron microscopy (SEM) studies

Surface morphology of pure indomethacin powder, Syloid® XDP 3150, Neusilin® US2, Florite® PS-200 and their corresponding solid SNEDDS powder formulations was studied using scanning electron microscopy. The micrographs obtained are presented in **Figures 4.11, 4.12 and 4.13**.

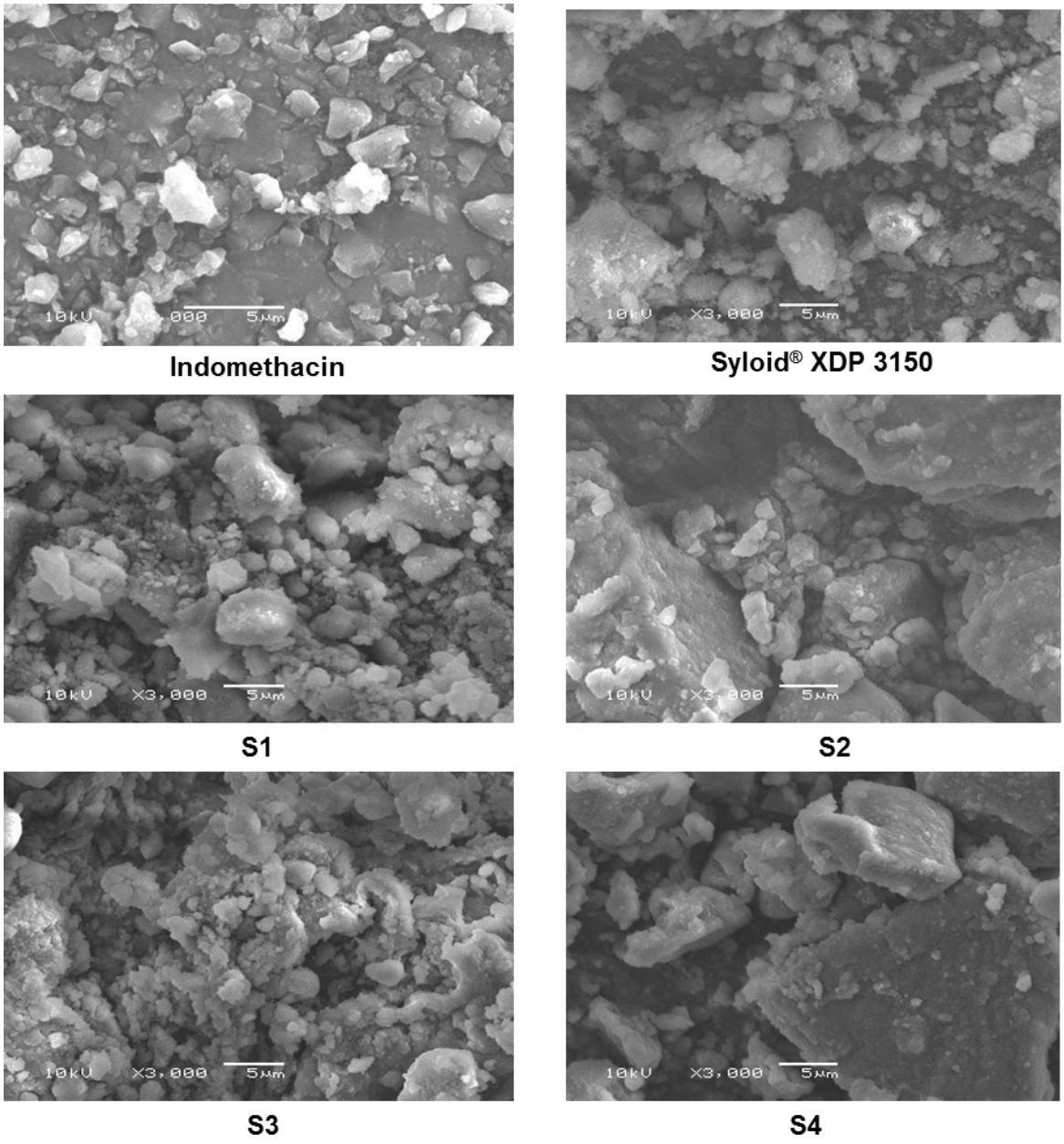


Figure 4.11 SEM micrographs of indomethacin, Syloid® XDP 3150 and various drug-loaded solid SNEDDS formulations.

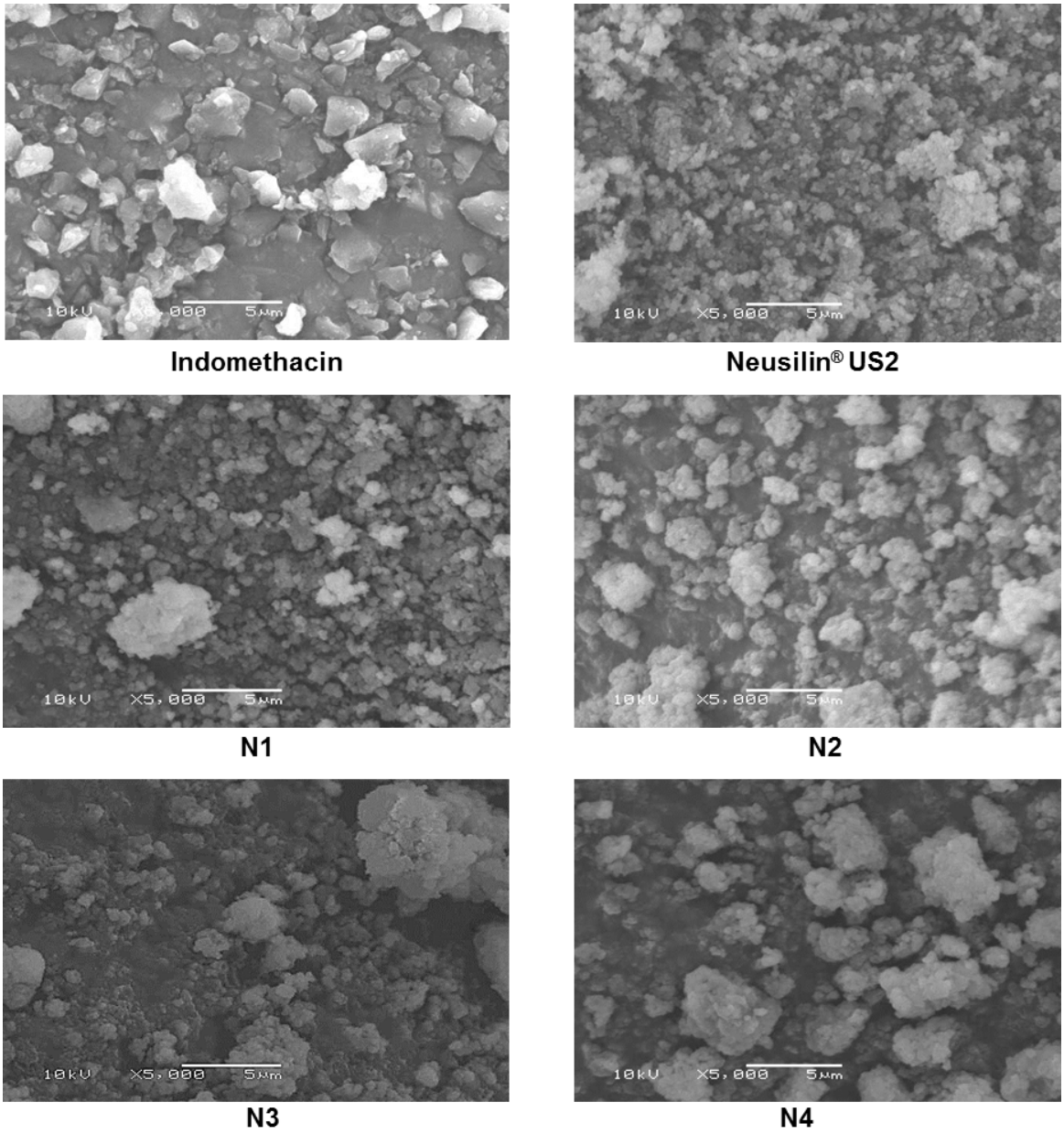


Figure 4.12 SEM micrographs of indomethacin, Neusilin® US2 and various drug-loaded solid SNEDDS formulations.

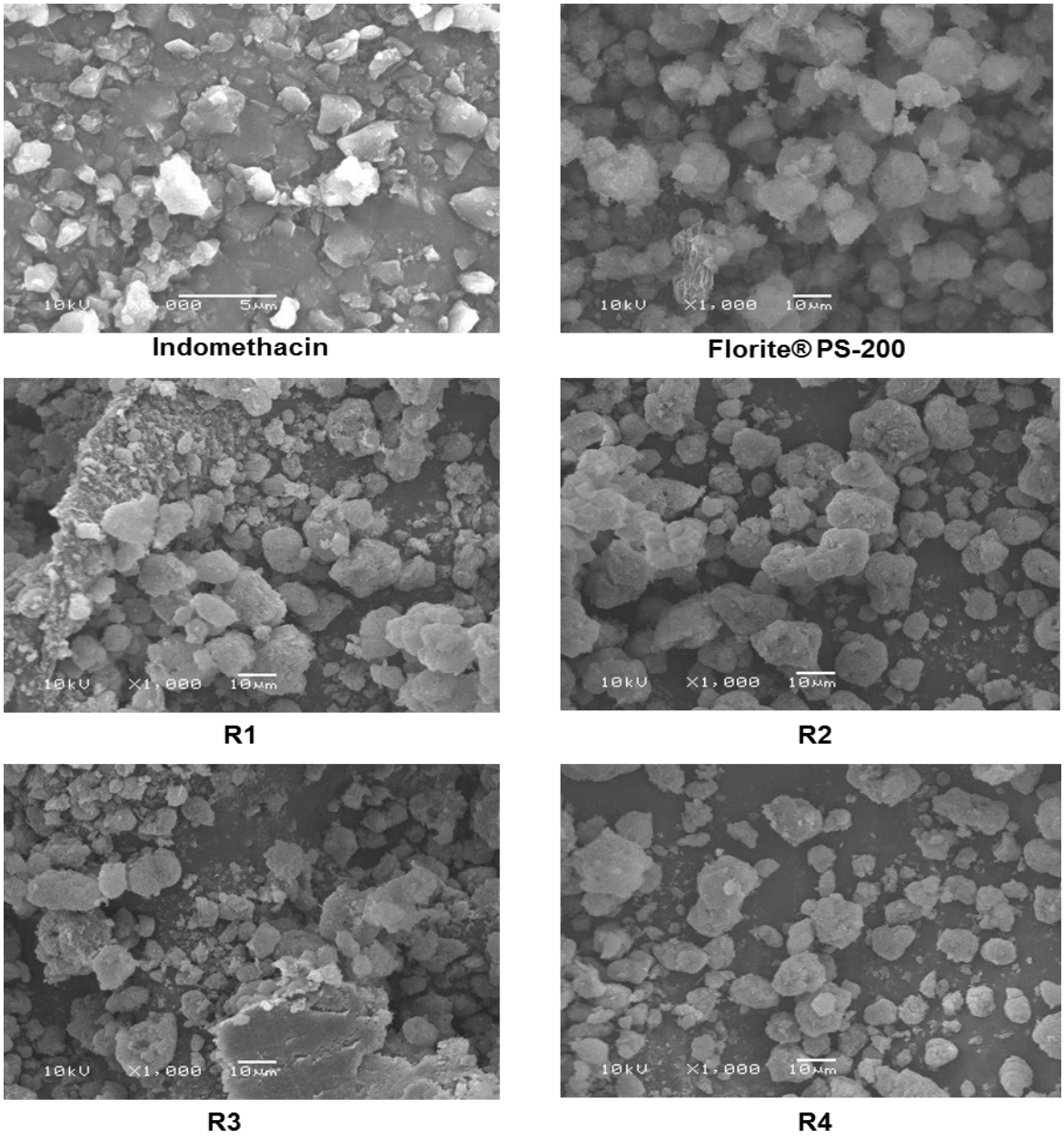


Figure 4.13 SEM micrographs of indomethacin, Florite® PS-200 and various drug-loaded solid SNEDDS formulations.

SEM of pure indomethacin particles revealed multi-faceted structures with smooth surfaced rectangular crystals. As depicted in **Figure 4.11,** irregularly shaped granules of various sizes were observed in SEM of Syloid® XDP 3150 particles and drug-loaded solid SNEDDS prepared with this adsorbent (S1 – S4). In addition, no crystals of indomethacin were obvious on the surface of solid SNEDDS powder formulations after adsorbing the liquid SNEDDS formulations onto Syloid® XDP 3150. Similar SEM observations were reported for glipizide-loaded solid SEDDS prepared by adsorption of optimized liquid SEDDS onto Syloid® 244FP (Agrawal et al., 2015).

Micrographs of Neusilin® US2 and its corresponding indomethacin-loaded solid SNEDDS formulations (N1 – N4) are presented in **Figure 4.12**. Neusilin® US2 appeared as highly porous granular material with a pore size that may reach up to 1µm in diameter. These large pores may classify this carrier as highly macroporous (Gumaste et al., 2013b). However, Neusilin® US2 has been classified as a mesoporous material with pore size in the range of 2 – 50 nm, while materials with pore size < 2 nm are considered microporous (Qian and Bogner, 2012). SEM of drug-loaded solid SNEDDS formulated with Neusilin® US2 (N1 – N4) showed rough surfaced particles which may be due to partial spreading of part of the liquid formulation on the surface of the carrier after adsorption of most of the liquid formulation into the mesopores and deep into the channels of the macropores (Gumaste et al., 2013b). Also, no obvious precipitation of indomethacin was noticed on the surface of these powder formulations. Similar SEM observations were reported for solid SNEDDS of darunavir (Inugala et al., 2015) and self-nanoemulsifying powder of isradipine (Ramasahayam et al., 2015) prepared using Neusilin® US2 as adsorbent.

Figure 4.13 shows the SEM micrographs of Florite® PS-200 and drug-loaded solid SNEDDS prepared with this carrier (R1 – R4). Florite® PS-200 appeared as granular and highly porous material. This carrier is considered as a mesoporous and macroporous adsorbent (Qian and Bogner, 2012, Weerapol et al., 2015b). The appearance in the micrographs of powder formulations prepared with Florite® PS-200 (R1 – R4) was similar to that obtained for the carrier raw material. This indicates that most of the liquid SNEDDS formulations were adsorbed deeply into the macropores and also into the mesopores of the carrier. Also, no evidence of crystals of indomethacin was observed in the micrographs of solid SNEDDS tested (R1 – R4). These findings are consistent with previous SEM results obtained for self-emulsifying powder formulations loaded with different drugs such as nifidipine, felodipine, manidipine and itraconazole and prepared using porous calcium silicate (Florite® RE) as adsorbent (Weerapol et al., 2015b).

Absence of any crystals of indomethacin on the surface of the drug-loaded solid SNEDDS prepared with the above mentioned carriers indicates that the drug remained in a molecularly dissolved state within formulations even after adsorption onto the surface of different carriers.

The effect of the addition of higher amounts of liquid SNEDDS formulation on the appearance of particles of different solid carriers was also examined in the SEM micrographs of various solid SNEDDS formulations presented in **Figures 4.11, 4.12 and 4.13**. When liquid SNEDDS formulation was mixed with the adsorbent in adsorbent: liquid SNEDDS ratio of 1: 1.5, the liquid formula adsorbed into most the pores and also deposited on the surface of the particles of the carrier and resulted in the surface roughness observed in the corresponding micrographs of S1, S3 (**Figure 4.11**), N1, N3 (**Figure 4.12**), R1 and R3 (**Figure 4.13**). Upon further addition of the liquid formulation to the carrier in an adsorbent: liquid SNEDDS ratio of 1: 2, additional adsorption into the pores and covering of the surface of the carrier were observed as the solid SNEDDS powder mixtures were still flowable and their corresponding micrographs of S2, S4 (**Figure 4.11**), N2, N4 (**Figure 4.12**), R2 and R4 (**Figure 4.13**) were still showing rough surfaced granules. These findings are in agreement with previous report (Gumaste et al., 2013b) that has found that increasing the lipid content in a mixture of Cremophor® EL and Neusilin US2 resulted in further deposition and covering of the surface of silicate carrier after adsorption of most of the liquid into the pores of the carrier as confirmed by the SEM analysis of the obtained powder mixtures.

4.4.7. In vitro dissolution studies

Dissolution of indomethacin from different drug-loaded solid SNEDDS prepared with different solid carriers was carried out in phosphate buffer pH 7.2 and compared to the dissolution from pure drug. The dissolution profiles obtained are shown in **Figures 4.14, 4.15 and 4.16**. It was observed that hard gelatin capsules disintegrated and released its content after 90 seconds of the start of the dissolution studies. Different solid SNEDDS showed maximum percentage of the drug release within 15 – 20 minutes, however, the dissolution studies were continued for 1 hour to detect any precipitation or variation that may occur over a period of time (Kallakunta et al., 2012). Pharmacopoeial standards of the dissolution of active substance filled in capsule dosage forms require not less than 80% of the active ingredient to go into solution within 45 minutes (British Pharmacopoeia, 2015). However, comparison of different dissolution profiles based on this approach of single point measurement may not sufficiently characterize the

dissolution process (Podczeck, 1993). Therefore, different dissolution parameters such as the mean dissolution time (MDT), the dissolution efficiency after 15 minutes (%DE_{15}) in addition to the % released after 15 minutes (%Q_{15}) were calculated for different tested formulations and are presented in **Table 4.11**.

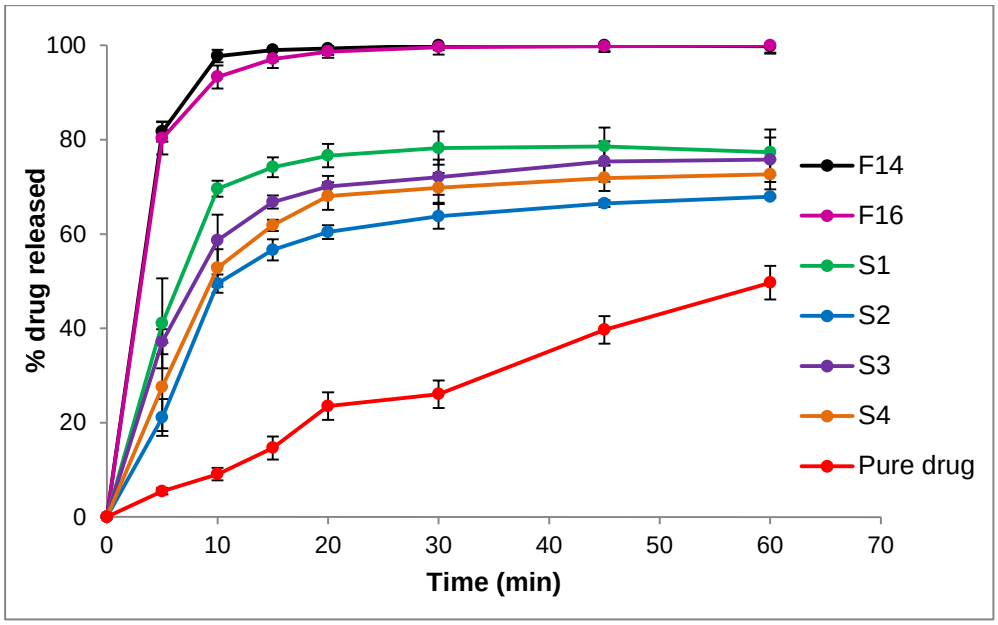


Figure 4.14 In vitro dissolution profiles of pure indomethacin, drug-loaded solid SNEDDS formulations (S1 – S4) and optimized liquid SNEDDS formulations (F14 & F16) in phosphate buffer pH 7.2 (mean ± SD, n=3).

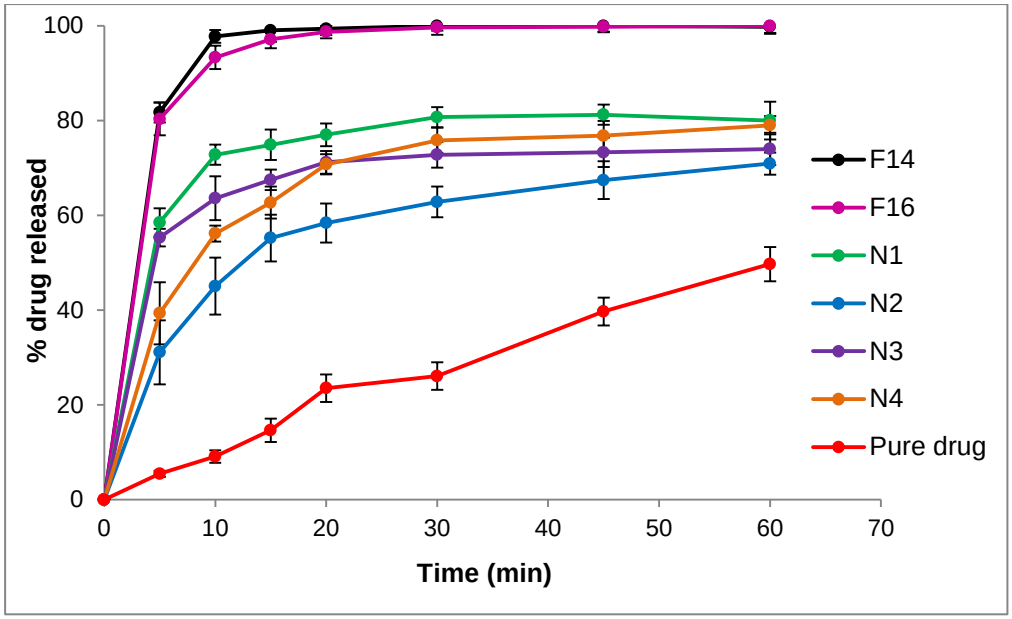


Figure 4.15 In vitro dissolution profiles of pure indomethacin, drug-loaded solid SNEDDS formulations (N1 – N4) and optimized liquid SNEDDS formulations (F14 & F16) in phosphate buffer pH 7.2 (mean ± SD, n=3).

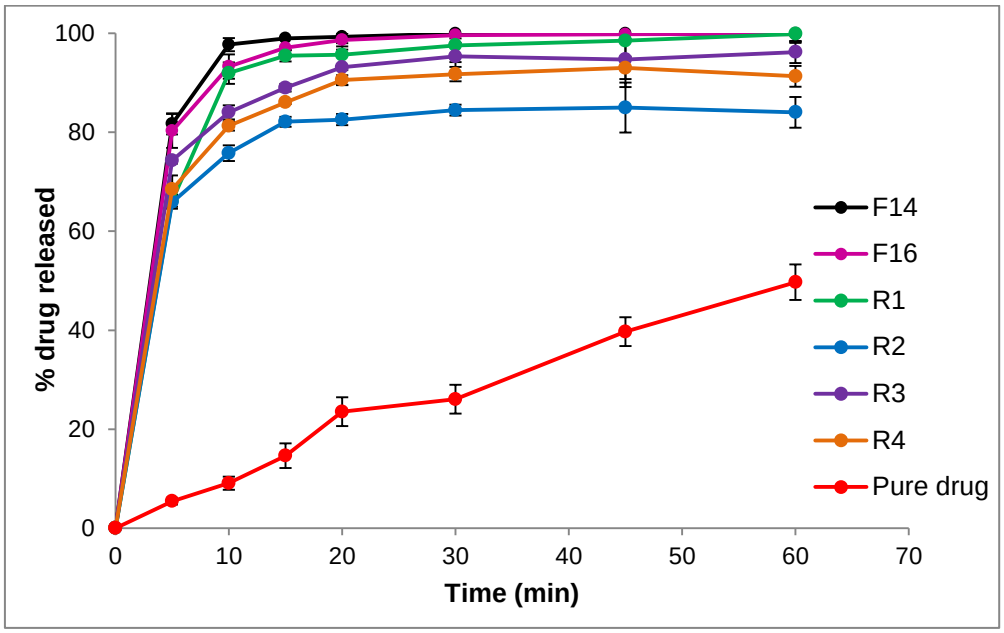


Figure 4.16 In vitro dissolution profiles of pure indomethacin, drug-loaded solid SNEDDS formulations (R1 – R4) and optimized liquid SNEDDS formulations (F14 & F16) in phosphate buffer pH 7.2 (mean ± SD, n=3).

Table 4.11 Mean dissolution time (MDT), mean dissolution efficiency ($\%DE_{15}$) and % released after 15 minutes ($\%Q_{15}$) calculated for pure indomethacin, drug-loaded solid SNEDDS formulations and optimized liquid SNEDDS formulations

Formulation Code	MDT (mean ± SD)	$\%DE_{15}$ (mean ± SD)	$\%Q_{15}$ (mean ± SD)
F14	3.54 ± 1.03	76.32 ± 3.71	99.01 ± 2.41
F16	4.12 ± 0.78	74.05 ± 2.85	97.10 ±1.87
S1	5.18 ± 1.84	49.24 ± 3.50	74.17 ± 2.11
S2	10.03 ± 0.66	32.96 ± 1.38	56.62 ± 2.25
S3	7.97 ± 1.32	43.07 ± 2.66	66.77 ± 1.40
S4	8.85 ± 0.44	37.11 ± 4.51	61.86 ± 1.20
N1	4.50 ± 1.89	56.23 ± 2.24	78.87 ± 3.19
N2	11.77 ± 1.70	34.57 ± 4.66	55.20 ± 4.95
N3	5.52 ± 1.19	50.88 ± 2.46	67.47 ± 2.14
N4	9.21 ± 0.87	42.27 ± 3.27	62.65 ± 3.38
R1	5.62 ± 0.98	68.63 ± 0.74	95.46 ± 1.11
R2	4.05 ± 1.60	60.91 ± 1.09	82.14 ± 1.02
R3	5.23 ± 1.11	67.62 ± 0.77	89.01 ± 0.80
R4	4.31 ± 0.43	64.27 ± 0.80	86.05 ± 0.32
Pure drug	27.32 ± 4.32*	7.28 ± 1.08*	14.64 ± 2.47*

*Significant difference at $p < 0.05$.

Indomethacin-loaded liquid SNEDDS formulations (F14 & F16) exhibited optimal dissolution performance when compared to other formulations. This was indicated by significantly higher $\%DE_{15}$ and significantly lower values of MDT ($p < 0.05$) when compared to those of solid SNEDDS formulations or to that obtained for pure drug filled in capsules (**Table 4.11)**. High dissolution profiles of liquid SNEDDS are due to quick formation of o/w nanoemulsions with small droplet size upon exposure to dissolution medium with gentle agitation. In addition, the presence of the drug in a dissolved state in liquid SNEDDS formulations avoids the dissolution rate-limiting step required for crystalline drugs (Agrawal et al., 2015). However, the dissolution of indomethacin from the pure drug was significantly lower and slower ($p < 0.05$), compared to that observed for all of the various drug-loaded liquid and solid SNEDDS formulations, because of poor aqueous solubility and poor wettability of the drug. Poor wetting properties of indomethacin could be due to high surface free energy which may lead to predominant cohesive forces between drug particles over the adhesive forces between the drug and

aqueous medium which then inhibits the development of an interface (Ramasahayam et al., 2015).

On the other hand, the dissolution of indomethacin from different solid SNEDDS formulations (**Figures 4.14 – 4.16**) was intermediate between the maximum dissolution shown by liquid SNEDDS formulations (F14 & F16) and the minimum dissolution exhibited by pure drug powder. Higher dissolution profiles were observed for solid SNEDDS formulations prepared with Florite® PS-200 (R1 – R4) (**Figures 4.16**) compared to that obtained for solid formulations prepared with Syloid® XDP 3150 (S1 – S4) (**Figures 4.14**) or Neusilin® US2 (N1 – N4) (**Figures 4.15**). These differences could be due to that part of the drug remained adsorbed within the formulations prepared with Syloid® XDP 3150 or Neusilin® US2 and when equilibrium was attained between the amount of the drug dissolved and the amount remained within the solid carrier, no further dissolution was observed.

It is evident from the dissolution parameters presented in **Table 4.11** that the dissolution efficiency ($\%DE_{15}$) of indomethacin from different solid SNEDDS formulations prepared with different carriers showed 4.5 – 9.4 fold increase in comparison to $\%DE_{15}$ of pure drug. Rapid drug release from solid SNEDDS powder formulations may be due to rapid emulsification caused by low surface free energy of self-emulsifying systems which may result in quick formation of an interface between oil droplets and the dissolution medium (Craig et al., 1995). Also, it was proposed that decreased droplet size caused by swelling of oil/surfactant/co-surfactant and water phases may lead to increased dissolution rate (Balakrishnan et al., 2009b). In addition, increased dissolution of the drug from solid SNEDDS formulations could be attributed to high surface area and high porosity of the carriers used in different formulations. High surface area of the carrier may improve wettability and molecular dispersion of the drug while high porosity may allow quick entrance of the dissolution medium into the pores with subsequent rapid emulsification. Furthermore, rapid drug release from solid SNEDDS powder formulations may be due to that the drug remained in a dissolved state after adsorption of the liquid SNEDDS on to adsorbents and this was apparent from the physicochemical characterization of various solid SNEDDS studied.

From the dissolution parameters calculated and presented in **Table 4.11**, it was observed that indomethacin-loaded solid SNEDDS formulations prepared with Florite® PS-200 (R1 – R4) exhibited higher dissolution profiles and, consequently, higher dissolution efficiency ($\%DE_{15}$) when compared to formulations prepared with Neusilin® US2 (N1 – N4) or Syloid® XDP 3150 (S1 – S4). These differences could be attributed to differences in specific surface area of the used carriers. Florite® PS-200 (calcium silicate)

possesses the lowest specific surface area (120 m^2/g) compared to Neusilin® US2; magnesium aluminometasilicate (300 m^2/g) or Syloid® XDP 3150; amorphous silicon dioxide (320 m^2/g). According to Ito et al. (2005), porous carriers with low specific surface area may enhance dispersion of the drug in the dissolution medium and lead to faster dissolution and hence, faster absorption. However, adsorbents with high specific surface area may not sufficiently disperse the drug and therefore may result in lower dissolution rate and bioavailability. Similar findings were reported for gentamycin sulfate dispersed in Labrasol® and adsorbed onto the surface of different silicate-based adsorbents (Ito et al., 2005). Higher dissolution rate and bioavailability of the drug was obtained from formulations prepared with the low specific surface area carrier; Florite® RE; compared to formulations prepared with Neusilin® US2 or Sylysia® 320 which exhibited higher specific surface area. However, the use of another grade of calcium silicate (Hubersorb® 5121), with low specific surface area (50 m^2/g), to adsorb SEDDS of griseofulvin resulted in slow dissolution profile of the drug compared to other solid SEDDS of griseofulvin prepared with larger surface area carriers such as Neusilin® US2 or silicon dioxide (Agarwal et al., 2009). This observation was ascribed to adsorption of larger amount of the formulation onto the surface of calcium silicate leading to increased contact and, hence, hydrophobic interaction between the drug and the adsorbent surface with subsequent precipitation of the drug (Agarwal et al., 2009).

The dissolution performance of indomethacin-loaded solid SNEDDS produced with Neusilin® US2 (N1 – N4) was intermediate (**Figure 4.15 & Table 4.11)** between those observed for formulations produced with Florite® PS-200 (R1 – R4) or Syloid® XDP 3150 (S1 – S4). This could be due to intermediate specific surface area of Neusilin® US2 (300 m^2/g), compared to other adsorbents, which may result in lesser dispersibility of the drug and hence, lower dissolution rate (Ito et al., 2005). Also, decreased dissolution profiles of formulations produced with Neusilin® US2 may be due to entrapment of liquid SNEDDS formulation in the characteristic long and narrow pores of this carrier which may reduce drug release. Greater pore length may reduce the rate of leaching of the formulation to the surrounding medium and also diminish access of the dissolution medium to rehydrate the entrapped liquid formulation (Agarwal et al., 2009). Similar findings were reported for the dissolution of loratadine from porous polystyrene beads (Patil and Paradkar, 2006) and also for dissolution of griseofulvin from solid SEDDS prepared using magnesium aluminometasilicate as adsorbent (Agarwal et al., 2009). The release of indomethacin from solid SNEDDS formulations increased gradually due to gradual access of the dissolution medium to the self-emulsifying formulation present in the pores of the carrier.

Solid SNEDDS formulations prepared with Syloid® XDP 3150 (S1 – S4) showed lower dissolution performance (**Figure 4.14 & Table 4.11**) compared to formulations prepared with Neusilin® US2 (N1 – N4) or Florite® PS-200 (R1 – R4). This may be due to high specific surface area of this carrier (320 m^2/g), compared to other adsorbents used, which may lead to insufficient dispersion of the drug and consequent lower dissolution rate (Ito et al., 2005). Also, possible gelation of silicon dioxide may lead to formation of a barrier that may retard drug release from the corresponding formulations (Weerapol et al., 2015a). Additionally, insufficient porosity of this carrier may cause ineffective wetting of drug particles entrapped in the pores (Krupa et al., 2015). Similar findings were reported for nifidipine-loaded self-emulsifying powder prepared with similar grade of porous silicon dioxide (Syloid® 244FP) (Weerapol et al., 2015a).

