Development, characterization and evaluation of solid lipid nanoparticles as a potential anticancer drug delivery system

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Development, Characterization and Evaluation of Solid Lipid Nanoparticles as a potential Anticancer Drug Delivery System

by

Meghavi Patel

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science Degree in Pharmaceutical Sciences with Industrial Pharmacy Option

________________________________________
Jerry Nesamony, PhD., Committee Chair

________________________________________
Sai Hanuman Sagar Boddu, PhD., Committee Member

________________________________________
Surya M. Nauli, PhD., Committee Member

________________________________________
Patricia R. Komuniecki, PhD, Dean
College of Graduate Studies

The University of Toledo

December 2012
An Abstract of
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Solid lipid nanoparticles (SLNs) consist of spherical solid lipid particles in the nanometer size range, which are dispersed in water or in an aqueous surfactant solution. SLN technology represents a promising new approach to deliver hydrophilic as well as lipophilic drugs. The commercialization of SLN technology remains limited despite numerous efforts from researchers. The purpose of this research was to advance SLN preparation methodology by investigating the feasibility of preparing glyceryl monostearate (GMS) nanoparticles by using three preparation methods namely microemulsion technique, magnetic stirring technique and temperature modulated solidification technique of which the latter two were developed in our laboratory. An anticancer drug 5-fluorouracil was incorporated in the SLNs prepared via the temperature modulated solidification process. Optimization of the magnetic stirring process was performed to evaluate how the physicochemical properties of the SLN was influenced by systematically varying process parameters including concentration of the lipid, concentration of the surfactant, type of surfactant, time of stirring and temperature of storage. The results demonstrated 1:2 GMS to tween 80 ratio, 150 ml dispersion medium
and 45 min stirring at 4000 RPM speed provided an optimum formulation via the temperature modulated solidification process. SLN dispersions were lyophilized to stabilize the solid lipid nanoparticles and the lyophilizates exhibited good redispersibility. The SLNs were characterized by particle size analysis via dynamic light scattering (DLS), zeta potential, transmission electron microscopy (TEM), differential scanning calorimetry (DSC), drug encapsulation efficiency and in vitro drug release studies. Particle size of SLN dispersion prepared via the three preparation techniques was approximately 66 nm and that of redispersed lyophilizates was below 500 nm. TEM images showed spherical to oval particles that were less dense in the core with a well-defined shell and the particle size was in agreement with the particle size analysis data obtained by DLS. DSC thermograms of the lyophilized SLNs indicate a reduction in the crystallinity order of GMS particles. The drug encapsulation efficiency was found to be approximately 30%. In vitro drug release studies from redispersed lyophilized SLNs showed that 17 % of the encapsulated drug was released within 2 h. The SLNs prepared in our lab demonstrated characteristics that can potentially be utilized in an anticancer drug delivery system. Future in vitro cell culture and in vivo animal model studies will delineate compatibility and utility of these formulations in biological systems.
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Chapter One

Solid Lipid Nanoparticles (SLNs)

1.1 Overview

For decades, various pharmaceutical dosage forms such as tablets, capsules, liquids, suppositories, creams, ointments, injections, aerosols, etc. have been used as drug delivery systems for treatments of acute and chronic diseases. Colloidal drug delivery systems namely oil-in-water emulsions, liposomes, micelles, microparticles and nanoparticles opened a new frontier for targeting drugs and pharmaceuticals. Nanoparticles are solid colloidal particles in which the active principles are dissolved, entrapped, and/or to which the active principle is adsorbed or attached. Nanoparticles offer several advantages in drug delivery owing to their small particle size, large surface area and the capability of changing their surface properties. In general, nanoparticles can be used to target the delivery of drugs, to sustain its effect, to improve bioavailability, to solubilize it for intravascular delivery and to improve its stability against enzymatic degradation [1]. Based on the type of the inactive ingredient used, there are four classes of nanoparticles: Lipid based nanoparticles [2], polymeric nanoparticles [3], metal based nanoparticles [4] and biological nanoparticles [5].
Solid lipid nanoparticles (SLNs) have been used as an alternative drug delivery system to colloidal drug delivery systems namely oil-in-water emulsions, liposomes, microparticles and polymeric nanoparticles. They consist of spherical lipid particles in nanometer size range. SLNs are used for the controlled and targeted delivery of drugs and for the incorporation of hydrophilic and lipophilic drugs. SLNs are made up of solid lipids, emulsifier and/or coemulsifier and water. A typical solid lipid that is used in such delivery systems melts at temperatures exceeding body temperature (37°C). Examples of some of the lipids that have been investigated are fatty acids, steroids, waxes, triglycerides, acylglycerols and their combinations. All classes of emulsifiers, either by itself or in combination have been utilized to stabilize the lipid dispersion. Examples of some of the emulsifiers that have been investigated are lecithin, bile salts such as sodium taurocholate, nonionic emulsifiers such as ethylene oxide/propylene oxide copolymers, sorbitan esters, fatty acid ethoxylates, and their combinations [7]. Deionized water is used as a dispersion medium.
<table>
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<td><strong>Triglycerides</strong></td>
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**Table 1.1**: Lipids for preparing solid lipid nanoparticles [8]

SLNs have been claimed to combine the advantages of some colloidal carriers and simultaneously avoid their disadvantages [8].

