FORMULATION AND OPTIMIZATION OF SOLID SNEDDS OF GLIMEPIRIDE: *IN VITRO* AND *EX VIVO* EVALUATION

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTERS OF PHARMACY (PHARMACEUTICS)

By

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Dedicated To My Parents,
My Supervisors
And
Supreme God

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Statement by the candidate

This is to submit that this written submission in thesis entitled **"*Formulation and Development of Solid SNEDDS of Glimepiride: In vitro and Ex vivo Evaluation*"** represents original ideas in my own words and where others' ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the school and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required.

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This thesis encompasses the information generated by me based on experimental work carried out in the Institute/Industry. I assure and hold full responsibility for its genuineness.

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The work described in this project report entitled ***"Formulation and Development of Solid SNEDDS of Glimepiride: In vitro and Ex vivo Evaluation"*** has been carried out by **Yadav Sarvi Rajesh, Reg.No.11502377** under my supervision. I certify that this is his bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

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Abstract

Development of self-nanoemulsifying drug delivery systems (SNEDDS) of glimepiride is reported with the aim to achieve its oral delivery. Lauroglycol® FCC, Tween-80, and ethanol were used as oil, surfactant and co-surfactant, respectively as independent variables. The optimized composition of SNEDDS formulation (F1) was 10% v/v Lauroglycol® FCC, 45% v/v Tween 80, 45%v/v ethanol, and 0.005% w/v glimepiride. Further, the optimized liquid SNEDDS were solidified through spray drying using various hydrophilic and hydrophobic carriers. Among the various carriers, Aerosil® 200 was found to provide desirable flow, compression, dissolution and diffusion. Both, liquid and solid-SNEDDS have shown release of more than 90% within 10 min. The formulation was found stable with temperature variation and freeze thaw cycles in terms of droplet size, zeta potential, drug precipitation and phase separation. Crystalline glimepiride was observed in amorphous state in solid SNEDDS when characterized through SEM, DSC and PXRD studies. The study revealed successful formulation of SNEDDS for glimepiride.

Keywords: Solid-SNEDDS; Box-Behnken Design; Spray Drying; Dissolution; Diffusion

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LIST OF SYMBOLS AND ABBREVIATIONS

Symbol/ Abbreviations	Full form
SNEDDS	Self nanoemulsifying drug delivery system
GIT	Gastrointestinal tract
L-SNEDDS	Liquid self nanoemulsifying drug delivery system
S-SNEDDS	Soild self nanoemulsifying drug delivery system
HLB	Hydrophilic lipophilic balance
XRD	X-ray diffraction
SEM	Scanning electron microscope
mL	Millilitre
mg	Milligram
%	Percentage
Rpm	Rotations per minute
HCL	Hydrochloric acid
℃	Degree celcius
DSC	Differential scanning calorimetry
Eg	Example
Hr	Hour
NaoH	Sodium hydroxide
nm	Nanometer
S.D	Standard deviation
min	Minute
TEM	Transmission electron microscope
PEG	Polyethylene glycol
NIDDM	Non-Insulin Dependent Diabetes Mellitus
ATP	Adenosine triphosphate
AUC	Area under curve

CHAPTER 1

INTRODUCTION

1.1 Delivery system

Oral drug delivery is most popular and convenient route of drug administration for the patients as well as manufacturers for the treatment of pathological state. Despite considerable attempts done in non- oral drug delivery system till date, oral route is commonly preferred route in today's commercial world because of its ease of administration, patient compliance, desired therapeutic effects. Though, half of the drug candidates given orally get diminished in the gastro-intestinal tract (GIT) due to poor aqueous solubility and high lipophilicity, it leads to poor oral bioavailability, high intra and inter subject variability and lack of dose proportionally. Besides this, oral bioavailability depends on some factors such as, stability on GI fluids, intestinal permeability, hepatic first pass effect and interaction with P-glycoprotein (efflux transporter) (Nasr et al., 2016; Beg et al, 2016; Singh et al., 2009).

There are a number of approaches that are reported to improve the dissolution rate limited bioavailability of poorly soluble drugs (Renuka et al., 2014). These approaches include increasing the surface area (Renuka et al., 2014), particle size reduction (Romero et al., 1999), formulation in a dissolved state (Brittain, 2007), liquid-solid compacts (Singh et al., 2012), preparation of inclusion complexes (Bond, 2009), solid dispersions (Cabri et al., 2007), use of pro drugs (Raw and Yu, 2004), and generation of metastable polymorphs (Bartolomei et al., 2007).

The Limitations of solid dispersion are method of preparation, problem in physical and chemical stability of drug and vehicle manufacturing conditions affect the physicochemical properties of solid dispersion, problems in scale up of manufacturing, problem in dosage form development (Serajuddin, 1999). Limitations of cyclodextrin complexation are complex formation lead to uncommon dissolution profiles, complex stability, physical properties of cyclodextrin also influence the complexation process (Szejtli, 1984). Limitations of micronization is particle size distribution is poorly controlled, dissolution rate is insufficient (Gupta et al., 2013). Hence, to overcome these problems lipid based drug delivery system (SNEDDS) is used that is prepared by

incorporation of liquid excipients into the powders by solidification. This is one of the promising drug delivery systems as it is more stable, being anhydrous it can be easily filled in hard gelatin capsules, also it helps in improving the bioavailability by its enhanced permeation across the intestinal membrane and its nano-metric droplet size increases the dissolution and helps in increasing absorption of the drug (Kamel and Mahmoud, 2013). Fig.1.1 depicts the mechanism of the physiological pathways leading to reduction in drug bioavailability through oral conventional dosage forms.
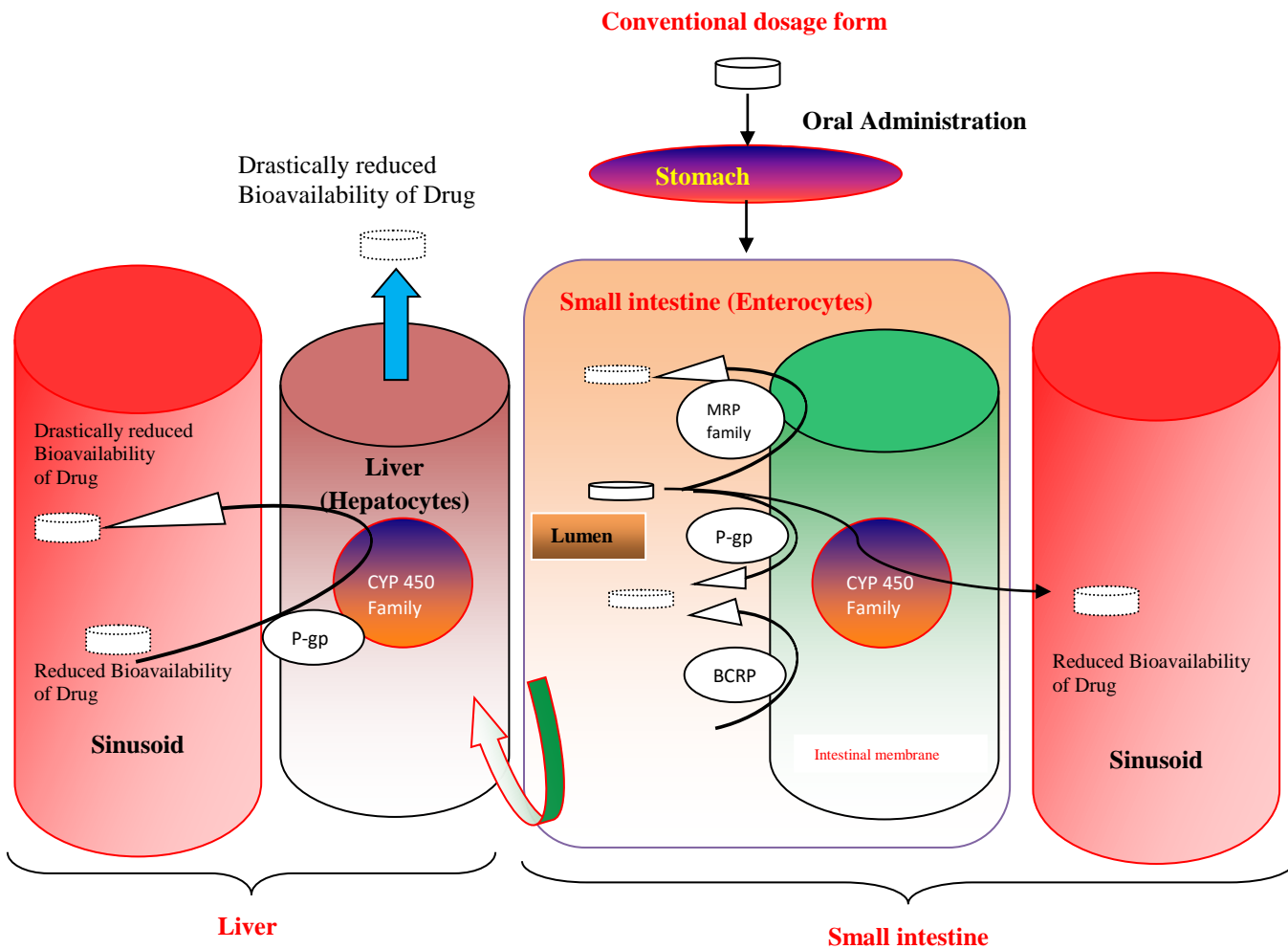
Fig.1.1. Physiological pathways leading to reduction in drug bioavailability through oral conventional dosage form (Singh et al., 2009)

SNEDDS are isotropic, thermodynamically stable and multi-component drug delivery systems composed of synthetic or natural oil, surfactant, co-surfactant that form fine oil-in-water nano-emulsion on addition to aqueous phase with mild agitation. This mixture during administration

comes in contact with the aqueous phase of gastrointestinal tract and forms an oil-in-water nano-emulsion (Nasr et al., 2016). Once the formulation enters GIT, in the presence of gastrointestinal (GI) fluids, it forms spontaneous emulsion. The drug present in the formulation gets solubilized and remains inside small droplets of isotropic mixture. This enhances absorption of drug through the GI membrane and reaches to systemic circulation. Therefore, bioavailability of prepared SNEDDS gets increased (Nasr et al., 2016). Fig. 1.2 represents the pathways through which this self-emulsifying formulation helps in increasing the bioavailability of the drug. SNEDDS has been investigated not only to improve the solubility and dissolution but also used to improve the permeability, hepatic first pass effect and by pass the P- glycoproteins efflux (Singh et al., 2009).
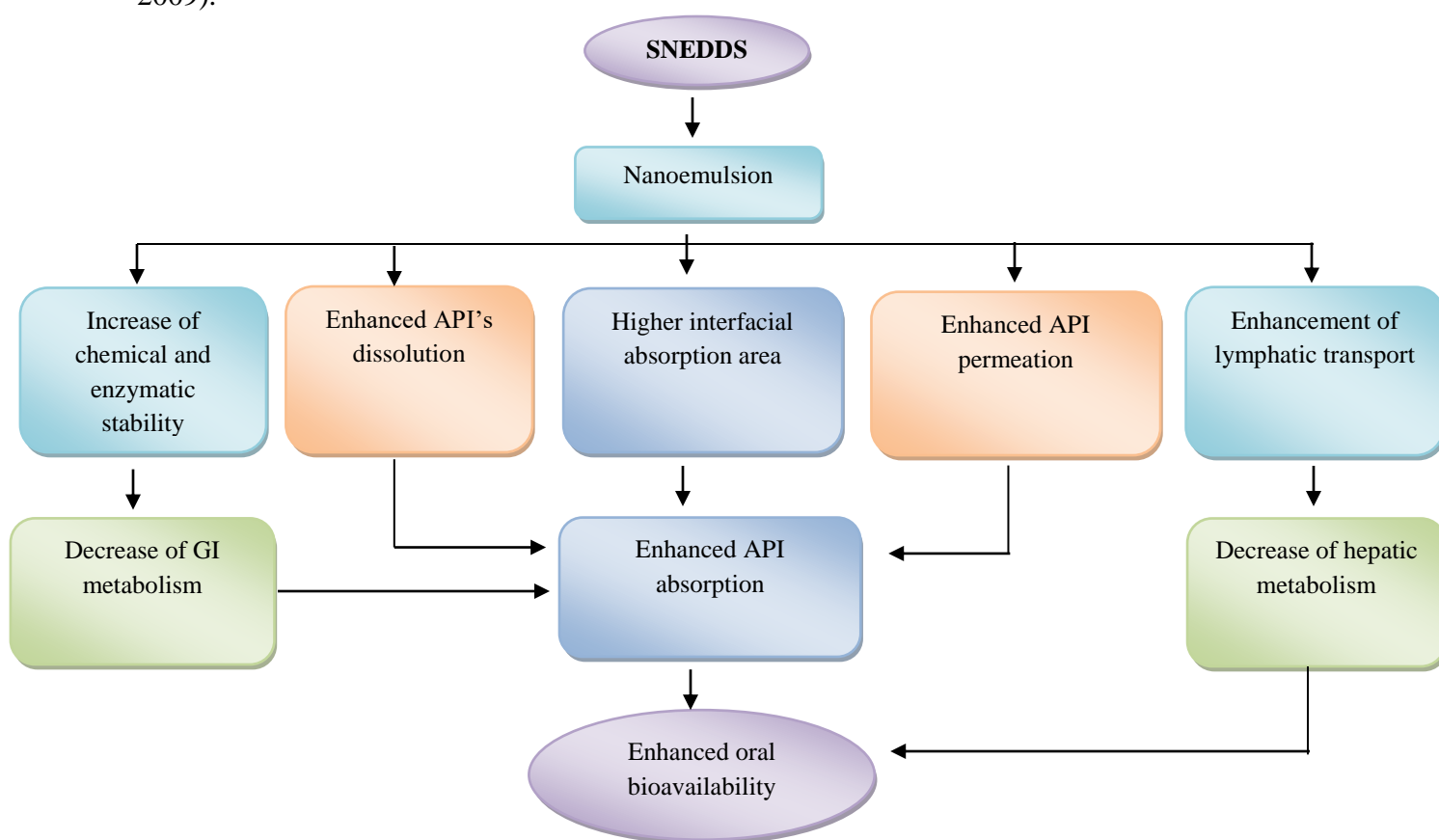
Fig.1.2 Represents the pathways through which this self-emulsifying formulation helps in increasing the bioavailability of the drug.

1.2 Components of SNEDDS

In order to achieve stable emulsion, it becomes important to select the components judiciously with the following objectives:

- Solubilization of the selected drug candidate.
- Achievement of minimum self-emulsification time and droplet size in the GIT for maximum absorption.
- Achievement of maximum drug loading.
- Reduction in drug's degradation in physiological milieu.
- Reduction of variation in the droplet size of emulsion (Rahman et al., 2013)

The various components of SNEDDS are as follows:

- Lipids/oils
- Surfactant
- Co-surfactant

1.2.1 Lipids/oil

: Lipids represent one of the important components of SNEEDS. They not only help in solubilizing the lipophilic drugs but also help in transport of lipophilic drug by the intestinal lymphatic system thereby help in increasing their GI absorption (Singh et al., 2009; Tanvi P et al., 2016). Lipids also protect the drug from chemical and enzymatic degradation (Garg et al., 2016). It is important to note that the selected lipid /oil should yield a nano-emulsion with small droplet size. Mixture of oils can also be used for solubility of the drug (Sakthi M et al., 2013). Natural edible oil consisting of medium chain triglycerides, are not used much because they are incapable of dissolving the lipophilic drug. Apart from this modified long and medium chain triglyceride oil are most commonly used for the preparation of SNEDDS formulations. These oils also provide advantages as their degraded product is similar to that of intestinal digestion end product. Commonly used oily phases are: (Sakthi M et al., 2013; Makadia et al., 2013; Singh et al., 2009). Various oils/lipids used for formulation of SNEDDS are listed in Table 1.1

Table.1.1 Various oils/lipids used for formulation of SNEDDS.

S.N.	Excipient	Trade name	HLB	Supplier
1	Glyceryl triacetate (Triacetin)	Captex® 500P Triacetic®	-	Abitec Co. Sigma Aldrich
2	Glyceryl mono and dicaprylate/ caprate	Capmul® MCM Imwitor® 742	3-4	Abitec Co Sasol
2	Glyceryl tricaprylate/ caprate (medium chain triglycerides)	Migloyl® 810 Migloyl® 812N Captex® 300 Captex® 355 Labrafac® CC	1	Sasol Sasol Abitec Co Abitec Co Gattefosse
4	Glyceryl monolinoleate	Maisine® 35-1	3	Gattefosse
5	Glyceryl monoleate	Capmul® GMO Peceol®	3	Abitec Co Gattefosse
6	Glyceryl mono-di- and tristearate	Imwitor® 900	3	Sasol
7	PEG- 6 glyceryl linoleate	Labrafil® M2125 CS	3-4	Gattefosse
8	PEG-6 glyceryl oleate	Labrafil® M 1944 CS	3-4	Gattefosse
9	PEG-8 glyceryl caprylate/ caprate	Labrasol® Acconon® MC-8	14	Gattefosse Abitec Co
10	PEG-35 castor oil	Cremophor® EL Etocas® 35 NF	12-14	BASF Croda
11	PEG-40 hydrogenated castor oil	Cremophor® RH-40	14-16	BASF
12	Propylene glycol	Lauroglycol® FCC	4	Gattefosse

1.2.2 Surfactant: It also plays an important role in preparation of SNEDDS. Surfactants are amphiphilic in nature and can solubilize large number of hydrophobic drugs and help in keeping both oil and water phase together in emulsion. HLB value plays an important role in selection of surfactant as well it gives information about the essential utility during the formation of SNEDDS. Non-ionic surfactants having high HLB value are suitable for preparation of SNEEDS because they allow immediate emulsification when come in contact with aqueous phase in GIT and this would allow the drug to remain on the absorption site for prolonged period of time (Singh et al., 2009)

Among all surfactants, non-ionic surfactants with high HLB value include solid or liquid Tween-80, polyoxyethylene (20) sorbitan monoleate (Tween-80) and Pluronic F127 (Singh et al., 2009). These non- ionic surfactants are safer than ionic surfactants. Surfactant concentration in the preparation of SNEDDS is kept to be 30-60% w/w because above this concentration it may lead to GIT irritation. Surfactants help in increasing the bioavailability by improving the dissolution

of the drug. Surfactants also help in increasing the permeability of drug across the epithelial cells and tight junctions. (Singh et al, 2009; Garg et al., 2016). Various surfactant used are given in Table 1.2.