In general, it appears that drug release from solid SNEDDS powder formulations depends on the physical characteristics of solid adsorbents in addition to the molecular interaction between the drug, lipid/surfactant/co-surfactant mixture and the solid carrier (Tan et al., 2013). According to Agarwal et al. (2009), different factors may affect the dissolution performance from solid SEDDS prepared by adsorption onto silica-based carriers. Increased length of the pores may decrease access of water to entrapped drug and diminish hydration of the formulation (Agarwal et al., 2009). The effect of specific surface area of the carrier on the dissolution profile of solid SEDDS formulations may be variable. Some authors (Agarwal et al., 2009) showed that low specific surface area may lead to adsorption of liquid formulation onto the surface of the carrier in the form of thin films rather than entrapping inside the pores. Presence of thin films of the formulation on the surface of the adsorbent may increase hydrophobic interaction between the drug and the adsorbent and therefore the drug precipitates on the surface of the adsorbent resulting in lower dissolution performance. However, Ito et al. (2005), have proposed that carriers with low specific surface area may facilitate dispersion of the drug in the dissolution medium leading to faster dissolution performance, while carriers with high specific surface area may not adequately disperse the drug and this may result in reduced dissolution behavior. In addition, high affinity of drug particles towards the carrier may increase the risk of drug precipitation and hence, reduce the extent of drug dissolution (Agarwal et al., 2009).

Higher values of dissolution efficiency (**Table 4.11**) were observed for solid SNEDDS formulations (S1, S3, N1, N3, R1 and R3) prepared from the liquid formula; F14, than that calculated for powder formulations (S2, S4, N2, N4, R2 and R4) produced from the liquid formula; F16. This may be attributed to smaller droplet size observed for F14 (20.68 ± 0.03 nm) in comparison to that observed for F16 (22.69 ± 0.06 nm), which

may lead to rapid emulsification of liquid formulation entrapped in the pores of the carrier with quick formation of an interface between oil droplets and dissolution medium (Balakrishnan et al., 2009a, Craig et al., 1995).

Further inspection of the dissolution parameters presented in **Table 4.11** revealed that increasing the amount of indomethacin-loaded liquid SNEDDS in formulation of solid SNEDDS powders S2, S4, N2, N4, R2 and R4 resulted in decreased dissolution performance. This was evident by decreased dissolution efficiency ($\%DE_{15}$) and increased MDT values of these formulation when compared to solid SNEDDS formulations prepared with lesser amount of liquid SNEDDS (S1, S3, N1, N3, R1 and R3). This could be due to the fact that increasing the amount liquid formulation will fill all the pores of the carrier and may result in difficult wetting of excess drug molecules entrapped in the pores of the solid carrier and this may lead to decreased drug dissolution (Krupa et al., 2015). These results are similar to those obtained for ibuprofen-loaded solid self-emulsifying powders prepared with different Neusilin® grades, where increasing the amount of liquid lipid formulation adsorbed on the surface of Neusilin resulted in a decrease in the amount of drug released (Krupa et al., 2015). However, increasing the amount of griseofulvin SEDDS adsorbed onto silicon dioxide resulted in increased dissolution profiles which was ascribed to different levels of pore filling and entrapment of the lipid formulation droplets within the pores with minimal interaction with the carrier (Agarwal et al., 2009).

It is also worthy to note that changes in MDT values upon incorporation of higher amounts of liquid SNEDDS formulations were less noticeable in formulations prepared with Florite® PS-200 (R1 – R4) even though the pattern of changes in the values of $\%DE_{15}$ or $\%Q_{15}$ was consistent with those calculated for other solid SNEDDS prepared with Neusilin® US2 (N1 – N4) or Syloid® XDP 3150 (S1 – S4). These insignificant changes in MDT may be due to high dissolution rate produced by Florite® PS-200 as a carrier and the slight differences observed between dissolution profiles of these formulations (R1 – R4)

4.5. Conclusions

In this part of the study, formulation of solid SNEDDS formulations from liquid SNEDDS was investigated in order to avoid different disadvantages associated with conventional filling of liquid formulations into capsules. Drug-loaded solid SNEDDS were developed by adsorption of liquid SNEDDS formulations onto solid carriers. Indomethacin-loaded liquid SNEDDS (F14 & F16) composed of appropriate concentrations of Capryol™ 90 as oil phase, Cremophor® RH40 as surfactant and

Transcutol® HP as co-surfactant were selected as optimized formulations because they exhibited good self-nanoemulsifying properties with robustness to dilution, rapid emulsification, thermodynamic stability and nano sized globules.

The optimized liquid SNEDDS (F14 & F16) were transformed into solid SNEDDS powder formulations by physical adsorption onto different solid carriers including Syloid® XDP 3150 (amorphous silicon dioxide), Neusilin® US2 (magnesium aluminometasilicate) and Florite® PS-200 (calcium silicate). Evaluation of different indomethacin-loaded solid SNEDDS powder formulations revealed good flowability and reasonable values of drug content. In addition, formulated solid SNEDDS preserved self-nanoemulsification properties and produced clear nanoemulsion with droplet size similar to that of the liquid SNEDDS formulations.

Physicochemical characterization of produced solid SNEDDS concluded the presence of the drug in a dissolved state within powder formulations, as indicated by DSC measurement and XRD analysis.

In addition, all drug-loaded solid SNEDDS formulated with different carriers exhibited improved dissolution profiles compared to that of the pure powder of drug because of their ability to introduce liquid SNEDDS formulations into the dissolution medium and subsequent formation of nanoemulsions by gentle agitation. Enhanced dissolution behaviour was dependent on the physicochemical properties of carriers used in formulations and this variation was supported by dissolution parameters calculated for different formulations. Powder formulations produced by adsorption of liquid SNEDDS formulation (F14) onto Florite® PS-200 (R1 & R3) showed optimum dissolution behaviour compared to other solid SNEDDS formulations tested. This was due to low specific surface area of the carrier which enhanced dispersion of the drug in the dissolution medium and led to rapid formation of spontaneous nanoemulsion with small droplet size. Therefore, properties of solid carriers have great impact on drug dissolution profile from solid SNEDDS and thus must be considered in rationalizing development of solid SNEDDS formulations.

Overall, it could be concluded that indomethacin-loaded solid SNEDDS formulations may successfully introduce the drug in an immediate-release capsule dosage forms with enhanced solubility and dissolution behaviour. Increased dissolution of indomethacin from solid SNEDDS formulations suggests that these formulations may represent promising systems for oral administration of poorly soluble (BCS class II) drugs such as indomethacin.

Chapter 5

Development and evaluation of Gelucire®-based solid SNEDDS formulations of indomethacin

5.1. Introduction

Lipid based formulations are one of the most widely investigated approaches for delivering BCS class II drugs that possess low solubility and high permeability and therefore, reduced oral bioavailability. Self-emulsifying systems were considered as a popular approach for administration of poorly water soluble drugs for which absorption is dissolution rate-limited. Self-emulsifying systems are isotropic mixtures of oil, surfactant and co-surfactant which emulsify spontaneously to give fine oil in water emulsion upon exposure to liquid medium under gentle agitation. Conventionally, these mixtures are filled in hard or soft gelatin capsules for ease of oral administration. However, interaction of the components of self-emulsifying systems with the capsule shell and possible leaking of the formulation from the capsule are common problems with liquid-filled capsules (Patil and Paradkar, 2006). Therefore, solidification of liquid formulations was proposed to avoid stability and handling problems. Various approaches of solidification have been reported in literature to formulate solid SEDDS such as using goat fat as an oil phase (Attama et al., 2003), loading into porous polystyrene beads (Patil and Paradkar, 2006) or formulation of gelled SEDDS that can act as an intermediate to develop solid sustained release SEDDS (Patil et al., 2004). In addition, different studies in literature have discussed transformation of liquid self-emulsifying systems into solid formulations using the method of blending liquid systems with solid excipients such as cellulose or silicate-based excipients to produce powder mixtures that are free-flowing and can be compressed into tablets (Agrawal et al., 2015, Inugala et al., 2015, Ramasahayam et al., 2015, Wang et al., 2010). However, these solidification methods require higher ratios of solidifying materials to SEDDS to produce solid mixtures with suitable processing characteristics, which may not be suitable for drugs with limited solubility in oil phase (Patil and Paradkar, 2006). Also, incomplete drug dissolution and low drug loading (Nazzal and Khan, 2006) in addition to poor flow characteristics of resulting powder (Agarwal et al., 2009) may be encountered with these different solidification approaches.

Solidification of liquid lipid formulations can be also achieved by direct dispersion of poorly water soluble drugs in solid or semi-solid carriers which could be lipids in nature or possess surface active properties. Such methods of solidification may exclude the need for filing liquid lipid formulation into hard or soft gelatin capsules. For example, direct dissolving of a poorly water soluble compound in a solid matrix of PEG 3350 and Polysorbate 80 resulted in physically and chemically stable formulation (Li et al., 2009). Also, utilization of a mixture of PEG 6000 and vitamin E (TPGS) as solubilizing carrier

matrix to formulate solubilizing solid dispersions of antimalarial drug, halofantrine, resulted in 5 – 7 folds increase in absolute oral bioavailability in beagle dogs (Khoo et al., 2000). However, the proportion of surfactants or lipids used in these studies was not adequate to maintain the drug in a solubilized state in the formulation or after dispersion in aqueous medium (Li et al., 2009). On the other hand, Shah and Serajuddin (2012) have demonstrated that mixing various lipids with PEG 8000 or Poloxamer 188 (Pluronic® F68 NF) produced solid systems depending on the composition of lipids used. Only lipids with high percentage of monoesters or monoglycerides in their structure were able to form solid products with PEG 8000 or Poloxamer 188, while lipids containing higher proportion of di- and tri-glycerides or propylene glycol diesters were unable to form solid systems. Addition of solid systems prepared with Poloxamer 188 to water resulted in emulsification and dispersion of oil globules, while solid systems prepared with PEG 8000 did not emulsify upon dispersion in water indicating that Poloxamer 188 possess surfactant properties that lead to emulsification (Shah and Serajuddin, 2012).

Therefore, utilization of solidifying vehicles that possess self-emulsifying or surface active properties may assist in producing solid self-emulsifying systems that may instantaneously self-emulsify upon dispersion in a liquid phase leading to improved solubility of incorporated poorly soluble drugs and therefore, enhanced dissolution and oral bioavailability.

Gelucires® are novel excipients that possess the ability to solidify upon cooling and to self-emulsify upon dispersion in aqueous medium. These excipients are inert, solid (or semisolid), waxy and amphiphilic materials with surface active properties that spontaneously form fine emulsions upon contact with water. These polymeric materials are saturated polyglycolized glycerides that consist of mono-, di- and tri-glycerides in addition to mono- and di-fatty acid esters of PEG. The proportion and type of each component can determine the hydrophobicity and hydrophilicity of the carrier and may control drug release properties from the dosage form. Different types of Gelucires® can be identified by two numeric values referring to their melting point (33°C – 65°C) and hydrophilic – lipophilic balance (HLB) values (1–18). The wide range of the melting points and different proportions of hydrophilic and lipophilic components of Gelucires® contribute to the wide applicability of these carriers in formulation of different dosage forms with different release characteristics (Kalpana et al., 2015).

Generally, Gelucire® grades with high HLB values are employed to enhance the solubility of poorly soluble drugs and subsequently improve their in vitro dissolution and in vivo bioavailability (Barker et al., 2003, da Fonseca Antunes et al., 2013, Karataş et

al., 2005, Yüksel et al., 2003), while those with low HLB values are used to formulate controlled release matrices (Dennis et al., 1990, Galal et al., 2004).

In this study, Gelucire® 44/14 and Gelucire® 48/16 were evaluated as self-emulsifying vehicles in formulation of solid SNEDDS of indomethacin. Each of these two carriers possesses unique properties upon contact with aqueous solutions due to their characteristic chemical composition. For instance, Gelucire® 44/14 combines mono and di-esters of PEG which may act as surfactants, monoglycerides which may perform as co-surfactants and di- and triglycerides which constitute the oily portion of the molecule (Chambin and Jannin, 2005). Gelucire® 44/14 spontaneously emulsifies upon contact with aqueous fluids due to its well-balanced proportions of short, medium and long chain fatty acids. The resultant fine oil in water emulsion droplets show a size ranging from few nanometers up to 300 nm (Chambin and Jannin, 2005). This carrier is considered as a non-ionic surfactant (Abdul-Fattah and Bhargava, 2002) that has the ability to form micelles at concentrations of 2 µg/ml or 10 µg/ml (Kawakami et al., 2004, Schamp et al., 2006). The ability of Gelucire® 44/14 to form micelles may allow this carrier to increase the solubility of poorly soluble compounds in aqueous media by the micellar solubilization approach. Also, Gelucire® 44/14 may decrease the interfacial tension between poorly soluble drugs and aqueous fluid resulting in decreased contact angle between drug particles and dissolution medium and hence, leading to improved wetting and dissolution by preventing aggregation of particles (Damian et al., 2000).

On the other hand, Gelucire® 48/16 is also a non-ionic surfactant that does not contain glyceride components. It is mainly composed of PEG esters of stearic and palmitic fatty acids. Similarly to Gelucire® 44/14, this carrier self-emulsifies into fine oil in water emulsions upon contact with aqueous medium. It also has the ability to form micelles at a concentration of 153 µg/ml and therefore, may improve solubility of poorly soluble drugs by micellar solubilization (Gattefossé, 2015).

Several previous studies have reported the use of different Gelucire® grades to enhance the solubility and oral bioavailability of various drugs. For example, Gelucire® 44/14 was successfully employed alone or in combination with other excipients to improve solubility and dissolution performance of piroxicam (Karataş et al., 2005), carbamazepine (da Fonseca Antunes et al., 2013), aceclofenac (Kalpana et al., 2015) and naproxen (Nagabandi et al., 2014). Also, improved oral bioavailability of α–tocopherol (Barker et al., 2003), halofantrine (Khoo et al., 2000), and piroxicam (Yüksel et al., 2003) upon dispersion in Gelucire® 44/14 was reported(Yüksel et al., 2003)(Yüksel et al., 2003). Further utilization of different grades of Gelucires® such as Gelucire® 50/13

to formulate solid dispersions of indomethacin (El-Badry et al., 2009), loratadine (Bandari et al., 2014) and carvedilol (Potluri et al., 2011) has resulted in significant increase in solubility and dissolution behavior of these poorly water soluble drugs. Moreover, investigation of the solubilizing properties of the newly introduced grade, Gelucire® 48/16, when used in formulation of solid dispersions of piroxicam or curcumin revealed significant increase in solubility of both compounds (Gattefossé, 2015).

Improvement of solubility and dissolution rate of poorly soluble drugs by different grades of Gelucires® was attributed to solubilizing effects and self-emulsifying properties of these carriers (Gattefossé, 2012, Karataş et al., 2005). It was reported that Gelucire® 48/16 self-emulsifies in aqueous liquids to form micellar solutions that may encapsulate drug particles within micelles leading to solubilization of poorly soluble drugs (Ganesh, 2016, Gattefossé, 2015). Gelucires® may also improve wetting of drug particles in the dissolution medium resulting in enhanced solubility and dissolution performance (El-Badry et al., 2009, Karataş et al., 2005, Potluri et al., 2011). Furthermore, enhancement of oral bioavailability of poorly soluble drugs when dispersed in Gelucires® was explained on the basis of improvement of lymphatic transport and intestinal permeability of drug particles (Hauss et al., 1998).

Literature data lack information on the development of solid self-nanoemulsifying formulations using Gelucires® alone. Therefore, based on the self-emulsifying properties of Gelucires® and their ability to solidify upon cooling, it was proposed to investigate the feasibility of using Gelucires® as single self-emulsifying carriers to produce solid SNEDDS of indomethacin by direct dispersion of the drug in the carrier. Because of the self-emulsifying and solidifying properties of Gelucires®, utilization of a certain grade of Gelucires® as a single carrier may replace the components of the liquid SNEDDS (lipid, surfactant and co-surfactant) that were investigated in **Chapter 3** of this project and also may replace using solid carriers utilized to solidify the liquid SNEDDS formulations that was described previously in **Chapter 4**. Application of Gelucires® as self-emulsifying and solidifying agents may bypass the problems associated with the production of liquid self-emulsifying formulations and exclude the need for further solidification processes because solidification can be simply achieved upon cooling of the melted mixture of the drug and the surface active excipient. Also, the use of a single excipient with self-emulsifying properties to formulate solid SNEDDS is expected to allow higher drug load to be incorporated in the formulation because no other liquid or solid excipients will be needed. Further, this work aims to investigate the applicability of the hot melt extrusion (HME) technique as a means of scaling up the production of Gelucire®-based solid SNEDDS. HME allows more efficient melting, mixing and temperature control to produce

stable formulations. Compared to traditional melting and solvent techniques, HME provides a continuous process with controlled processing parameters, doesn't require solvents and can be scaled to produce appropriate pharmaceutical batch sizes.

As a part of this study, the potential of using Gelucire® as a self-emulsifying vehicle was assessed by evaluating the self-nanoemulsification efficiency, droplet size and dissolution profiles of the produced indomethacin-loaded solid SNEDDS formulations. Further, various solid SNEDDS formulations produced were physically characterized to determine the effect of the used carrier on crystallinity of the drug as well as the in vitro dissolution.

5.2. Materials

- Indomethacin was obtained from Sigma-Aldrich Chemie GmbH, Germany.
- Gelucire® 44/14, Gelucire® 50/13 and Gelucire® 48/16 were kindly provided by Gattefossé Co., France.
- Neusilin®US2 (magnesium aluminometasilicate) was kindly provided by Fuji Chemical Industry (Japan).
- Florite®PS-200 (calcium silicate) was kindly provided by Tomita Pharmaceutical Co. (Japan).
- Potassium dihydrogen orthophosphate anhydrous was obtained from Loba Chemie Pvt. Ltd. (Mumbai, India).
- Sodium hydroxide was obtained from Fluka Chemie GmbH (Germany).
- Calcium chloride was obtained from Sigma Aldrich (U.K.).
- Methanol was obtained from Fisher Scientific UK Limited (Leicestershire, UK).
- Deionized water was purified using an Ultra-purification Water System, Millipore Co. Ltd. (Bedford, MA, USA).
- Hard gelatin capsules were obtained from pharma tradechem (Mumbai, India).

5.3. Methods

5.3.1. Construction of a standard calibration curve of indomethacin in phosphate buffer pH 7.2

The standard calibration curve of indomethacin constructed in phosphate buffer pH 7.2, as described in **Chapter 4,** was utilized for this part of the study. The inter-day accuracy as well as the intra-day and inter-day precision (reproducibility) of the assay procedure for determination of indomethacin concentrations in phosphate buffer were evaluated as previously presented in **Chapter 3**.

5.3.2. Phase solubility study of indomethacin in different Gelucires®

Phase solubility studies were conducted according to the method of Damian et al. (2000) to determine the grade of Gelucires® (Gelucire® 44/14, Gelucire® 50/13 or Gelucire® 48/16) that may exhibit maximum solubilizing potential for indomethacin. Different concentrations of each Gelucire® grade (1%, 3%, 5%, 10% and 15%) were prepared in purified water. An excess amount of the drug was added to each of these concentrations. All systems were mixed and kept shaking for 48 hours in an isothermal shaking water bath adjusted at 25^{o}C. After equilibrium, samples were centrifuged at 3000 rpm for 15 minutes to remove undissolved drug and then filtered through a 0.45 µm membrane filter. Parts of the filtered samples were suitably diluted and assayed for the drug at 320 nm against a blank prepared from each carrier in purified water. Three determinations of indomethacin solubility were carried out for each sample and the results are presented as mean ± SD.

From the results of phase solubility studies, Gelucire® grades that showed maximum solubility of the drug were selected for further investigation.

5.3.3. Thermogravimetric analysis (TGA)

Thermal stabilities of indomethacin, Gelucire® 44/14, Gelucire® 48/16, Neusilin® US2 and Florite® PS-200 were determined in the temperature range of 30^{o}C to 250^{o}C, at a heating rate of 10^{o}C/min using TGA 7 (Perkin Elmer, USA) fitted with Pyris manager software (version 5.00.02, Perkin Elmer, USA). Samples of approximately 10 mg were used and the percentage loss in weight was calculated at different temperatures.

5.3.4. Formulation of Gelucire®-based solid SNEDDS of indomethacin

Based on the results of phase solubility studies, Gelucire® 44/14 and Gelucire® 48/16 were selected to formulate Gelucire®-based solid SNEDDS of indomethacin. Different physical mixtures of indomethacin and any Gelucire® were prepared at the drug: carrier ratios of 0.5: 10, 1: 10, 2: 10 and 3: 10. The amount of the carrier in each formulation was kept constant in order to evaluate the effect of increasing drug load on the self-emulsifying properties as well as the dissolution performance of different formulations. Further investigation of the effect of addition of solid adsorbents on dissolution behaviour of Gelucire®-based solid SNEDDS formulations was also proposed. Therefore, adsorbents such as Neusilin® US2 or Florite® PS-200 (optimized from **Chapter 4**) were added to the optimized Gelucire®-based formulations. For this purpose, new physical mixtures of indomethacin, Gelucire® 48/16 and any of the proposed adsorbents were also prepared in the ratios of 2: 10: 0.12 and 2: 10: 0.36, respectively. The added amounts of the adsorbent constituted about 1% w/w or 3% w/w of the total formulation.

HME processing of different physical mixtures prepared with Gelucire® 44/14 was carried out at 40^{o}C, while mixtures prepared with Gelucire® 48/16 were processed at both 40^{o}C and 50^{o}C. Therefore, the temperature of the extruder barrel was adjusted at either 40^{o}C (below the melting point of both Gelucire® grades) or at 50^{o}C which corresponds to the melting point of Gelucire® 48/16, while the rotational speed of the screws was adjusted at 30 rpm. Each physical mixture was introduced into a co-rotating twin screw extruder (micro-compounder, MC15, Xplore Instruments BV, Sittard, The Netherlands) through the hopper. The introduced mass was mixed for 5 minutes inside the barrel before extrusion through a die with 1 mm diameter. The collected mass was allowed to cool at room temperature, then cut or crushed into small pieces. The crushed mass was then sieved through a 500 µm sieve and the obtained granular product was stored in a desiccator over anhydrous calcium chloride until further evaluation. Codes and composition of different indomethacin solid SNEDDS formulations produced by HME using Gelucire® 44/14, Gelucire® 48/16 and adsorbents are presented in **Table 5.1**.

Table 5.1 Codes and composition of different Gelucire®-based solid SNEDDS of indomethacin produced by HME technique at different temperatures

Code of formulation	Extrusion temperature (^oC)	Carrier used	Adsorbent used	Ratio of indomethacin : Gelucire® : adsorbent
G1	40	Gelucire® 44/14	…….	0.5: 10: 0
G2				1: 10: 0
G3				2: 10: 0
G4				3: 10: 0
G5	40	Gelucire® 48/16	…….	0.5: 10: 0
G6				1: 10: 0
G7				2: 10: 0
G8				3: 10: 0
G9	50	Gelucire® 48/16	…….	0.5: 10: 0
G10				1: 10: 0
G11				2: 10: 0
G12				3: 10: 0
G13	40	Gelucire® 48/16	Neusilin® US2	2: 10: 0.12
G14				2: 10: 0.36
G15	40	Gelucire® 48/16	Florite® PS-200	2: 10: 0.12
G16				2: 10: 0.36

5.3.5. Evaluation of produced Gelucire®-based solid SNEDDS of indomethacin

5.3.5.1. Determination of drug content

As previously mentioned in **Chapter 2**, drug content was determined for Gelucire®-based solid SNEDDSs to ensure equal distribution of the powdered drug within different formulations produced (Gumaste et al., 2013a). An accurately weighed amount of each formulation was dispersed in a suitable quantity of methanol and mixed thoroughly for 10 minutes to confirm dissolution of the drug into the methanol. The samples were filtered through a 0.45 µm membrane filter, suitably diluted and assayed for the drug at 320 nm against a reference standard. The drug content in each sample was calculated as previously described in **Chapter 4** using the following equation:

$$\%\, drug\ content = \frac{drug\ content\ of\ the\ formulation}{weight\ of\ the\ formulation\ taken} \times 100 \qquad \text{(Equation 5.1)}$$

All experiments were repeated in triplicates and the results were presented as mean ± SD.

5.3.5.2. Self-nanoemulsification efficiency tests

Self-nanoemulsification tests were carried out to determine the possibility of drug precipitation that may take place upon dilution of formulations with purified water. These tests were carried out using a method similar to that adopted for liquid SNEDDS formulations described previously in **Chapter 3**. An amount equivalent to 25 mg of indomethacin was weighed from each Gelucire®-based solid SNEDDS formulation and added to 200 ml of purified water (maintained at 37^{o}C) in dissolution apparatus and stirred at 50 rpm until complete dissolving. Signs of precipitation of the drug in each formulation sample were recorded and self-nanoemulsification performance of the formulation was evaluated visually according to different grading scales defined by Khoo et al. (1998) and described earlier in **Chapter 3**.

5.3.5.3. Determination of droplet size

Redispersibility and droplet size of all Gelucire®-based solid SNEDDSs of indomethacin were assessed according to the method of Kanaujia et al. (2014) using Malvern Zetasizer nano-ZS (Malvern Instruments, Worchestershire, UK). Each formulation sample was dispersed in deionized water to obtain a final drug concentration of 100 µg/ml. Mixtures were shaken gently for 5 minutes, centrifuged at 3000 rpm for 15 minutes and then filtered through a 0.45 µm syringe filter. The average globule size, polydispersity index and zeta potential of the nanoemulsion formed from Gelucire®-based solid SNEDDSs were determined by Malvern Zetasizer. All measurements were made in triplicates and the results are presented as mean ± SD.

5.3.5.4. Determination of solubility of the drug in Gelucire®-based solid SNEDDS formulations

Measurement of the solubility of the drug in different formulations obtained was carried out according to the method of El-Badry et al. (2009). An excess amount of each Gelucire®-based solid SNEDDS formulation was added to phosphate buffer pH 7.2. The samples were mixed and shaken for 48 h at 37^{o}C in an isothermal shaking water bath. After equilibrium, samples were filtered through 0.45 µm membrane filter, suitably diluted and assayed for the drug at 320 nm against a blank prepared with the excipient used. All determinations were repeated in triplicates and the results were presented as mean ± SD.

5.3.6. Solid state characterization of Gelucire®-based solid SNEDDS of indomethacin

5.3.6.1. Fourier transform infrared spectroscopy (FTIR)

In order to identify possible interactions between indomethacin, different Gelucires® and adsorbents used in formulation of Gelucire®-based solid SNEDDSs, FTIR spectra of the different formulations obtained were recorded in the scanning range of 4000 – 400 cm^{-1} using a Fourier transform infrared (FTIR) spectrophotometer (Nicolet 6700, Thermo Fisher Scientific, USA). The individual FTIR spectra of indomethacin, Gelucires® and the adsorbents were also obtained for the purpose of comparison. The test was performed by mixing an amount of each formulation sample (4 mg) with dry potassium bromide (IR grade, 200 mg) and compaction of the lightly ground mixture into a disc using hydraulic press. Scanning was performed at a speed of 4 scans/second.

5.3.6.2. Differential scanning calorimetry (DSC)

In order to determine the physical state of indomethacin in the formulated Gelucire®-based solid SNEDDS, the thermal behavior of indomethacin, Gelucires®, adsorbents and different formulations was studied using conventional DSC analysis (DSC 4000, Perkin Elmer, US). An accurately weighed sample (5 – 10 mg) was sealed in a flat bottomed standard aluminum pan and scanned at a heating rate of 10^{o}C/ minute from 25 – 300 ^{o}C under a nitrogen gas flow rate of 20 ml/min.

5.3.6.3. Powder X-ray diffraction (XRD)

To characterize the physical state of the drug in different formulations obtained, the X-ray diffraction studies were performed for indomethacin, Gelucires®, adsorbents and Gelucire®-based solid SNEDDS formulations using an Ultima IV-diffractometer (Rigaku Co., Ltd., Tokyo, Japan) equipped with a copper X-ray source maintained at 40 kV of tube voltage and 40 mA of tube current to produce emissions of 0.15406 nm. The samples were scanned at 3 − 60° 2θ range at a scanning speed of 0.5 deg. /min. Data were collected using a step scan mode with step size of 0.02^{o} and counting time of 1 second per step.

5.3.6.4. Scanning electron microscopy (SEM)

The surface morphology of indomethacin, Gelucires®, and Gelucire®-based solid SNEDDS formulations were investigated using a JSM-6060LV scanning electron

microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 10 kV. Samples were lightly sprinkled onto double-sided sticky tape which then was affixed to an aluminum stub and made electrically conductive with a gold coating (13 – 14 nm/min; 45 s; 20 mA) under vacuum using JFC-1600 Auto Fine Coater (Jeol, Tokyo, Japan). Micrographs at different magnifications were recorded and analyzed for the surface morphological properties.

5.3.7. In vitro dissolution studies

In vitro dissolution studies of Gelucire®-based solid SNEDDS formulations were performed according to British Pharmacopoeia (2015) as previously described in **Chapter 4**. Briefly, an amount of each formulation equivalent to 25 mg of indomethacin was filled in a hard gelatin capsule and used for dissolution studies. Samples (5 ml) were withdrawn at predetermined time intervals, filtered through a 0.45 µm syringe filter and assayed for indomethacin at 320 nm. An equal volume of fresh dissolution medium kept at 37^{o}C was added to keep constant volume during dissolution study. For the purpose of comparison, the experiment was repeated with the same quantity of pure indomethacin powder (25 mg) filled in hard gelatin capsules. All experiments were performed in triplicates and results were averaged ± SD.

Different dissolution parameters such as dissolution efficiency after 15 minutes (DE_{15min}), mean dissolution time (MDT) and % released after 15 minutes ($\%Q_{15min}$) were used to compare the dissolution performance of different Gelucire®-based solid SNEDDS formulations. The DE_{15min} was determined for the time intervals of dissolution study (from 0 – 15 min) while MDT was calculated for all dissolution time interval (from 0 – 60 min) using DDSolver as Excel add inn.

5.3.8. Statistical analysis

Differences between the data of interest were detected using one way analysis of variance (ANOVA) and t-test. Significant differences were determined at a 5% significance level, unless otherwise stated elsewhere. Statistical differences yielding ($p < 0.05$) were considered significant.

5.4. Results and discussion

5.4.1. Phase solubility study of indomethacin in different Gelucires®

The aqueous solubility of a drug is an important factor that determines its dissolution rate and hence, its bioavailability. Therefore, poorly water soluble drugs with aqueous solubility lower than 0.1 mg/ml show poor oral absorption due to limited dissolution (Horter and Dressman, 2001). Indomethacin, a weakly acidic drug, with a p*K*a value of 4.5, is considered as "practically insoluble" in simulated gastric fluid (pH 1.2) and "slightly insoluble" in simulated intestinal fluid (pH 7.4) (Nokhodchi et al., 2005). Variable or inconsistent estimations of aqueous solubility of indomethacin in purified water were reported in different studies (Palanisamy and Khanam, 2014, Shakeel et al., 2013a, Yadav and Yadav, 2009). These studies have reported aqueous solubility values for indomethacin, in water at 25°C, ranging between 0.00094 – 0.367 mg/ml. The reported aqueous solubility of indomethacin in water was found to increase gradually with increasing temperature (Palanisamy and Khanam, 2014, Shakeel et al., 2013a). In this study, and as indicated previously in **Chapter 3**, the solubility of indomethacin in distilled water at 25°C was found to be 0.02 ± 0.01 mg/g, and therefore, improvement of solubility by the use of different grades of Gelucires® was investigated. Different types of Gelucires® increased the solubility of indomethacin based on their HLB values and concentrations used, as can be seen in the phase solubility diagrams of the drug depicted in **Figure 5.1**.