### 1.1.1 Advantages of SLNs over microparticles

- Smallest blood capillaries in body are approximately 5-6 μm and hence particles should be less than 5 μm in the blood stream without forming aggregates to minimize embolism. Therefore SLNs are better suited for I.V. delivery.

- Size of the microparticles is a limitation to cross the intestinal lumen into lymphatic system following oral delivery of vaccines, peptides, and other biomacromolecules. Microparticles remain in Peyer’s patches while SLNs are disseminated systematically [2].
1.1.2 Advantages of SLNs over liposomes

- Avoidance of organic solvents when desired
- Excellent reproducibility and feasible large scale production
- Unique ability to create controlled release and drug targeting by coating/attaching ligands to SLNs [9]
- Increased product stability of about 1 year

1.1.3 Advantages of SLNs over polymeric nanoparticles

- Lipids are biodegradable and hence have better biocompatibility [8]
- Avoidance of organic solvents when desired
- Feasibility of large scale production and sterilization
- Excellent reproducibility with cost effective high pressure homogenization method as the preparation method [10]
- Increased stability of the active ingredient [8]

1.2 Solid Lipid Nanoparticles production techniques:

For several years, solid lipids have been used in the form of pellets to achieve delayed drug release [8]. In the early 80s, Speiser and coworkers developed spray dried and congealed micropellets [11] and nanopellets of lipids for oral administration [12]. Nanopellets developed by Speiser [12] often contained high amounts of microparticles. Domb produced lipospheres by high shear mixing or ultrasonication [13]. But both the nanopellets and lipospheres produced by Speiser and Domb respectively were
contaminated by microparticles. Since the last decade several scientists have realized the potential of SLNs technology and their research efforts have brought about improvement in solid lipid nanoparticles synthesis.

1.2.1 High pressure homogenization

Muller and Lucks were the first to prepare solid lipid nanoparticles by applying high pressure homogenization (HPH) technique [14]. Homogenizers have been used commercially for several years now for the production of nanoemulsions for parenteral nutrition, such as Intralipid® and Lipofundin® [15]. Thus, scaling up represents fewer problems when compared to other techniques and it is cost effective. Naturally, a lot of research has been done utilizing this method to produce better solid lipid nanoparticles by several research groups. A homogeneous dispersion with narrow size distribution is desirable to increase the physical stability of the aqueous dispersion. In this technique the liquid is forced at a high pressure (100-2000 bar) through a narrow gap of few microns. The resulting high shear stress and cavitation forces decrease the particle size. If the particles localized at different positions in the dispersion volume experience different forces then the degree of particle disruption will vary. The two basic production methods to HPH are the hot and the cold homogenization techniques.

1.2.1.1. Hot homogenization technique

In this method the active ingredient is first dissolved in the lipid melt. A coarse pre-emulsion is made by dispersing the lipid melt into hot surfactant solution which is heated to a temperature above the melting point of the lipid while applying stirring [16]. The pre-emulsion is then passed through a high pressure homogenizer for 3 to 5 cycles and applying a pressure of about 500-1500 bar [17]. The obtained nanoemulsion is then
allowed to cool to room temperature or lower. The lipid nanodroplets solidify while cooling and forms an aqueous dispersion of solid lipid nanoparticles. Homogenization pressure and the number of cycles should not be higher than that required to achieve the desired effects because this increases the cost of production and the chances of metal contamination as well as in some cases it would result in increase in particle size due to aggregation as a result of the high surface free energy of the particles [8]. This technique is performed at a high temperature and thus cannot be used for temperature sensitive drugs. Moreover it has been reported that the lipids remain as a super-cooled melt for several months owing to their small particle size and the presence of emulsifier. This method is also not suitable for hydrophilic drugs [2].