Table.1.2. Various surfactant used to formulate SNEDDS (Makadia et al., 2013).

S.N.	Chemical name	HLB	Brand name	Supplier
1	PEG-4 lauryl ether	9.7	Brij®-30	Atlas/ ICI
2	PEG-6 corn oil	4	Labrafil® M2125CS	Gattefosse
3	PEG-6 apricot kernel oil	4	Labrafil® M 1944 CS	Gattefosse
4	PEG-8 caprylic/ capric glycerides	14	Labrasol®	Gattefosse
5	PEG-8 caprylic/ capric glycerides	>10	Labrafac® CM 10	Gattefosse
6	Polyoxyethylene-polyoxypropylene copolymer	18-23	Pluronic® F 127	BASF
7	PEG-8 corn	6-7	Labrafil® WL 2609 BS	Gattefosse
8	PEG-20 sorbitane monoleate	15	Tween -80	Atlas/ICI
9	PEG-20 sorbitane trioletae	11	Tween 85	Atlas/ ICI
10	PEG-35 castor oil	12-14	Cremophor®EL Cremophor® ELP	BASF BASF
11	PEG- 35 hydrogenated castor oil	13	Cremophor® RH-40	BASF
12	Sorbitane mono-oleate	4.3	Span 80	Atlas/ ICI
13	Polyoxy-40- hydrogenated castor oil	13	Cremophor® RH-40	BASF
14	Glyceryl monoleate	3-4	Peceol®	Gattefosse

1.2.3 Co- surfactant: For the preparation of optimum SNEDDS surfactants are needed in high concentrations. Co-surfactant is added in SNEEDS for the pharmaceutical uses and are as follows: (Makadia et al., 2013**;** Tanvi et al., 2016)

- For increasing the drug loading in SNEDDS.
- To improve the droplet size of nano-emulsion.
- To improve the self-emulsification time of SNEDDS

For improving the droplet size, stability as well as payload of active ingredients, co-solvents are used in formulation of SNEDDS. These include Transcutol® HP, ethanol, propylene glycol and polyethylene glycol. Co-surfactant decreases the bending stress of interface and allows sufficient flexibility to take up different deviations (curvature) required to form nano–emulsion. Volatile co-solvents lead to precipitation of drug because it evaporates in the shells of the soft gelatin capsules or hard or sealed gelatin capsules. Co-solvents like Transcutol®P and Glycofurol® provide better stability and less volatility as compared to traditional ones

(Gupta et al., 2013). Sometimes addition of co-solvent may decrease the solubility of the drug, for example in case of Cinnarizine, SNEDDS prepared by optimizing the oral bioavailability, presence of propylene glycol as co-solvent decreases the solubility of the drug to remarkable extent (Shahba et al., 2012)**.** Commonly used co-surfactants are given in Table 1.3 (Singh et al., 2009; Garg et al., 2016)**.**

Table.1.3. List of co-surfactants used for formulation of SNEDDS.

S.N.	Chemical name	HLB	Brand name	Manufacturer/ supplier
1	Polyglyceryl -6 dioleate	6	Plurol Oleique®CC497 Caprol® 6G20 Hodag® PGO-62	Gattefosse Abitec Co Calgene
2	PEG- 6 apricot Kernel oil	4	Labrafil®1944CS	Gattefosse
3	Sorbitane mono-oleate	4.3	Span 80	Atlas/ICI
4	Propylene glycol monolaurate	5	Lauroglycol® 90	Gattefosse
5	PEG-60 hydrogenated castor oil	14	HCO®60	Nikko
6	Sodium lauryl sulfate	40	Sodium Lauryl Sulphate®	Canadian Alcolac
7	Propylene glycol monolaurate	4	Lauroglycol®FCC	Gattefosse
8	PEG-60 hydrogenated castor oil	14	HCO®60	Nikko
9	Propylene glycol monolaurate	4	Lauroglycol®FCC	Gattefosse
10	Diethyl glycol mono-ethyl ether	-	Transcutol® P	Gattefosse
11	Glyceryl caprylate	5-6	Capmul®MCM-C8	ABITEC
12	Caprylic/ Capric glycerides	5-6	Akoline®MCM	Aarhuskarlshamn
13	Diethyl glycol monoethyl ether	-	Carbitol®	Dow chemicals
14	Polaxomer 188	29	Lutrol®f 68	BASF
15	Methyl- oxirane polymer with oxicrane	12-18	Pluronic®L64	BASF

1.3 Mechanism of Self emulsification

Mechanism of self-emulsification is not well understood. However, it is assumed that when the entropy change (that favors dispersion) is more than the energy required to increase the surface area between oil and aqueous phase then self-emulsification takes place. The change in free energy (ΔG) is associated with process of emulsification, ignoring the free energy of mixing, and it is expressed by equation 1.1:

$$\Delta G = \sum N_i 4\pi r_i^2 \, \sigma \qquad\qquad \text{Eq. 1.1}$$

Here, ΔG= free energy related to process; r_i = radius of droplets; N_i = number of droplet; σ= interfacial energy.

Two phases of emulsion tend to get separated with span of time to lower the interfacial tension and also minimize the free energy of the system. The emulsifying agents stabilize the emulsion by forming a monolayer around the emulsion droplets and reduce the interfacial energy thereby form a barrier to coalescence. Emulsification also occurs rapidly with self-emulsifying formulations because the free energy required to form emulsion is low, whether positive or negative. For emulsification, it is necessary for the interfacial structure to show no resistance against surface shearing.

Water penetration into liquid crystals or gel phases formed on the surface of the droplet plays an important role for emulsification. The interface between the oil and aqueous continuous phase is formed upon addition of a binary mixture (oil/ surfactant) to water. Further, solubilization takes place within the oil phase because of the aqueous penetration to the surface and this process is continued until the solubilization limit reaches close to the interface. Although everything that is in close contact with the interface will be the liquid crystal, and actual amount of it depends on the emulsifier concentration in binary mixture. Hence, by gentle agitation of the self-emulsifying system, water quickly enters the aqueous phase leading to disruption of the interface and there is formation of droplet (Gupta et al., 2013; Singh et al., 2009). The steps involved in formulation of SNEDDS are shown in Fig.1.3.

1.4 Method of solidification of L-SNEDDS

- Spray drying
- Spay cooling
- Adsorption on to carrier
- Melt Granulation
- Supercritical fluid method

1.4.1 Spray drying: In this process, liquid SNEDDS are solidified using various porous carriers that could be hydrophobic or hydrophilic in nature. The hydrophilic carriers include, polyvinyl alcohol (PVA), Sodium Carboxy Methyl Cellulose (Na-CMC), Hydroxy Propyl Beta Cyclodextrin (HP- β-CD) and hydrophobic carriers include silicon dioxide (Aerosil®) and

magnesium stearate (MS), etc. Initially liquid SNEDDS are adsorbed on the surface of porous carriers and then dissolved/dispersed in the suitable solvent. Further, the dispersion is spray dried to achieve free flowing powder (Kang et al., 2012; Tang et al., 2008).

During spray drying, liquid when passed through heater gets heated and then reaches to spray nozzle using peristaltic pump where it is atomized into spray of droplets. The volatile phase (ethanol or water) gets evaporated as it reaches the drying chamber forming dry particle at suitable temperature and air flow conditions. Crucial parameters of spray dryer includes, inlet temperature, outlet temperature, solid content, surface tension, feed temperature, volatility of solvent, and nozzle material. Then powder is further prepared into tablet and capsule (Selvam et al., 2011). The scheme for spray drying is shown in Fig.1.4
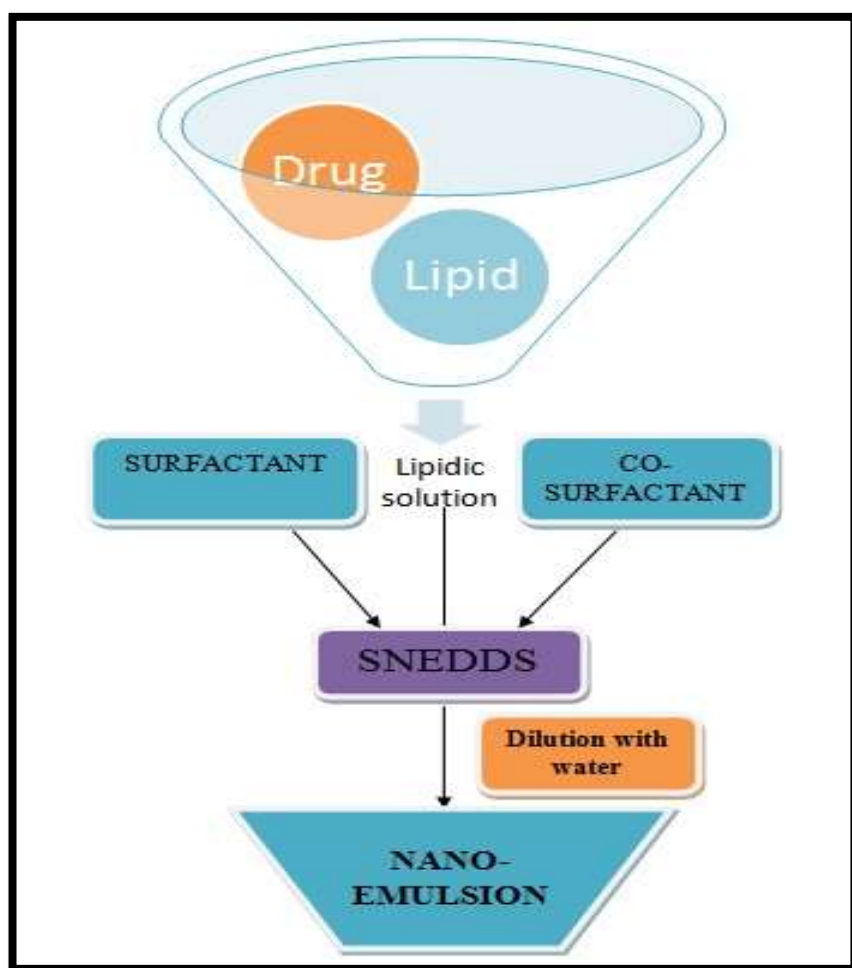
Fig.1.3 Steps involved in formation of self-emulsifying drug delivery system.
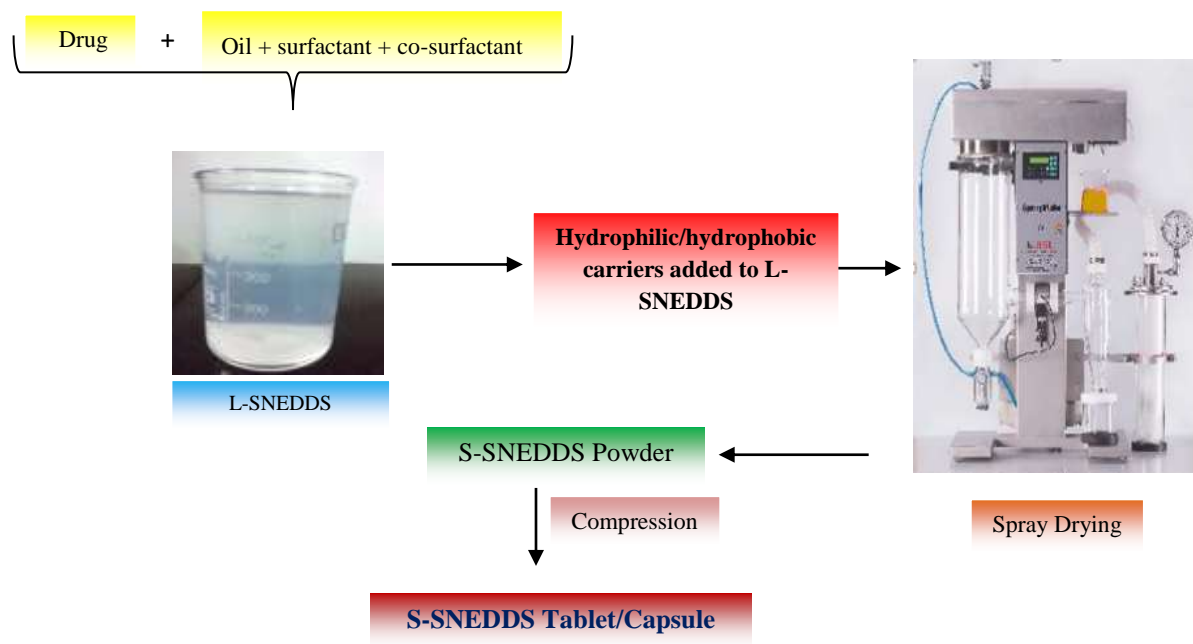
Fig.1.4 Scheme for solidification of SNEDDS through spray drying.

Advantages of Spray dryer

- The process is very rapid.

- It can be designed for any required capacity.

- It is available in different design to meet product specification.

- It can be used for both heath labile and heat sensitive products.

- It is fully automatic system and allows constant monitoring and recording of various process variables.

- It provides precised control on particle size, bulk density, degree of crystallinity, organic volatile impurity and residual solvent.

- Same quality of powder was produced during entire cycle (Selvam et al., 2011)

Limitations of Spray dryer

- The instrument is very bulky and expensive

- Thermal efficiency is low (Selvam et al., 2011)

1.4.2. Spray cooling: It is also called spray chilling or spray congealing (Kini et al., 2011). In this process, molten formulation is sprayed into cooling chamber. The time when this molten

formulation comes in direct contact with the cooling air then these molten droplets congeal and recrystallize into spherical solid particles and that appear as fine powder, when fall at the bottom of the chamber. This fine powder is used for the formulation of solid dosage forms such as tablets and capsules (Sapra et al., 2012; Selvan et al., 2011).

For spraying the liquid mainly three types of devices are available such as rotary or centrifugal atomizer, airless nozzles and air or two fluid nozzles. Recently ultrasonic atomizers are used for spraying the liquid (Passerini et al., 2002). The excipients used for spray congealing technique are polyoxyglycerides and mostly used is stearoyl polyoxyglycerides Gelucirine 50/13 because helps in producing the microparticles with narrow size which helps in increasing the drug release of poorly soluble drugs (Passerine et al; 2002)

Advantages of spray cooling: (Selvam et al., 2011)

- Spray cooling has easy and continuous operation
- Spray cooling is fully automatic machine and has fast response time.
- During cooling process powder quality or specifications remains constant throughout.
- Whether the feed stock is either melted or corrosive it can be handled and pumpable by spray cooling.
- Wide range of designs is available for spray cooling (Selvam et al., 2011)

Disadvantages **of spray cooling:** (Selvam et al., 2011)

- Installation cost is high
- They are expensive.

1.4.3 Adsorption on carriers: Free flowing powder of S-SNEDDS is obtained from liquid-SNEDDS by its adsorption onto the solid carrier. This process is simple and involves 2 steps:

- The addition of L-SNEDDS onto the solid carrier by mixing in a blender or, mortar pestle and then passed through sieve 30 to obtain free flowing powder (Beg et al., 2016)**.**
- The resultant mixture was directly filled in capsules shells or by addition some excipients it is compressed into tablets (Selvam et al., 2011)**.**

Gravimetric method was used for the determination of oil adsorption capacity by porous carriers. In this, increasing amount of carriers was added into liquid oily formulation until free-flowing powder is obtained and simultaneously drug content is also evaluated (Krupa et al., 2015)**.** The

prepared free flowing powder undergoes micromeritic properties like bulk density, tapped density, angle of repose and Carr's index (Selvam et al., 2011).

Solid carriers used can be of different types:

- Hydrophobic carriers: Silicon dioxide (eg: Aerosil 200, Sylysia), magnesium stearate, aluminum silicate (eg: Neusilin), calcium silicate (eg: Florite, Hubersob), micronized porous silica (eg: Syloid), precipitated silica (eg: Neosyl), fumed silica (eg: Aeroperl 300 and dibasic calcium phosphate (eg: Fujicalin SG) (Beg et al., 2016).
- Hydrophilic carriers: PVA, Na-CMC and HPβCD (Kang et al., 2012)

1.4.4. Melt granulation: It is also called Pelletization and is a single step operation that allows formation of granules obtained from powder mix (Jannin et al., 2008). In this technique binder is also added that is melted at low temperature and after melting acts binding agent (Schaefer et al., 1990). This meltable binder is when sprayed in molten state on powder mix then this technique is named as "pump on technique". Secondly, the meltable binder blended in the powder mix generates heat due to the friction between the particles and requires high shear mixing which lead to formation of liquid bridges among the powder particles that lead to formation of small granules (Jannin et al., 2008).