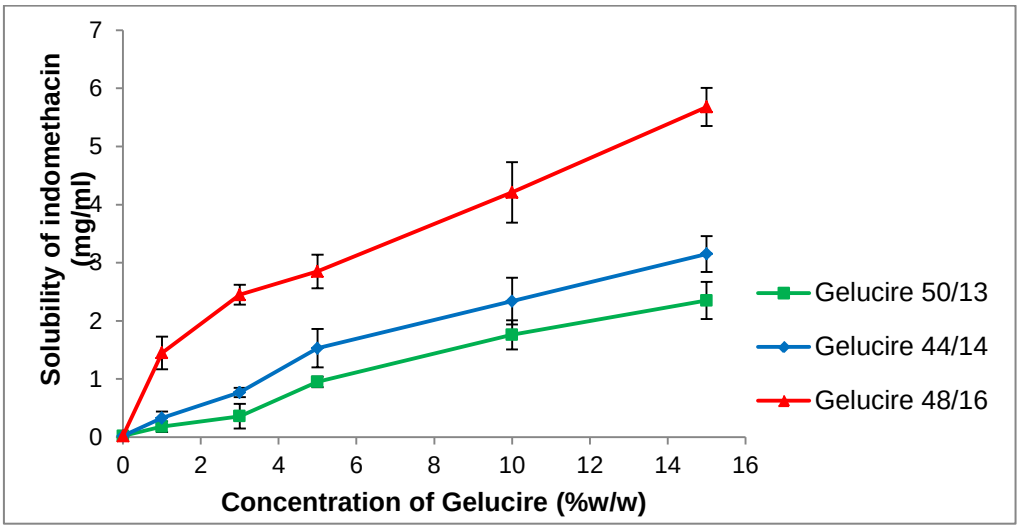


Figure 5.1 Phase solubility diagram of indomethacin in aqueous solutions of different Gelucires® at 25°C

It can be observed that at 25°C the solubility of indomethacin in purified water (0.02 ± 0.01 mg/ml) was significantly increased in aqueous mixtures prepared with increased concentrations of Gelucires®. For example, 5% aqueous solutions prepared with Gelucire® 50/13, Gelucire® 44/14 or Gelucire® 48/16 resulted in increased solubility of indomethacin by 47.5, 76.5 or 142.5 folds, respectively. An almost linear increase in solubility of the drug was observed with increasing the concentration of each Gelucire® grade.

Improvement of the solubility of indomethacin in the presence of these amphiphilic carriers could be attributed to increased wettability and micellar solubilization of the drug (Damian et al., 2000, Horter and Dressman, 2001, Leuner and Dressman, 2000). An indication of the transfer process of indomethacin from pure water to the aqueous solutions of different Gelucires® was obtained from the values of the Gibbs free energy change. The Gibbs free energy of transfer (ΔG^{o}_{tr}) of indomethacin from pure water to aqueous solutions of Gelucires® can be calculated from the equation below (Damian et al., 2000):

$$\Delta G^{o}_{tr} = -2.303\ RT\ log\ \frac{S_o}{S_s} \qquad \text{(Equation 5.1)}$$

where S_o/S_s is the ratio of molar solubility of indomethacin in aqueous solutions of Gelucires® to that in pure water. The calculated values of Gibbs free energy presented in **Table 5.2** provide information of increased solubility of indomethacin in the presence of different Gelucires®. The negative values of ΔG^{o}_{tr} indicate spontaneous solubilization of indomethacin, and the decrease of these values with increasing the concentration of each Gelucire® grade indicates that the reaction became more favourable as the concentration of Gelucires® increased (Damian et al., 2000, Potluri et al., 2011).

Table 5.2 Solubility and thermodynamic parameters of indomethacin in aqueous solutions of different Gelucires® prepared at 25°C.

Gelucire® (% w/v)	Gelucire® 50/13		Gelucire® 44/14		Gelucire® 48/16	
	Solubility of indomethacin (mg/ml)	ΔG^{o}_{tr} (kJ/mol)	Solubility of indomethacin (mg/ml)	ΔG^{o}_{tr} (kJ/mol)	Solubility of indomethacin (mg/ml)	ΔG^{o}_{tr} (kJ/mol)
0%	0.02 ± 0.01	…..	0.02 ± 0.01	…..	0.02 ± 0.01	…..
1%	0.18 ± 0.09	-5.45	0.33 ± 0.11	-6.95	1.45 ± 0.28	-10.62
3%	0.36 ± 0.21	-7.16	0.77 ± 0.08	-9.05	2.45± 0.17	-11.92
5%	0.95 ± 0.09	-9.57	1.53 ± 0.33	-10.75	2.85 ± 0.29	-12.29
10%	1.76 ± 0.25	-11.10	2.34 ± 0.40	-11.80	4.21 ± 0.52	-13.26
15%	2.35 ± 0.32	-11.81	3.15 ± 0.31	-12.54	5.68 ± 0.33	-14.00

In addition, the presence of Gelucires® in aqueous solutions may decrease the contact angle between drug particles and water leading to improved wettability of drug particles and hence, improved solubility. It was reported that larger contact angles (> 65°) between particles and tested medium indicates a hydrophobic surface, while smaller contact angles (< 65°) represent a hydrophilic surface (Chambin et al., 2009). This suggests that when a poorly soluble drug is present in contact with a hydrophilic carrier, less hydrophobicity of the surface of drug particles is produced and this may promote contact with aqueous medium leading to increased solubility (Kallakunta et al., 2013).

From **Figure 5.1**, it was also noticed that enhancement of indomethacin solubility in the presence of different grades of Gelucires® showed the following order: Gelucire® 48/16 > Gelucire® 44/14 > Gelucire® 50/13. Among different amphiphilic carriers used, Gelucire® 48/16 showed the maximum solubilizing effect of indomethacin when compared to Gelucire® 44/14 or Gelucire® 50/13. Differences in enhancement of drug solubility by different grades of Gelucires® may be due to differences in the composition and HLB values of used carriers. Gelucire® 50/13 comprises a high percentage of long chain palmitic (C_{16}) and stearic (C_{18}) fatty acids, while Gelucire® 44/14 includes high proportions of lauric (C_{12}) and myristic (C_{14}) fatty acids (Gattefossé, 2012). On the other hand, Gelucire® 48/16 is mainly a PEG ester of palmitic (C_{16}) and stearic (C_{18}) fatty acids (Gattefossé, 2015). Differences in chemical compositions may influence the degree of hydrophobic interaction between drug particles and the core of the micelles produced by these surface active carriers. Additionally, it has been proposed that Gelucire® 50/13 is

able to exist in different crystalline forms which may influence the solubilizing properties of this carrier (Gattefossé, 2012, Karataş et al., 2005), while Gelucire® 44/14 possesses surface active properties and has the ability to self-emulsify when in contact with aqueous medium to produce fine emulsions (da Fonseca Antunes et al., 2013, Gattefossé, 2012). Similarly, Gelucire® 48/16 can self-emulsify when in contact with an aqueous fluid leading to formation of micellar solutions that may encapsulate drug particles within micelles (Gattefossé, 2015).

Differences in the HLB values of different grades of Gelucires® may also be responsible for differences in their solubilizing potential of indomethacin. Enhancement of indomethacin solubility was more pronounced with aqueous solutions of Gelucire® 48/16 than that obtained from aqueous solutions of Gelucire® 44/14 or Gelucire® 50/13. This could be due to higher HLB values of Gelucire® 48/16 which may enhance miscibility and dispersibility of the drug within the carrier leading to increased solubility (Kalpana et al., 2015).

Based on the results obtained from phase solubility studies, Gelucire® 44/14 and Gelucire® 48/16 were selected to formulate Gelucire®-based solid SNEDDS of indomethacin by direct dispersion of the drug in these carriers. These carriers showed the maximum solubilizing potential for indomethacin due to their self-emulsifying properties, high HLB value and formation of micellar solutions that may entrap the poorly soluble drug molecules inside the micelles. Gelucire® 50/13 was not employed for further formulations because its solubilizing potential for indomethacin was lower than that obtained by Gelucire® 48/16 or Gelucire® 44/14. Also Gelucire® 50/13 does not possess self-emulsification properties when in contact with aqueous fluids and hence, will not be useful to formulate self-emulsifying formulations.

5.4.2. Thermogravimetric analysis (TGA)

TGA was performed to evaluate thermal stability of the drug and the carrier prior to their use in hot melt extrusion to produce carrier-based solid SNEDDS formulations. In the heating range (30^{o}C – 250^{o}C), an almost constant weight of indomethacin was observed until the temperature of 170^{o}C which may indicate that the drug is stable and non-hygroscopic. However, about 0.5% loss in weight was observed at 200^{o}C. In literature, it was reported that indomethacin is stable up to 248^{o}C (Rusu et al., 2000). Also, a constant weight was maintained for both Gelucire® 44/14 and Gelucire® 48/16 until 100 ^{o}C, while loss of weight by about 1.3% and 4% were observed for both carriers at 200 ^{o}C, respectively. Based on these TGA results, an optimum temperature of melting

at 50 °C (which approximates the melting point of Gelucire® 48/16) was selected for hot melt extrusion of different blends of indomethacin and Gelucire® 48/16. HME processing of different mixtures of indomethacin and Gelucires® at 40 °C (below the melting point of both forms of Gelucire®) was also proposed for the purpose of comparison of the two carriers and evaluation of the effect of temperature on the extrudability of the mixtures as well as their dissolution behavior and crystalline state of the drug.

TGA plots of the adsorbents, Neusilin® US2 and Florite® PS-200, revealed about 1.5% loss in weight (that is possibly due to water evaporation) at the maximum proposed temperature of melting (50°C) which indicate thermal stability of these adsorbents at selected temperatures for hot melt extrusion process.

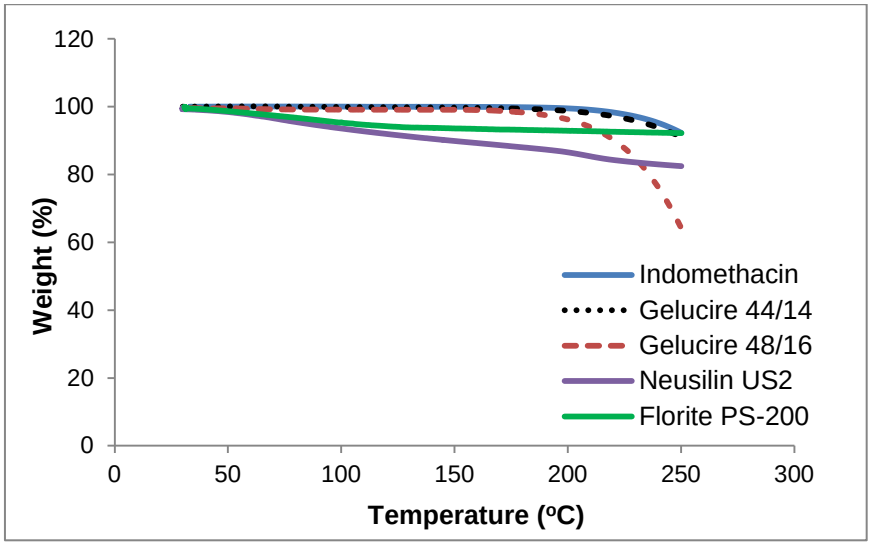


Figure 5.2 TGA plots of indomethacin, Gelucire® 44/14, Gelucire® 48/16, Neusilin® US2 and Florite® PS-200.

5.4.3. Hot melt extrusion of Gelucire®-based solid SNEDDS of indomethacin

Extrusion of physical mixtures of indomethacin and any of the Gelucires® was carried out at two different temperatures (below and approximately at the melting point of the carrier) in order to evaluate the effect of different extrusion temperatures on the drug-carrier interaction, drug crystallinity and drug dissolution performance. Extrusion processing of physical mixtures of indomethacin with Gelucire® 44/14 or Gelucire® 48/16 below the melting point of the excipients may also allow comparison of the self-emulsifying formulations prepared with the two Gelucires® in addition to investigation of the possibility of processing both Gelucires® by softening instead of complete melting.

In this study, two temperatures (40°C and 50°C) were selected for HME processing of Gelucire®-based solid SNEDDS formulations of indomethacin. Preliminary

determination of the melting point of untreated Gelucire® 48/16 by conventional DSC revealed an onset of melting at 41.53 ± 0.34°C and a peak of melting recorded at 50.74 ± 0.15°C. On the other hand, the onset of melting of untreated Gelucire®44/14 was observed at 36.78 ± 0.28°C while a peak of melting was shown at 46.49 ± 0.25°C.

A residence time of 5 minutes was sufficient to allow thorough softening of mixtures of indomethacin and both Gelucires® when the temperature of the barrel was adjusted to 40°C and the speed of rotation adjusted to 30 rpm. All formulations prepared from indomethacin and Gelucire® 44/14 at different drug: carrier ratios (G1 – G4) were successfully extruded at 40°C through the die in the form of curled threads which solidified upon cooling. In the case of formulations prepared from indomethacin and Gelucire® 48/16, only the mixtures prepared in drug: carrier ratios of 0.5: 10 or 1: 10 (G5 and G6) were successfully extruded at 40°C and formed a product of curled threads which solidified upon cooling, while the mixtures (G7 and G8) prepared at higher drug: carrier ratios (2: 10, or 3: 10) did not extrude through the die and required removal of the die part to collect the melt in the form of a transparent viscous mass that hardened at room temperature. Visually, the formulations with high drug contents (G7 and G8) had a thicker consistency and appeared more viscous than the formulations with lower drug contents (G5 and G6). Although not measured experimentally, this increased viscosity is likely to be too great for the extrusion processing parameters used here; hence the formulations could not be extruded through the die here. Extrusion may be possible if the shearing force inside the HME barrel could be increased, for example, by increasing the rotational speed and barrel pressure. On the other hand, all formulations of indomethacin and Gelucire® 48/16 (G9 – G12) prepared at different drug: carrier ratios and processed by HME at 50°C did not extrude in the form of threads but remained as a thin liquid in the barrel and collected in the form of a transparent liquefied mass which transformed into a solid opaque mass on cooling. Processing of mixtures at a temperature that corresponds to the melting point of the carrier resulted in complete melting of the carrier and formation of a liquefied mass inside the extruder barrel which in turn may require increased pressure to force the melt through the die, which did not work in this case and removal of the die was crucial.

Preparation of different Gelucire®-based solid SNEDDS with different drug loads may allow evaluation of the effect of increased drug loading on the dissolution performance and self-emulsifying properties of different formulations.

5.4.4. Evaluation of Gelucire®-based solid SNEDDS of indomethacin produced by HME

5.4.4.1. Determination of drug content

Drug content was determined for Gelucire® 48/16-based solid SNEDDSs to confirm equal distribution of the drug within the carrier used to produce different formulations. The results of drug content calculated as both % w/w and mg/g for different Gelucire®-based solid SNEDDS formulations of indomethacin prepared at different temperatures are illustrated in **Table 5.3**. All tested formulations prepared at 40°C or 50°C showed good drug content values ranging from 95.40 ± 0.91% to 99.95 ± 1.28% with no significant difference ($p < 0.05$) observed between formulations prepared at different temperatures. As mentioned previously in **Chapter 4,** the U.S. FDA (2003) indicates that a given batch of a blend may pass drug content uniformity test if a relative standard deviation (RSD) ≤ 4% was obtained from the assay of 60 samples or more from that batch.

Although analysis of large number of samples was not possible with lab scale experiments, the results of drug content obtained from the assay of 3 samples of Gelucire®-based solid SNEDDS formulations (**Table 5.3**) showed acceptable values of RSD <3% which may indicate that the adopted hot melt extrusion process produced Gelucire®-based solid SNEDDS formulations with reasonable values of drug content. Less than the expected 100% drug content values that were observed for the tested formulations may be due to loss of the drug during extrusion process which in turn can be ascribed to adherence of drug particles to the sides of the barrel of the extruder during mixing of different blends. Similar interpretation was given by Gumaste et al. (2013a) to explain the less than 100% drug content values obtained for various powder formulations prepared by adsorption of liquid SEDDS of the model drug, probucol, onto Neusilin® US2 at 1:1 w/w ratio. The authors proposed that increasing the batch size of a formulation may reduce the drug loss and hence improve the drug content of different formulations.

Table 5.3 Results of mean drug content (calculated as % w/w and mg/g) ± SD obtained for different Gelucire®-based solid SNEDDS formulations of indomethacin prepared at different temperatures

Code	Processing temperature (°C)	Mean drug content ± SD		RSD (%)
		(mg/g)	(% w/w)	
G1	40	47.71 ± 1.17	95.42 ± 2.35	2.46
G2		97.49 ± 0.94	97.49 ± 0.94	0.96
G3		191.67 ± 2.71	95.84 ± 1.35	1.41
G4		292.38 ± 4.13	97.46 ± 1.38	1.41
G5	40	48.88 ± 0.59	97.77 ± 1.17	1.20
G6		96.67 ± 1.63	96.67 ± 1.63	1.68
G7		192.34 ± 1.40	96.17 ± 0.70	0.73
G8		288.02 ± 1.83	96.01 ± 0.61	0.63
G9	50	48.30 ± 1.02	96.95 ± 2.03	2.10
G10		99.95 ± 1.28	99.95 ± 1.28	1.28
G11		193.68 ± 3.31	96.84 ± 1.65	1.71
G12		292.38 ± 4.00	97.46 ± 1.33	1.37
G13	40	195.00 ± 3.05	97.50 ± 1.53	1.57
G14		194.25 ± 3.65	97.13 ± 1.83	1.88
G15	40	192.07 ± 3.34	96.03 ± 1.67	1.74
G16		190.80 ± 1.83	95.40 ± 0.91	0.96

5.4.4.2. Self-nanoemulsification efficiency tests

Since Gelucire® 44/14 and Gelucire® 48/16 are considered as self-emulsifying vehicles (Gattefossé, 2015); self-nanoemulsification efficiency tests (or dispersibility tests) were conducted to evaluate any tendency of the formulated Gelucire®-based solid SNEDDS to show drug precipitation or phase separation when in contact with aqueous fluids. The results of these tests are shown in **Table 5.4**. Formulations prepared with Gelucire® 44/14 (G1 – G4) showed grade B appearance that indicates rapid formation of hazy or less clear bluish white nanoemulsions after dilution and mixing with purified water. On the other hand, Gelucire® 48/16- based SNEDDS formulations prepared at

40°C (G5 – G7) or 50°C (G9 – G11) using the drug: carrier ratios of 0.5: 10, 1: 10 and 2: 10 showed rapid formation of clear nanoemulsions (grade A) upon dilution with water. This could be due to higher HLB value of Gelucire® 48/16, compared to Gelucire® 44/14, which may facilitate dispersibility of the drug within the carrier leading to increased solubility (Kalpana et al., 2015). Formulations prepared with Gelucire® 48/16 and containing adsorbents (G13 – G16) showed grade B appearance which may be attributed to the intense mixing of the adsorbent with the drug and the carrier that took place inside the extruder barrel and this might cause embedding of the adsorbent within the carrier and led to the formation of less clear nanoemulsion. Signs of precipitation were observed in Gelucire®-based solid SNEDDS prepared with Gelucire® 48/16 or Gelucire® 44/14 at the drug: carrier ratio of 3: 10 (G4, G8 and G12). The higher content of indomethacin in these formulations may have exceeded the solubilizing ability of the carriers leading to precipitation of excess drug upon dilution.

Table 5.4 Results dispersibility tests and mean solubility ± SD obtained for different Gelucire®-based solid SNEDDS formulations of indomethacin prepared at different temperatures

Code	Processing temperature (°C)	Dispersibility tests	Precipitation	Solubility in phosphate buffer (mg/ml)
G1	40	B*	No	1.53 ± 0.02
G2		B	No	1.79 ± 0.05
G3		B	No	1.85 ± 0.05
G4		B	Yes	2.11 ± 0.07
G5	40	A**	No	2.10 ± 0.02
G6		A	No	2.25 ± 0.08
G7		A	No	3.08 ± 0.03
G8		B	Yes	2.81 ± 0.08
G9	50	A	No	2.38 ± 0.04
G10		A	No	2.46 ± 0.02
G11		A	No	3.13 ± 0.02
G12		B	Yes	3.01 ± 0.02
G13	40	B	No	3.11± 0.06
G14		B	No	2.88 ± 0.03
G15	40	B	No	3.09 ± 0.03
G16		B	No	3.03 ± 0.03
Drug	…	…	...	1.07 ± 0.07

* Rapid formation of less clear or bluish white nanoemulsion that emulsifies within 2 minutes.

** Rapid formation of clear or slightly bluish nanoemulsion that emulsifies within 1 minute.

5.4.4.3. Determination of droplet size

The droplet size is an important factor in spontaneous self-emulsification behaviour, because it provides information on the rate and extent of in vitro drug dissolution and the likely in vivo absorption. In general, smaller droplets allow faster dissolution rates and at the same time provide larger interfacial surface area for in vivo drug absorption (Weerapol et al., 2015a).

The results of measurement of droplet size, polydispersity index and zeta potential obtained for different Gelucire®-based solid SNEDDS formulations prepared by HME using Gelucire® 44/14 (G1 – G4), Gelucire® 48/16 (G5 – G12) as well as those prepared using Gelucire® 48/16 and different adsorbents (G13 – G16) are presented in **Table 5.5**.

Table 5.5 Mean droplet size, PDI and zeta potential of formulated Gelucire®-based solid SNEDDS of indomethacin

Code	Mean droplet diameter (nm) ± SD	Mean PDI ± SD	Zeta potential (mV)	Zeta deviation (mV)
Gelucire® 44/14	15.47 ± 0.16	0.26 ± 0.01	---	---
G1	18.46 ± 0.24	0.40 ± 0.03	-21.9	6.21
G2	26.96 ± 1.22	0.57 ± 0.07	-20.7	5.18
G3	45.33 ± 0.37	0.67 ± 0.01	-20.6	9.56
G4	34.09 ± 6.67	0.68 ± 0.20	-29.3	5.42
Gelucire® 48/16	11.78 ± 0.12	0.11 ± 0.01	---	---
G5	19.62 ± 0.42	0.53 ± 0.020	-23.4	7.93
G6	29.41 ± 1.26	0.78 ± 0.010	-23.1	7.46
G7	60.23 ± 11.12	0.86 ± 0.060	-22.4	3.35
G8	30.32 ± 1.88	0.81 ± 0.001	-34.4	3.62
G9	29.38 ± 2.16	0.68 ± 0.004	-27.3	5.84
G10	75.76 ± 1.57	0.43 ± 0.010	-23.6	6.59
G11	125.90 ± 3.25	0.34 ± 0.040	-24.5	4.08
G12	110.95 ± 3.75	0.28 ± 0.010	-29.2	8.66
G13	78.59 ± 1.12	0.75 ± 0.080	-21.0	6.94
G14	182.25 ± 1.06	0.51 ± 0.001	-19.6	6.35
G15	92.01 ± 13.42	0.87 ± 0.180	-20.1	6.78
G16	136.45 ± 1.48	0.73 ± 0.010	-19.8	6.22

The sizes of Gelucire® 44/14 and Gelucire® 48/16 globules produced in the dispersion medium (purified water) showed values of 15.47 ± 0.16 nm and 11.78 ± 0.12 nm, respectively. The presence of the drug in different solid SNEDDS formulations prepared with these carriers resulted in significant increase ($p < 0.05$) in the droplet size.

It can be observed that increasing the drug load of different Gelucire®-based SNEDDS formulations (G1 – G12) resulted in larger droplets diameter. Based on the surface active properties of both Gelucires® used, higher drug loading possibly led to formation of multiple micelles or closely packed Gelucire® molecules around the dispersed particles which in turn may have led to formation of larger dense particles. When the drug content was increased beyond the drug: carrier ratio of 2: 10, a decrease in the droplet size was noticed for formulations G4, G8 and G12. This may be ascribed to breakage or rupture of the micelles entrapping excessive amount of drug particles and hence, ejection of excess drug particles into the aqueous phase and production of smaller droplets.

Gelucire®-based solid SNEDDS formulations prepared with Gelucire® 48/16 (G5 – G12) exhibited larger droplet size compared to formulations prepared with Gelucire® 44/14 (G1 – G4). This could be explained on the basis that different surface active Gelucires® with different HLB values may produce different droplet size of the nanoemulsion upon dilution. Similarly, smaller droplets were observed for nanoemulsion formulations of Vitamin D (Guttoff et al., 2015) and Vitamin E (Saberi et al., 2013) prepared with Tween® 80 compared to those prepared using Tween® 20.

Also, differences in the molecular geometry (or critical packing parameter) of molecules of both Gelucires® may explain the larger droplets produced by Gelucire® 48/16-based SNEDDS formulations compared to those observed for formulations prepared using Gelucire® 44/14. Critical packing parameter that relates the cross sectional area of the tail group to that of the head group of a surfactant, may affect packing of the surfactant molecules at the oil-water interface and in turn may reflect differences in interfacial properties such as surface energy which influence the formation of ultrafine droplets (Guttoff et al., 2015, Saberi et al., 2013).

Further, differences in chemical structure between Gelucire® 48/16 and Gelucire® 44/14 may affect packing of the surfactant molecules at the boundaries between drug particles and aqueous medium (Guttoff et al., 2015, Marasini et al., 2012, Saberi et al., 2013). Gelucire® 44/14 is composed of fatty acid (C_8, C_{10}, C_{12}, C_{14}, C_{16} and C_{18}) esters of glycerol with high proportions of lauric (C_{12}) and myristic (C_{14}) fatty acids, PEG esters and free PEG (Chambin and Jannin, 2005, Gattefossé, 2012). On the other hand, Gelucire® 48/16 is mainly a PEG ester of palmitic (C_{16}) and stearic (C_{18}) fatty acids and

contains no glyceride portion (Gattefossé, 2015). It might be possible that absence of glyceride portion in Gelucire® 48/16 led to enhanced spontaneous formation of ultrafine droplets upon dilution of the formulation.

Larger droplets observed for Gelucire® 48/16-based SNEDDS formulations prepared at 50°C (G5 – G8) compared to formulations prepared at 40°C (G9 – G12) may be attributed to more efficient incorporation and dissolving of drug particles in carrier molecules that may have occurred at elevated temperatures more than that occurred at lower temperature of melting. Consequently, increased encapsulation of drug particles inside micelles led to formation of larger droplets (Rangel-Yagui et al., 2005).

Incorporation of adsorbents in Gelucire® 48/16-based solid SNEDDS formulations such as Neusilin® US2 (in formulations G13 and G14) or Florite® PS-200 (in formulations G15 and G16) resulted in increased droplet size compared to the formulation prepared without adsorbents (G7). These two adsorbents could have been embedded in the carrier along with the incorporated drug particles (Vithani et al., 2013) leading to formation of larger droplets.

5.4.4.4. Determination of solubility of the drug in Gelucire®-based solid SNEDDS formulations

Measurement of solubility of the drug in different Gelucire®-based solid SNEDDS formulations was carried out to investigate the effect of the type of the carrier as well as the drug loading on the solubility of indomethacin upon incorporation into different Gelucires®. The results of drug solubility in various formulations are presented in **Table 5.4**. It was observed that solubility of indomethacin in phosphate buffer pH 7.2 from all Gelucire®-based solid SNEDDS formulations was increased by 1.4 – 2.9 folds compared to the solubility of pure indomethacin in the same buffer (1.07 ± 0.07 mg/ml). Enhanced solubility of indomethacin from Gelucire®-based solid SNEDDS formulations could be attributed to decreased contact angle, increased wettability and micellar solubilization of drug particles by the carriers adopted in different formulations (Chambin et al., 2009, Damian et al., 2000, Horter and Dressman, 2001, Leuner and Dressman, 2000). A significant difference ($p < 0.05$) in the solubility of the drug was detected when formulations prepared with Gelucire® 44/14 (G1 – G4) were compared to formulations prepared with Gelucire® 48/16 (G5 – G8) using one way ANOVA. However, statistical comparison of formulations prepared with Gelucire® 48/16 either at 40°C (G5 – G8) or 50°C (G9 – G12) yielded no significant difference ($p > 0.05$) in the solubility of the drug. Only slight reductions in the solubility of indomethacin from Gelucire® 48/16-based solid

SNEDDS formulations containing Neusilin® US2 (G13 and G14) or Florite® PS-200 (G15 and G16) were observed.

5.4.5. Solid state characterization of Gelucire®-based solid SNEDDS of indomethacin

5.4.5.1. Fourier transform infrared spectroscopy (FTIR)

FTIR studies were carried out to detect any incompatibility and/or interaction between the drug, the carriers and the adsorbents used in preparation of Gelucire®-based solid SNEDDS formulations of indomethacin. As mentioned earlier in **Chapter 4**, FTIR is used for this purpose because each compound absorbs specific radiation frequencies according to its molecular structure. Therefore, formulations comprising the drug and different excipients may exhibit changes in their FTIR spectrum. These changes may appear in the form of disappearance, shifting or broadening of the characteristic peaks of each component used in the formulation (Lim et al., 2013).

FTIR spectra of indomethacin, Gelucire®-based solid SNEDDS formulations and the corresponding carriers and adsorbents used in different formulations are presented in **Figures 5.3 – 5.6**.

As explained earlier in **Chapter 4**, the obtained FTIR spectrum of pure indomethacin showed specific peaks for the γ-polymorph of the drug (Dupeyrón et al., 2013). Two strong peaks were observed at 1716 cm^{-1} related to asymmetric acid C=O of a cyclic dimer, while the peak showing at 1692 cm^{-1} related to the benzoyl C=O (Taylor and Zografi, 1997). Other absorption peaks were recorded at 2925 cm^{-1} (C-H stretching vibrations), 1223 cm^{-1} (asymmetric aromatic O-C stretching), and 1068 cm^{-1} (symmetric aromatic O-H stretching). Complex absorption patterns were observed in the region of 1300 – 650 cm^{-1} which is related to the aromatic structure of indomethacin. Therefore, strong or medium intensity bands appearing in this region may be less useful for structural characterization, while a weak intensity band in the region above 2000 cm^{-1} may be group-specific (Dupeyrón et al., 2013).

Gelucire® 44/14 showed principal peaks at 2887, 1737, 1460, 1341, 1106, 957 and 841 cm^{-1} (**Figure 5.3**). The FTIR spectrum of all solid SNEDDS formulations prepared with this carrier at 40°C (G1 – G4) exhibited all characteristic peaks of Gelucire® 44/14 in addition to the characteristic peaks of the drug at 1716, 1223 and 1068 cm^{-1}. Presence of specific drug peaks in the spectra of different Gelucire® 44/14-based solid SNEDDS formulations indicates that the molecular structure of indomethacin remained intact during melt processing at 40°C. Absence of some drug peaks in the spectra of these solid SNEDDS formulations may be because these peaks are more likely to be hidden in the baseline of the corresponding spectra. In addition, no new extra peaks were observed in the FTIR spectra of drug-loaded solid SNEDDS formulations (G1 – G4) which indicate that no chemical interaction occurred between the drug and the carrier.

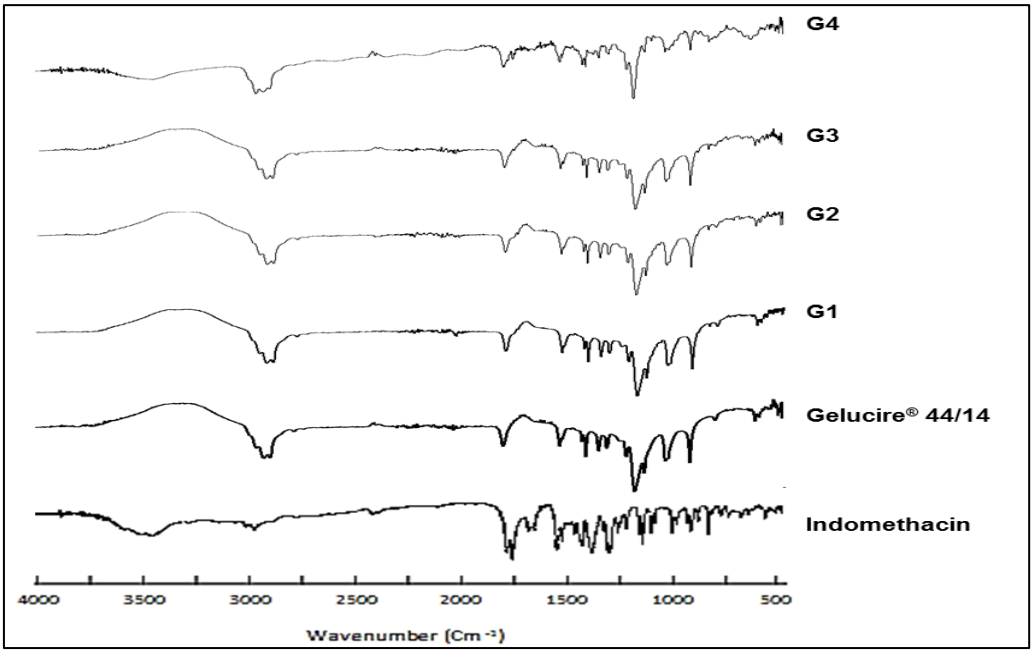


Figure 5.3 FTIR spectra of indomethacin, Gelucire® 44/14 and various Gelucire® 44/14-based solid SNEDDS of indomethacin prepared by HME at 40°C.