1.2.1.2. Cold homogenization technique

In this process the active ingredient is first dissolved in the lipid at a temperature above the melting point of the lipid. The mixture is rapidly cooled with the help of liquid nitrogen or dry ice. Rapid cooling procedure helps in the homogeneous distribution of the active ingredient. This solidified mixture is milled to about 50-100 µm particles using a ball or mortar mill [15]. The lipid microparticles obtained by milling are suspended in a surfactant solution to obtain a suspension. This suspension is then passed through a high pressure homogenization at or below room temperature to obtain solid lipid nanoparticles. The cold homogenization technique reduces the chances for temperature induced drug degradation and thus thermo sensitive drugs can be used. Since the solid lipid is milled the complexity arising due to lipid modification can be avoided [8]. Chances of drug distribution into the aqueous phase are limited and hence this method can be used for hydrophilic drugs as well as lipophilic drugs. Lipid nanoparticles
prepared via this technique possess a slightly larger particle size and polydispersity when compared to the ones obtained by hot homogenization technique, using the same lipid at similar homogenization parameters (pressure, temperature and the number of cycles). A higher number of homogenization cycles can be applied to reduce the particle size [18].

1.2.2 Microemulsion based technique

Gasco and coworkers were the first to develop solid lipid nanoparticles based on the dilution of microemulsions [19]. Microemulsions are thermodynamically stable, clear and isotropic mixtures usually composed of an oil or lipid, emulsifier and/or co-emulsifier and water. Lipids used to prepare SLNs are solids at room temperature and hence the microemulsion is prepared at a temperature above the melting point of the lipid. Both the lipid and the aqueous phase containing the emulsifier are mixed in appropriate ratios and stirred so that it will produce a microemulsion. The hot microemulsion is then diluted with cold water (2-8°C) while stirring. The ratio of the hot microemulsion to cold water is usually in the range of 1:25 to 1:50. It has been noted in literature that a droplet structure is already present in the microemulsion and therefore, no external energy is required to achieve the small particle size. When the microemulsion is diluted by cold water, the lipid droplets solidify as the temperature decreases. The temperature gradient and pH value determine the quality of the product in addition to the composition of the microemulsion. The major limitation of this technique is its sensitivity to minor changes in composition or thermodynamic variables, which can lead to phase transitions. Lack of robustness of the microemulsion technique can lead to high production costs. Moreover, solidification of the lipids shifts the system to a thermodynamically unstable state. Due to the dilution of microemulsion the
concentrations of particle content are below 1% and, therefore, large amount of water has
to be removed in order to process to a final dosage form. The high concentration of
surfactants used may produce toxicity. This necessitates removal of excess surfactants
using ultracentrifugation, ultrafiltration or dialysis.

1.2.3 Solvent emulsification-evaporation technique
Sjöström and Bergenståhl were the first to describe the production of solid lipid
nanoparticles by solvent emulsification-evaporation technique [20]. The solid lipid is
dissolved in a water immiscible organic solvent (ex. cyclohexane, chloroform, ethyl
acetate, methylene chloride, etc.) and the drug is dissolved or dispersed in the solution
[21]. This organic phase containing the drug is emulsified in an aqueous solution of a
surfactant by mechanical stirring. The organic solvent is then removed from the emulsion
under mechanical stirring or reduced pressure (40-60 mbar) [8, 21]. Lipid nanoparticle
dispersion is formed by the precipitation of the lipid phase in the aqueous surfactant
medium. Aggregation of the particles can be avoided in this technique by removing the
solvent at a faster rate [2]. This technique can be used to incorporate hydrophilic drugs by
preparing a w/o/w emulsion and dissolving the drug in the internal water phase [22, 23].
Thermosensitive drugs can be incorporated via this technique as it avoids thermal stress.
Trace amounts of organic solvent remaining in the final product can potentially create
toxicity problems. Moreover, increasing the lipid content decreases the efficiency of
homogenization due to the high viscosity of the dispersed phase and hence the
dispersions are very dilute and have very low lipid particle content (0.1g/l) [8]. A large
quantity of water has to be removed during the final processing of the formulation [24,
25].
1.2.4 Solvent displacement technique

Fessi et al. were the first to describe this technique for the preparation of polymeric nanoparticles by polymerization in solution [26]. Recently, this technique has been modified and used for the preparation of SLN’s [27, 28]. In this technique, lipid and the active ingredient is dissolved in a water miscible solvent such as ethanol, isopropanol, acetone, or methanol [29]. The mixture is then dispersed into an aqueous solution of a surfactant with mild mechanical stirring producing a suspension of lipid nanoparticles [27]. The solvent is subsequently removed by distillation. Ultracentrifugation, ultrafiltration or lyophilization can be used for isolating the lipid nanoparticles.