Mostly lipid based binders are used within 15% and 25% level depending on the fineness of powder mix. Lipids with low HLB value and high melting point are mostly preferred for sustain release formulations. Formulation parameters required to be considered are: drug particle size, shape, solubility in binder, concentration of binder, binder's melting point and thermoplastic behavior (Jannin et al., 2008).

Advantages of melt granulation:

- In this process, there is uniform distribution of the particles.
- The process is less time consuming due to elimination of drying step.
- In this process, there is no use of solvent or water.
- This method acquires good stability at different pH and moisture level.

Disadvantages of melt granulation:

- Higher energy is required in this process
- This process is not applicable for heat sensitive materials (Selvam et al., 2011; Janninet al., 2008).

1.4.5 Super critical fluid method: Lipids are used for coating of drug particle or for producing the solid dispersion in super critical method. Solid particles are obtained by adding the lipid based excipients and drug in methanol (organic solvent) and then subjected to supercritical fluid followed by lowering the temperature and pressure conditions to decrease their solubility in the fluid. Lipid based excipients used for controlled release are as follows: glyceryl trimyristate (Dynasan 114) and stearoyl polyoxyglycerides (Gelucire 50/02).

The useful consideration for SCF method includes: firstly, the solubility of excipient as well as the active substance in SCF, stability is also checked in the process condition. The energy and environmental condition should be checked due to evaporation of solvent. Mostly used SCF solvents are carbon dioxide, nitrous oxide, ethylene, propylene, propane, n- pentane, ethanol, ammonia and water. The SCF methods are used for highly potent drugs, low solubility drugs (because of less drug loading capacity) and drugs having higher lipid exposure potential (Schaeferet al., 1990; Yasuji et al., 2008).

Techniques involved in SCF method includes (Maulvi, 2015).

- Rapid expansion of supercritical solutions
- Gas anti-solvent recrystallisation
- Precipitation with compressed fluid anti-solvent
- Impregnation or infusion of polymers with bio-active materials
- Solution enhanced dispersion by supercritical fluid.

1.5 Advantages of SNEDDS (Beg et al., 2016)

- Improve oral bioavailability lead to reduction in dose
- Lowers production cost
- Higher stability
- Improve patient compliance and safety
- Better portability
- Provide large interfacial areas.
- It can protect the drug from hydrolysis and enzymatic degradation.
- Reduction in intra and inter subject variability and food effects.
- Quick onset of action.
- Ease of manufacture and scale up.

1.6 Limitations of SNEDDS (Nasr et al., 2016)

- Affected by temperature and pH.

- High production costs.

- Incompatibility problems with capsule shells.

1.7 Applications of S-SNEDDS

Table 1.4.Various SNEDDS prepared till date:

Drug	Composition of L-SNEDDS	Techniques of solidification	Formulation prepared	Carrier Used	Stage of development	References
Loratidin	Liquid paraffin, Capriole, Span 20 and Transcutol®	Extrusion Spheronization	S-SNEDDS	Aerosil	Formulation and development	(Abbaspour et al., 2014)
Carvedilol	Capmul® MCM, Nikkol® HCO 50,	Congealing	S-SNEDDS	Nikkol HCO 50	Preclinical phase	(Singh et al., 2013)
Lovastatin	Capmul® MCXM, Nikkol® HCO-50, Lutrol® F127	Melting method	S-SNEDDS	-	Preclinical phase	(Beg et al., 2015)
Loratadine	Captex® 200, Capmul ® MCM, Cremophor® - EL, Cremophor® EL	Bead formation by evaporation	S-SNEDDS	Porous polystyrene	Clinical phase	(Han et al., 2004)
Nifedipine	Imwitor© 742	Physical adsorption by trituirate	S-SNEDDS	Aerosil 200	Formulation and development	(Weerapol et al., 2014)
Vitamin A acetate	Soyabean oil, Capmul® MCM-C8, Cremophore® EL	Mixing and compression into tablets	SNEDDS tablets	Avicel	Formulation and development	(Taha et al., 2009)
Darunavir	Capmul® MCM, Tween 80, Transcutol® P,	Physical adsorption	S-SNEDDS	Neusilin US2	Preclinical phase	(Inugala et al., 2015)
Cilostazol	Peceol, Tween 20, Labrasol	Spray dried	S-SNEDDS	Calcium silicate	Preclinical phase	(Mustapha et al., 2017)
Embelin	Capryol® 90, Acrysol® EL 135,PEG 400	Physical adsorption	S-SNEDDS	Aerosil, Neusilin US2	Formulation and development	(Parmar et al., 2015)
Rosuvastatin calcium,	Garlic oil, olive oil, Tween-80, PEG 400	Physical mixing	Solid supersaturable SNEDDS	Maltodextrin and MCC 102	Priclinical phase	(Abo Enin and Abdel-Bar, 2016)
Tacrolimus	Capryol® PGMC, Transcutol ® HP, Labrasol®	Absorption method	S-SNEDDS	Colloidal silica	Preclinical phase	(Seo et al., 2015)
Valsartan	Capmul® MCM, Labrasol®, Tween 20	Adsorption method	S-SNEDDS	Aerosil 200, Sylysia (350,550,730), Neusilin US2	Preclinical phase	(Beg et al., 2012)

Loratidine	Solutol® HS 15,Capmul® MCM C8	Adsorption method	S-SNEDDS	Aerosil (A200), Aerosil (AR972)	Preclinical phase	(Verma et al., 2016)
Celecoxib	Capryol® 90, Cremophor® RH 40, Propylene glycol	-	SNEDDS	-		(Kaur et al., 2013)
Rosuvastatin	Capryol® 90, poloxamer 407, Transcuto®l P	Spray dried	S-SNEDDS	Mannitol	Formulation and development	(Kamel and Mahmoud, 2013)
Flurbiprofen	Labrafill® M 1944, Labrasol, Transcutol® HP	Spray dried	S-SNEDDS	Hydrophobic and hydrophilic carriers	Formulation and development	(Kang et al., 2012)
Glimepiride	Tween® 80, PEG and Mygliol ®812	Physical adsorption	S-SNEDDS	Aerosol® 200	Preclinical phase	(Mohd et al., 2015)
Olmesartan medoxomil	Oelic acid, Tween 80 and Transcutol ®HP	Surface adsorption method	S-SNEDDS	Aerosil 200, Aeroperl GT, Sylysia 550, Neusilin US2 and Fujicalin SG	Preclinical phase	(Beg et al., 2016)
Repaglinide	Olive oil, Miglyol® Cremophore® RH 40, Capryol® 90 and Labrasol®	Adsorption technique	S-SNEDDS	Neusilin US2	Formulation and development	(Reddy et al., 2014)
Erlotinib	Labrafil® M2125CS, Labrasol, and Transcutol ®HP	Spray dried	S-SEDDS	Dextran or Aerosil	Preclinical phase	(Truong et al., 2016)
Docetaxel	Capryol® 90, Cremophore EL and Transcutol® HP	Absoption method	S-SNEDDS	Colloidal silica	Preclinical phase	(Quan et al., 2012)
Simvastatin	Capryol® 90, Cremophore® RH 40, Transcutol ® HP	Adsorption technique	S-SNEDDS	Crospovidone	Formulation and development	(Sunitha Reddy and Sowjanya, 2015)
Irbesartan	Capryol ®90, Cremophor® RH40 and Transcutol ® HP	Spray dried	S-SNEDDS	Aerosil 200	Research	(Nasr et al., 2016)
Glipizide	Captex ®355, Solutol® HS15 and Imwitor® 988	Physical mixing	S-SNEDDS	Calcium carbonate	Formulation and development	(Dash et al., 2015)

1.8 Patents of S-SNEDDS:

Table 1.5 Patents related to S-SNEDDS

S.N.	Title / Year	Patent number	Inventors	References
1	The self-emulsifying formulation consists of effective amount of curcuminoids, an oil phase, a surfactant, and a co-surfactant. The composition may comprise of additives	US 20110294900A1	Kohli, K., Chopra, S., Arora, S., Khar, RK., Pillai, K.K.	Tarate et al., 2013
2	A eutectic based SNEDDS is formulated from essential oils, and pharmacologically effective drug. The drug is poorly water soluble, such as ubiquinone. The SNEDDS can be further incorporated into a powder to produce a solid dosage form. The solid dosage form contains the SNEDDS, a copolymer of vinylpyrrolidone® and vinyl acetate, maltodextrin and microcrystalline cellulose	7588786	Khan et al., 2009	Agrawal et al., 2012
3	Eutectic based SNEDDS formulated from Cremophor, Capmul, essential oil, and poorly soluble drug. The SNEDDS may be converted into solid dosage form by adding the ingredients comprising Kollidon® VA 64, maltodextrin, and MCC	US20100166873A1	Khan, M.A., Nazzal, S.	Tarate et al., 2013

1.9 Drug profile

Glimepiride is an oral hypoglycemic agent that belongs to the class of third generation sulfonylureas. It may act by extrapancreatic mechanisms as well. It is a drug of choice in patients suffering from Non-Insulin Dependent i.e. Type II Diabetes Mellitus (NIDDM). It may be combined with insulin for patients associated with secondary sulfonylurea failure (https://www.ncbi.nlm.nih.gov/pubmed/9561345).

Compared to other generations of sulfonylureas, it has higher potency and acts for a longer time. It undergoes metabolism by the enzyme CYP2C9 and agonizes the activity of peroxisome proliferator-activated receptor gamma (PPARgamma).

(https://ncit.nci.nih.gov/ncitbrowser/ConceptReport.jsp?dictionary=NCI_Thesaurus&ns=NCI_Thesaurus&code=C29073).

The role of glimepiride in reducing glucose levels in blood apparently depends on the stimulation of release of insulin from functional beta cells in pancreas, and sensitizing the peripheral tissues towards insulin. It likely binds to ATP sensitive K^+ channel receptors on the surface of pancreatic cells, decreasing the conductance of potassium ions thereby depolarizing the membrane. It in turn stimulates influx of Ca^{++} through voltage-sensitive Ca^{++} channels. This elevation in the intracellular concentration of Ca^{++} induces the secretion of insulin (http://www.hmdb.ca/metabolites/HMDB14367)

1.9.1 Complete profile of Glimepiride (https://www.drugbank.ca/drugs/DB00222, accessed on 5/7/2017)

Characteristic	Description
Drug name	Glimepiride
Category	Antidiabetic
Formula	$C_{24}H_{34}N_4O_5S$
Molecular weight	490.619 g/mol
Synonyms	Amaryl, glimepiride,glimepiride, glimepiridum
IUPAC name	3 ethyl-4-methyl-N- {2-[4({[(4-Methyl cyclohexyl) carbonyl] amino} sulfonyl)phenyl] ethyl}-2-oxo-2,5-dihydro-1H –Pyrrole -1- carboxamide.

Chemical structure

Solubility	Insoluble
Melting point	207℃
Log P	3.5
Absorption	100%
Protein binding	More than 99.5%
Half life	Approx 5 hours

CHAPTER 2

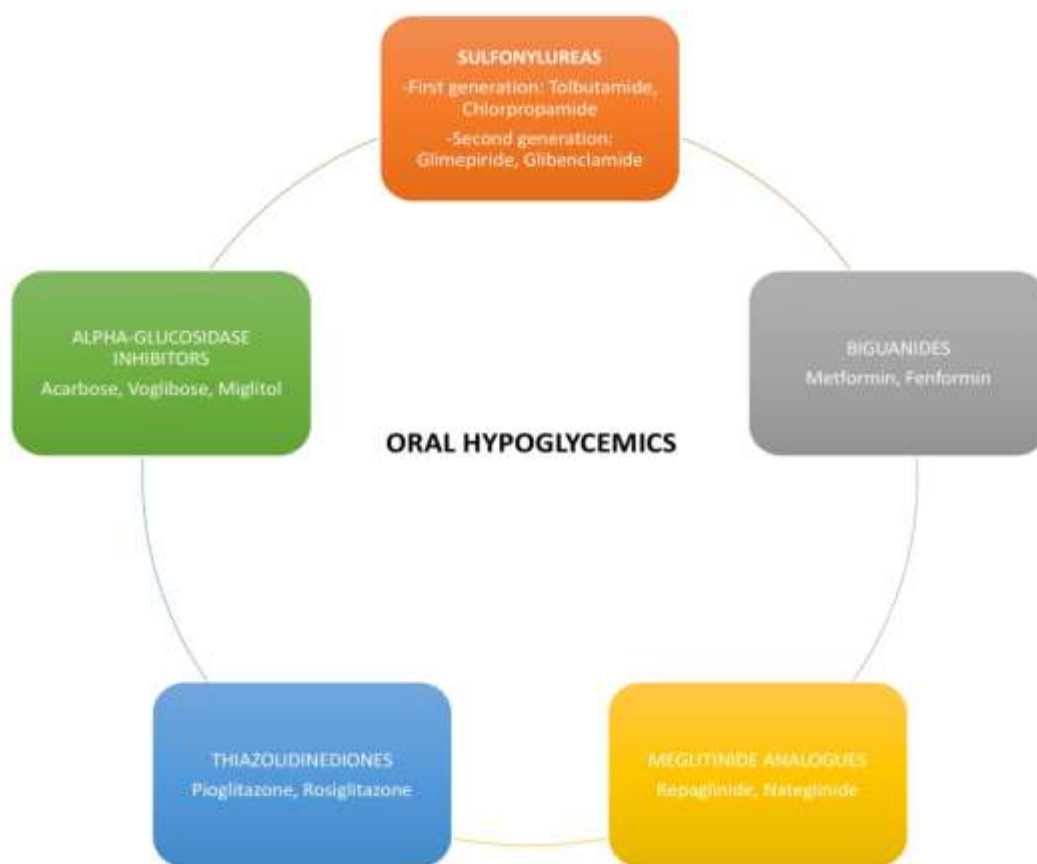# LITERATURE REVIEW

2.1 Literature review of diabetes

Diabetes mellitus is one of the oldest diseases and about 3000 years ago it was first reported in manuscripts of Egyptians (Ahmed, 2002). Treatment of diabetes is known since middle ages and explanation of its pathogenesis was found in 20th century (Piero, 2015). It is a principle cause of persistent ill health and mortality, moreover, it takes more lives per year as compared to HIV-AIDS with almost 1 death in every 10seconds (Kaul et al., 2013).

Globally, due to rise in obesity, diabetes became a global epidemic and continued to increase every year (King et al., 1998). Recent survey from the fact sheet of WHO predicted that the number of people suffering from diabetes has increased from 108 million in 1980 to 422 million in 2014. Among adults over 18 years of age the global prevalence of diabetes has increased from 4.7% in 1980 to 8.5% in 2014. Its prevalence is increasing rapidly in middle and low economic countries and is major cause of kidney failure, blindness, heart attacks, stroke and amputation of lower limb. In the year 2012, about 1.5 million deaths were reported that are caused by diabetes and about 2.2 million deaths were because of high blood glucose. WHO projects that diabetes will be the 7th leading cause of death in 2030. There are many ways to prevent or delay the onset of type 2 diabetes such as healthy diet, regular physical activity, maintaining a normal body weight and avoiding tobacco use (http://www.who.int/diabetes/global-report/en/).

Diabetes mellitus is classified into three categories such as type 1diabetes, type 2 diabetes and gestational diabetes. Type1diabetes is also called juvenile onset diabetes or autoimmune diabetes (Kaul et al., 2013). It is characterized by beta cells destruction because of autoimmune process which results in absolute insulin deficiency. Due to complete insulin deficiency, the pancreatic alpha cells function is also abnormal and there is excessive production of glucagon in type 1 diabetes mellitus (Maraschin, 1997). If there is complete insulin deficiency then ketoacidosis may be developed and patient may enter in coma and finally death. The problem faced during treatment of diabetes mellitus is to maintain normal blood glucose level, hence, patient with type

1 diabetes mellitus are treated with insulin which can be given orally, or in inhaled form or in form of injectable or with novel drug delivery system (Chaillous et al., 2000; Card and Magnuson, 2011; Zarogoulidis et al., 2011).

Type 2 diabetes mellitus is also known as adult onset diabetes and it is very common type of diabetes and comprise of 85-90% cases worldwide (Diabetes, 2011). Type 2 diabetes is characterized by problem in insulin secretion and sensitivity. Type 2 diabetes is not easily recognized by the patients up to many years because there is gradual development of hyperglycemia and in starting stage it is not very severe for the patient to notice its symptoms. Initially in type 2 diabetes mellitus patients do not require treatment of insulin to survive. Etiology of type 2 diabetes mellitus is not known and autoimmune destruction of beta cells does not take place (Diabetes, 2011). First line defense against type 2 diabetes mellitus is to control blood glucose levels and blood pressure as well as changes in living standard, moreover, diet and weight control should also be followed. If a patient does respond to the above practices and still there is increased blood glucose then oral hypoglycemic is used. Third type of diabetes mellitus is known as gestational diabetes mellitus. It is defined as glucose intolerance and occurs during pregnancy and gestational period. There are mainly 5 types of oral hypoglycemic agents and they are as follows:

Fig.2.1. Classification of oral hypoglycemics.