The FTIR spectrum of Gelucire® 48/16 is shown in **Figures 5.4 – 5.6** and is characterized by the main peaks appearing at 2887, 1735, 1467, 1343, 1113, 963, 842 and 529 cm^{-1}. As presented in **Figure 5.4 and Figure 5.5**, all solid SNEDDS formulations prepared with Gelucire® 48/16 either at 40°C (G5 – G8) or at 50°C (G9 – G12) showed the absorption peaks of the carrier at the same position as well as the specific peaks of indomethacin mentioned earlier. Also, no chemical interaction was obvious within these Gelucire®-based solid SNEDDS formulations as evidenced by absence of new extra peaks in their corresponding spectra.

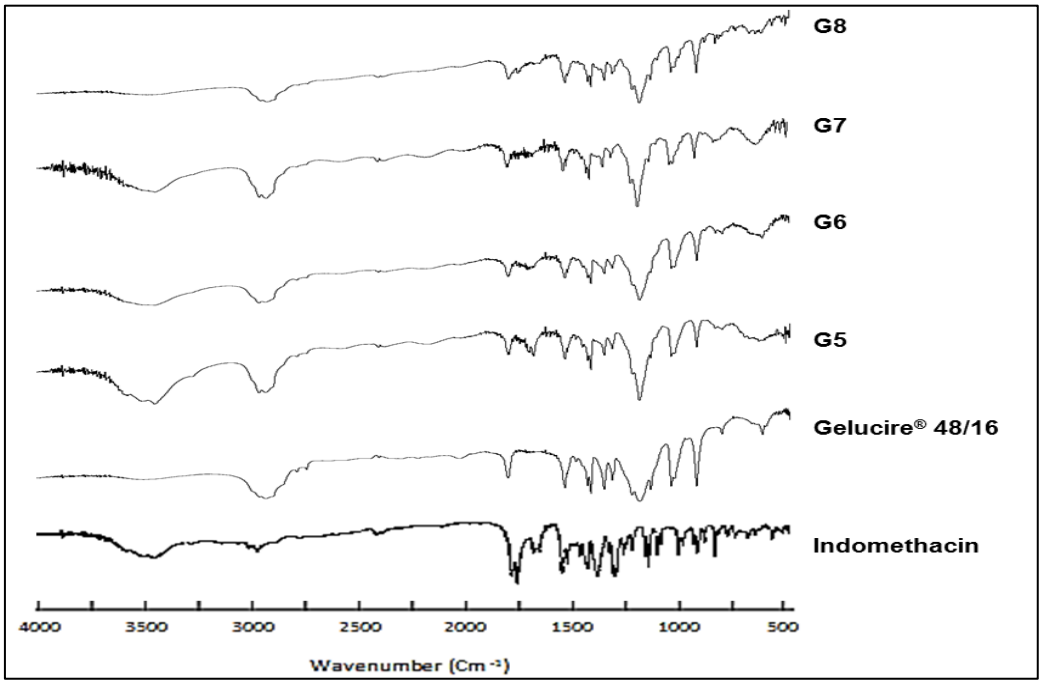


Figure 5.4 FTIR spectra of indomethacin, Gelucire® 48/16 and various Gelucire® 48/16-based solid SNEDDS of indomethacin prepared by HME at 40°C.

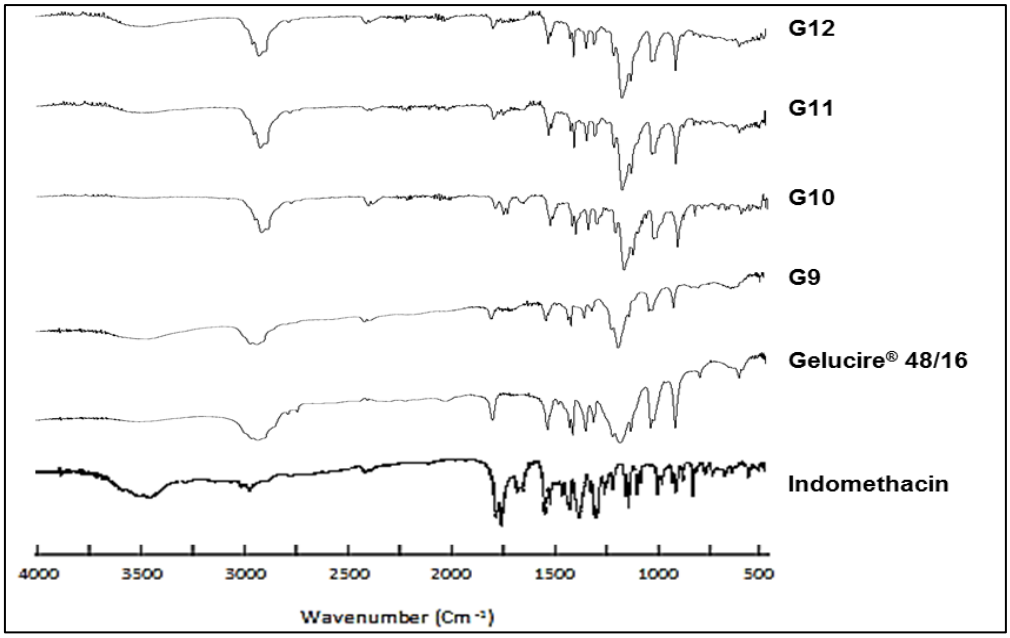


Figure 5.5 FTIR spectra of indomethacin, Gelucire® 48/16 and various Gelucire® 48/16-based solid SNEDDS of indomethacin prepared by HME at 50°C.

The FTIR spectra of Gelucire® 48/16-based solid SNEDDS of indomethacin in which Neusilin® US2 (G13 & G14) or Florite® PS-200 (G15 and G16) were incorporated are shown in **Figure 5.6**. Formulations G13 and G14 showed the characteristic peaks of Gelucire® 48/16 and the drug at the same frequencies, in addition to the specific peaks of Neusilin® US2 (magnesium aluminometa silicate) appearing at 3444, 1638, 1380, 1041 and 472 cm^{-1}. Also, the characteristic peaks of Florite® PS-200 (calcium silicate) observed at 3448, 1637, 1348, 1039, 784, 607 and 467 cm^{-1} in addition to absorption peaks of the drug and the carrier were noticed in formulations G15 and G16 prepared with these three components. The presence of the absorption peaks specific for the drug, the carrier and the adsorbent in Gelucire® 48/16-based solid SNEDDS (G13 – G16) indicates compatibility of components used in different formulations.

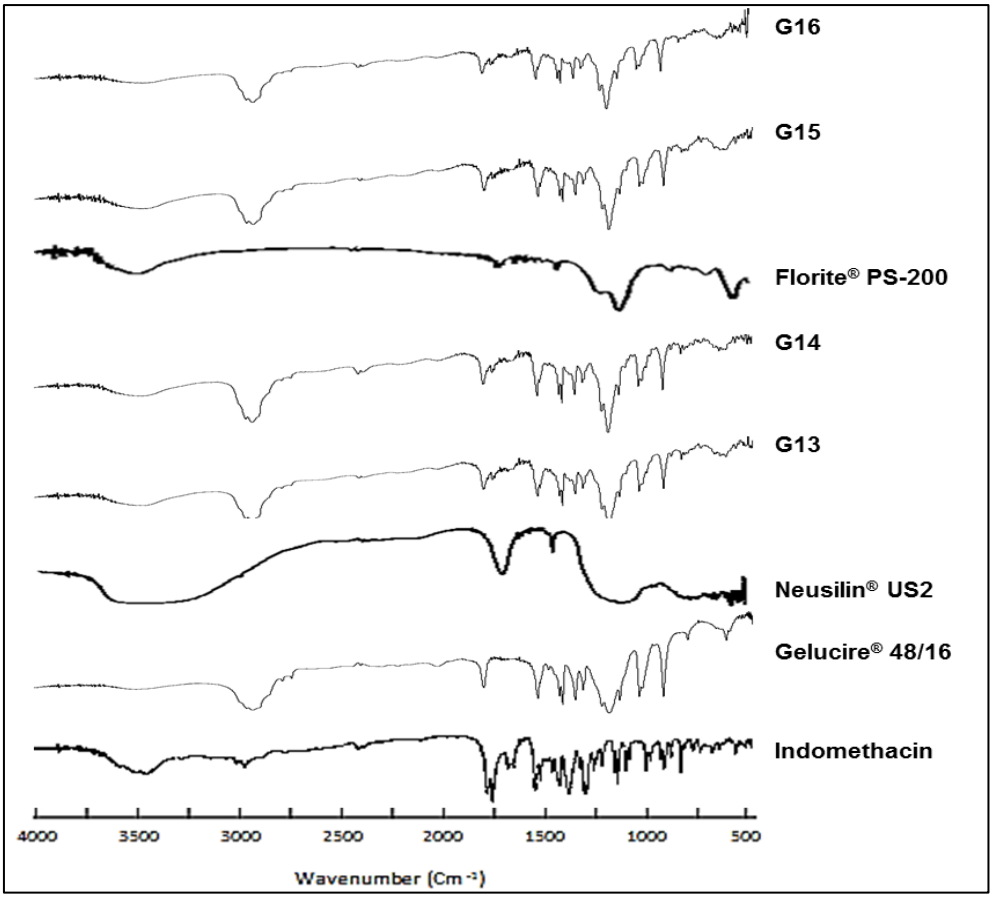


Figure 5.6 FTIR spectra of indomethacin, Gelucire® 48/16, Neusilin® US2, Florite® PS-200 and various Gelucire® 48/16-based solid SNEDDS of indomethacin prepared by HME at 40°C.

Similar FTIR findings were reported for solid dispersions of flurbiprofen prepared in Gelucire® 44/14, where the optimized formulation showed absorption peaks of the drug and the carrier at the same frequencies indicating compatibility and absence of interaction between the drug and the carrier (Daravath et al., 2015).

5.4.5.2. Differential scanning calorimetry (DSC)

Thermal behaviour and physical state of a drug within a formulation can be determined by DSC studies (Kallakunta et al., 2012, Ramasahayam et al., 2015). DSC traces of different Gelucire®-based solid SNEDDS formulations of indomethacin are depicted in **Figures 5.7 – 5.10**.

Pure indomethacin exhibited a sharp endothermic peak, that corresponds to its melting point, at 162.31°C and this indicates that the drug is present in a crystalline form. The value of ΔH (enthalpy) was found to be equal to 106.2950 J/g. In **Figure 5.7**, Gelucire® 44/14 showed an endothermic melting peak at 46.49°C which is also the only peak that appeared in the DSC trace of different solid SNEDDS formulations prepared with this carrier (G1 – G4) with no peaks representing indomethacin.

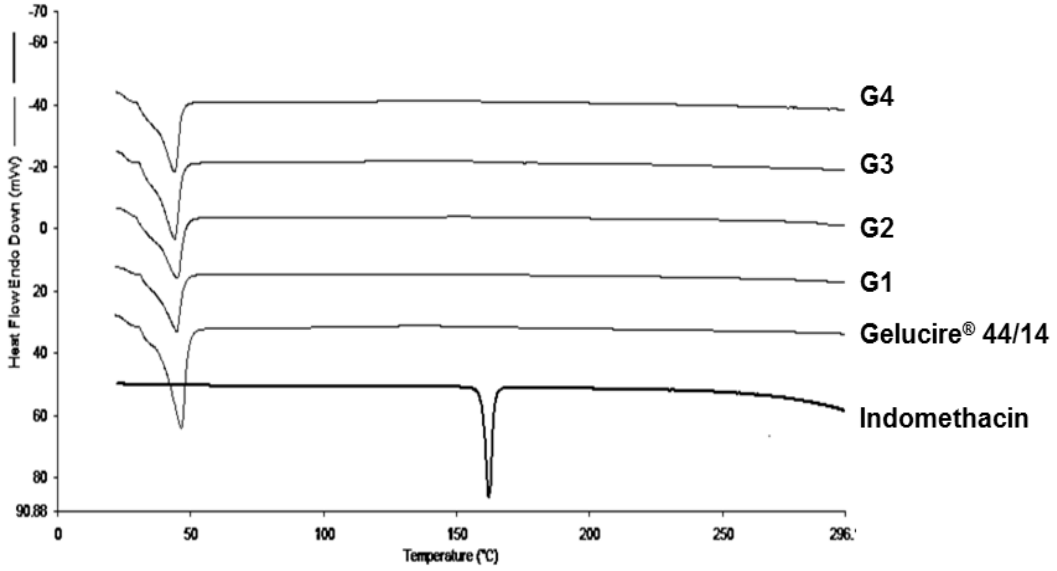


Figure 5.7 DSC traces of indomethacin, Gelucire® 44/14 and various Gelucire® 44/14-based solid SNEDDS of indomethacin prepared by HME at 40°C.

As can be seen in **Figures 5.8, 5.9 and 5.10**, Gelucire® 48/16 exhibited an endothermic melting peak at 51.10°C. As can be observed in **Figure 5.8 & Figure 5.9**, all solid SNEDDS formulations of indomethacin prepared using Gelucire® 48/16 at 40°C (G5 – G8) or at 50°C (G9 – G12) did not show an obvious endothermic peak corresponding to the melting of crystalline indomethacin. In addition, a kink (step change) appeared in the DSC traces of formulations G10, G11 and G12 (**Figure 5.9**) below the main melting point of Gelucire® 48/16 which possibly indicates a T_g of

indomethacin (42°C). Therefore, these formulations probably contain an amorphous component of the drug which wasn't observed in the other formulations.

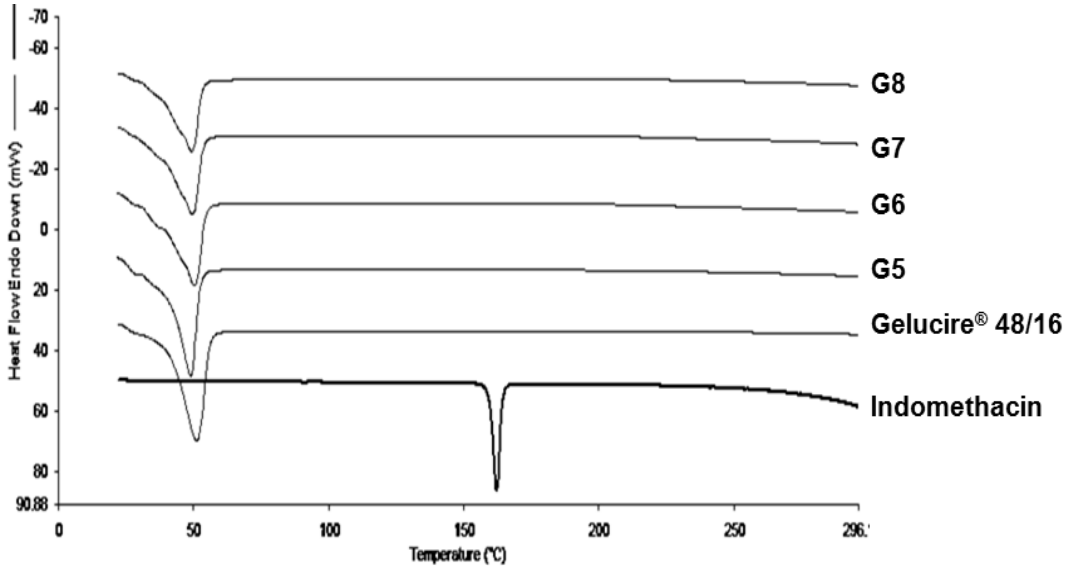


Figure 5.8 DSC traces of indomethacin, Gelucire® 48/16 and various Gelucire® 48/16-based solid SNEDDS of indomethacin prepared by HME at 40°C.

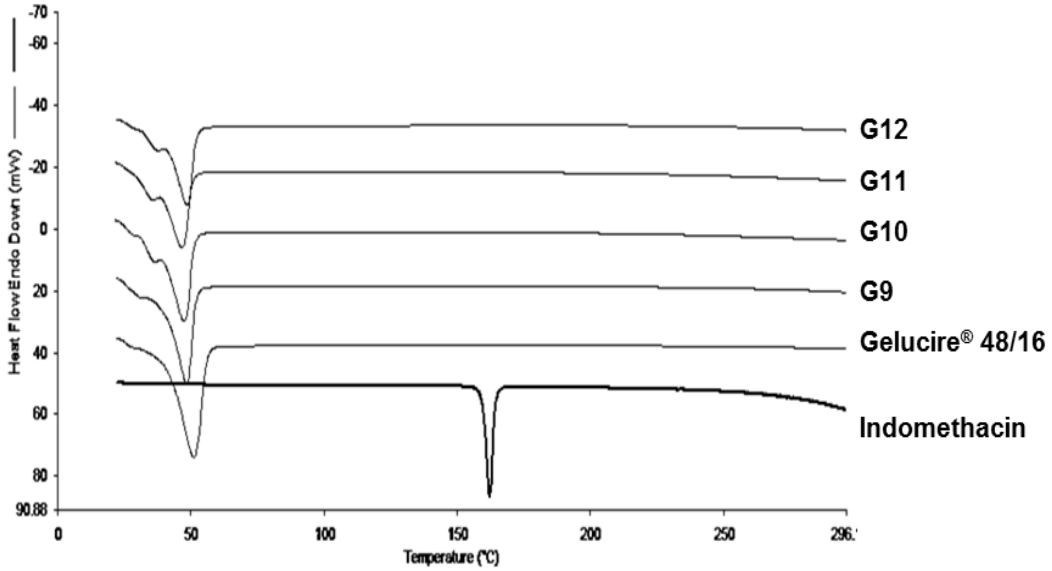


Figure 5.9 DSC traces of indomethacin, Gelucire® 48/16 and various Gelucire® 48/16-based solid SNEDDS of indomethacin prepared by HME at 50°C.

In **Figure 5.10**, Neusilin® US2 (magnesium aluminometa silicate) showed a broad endothermic peak at 228.61°C, while Florite® PS-200 (calcium silicate) did not show any prominent peak over the entire range of the tested temperature. In addition, no peaks of crystalline indomethacin were noticed in solid SNEDDS formulations prepared with Gelucire® 48/16 at 40°C and incorporated Neusilin® US2 (G13 and G14) or Florite® PS-200 (G15 and G16).

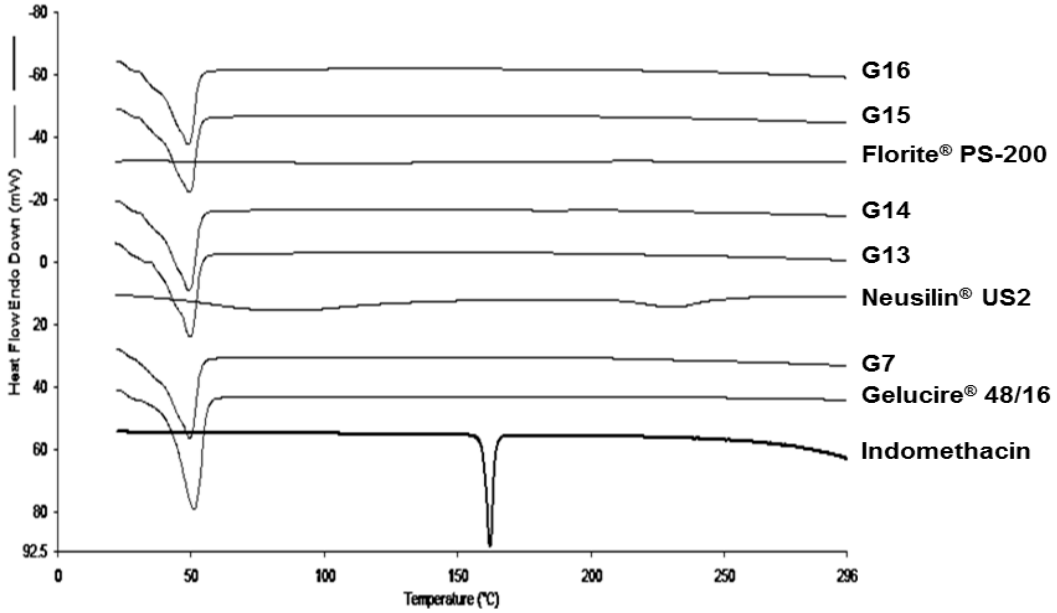


Figure 5.10 DSC traces of indomethacin, Gelucire® 48/16, Neusilin® US2, Florite® PS-200 and various Gelucire® 48/16-based solid SNEDDS of indomethacin prepared by HME at 40°C.

Therefore, the absence of a specific endothermic peak corresponding to the melting of crystalline indomethacin in all drug-loaded solid SNEDDS formulations prepared with Gelucire® 44/14 or Gelucire® 48/16 at different temperatures (except formulations G10 – G12 which showed a possible Tg of the drug as observed in **Figure 5.9**) may indicate that the drug exists in a molecularly dispersed state within the carrier. This assumption can be supported by the measured solubility values presented earlier in **Table 5.4**.

Overall, these results of DSC analysis are not conclusive to determine if the drug is present in crystalline or amorphous states within different Gelucire®-based formulations. If a crystalline drug is present in the final formulation, it will dissolve in the melted Gelucire® during heating of the sample in the DSC and hence no melting peak of the crystalline drug can be observed. On the other hand, if the drug is dissolved in the carrier and then solidified in an amorphous form, the expected T_g of the drug will be

obvious in the DSC trace of the formulation. In the case of Gelucire® formulations of indomethacin and because the T_g of the drug is about 42°C, it is expected that the peak corresponding to the Tg of indomethacin will be overlapped with the broad peak of melting of Gelucire®. Therefore, the results of DSC should be combined with the results of other characterization tests such as the X-ray diffraction and the SEM to determine the presence of crystalline drug within different Gelucire®-based formulations.

Also, it is important to note that although the DSC analysis is a widely used technique to determine the crystallinity of amorphous formulations, it still has a low limit of sensitivity to detect smaller amounts of crystals that is less than 10% (w/w) (Nagapudi and Jona, 2008, Saleki-Gerhardt et al., 1994).

DSC analysis of various drugs dispersed in Gelucire® 44/14 or Gelucire® 48/16 have shown that absence of the endothermic melting peak of the drug may indicate transformation of the crystalline drug to an amorphous state. For example, the DSC analysis of solid lipid dispersion formulation of rivaroxaban (BCS Class II drug) prepared by spray drying method using Gelucire® 48/16, Compritol HD5 and Labrasol as the dispersion matrix, showed that the endothermic peak of the drug disappeared in the DSC traces of the solid lipid dispersion formulation and this was attributed to conversion of the drug into an amorphous form (Ganesh, 2016). Also, the DSC studies of semi-solid dispersions of piroxicam prepared in Gelucire® 44/14 showed absence of the crystalline melting peak of the drug and appearance of a new sharp melting peak. Absence of crystalline piroxicam melting peak was explained on the basis that the drug was present in amorphous state rather than a crystalline phase within the semi-solid dispersion formulation, while the resulting new sharp melting peak was attributed to a chemical reaction between –OH group on the benzothiazine ring of piroxicam and the fatty acids of Gelucire® 44/14 that took place because of heating applied during preparation of semi-solid dispersion formulation (Karataş et al., 2005). In addition, solid self-emulsifying formulation of lercanidipine hydrochloride prepared by adsorption of liquid self-emulsifying formulations of the drug (comprising Gelucire® 44/14 as an oil phase, labrasol® as a surfactant and Transcutol®-P as a co-surfactant) onto Neusilin®US2 showed a DSC trace without representative peak of the drug and this was related to maintaining of the drug in a dissolved state within the emulsifying ingredients and hence, inhibiting drug recrystallization (Kallakunta et al., 2013).

5.4.5.3. X-ray diffraction (XRD)

The degree of crystallinity and amorphous content of pharmaceutical formulations can be detected by XRD analysis. This technique is also useful to determine the percentage of components in a formulation so it can be applied for quantitative analysis of pharmaceutical mixtures (Gilmore, 2011).

Qualitative XRD analysis was conducted in this study to confirm crystallinity of the drug within different formulations. The XRD diffractograms obtained for different Gelucire®-based solid SNEDDS of indomethacin prepared with different Gelucires® and adsorbents are presented in **Figures 5.11 – 5.14**. The XRD patterns of indomethacin, carriers and adsorbents employed in different formulations were also obtained for the purpose of comparison.

In the indomethacin diffractogram, the characteristic narrow and sharp diffraction peaks appearing at 2θ values of 11.9°, 13°, 17.2°, 19.9°, 20.6°, 21.2°, 22.1°, 23.4°, 24.3°, 26.9°, 29.7° and 31.9° indicate the crystalline nature of the drug. The resulting diffractogram of indomethacin is similar to those reported in previous studies (Dupeyrón et al., 2013, Lim et al., 2013).

The XRD pattern of Gelucire® 44/14 presented in **Figure 5.11** showed intrinsic sharp peaks at 2θ values of 19.2°, 23.4°, 26.5°, 32.7°, 36.3° and 39.8°. Also, it was observed that solid SNEDDS formulations prepared with Gelucire® 44/14 at 40°C (G1 – G4) showed the peaks noticed for the carrier. Formulations G1 – G3 exhibited an absence of representative crystalline peaks of the drug which indicates that the drug remained in a molecularly dissolved state within the carrier. However, formulation G4 prepared with higher ratio of drug: Gelucire® 44/14 (3: 10) showed sharp peaks related to crystalline indomethacin at 2θ values of 11.9°, 17.2°, 21.2° and 29.7°. It could be possible that the presence of excess drug particles in this formulation have exceeded the solubilizing capacity of the carrier at 40°C and therefore, remained incompletely dissolved in the carrier. Also, the absence of an amorphous halo and the appearance of extra peaks of the drug in the XRD diffractogram of formulation G4 may suggest that the formulation is semi-crystalline. Combining the results of XRD analysis for formulations G1 – G4 (**Figure 5.11**) together with the DSC results of the same formulations (**Figure 5.7**) may suggest that the drug exists in a molecularly dispersed state within the carrier especially when added at lower concentrations (as in formulations G1 – G3) but not when added at higher concentrations (as in formulation G4).

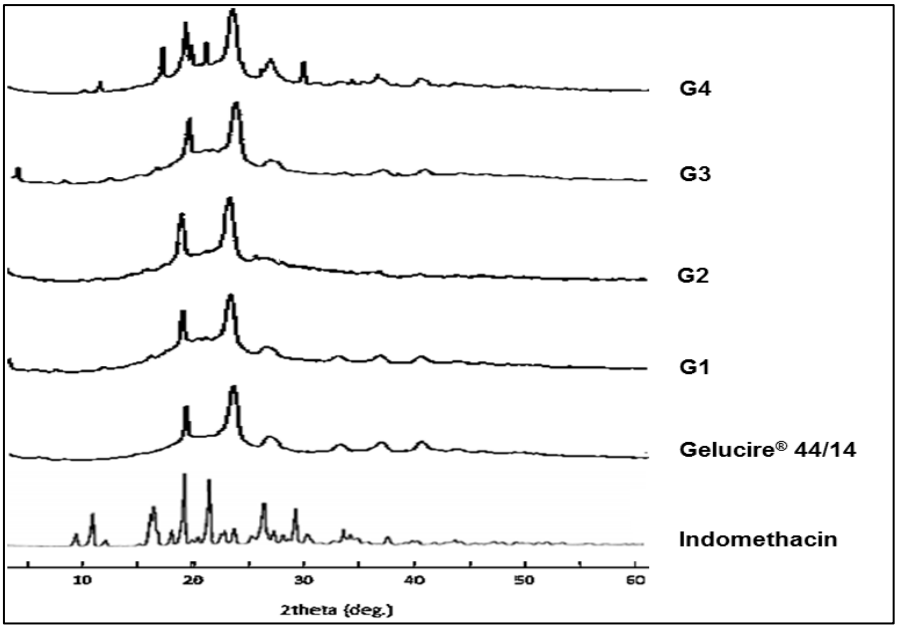


Figure 5.11 XRD diffractograms of indomethacin, Gelucire® 44/14 and various Gelucire® 44/14-based solid SNEDDS of indomethacin prepared by HME at 40°C.

On the other hand, the XRD pattern of Gelucire® 48/16 presented in **Figure 5.12, 5.13 and 5.14** exhibited high intensity peaks at 2θ values of 19.4° and 23.5 °. Solid SNEDDS formulations prepared with Gelucire® 48/16 at 40°C (G5 – G8) or at 50°C (G9 – G12) showed the characteristic peaks of the carrier and no crystalline peaks related to the drug. However, formulations G8 (**Figure 5.12**) and G12 (**Figure 5.13**) prepared with increased drug load (drug: carrier ratio 3: 10) showed peaks of crystalline drug at 2θ values of 11.9° and 26.9°. This could be due to that excess drug may have exceeded the solubilizing potential of the carrier at either 40°C or 50°C and hence, excess crystalline drug particles remained incompletely dissolved. Again, visualizing the XRD diffractograms of formulations G5 – G12 (**Figures 5.12 and 5.13**) with the DSC traces of the same formulations (**Figures 5.8 and 5.9**) may indicate that the drug exists in a molecularly dispersed state within the carrier only when added at lower concentrations (as in formulations G5 – G7 and G9 – G11) but not at higher concentrations (as in formulations G8 and G12).

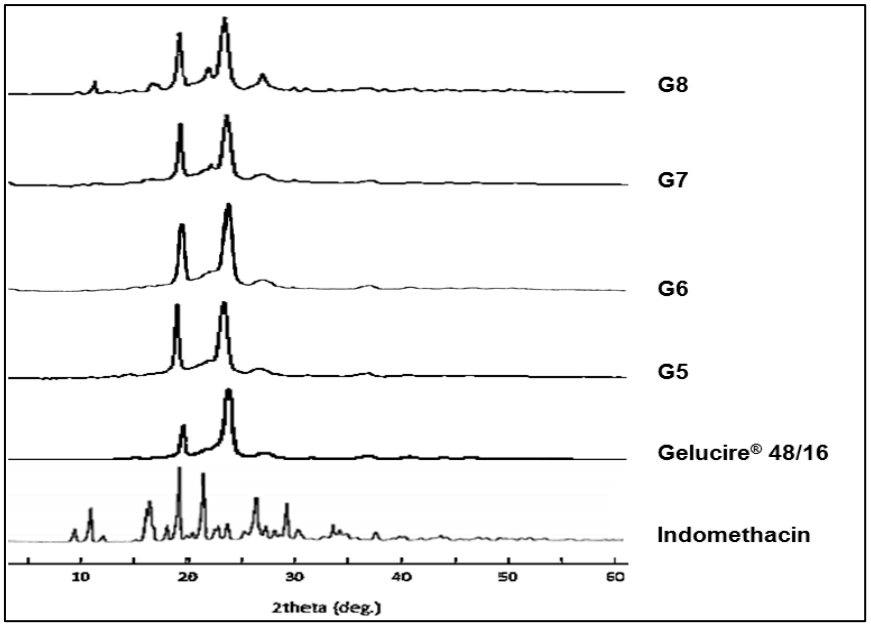


Figure 5.12 XRD diffractograms of indomethacin, Gelucire® 48/16 and various Gelucire® 48/16-based solid SNEDDS of indomethacin prepared by HME at 40°C.

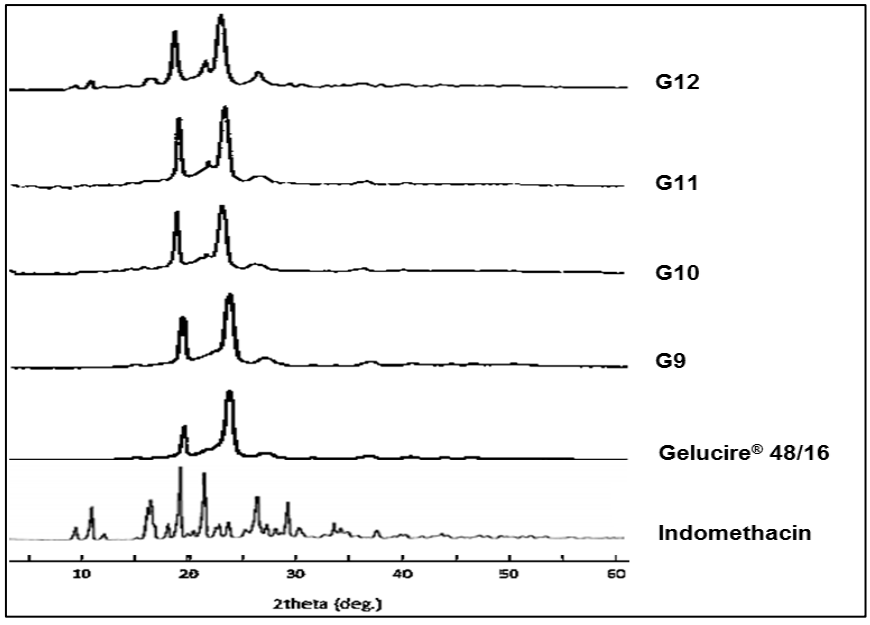


Figure 5.13 XRD diffractograms of indomethacin, Gelucire® 48/16 and various Gelucire® 48/16-based solid SNEDDS of indomethacin prepared by HME at 50°C.