1.2.5 Emulsification-diffusion technique

Quintanar-Guerrero et al. were the first to describe this technique for the preparation of polymeric nanoparticles [30]. Recently this technique has been modified by various research groups for the preparation of SLN’s [31-33]. In this technique the lipid is dissolved in a partially water miscible solvent such as benzyl alcohol, isobutyric acid, or tetrahydrofuran which is previously saturated with water at room temperature or at a controlled temperature. The mixture is then emulsified in an aqueous solution of a surfactant by mechanical stirring at the temperature used to dissolve the lipid producing an o/w emulsion. This o/w emulsion is then diluted with excess water at a controlled temperature which causes the diffusion of the solvent into the external phase and subsequent precipitation of the lipid nanoparticles. The solvent can be removed either by distillation or by ultrafiltration. The concentration and the nature of the lipid and surfactant, stirring rate and the processing temperature are critical variables in this technique [31].
1.3 Separation and Purification of Solid Lipid Nanoparticles

Depending on the method of preparation, potentially toxic impurities such as surfactant micelles, residual monomers, polymers, metallic impurities and organic solvents can be present in the SLN dispersion. For an effective SLN drug delivery system, it should be free from any unencapsulated drug or impurities. SLNs can be separated and purified using diafiltration, ultracentrifugation, dialysis, gel filtration and crossflow microfiltration [34-38].

1.4 Effect of Lipids and Surfactants

The particle size of SLNs significantly affects the physical stability of the formulation, release rate of the drug and the fate of the particles in-vivo. The particle size is affected by various parameters such as the properties of the lipid and the surfactant, production technique and processing conditions (such as time, temperature, pressure, number of cycles). For both high pressure homogenization and high shear homogenization techniques, the average particle size of the SLN dispersion increases with increase in the melting point of lipids. Increase in the viscosity of the dispersed phase with increase in the melting point of the lipids has been suggested as the reason for larger particle sizes. Other parameters such as crystallization rate, lipid structure and size will vary for each type of lipid. The quality of the SLN dispersion is considerably affected by the composition of the lipid. Moreover, most of the lipids used are a mixture of several chemical compounds and hence their composition varies from different suppliers and batches. Lipid content over 5–10% results in an increased particle size and increased polydispersity index due to increased viscosity of the liquid SLN dispersion which affects the homogenization efficiency and increased rate of particle agglomeration [8].
Properties and concentration of the surfactant affects the size and the efficacy of the SLNs as a drug delivery system. Smaller particle sizes result in an increase in the SLN surface area. This increased surface area leads to thermodynamic instability and results in phase separation from the Ostwald ripening phenomenon [8]. The concentration of the surfactant should be sufficient to cover all the newly formed surfaces following SLN preparation. Surfactants inhibit the occurrence of phase separation by lowering the interfacial tension between the lipid and aqueous phases. Excess surfactant might be present in the formulation in various forms such as monomer, micelles or liposomes. A few patterns related to surfactant concentration and the quality of SLNs has been observed by research groups and the optimum concentration of the surfactant in a particular formulation should be determined for each case. Also SLNs stabilized with a surfactant/cosurfactant mixture have been observed to possess lower particle size and better stability when compared to those prepared with a surfactant alone. Siekmann et al. reported that 10 %w/v tyloxapol was required to stabilize 10 %w/v tripalmitin dispersion [21]. Cavalli et al. demonstrated that an SLN dispersion stabilized by an ionic surfactant showed lower particle size (70 ± 2 nm) when compared to an SLN dispersion stabilized by a nonionic surfactant (200 ± 5 nm) [39]. The homogenization parameter required in a particular formulation is dependent on the type of surfactant used in the SLN. Homogenization under a pressure of 500 bar and three cycles was optimum for poloxamer 188 stabilized systems while 1500 bar pressure was necessary for lecithin stabilized systems.
1.5 Stability of the Drug and Solid Lipid Nanoparticles

Stability considerations relevant to SLNs include the chemical stability of the drug and the physical stability of the SLNs. Prevention of degradation reactions such as hydrolysis is an important chemical stability parameter and examples for physical stability issues include the prevention of particle size growth and polymorphic changes of the solid lipid. Lipids and surfactants must be chosen carefully and should be mutually compatible to improve chemical stability. Lim et al. reported improvement of stability of all-trans retinol encapsulated into SLN upon irradiation with 60W incandescent bulb. A 43% improvement of stability was attained by incorporating a small amount of antioxidant in the SLN preparation [40].

The biodistribution, shelf-life, reticulo-endothelial system (RES) clearance mechanisms, and the route of administration are determined by the particle size distribution of the SLN formulation. The SLN dispersion should possess a narrow size distribution to avoid particle size growth due to Ostwald ripening. Ostwald ripening is a thermodynamically driven process, in which smaller particles dissolve and redeposit onto the surface of larger particles. This process occurs because smaller particles have larger surface area and higher surface energy and hence higher Gibbs free energy than the larger particles. All systems tend to attain lowest Gibbs free energy. In other words, larger particles are more energetically stable and favored over smaller particles. Ostwald ripening can be reduced by minimizing polydispersity in the particle size but it cannot be prevented.