2.2 Literature review of SNEDDS

Taha et al., (2009) formulated SNEDDS of vitamin A and was easily converted to solid state by proper mixing with Avicel® (directly compressible vehicle) and by using 4% talc powder which helped in enhancing the flowability of powder and finally compressed into tablets. Vitamin A SNEDDS consisted of Cremophor® EL, soyabean oil, and Capmul® MCM- C8 showed relative higher bioavailability as compared to unprocessed vitamin A oily solution. Further, significant difference was observed in AUC and C_{max} of vitamin A SNEDDS tablets and unprocessed vitamin A oily solution.

Kang et al., (2012) examined the effects of different solid carriers on the dissolution, crystalline properties and bioavailability of flurbiprofen. Liquid SNEDDS were spray dried to form S-SNEDDS using different carriers. L-SNEDDS contained Labrafil®M 1944 CS, Labrasol®, Transcutol® HP and flurbiprofen. Hydrophobic carriers such as silicon dioxide produced

excellent S-SNEDDS with droplet size less than 10 nm and magnesium stearate showed largest diameter and formed eutectic mixture with improved oral bioavailablity and dissolution rate. S-The hydrophilic carriers such as polyvinyl alcohol (PVA), sodium carboxy methyl cellulose (NA- CMC) almost improved the dissolution rate but they were found comparatively less than that of S-SNEDDS formed by using silicon dioxide.

Beg et al., (2012) formulated solid SNEDDS of valsartan by using Capmul® MCM, Labrasol® and Tween® 20. Solubility studies and pseudo ternary phase diagram were constructed. Box Behnken design was used for optimizing SNEDDS using the principles of surface response methodology. Porous carriers like Aerosil® 200, Sylysia® (350, 550 and 730) and Neusilin® US2 were used to form free flowing granules for selected L-SNEDDS. Authors reported that because of enhance solubility, in-vitro dissolution studies showed 3-3.5 folds increased in dissolution rate of valsartan. Further, *in-vivo* test performed for S-SNEDDS decreased in systolic blood pressure in Wistar rats. Powder XRD performed revealed lack of drug interaction with porous carriers and lipidic excipient. Accelerated stability studies performed for 6 months revealed S-SNEDDS was stable with no change in physiochemical properties.

Quan et al., (2012) developed an alternative dosage for marketed injectable docetaxel product. Hence, they formulated docetaxel S-SNEDDS using spray drying. Colloidal silica was used solidify L-SNEDDS. L-SNEDDS were comprised of Capryol® 90, Cremophore® EL and Transcutol® HP. It was reported that S-SNEDDS containing 3.3% (w/v) docetaxel produced nano-emulsion which showed absolute bioavailability about 12.5% in rats.

Kamel and Mahmoud, (2013) prepared spray dried SNEDDS tablets of rosuvastatin using mannitol as a carrier. SNEDDS consisted of Capryol® 90 (oil), poloxamer 407 (surfactant) and Transcutol® or triacetin (co- surfactant). Characterization was performed for prepared SNEDDS for droplet size and polydispersity index. Caco-2 cells lines were used for the evaluation of cytotoxicity study. During spray drying, it was reported that formulation prepared using Transcutol® was found to be soft and sticky on the wall of dryer, hence, SNEDDS prepared using triacetin were used for further studies. The results of Caco-2 cells revealed that the formulation was safe to be used. The comparative bioavailability study conducted revealed better self nano-emulsifying capacity by using triacetin as co- surfactant. Droplet size analysis revealed droplet size less than 50 nm and polydispersity index between 0.127-0.275. It was reported that by

incorporation of rosuvastatin into SNEDDS, its anticancer effect enhanced because of it penetration inside the cells through SNEDDS.

Sakthi et al., (2013) reported that SNEDDS are in demand nowadays because of their benefits such as good portability, high stability, higher drug loading and economic production and also used to enhance the solubility of lipophilic drugs. It was also reported that SNEDDS play an important role in improving the oral bioavailability of some poorly soluble drugs.

Abbaspour et al., (2014) prepared solid SNEDDS of poorly soluble loratadine using extrusion-spheronization method. L-SNEDDS consisted of loratadine, liquid paraffin, span 20, Transcutol® and Capriole®. For formulation of SNEDDS a multilevel factorial design was employed. Optimized SNEDDS pellets were compared with that of marketed loratidine SNEDDS and powder tablets. Results showed that the self nano-emulsifying pellets have uniform shape and size and in vitro release of SNEDDS pellets was found to be more as compared to the liquid SNEDDS and powder tablets.

Seo et al., (2015) formulated S-SNEDDS of tacrolimus to enhance its oral bioavailability. L-SNEDDS composed of Capryol® PGMC, Transcutol® HP and Labrasol®, were spray dried by using colloidal silica as a carrier. The S-SNEDDS containing 5%w/v of tacrolimus was found to increase its oral bioavailability and dissolution rate.

Dash et al., (2015) formulated S-SNEDDS of glipizide for improving its solubility and dissolution profile. Pre-concentrate of SNEDDS was optimized using central composite design and it consists of Captex® 355, Solutol® HS and Imwitor® 988. The PXRD, DSC, and SEM results indicated that glipizide is present in amorphous and in molecular dispersion state within solid SNEDDS. Authors reported that S-SNEDDS helped in preserving the self-emulsifying property of L-SNEDDS and as a result there is increase in dissolution of glipizide as compared to marketed and pure drug.

Nasr et al., (2016) prepared solid SNEDDS for enhancing the solubility as well as dissolution. Aerosil® was used as carrier. Optimized batch was prepared using Capryol® 90, Cremophor® RH40 and Transcutol® HP. Characterization and evaluation results revealed droplet size was in nanometric range and poly-dispersity value was also in acceptable range. The prepared batches showed high stability, good optical clarity, rapid emulsification time and high amount of drug

content. Further, TEM showed spherical size particle and droplet size less than 50 nm. *In-vitro* release showed 90% of drug released in 90 minutes and on basis of results obtained, optimized batch was chosen for spray drying. The prepared S-SNEDDS was evaluated and result obtained showed high amount of drug content and good flow properties.

Beg et al., (2016) developed S-SNEDDS of olmesartan medoximil by using porous carriers for increasing their oral bioavailability. On the basis of solubility studies and pseudo ternary phase diagrams, oleic acid, Tween® 40 and Transcutol® were selected as oil, surfactant and co-surfactant. Porous carriers like Aerosil® 200, Sylysia® 550, Aeroperl® 300, Neusilin® US2 and Fujicalin SG® were adsorbed on to the L-SNEDDS to form S-SNEDDS. From these, Neusilin® US2 was selected due to its good oil adsorption capacity, micromeritic properties and excellent flowability and compactibility. About 2.6 folds increase in drug release rate was observed for optimized S-SNEDDS as compared to raw drug. Whereas, there was no tangible difference in the drug release was observed between L-SNEDDS and S-SNEDDS. *In-vivo* pharmacokinetic studies were performed on Wistar rats and it showed about 2.32 and 3.27 folds increase in Cmax and AUC of the drug present in S-SNEDDS as compared to its raw form.

Chai et al., (2016) developed SNEDDS of dabigatran etexilate for the inhibition of stroke and thromboembolism. Ternary phase diagram was used to optimize SNEDDS and then solidified into dispersible tablets. Phase diagram study was used to analyze *in-vitro* dissolution rate. The results showed that 60% of dabigatran etexilate (DE) was present in oil. *In vivo* study was performed in male Sprague-Dawley rats. Dissolution rate was increased without any precipitation of drug in gastric fluid. Relative bioavailability of optimized SNEDDS was 531.80 in comparison to marketed product of DE.

2.3 Literature Review of drug

Bhagat and Sakhare, (2012) used solid dispersion technique for improving the solubility of glimepiride by using poloxamer 188 (PXM 188). From optimized solid dispersion batch, solid dispersion tablet was prepared by using croscarmellose. The results revealed that solid dispersion containing drug to polymer in ratio of 1:4 gave best dissolution profile. Formulation containing 5% croscarmellose gave good dissolution and disintegration results as compared to other formulations.

Chaudhari et al., (2012) used solid dispersion technique for improving the solubility as well as dissolution of poorly soluble drug glimepiride. Solvent evaporation method was used to prepare the solid dispersion (SD) of glimepiride in PVP K30 and solid dispersion tablet was prepared using cross povidone from the optimized batch of the solid dispersion formulation. Result revealed that the SD prepared from PVP K30 in the ratio 1:5 gives excellent dissolution profile and formulations containing 5% cross povidone gave best dissolution and disintegration results as compared to other formulations.

Kamble et al., (2012) formulated and optimized SNEDDS of glimepiride and converted to S-SNEDDS by using Aersoil® 200 as carrier. Optimized formulation of L-SNEDDS having glimepiride was developed with the help of ternary phase diagram and D-optimal mixture design. S-SNEDDS showed 99.5% drug release as compare to marketed glimepiride. The results revealed that stable S-SNEDDS can be formed for drugs that are poorly water soluble and also helped in improving the solubility and dissolution.

Shah et al., (2013) designed SNEDDS of glimepiride and optimized it. Solubility of glimepiride was determined in various vehicles. Oils, surfactant and co-surfactant was used for construction of ternary phase diagrams. A three level Box –Behnken design (BBD) was used to check the interaction effect of dependent and independent variables. Suitable ternary system selected SNEDDS composed of Capmul® MCM, Akcrysol® K 140 and Transcutol®. This system revealed release of 80% drug within 5 minutes.

Mohd et al., (2015) developed S-SNEDDS of glimepiride for improving its oral delivery and therapeutic efficacy in albino rats. It was reported that S-SNEDDS rapidly got emulsified into oil in water nano-emulsion. The prepared S-SNEDDS were characterized by SEM, DSC and X- ray studies wherein the results revealed that the glimepiride was present in amorphous state in S-SNEDDS. The *in vitro* results showed that S-SNEDDS has shown rapid dissolution rate as compared to pure glimepiride.

Li et al., (2015) utilized hydrotropy technique for improving the solubility and bioavailability of glimepiride. Meglumine was used here as hydrotrope. Lyophilization technique was used to prepare glimepiride meglumine (GLMP- MU) complex powder. FTIR, XRD and DSC results showed that due to presence of large number of hydrogen bonds the GLMP-MU complex was

converted to amorphous state. *In vitro* and *in -vivo* results of GLMP-MU complex showed rapid dissolution rate and improved bioavailability.

Li et al., (2016) utilized micro-emulsion technique for increasing the oral bioavailability of glimepiride. On the basis of solubility study, pseudo ternary phase diagrams and Box- Behnken design, glimepiride micro-emulsion was prepared and optimized. Optimized micro-emulsion composed of Capryol® 90, Cremophor® RH 40 and Transcutol® enhanced the solubility of glimepiride. *In –vivo* pharmacokinetic and pharmacodynamic studies revealed that glimepiride micro-emulsion helped in controlling the glucose blood level in diabetic mice and also helped in improving the bioavailability of glimepiride.

CHAPTER 3

RESEARCH ENVISAGED & PLAN OF WORK

3.1 Rationale

Glimepiride is a potent sulfonylurea and has established potential benefits such as lower dose, rapid onset, low insulin levels and less-pronounced glucagonotropic effects, insulin-sensitizing and insulin-mimetic affects. However, it is a poorly soluble drug (< 8 μg/mL in pH 7.4 phosphate buffer) with relatively high permeability through CaCo-2 cell monolayer's which warrants it to be classified under BCS Class II classification.

Glimepiride administration under fasting condition significantly increases the area under curve for 24 h and increases the maximum concentration of glimepiride in blood compared to its administration under feeding condition, moreover, the lag time was significantly reduced in fasting condition compared to feeding condition suggesting that glimepiride is effectively absorbed from the gastrointestinal tract, but the presence of food, and certain dietary supplements interfere with its dissolution and in turn its absorption (Hardman and Limbird, 2001).

In view of the time required to reach an optimal concentration in plasma, glimepiride may be more effective if given 30 min prior to meal (Hardman and Limbird, 2001).Conversely, this might reduce patient compliance since after taking the drug if the patient is not able to have the meal it would result in severe hypoglycemia and if taken with meal, food would interfere sequentially with its absorption. Hence, improving the dissolution characteristics of glimepiride might allow its concomitant dosing with food. Several attempts have been tried to improve the dissolution rate of glimepiride starting from the conventional approaches like solid dispersions, complexation to the novel approaches like nanotechnologies. Each approach tried was having some limitations like stability, cost in scale up or regulatory approvals. However, SNEDDS of glimepiride is the area which was untouched by the scientists either in terms of research publications or patents. In modern year, Self-Nano Emulsifying Drug Delivery Systems (SNEDDS) have emerged as a novel approach to overcome the problem of bioavailability and dissolution. SNEDDS is defined as an isotropic mixture of oil, surfactant and co-surfactant that

have ability to form oil in water nano-emulsion upon mild agitation followed by dilution in aqueous media that is GI fluid.

Hence, in the present study it was sought to prepare SNEDDS of glimepiride in order to enhance its dissolution rate and permeability.

3.2 Aim and objective

3.2.1 Aim

Formulation and evaluation of Solid SNEDDS of glimepiride: *In vitro and ex vivo* evaluation

3.2.2 Objective of study

- Formulation of S- SNEDDS of glimepiride.
- Characterization of developed L-SNEDDS and S-SNEDDS.
- Improvement of solubility, dissolution rate and permeability of glimepiride through SNEDDS.

3.3 Plan of work

Literature review

Solubility of Glimepiride in various oil, surfactant and co-surfactant

Preliminary study in order to select best oil, surfactant and co-surfactant

Formulation ,development ,optimization and characterization of L-SNEDDS of glimepiride

Development of S-SNEDDS by spray drying using porous carriers

Characterization of S-SNEDDS

In vitro and ex vivo study of optimized batch of glimepiride

CHAPTER 4

EXPERIMENTAL WORK

4.1 Materials

Table 4.1 List of materials used in study

Chemicals	Manufacturers
Glimepiride	Micro Labs, India
Acetonitrile HPLC Grade	Lobachemie Pvt. Ltd., Mumbai, India
Sodium Hydroxide pellets	Central drug house (P) Ltd, New Delhi
Orthophosphoric acid	Lobachemie Pvt. Ltd., Mumbai, India
Triethylamine	Lobachemie Pvt. Ltd., Mumbai, India
Ethanol	Central drug house (P) Ltd, New Delhi
Aerosil 200	Central drug house (P) Ltd, New Delhi
Potassium Dihydrogen Orthophosphate	Central drug house (P) Ltd, New Delhi
Hydrochloric acid	Lobachemie Pvt. Ltd., Mumbai, India
Ammonium acetate	Lobachemie Pvt. Ltd., Mumbai, India
Millipore water	Bio-Age Equipment Ltd., Mohali, Punjab, India
Hydrochloric acid	Lobachemie Pvt. Ltd., Mumbai, India
Lauroglycol® FCC	Gattefosse India Pvt. Ltd
Tween (80,20 and 60)	Central drug house (P) Ltd, New Delhi
Span(20,40,60 and 80)	Central drug house (P) Ltd, New Delhi
PEG (200,400,600 and 800)	Central drug house (P) Ltd, New Delhi
Pluronic ®F-68	Central drug house (P) Ltd, New Delhi

Chemicals	Manufacturers
Sesame oil	Central drug house (P) Ltd, New Delhi
Peanut oil	Central drug house (P) Ltd, New Delhi
Sunflower oil	Central drug house (P) Ltd, New Delhi
Cotton seed oil	Central drug house (P) Ltd, New Delhi
Soyabean oil	Central drug house (P) Ltd, New Delhi
Mustard oil	Central drug house (P) Ltd, New Delhi
Oleic acid	Central drug house (P) Ltd, New Delhi
Olive oil	Central drug house (P) Ltd, New Delhi
Eucalyptus oil	Central drug house (P) Ltd, New Delhi
Castor oil	Central drug house (P) Ltd, New Delhi
Hydroxy propyl beta cyclodextrin (HPBCD)	Central drug house (P) Ltd, New Delhi
Polyviny alcohol (PVA)	Central drug house (P) Ltd, New Delhi
Sodium carboxy methyl cellulose (NA-CMC)	Central drug house (P) Ltd, New Delhi
Formic acid	Lobachemie Pvt. Ltd., Mumbai, India
Trehalose	Lobachemie Pvt. Ltd., Mumbai, India
Mannitol	Lobachemie Pvt. Ltd., Mumbai, India
Sorbitol	Lobachemie Pvt. Ltd., Mumbai, India
Labrafac® CC	Gattefosse India Pvt. Ltd
Labrafil® MI944CS	Gattefosse India Pvt. Ltd
Labrafil® M2125	Gattefosse India Pvt. Ltd

Chemicals	Manufacturers
Labrasol®	Gattefosse India Pvt. Ltd
Maisine® 35-1	Gattefosse India Pvt. Ltd
Capryo®l 90	Gattefosse India Pvt. Ltd
Miglyol ® 812N	Cremer Ole GmbH& Co.KG, Germany
Syloid XDP3150	Grace Material Technologies, Discovery Sciences, Pune ,India
Capmul® MCM	M/S Abitec Corp., Ohio
Transcutol®P	Gattefosse India Pvt. Ltd
Syloid 244 FP	Grace Material Technologies, Discovery Sciences, Pune ,India
Cithrol® GMS	Croda India Company Pvt. Ltd, India
Triacetin	Sigma Aldrich, USA
Egg phosphatidyl Choline	Lipoid GmbH, Germany
Soya phosphatidyl Choline	Sigma Aldrich, USA
Lactose	Lobachemie Pvt. Ltd., Mumbai, India

4.2 EQUIPMENTS

Table 4.2 List of equipments used in the study

Equipments	Model/Manufacture
Electronic weighing balance	CY360, Shimadzu Co. Ltd., Japan
Tablet Dissolution apparatus	DS 8000 (Manual) LABINDIA, Maharashtra, India
pH meter	Phan, LABINDIA, Thane West, Maharashtra, India
High Performance Liquid Chromatography	HPLC LC-20AD, Shimadzu Co. Ltd., Japan
UV Spectrophotometer	UV-1800, Shimadzu Co. Ltd., Japan
Spray drier	JISL Spray Mate
Ultrasonication bath	LOBA LIFE, Loba Chemie, Mumbai, India
Hot air oven	Cadmach Drying Oven, Cadmach Machinary Ltd., Ahmadabad, India
Sieves	Sieve No. 44, Bhushan Engineering & Scientific Traders, Ambala
Magnetic Stirrer	Remi 5MLH, Vasai, Mumbai, India
FTIR Spectrophotometer	Shimadzu Co. Ltd., Japan
Stability chamber	Remi CHM 10S
Differential scanning calorimeter	DSC Q200 V24.4 Build 116
Scanning electron microscope	Hitachi S-3400N
Transmission electron microscope	FEI Tecnai G 2 F20 model, The Netherlands
XRD analyzer	PAN analytical X'pert 3 Pro, The Netherlands
Zeta sizer	Beckman Coulter Delsa™Nano
Partilce size	Zetasizer ,Malvern Instruments Ltd

4.3. Experimental

4.3.1. HPLC method development of glimepiride

The HPLC system consisted of a mobile phase delivery pump (LC-20 AD; Shimadzu, Japan), a photodiode array detector (SPDM20A; Shimadzu, Japan), a 20µL loop (Rheodyne) and LC Solution software. A C-18 reverse-phase column (Nucleodur C18, 250 mm × 4.6 mm i.d.,5µ) was utilized for estimation and separation of glimepiride, using acetonitrile - 5% ammonium acetate buffer pH 5 (60:40, v/v) as mobile phase. The flow rate was 1.0 mL min^{-1} and detection wavelength was 228 nm. Standard solutions (5, 10, 15, 20 and 25 µg/mL for glimepiride, respectively) were prepared in mobile phase and analysed. The developed method was validated as per ICH Q2 (R1) guidelines.