The XRD diffractograms of Gelucire® 48/16-based solid SNEDDS formulations of indomethacin prepared at 40°C and incorporated Neusilin® US2 (magnesium aluminometa silicate) or Florite® PS-200 (calcium silicate) as adsorbents are shown in **Figure 5.14**. Neusilin® US2 diffractogram showed two halo peaks centered on 2θ values of 20.6° and 35° with no high intensity peaks, while Florite® PS-200 diffractogram showed sharp diffraction peaks at 2θ values of 5.6°, 10.6°, 21.2°, 28.5°, 38.1°, 44.3° and 50° which indicate partial crystalline nature of this adsorbent. Diffractograms of formulations that contained different amounts of Neusilin® US2 (G13 and G14) or Florite® PS-200 (G15 and G16) exhibited the specific peaks of Gelucire® 48/16 in addition to crystalline peaks of indomethacin appearing at 2θ values of 11.9° and 26.9°. Adsorbents added to these formulations might have been embedded within Gelucire® 48/16 and therefore, the amount of the carrier available to dissolve the drug is reduced and part of the crystalline drug remained undissolved. In addition, absence of the peaks of the two adsorbents in the diffractograms of their corresponding formulations may be due to their small amounts present relative to the amount of the carrier in formulations examined. Appearance of the peaks of crystalline indomethacin in the XRD diffractograms of Gelucire® 48/16-based solid SNEDDS formulations containing Neusilin® US2 (G13 and G14) or Florite® PS-200 (G15 and G16) (**Figure 5.14**) contradicts the DSC traces of the same formulations (**Figure 5.10**) and this confirms that XRD and DSC analyses should be visualized together to indicate any crystallinity of the drug in the final tested formulations.

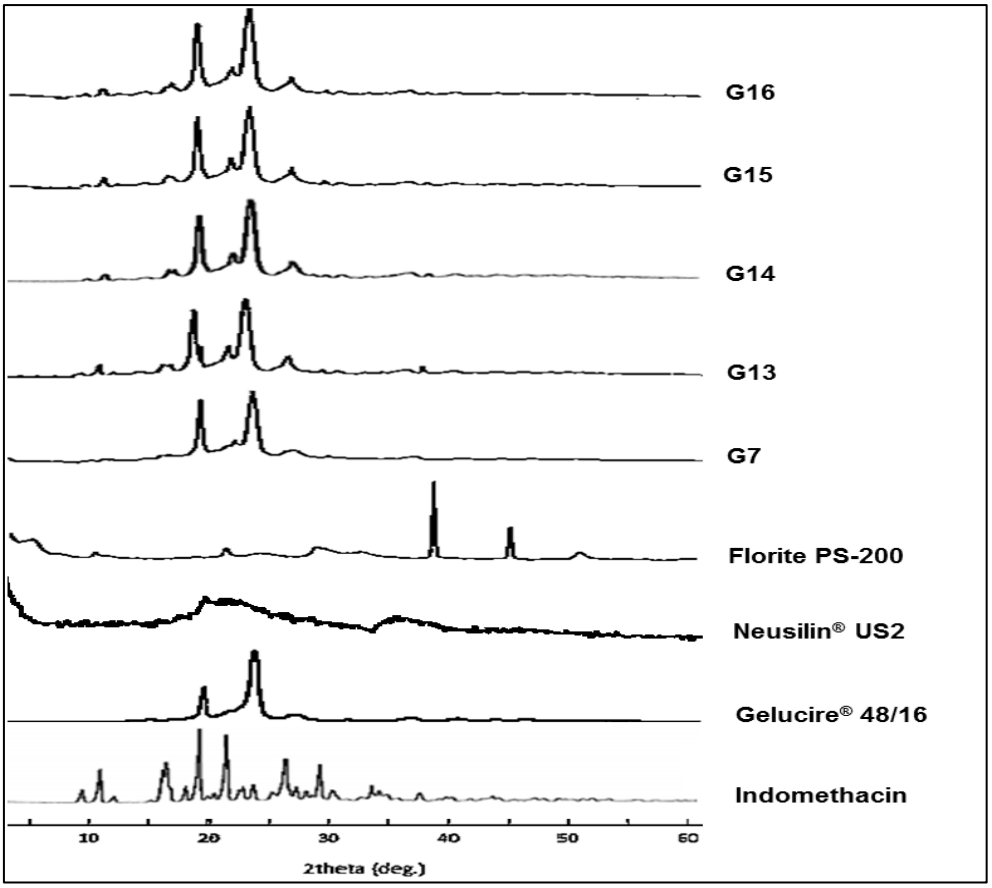


Figure 5.14 XRD diffractograms of indomethacin, Gelucire® 48/16, Neusilin® US2, Florite® PS-200 and various Gelucire® 48/16-based solid SNEDDS of indomethacin prepared by HME at 40°C.

5.4.5.4. Scanning electron microscopy (SEM)

Surface morphology of indomethacin, Gelucires®, Neusilin® US2, Florite® PS-200 and their corresponding Gelucire®-based solid SNEDDS formulations were investigated by scanning electron microscope. The micrographs obtained are presented in **Figures 5.15 – 5.18**.

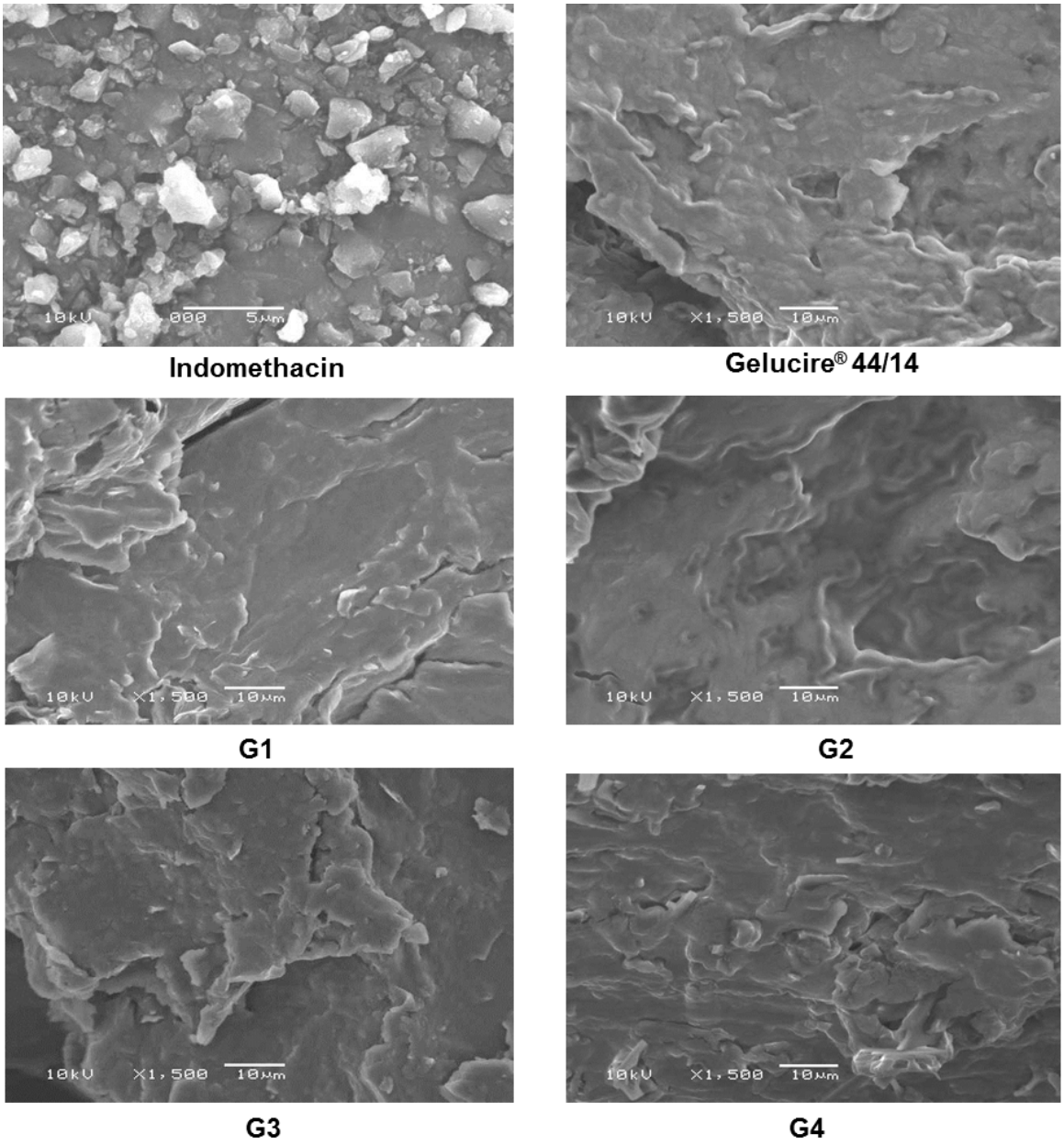


Figure 5.15 SEM micrographs of indomethacin, Gelucire® 44/14 and various Gelucire® 44/14-based solid SNEDDS of indomethacin prepared by HME at 40°C.

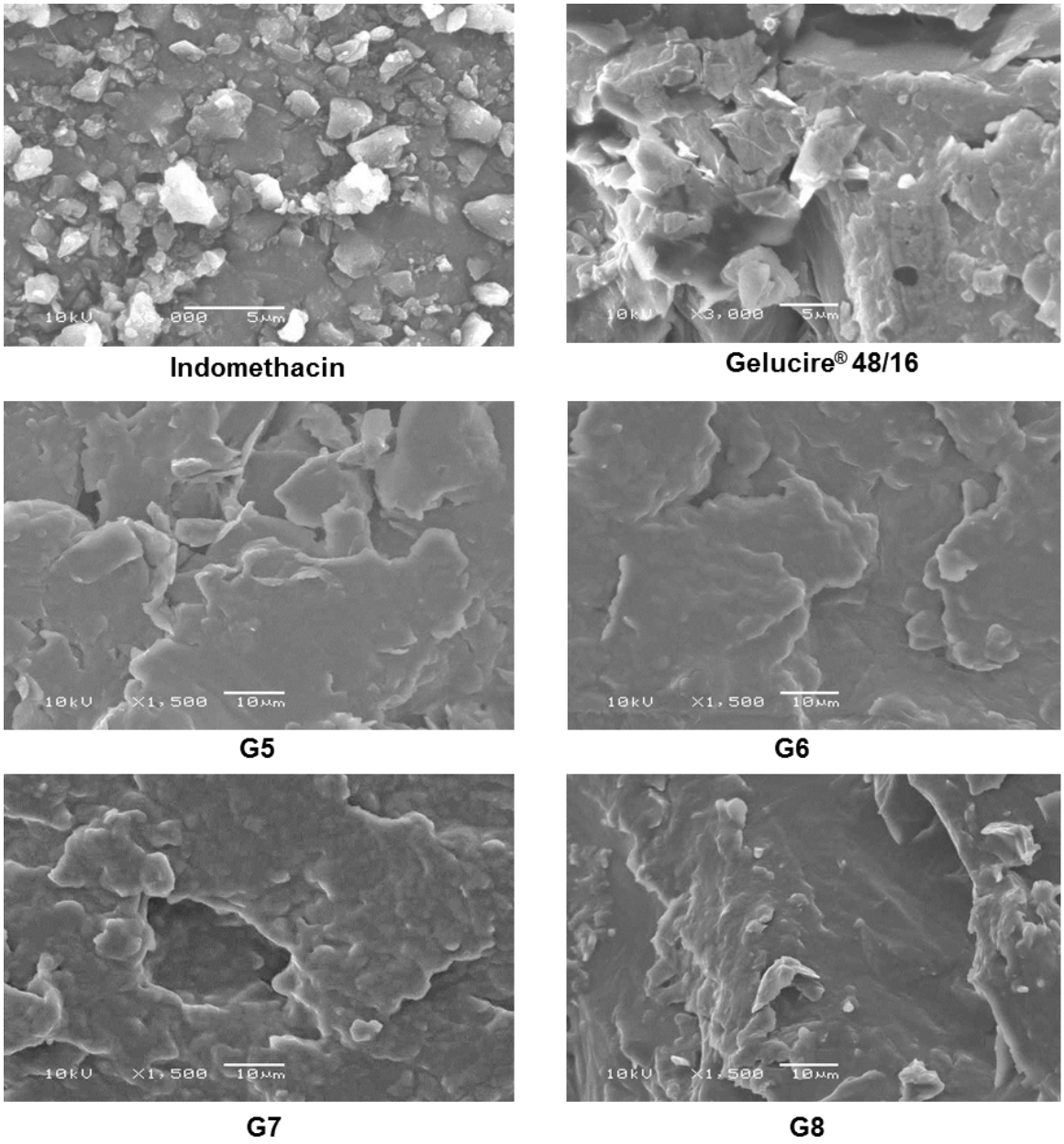


Figure 5.16 SEM micrographs of indomethacin, Gelucire® 48/16 and various Gelucire® 48/16-based solid SNEDDS of indomethacin prepared by HME at 40°C.

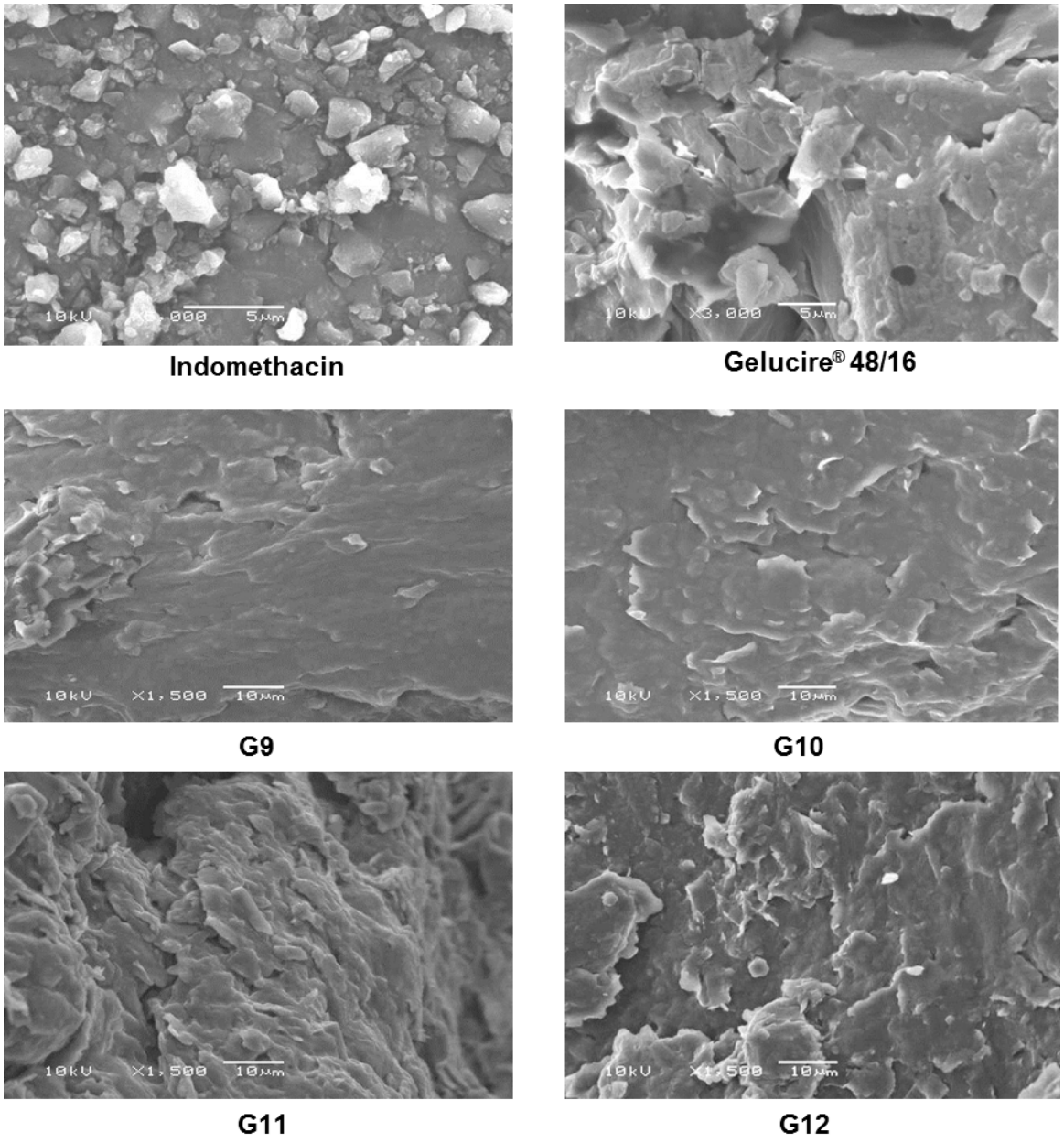
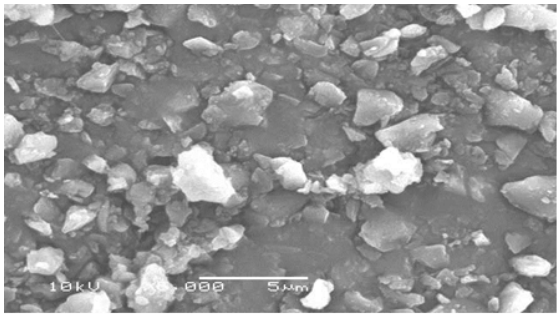
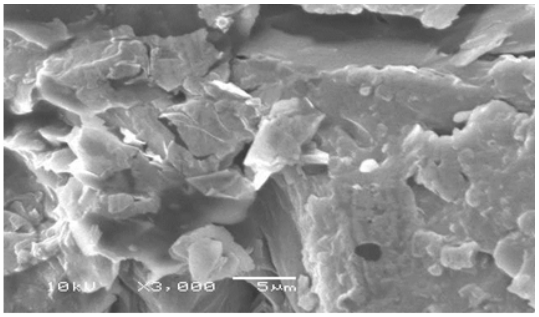


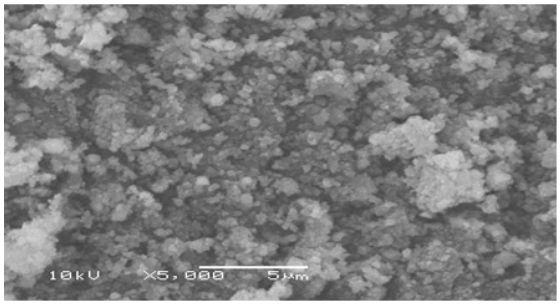
Figure 5.17 SEM micrographs of indomethacin, Gelucire® 48/16 and various Gelucire® 48/16-based solid SNEDDS of indomethacin prepared by HME at 50°C.



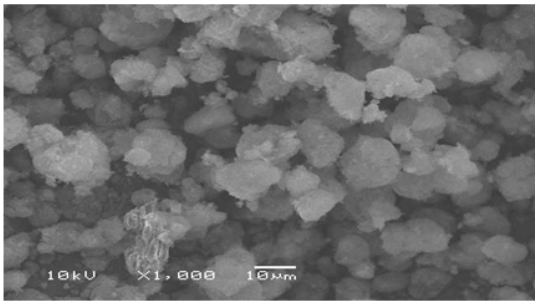
Indomethacin



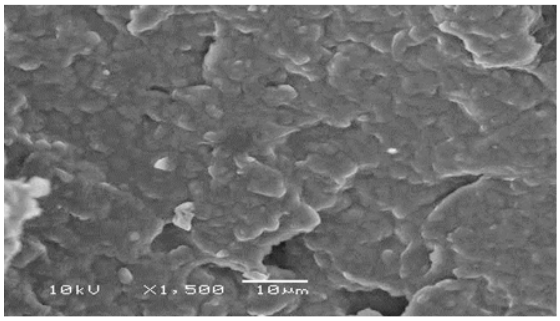
Gelucire® 48/16



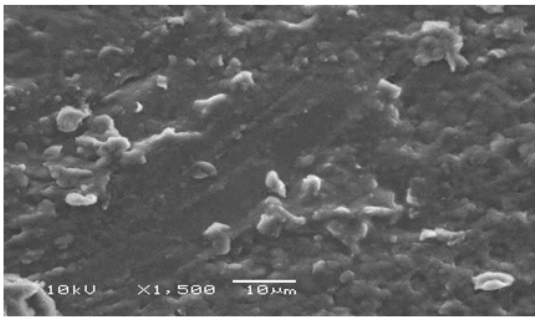
Neusilin® US2



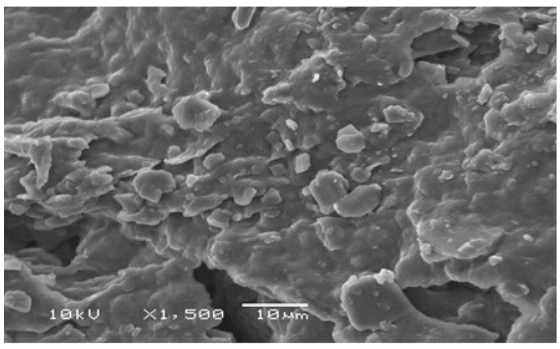
Florite® PS-200



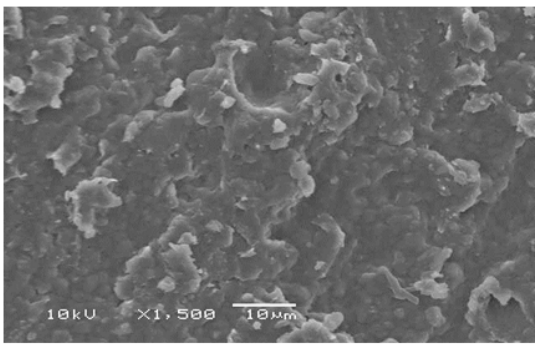
G13



G14



G15



G16

Figure 5.18 SEM micrographs of indomethacin, Gelucire® 48/16, Neusilin® US2, Florite® PS-200 and various Gelucire® 48/16-based solid SNEDDS of indomethacin prepared by HME at 40°C.

Scanning electron micrograph of indomethacin particles revealed multifaceted structures with smooth surfaced rectangular crystals. The surface topographies of Gelucire® 44/14 (**Figure 5.15**) and Gelucire® 48/16 (**Figure 5.16, 5.17 & 5.18**) appeared similar with comparatively smooth textures despite differences in their consistency at room temperature, where Gelucire® 44/14 exists as a sticky and semi-solid mass while Gelucire® 48/16 exists in the form of solid pellets. Micrographs of solid SNEDDS formulations prepared at 40°C using Gelucire® 44/14 (G1 – G4) showed relatively homogenous mixtures that looked like a matrix due to dispersion of the drug in the softened carrier at the molecular level. However, formulation G4 showed some crystals of the drug and this confirms its XRD studies.

In addition, scanning electron microscopy of formulations prepared using Gelucire® 48/16 at 40°C (**Figure 5.16**) or at 50°C (**Figure 5.17**) revealed that the drug was homogenously dispersed within the carrier at both melting temperatures as indicated by the absence of drug particles in different micrographs obtained for formulations G5 – G7 and formulations G9 – G11. On the other hand, micrographs obtained for formulations G8 and G12 prepared with increased drug loading at 40°C or at 50°C, respectively, showed drug particles that did not disperse completely within the carrier possibly due to increased viscosity of both formulations due to the presence of high drug loadings. These observations for G8 and G12 support their XRD studies.

SEM micrographs of Neusilin® US2 and Florite® PS-200 (**Figure 5.18**) showed highly porous granular materials. Formulations prepared with Gelucire® 48/16 that included adsorbents like Neusilin® US2 (G13 and G14) or Florite® PS-200 (G15 and G16) exhibited the matrix appearance observed for different formulations prepared with Gelucire® 48/16 with no adsorbents added (G5 – G8). The matrix-like appearance observed may be due to homogenous dispersion of the drug within the carrier at the melting temperature in addition to the relatively small amount of adsorbent incorporated if compared to the amount of the carrier used in different formulations. Even with the matrix-like appearance, some drug particles did not disperse completely within the carrier and these particles were obvious in the micrographs of G13 – G16 (**Figure 5.18**). These observations appear to be in accordance with the XRD analysis of these formulations (**Figure 5.14**).

SEM observations were previously reported for different solid dispersions prepared for various drugs in different Gelucire® grades. For instance, scanning electron

micrographs of carvedilol (Potluri et al., 2011), glimepiride (Makar et al., 2013) and indomethacin (El-Badry et al., 2009) solid dispersions prepared using Gelucire® 50/13 revealed homogenous formulations which were attributed to dispersion of the drug in the molten carrier.

5.4.6. In vitro dissolution studies

Dissolution of indomethacin from different Gelucire®-based solid SNEDDS formulations was conducted in phosphate buffer pH 7.2 and compared to the dissolution from pure drug. The dissolution profiles obtained are presented in **Figures 5.19 – 5.22**. Although the tested formulations showed maximum percentage of drug release within 15 – 20 minutes, the dissolution studies were continued for 1 hour, as suggested by Kallakunta et al. (2012), to observe any precipitation or changes that may develop over a period of time. Different dissolution parameters such as the mean dissolution time (MDT), the dissolution efficiency after 15 minutes ($\%DE_{15}$) in addition to the % drug released after 15 minutes ($\%Q_{15}$) were calculated to compare different dissolution profiles obtained for different formulations. These parameters are presented in **Table 5.6**. Comparison based on these dissolution parameters was considered, as indicated by Podczeck (1993), to avoid inadequate characterization of the dissolution process that may take place if dissolution profiles are compared based on single point measurement as specified in the British Pharmacopoeia (2015) for the dissolution of active substance filled in capsule dosage forms.

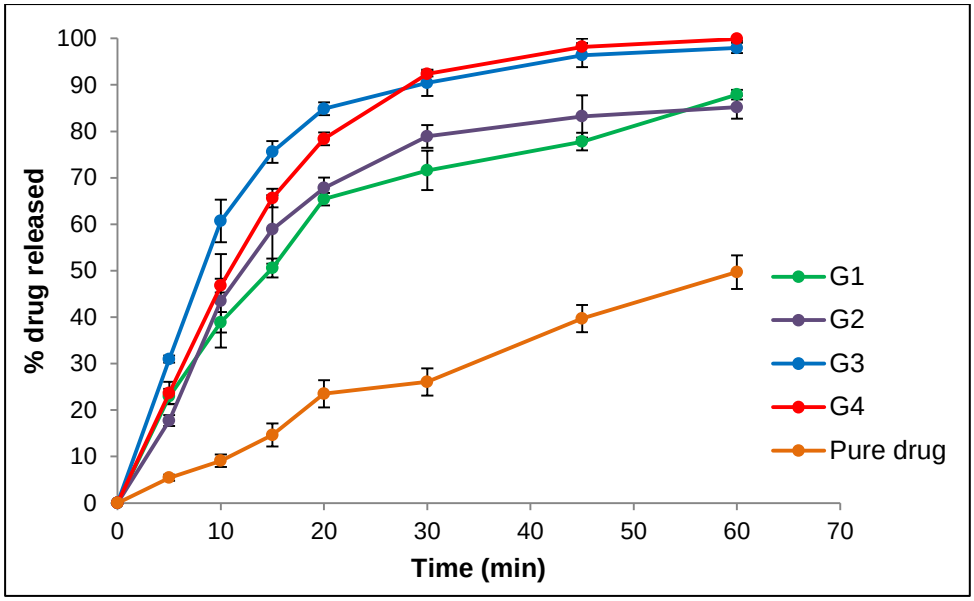


Figure 5.19 In vitro dissolution profiles of pure indomethacin, Gelucire® 44/14-based solid SNEDDS formulations prepared by HME at 40°C (G1 – G4) in phosphate buffer pH 7.2 (mean ± SD, n=3).

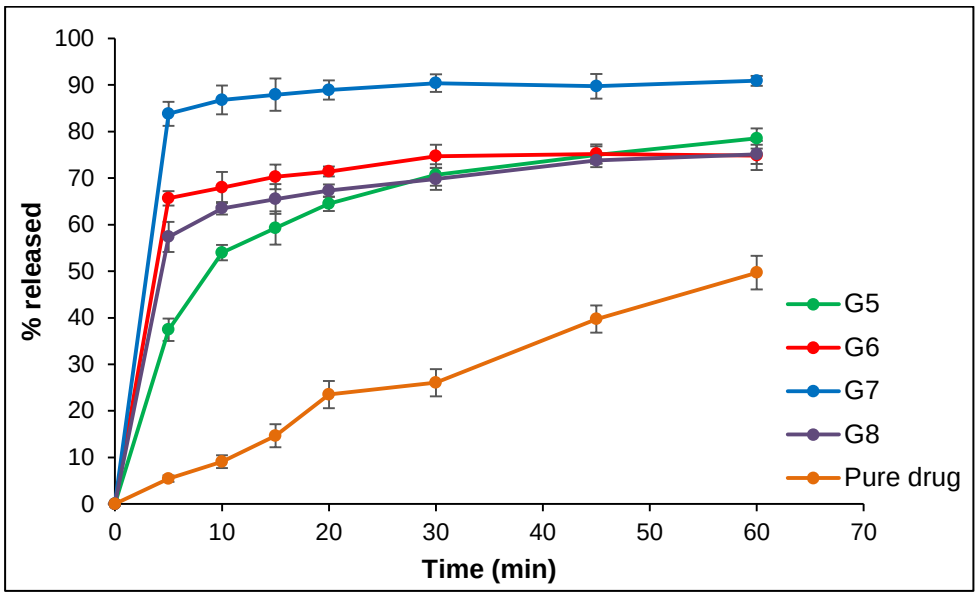


Figure 5.20 In vitro dissolution profiles of pure indomethacin, Gelucire® 48/16-based solid SNEDDS formulations prepared by HME at 40°C (G5 – G8) in phosphate buffer pH 7.2 (mean ± SD, n=3).

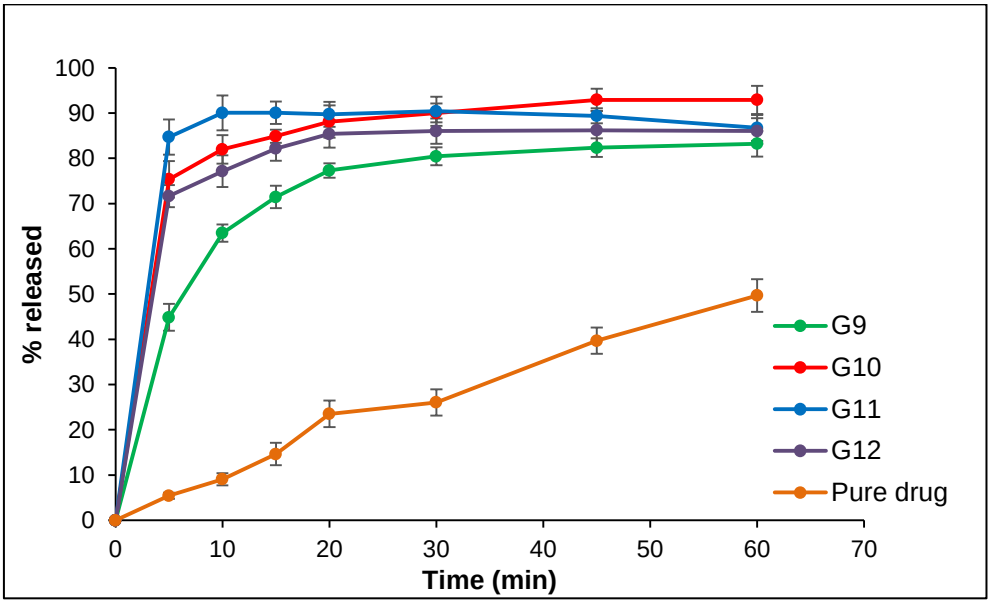


Figure 5.21 In vitro dissolution profiles of pure indomethacin, Gelucire® 48/16-based solid SNEDDS formulations prepared by HME at 50°C (G9 – G12) in phosphate buffer pH 7.2 (mean ± SD, n=3).