There are three other types of instabilities in SLN dispersions: creaming, flocculation and coalescence. Creaming is a process in which the less dense phase
migrates to the top of the dispersion under the influence of buoyancy or centripetal force. Creaming brings the SLN particles close to each other aiding Ostwald ripening, flocculation and coalescence. This is of significance to the formulator since a centrifuge is usually used to separate the nanoparticles from the liquid SLN dispersion. Creaming can be prevented by matching the density of the lipid and aqueous phases. Flocculation is a process in which the nanoparticles are held together in loose associations by weak van der Waals forces. Coalescence is a process in which the nanoparticles fuse to form larger particles. The electrostatic repulsion and steric hindrance between particles produced in the presence of surfactants have been found to inhibit flocculation [41, 42].

Electrostatic repulsion produces an electrical double layer around each nanoparticle in SLN dispersion. The electrical double layer comprises of two parts: an inner region (stern layer), in which the ions are tightly bound and an outer diffuse region, in which the ions are less firmly attached. A notional boundary forms between particles and ions within this diffuse layer. Ions within the boundary move with the particle and the ions outside the boundary do not move with the particle. This notional boundary is called as slipping plane. The potential at the slipping plane is known as zeta potential. The magnitude of the zeta potential is an important determinant of the stability of SLN dispersions. As the zeta potential increases, the magnitude of electrostatic repulsion between the particles also increases, hence the particles will tend to repel each other and there is no tendency to flocculate. Colloidal dispersions with a zeta potential more positive than +30 mV and more negative than -30mV are considered to be stable [43].

Steric effects also play an important role in the stability of SLN dispersion by hindering the particles from coming close to each other and thus preventing flocculation.
and coalescence. The polyoxyethylene chain present in nonionic surfactants extends in the aqueous medium in the form of a coil and providing steric hindrance. Optimum surfactant concentration and sufficient chain length (≥ 20 ethylene oxide units) will impart steric effect mediated formulation stability [41]. For long-term stability a balance between electrostatic repulsion and steric effect must be obtained.

**Figure 1-2:** A schematic of electrical double layer theory modified from [44]

Lipid crystallization is important for the stability of lipid nanoparticles. It significantly affects the drug incorporation and release rates. Polymorphic transition is the ability to
form a different unit cell structure in crystals due to different molecular conformations and packing patterns. SLNs do not completely crystallize during their storage and contain various polymorphic forms such as α, β’ and β. The main difference between the polymorphic forms is the molecular distance. “α” form is unstable and is characterized by the hexagonal structure with the largest molecular distance. “β” form is stable and is characterized by the tightest triclinic packing pattern. Presence of residual liquids in lipid nanoparticles promote the crystallization of the stable form because unstable crystals may redissolve and recrystallize to the more stable form [8]. Increase in particle size, change in particle shape, and drug expulsion occurs when lipids undergo polymorphic modifications. An increase in thermodynamic stability and decrease in the drug incorporation rate was observed in the following order [2]: supercooled melt < α-modification < β’-modification < β-modification.

Differential scanning calorimetry (DSC) and X-ray scattering are widely used to study lipid polymorphic transitions. Different lipid forms possess different melting points and enthalpies and thus can be detected by DSC. X-ray scattering can be used to detect the length of long and short spacings of the lipid lattice [2].

1.5.1 Lyophilization or freeze drying of solid lipid nanoparticles

The stability of SLN dispersions has been reported to be in the range of 12 to 36 months [8]. But in most formulations the particle size increases within a short period of time and hence lyophilization is a way to increase the stability of SLNs. Ostwald ripening as well as hydrolysis can be avoided by lyophilization. Moreover it also makes SLNs feasible to be incorporated into various dosage forms such as tablets, capsules, pellets, parenteral redispersion, etc.
Lyophilization involves freezing the SLN dispersion followed by the evaporation of the water under vacuum. The lyophilization parameters to be considered are freezing out effect which leads to changes in osmolarity and pH. Low water and high particle content produces high osmotic pressure which in turn favors particle aggregation and hence the lipid content of the SLN dispersion should not exceed 5% [8]. Cryoprotectants such as mannitol, sorbitol, trehalose, glucose and polyvinylpyrrolidone are usually added to decrease particle aggregation and to obtain better redispersion of the lyophilizates. Cryoprotectants help in SLN stability by decreasing osmotic activity of water and crystallization and favoring the formation of glassy state of the frozen sample [45-48]. They prevent direct contact between lipid particles and they also interact with the polar groups of the surfactants and serve as a pseudo hydration shell [49]. Trehalose has been reported to give the best results as cryoprotectant for SLN lyophilization. Cryoprotectants are usually used in a concentrations of 10-15% [8].