4.3.2 Solubility studies of raw glimepiride in various oils, surfactants and co-surfactants

In order to select the best oil, surfactant and co-surfactant for the formulation of SNEDDS, the solubility studies have been performed for raw glimepiride in oils (castor oil, sesame oil, coconut oil, peanut oil, sunflower oil, eucalyptus oil, cotton seed oil, oleic acid, sunflower oil, Labrafac®, olive oil, Labrafil®1944CS, Capmul® MCM, Labrafil® M2125, Soyabean oil, Capryol® 90, Lauroglycol® FCC, Maisine® 35-1, Miglyol® 812N, mustard oil, Triacetin® and Cithrol® GMS), surfactants (PEG 400, PEG 200, PEG 600, PEG 800, PG, Tween 80, Tween 20, Tween 60, Span 20, Span 60, Span 80, Span 40, Egg phosphatidyl choline [1% w/v in water: ethanol mixture (50:50 v/v)], soya phosphatidyl choline [1% w/v in water: ethanol mixture (50:50 v/v)] and Labrasol®) and co-surfactants (Transcutol® P, ethanol) respectively. To 1mL of each oil, surfactant and co-surfactant, 100 mg of raw glimepiride was taken separately in 5 mL clean glass vials and vortexed (CM 101 CYCLO MIXER, REMI, India) for 2 min for proper mixing of glimepiride with the vehicle. The vials were stoppered and agitated for 48h at 37±0.2°C in a shaking water bath. Upon equilibration, all the samples were centrifuged at 10000 rpm for 15 min for removal of the undissolved glimepiride from saturated solutions (Inugala, et al. 2015). The supernatants were accurately measured and appropriately diluted with ethanol and glimepiride concentration was estimated by HPLC at 228 nm.

4.3.3 Preparation of L-SNEDDS

Based on the results of solubility studies, Lauroglycol® FCC was selected as oil, Tween-80 as surfactants and ethanol as co-surfactant, respectively. A total of 27 SNEDDS prototypes were prepared by varying the ratios of oils, surfactants and co-surfactants. The concentration of

Lauroglycol® FCC (oil) was varied from 10 to 90 % v/v and mixture (S_{mix}) of surfactant (i.e. Tween-80) and co-surfactants (i.e. ethanol) was varied from 10 to 90% v/v in the ratio of 1:1, 2:1 and 1:2, respectively. In small increments, glimepiride (10 mg) was added individually to all the prepared formulations and mixed using vortex mixer to form a monophasic system and stored in clean glass vials (screw capped) at room temperature until their further evaluation (Inugala et al. 2015).

4.3.4. Construction of ternary phase diagram

Selected oil, surfactant and co-surfactant were mixed in various ratios and ternary-phase diagram was plotted to achieve spontaneous and stable self-emulsification zone. In order to assess the self-emulsification properties, the prepared emulsions were verified visually through test reported by Craig et al. (1995) and Inugala, et al. (2015) with minor modification. Ternary phase diagram was constructed by considering the factors like tendency to form emulsion, phase separation, clarity, coalescence of droplets and drug precipitation. The prepared L-SNEDDS (200 µL) were dropped in glass beaker containing 500 mL distilled water that was maintained at $37\pm0.2°C$, which was continuously stirred at 100 rpm using magnetic stirrer. The resulting emulsions were observed visually for the relative turbidity. The stability of formed emulsions was confirmed by visual inspections such as extemporary emulsification, drug precipitation, phase separation, cracking of the emulsion on storage (48 h) at room temperature. The formulations were considered unstable when no emulsion formed or, emulsion formed with immediate coalescence of droplets along with phase separation and drug precipitation (Craig et al., 1995; Inugala, et al. 2015).

From the pseudo ternary phase diagram, nanoemulsion region was selected. The results revealed that Lauroglycol® FCC, Tween-80 and ethanol were used in varying ratios of 1:1 (F_{1-4}), 1:2 (F_{10-18}) and 2:1 (F_{19-21}) exhibited the largest nanoemulsion area. Moreover, it was also observed that increasing the amount of Lauroglycol® FCC above 40% caused increase in droplet size as well as PDI, whereas, increase in surfactant and co-surfactant percentage above 60 revealed in decrease in droplet size and PDI.

4.3.5. Evaluation of optimized L-SNEDDS formulation for thermodynamic stability studies and cloud point

Stability of the optimized L-SNEDDS formulation was evaluated at different stress conditions such as heating cooling cycles (4°C and 40°C) and freeze thaw cycles (-21°C and +25°C) along with storage at specified temperature for 48 h. In order to carry out centrifugation stress study, 1 mL of the formulation was diluted to 100 mL with distilled

water and centrifuged at 10000 *g* for 20 min and visually observed for any phase separation (Kallakunta et al., 2012; Inugala, et al. 2015). In order to determine cloud point temperature, 10 mL of diluted L-SNEDDS formulation were gradually heated on a water bath and observed for cloudiness using thermometer. The temperature at which cloudiness appeared was denoted as cloud point (Zhang et al., 2008; Inugala et al. 2015).

4.3.6. Solidification of optimized batch of SNEDDS

4.3.6.1. Oil adsorption capacity

In order to enhance the stability of L-SNEDDS formulation it was further solidified by using array of porous carrier. Both hydrophobic like Aerosil®-200 (A-200), Syloid® 244FP (SFP), Syloid® XDP 3150 (SXDP), Magnesium stearate (MS), Micro Crystalline Cellulose (MCC) PH102 and lactose and hydrophilic carriers like Poly vinyl alcohol (PVA), Na-CMC and HP-β-CD, were used. In order to achieve better flow and compaction the oil adsorption capacity (OAC) of carriers should be high, hence, selected carriers were subjected for estimation of their OAC. Gravimetric method was used to carry out OAC, where the amount of porous carrier required to transform the unit dose of oily liquid formulation into the free-flowing powder was calculated (Modasiya et al. 2009; Malaysia 2012).

4.3.6.2. Preparation of solid SNEDDS (S-SNEDDS) using spray drying

Different batches of S-SNEDDS were formulated using spray dryer. The hydrophobic carriers (1g) such as A-200, SFP, SXDP, MCC PH 102 and MS, were each suspended in 100 mL ethanol. Similarly, hydrophilic carriers (1g) PVA, Na-CMC and HPBCD, were each dissolved in 100 mL water. The L- SNEDDS (1 mL) was added to the prepared solutions/dispersions with constant stirring on a magnetic stirrer at 100 rpm and kept stirred for homogenous dispersion. Each dispersion was subjected to spray drying through 0.7 mm diameter nozzle at a peristaltic pump flow rate of 16 mL/min, atomization air pressure 4 Kg/cm^2, aspirator filter pressure -25 mbar, inlet temperatures of 70°C (for ethanolic dispersions) and 100°C (for aqueous dispersions) and recorded outlet temperatures of 35 and 50°C, respectively.

4.3.7. Characterization of developed S-SNEDDS formulation

The S-SNEDDS powders were further subjected to micromeritic characterization for true, bulk, and tapped density, flow rate, angle of repose, Carr's compressibility index.

4.3.7.1. Flow rate and Angle of repose

The flow rate of the powders was determined as the ratio of mass (g) to time (s) using glass funnel with an orifice diameter of 10 mm (n = 3). The procedure was followed as per our previously reported study for glimepiride-solid dispersion with minor modifications (Kaur et al. 2015a) using fixed funnel and free-standing cone method. On a flat horizontal surface a graph paper was placed and a funnel was clamped above a graph paper by maintaining about 7 cm gap between paper and tip of funnel. Accurately weighed powders were poured through the funnel until the apex of the cone, thus formed, just reached the tip of the funnel. Average diameters of the base of the powder cones were determined and tangent of the angle of repose calculated using Eq. (4.1) (Kaur et al. 2015a):

$$\tan \alpha = 2h/D \hspace{3cm} \text{Eq. (4.1)}$$

Here, h = Height of the heap of powder; D = Diameter of the base of the heap of powder

4.3.7.2. Bulk Density

A graduated measuring cylinder was taken and accurately weighed powder of S-SNEDDS was poured through it and bulk density was calculated by the formula given in Eq. 4.2 (Kaur et al. 2015).

$$\rho b = M/Vb \hspace{3cm} \text{Eq. (4.2)}$$

Here, ρb = Bulk density; Vb = Bulk volume; M = Weight of powder

4.3.7.3. Tapped Density

Accurately weighed S-SNEDDS powder was taken in a measuring cylinder and the cylinder was tapped 100 times. Tapped density (ρt) was calculated using the following formula (Kaur et al. 2015).

$$\rho t = M/ Vt \hspace{3cm} \text{Eq. (4.3)}$$

Where, Vt = Minimum volume occupied by the blend in the cylinder; M = Weight of the blend.

4.3.7.4. Compressibility Index

Carr's compressibility index (CI) was calculated using the formula given in Eq. (4.4) (Kaur et al. 2015).

$$CI = \text{Bulk density} - \text{Tap density/Bulk density} \times 100 \hspace{2cm} \text{Eq. (4.4)}$$

4.3.8. Calculation of drug loading

SNEDDS were prepared by adding 30 mg of glimepiride to each batch containing 1 mL mixture of Lauroglycol® FCC, Tween-80 and ethanol as per the design mentioned in DOE (Table 1). These were vortexed using vortex mixer for 15 min and then added to 500 mL of

double distilled water being stirred at 500 rpm at a temperature of 37ºC. Sample (5 mL) was withdrawn and centrifuged at 10000 rpm for 15 min for removal of the un-dissolved glimepiride. The supernatants were accurately measured and appropriately diluted using distilled water and glimepiride concentration was estimated by HPLC at 228nm. The percentage drug loading was calculated as per the formula given in Eq. (4.5).

$$\% \text{ Drug Loading} = \frac{\text{Absorbance of test drug present in SNEDDS} \times 100}{\text{Absorbance of known standard}} \qquad \text{Eq. (4.5)}$$

4.3.9. Emulsion droplet size and zeta potential analysis

Droplet size, PDI and zeta potential of SNEDDS were determined by Photon Correlation Spectroscopy (PCS) using Malvern zeta sizer nano ZS90 (Malvern Instruments Ltd., UK). Recordings were doneusing 50 mV laser at fixed angle of 90° at 25°C in disposable polystyrene cells. L-SNEDDS/S-SNEDDS sample (100μL) was diluted with 100mL double distilled water. Each run underwent 12 sub-runs for a period of 2 minutes. Each study was repeated in triplicate and mean data was recorded (Sood et al. 2014).

4.3.10. Transmission electron microscopy (TEM)

TEM studies were performed in order to detect the droplet morphology of the selected S-SNEDDS formulation. The model used for scanning was H-7500, Hitachi, Japan. The procedure was carried out as reported by Inugala et al. (2015). Optimized SNEDDS formulation (100 μL) was diluted with 10 mL of double distilled water. For negative staining of sample, a drop of emulsion was placed onto a carbon-coated copper grid to leave a thin film and excess of solution was drained off by using filter paper. After 10 min, one drop of 2%w/v phosphotungstic acid (PTA) solution was dripped on the copper grid for about 1 min and excess of solution was drained. The grid was allowed for air drying and sample was analyzed through TEM (Inugala et al. 2015).

4.3.11. Scanning electron microscopy (SEM) of S-SNEDDS

The surface morphology of the raw glimepiride, A-200, physical mixture and S- SNEDDS were visualized through scanning electron microscopy (SEM) as per the procedure discussed in Kaur et al. (2015a) and Renuka et al. (2014). In brief, a double-sided conductive tape of 12 mm diameter was taken and placed on a metallic stub. Then the samples were fixed over it. The data station used was - Supra 35VP (Oberkochen, Zeiss, Germany) having an acceleration voltage of 1.00 kV.

4.3.12. Powder X-ray Diffraction (PXRD) studies

The PXRD pattern of raw glimepiride, A- 200, their physical mixture and S- SNEDDS powder was recorded using an X-ray diffractometer (Bruker axs, D8 Advance, Coventry, U.K.), using copper line as the source of radiation. Samples were scanned at scanning rate of $0.010°min^{-1}$ over a 2θ range of 3-45°. About 40-kV voltage and 40-mA current was used to record the diffractograms (Kaur et al., 2015a).

4.3.13. DSC analysis

The thermograms for raw glimepiride, A- 200, Physical mixture and S- SNEDDS powder were recorded using DSC Q200 TA, Universal V 24.4 software, Bangalore, India, as per the procedure discussed Kaur et al. (2015a). In brief, 3 mg of samples were crimped separately in an aluminum pan and heated from 0 to 300°Cat a heating rate of 10°C/min. During the scanning nitrogen gas at a flow rate of 50 mL/min was continuously provided. An empty aluminum pan was used as reference. The melting points (T_m) were determined using TA-Universal Analysis 2000 software (version 4.7A).

4.3.14. In vitro dissolution studies

Raw glimepiride, L-SNEDDS, and selected batch of S-SNEDDS powder, containing an amount equivalent of 5 mg glimepiride, were subjected for *in vitro* dissolution studies in USP type I dissolution apparatus employing 500 mL of simulated gastric fluid (SGF) (pH 1.2) maintained at 37 ± 0.5°C, at a stirring speed of 50 ± 4 rpm. Raw glimepiride, S-SNEDDS powder and L-SNEDDS were weighed and filled into size "0" hard gelatin capsules and kept in basket and then subjected to dissolution apparatus. Samples (5 mL) were withdrawn after 5, 10, 15, 30, 45 and 60 min, filtered using a 0.2 μm membrane filter. Filtered solutions were then centrifuged at 10000 rpm for 15 min. Supernatant was collected and analyzed at 228 nm using HPLC for glimepiride. The study was carried out six times and mean data ($\pm$ s.d.) was recorded.

4.3.15. Ex vivo diffusion studies

Ex vivo diffusion study of L-SNEDDS, S-SNEDDS and glimepiride suspension was carried out by using freshly isolated goat intestine membrane, collected from slaughter house in SIF (pH 6.8). The membrane from duodenal part of the small intestine was isolated. The tissue was then washed with distilled water to remove the mucous and other adhered matrices. Tissues of about 0.2 mm thickness and 3 cm length were mounted on Franz diffusion cell having surface area of $1.79cm^2$ and volume of 25 mL. The tissue was stabilized using SIF (pH 6.8) in both, donor and receptor compartments with magnetic stirring for 30 min. At the

end of 30 min, the existing buffers in both the compartments were replaced with fresh SIF. Optimized L-SNEDDS and S-SNEDDS powders were diluted individually to 1mL using SIF (containing glimepiride 5 mg/mL) and placed on to the donor compartment. The study was carried out for 3h and at predetermined time intervals, samples were withdrawn from the receptor compartment. Withdrawn samples were filtered through 0.45µm membrane filter and analyzed for drug concentration using HPLC at 228 nm. Each study was carried out in triplicate and mean data was recorded. Similarly, raw glimepiride (5 mg/mL) was suspended in 1 mL solution of 0.1% w/v carboxy methyl cellulose (CMC), prepared in SIF and mounted on to the donor compartment. Study was carried out in a similar pattern as that of L-SNEDDS and S-SNEDDS powders. The permeation profile was constructed by plotting amount of drug permeated per unit skin surface area ($\mu g/cm^2$) versus time (h). The steady state flux (Jss, mcg/cm^2 h) was calculated from slope of the linear portion of the plot using linear regression analysis (Sood et al., 2014).