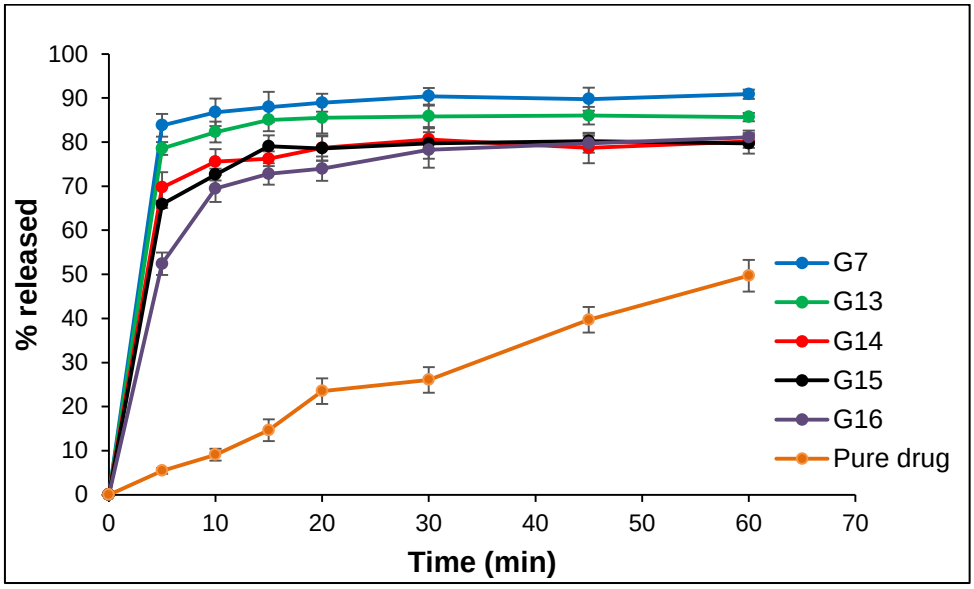


Figure 5.22 In vitro dissolution profiles of pure indomethacin, Gelucire® 48/16-based solid SNEDDS formulations prepared by HME at 40°C incorporating different amounts of Neusilin® US2 (G13 and G14) or Florite® PS-200 (G15 and G16) in phosphate buffer pH 7.2 (mean ± SD, n=3).

Table 5.6 Mean dissolution time (MDT), mean dissolution efficiency (%DE_{15}) and % released (%Q_{15}) after 15 minutes calculated for pure indomethacin and Gelucire®-based solid SNEDDS formulations

Formulation Code	MDT (mean ± SD)	%DE_{15} (mean ± SD)	%Q_{15} (mean ± SD)
G1	17.07± 1.43	29.04 ± 1.61	50.58 ± 2.05
G2	13.25 ± 1.05	30.23 ± 4.96	58.90 ± 2.02
G3	11.16 ± 0.83	43.16 ± 2.16	75.59 ± 2.35
G4	13.50 ± 1.24	34.42 ± 1.64	65.63 ± 7.36
G5	11.17 ± 1.09	40.35± 1.37	59.27 ± 3.21
G6	4.19 ± 1.76	56.25 ± 1.94	70.26 ± 3.58
G7	3.70 ± 1.53	71.51 ± 2.35	87.92 ± 2.67
G8	7.02 ± 1.34	51.21 ± 1.85	65.49 ± 3.45
G9	7.80 ± 1.12	48.02 ± 1.25	71.46 ± 2.48
G10	5.25 ± 1.23	66.60 ± 1.23	84.88 ± 1.45
G11	0.98 ± 0.53	73.27 ± 1.88	90.07 ± 2.46
G12	4.11 ± 1.05	63.29 ± 2.13	82.16 ± 2.68
G13	3.09 ± 1.03	67.77 ± 1.46	85.02 ± 2.58
G14	4.01 ± 1.12	61.13 ± 2.33	76.22 ± 1.69
G15	3.87 ± 1.06	59.35 ±1.58	79.05 ± 2.44
G16	6.83 ± 1.30	52.76 ±1.77	72.78 ± 2.46
Pure drug	27.32 ± 4.32*	7.28 ± 1.08*	14.64 ± 2.47*

*Significant difference at $p < 0.05$.

All Gelucire®-based solid SNEDDS formulations of indomethacin exhibited significantly higher dissolution performance as presented by significantly higher %DE_{15} and significantly lower values of MDT ($p < 0.05$) compared to that obtained for pure drug filled in capsules (**Table 5.6)**. For example, it is evident from the data presented in **Table 5.6** that the %DE_{15} calculated for Gelucire®-based solid SNEDDS formulations showed 3.99 – 9.82 folds increase in comparison to %DE_{15} of pure drug.

High dissolution profiles of Gelucire®-based solid SNEDDS formulations could be attributed to increased wettability and micellar solubilization of drug in the presence of Gelucires® (Damian et al., 2000, Horter and Dressman, 2001, Leuner and Dressman, 2000) resulting in improved solubility of indomethacin. The presence of Gelucires® in aqueous solutions may decrease the contact angle between drug particles and water leading to improved wettability of drug particles and therefore, enhanced dissolution rate. Also, the presence of hydrophilic carriers, such as Gelucire®44/14 and Gelucire®48/16, along with poorly soluble drug may lead to decreased hydrophobicity of the surface of

drug particles and then increased contact with the aqueous medium resulting in increased dissolution rate (Kallakunta et al., 2013). In addition, rapid drug dissolution from Gelucire®-based SNEDDSs may be ascribed to the low surface free energy of these self-emulsifying systems leading to rapid emulsification and quick formation of an interface between drug particles and the dissolution medium (Craig et al., 1995). Furthermore, increased dissolution of the drug from Gelucire®-based SNEDDSs could be attributed to the micellar solubilzation of the drug. Both Gelucire® 44/14 and Gelucire® 48/16 possess surface active properties and have the ability to self-emulsify when in contact with aqueous medium to produce fine emulsions or micellar solutions (da Fonseca Antunes et al., 2013, Gattefossé, 2015) which may entrap poorly soluble drug particles within micelles leading to solubilization.

The obtained dissolution performance of indomethacin from pure drug was significantly low ($p < 0.05$) compared to dissolution performance of different Gelucire®-based solid SNEDDS formulations. This is due to poor aqueous solubility and poor wettability of the drug. Poor wetting of indomethacin particles could be due to high surface free energy that may lead to increased cohesion between drug particles; than the adhesion between drug particles and dissolution medium; which then inhibits the formation of an interface (Ramasahayam et al., 2015).

From the dissolution parameters calculated and presented in **Table 5.6**, it was observed that Gelucire®-based solid SNEDDS formulations prepared with Gelucire® 48/16 (G5 –G12) exhibited higher dissolution profiles and hence, higher dissolution efficiency ($\%DE_{15}$) when compared to SNEDDS formulations prepared with Gelucire® 44/14 (G1 –G4). These differences could be ascribed to differences in the HLB values of each Gelucire® grade. Gelucire® 48/16 possesses higher HLB value compared to Gelucire® 44/14 and therefore, this may enhance dispersion and miscibility of the drug within the carrier resulting in increased solubility and faster dissolution (Kalpana et al., 2015). These dissolution observations are supported by the results obtained previously in **Section 5.4.1** for phase solubility study of indomethacin in different Gelucires® as well as the results of determination of solubility of the drug in Gelucire®-based solid SNEDDS formulations presented in **Section 5.4.4.4**.

Similar findings were reported for solid dispersions of flurbiprofen prepared by solvent evaporation method using Gelucire® 44/14 and Gelucire® 50/13 (Daravath et al., 2015). Formulations prepared with Gelucire® 44/14 showed enhanced solubility and dissolution rate of the drug more than that obtained from formulations prepared with Gelucire® 50/13. These differences were attributed to differences in wettability and emulsifying properties of both carriers used (Daravath et al., 2015).

The dissolution performance of Gelucire®-based solid SNEDDS formulations prepared with Gelucire® 48/16 at 50°C (G9 – G12) was numerically higher than that observed for formulations prepared at 40°C (G5 – G8) even though no significant difference ($p > 0.05$) was observed between the dissolution parameters of these formulations (**Figures 5.20, 5.21 and Table 5.6**). Increased heating during extrusion processing may have led to increased dispersion of drug particles inside the matrix of the carrier and resulted in enhanced solubilization of the drug at the molecular level and hence, increased dissolution. From this observation, it appears that melt extrusion processing of different formulations at temperatures that are close to or higher than the melting point of the carrier may result in improved dissolution profiles of the drug compared to extrusion processing carried out by only softening of the carrier at a temperature that is well below its melting point.

Similarly, the effect of extrusion conditions on drug crystallinity, drug-lipid interaction and dissolution patterns were evaluated and compared in the manufacture of sustained release tablets of diclofenac sodium based on solid lipid matrices of the drug (Vithani et al., 2014, Vithani et al., 2013). Mixtures of diclofenac sodium and Compritol® 888 ATO were extruded either with "cold" extrusion process; where the barrel temperature was kept below the lipid melting point of 70°C, or by hot melt extrusion where the barrel segments heated to temperatures above the melting point of the solid lipid. The drug dissolution studies revealed that tablets prepared with lipid matrices that were developed by cold extrusion process showed faster dissolution rate of the drug compared to tablets obtained from lipid matrices produced by hot melt extrusion process (Vithani et al., 2013). The authors concluded that extrusion conditions may play an important role in determination of drug dissolution behavior. In another study, this group of authors demonstrated that tablets composed of extrudates of diclofenac sodium and Compritol® 888 ATO produced by hot melt extrusion showed slightly faster dissolution rate; although no significant difference was observed upon comparison to the dissolution rate of tablets developed from "cold" processed extrudates (Vithani et al., 2014). In addition, it was reported that extrudates prepared from mixtures of Dynasan 114® and theophylline and extruded at a temperature above the melting point of the solid lipid showed faster dissolution profile compared to extrudates prepared at temperatures below the melting point of the carrier. This result was attributed to the highly porous surface structure of the extrudates obtained upon extrusion above the melting point of the carrier (Reitz and Kleinebudde, 2007). Furthermore, the dissolution performance of hot melt extruded solid dispersions of 17β-estradiol-hemihydrate prepared in

polyvinylpyrrolidone by extrusion at 180^{o}C was higher than that obtained for solid dispersions extruded at lower temperatures (100^{o}C or 160^{o}C) (Hülsmann et al., 2001).

From the dissolution profiles presented in **Figure 5.22** and the dissolution parameters shown in **Table 5.6**, it was observed that incorporation of different adsorbents into Gelucire®-based solid SNEDDS formulations such as Neusilin® US2 (G13 and G14) and Florite® PS-200 (G15 and G16) resulted in lower dissolution performance of the drug compared to the Gelucire®-based solid SNEDDS formulation prepared without adsorbent (G7). Also, it was noticed that increased content of the adsorbent in the formulation led to further reduction of the dissolution behavior of corresponding formulations. This may be explained on the basis of embedding of these adsorbents (Neusilin® US2 or Florite® PS-200) within Gelucire® matrix during thermal processing and this may have led to molecular immobilization and decreased matrix wetting which then resulted in decreased drug dissolution (Vithani et al., 2013). This explanation may be supported by the observation of further reduction of drug dissolution profile upon increasing the amount of incorporated adsorbent.

This possible explanation may be supported by the XRD diffractograms of these formulations (G13 – G16), presented previously in **Figure 5.14**, where crystalline peaks of indomethacin were observed which may indicate that the drug did not dissolve completely in the carrier probably because of embedding of the adsorbent in the carrier and consequent reduction of the amount of the carrier available for dissolving the drug.
Similar findings were reported for sustained release tablets produced by HME of pre-mixed solid lipid matrices of diclofenac sodium/Compritol® 888 ATO with Neusilin® US2, Fujicalin® and magnesium stearate. These pre-mixed extruded tablets showed sustained dissolution profiles compared to the dissolution profile obtained for tablets prepared from lipid matrices and excipients without co-extrusion of tablet ingredients (Vithani et al., 2013). The authors suggested that thermal processing may have accelerated embedding of tablet excipients within the hydrophobic lipid matrix and led to decreased matrix wetting and hence, reduced dissolution profiles.

From the dissolution parameters calculated and presented in **Table 5.6**, it was observed that increase of the drug loading in Gelucire®-based solid SNEDDS formulations (G1 – G12) prepared at 40^{o}C or 50^{o}C, using drug: Gelucire® ratios of 0.5: 10, 1: 10 and 2: 10, resulted in increased dissolution performance of the corresponding formulations. On the other hand, formulations prepared using higher drug loadings (drug: Gelucire® ratios of 3: 10) showed reduced dissolution performance.

These observations could be explained based on the surface active properties of Gelucires® used and therefore, their ability to increase the solubility of poorly soluble

compounds in aqueous medium through micelle formation (Kalpana et al., 2015). Micellar solubilization occurs at surfactant concentrations above the critical micelle concentration (CMC) and involves the spontaneous dissolving of a substance by interaction with the micelles in water to produce thermodynamically stable isotropic solutions (Rangel-Yagui et al., 2005). The improved dissolution performance of Gelucire®-based solid SNEDDS formulations could be attributed to micellar solubilization of indomethacin by the surface active carriers. Also, the increased dissolution profiles of Gelucire®-based SNEDDS formulations obtained upon increasing the drug load could be related to the capacity of the surface active carrier to solubilize drugs as well as some important parameters of micelles.

Generally, the number of drug molecules that can be solubilized in each micelle increase with increasing aggregation number of micelles or when micelles grow from a spherical shape to an elongated or disc like structure. Elongated or disc-like micelles are considered as larger micelles and may readily solubilize more than one drug molecule (Tehrani-Bagha and Holmberg, 2013). Usually, the aggregation number of micelles, which corresponds to the average number of surfactant monomers in each micelle, is approximately constant over a wide concentration range that might reach up to 100 times the CMC. However, in some cases when micelles grow in shape, the aggregation number may vary as well. Because micelles are labile and unstable entities, they can change their shape and size depending on several factors such as their chemical structure, their concentration, in addition to solution conditions including the temperature, ionic strength and pH. In particular, the surfactant type and the solution conditions may determine the change of the spherical micelles into cylindrical or discoidal ones (Rangel-Yagui et al., 2005).

Moreover, the extent of solubilization of a drug into a particular micelle depends on the shape of the micelle which is determined by the critical packing parameter (CPP). The CPP relates the geometry of the surfactant molecule to its ability to form particular aggregates, and can be calculated from the following equation (Lawrence and Rees, 2000):

$$CPP = v/a.l_c \qquad \text{(Equation 5.1)}$$

where, v is the molar volume of the hydrophobic portion of surfactant, a is the optimal head group area and l_c is the length of surfactant tail (or the critical length of the hydrophobic chain) which is generally assumed to be 70 – 80% of its full extended length. The CPP provides a measurement of the preferred geometry adopted by the surfactant and therefore, predicts the type of the aggregates that it is likely to form

(Lawrence and Rees, 2000). As the value of CPP increases, the micelles become more asymmetrical and the volume of the inner core increases relative to that of the outer portion. Therefore, the solubilization of the drug in the core of the micelle will increase while the solubilization in the outer region will decrease with increased asymmetry (Rangel-Yagui et al., 2005).

Furthermore, the temperature and the pH of micellar solutions can affect the extent of micellar solubilization. The amount of drug solubilized in the inner core of micelles increases as the temperature of micellar systems is increased. Increasing the temperature may lead to an increase in the space available for solubilization in the micelles and also may result in micellar growth. The pH of the micellar solutions can also influence the extent of solubilization of drugs because it may change the equilibrium between ionized and non-ionized forms of the drug. Weakly acidic drugs may exhibit increased solubility at elevated pH values due to increase in the ionized form of the drug (Rangel-Yagui et al., 2005).

Based on the overview above, it could be assumed that increased solubilization of indomethacin in Gelucire®-based solid SNEDDS formulations may be due to the fact that thermal processing of different mixtures of the drug and Gelucires® may have facilitated incorporation of the drug molecules within the surfactant molecules because of increased space available for solubilization in the micelles imparted by heating which also performed to increase the solubility of the drug within the carrier. Subsequently, possible changes in the shape of micelles influenced by increased drug load along with thermal treatment have occurred. Changes in micelles shape and hence their size, may lead to increased CPP and this probably have allowed encapsulation and solubilization of more drug particles within the micelles. The role of thermal processing in increasing the inclusion and solubilization of drug within micelles can be observed in the enhanced dissolution performance of Gelucire®-based SNEDDS formulations prepared with Gelucire 48/16 at 50^{o}C (G9 – G12) compared to the dissolution profiles obtained for similar formulations prepared at 40^{o}C (G5 – G8), even though no significant difference ($p > 0.05$) existed between their dissolution parameters.

Decreased dissolution profiles of Gelucire®-based SNEDDS formulations of indomethacin (G4, G8 and G12) prepared with higher drug loads (at drug: Gelucire® ratios of 3: 10) compared to other formulations could be due to exceeding the solubilizing capacity of the carrier for excess drug (as indicated in the XRD diffractograms of the corresponding formulations). Also, increased dug loading in these formulations resulted in formulations with thick consistency which possibly retarded the passage of the dissolved drug particles into the dissolution medium.

The results of measurement of droplet size of nanoemulsions generated from different Gelucire®-based solid SNEDDS formulations which were presented in **section 5.4.4.3** may coincide with the results of dissolution studies of these formulations. As explained in that section, increased drug loading between different formulations resulted in the formation of larger dense particles probably because increased amount of drug particles were solubilized during thermal processing of different mixtures of the drug and the carrier.

5.5. Conclusions

In this part of the study, formulation of solid SNEDDS of indomethacin was carried out using different grades of Gelucires® as single self-emulsifying vehicles that possess the ability to solidify upon cooling to room temperature. Based on the results of phase solubility studies conducted using different grades of Gelucires®, it was found that Gelucire®44/14 and Gelucire®48/16 exhibited highest solubilizing potential of the drug and therefore, these two carriers were selected as self-emulsifying vehicles to formulate solid SNEDDS of indomethacin adopting the HME technique. Different Gelucire®-based solid SNEDDS formulations were prepared at different drug: carrier ratios in which the amount of the drug was increased relative to a constant amount of the carrier. The effect of incorporation of different types and concentrations of adsorbents on the dissolution behaviour of selected formulations was also investigated. Hot melt extrusion of different physical mixtures prepared from the drug, specific Gelucire® grade with or without adsorbent was carried out at a barrel temperature adjusted at 40^{o}C or at 50^{o}C with rotational speed of 30 rpm.

Evaluation of different Gelucire®-based solid SNEDDS formulations prepared by HME at barrel temperature of 40^{o}C or 50^{o}C revealed good drug content with acceptable values of RSD <3%. Also, these formulations manifested good self-nanoemulsifying properties as indicated by the clear to hazy nanoemulsion produced upon dilution with liquid medium. No signs of precipitation were observed upon dilution except with formulations prepared with high drug: carrier ratios (3: 10). Further, all Gelucire®-based solid SNEDDS formulations showed increased solubility of the drug in phosphate buffer pH 7.2 when compared to the solubility of pure drug in the same medium.

DSC studies and XRD analysis of different Gelucire®-based solid SNEDDS formulations demonstrated that the drug might have remained in a molecularly dissolved state within the carrier.

All Gelucire®-based solid SNEDDS formulations of indomethacin showed improved dissolution profiles when compared to that obtained for the pure powder of the drug. Enhanced dissolution performance of these formulations was due to increased wettability, micellar solubilization of drug particles in addition to decreased hydrophobicity of the surface of drug particles influenced by the presence of Gelucires®. Also, solid SNEDDS formulations prepared with Gelucire® 48/16 (G5 – G12) exhibited higher dissolution profiles when compared to formulations prepared with Gelucire® 44/14 (G1 – G4). These differences could be ascribed to differences in the HLB values of both carriers. In addition, the dissolution performance of Gelucire®-based solid SNEDDS formulations prepared with Gelucire® 48/16 at 50^{o}C (G9 – G12) was higher than that observed for formulations prepared at 40^{o}C (G5 – G8). This was attributed to enhanced solubilization of the drug in the completely melted carrier obtained upon increasing the processing temperature. Moreover, incorporation of different types and amounts of adsorbents into Gelucire®-based solid SNEDDS formulations such as Neusilin® US2 (G13 and G14) and Florite® PS-200 (G15 and G16) led to reduced dissolution of the drug compared to the formulation prepared without adsorbent (G7). The incorporated adsorbents might have been embedded within Gelucire® matrix during thermal processing and therefore, resulted in reduced wetting of the carrier and consequently decreased drug dissolution.

Furthermore, the dissolution performance of Gelucire®-based solid SNEDDS formulations prepared at 40^{o}C (G1 – G3, G5 – G7) and those prepared at 50^{o}C (G9 – G11) increased with increasing the drug load. This was explained based on micellar solubilization of indomethacin by the surface active carriers in addition to some important properties of micelles such as their aggregation number, shape, size and critical packing parameter that might be affected by the temperature and the pH of micellar solutions.

On the other hand, reduced dissolution performance was observed for Gelucire®-based solid SNEDDS formulations when the drug load was increased to the drug: carrier ratio of 3: 10 (G4, G8 and G12). This was assumed to be due to exceeding the solubilizing capacity of the carrier influenced by increased drug load which resulted in incomplete solubilization of excess drug by the melted or softened carrier and hence led to reduced dissolution performance. Also high viscosity of these formulations may have retarded the passage of the dissolved drug particles into the dissolution medium. Determination of droplet size of nanoemulsions generated from different Gelucire®48/16-based solid SNEDDS formulations (G1 – G12) was consistent with the results of dissolution studies obtained for these formulations.

Overall, it appears that production of Gelucire®-based solid SNEDDS of indomethacin by HME requires careful selection of the barrel temperature at which the carrier will be completely melted. Also, monitoring the drug load in each formulation is essential to ensure complete molecular solubilization of the drug molecules in the completely melted carrier. This will then lead to a higher self-emulsification efficiency of the formulation, in addition to enhanced solubility and improved dissolution performance of the poorly water soluble drug.

Chapter 6

Stability studies of selected Gelucire®-based solid SNEDDS formulations of indomethacin

6.1. Introduction

Drug stability is one of the most critical quality attributes that need to be evaluated during pharmaceutical development. Stability studies provide information on different changes in the quality of drug substance or drug product that may develop with time under controlled conditions of temperature and relative humidity (RH). This information will help to establish the shelf-life of a drug substance or drug product, or define the storage conditions suitable for the formulation product (Guo et al., 2013).

Development of a stable formulation that will exhibit minimal or no degradation of the active compound throughout the formulation's shelf life is of a great importance for any dosage form. Incorporation of numerous excipients in formulating conventional dosage forms may increase the potential for drug-excipients incompatibilities which in turn may lead to drug degradation. Also, the rate of chemical reaction and hence drug decomposition within a formulation is greatly influenced by the temperature at which the formulation was produced. Therefore, production of formulations at the lowest possible temperature is advisable to avoid chemical instability (Maddineni et al., 2015).

Investigation of physical stability is required to ensure that the dosage form will maintain its proposed performance during the shelf-life and that the drug potency is not altered so that the effectiveness of the formulation will be preserved. Therefore, an understanding of the physical and chemical mechanisms of any physical change is important in achieving high quality and stable drug formulations. Physical instability of some pharmaceutical dosage forms may be due to the solid-state physical instability of drug substances, such as recrystallization of an amorphous drug, or may be due to changes in the formulation matrix itself, or a combination of both issues.

More novel drug delivery approaches such as the lipid-based formulations, nano-based systems and solid dispersions often face greater physical stability challenges compared to conventional oral drug formulations. Many of these formulation technologies are based on supersaturated drug delivery systems, and hence maintaining this supersaturation is important to maintaining product performance. Proper understanding of the solid-state properties of the formulation and how these may be affected by the manufacturing process and storage conditions is fundamental for the development of highly stable formulations (Guo et al., 2013).

During pharmaceutical manufacturing processes, crystalline drug substances may be converted either partially or totally into amorphous forms which possess increased apparent solubility, dissolution rate and possibly bioavailability. Conversely, an initial amorphous form may revert back to the stable crystalline form during

manufacturing or during storage (Guo et al., 2013), altering its physical behaviour and likely bioavailability.

Hot melt extrusion (HME) technology has been successfully used to enhance the aqueous solubility of poorly water-soluble drugs (Alshahrani et al., 2015, Lee et al., 2015, Liu et al., 2010, Tho et al., 2010). In a HME product, the poorly soluble drug is mixed with a polymeric (or lipid) carrier matrix to form a solid dispersion, with the drug being present either in the monomolecular state, i.e., a solid solution, or as amorphous clusters within the polymeric matrix. Enhancement of solubility then results from one or more of the following effects: increased drug specific surface area, higher saturation solubility and increased free energy. Successful solubilization by HME is determined by several factors related to the physicochemical properties of the drug and the carrier in addition to manufacturing considerations such as the processing temperature, shearing forces and other operating conditions. For example, the ideal processing temperature is selected to allow softening of the carrier and often melting so that its low viscosity permits extrusion (Shah et al., 2013).

Therefore, in formulations produced by HME technology the drug is either converted from its crystalline form into an amorphous state or involved in the formation of a solid solution or molecular dispersion in hydrophilic carriers (Alshahrani et al., 2015). Crystalline drugs are commonly converted to amorphous state by the effect of heating and high shear forces which are typically utilized in hot melt extrusion process (Alsulays et al., 2015). High shear mixing during HME process contributes to high drug–polymer interactions and consequently high solubility (Sarode et al., 2014). Compared to crystalline drugs, increased solubility and dissolution rate can be achieved from an amorphous drug because no energy is required to break up the crystalline lattice (Vasconcelos et al., 2007). However, increased free energy of amorphous drugs may influence their thermodynamic stability upon storage, and this may lead to recrystallization of drug particles which may result in decreased dissolution rate and solubility of the drug and hence, decreased pharmacological efficacy. Therefore, amorphous solid dispersion systems are thermodynamically unstable and tend to change to stable state through recrystallization process. Reduced physical stability of amorphous dispersions could be responsible for the limited number of products formulated by extrusion that can be found in the market. For this reason, formulation of stable solid dispersions is of a prime importance as that required for solubility enhancement (Alshahrani et al., 2015).

A number of different factors may play an important role in the physical and chemical stability of amorphous solid dispersions. These factors may include: molecular

mobility, environmental stress, thermodynamic properties and preparation methods and conditions (Baghel et al., 2016).

The crystallization process of amorphous solid dispersions takes place in two steps that occur simultaneously: nucleation which occurs at lower temperatures and crystal growth which needs higher temperatures. Nucleation of supersaturated solutions will occur only at a certain degree of supersaturation in order to overcome the high interfacial tension between small particles. The range of supersaturated concentrations where no nucleation occurs is defined as the metastable zone. Polymeric carriers that may extend this region by increasing the degree of supersaturation or decreasing the interfacial energy are considered more suitable to prevent crystallization (Baghel et al., 2016). Polymeric carriers that increase the aqueous solubility, and hence inhibit the precipitation of dissolved drug, can retard the rate of nucleation by reducing the amount of free drug available to form nuclei (Surwase et al., 2015). Also, polymers increase the viscosity of the dispersion system and therefore, alter the frequency of molecular transport at the surface of nucleus. In addition, high molecular weights of polymers and their ability to exist in different conformations may play a role in reducing the tendency of drug recrystallization by reducing the free energy of amorphous solid dispersions (Baghel et al., 2016).

Intermolecular interactions between the drug and the carrier molecules that take place through H-bonding, ionic bonding or weak van der Waals forces contribute to the physical stability of the systems by limiting the molecular mobility of drug molecules in the polymer matrix. Also, drug-polymer interactions play an important role in inhibiting crystallization by interfering with the process of nucleation or by inhibiting the crystal growth (Baghel et al., 2016). The magnitude of these intermolecular interactions was reported to be dependent on the miscibility of the drug and the polymer in addition to the drug / polymer ratio in the formulation (Maniruzzaman et al., 2013a).

Physical stability of amorphous systems is also affected by the molecular mobility of drug molecules. Restriction of the molecular mobility of the amorphous drug can be achieved by the polymer molecules included in amorphous formulations. The polymer matrix may act as a physical barrier to the molecular motion leading to increased stability (Baghel et al., 2016). Generally, utilization of polymers having high glass transition temperature (T_g) in formulating amorphous solid dispersions may contribute to reducing the molecular mobility of the drug and hence reducing the tendency for recrystallization at specific storage temperatures. Also, preservation of the drug-polymer intermolecular interactions is important for stability of solid dispersions. These considerations require the drug to be soluble at the molecular level in the polymer (Qian et al., 2010).

Therefore, physical stability of amorphous solid dispersions is determined by the molecular level of interaction that occurs between the drug molecules and the carrier. Other factors that must be considered to achieve physically stable formulation may include: (1) the physicochemical properties of the carrier such the molecular weight, melting point or glass transition temperature, hydrophilicity, hygroscopicity, capability of H-bonding and acidic or basic functional groups available for ionic interaction; (2) the drug to carrier ratio or the drug load used in the formulation, where low drug loading lead to more physically stable formulation. Low drug loading will minimize interaction between drug molecules themselves and hinder drug recrystallization. However, chemical instability or degradation is usually linked with low drug concentrations. In this case, a balance between physical and chemical stability should be determined especially for chemically labile drugs; and (3) the manufacturing method should be monitored for both process development and in-process control because crystallization of the amorphous form during manufacturing process will significantly affect the performance of the solid dispersion and the subsequent dosage form (Guo et al., 2013).

Formulation of solid dispersions by hot melt extrusion (HME) is similar to the traditional melting (fusion) method and involves intense mixing of the drug and the carrier inside the extruder. HME offers the possibility to shape the molten mixture into pellets, implants or oral dosage forms and requires complete miscibility of the drug and the carrier in the molten phase. Intense mixing at high shear forces and temperatures may result in uniform distribution of drug molecules in the carrier matrix leading to formation of dispersions at the molecular level (Baghel et al., 2016). Theoretically, a homogenous blend of the drug and the carrier forms a stable one-phase system in which the molecularly dispersed drug does not require the breakdown of the lattice structure before dissolution (Chan et al., 2015). In HME, different process parameters such as the heating temperature, the screw speed and residence time play an important role in the extrusion process as well as the properties of resulting solid dispersion. The influence of these parameters is dependent on the physicochemical properties of the drug and the carrier (Maddineni et al., 2015).

In this part of the project, investigation of physical stability of selected Gelucire® 48/16-based solid SNEDDS of indomethacin was carried out. These formulations were selected based on their different drug: carrier ratios (or different drug loading) in addition to different HME processing temperatures. These formulations had shown high dissolution performance as determined from the in vitro dissolution studies conducted in **Chapter 5**. In order to evaluate the physical stability, these formulations were stored after manufacturing at controlled conditions of temperature and relative humidity (RH) for

6 months. Assessment of physical stability of stored formulations was carried out after 1, 3 and 6 months of storage using the XRD, SEM and dissolution studies. The results were compared to those obtained for the corresponding initial formulations that were evaluated immediately after production.

6.2. Materials

- Indomethacin was obtained from Sigma-Aldrich Chemie GmbH, Germany.
- Gelucire® 48/16 was kindly provided by Gattefossé Co., France.
- Potassium dihydrogen orthophosphate anhydrous was obtained from Loba Chemie Pvt. Ltd. (Mumbai, India).
- Sodium hydroxide and sodium chloride were obtained from Fluka Chemie GmbH (Germany).
- Hard gelatin capsules were obtained from pharma tradechem (Mumbai, India).

6.3. Methods

6.3.1. Construction of a standard calibration curve of indomethacin in phosphate buffer pH 7.2

The standard calibration curve of indomethacin constructed in phosphate buffer pH 7.2, as described in **Chapter 4,** was utilized for this part of the study. The accuracy as well as the precision (reproducibility) of the assay procedure for determination of indomethacin concentrations in phosphate buffer were evaluated as previously presented in **Chapter 3**.

6.3.2. Formulation of Gelucire®-based solid SNEDDS of indomethacin

Gelucire® 48/16-based solid SNEDDS formulations of indomethacin described previously in **Chapter 5** were selected for investigation of the physical stability. These formulations were prepared by HME at 40^{o}C (G5 – G7) or at 50^{o}C (G9 – G11) using different drug: carrier ratios. The selection of these formulations for physical stability studies was based on their relatively high dissolution performance, good drug content with acceptable values of %RSD and good self-nanoemulsifying properties. In addition, the XRD diffractograms of these formulations demonstrated that the drug existed in a molecularly dissolved state within the carrier following manufacture. On the other hand, Gelucire® 48/16-based solid SNEDDS formulations of indomethacin prepared at 40^{o}C (G8) or at 50^{o}C (G12) using higher drug: carrier ratio (3: 10) were excluded from physical

stability studies because these formulations showed crystalline drug particles that remained undissolved in the carrier as demonstrated by their XRD diffractograms and SEM micrographs. Codes and composition of formulations subjected to physical stability studies are given in **Table 6.1**. Selection of formulations that were processed at two different temperatures and contained different drug loading would be useful to identify the effect of different processing temperatures as well as different drug loadings on stability of obtained formulations.

Compounding of Gelucire® 48/16-based solid SNEDDS of indomethacin for the purpose of evaluation of physical stability was carried out in the same manner as described in **Chapter 5**. Briefly, different physical mixtures of indomethacin and Gelucire®48/16 were prepared at the drug: carrier ratios of 0.5: 10, 1: 10 and 2: 10. Each physical mixture was introduced into co-rotating twin screw extruder (micro-compounder, MC 15, Xplore Instruments BV, Sittard, The Netherlands) through the hopper. The barrel temperature was adjusted at either 40°C (below the melting point of Gelucire® 48/16) or 50°C (at the melting point of Gelucire® 48/16) while the rotational speed was fixed at 30 rpm. The introduced blend was mixed for 5 minutes inside the barrel before extrusion through a die with 1 mm diameter. The collected mass was allowed to cool at room temperature, then cut or crushed into small pieces which were sieved through 500 µm sieve to obtain the granular product.