Schwarz et al. reported that the particle size of reconstituted lyophilizates of Compritol® SLN was 330 nm when compared to 160 nm prior to lyophilization of the liquid dispersion [48]. Increase in particle size of approximately 1.5-2.4 times has been observed following lyophilization with the particles still in the submicron range. The time of addition of the cryoprotectant affects the quality of the lyophilizates. Addition of cryoprotectant prior to homogenization helps in reducing the increase in the particle size. Better particle size results are obtained when SLN lyophilizates are redispersed using a bath sonicator as opposed to simple hand shaking. The removal of water and increase in particle concentration during lyophilization compromises the protective effect of the
surfactant and hence favors particle aggregation. Mehnert et al. recommends a sugar/lipid weight ratio of 2.6-3.9 [8].

Extensive research has been done in optimizing the lyophilization procedure of SLN dispersions. Results on the rate of freezing (Slow freezing in a deep freeze at -70°C, rapid freezing in liquid nitrogen) are ambiguous and hence the procedure has to be optimized on a case-by-case basis. Thermal treatment (2 h at -22°C followed by 2 h at -40°C) of the frozen SLN dispersion has also been reported to improve the results [8]. Rapid cooling helps to decrease freezing out effects by forming small and heterogeneous crystals.

1.5.2 Spray drying

Although rarely used, spray drying is another technique that can be used to transform an aqueous SLN dispersion into a dry product. The production cost is lower with spray drying when compared to lyophilization. Spray dryers utilize hot gases and atomizers or spray nozzles to disperse the SLN dispersion and hence cause aggregation and partial melting of the SLN particles. Freitas suggests the use of high melting point lipids (>70°C), low lipid content in the dispersion, ethanol-water mixtures (10/90 v/v) as the dispersion medium, addition of about 20-30 % carbohydrates such as trehalose to control particle aggregation during spray drying [8].

1.6 Drug Loading and Release in Lipid Nanoparticles

1.6.1 Drug loading

Hydrophilic as well as lipophilic drugs can be incorporated into the SLN system. Ideally a nanoparticulate system should have high drug loading and long-term incorporation for
efficacy and efficiency reasons. Factors affecting the drug loading are drug/lipid ratio and solubility, partition coefficient, chemical and the physical structure of the solid lipid matrix and polymorphic state of the lipid [2]. The drug gets positioned between the lipid layers, imperfections or fatty acid chains. In a lipid matrix the drug is located either in the core or the shell or is molecularly dispersed throughout the matrix. However, drug can also be entrapped in micelles, mixed micelles, liposomes, super-cooled melts and other lipid modifications [8]. The drug loading capacity is generally expressed as percentage and is calculated as the ratio of drug to the lipid phase (matrix lipid + drug).

The drug should be highly soluble in the lipid to obtain sufficient loading capacities. The solubility of the drug decreases when cooling down the melt and is lower in the solid lipid [2]. Solubilizers can also be added to increase the solubility. The chemical properties of the lipid are an important factor affecting loading capacity as the drug gets expelled from highly crystalline particles with perfect lattice. The presence of mono-, di- and triglycerides in the lipid will form less perfect crystalline structures with many imperfections and possess higher loading capacities.

### 1.6.2 Drug release

The release profiles from SLNs can be modulated to obtain burst release, prolonged release (with no initial burst release) and different percentages of burst release followed by prolonged release [2]. The release rate of the SLN can be modified by proper choice of the lipid type, surfactant concentration and production parameters [50]. One important application of SLNs is the sustained and controlled delivery of the drugs. A major limitation with SLNs is the observance of burst release kinetics. Burst release was found to be independent of the production technique when incorporating tetracaine and
etomidate [15]. Controlled release of the drug was first reported by Müller when incorporating prednisolone into SLN produced by high pressure homogenization [51].

As depicted in figure 1-3, drug partitions from the liquid lipid phase to the aqueous phase when hot homogenization is the production technique. As the water solubility of the drug increases, the amount of the drug partitioning into the water phase also increases. The saturation solubility of the drug in the water phase increases with increasing processing temperature and surfactant concentration. As the temperature of the system decreases the solubility of the drug in the water decreases and the drug tends to repartition into the lipid phase. A solid lipid core containing the drug in it starts forming at the recrystallization temperature and the hydrostatic pressure on the drug further increases the drug repartition into the lipid phase. As the system continues to cool the solid lipid core is not accessible to the drug and the drug molecules tend to solubilize more in the liquid lipid outer shell of the SLN. The drug in the lipid core exhibits prolonged release kinetics whereas the drug in the shell exhibits burst release kinetics.