4.3.16. Statistical analysis

All the experimental data are expressed as mean ± standard deviation (SD), respectively. Statistical analysis of obtained data was carried out either by analysis of variance or Tukey's multiple comparison test using GraphPad Prism version 7.0 (GraphPad Software Inc., CA, USA). A value of $P < 0.05$ indicated significant difference in the obtained results. The dissolution profiles were compared using model independent analysis (F_2 comparison) as discussed in Shah et al. (1998).

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 RESULT AND DISCUSSION

5.1.1. HPLC method development of glimepiride

The retention time of glimepiride was found to be 6.8 min. It was found linear in the range of 5-25 µg/mL with coefficient of regression (r^2) 0.9986. The chromatogram is shown in Fig. 5.1 and calibration curve is shown in Fig. 5.2.
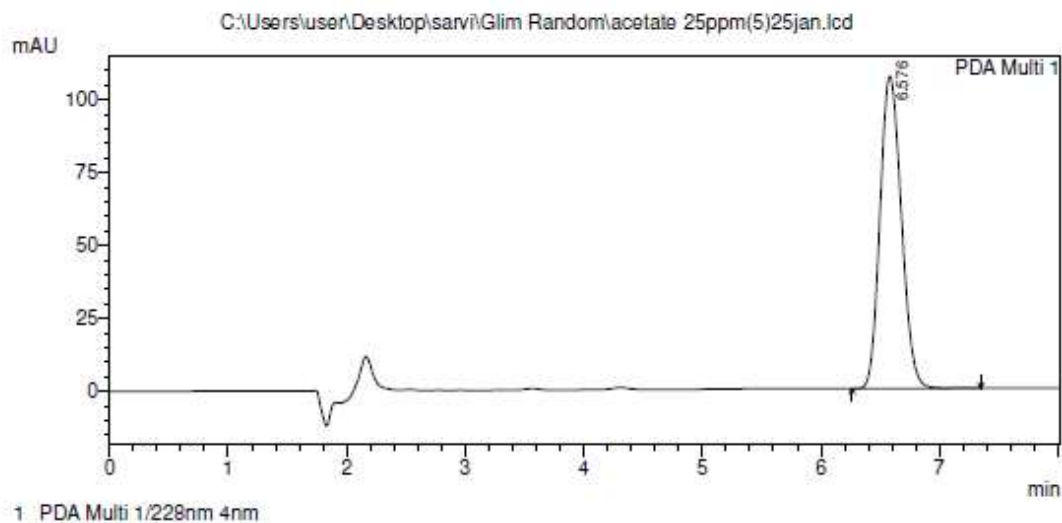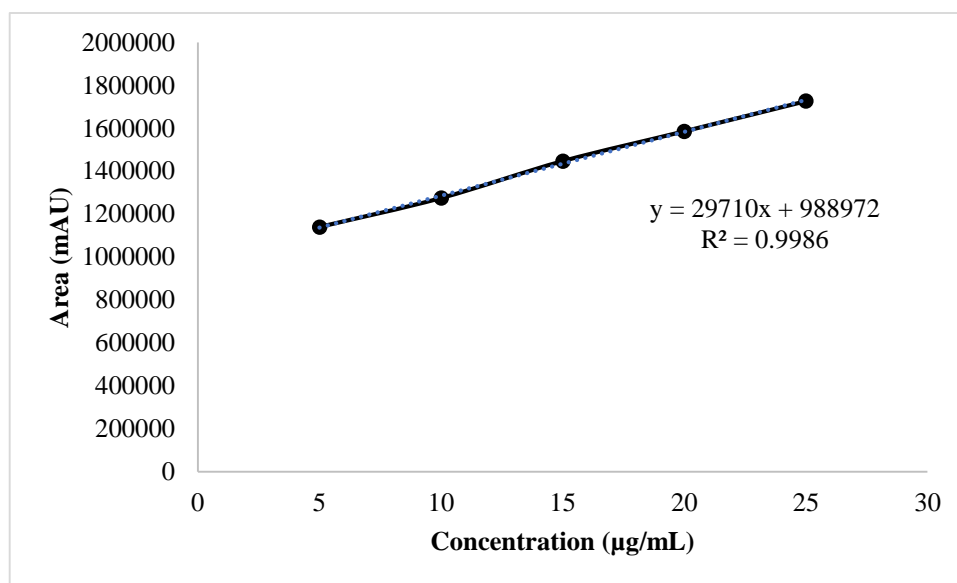

Fig. 5.1. Chromatogram of glimepiride.

Fig.5.2. Calibration plot of glimepiride.
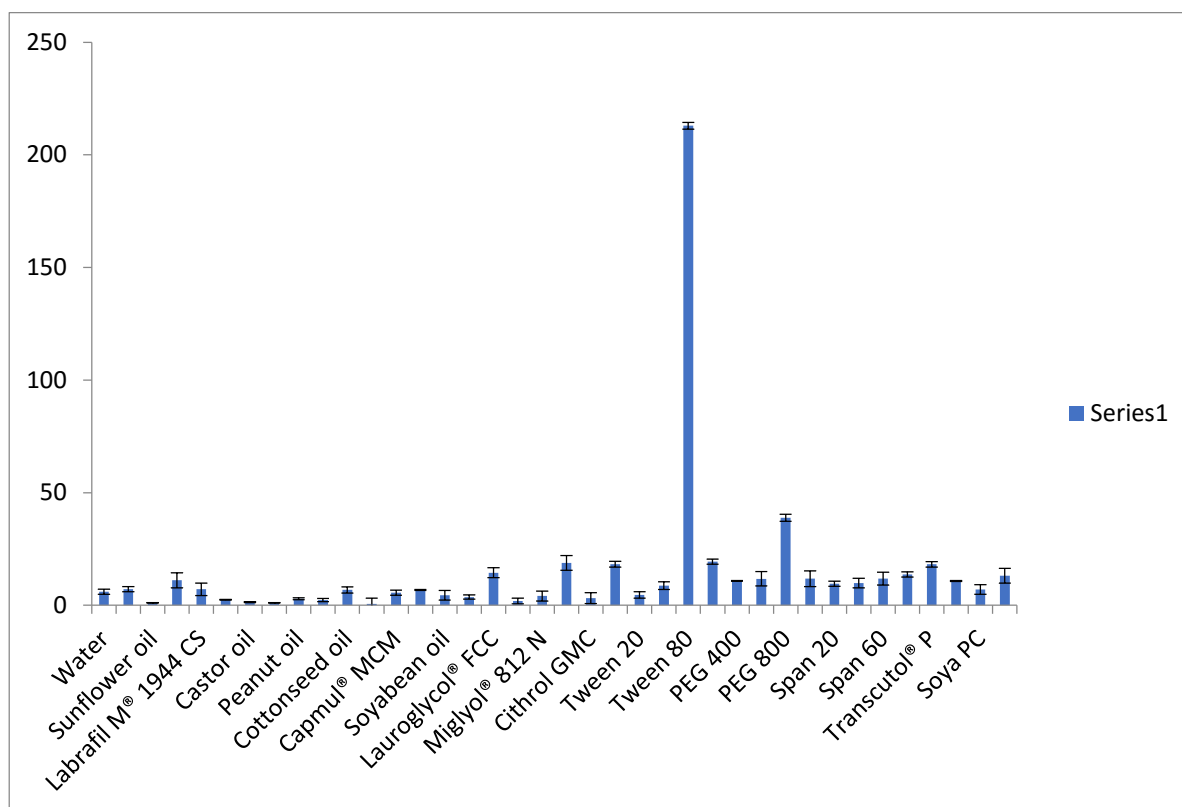
5.1.2. Solubility studies

The solubility of glimepiride was determined in oils, surfactants, co-surfactants, mixture of oils and mixture of surfactants. The results are shown in table 5.1 and figure is shown in 5.3. Among the selected oils, glimepiride had revealed highest solubility in Lauroglycol® FCC (14.46 ± 2.18 µg/mL) Among surfactants, glimepiride showed maximum solubility in Tween-80 (212.92 ± 1.48µg/mL) and among co-surfactants, drug showed highest solubility in ethanol (10.75 ± 0.18µg/mL) for raw glimepiride. In order to form clear nanoemulsion judicious selection of oil, surfactant, co-surfactant and oil to surfactant/co-surfactant ratio is very important. In order to achieve this, it is recommended that a surfactant should have hydrophilic-lipophilic balance (HLB) value more than 10 to form an o/w emulsion. Among surfactants, glimepiride revealed highest solubility in Tween 80 and it also has HLB value of 15, hence, it was selected as surfactant. It has also been reported that most of the surfactants used in formulation of nanoemulsion are generally single chain surfactants and fail to lower interfacial tension sufficiently to form nanoemulsion. Hence, a co-surfactant is required to be added to the system (Bali et al. 2010; Sood et al. 2014). In present study, ethanol (HLB 4.2)

was chosen as co-surfactant as it intercalates between surfactant molecules, thereby polar head group interactions get decreased.

Table.5.1. Solubility of glimepiride in various vehicles (each value represents the mean ± SD, n = 3).

| Vehicle	Solubility of raw glimepiride ($\mu g/mL$)	Vehicle	Solubility of raw glimepiride ($\mu g/mL$)
Water	6 ± 1.16		
Oil		**Surfactants**	
Oleic acid	7.21± 1.15	**Tween 80**	**212.92 ± 1.48**
Sunflower oil	1.11 ± 0.09	PEG 200	19.44 ± 1.15
Olive oil	11.07 ± 3.33	PEG 400	10.85± 0.19
Labrafil M® 1944 CS	7.13 ± 2.8	PEG 600	11.76 ± 3.18
Labrafac® CC	2.44 ± 0.18	PEG 800	38.81 ± 1.56
Castor oil	1.38 ± 0.17	PG	11.78 ± 3.47
Sesame oil	1.11 ± 0.02	Span 20	9.54 ± 1.16
Peanut oil	2.87 ± 0.45	Span 40	9.81 ± 2.12
Eucalyptus oil	2.33 ± 0.76	Span 60	11.81 ± 2.87
Cottonseed oil	6.82± 1.34	Span 80	13.66 ± 1.16
Mustard oil	0.6 ± 2.54	Transcutol® P	18.21 ± 1.22
Capmul® MCM	5.6 ± 1.18	**Ethanol**	**10.75 ± 0.18**
Labrafiil® M 2125 CS	6.8± 0.18	Soya PC	07.01± 2.12
Soyabean oil	4.4± 2.14	Egg PC	13.13± 3.22
Maisine® 35-1	3.66 ± 0.95		
Lauroglycol® FCC	**14.46 ± 2.18**		
Triacetin	1.85 ± 1.26		
Miglyol® 812 N	4.11 ± 2.23		
Surfactants			
Capryol™ 90	18.8 ± 3.22		
Cithrol GMC	3.17± 2.43		
Labrasol®	18.23±1.24		
Tween 20	4.64 ± 1.41		
Tween 60	8.72 ± 1.67		

Fig 5.3 Bar graph of solubility of glimepiride in various vehicles

5.1.3. Construction of ternary phase diagram

Different batches of SEDDS were formulated and visually observed for their self-emulsifying properties. The formed emulsions were judged as SNEDDS, SMEDDS and normal emulsion on the basis of their turbidity measurements and visual observations for transparency. In order to identify the self-emulsifying region and optimize the concentration of oil, surfactant and co-surfactant in the formulation, ternary phase diagram was constructed in the presence of glimepiride (Fig.5.4). The concentration of components was expressed as percent volume/volume (%v/v) in ternary phase diagram. The results revealed that Lauroglycol®FCC, Tween-80 and ethanol were used in varying ratios of 1:1 (F_{1-3}), 1:2 (F_{10-12}) and 2:1 (F_{19-21}) exhibited largest nanoemulsion area and shortest emulsification time (less than 1 min). The colored region in the enclosed area of the diagrams reveals SNEDDS region which formed clear transparent oil in water emulsion upon gentle agitation. It was observed that with

increase in the ratio of the ethanol, spontaneity of the self-emulsification process got increased. It was noted that higher concentration of surfactant mixture (S_{mix}) (i.e. Tween 80/ethanol; >70%) or lower concentration of oil (Lauroglycol FCC; < 30%) resulted in formation of clear transparent emulsions with nanosized droplets. This could be due to higher HLB value of Tween 80 and better solubilization of glimepiride in ethanol. The transparent emulsions ($F_{1-3, 10-12 and 19-21}$) were visually evaluated for clarity and stability after 48h at room conditions. All tested emulsions remained clear transparent even at the end of 48h. Moreover, these formulations were diluted with SGF (pH 1.2) as well as distilled water to 10, 100 and 1000 times and found clear transparent without any phase separation and precipitation in both the medium. This indicated stability of formed emulsions at various dilutions and pH conditions that mimics *in vivo* situation (Inugala et al. 2015). Hence, these formulations have been selected for further studies.
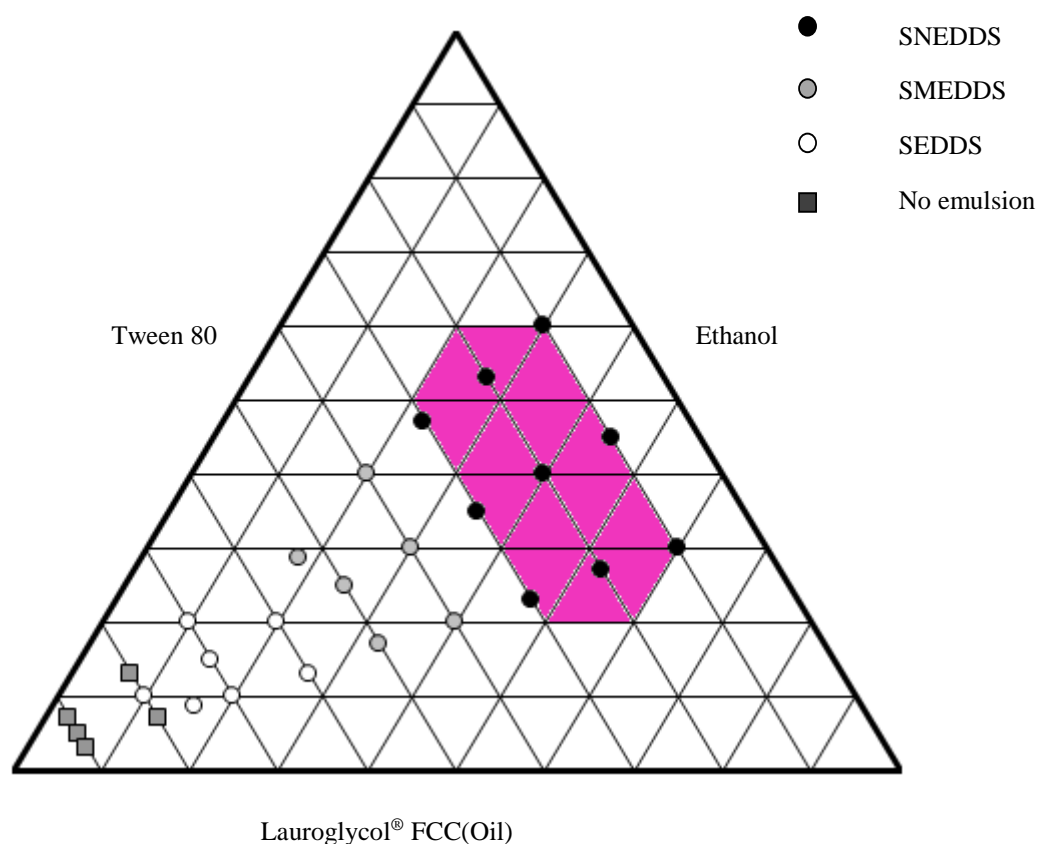
Fig. 5.4. Pseudo-ternary phase diagram plot depicting nano-emulsion region of F_{1-27} which include various ratios of Lauroglycol® FCC, Tween-80 and ethanol.

5.1.4. Thermodynamic stability and cloud point determination

In order to identify and avoid formation of metastable L-SNEDDS formulation, thermodynamic stability study was conducted for formulations: $F_{1-4, \, 10-12 \, and \, 19-21}$. All the formulations passed the thermodynamic stability studies without any signs of phase separation and precipitation during alternative temperature cycles (4°C and 40°C), freeze thaw cycles (-21°C and +25°C) and centrifugation at 10,000 g indicating good stability of formulations and their emulsions. In the present study Tween-80 has been used as surfactant to formulate L-SNEDDS, which is a non-ionic surfactant. Determination of cloud point is an essential parameter for the selection of a stable L-SNEDDS particularly when composed with non-ionic surfactants (Itoh et al., 2002; Inugala et al. 2015). "The cloud point temperature (lower consolute temperature) indicates the temperature at which the transparent monophasic system was transformed into cloudy biphasic system as dehydrated surfactant molecules associated together as precipitate, which can affect the formulation adversely (Chen et al., 2000; Warisnoicharoen et al., 2000). It is recommended that the cloud point for SNEDDS should be higher than body temperature (37°C) (Chen et al., 2000; Warisnoicharoen et al., 2000), which will avoid phase separation occurring in the gastrointestinal tract. The cloud point temperature of the tested L-SNEDDS was found to be in the range of 75-97°C (Table 5.2). Thus, it can be inferred that the developed formulation was stable and do not require a precise storage temperature and it develops a stable emulsion upon administration at physiological temperature *in vivo* (Zhang et al., 2008; Inugala et al. 2015).