Table 6.1 Codes and composition of different Gelucire®48/16-based solid SNEDDS of indomethacin involved in physical stability studies

Code of formulation	Extrusion temperature (°C)	Ratio of indomethacin : Gelucire®48/16
G5	40	0.5: 10
G6		1: 10
G7		2: 10
G9	50	0.5: 10
G10		1: 10
G11		2: 10

6.3.3. Stability studies of Gelucire®-based solid SNEDDS of indomethacin

Stability studies were conducted at 30°C/75% RH for 6 months. According to the International Conference on Harmonization (ICH) guidelines, the standard elevated storage conditions for accelerated stability of a pharmaceutical formulation are 40°C/75%

RH for 6 months (ICH, 2003). However, the physical stability of different Gelucire®48/16-based solid SNEDDS formulations of indomethacin was evaluated here at 30°C/75% RH for 6 months to avoid any issues relating to softening and melting of the Gelucire® 48/16 carrier that might be expected given its nominal melting point and observed physical behaviour (**Chapter 5**). Re-softening of formulations had been observed in a preliminary experiment upon storage of samples at 40°C. All samples were therefore stored at 30°C (which is 10 degrees below the minimal processing temperatures used) to avoid possible re-softening of formulations, especially those produced at a barrel temperature of 40°C, during storage period. The humidity condition of 75% RH was maintained to be as close as possible to ICH guidelines.

Manufactured formulations of Gelucire®48/16-based solid SNEDDS of indomethacin were placed in closed glass vials and stored at 30°C in a desiccator containing a saturated salt solution of sodium chloride to generate the required relative humidity. Stored samples were removed after 1, 3, and 6 months and tested for dissolution behaviour as well as for crystallinity of the drug using XRD and SEM. The results of different evaluation tests obtained for stored samples were compared to the results obtained for the initial formulations tested immediately after production.

6.3.4. Evaluation of stored Gelucire®48/16-based solid SNEDDS of indomethacin

6.3.4.1. Powder X-ray diffraction (XRD)

The physical state of the drug in the stored formulations was determined using X-ray diffraction after 1, 3 and 6 months of storage. The results were compared to the X-ray diffraction pattern obtained previously for the initial Gelucire®48/16-based solid SNEDDS formulations directly after production. X-ray diffraction studies were performed using Ultima IV diffractometer (Rigaku Co., Ltd., Tokyo, Japan) equipped with a copper X-ray source maintained at 40 kV of tube voltage and 40 mA of tube current to produce emissions of 0.15406 nm. The samples were scanned at 3 − 60° 2θ range at a scanning speed of 0.5 deg./min. Data were collected using a step scan mode with step size of 0.02° and counting time of 1 second per step.

6.3.4.2. Scanning electron microscopy (SEM)

The surface morphology of stored solid SNEDDS formulations was investigated after 1, 3 and 6 months of storage using JSM-6060LV scanning electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 10 kV. Samples were lightly sprinkled

onto double-sided sticky tape which then was affixed to aluminum stub and made electrically conductive with a gold coating (13 – 14 nm/min; 45 s; 20 mA) under vacuum using JFC-1600 Auto Fine Coater (JEOL, Tokyo, Japan). Micrographs obtained for stored formulations were recorded and compared to those obtained for fresh solid SNEDDS formulations directly after manufacture.

6.3.4.3. In vitro dissolution studies

In vitro dissolution studies of stored Gelucire®48/16-based solid SNEDDS formulations were performed after 1, 3 and 6 months of storage according to British Pharmacopoeia (2015) as previously described in **Chapter 4**. Briefly, an amount of each formulation equivalent to 25 mg of indomethacin was filled in hard gelatin capsule and used for dissolution studies in phosphate buffer pH 7.2. Samples (5 ml) were withdrawn at fixed time intervals, filtered through a 0.45 µm syringe filter, suitably diluted and assayed for the drug at 320 nm. An equal volume of fresh dissolution medium kept at 37^{o}C was added to keep constant volume during dissolution study. All experiments were performed in triplicates and results were averaged ± SD.

Different dissolution parameters such as dissolution efficiency after 15 minutes (DE_{15min}), and % released after 15 minutes ($\%Q_{15min}$) were calculated using DDSolver (Excel add inn) and used to compare the dissolution performance of different Gelucire®-based solid SNEDDS formulations after storage. Evaluation of physical stability by dissolution test was carried out by comparing the dissolution parameters obtained for stored samples to those obtained for initial formulations.

6.3.4.4. Statistical analysis

One way analysis of variance (ANOVA) and t-test were used to detect differences between the data of interest. Significant differences were determined at a 5% significance level, unless otherwise stated elsewhere. Statistical differences yielding ($p < 0.05$) were considered significant.

6.4. Results and discussion

Different Gelucire® 48/16-based solid SNEDDS of indomethacin that showed optimum dissolution performance (**Chapter 5**) were taken for further evaluation of physical stability. Specifically, formulations prepared with different drug: carrier ratios using Gelucire® 48/16 that were processed at 40^{o}C (G5 – G7) or at 50^{o}C (G9 – G11) were selected to conduct stability studies at 30^{o}C/75% RH for 6 months. Stored samples

were removed after 1, 3, and 6 months of storage and tested for crystallinity of the drug using XRD and SEM as well as for the dissolution performance.

In this part of the study, the differential scanning calorimetry (DSC) and the Fourier Transform Infrared (FTIR) analyses were not adopted for evaluation of physical stability of different Gelucire® 48/16-based solid SNEDDS of indomethacin. As shown in **Chapter 5**, the conventional DSC analysis of different Gelucire® formulations was not reliable to detect the presence of crystalline particles of the drug within different formulations, and therefore the DSC results were combined with the results of other characterization tests such as the XRD and SEM analyses to confirm the presence of crystalline drug. On the other hand, the FTIR analysis was used in **Chapter 5** to detect any chemical interaction between the drug and the carrier in different formulations. Since only physical stability is to be studied in this Chapter, the FTIR analysis was not adopted.

The results of XRD and SEM as well as the dissolution performance obtained for stored samples compared to those obtained for initial formulations are presented in the following sections.

6.4.1. X-ray diffraction (XRD)

The XRD diffractograms of stored Gelucire®48/16-based solid SNEDDS formulations prepared at 40°C (G5 – G7) and at 50°C (G9 – G11) are presented in **Figures 6.1 and 6.2**, respectively.

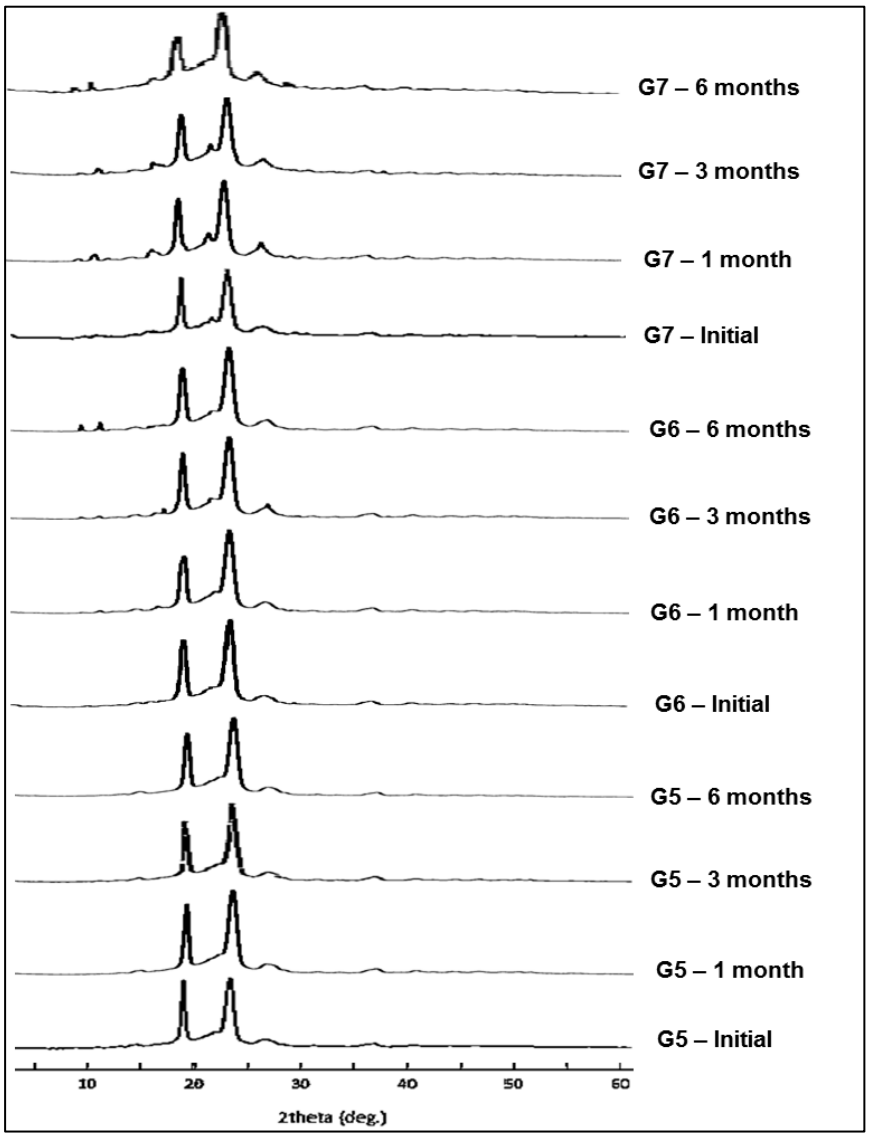


Figure 6.1 XRD diffractograms of initial and stored Gelucire® 48/16-based solid SNEDDS formulations of indomethacin prepared by HME at 40°C.

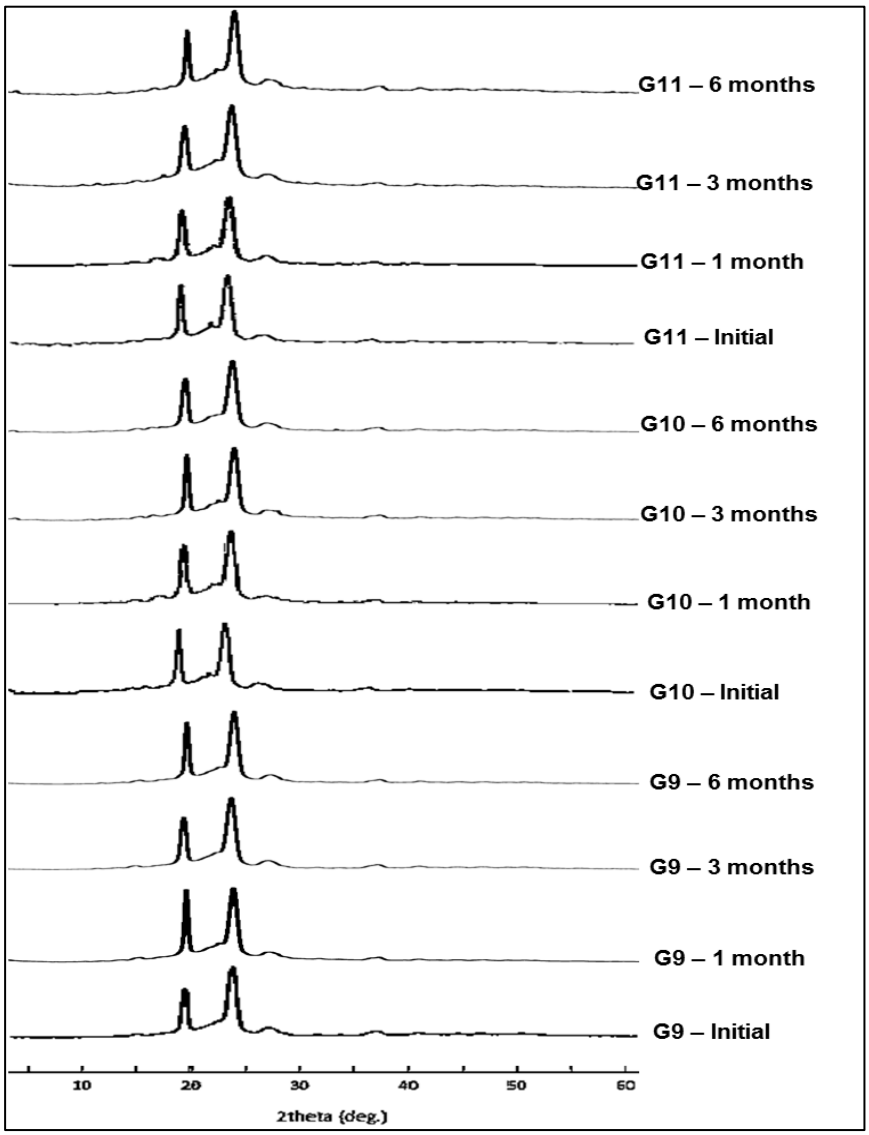


Figure 6.2 XRD diffractograms of initial and stored Gelucire® 48/16-based solid SNEDDS formulations of indomethacin prepared by HME at 50°C.

As depicted in **Figure 6.1**, it is obvious that Gelucire®48/16-based solid SNEDDS formulations prepared by HME at 40°C (G5 – G7) behaved differently upon storage at 30°C/75% RH. The diffractograms of formulation G5 showed no signs of drug crystallization during the 6 months storage, while the diffractograms of formulations G6 and G7 showed peaks of crystalline indomethacin during storage for the same period. On the other hand, the XRD diffractograms of all Gelucire®48/16-based solid SNEDDS formulations prepared by HME at 50°C (G9 – G11) and stored at 30°C/75% RH, as presented in **Figure 6.2,** did not show any specific peaks related to crystalline indomethacin.

The XRD diffractogram of stored formulations G6 and G7 (**Figure 6.1**) exhibited specific peaks of crystalline indomethacin at 2θ values of 11.9^{o}, 17.2^{o} and 26.9^{o} at all time points, although these peaks were more pronounced for the higher drug loading formulation and for the later time points, indicating progressive indomethacin crystallization. Therefore, it appears that Gelucire®48/16-based solid SNEDDS prepared by softening of the carrier at 40^{o}C (G6 & G7) were not stable at 30^{o}C/75 RH for 6 months. Since formulations G6 and G7 were prepared with higher drug loading (drug: carrier ratios of 1: 10 and 2: 10, respectively), it could be possible that the higher drug concentration may have led to close contact and interaction between drug molecules themselves which in turn initiated recrystallization during storage period. This assumption can be supported by the absence of crystalline peaks of the drug in the X-ray diffraction of formulation G5 (**Figure 6.1**) that was prepared with lower drug concentration (drug: carrier ratio of 0.5: 10) and stored for the same period of time under the same storage conditions. Low drug loading allowed drug molecules to remain completely dissolved at the molecular level within the softened carrier during storage period because of the high shear forces applied in the extruder. Molecular level of mixing of the drug and the carrier may lead to the formation of homogenous solid solution structure (Wlodarski et al., 2015). Also, the presence of low drug loading will minimize interaction between drug molecules themselves and hinder drug recrystallization leading to more physically stable formulation (Guo et al., 2013).

On the other hand, the absence of signs of crystallization in the XRD diffractograms of all Gelucire®48/16-based solid SNEDDS prepared by HME at 50^{o}C (G9 – G11) and stored at 30^{o}C/75% RH, which are presented in **Figure 6.2**, confirms that all of these formulations were stable at 30^{o}C/75% RH for 6 months.

Differences in the XRD diffractograms of stored Gelucire®48/16-based solid SNEDDS formulations prepared by HME at 40^{o}C (**Figure 6.1**) or at 50^{o}C (**Figure 6.2**) indicate that processing mixtures of indomethacin and Gelucire®48/16 at a temperature that corresponds to the melting point of the carrier (50^{o}C) is preferably required to solubilize high drug loadings at a molecular level within the completely melted carrier and to produce a physically stable single phase homogenous formulations. It was reported that high shear mixing during HME process will lead to high drug-carrier interaction and therefore, high solubility (Sarode et al., 2014). Intermolecular interactions occurring between drug and carrier molecules via hydrogen or ionic bonding contribute to the physical stability of the system and prevention of crystallization by hindering the nucleation process and thus inhibiting the crystal growth (Baghel et al., 2016).

6.4.2. Scanning electron microscopy (SEM)

Scanning electron microscopy was also used to assess the stability of selected Gelucire®48/16-based solid SNEDDS formulations of indomethacin processed at both 40°C (G5 – G7) and at 50°C (G9 – G11). Different micrographs obtained for initial and stored formulations are presented in **Figure 6.3** and **Figure 6.4**.

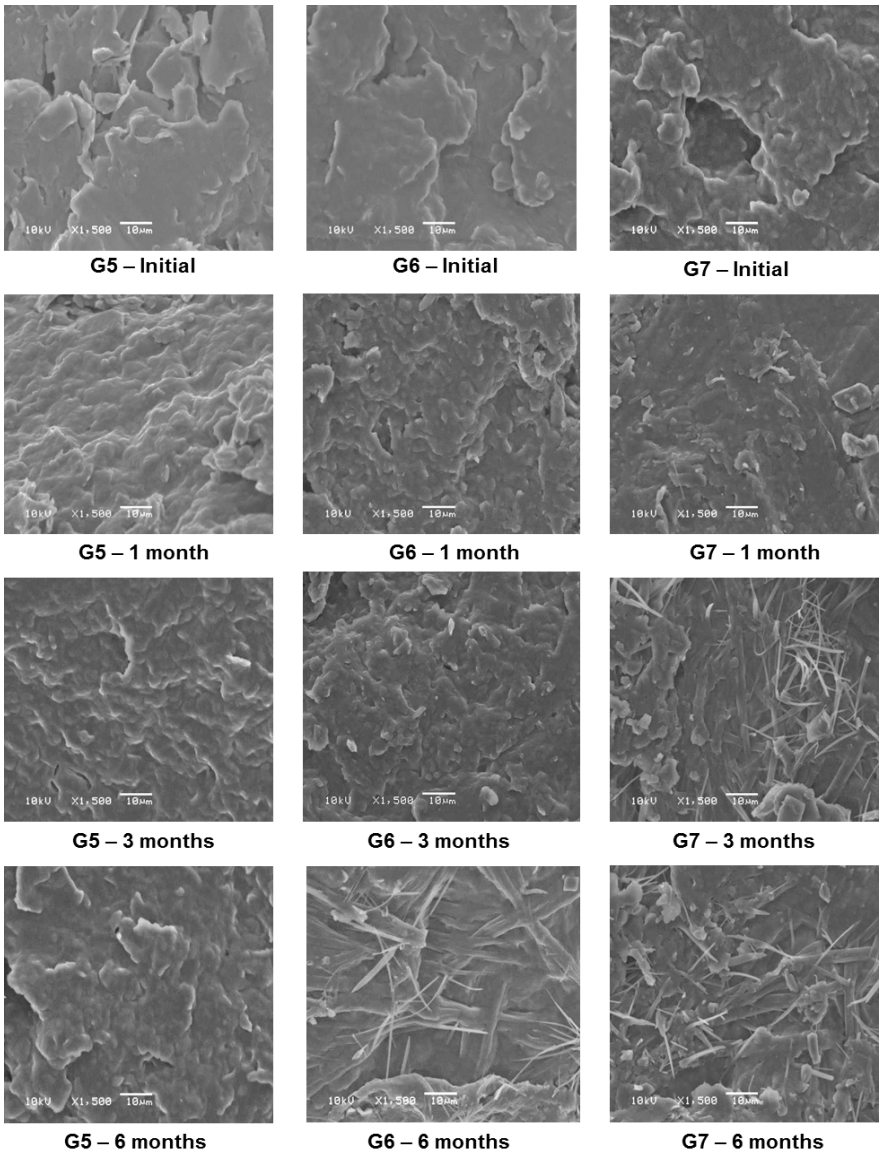


Figure 6.3 SEM micrographs of initial and stored Gelucire® 48/16-based solid SNEDDS formulations of indomethacin prepared by HME at 40°C.

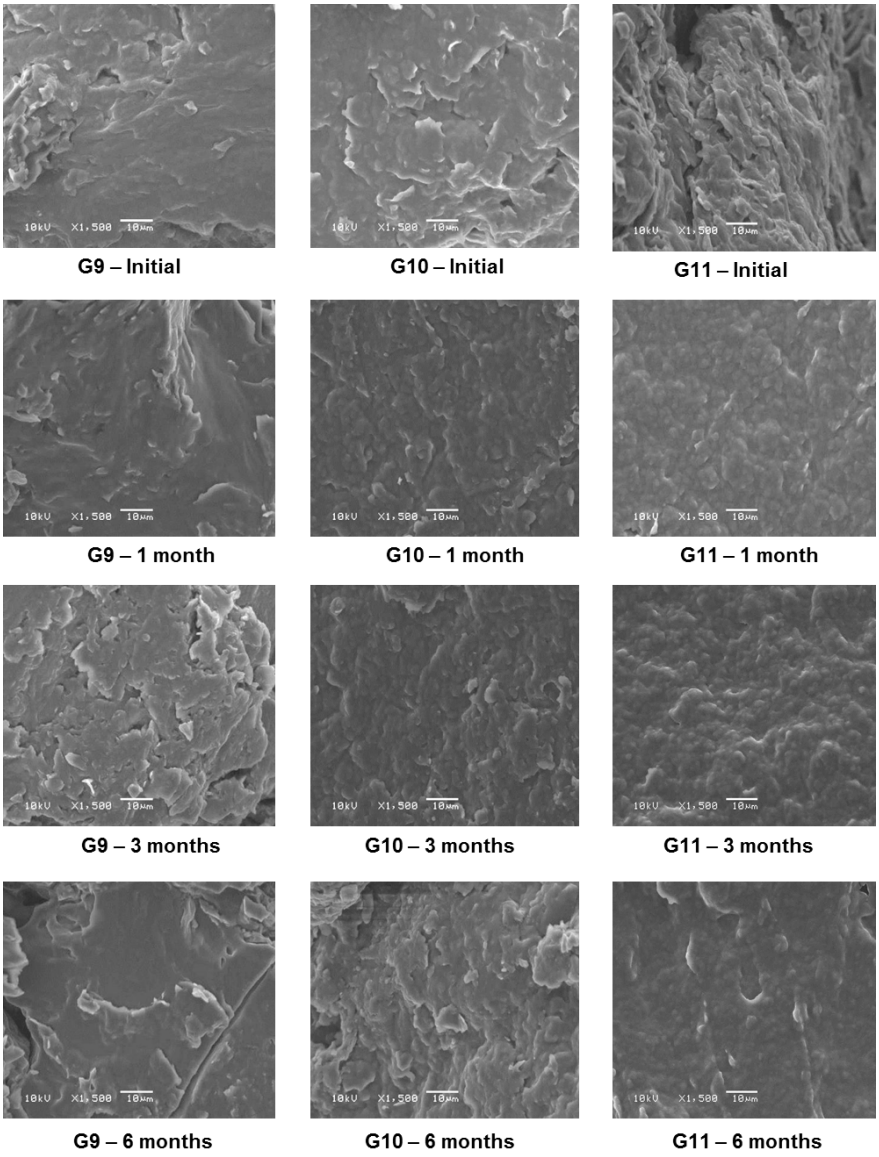


Figure 6.4 SEM micrographs of initial and stored Gelucire® 48/16-based solid SNEDDS formulations of indomethacin prepared by HME at 50°C.

Scanning electron micrographs obtained after 1, 3 and 6 months of storage of formulations prepared by HME at 40°C (G5 – G7) are shown in **Figure 6.3**. The absence of drug crystals in different micrographs obtained for formulation G5 throughout the storage period indicates that this formulation retained its homogeneity during storage and was physically stable at 30°C/75% RH for 6 months. On the other hand, inspection of the SEM micrographs obtained for stored formulations G6 and G7 revealed the presence of crystals of the drug inside the Gelucire® matrix after 1 and 3 months of storage, while a high degree of crystallization was obvious in the SEM micrographs of these two formulations after 6 months of storage. These observations coincide with the

XRD diffractograms obtained for formulations G5 – G7 after storage for the same time period (**Figure 6.1**).

Scanning electron micrographs recorded after 1, 3 and 6 months of storage of Gelucire® 48/16-based solid SNEDDS prepared by HME at 50°C (G9 – G11) are presented in **Figure 6.4**. It was obvious that none of these formulations developed any crystallization during 6 months of storage which indicates that these formulations preserved their homogenous dispersion characteristic. This observation for stored formulations G9 – G11 is consistent with their XRD diffractograms obtained during storage period (**Figure 6.2**) that showed an absence of peaks of crystalline drug. Therefore, it can be concluded that solid SNEDDS prepared by HME at the melting point of Gelucire® 48/16 are physically stable at 30°C and 75% RH for 6 months. Absence of crystallization in formulations G9 – G11 during storage could be due to complete solubilization of the drug in the completely melted carrier with consequent intermolecular interactions between the drug and the carrier molecules. High intermolecular interactions may inhibit recrystallization by inhibiting nucleation and crystal growth (Baghel et al., 2016, Sarode et al., 2014).

An alternative explanation for the appearance of crystals in formulations G6 and G7 is that not all the drug was completely dissolved initially during processing at 40°C, leaving extremely small drug deposits which then acted as foci for nucleation and growth on storage. These deposits would have been below the limit of detection for the techniques used, i.e., SEM and XRD. It was reported that the longest dimension of indomethacin unit cell is about 25 Angstrom (or 2.5 nm) and therefore, a crystal of at least 50 nm in length can be detected by microscopy analysis (Chen, 2002). Based on this, it can be assumed that crystals less than 10 nm in length may not be detected by SEM. In addition, the poor limit of detection by XRD analysis may render this technique unreliable to detect an amount of crystals that is less than 10% (w/w) (Saleki-Gerhardt et al., 1994). On the other hand, the higher temperature (50°C) used in processing of formulations G9 – G11 allowed a greater degree of dissolution of the drug initially, hence no recrystallization was seen on storage for 6 months.

6.4.3. In vitro dissolution studies

The dissolution performance of Gelucire® 48/16-based solid SNEDDS formulations that were subjected to stability testing was evaluated after 1, 3 and 6 months of storage and compared to the dissolution properties obtained for the corresponding formulations right after manufacturing. Comparison of the dissolution behavior of different formulations was based on the % drug released after 15 minutes

($\%Q_{15}$) in addition to the dissolution efficiency after 15 minutes ($\%DE_{15}$). For the purpose of clarity, these dissolution parameters were found to be best presented in the bar graphs shown in **Figure 6.5** and **Figure 6.6**, respectively.

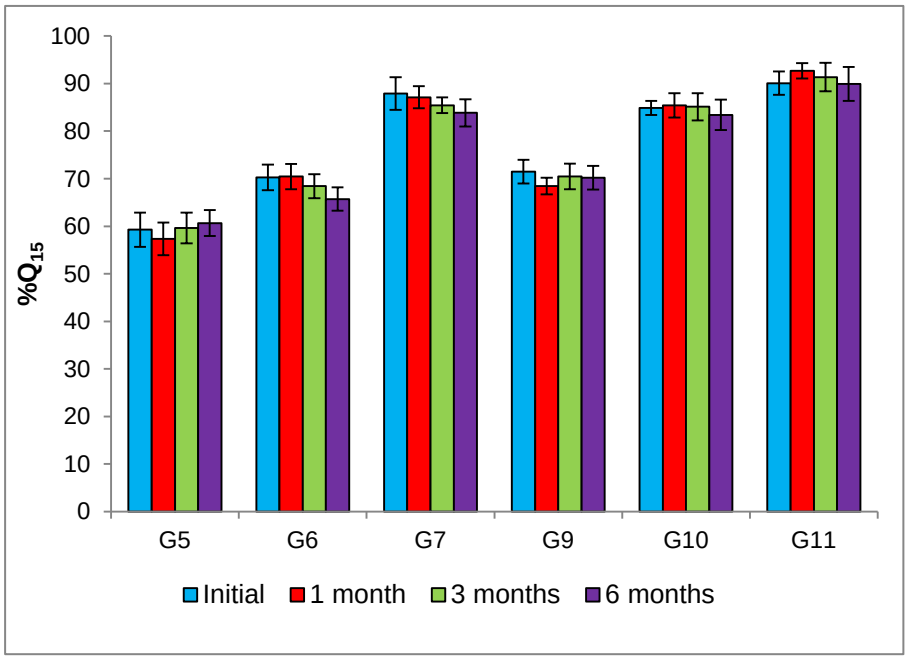


Figure 6.5 Comparison of mean % drug released after 15 minutes ($\%Q_{15}$) from initial and stored Gelucire®48/16-based solid SNEDDS formulations of indomethacin prepared by HME at 40°C and 50°C.

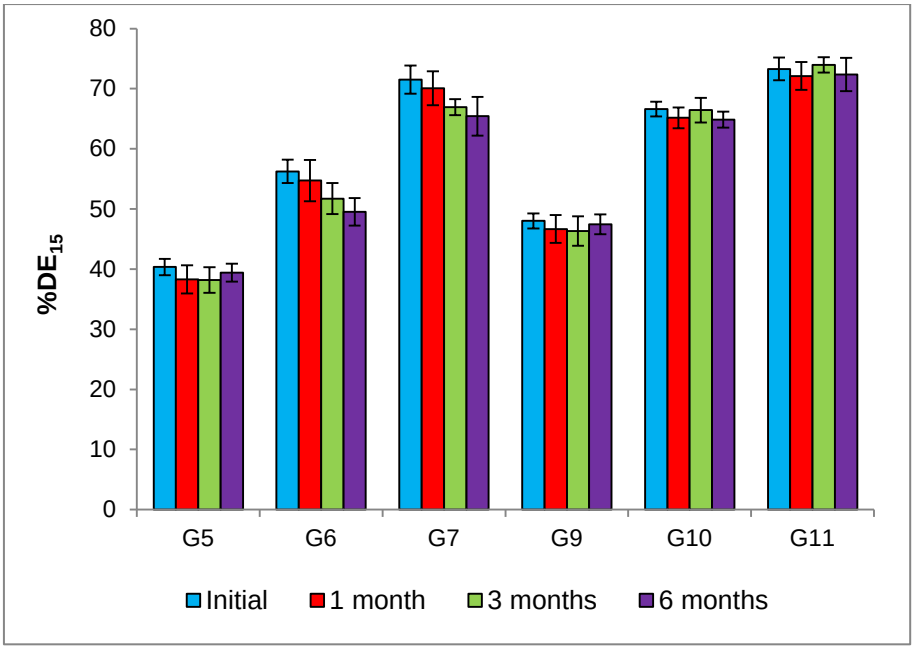


Figure 6.6 Comparison of mean dissolution efficiency after 15 minutes ($\%DE_{15}$) from initial and stored Gelucire® 48/16-based solid SNEDDS formulations of indomethacin prepared by HME at 40°C and 50°C.

From the results shown in **Figure 6.5** and **Figure 6.6**, it was noticed that Gelucire® 48/16-based solid SNEDDS formulations of indomethacin processed at 40°C (G5 – G7) showed slight decreases in the % drug released after 15 minutes (% Q_{15}) as well as the dissolution efficiency after 15 minutes ($\%DE_{15}$) upon storage at 30°C/75% RH for 6 months, although no significant difference ($P>0.05$) was detected between these dissolution parameters calculated for the initial and stored formulations. Reduced values of dissolution parameters during storage were more prominent in formulations G6 and G7. Decreased dissolution of these formulations could be attributed to recrystallization of the drug that occurred during storage possibly because of high drug loading that initiated interaction between the adjacent drug molecules themselves. The presence of crystalline peaks of indomethacin in the XRD diffractograms (**Figure 6.1**) as well as the appearance of crystals of the drug in the SEM micrographs (**Figure 6.3**) of formulations G6 and G7 confirm the recrystallization process that occurred during storage. Presence of crystalline drug within these formulations during storage probably has reduced the amount of molecularly dissolved drug available for dissolution and resulted in reduced dissolution profiles.

On the other hand, Gelucire® 48/16-based solid SNEDDS formulations of indomethacin processed at 50°C (G9 – G11) did not exhibit significant changes in the % drug released after 15 minutes (% Q_{15}) (**Figure 6.5**) or in the dissolution efficiency after 15 minutes (%DE_{15}) (**Figure 6.6**) when stored at 30°C/75% RH for 6 months. This indicates that these formulations were physically stable for 6 months at the storage conditions. Physical stability of formulations G9 – G11 may be due to intimate mixing of the drug and the carrier in these formulations which was achieved by HME processing at a higher temperature (50°C) and in turn, this may have contributed to complete molecular solubilization of the drug within the completely melted carrier. Inhibition of recrystallization was due to high intermolecular interactions between drug and carrier molecules obtained upon molecular solubilization of the drug (Baghel et al., 2016, Sarode et al., 2014).