![Figure 1-3: Drug partitioning when using hot homogenization as the production technique (modified after [2])](image)
Mehnert et al. proposed three drug incorporation models: 1. Solid solution, 2. Drug enriched shell/lipid core model and 3. Drug enriched core/ lipid shell model (Figure 1-4) [2]. Controlled release kinetic profile is obtained with solid solution model. In the cold homogenization technique the drug is molecularly dispersed in the solid lipid and mechanical force is used to reduce the particle size thus SLNs produced by this technique typically exhibit controlled release. SLNs produced by hot homogenization technique exhibit drug enriched shell/lipid core model and show burst release profiles. Drug enriched core/ lipid shell model is exhibited when the drug precipitates first before the lipid crystallizes [2]. In such systems when the solubility of the drug is close to its saturation solubility prolonged drug release is observed.

![Proposed models of incorporation of drug into SLN](modified after [2])

**Figure 1-4:** Proposed models of incorporation of drug into SLN (modified after [2])

### 1.7 Application of SLNs in drug delivery system

SLNs are composed of physiological lipids and hence the pathways for lipid transportation and metabolism already present in the body determine the *in vivo* fate of the carrier. Enzyme lipases are most important for SLN degradation.
SLNs are stable for a long period of time and easy to scale up when compared to other colloidal systems and thus may be important for many modes of targeting. Anticancer agents are usually delivered systemically. SLNs can be administered intravenously owing to their small size. They have been reported to be useful as drug carriers to treat tumors. They provide a novel and a unique drug delivery system to prevent rapid clearance by the immune system. Stealth nanoparticles can be used to target specific tissues in accessible cells. Fluorescent SLNs prepared using fluorescent markers and drugs have been successfully tested in animal models [52]. Tumor targeting has been reported with SLNs loaded with methotrexate [53] and camptothecin [54]. Longer circulation times have been reported to be achieved with paclitaxel [55].

SLNs can penetrate the BBB due to adsorption of blood proteins such as apolipoproteins on lipid nanoparticles surface which in turn may lead to interactions with endothelial cells that facilitate crossing the BBB. Such properties have been reported for the drugs such as tobramycin, doxorubicin and idarubicin [56-58].

SLN can be used in the formulation for delivery of gene vector [59]. DNA degradation can be avoided and target specific delivery can be achieved by its incorporation in the SLN. Increase in the bioavailability and decrease in the dosing frequency has been reported to be achieved by incorporating antitubercular drugs such as rifampicin, isoniazid, and pyrazinamide in the SLNs [60].

SLNs have been used for topical application of various drugs as it gives potential advantage of delivering the drug directly to the site of action [61]. Research has been done for the incorporation of active ingredients such as anticancer drugs [62, 63], imidazole antifungals [64, 65], DNA [59, 66], flurbiprofen [67], glucocorticoids [68],
isotretinoin [69], triptolide [70], and Vitamin A [71, 72] into the SLNs. SLNs are known to be suitable as carriers for UV-blockers due to their particulate character and adhesive properties [73]. SLNs aid in achieving better localization, occlusiveness [71], controlled release and increased skin hydration in topical formulations [74].
Chapter 2

Instrumentation

2.1 Dynamic Light Scattering

Dynamic light scattering (also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering (QELS)) is one of the most popular technique used to determine the size and size distribution of molecules or particles in the submicron region. The various types of samples that can be characterized are proteins, polymers, micelles, vesicles, carbohydrates, nanoparticles, colloidal dispersions, emulsions, microemulsions, liquid crystals, gels, DNA, and pigments. [75]. DLS can be used to measure particles in the size range from 3 nm to several microns [76].

Principle: Particles in solution or suspension undergo Brownian motion which causes fluctuations of local concentration of the particles. This results in local in homogeneities of the refractive index and causes fluctuations of intensity of the scattered light when the particles are placed in the path of laser light. These fluctuations may be recorded and detected in the form of intensity versus time profile by detectors. The digital output from the detectors enables creation of an auto-correlation function by the autocorrelator of the DLS instrument. The autocorrelation function is an exponentially decaying time function. The decay constant of this function is inversely proportional to the diffusion coefficient.
of the particles. This inverse relationship enables using the Stokes-Einstein equation to calculate the particle size.

The technique is essentially based on two assumptions: the particles are in Brownian motion and that the samples used in the experiment are spherical particles whose diameter is small compared to the molecular dimensions [77]. If the above two assumptions are followed then it is possible to apply the Stoke-Einstein relation and obtain the diffusion coefficient.

\[
D = \frac{k_B T}{6\pi \eta a}
\]

Equation 2.1

Where, \(a\) is the hydrodynamic radius of the particles, \(k_B\) is the Boltzmann constant, \(T\) is the temperature in Kelvin and \(\eta\) is the viscosity of the medium.