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Table 5.2 Composition of selected batches of glimepiride loaded L-SNEDDS (% w/w) and evaluation parameters.

Formulation code	S/CS (Smix) (%w/w)	Oil/Smix (%w/w)	Mean droplet size (nm)	PDI	Cloud point (°C)	Appearance	Phase separation after 48h	Phase separation after centrifugation
F_1		10:90	117.91	0.436	93.16	TP*		
F_2	1:1	20:80	262.45	0.561	93.54	TL**		
F_3		30:70	346.66	0.662	91.18	TL		
F_{10}		10:90	152.41	0.448	96.54	TP		
F_{11}	1:2	20:80	276.34	0.566	91.48	TL	No	No
F_{12}		30:70	564.16	0.680	88.18	TL		
F_{19}		10:90	167.22	0.467	99.16	TP		
F_{20}	2:1	20:80	294.36	0.654	87.38	TL		
F_{21}		30:70	528.88	0.718	81.16	TL		

TP* - Transparent; TL**- Translucent

5.1.5. Droplet size and polydispersity index (PDI) of L-SNEDDS

The selected L-SNEDDS formulations ($F_{1-3,\ 10-12\ and\ 19-21}$) were subjected for droplet size and polydispersity index analysis. It was observed that formulations F_1containing S_{mix} in the ratio of 1:1 revealed very good droplet size having z-average less than 100 nm along with PDI less than 0.5. The other formulations ($F_{10-13\ and\ 19-21}$) have also shown droplet size in nanometer range with greater PDI values (Table 5.2). It was also observed that increasing the amount of Lauroglycol® FCC above 30% (300 µL) caused increase in droplet size as well as PDI, whereas, increase in surfactant and co-surfactant percentage above 70 revealed in decrease in droplet size and PDI. The increasing order of droplet size and PDI was:

$F_1< F_{10}< F_{19}< F_2< F_{11}< F_{20}< F_3< F_{12}< F_{21}$

Formulation "F_1" showed least droplet size and PDI, hence, it was selected as the best batch and study was continued further on "F_1".

5.1.6. Oil adsorption capacity

It was observed that oil adsorption capacity of Aerosil® 200 was found better as compared to any other carriers used while it was found minimum with MS. The oil adsorption capacity for various carriers was found to be decreasing in the order of:

Aerosil® 200 (200 mg) > SFP (240 mg) > SXDP (300 mg) > MCC PH 102 (400 mg) > HP-β-CD (560 mg) > Na-CMC (575 mg) >Lactose (1500 mg) > MS (1625 mg)

It is important to note that values (in mg) indicate amount of carrier required for adsorbing unit dosage of optimized SNEDDS formulation.

5.1.7. Droplet size and PDI analysis of solid-SNEDDS (S-SNEDDS)

L-SNEDDS were solidified by spray drying (SD) using various hydrophobic and hydrophilic carriers. The average droplet diameter and PDI of the S-SNEDDS and L-SNEDDS formulation is presented in Table 5.3. The average droplet size of optimized L-SNEDDS was 117.91 nm with very good PDI of 0.436. It was recorded that the average droplet size and PDI were greatly depended on solidification techniques as well as solid carriers. Spray dried S-SNEDDS powder shown rapid dispersion (within 30 sec) during dilution in water. It is also important to mark that the hydrophobic carriers have showed better results as that of hydrophilic carriers. It was investigated that the lactose, magnesium stearate, Na-CMC and HPβ-CD have shown larger droplet size. Only Aerosil® 200 had shown the value of droplet diameter closer to that of L-SNEDDS. The average droplet diameter of S-SNEDDS prepared by using various solid carriers was:

Aerosil®200 < SXDP < SFP < MCC PH102 < HPβ-CD < Na-CMC < MS< Lactose

Table 5.3 Droplet size and PDI of various carriers.

Formulations/S-SNEDS prepared using different carriers	Droplet size (nm)	Polydispersity Indices (PDI)
L-SNEDDS	117.91 ± 1.18	0.436 ± 0.06
Aerosil® 200	126.18 ± 3.38	0.456 ± 0.09
SXDP	144.19 ± 2.31	0.56 ± 0.012
SFP	181.18 ± 1.16	0.59 ± 0.021
MCC PH102	266.67 ± 1.46	0.66 ± 0.028
HPβ-CD	387.26 ± 3.23	0.42 ± 0.001
Na-CMC	418.16 ± 1.34	0.51 ± 0.02
MS	486.18 ± 9.69	0.42 ± 0.021
Lactose	566.18 ± 9.69	0.65 ± 0.034

5.1.8. Micromeritic characteristics of S-SNEDDS

The results of micromeritic properties of S-SNEDDS prepared using different porous carriers by spray drying (SD) is shown in Table 5.4. The bulk density was found to be ranging between bulk density from 0.201 ± 0.22 and 0.303 ± 0.09 g/cm^3, and tapped density from 0.225 ± 0.22 and 0.425 ± 0.03 g/cm^3, respectively. The flow rate was found to be ranging between 0.55 ± 0.31 and 4.88 ± 0.65 g/s, angle of repose from 19.35 ± 1.16 and 44.16 ± 2.22 (Θ), and Carr's index from 11.94 ± 0.04 and 40.26 ± 0.81, respectively. The high degree of variability in the micromeritic properties of various porous carriers can be ascribed owing to the differences in the physiochemical properties and oil adsorption capacity of the materials. Among the carriers used, higher values of density, angle of repose and lower flow rate could be due to their poor oil adsorbing capacities of MS, Lactose, Na-CMC, and HP-β-CD as compared to SFP, SXDP, MCC PH 102 and Aerosil® 200. The density and angle of repose for various carriers was found to be decreasing in the order of:

MS >Lactose>Na-CMC > HP-β-CD > SFP > MCC PH 102 > SXDP > Aerosil® 200

Overall, the studies revealed that Aerosil® 200 exhibited promising micromeritic behavior as compared to S-SNEDDS prepared by using any other carrier. Based on the results, S-SNEDDS prepared by using other carriers were discontinued from further studies.

Table 5.4 Micromeritic characteristics of S-SNEDDS.

Component	Flow rate (g/s)	Angle of repose (θ)	Bulk Density (g/cm^3)	Tap Density (g/cm^3)	Carr's index
Aerosil® 200	4.88 ±0.65	19.35±1.16	0.201±0.22	0.225±0.22	11.94±0.04
SFP	1.96±0.56	35.26±1.22	0.287±0.09	0.360±0.01	25.43±1.38
SXDP	2.78±0.22	23.54±1.18	0.236±0.09	0.290±0.04	22.88±1.84
MS	0.55±0.31	44.16±2.22	0.303±0.09	0.425±0.03	40.26±0.81
MCC PH102	2.96±0.22	33.42±1.18	0.250±0.14	0.304±0.22	21.60±0.90
Na-CMC	0.89±0.10	39.16±1.87	0.294±0.07	0.401±0.09	36.39±1.30
HPβ-CD	1.14±0.21	36.18±1.44	0.290±0.03	0.406±0.08	40.00±1.54
Lactose	0.95±0.18	39.22 ± 1.23	0.299±0.18	0.416±0.22	39.13±2.10

**5.1.9 Dissolution studies of S-SNEDDS powder (Aerosil)**

The *in vitro* dissolution studies revealed nearly superimposable drug release profiles for S-SNEDDS powders vis-à-vis the L-SNEDDS, respectively ($P > 0.05$). All the formulations exhibited faster drug release characteristics ($> 90\%$) within 10 min and almost complete drug release in 15 minutes (Fig. 5.5). On the contrary, the raw drug showed only a maximum of 48 % release in 60 min time period. Nearly 2.08-folds improvement in the dissolution rate was, therefore, revealed by the prepared formulation vis-à-vis raw glimepiride. Besides, stronger physical interactions of the SNEDDS with hydrophobic surface of silica particles of Aerosil®200 are also responsible for impeding the dissolution rate of the drug at initial time points (Ahuja and Pathak, 2009; Abbaspour et al. 2014; Beg et al. 2016). Overall, the statistical analysis of dissolution behavior through profile comparison tests by calculating similarity factor (f2) showed a value of 70.21 for S-SNEDDS powder vis-à-vis the L-SNEDDS, respectively. In both the cases, f2 values > 50 confirmed the analogous drug release profiles from the liquid and solid SNEDDS formulations, indicating immediate release nature of both the formulations.

5.1.10 Zeta Potential

The zeta potential of S-SNEDDS [Aerosil®] was found to be -18.16 mV.

5.1.11 TEM analysis

The TEM images (Fig. 5.6) clearly indicated spherical droplets of S-SNEDDS at a scale of 200 nm (0.2μm). This image confirmed that the droplets were unagglomerated, distinct, and spherical in nano size and correlated with results of photon correlation spectroscopy done for droplet size analysis.
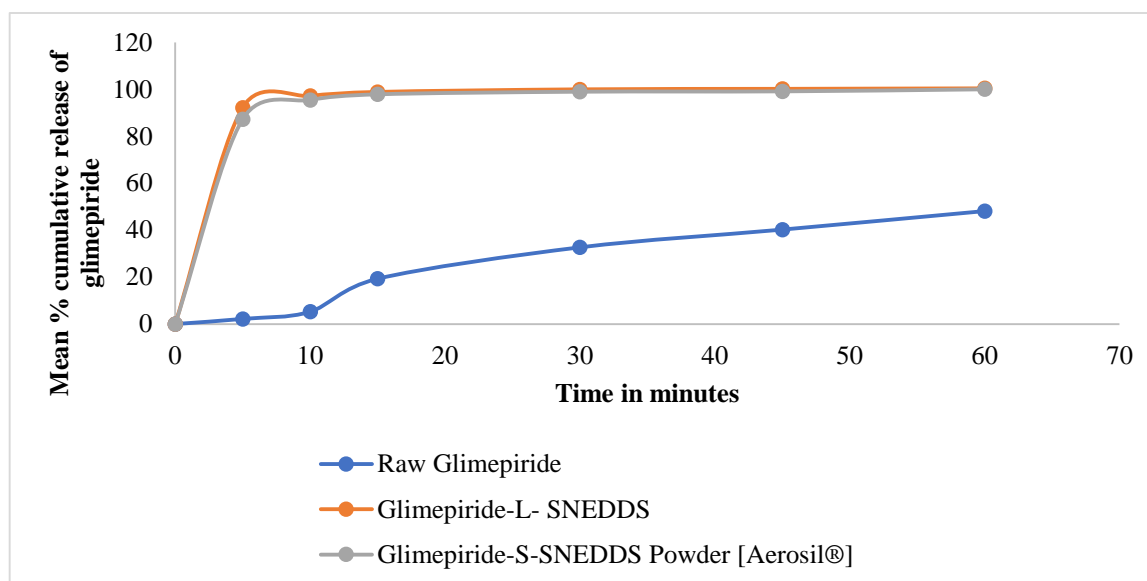
Fig.5.5. *In vitro* **drug release profile from various SNEDDS formulations and raw drug; Data expressed as mean ± S.D. (n = 6).**
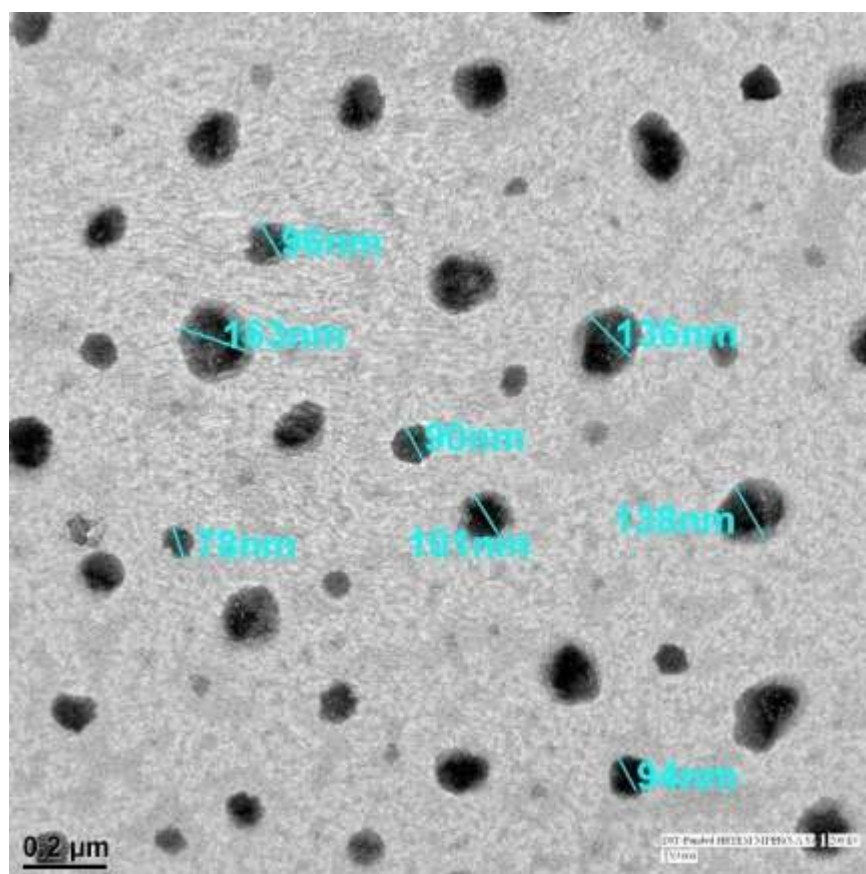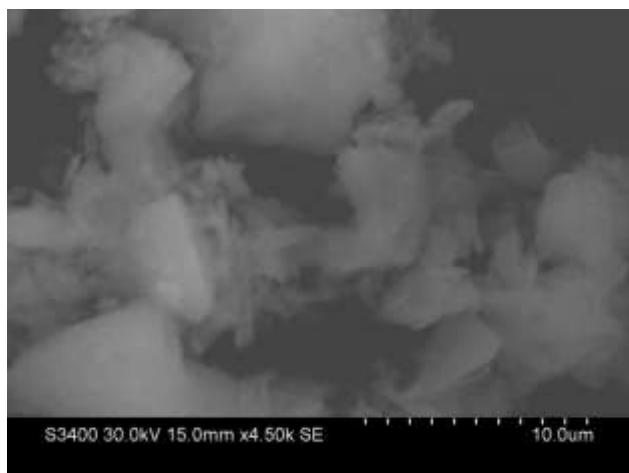

Fig5.6 TEM images of glimepiride SNEDDS.

5.1.12 Scanning Electron Microscopy (SEM)

The scanning electron micrographs of glimepiride, Aerosil® 200 powders, as well as their

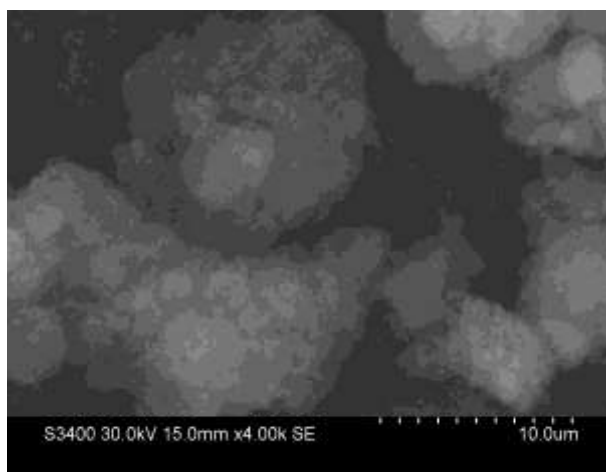solid SNEDDS formulations {glimepiride-S-SNEDDS Powder [Aerosil®] are shown in Fig.

5.7.Glimepiride appeared as flat, blade like smooth-surfaced rectangular crystals in shape with sharp irregular edges (Fig.5.8). Aerosil® 200 showed an unorganized mass with no discernible crystallinity owing to its amorphous nature (Fig.5.9). The S-SNEDDS appeared as rough-surfaced particles with porous and irregular aperture indicating that the liquid SNEDDS was absorbed or coated inside the pores of Aerosil® 200 (Fig.5.10.).

Fig5.8. Raw glimepiride
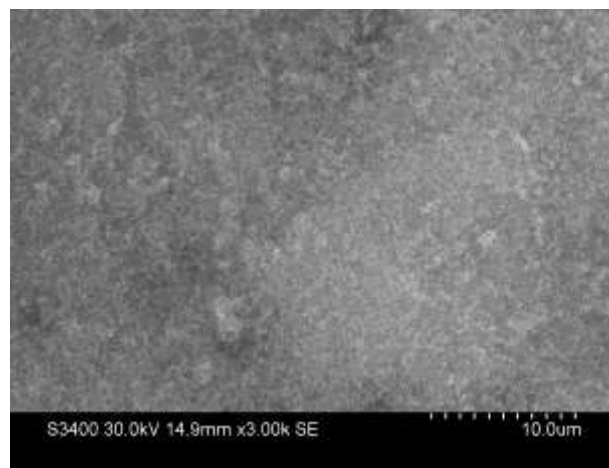
Fig 5.9. Aerosil® 200

Fig 5.10. S-SNEDDS

Fig.5.7. SEM images of raw glimepiride, Aerosil® 200 and S-SNEDDS [Aerosil] powder.