Similar findings were reported for hot melt extruded solid dispersions of 17β-estradiol-hemihydrate prepared in polyvinylpyrrolidone at different extrusion temperatures (Hülsmann et al., 2001). It was found that batches extruded at higher temperature (180°C) showed higher dissolution profiles compared to batches prepared at lower temperatures (100°C or 160°C). In addition, the dissolution profiles of formulations prepared at higher temperature did not change significantly during storage while formulations prepared at lower extrusion temperatures exhibited decreased dissolution on storage. This was ascribed to the fact that the drug remained dissolved in the carrier when processed at higher temperature and therefore, dissolution profiles remained unchanged, while formulations prepared at lower temperatures developed crystals that acted as nuclei and resulted in recrystallization and hence, reduced dissolution on storage (Hülsmann et al., 2001).

Overall, it appears that Gelucire® 48/16-based solid SNEDDS formulations processed by HME at 50°C were more physically stable than formulations processed at 40°C upon storage at 30°C/75% RH for 6 months. The results obtained from the XRD analysis, SEM as well as the dissolution studies conducted for stored formulations processed at 50°C indicated that processing of mixtures of indomethacin and Gelucire® 48/16 at high temperature is preferable for complete melting of the carrier so that molecular solubilization of the drug in the melted carrier can be achieved and hence, physically stable formulations can be produced.

6.5. General reflection on adsorbent-based and Gelucire®-based solid SNEDDS formulations of indomethacin

At this point, it may be useful to highlight on the overall performance of optimum solid SNEDDS of indomethacin produced in this study either as adsorbent-based (presented in **Chapter 4**) or as carrier-mediated (presented in **Chapter 5**) formulations. Specifically, solid SNEDDS formulations of indomethacin (R1 – R4) prepared by adsorption of drug-loaded liquid SNEDDS formulations onto the inert carrier, Florite® PS-200 (**Chapter 4**) in addition to Gelucire® 48/16-based solid SNEDDS formulations (G9 – G12) produced by HME technology (**Chapter 5**) were selected for this focus and comparison.

All adsorbent-based and carrier-mediated solid SNEDDS formulations mentioned above showed reasonable drug content and preserved the self-nanoemulsification properties of the original liquid SNEDDS or the solid self-emulsifying carrier which were used originally in their production. These formulations produced clear nanoemulsions upon dilution with an aqueous medium. The exception here is for formulation G12 that showed precipitation upon dilution because of its high drug loading that might exceeded the solubilizing ability of the carrier (Gelucire® 48/16).

Physicochemical characterization of both adsorbent-based (R1 – R4) and carrier-mediated (G9 – G12) formulations revealed that these two types of formulations maintained the drug in a dissolved state as indicated by their DSC traces, XRD diffractograms and SEM micrographs (except for formulation G12 which showed crystals of indomethacin in its XRD diffractogram as well as the SEM micrographs possibly due to the high drug loading in this formulation).

In **Chapter 4**, solid SNEDDS formulations of indomethacin prepared by adsorption of drug-loaded liquid SNEDDS formulations onto the inert carrier, Florite® PS-200 (R1 – R4) showed optimum dissolution behaviour compared to other powder formulations produced using different adsorbents such as Neusilin® US2 or Syloid® XDP 3150. This was due to the low specific surface of Florite® PS-200 which enhanced the dispersion of the drug in the dissolution medium leading to rapid formation of spontaneous nanoemulsions with small droplet size. On the other hand, solid SNEDDS formulations of indomethacin prepared by HME at 50^{o}C (G9 – G12) (**Chapter 5**) exhibited optimum dissolution performance compared to other Gelucire® 48/16-based formulations produced by melting at 40^{o}C using HME technology. High dissolution behaviour obtained for these formulations was due to micellar solubilization of the drug within the carrier which increased with increasing the melting temperature to 50^{o}C.

The dissolution parameters of Florite® PS-200-based (R1 – R4) and Gelucire® 48/16-based (G9 – G12) solid SNEDDS formulations are summarized in **Table 6.2**. It can be observed that the dissolution efficiency (%DE_{15}) of solid SNEDDS formulations prepared with Gelucire® 48/16 using HME was generally higher than that noticed for powder formulations prepared by adsorption of liquid SNEDDS onto Florite® PS-200. Although the drug was in a completely dissolved state within the liquid SNEDDS before adsorption onto the solid carrier, reduced dissolution behaviour of formulations prepared by adsorption of liquid SNEDDS onto Florite® PS-200 (R1 – R4) could be due to the high affinity of the adsorbent to the drug, which therefore may retard drug dispersion in the dissolution medium. However, formulation R1 showed high dissolution efficiency (%DE_{15}) compared to other Florite® PS-200-based formulations which could be due to its low content of liquid SNEDDS (adsorbent: liquid SNEDDS ratio of 1: 1.5) which was released from the pores of the carrier at a much faster rate. On the other hand, higher dissolution performance observed for Gelucire® 48/16-based formulations (G9 – G12) may be due to direct availability of the molecularly dissolved drug from the micelles of the carrier.

Table 6.2 Mean dissolution time (MDT), mean dissolution efficiency (%DE_{15}) and % released (%Q_{15}) after 15 minutes calculated for pure indomethacin, Florite® PS-200-based (R1 – R4) and Gelucire®48/16-based (G9 – G12) solid SNEDDS formulations

Formulation Code	MDT (mean ± SD)	%DE_{15} (mean ± SD)	%Q_{15} (mean ± SD)
R1	5.62 ± 0.98	68.63 ± 0.74	95.46 ± 1.11
R2	4.05 ± 1.60	60.91 ± 1.09	82.14 ± 1.02
R3	5.23 ± 1.11	67.62 ± 0.77	89.01 ± 0.80
R4	4.31 ± 0.43	64.27 ± 0.80	86.05 ± 0.32
G9	7.80 ± 1.12	48.02 ± 1.25	71.46 ± 2.48
G10	5.25 ± 1.23	66.60 ± 1.23	84.88 ± 1.45
G11	0.98 ± 0.53	73.27 ± 1.88	90.07 ± 2.46
G12	4.11 ± 1.05	63.29 ± 2.13	82.16 ± 2.68

Overall, utilization of HME in the production of solid SNEDDS formulations of indomethacin using Gelucire®48/16 as a single self-emulsifying carrier, which solidifies on cooling to room temperature, appears to be direct and more advantageous over the use of adsorption method to adsorb a drug-loaded liquid SNEDDS onto inert solid adsorbents. Higher drug loadings could be directly dissolved to the molecular level in Gelucire®48/16 by the effect of controlled heating at 50^{o}C and efficient mixing that can be obtained during HME processing of different formulations. Selection of the most appropriate HME conditions such as the melting temperature, the speed of rotation and the pressure in the barrel may contribute to the production of highly physically stable formulations. In addition, adoption of HME to manufacture solid SNEDDS of indomethacin offers a scalable method that can be taken to an industrial level.

On the other hand, production of solid SNEDDS formulations of indomethacin using the adsorption method resulted in high dissolution performance of different formulations depending on the type of the adsorbent used. However, adsorption method is an indirect method that first involves the formulation of a liquid SNEDDS which is then adsorbed onto the inert adsorbent. Also, many formulation ingredients that are used to produce liquid SNEDDS formulations may interact and increase the susceptibility of the final solid formulation to chemical or physical instability. In addition, filling of solid SNEDDS powder formulations into hard gelatin capsules may be restricted by the bulk

density of the adsorbent used. The use of adsorbents with low bulk density may in turn lead to reduced therapeutic dose of the drug that can be filled in each capsule.

6.6. Conclusions

In this part of the study, investigation of physical stability of different Gelucire®48/16-based solid SNEDDS of indomethacin prepared by HME at different extrusion temperatures was carried out. For this purpose, formulations G5 – G7 processed at 40°C and formulations G9 – G11 processed at 50°C were stored at 30°C/75% RH for a period of 6 months. Physical stability of these formulations was assessed using XRD analysis, SEM and dissolution studies after 1, 3, and 6 months of storage.

All formulations prepared by HME at 50°C (G9 – G11) appeared to be stable over the study period, with no sign of recrystallization on XRD or SEM analysis and their dissolution parameters did not change after 6 months of storage. Of the formulations processed at 40°C, only that with the lowest drug loading appeared to be equally stable. The formulations with higher drug loadings showed evidence of recrystallization over time by XRD and SEM analysis; however, although the dissolution parameter showed a decreasing trend, this was statistically insignificant.

The results obtained revealed that formulations prepared at 50°C (G9 – G11) were more physically stable at 30°C/75% RH for 6 months than formulations processed at 40°C. This indicates that processing of mixtures of indomethacin and Gelucire® 48/16 by HME at a temperature that corresponds to the melting point of the carrier is required for complete melting in order to obtain complete molecular solubilization of the drug in the melted carrier.

Chapter 7

Outlook and future work

This project aimed to explore different self-nanoemulsifying drug delivery systems (SNEDDSs) prepared in liquid, solid and carrier-based formulations and to evaluate the effect of these types of formulations on the solubility and dissolution performance of a poorly water soluble drug. For this purpose, indomethacin (BCS Class II compound) was utilized as the model drug in this study.

It is well documented that the aqueous solubility of an active pharmaceutical ingredient is the major factor that determines its dissolution properties and consequently its oral bioavailability. Poorly water soluble drugs that exhibit an aqueous solubility of less than 100 µg/ml also exhibit slow dissolution rate and incomplete bioavailability. Therefore, improvement of solubility of these compounds by different formulation approaches is crucial for their oral absorption and bioavailability (Horter and Dressman, 2001, Kawabata et al., 2011).

According to the biopharmaceutics classification system (BCS), class II drugs are characterized by their low solubility and high permeability. Oral bioavailability of this class of drugs is dissolution rate limited and enhancement of the dissolution rate of the drug will maximize its oral absorption (Kawabata et al., 2011, Pouton, 2006). Therefore, different formulation approaches can be applied to improve the solubility, dissolution rate and hence, bioavailability of BCS class II drugs either during preformulation studies or during development of formulation product (Kawabata et al., 2011). The lipid formulation approach appears as a promising approach that can be utilized for improving the solubility, dissolution properties and oral bioavailability of poorly soluble drugs.

Lipid-based formulations may enhance the solubility of poorly soluble drugs by different attributes. The presence of these formulations in the gastrointestinal tract (GIT) promotes secretion of biliary products which, together with gastric movement, leads to formation of fine emulsions that improve the solubility of poorly soluble drugs. Also, this type of formulations may undergo enzymatic degradation in the GIT and the resulting hydrolytic products may interact with biliary secretions to form micellar structures that could dissolve the poorly soluble drug. In addition, the surfactant content of these lipidic formulations may contribute to dissolving the poorly soluble drug. Furthermore, prolonged residence time of these formulations in the GIT may contribute to increased dissolution at the site of absorption and consequently, increased absorption (Dahan and Hoffman, 2008).

Self-nanoemulsifying drug delivery systems (SNEDDSs) are lipid-based formulations that consist of a drug dissolved in a mixture of excipients including oil, surfactant and co-surfactant. This anhydrous formulation can rapidly form fine oil in water nanoemulsions upon dispersion in the gastrointestinal fluids under mild agitation

imparted by the gastric motility (Hauss, 2007, Neslihan Gursoy and Benita, 2004, Porter et al., 2008). Formation of submicron droplets upon dilution produce a large interfacial surface area for transfer of the drug which may result in increased rate and extent of absorption and hence, improved bioavailability (Chakraborty et al., 2009). Also, it possesses the potential of increased drug loading capacity because of increased solubility of poorly soluble drugs in the amphiphilic surfactants, co-surfactants and co-solvents constituents of the formulation (Pouton, 2000). These formulations maintain the drug in a dissolved state throughout the GI tract and therefore, may enhance the bioavailability of poorly soluble drugs, for which absorption is dissolution rate limited (Chakraborty et al., 2009, Tang et al., 2008).

The main objectives of this study were generally focused on enhancement of the solubility of indomethacin to improve the dissolution performance which consequently, may reflect on enhancement of oral bioavailability of this poorly water soluble drug. In this context, this research was designed to investigate the effect of different formulations of SNEDDSs of indomethacin on drug solubility as well as dissolution performance. For this purpose, different liquid, solid and carrier-based SNEDDS formulations were adopted to incorporate the drug.

Liquid SNEDDS formulations are considered as anhydrous preconcentrates of nanoemulsions that can be filled into hard or soft gelatin capsules for improved palatability and patient compliance in addition to physical and chemical stability upon long term of storage (Date et al., 2010). Liquid SNEDDSs of indomethacin were successfully developed as described in **Chapter 3**. The type and the concentration of the components used in formulating liquid SNEDDS greatly affect the properties of the final nanoemulsion produced upon dilution such as the droplet size, polydispersity index and self-nanoemulsification efficiency. Therefore, selection of components of SNEDDS is based on their ability to dissolve the drug and to quickly produce spontaneous fine nanoemulsions upon contact with aqueous fluids. In addition, the phase behaviour of the constituents should be evaluated to determine different phases and phase transitions by plotting ternary phase diagrams to determine the areas of self-nanoemulsification where spontaneous nanoemulsions with droplet size of less than 100 nm can be produced. In this section, it was demonstrated that indomethacin exhibited significant increase in solubility upon incorporation into different blends of oil (Capryol™ 90), surfactant (Cremophor® RH 40) and co-surfactant (Transcutol® HP). These three constituents were optimized according to their maximum solubilizing abilities of the drug compared to other relative components tested, and also according to their ability to improve emulsification of different systems so that clear nanoemulsions can be produced. Ternary phase

diagrams plotted for ternary formulations produced from these three components at the most effective surfactant/co-surfactant ratios showed the maximum self-nanoemulsifying regions within which spontaneous nanoemulsion was produced upon contact with aqueous medium.

Incorporation of indomethacin in the optimized SNEDDS formulations resulted in significant enhancement of the solubility of the drug, compared to its solubility in water, which was directly proportional to the content of both oil and surfactant in the self-nanoemulsifying formulation.

All drug-loaded liquid SNEDDSs were prepared with 25 mg of indomethacin to represent the therapeutic dose and to ensure spontaneous emulsification of the formulation without drug precipitation upon aqueous dilution. It was shown that these formulations were thermodynamically stable and exhibited self-nanoemulsification properties.

Compared to different formulations produced with different surfactants, formulations F13 – F16 prepared with Capryol® 90, Cremophor® RH 40 and Transcutol® HP produced the maximum self-nanoemulsifying regions when formulated at effective surfactant/co-surfactant ratios of 2:1 and 3:1, and also showed smaller PDI values and a significantly smaller droplet size. Therefore, formulations F13 – F16 were employed in further studies.

The work reported in this chapter described the effect of various oils, surfactant and co-surfactant combinations to increase the solubility of indomethacin. Enhancement of solubility of indomethacin has been extensively investigated in the literature but this study focused on different blends of constituents that can be used to produce liquid SNEDDS formulations that improved the solubility of the poorly soluble drug and proved to be thermodynamically stable. It is possible that further investigation of a wider range of blends prepared from different oils, surfactants and co-surfactants available from different sources may identify other optimum constituents that may further improve the solubility of the drug. At this point, it can be suggested that the utilization of in vitro lipolysis model, which simulates digestion in the small intestine, could provide information on the digestion of SNEDDS formulations in addition to their tendency to precipitation. The importance of the in vitro lipolysis test comes from the fact that lipids can be digested in the GIT by the effect of lipases and their digestion may lead to loss of solvent capacity and subsequent reduction of solubility of the drug in the GIT which may result in precipitation of the drug and reduction of the absorption rate (Pouton, 2000, Pouton and Porter, 2008). Although the presence of surfactants in lipid formulations may inhibit the digestion of the oil within these formulations (Pouton, 2000), the necessity for

in vitro lipolysis testing to evaluate different types of lipid-based formulations is due to the fact that surfactants may also undergo digestion (Pouton and Porter, 2008).

Application of in vitro lipolysis model could be useful to optimize SNEDDS formulations before taking them into in vivo studies because it may predict the possibility of in vivo precipitation (Dai, 2010, Dahan and Hoffman, 2008)(Dai, 2010, Dahan and Hoffman, 2008)(Dai, 2010, Dahan and Hoffman, 2008). Also, in vitro – in vivo correlation of different SNEDDS formulations can be established by application of in vitro lipolysis assay (Date et al., 2010).

Indomethacin-loaded liquid SNEDDS formulations (F13 – F16) developed in **Chapter 3**, using different blends of Capryol® 90, Cremophor® RH 40 and Transcutol® HP at the most effective ratios of surfactant/co-surfactant of 2:1 and 3:1, were used in further studies conducted in **Chapter 4** to investigate the possibility of conversion of liquid SNEDDS into solid powder formulations that will maintain the self-nanoemulsifying properties of the original liquid formulations and at the same time provide an alternative technique to avoid different limitations associated with encapsulation of liquid SNEDDS that is essential for ease of oral administration. Solid SNEDDS produced from liquid SNEDDS combine the advantages of liquid SNEDDS formulations like improved solubility and bioavailability in addition to those advantages related to the solid dosage forms such as high stability and better patient compliance. For this purpose, adsorption of liquid SNEDDS of indomethacin onto solid adsorbents was utilized as a solidification method with anticipation of achieving high level of drug loading and good drug content.

It was demonstrated that different indomethacin-loaded solid SNEDDS powder formulations prepared with specific adsorbents (Syloid® XDP 3150, Neusilin® US2 and Florite® PS-200) showed good flow properties. Microcrystalline cellulose (Avicel PH 102) and Aerosil® 200 (silicon dioxide) failed to produce free flowing powder formulations and were difficult to manipulate especially at high adsorbent: liquid SNEDDS ratios. This indicated that careful selection of adsorbents with the most suitable physical properties is important to achieve highly flowable formulations. Utilization of Syloid® XDP 3150 (amorphous silicon dioxide), Neusilin® US2 (magnesium aluminometasilicate) and Florite® PS-200 (calcium silicate) as adsorbents resulted in solid SNEDDS powder formulations that exhibited reasonable drug content and maintained the self-nanoemulsification properties of the original liquid SNEDDS formulations. In addition, physicochemical characterization of these powder formulations by DSC and XRD indicated that the drug remained in a dissolved state after adsorption onto different solid carriers. Moreover, improved dissolution profiles of these solid SNEDDS compared to pure powder of the drug indicated the ability of these formulations to deliver the liquid

SNEDDS to the dissolution medium with spontaneous formation of nanoemulsions. It was shown that this enhancement of dissolution profiles was dependent on the physicochemical properties of the adsorbents used in development of different formulations.

Even though powder formulations are designed primarily for filling into hard gelatin capsules, a limitation related to the amount of the powder that can be filled into a capsule may occur. This limitation has been encountered during this study when the free flowing solid SNEDDS powder formulations needed to be filled into capsules for the purpose of conducting the in vitro dissolution studies. Larger amounts of the produced powder formulations were needed to achieve the therapeutic dose of the drug and these high amounts could not be filled into a single capsule (size 000) because most of the silicate adsorbents possess low bulk density. The need for more than one capsule to achieve the therapeutic dose of the drug means that a reduced drug load is present in each unit dose and additionally that patient compliance is likely to be low.

Therefore, compression of solid SNEDDS powder formulations into tablets may present a better alternative than filling into hard gelatin capsules for delivering SNEDDS formulations in a solid dosage form. Tablet dosage forms are more preferable by patients and more economical for the manufacturer than gelatin capsules. Good physical and chemical stability can be achieved from both tablet and capsule dosage forms. However, higher drug loading can be achieved with tablets because more powder can be compressed into tablets than can be filled into hard gelatin capsules. Despite the advantages of tablets over capsule dosage forms, there are still only limited studies in the literature discussing the development of tablet dosage forms for self-emulsifying systems, which may be due to different obstacles.

In this context, it is possible to suggest extension of this study to formulate self-nanoemulsifying tablet dosage forms from the produced powder formulations. Finding a suitable carrier that can adsorb adequate amount of the liquid SNEDDS formulation and at the same time possess good flowability and tabletability may be the most challenging factor that need to be overcome. Also, low compressibility of silicates and the possibility for the adsorbed liquid lipid formulation to leak out of the porous adsorbent upon compression may need to be addressed prior to tablet formulation. With various grades of silicates used in this study as adsorbents that possessed different physical properties and proved to adsorb reasonable amounts of the liquid SNEDDS, it could be possible to overcome some of the proposed limitations associated with production of self-emulsifying tablets.

In **Chapter 5**, another method was proposed to formulate solid SNEDDS of indomethacin in a direct way which excludes the need to formulate liquid SNEDDS and then fill it into hard gelatin capsules or adsorb it on to solid adsorbents. This came from the fact that some vehicles may possess surface active properties (like Poloxamer 188) and can form solid systems with certain types of lipids. With the increased availability of self-emulsifying vehicles that are solid or semisolid at room temperature, it was assumed that direct dispersion of the drug in vehicles that possess the properties of self-emulsification upon contact with aqueous fluids and at the same time have the ability to solidify upon cooling may be beneficial to achieve stable solid self-emulsifying formulations of the drug. These solid self-emulsifying formulations may quickly self-emulsify upon contact with liquid phase leading to improved solubility and consequently, dissolution and bioavailability of the incorporated poorly soluble drug. The directly formed solid self-emulsifying formulations could be further processed into solid dosage forms such as tablets or capsules.

In order to investigate this idea, Gelucires® which possess the ability to solidify upon cooling to room temperature and to self-emulsify upon dispersion in aqueous media were suggested as possible vehicles or carriers to formulate Gelucire®-based solid SNEDDS of indomethacin. Gelucires® also possess the ability to dissolve poorly soluble drugs due to their surface active properties. Therefore, utilization of Gelucires® in this study was primarily to affect solubilization of indomethacin when in melted form and then to produce solid self-emulsifying systems upon cooling. The use of Gelucires® as single vehicles to develop solid SNEDDS formulations would replace the need for a combination of oil, surfactant and co-surfactant to produce liquid SNEDDS formulations and consequently, exclude the need for solid adsorbents to solidify the resulting liquid SNEDDS by adsorption technique. Development of a formulation with the least number of components may help to achieve a chemically stable product. Also, the use of a single excipient to formulate solid SNEDDS is assumed to allow higher drug load to be incorporated in the formulation because no other liquid or solid excipients will be needed.

Solubilization of the drug in different Gelucires® was carried out by the melting method. Hot melt extrusion (HME) technology was applied for this purpose and was proposed to be more effective over traditional melting methods to produce stable formulations. This is justified by the controlled processing parameters of heating and mixing that can be set for an operation in addition to the possibility of scaling up the process to produce pharmaceutical batch sizes.

In **Chapter 5**, Gelucire®44/14 and Gelucire®48/16 were used as carriers to produce Gelucire®-based solid SNEDDS of indomethacin by the HME technique

because these two carriers exhibited high solubilizing potential of the drug. It was shown that different Gelucire®-based solid SNEDDS of indomethacin manufactured by the HME technique at a barrel temperature of 40°C or 50°C and a rotational speed of 30 rpm manifested acceptable drug content and showed good self-nanoemulsifying properties without signs of precipitation upon dilution with liquid medium, except in formulations prepared with high drug: carrier ratios (3: 10). Also, increased solubility of the drug in phosphate buffer pH 7.2 was obtained from these Gelucire®-based formulations compared to the solubility of pure drug in the same buffer. Physicochemical characterization of different Gelucire®-based solid SNEDDS formulations demonstrated that the drug remained in a molecularly dissolved state within the carrier as indicated by DSC studies and XRD analysis.

Improved drug dissolution profiles were obtained from all Gelucire®-based solid SNEDDS formulations of indomethacin and this was attributed to increased wettability and micellar solubilization of drug particles influenced by the presence of Gelucires®. The type of Gelucire®, HME processing temperature and drug loading obviously affected the degree of enhancement of drug dissolution profiles of differently manufactured formulation. Higher dissolution profiles observed for formulations prepared with Gelucire® 48/16 compared to those prepared with Gelucire® 44/14 were due to differences in hydrophile lipophile balance (HLB) values of these two carriers. In addition, HME processing of Gelucire®48/16-based solid SNEDDS formulations at a barrel temperature of 50°C produced better dissolution performance than formulations processed at 40°C. This indicated that complete melting of the carrier at 50°C, rather than softening at 40°C, is more preferable for complete molecular dissolving of the drug. Moreover, it was demonstrated that the dissolution performance of Gelucire®-based formulations of indomethacin increased with increasing the drug load up to the drug: carrier ratio of 2: 10 in different formulations. However, further increase in drug loading exceeded the solubilizing capacity of the carriers and resulted in viscous formulations with reduced dissolution. Further, it was shown that incorporation of different types and amounts of adsorbents into Gelucire®48/16-based solid SNEDDS formulations may not improve the dissolution performance of the corresponding formulations depending on the interaction of the adsorbent with the drug and/or the carrier.

Although Gelucire®-based formulations could have been prepared with high drug loadings, careful setting of optimum HME processing conditions need to be considered to improve the extrudability of Gelucires®-based formulations without affecting the performance of final formulations. Processing different mixtures at 40°C resulted in variation in the extrudability of various mixtures, where mixtures with low drug loading

passed easily through the die part while those with high drug loading could not be extruded through the die part but collected in the form of viscous masses. Increased risk of recrystallization of undissolved drug after extended period of storage is possible for these formulations prepared with high loads of the drug and processed at 40^{o}C. Similarly, mixtures that were processed at 50^{o}C were retrieved from the extruder in the form of a transparent melt indicating complete melting of the carrier and consequently complete dissolving of the drug. From this observation, it could be possible to suggest careful monitoring of the processing temperature as well as the speed of rotation and die diameter to obtain an extrudable mixture in which the carrier is completely melted and the drug is maximally solubilized.

The observed effect of different drug loading on the in vitro dissolution and the physicochemical characterization of differently prepared Gelucire®-based formulations may require further investigation of micellar solubilization of the drug by these surface active carriers. Different properties of micelles that can be characterized by different parameters such as the aggregation number, critical packing parameter, solubilization capacity and micelle-water partition coefficient may contribute to micellar solubilzation of poorly soluble drugs. Careful estimation and calculation of these parameters may provide better understanding of micellar solubilzation of indomethacin by Gelucires® as well as the molecular interactions occurring between the drug and the carrier.

Overall, the research presented in **Chapter 5** indicates that there is a possibility to formulate solid SNEDDS by HME technology using solid or semi-solid vehicles. Adjustments of process variables and formulation parameters are crucial to obtain a stable product with high self-emulsification efficiency. It also proved that Gelucire®48/16 maintained its maximum self-emulsification properties when completely melted inside the extruder.

All observations recorded from the in vitro dissolution studies performed on Gelucires®-based formulations revealed the importance of melting temperature and the drug load to ascertain maximum solubilization of the drug in the carrier. Controlling these two parameters may retard recrystallization of dissolved drug and hence, sustain the physical stability of produced formulations.

Lipid-based formulations are often susceptible to physical stability problems and therefore, require proper understanding of the solid-state physical instability of drug substance that occurs during shelf-life of the formulation in addition to the manufacturing process parameters and the storage conditions in order to produce highly stable formulations. The work conducted in **Chapter 6** was carried out to investigate the effect of different formulation and process parameters on the physical stability of the final solid

SNEDDS formulation. This was performed because HME parameters (like heating temperature and mixing) as well as the formulation parameters (such as the drug load) could greatly influence the physical stability of different formulations produced. For this purpose, some Gelucire® 48/16- based solid SNEDDS that were produced at 40°C or at 50°C using different drug: carrier ratios were adopted for physical stability studies that were carried at 30°C/75% RH for 6 months.

It was shown that all Gelucire® 48/16- based solid SNEDDS produced at 50°C with drug: carrier ratios up to 2: 10 were more physically stable at 30°C/75 RH for 6 months than formulations produced at 40°C because of maintaining of complete molecular solubilization of the drug within the completely melted carrier during storage. Formulations developed by softening of the carrier at 40°C with low drug loading (drug: carrier ratio of 0.5: 10) were more physically stable at 30°C/75 RH for 6 months than formulations prepared with higher drug loadings (drug: carrier ratios of 1: 10 and 2: 10) which showed crystallization after 3 months of storage. High drug concentrations in these formulations possibly led to interaction between the adjacent molecules of the drug and hence initiated crystal growth during storage. Alternatively, processing of these formulations at 40°C may have left extremely small drug deposits, which were below the limit of detection of the analyzing techniques initially, and these deposits acted as foci for nucleation and crystal growth on storage. These observations concluded that different processing temperatures during HME manufacturing of Gelucire® 48/16- based solid SNEDDS formulations affected the physical stability of the final products especially when high drug loadings were used. HME processing temperatures at which the carrier will be completely melted is essentially required to achieve and maintain complete molecular dissolving of the drug.

Conductions of accelerated stability studies for a short period of time may not be adequate to provide a complete picture of changes in the formulation throughout its shelf-life. Unlike chemical stability, changes that may occur due to physical instability are less quantitative and their predictability is low. Also, prediction of physical stability at ambient storage conditions using the results obtained from accelerated physical stability is often unreliable. Therefore, achievement of robust drug formulation development requires important understanding of the chemical and physical processes that may lead to physical instability (Guo et al., 2013). In this context, it is possible to suggest that physical stability studies be conducted for longer periods of time to assess physical changes that may develop during the shelf-life of the formulation.

Because of complexity of the analysis of solid state products and because there is no single analytical method that could provide all the information required on a drug

product or drug substance, most of these methods are used together to complement each other (Guo et al., 2013).

The XRD analysis and SEM used in this study to follow the changes due to physical instability provided good information on the solid state of the formulation. However, there is still a need for further assessment of physical stability, adopting other analytical techniques such as the thermal analysis methods. These methods like high speed DSC and modulated DSC may provide information on the rate and extent of drug crystallization, molecular mobility of the drug as well as miscibility of the drug in the excipients. Combining thermal analysis techniques with other methods such as hot stage microscopy may allow identification of the effect of temperature change on real-time solid state of drug product. Also, the atomic force microscopy (AFM) enables visualization of a surface at the nanoscale resolution and at the same time record thermal behavior of the surface when heat is applied locally (Guo et al., 2013).

In this study, investigation of physical stability of different samples of Gelucire® 48/16- based solid SNEDDS of indomethacin was carried out at 30^{o}C/75 RH for 6 months. The results of this accelerated stability study were obtained while the samples were stored in closed vials at the proposed conditions. Although formulations produced by HME do not contain water and therefore could be more physically stable, investigation of physical stability of these formulations while exposed to different relative humidity values may allow understanding of the interaction of water with the formulation components and its subsequent effects on molecular interactions present between different ingredients.

The most original point in this research was the applicability of hot melt extrusion technique to produce solid self-emulsifying systems by direct dispersion of the poorly soluble drug in the melted carrier. This work showed that this was possible with the availability of self-emulsifying vehicles that exist in a solid form and has the ability to solidify upon cooling of the molten phase.

Different studies conducted in this research project may remain to be improved or extended for further investigations. The following points may be considered:

1. Liquid SNEDDS formulations evaluated in this study may be better developed by utilizing the in vitro lipolysis model to elucidate the behavior of the formulation upon exposure to conditions similar to those in the real GIT. This is because lipids and surfactants may undergo digestion by lipase enzymes in the GIT which may lead to loss of solvent capacity and subsequent precipitation of the drug and reduced

absorption. Precipitation that occurs during the in vitro lipolysis tests may predict the possibility of in vivo precipitation.

2. Solid SNEDDS powder formulations developed by adsorption methods may need to be compressed into tablet dosage forms to obtain high drug load per unit dose. Utilization of highly porous adsorbents that possess good flow and high compressibility may be useful for this purpose.
3. HME processing parameters such as the barrel temperature and rotational speed may need to be optimized to ensure more extrudability of mixtures of the drug and the solid self-emulsifying carrier and at the same time to ensure that the drug is completely solubilized in the completely melted carrier.
4. The characterization methods conducted to investigate physical stability of different formulations may need to be extended for thermal analysis techniques such as modulated DSC and hot stage microscopy. This would provide information on the rate and extent of crystallization of the drug that occur during storage in addition to understanding the immediate effects of temperature change on real-time solid state of drug formulation.
5. As the ultimate goal in formulation development of poorly water-soluble drugs is to enhance oral absorption, an in vivo evaluation of the oral bioavailability of indomethacin from a conventional formulation (tablet or suspension) and from selected SNEDDS and S-SNEDDS formulations developed here would be useful. The improved solubility of indometacin in the formulations studied here, compared to the drug in water alone, and the functionality of the excipients used, gives confidence that an improved bioavailability will be observed but this remains to be definitively proved.

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