5.1.13 Differential Scanning Calorimetry (DSC) The DSC curves of raw glimepiride, Aerosil® 200, and S-SNEDDS {Glimepiride-S-SNEDDS Powder [Aerosil®]} formulations are shown in Fig 5.11, 5.12, 5.13. Raw glimepiride showed sharp endothermic peaks at about

102.26°C, corresponding to their melting points and indicating their crystalline nature. Aerosil® 200 showed flat line with no melting endotherm, owing to its amorphous nature. It is important to note that the endothermic peaks of the drugs were absent in the S-SNEDDS formulations prepared with Aerosil® 200 as carrier. This showed that the glimepiride have got dissolved completely in the formulation. In addition to this, the oil-surfactant-co-surfactant system has provided sufficient stabilization to the drug, because the precipitation of drug in the formulation would have shown the crystalline melting of glimepiride in the thermogram of formulation. Moreover, adsorption of glimepiride loaded L-SNEDDS on amorphous Aerosil®200 through spray drying would have further resulted in creation of complete amorphous state of the formulation. In order to have better insight, the DSC results were correlated with PXRD studies.
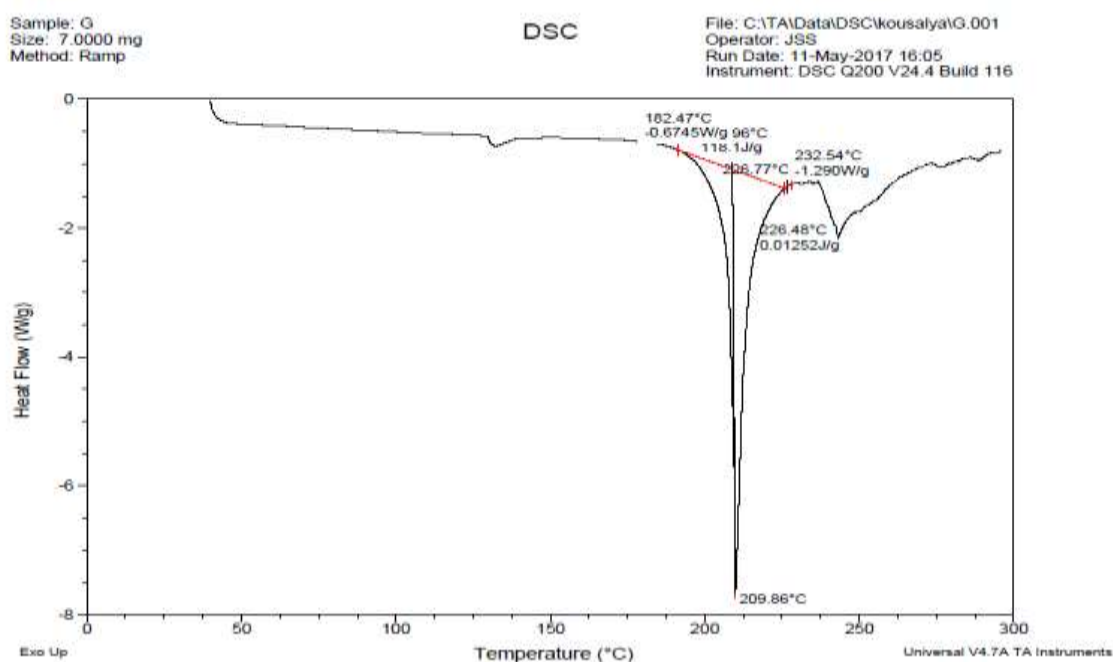

Fig.5.11. Raw glimepiride
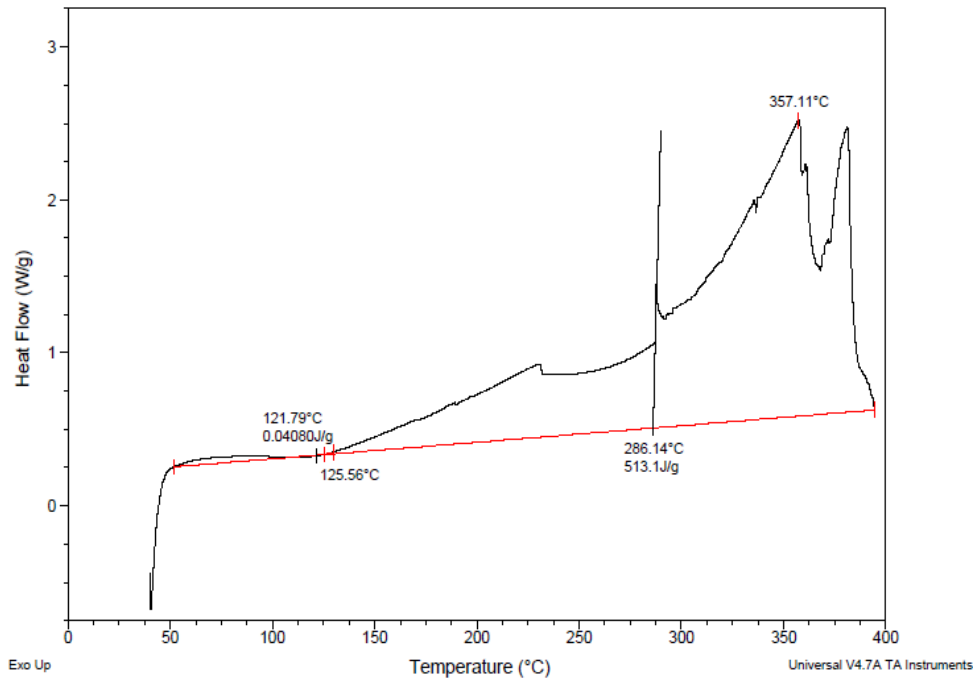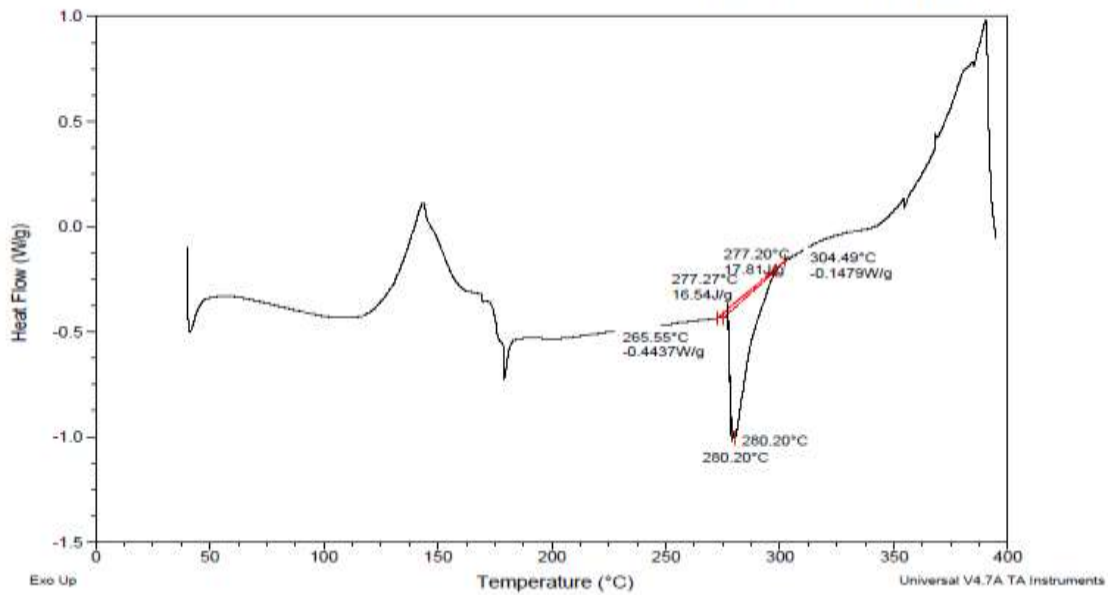
Fig.5.12.Aerosil® 200

Fig.5.13.. S-SNEDDS powder [Aerosil]

5.1.14. PXRD studies

The PXRD patterns are presented in Fig.5.14. glimepiride had shown sharp endothermic peaks at the diffraction angles showing a typical crystalline pattern (Fig.5.15). On the contrary, Aerosil® 200 showed no intrinsic peaks (Fig.5.16). The S-SNEDDS formulation showed no peaks at diffraction angles, showing an amorphous pattern (Fig.5.17). Thus, similar to DSC results, glimepiride was present in a changed amorphous state in the SNEDDS formulations prepared with Aerosil® 200 as carrier
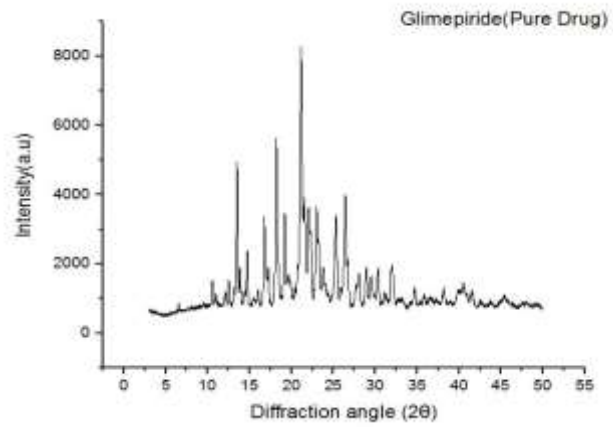
Fig.5.15
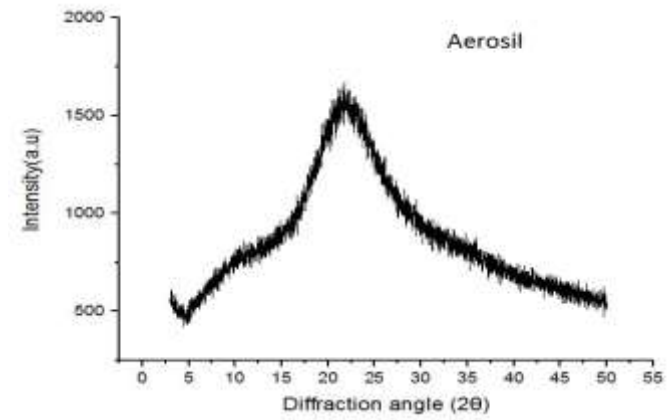
Fig. 5.16
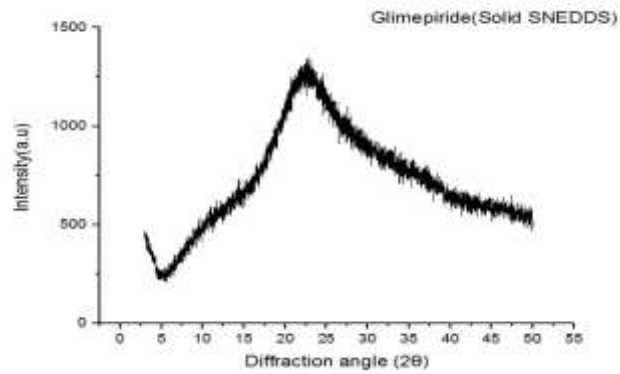
Fig. 5.17

5.1.15 . *Ex-vivo diffusion studies*

The diffusion studies revealed nearly superimposable drug permeation profiles for S-SNEDDS powders vis-à-vis the L-SNEDDS, respectively ($p > 0.05$). Whereas, the raw glimepiride suspension showed significantly lower ($p < 0.001$) flux and permeation compared to L-SNEDDS and S-SNEDDS. The results are shown in Fig. 5.18.. Flux for raw PPK suspension, L-SNEDDS and S-SNEDDS were found to 285.29 ± 18.22, 387.43 ± 22.18 and 378.61 ± 14.82 µg/cm^{2}h respectively. There was no significant difference ($p > 0.05$) in flux of SNEDDS formulations. At the end of 3rd hour, about 5.46 folds increase in drug permeation was observed in case of L-SNEDDS and S-SNEDDS as compared to raw glimepiride suspension
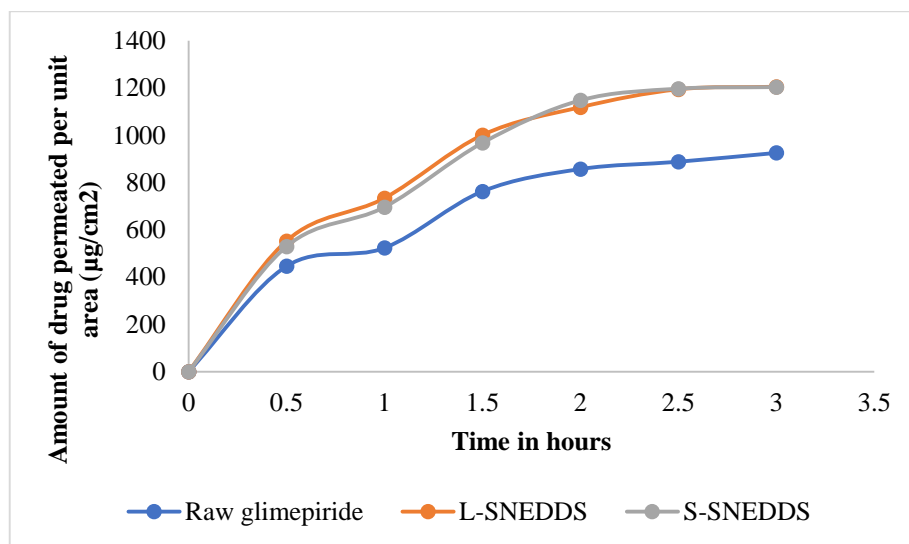
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Fig.5.18. *Ex-vivo* skin permeability results of raw glimepiride, L-SNEDDS and S-SNEDDS.

CHAPTER 6

SUMMARY AND CONCLUSION

A SNEDDS of glimepiride was successfully developed with Lauroglycol FCC, Tween 80, and ethanol as the components. Box Behnken design was used to optimize the formulation variables. Further the optimized batch of L-SNEDDS was solidified by using hydrophilic and hydrophobic solid carriers form solid SNEDDS (S-SNEDDS) using spray drying technique. This was followed by their detailed investigation through micromeritic, biopharmaceutics' and stability studies. It was observed that flow and compression properties were dependent on carrier and spray drying technique used. The formulated S-SNEDDS prepared by using Aerosil$^®$ 200 as hydrophobic carrier and spray drying, have provided nanoemulsions with unchanged droplet size and drug release when subjected at different stress conditions such as thermodynamic stress and freeze thaw cycles. In vitro dissolution studies revealed that the L-SNEDDS and S-SNEDDS were found to be remarkably superior over the raw glimepiride. SEM, DSC and PXRD revealed crystalline glimepiride was present in a changed amorphous state in the SNEDDS formulations prepared with Aerosil$^®$ 200 as carrier. The findings of current study, therefore, ratified successful selection of solidification process for L-SNEDDS and forecasts production of S-SNEDDS at larger scale using spray drying.

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APPENDIX

CrossMark

Research Article

Stable Co-crystals of Glipizide with Enhanced Dissolution Profiles: Preparation and Characterization

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Abstract. Present study deciphers preparation of co-crystals of lipophilic glipizide by using four different acids, oxalic, malonic, stearic, and benzoic acids, in order to achieve enhanced solubility and dissolution along with stability. All co-crystals were prepared by dissolving drug and individual acids in the ratio of 1:0.5 in acetonitrile at 60-70°C for 15 min, followed by cooling at room temperature for 24 h. FT-IR spectroscopy revealed no molecular interaction between acids and drug as the internal structure and their geometric configurations remain unchanged. Differential scanning calorimetry revealed closer melting points of raw glipizide and its co-crystals, which speculates absence of difference in crystallinity as well as intermolecular bonding of the co-crystals and drug. PXRD further revealed that all the co-crystals were having similar crystallinity as that of raw glipizide except glipizide-malonic acid co-crystals. This minor difference in the relative intensities of some of the diffraction peaks could be attributed to the crystal habit or crystal size modification. SEM revealed difference in the crystal morphology for all the co-crystals. Micromeritic, solubility, dissolution, and stability data revealed that among all the prepared co-crystals, glipizide- stearic acid co-crystals were found superior. Hence, it was concluded that glipizide-stearic acid co-crystals could offer an improved drug design strategy to overcome dissolution and bioavailability related challenges associated with lipophilic glipizide.

INTRODUCTION

It is a well-known fact that crystalline active pharmaceutical ingredients (API) have well-defined external and internal structures (1). The internal structure reveals the arrangement of molecules in the crystal lattice and deals with polymorphism, whereas the external structure reveals the habit of crystal, stating the shape of crystal without changing the internal structure of API (1). It is also a well-known fact that the crystalline state of API is susceptible to polymor- phism, where different crystal forms of the same API are generated depending upon the type of solvent used, applied temperature, and crystallization conditions (2,3). This alter- ation of external and internal structure of API may alter its crystalline form that could further affect the physicochemical

stability of the dosage form (2). This could further affect solubility, melting behavior, pre-compression, micromeritic properties, dissolution, compression, and syringeability (2,4-7). Thus, search for the crystal form with desirable solubility, dissolution, and stability is a consistent challenge for the pharmaceutical industries and the agencies that regulate them (2,8,9). The fact cannot also be denied that there is absence of existence of a single reliable method that could legally secure all possible dosage forms (4). Hence, it is mandatory for innovator as well as generic drug manufacturers to put their possible endeavors in order to discover new and efficient screening methods to generate new crystal forms of drugs, both in terms of time and resources.

Another challenge related to the generation of crystal form is related to their aqueous solubility. It is important to highlight here that most of the crystals that are generated by combinatorial chemistry possess poor aqueous solubility. Solubility of a drug affects the dissolution rate and mass transport. Hence, it becomes an important factor in under-standing how the drug is absorbed from its dosage forms (2,10-12). Persons involved in commercial production and marketing of such products need to understand the physico- chemical nature of the material being processed, its stability,

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APPENDIX