**Instrumentation:** The DLS instrument comprises of a laser source, laser delivering optics, sample holder, scattered light collecting optics, photon detector, and autocorrelator (AC). A schematic diagram of the instrument is shown in figure 2-1. A monochromatic He-Ne laser of a known wavelength is passed through a dilute sample. The random movement of sample particles produces constructive and destructive interferences in the scattered laser light. This creates fluctuations in the intensity of scattered light with time. The light collecting optics collects the scattered light at a specific angle. The intensity fluctuations of the scattered light are converted into electrical pulses using an avalanche photodiode detector or photomultiplier tube. These are fed into a digital correlator, which generates the autocorrelation function, from which the appropriate data analysis is performed.
Sample preparation: Several parameters such as solvent viscosity, refractive index, and sample temperature affect the measurements by DLS. Diluted samples should be used to suppress interparticulate interactions. The scattering intensity depends on the mass of the particles, its concentrations and differences in the refractive index of the particle and the solvent. Air bubbles and dust particles may be removed by ultrasonication, centrifugation, or filtration. Sample holders and cuvettes should be thoroughly cleaned and dried before placing the sample.

Application: DLS is used to characterize particle size of various samples such as nanoparticles, emulsions, proteins, micelles, polymers, etc. The advantages of DLS include easy sample preparation, rapid measurements, non destructive technique, and low sample volume [76].

2.2 Electrophoretic Light Scattering

Electrophoretic light scattering (ELS) is used to calculate the zeta potential or the charge on the surface of colloidal particles in a liquid suspension. Zeta potential can be used to

Figure 2-1: Schematic of NICOMP 380 DLS particle size analyzer modified from [76]
estimate the stability of the colloidal dispersions against aggregation. The physical mechanism used to stabilize colloidal systems is electrostatic repulsion. The surface charge produces the electrostatic repulsion producing the electrical double layer which in turn determines the colloid stability [78]. The electrical double layer comprises of two parts: an inner region (stern layer), in which the ions are tightly bound and an outer diffuse region, in which the ions are less firmly attached. A notional boundary forms between the particles and ions within this diffuse layer. Ions within the boundary move with the particle and the ions outside the boundary do not move with the particle. This notional boundary is called as slipping plane. The potential at the slipping plane is known as zeta potential. As the zeta potential increases, the magnitude of electrostatic repulsion between the particles also increases, hence the particles will tend to repel each other and there is no tendency to flocculate. Colloidal dispersions with zeta potentials more positive than +30 mV and more negative than -30mV are considered to be stable [79].

**Principle:** Charged particles suspended in an electrolyte are attracted towards the electrode of opposite charge upon application of electric field across an electrode. The particles move with a constant velocity after achieving equilibrium. ELS measure the electrophoretic mobility which is the velocity of a particle in an electric field. The zeta potential of the particle can be obtained by the application of Smoluchowski equation.

\[ \mu = \zeta \frac{\varepsilon}{\eta} \]  

**Equation 2.2**

Where \( \mu \) is the electrophoretic mobility of the particles, \( \zeta \) is the zeta potential, \( \varepsilon \) is the electrical permittivity and \( \eta \) is the viscosity of the solution.
Instrumentation: The ELS instrument comprises of a laser source, beam splitter, lens, ND filter, optical fiber, PMT detector, autocorrelator and sample holder. A schematic diagram of an ELS instrument is shown in figure 2-2. The original laser light beam is split by the beam splitter 1, mirror 2 (referred to as local oscillator) and directed at a beam splitter 2. A small fraction of the local oscillator (LO) light wave is reflected by beam splitter 2 into the optical fiber (OF) pick up, where it mixes with scattered light (ELS) wave. The resulting superposition of light signals is then transmitted to the PMT.
detector by the optical fiber. The PMT photocurrent signal, consisting of individual
photopulses of average frequency 2000–4000 kHz, is then passed to a digital
autocorrelator (AC).

**Application:** Zeta potential can be used to optimize the formulation of emulsions and
suspensions [81, 82]. It can also help in predicting long term stability of colloidal
dispersions [15]. There are several advantages of ELS such as rapid and accurate
measurements, the ability to use a wide range of concentration and good sensitivity.

### 2.3 Transmission Electron Microscopy

Transmission electron microscope (TEM) utilizes electrons instead of light as “light
source” and their much lower wave length makes it possible to get a visual resolution a
thousand times better than with a light microscope. TEM allows to see objects to the
order of a few angstrom (10^{-10} m)-even as small as a single column of atoms. TEM is
utilized in medical, biological and material science research.

**Principle [80]:** A source at the top of the TEM emits electrons that travel through
vacuum in the column of the microscope. Instead of glass lenses focusing the light in the
light microscope, the TEM uses electromagnetic lenses to focus the electrons into a very
thin beam, which then travels through the specimen. The transmission of electron beam is
highly dependent on the properties of material being examined such as density,
composition, etc. Porous material allows more electrons to pass through while dense
material allows fewer electrons to pass through. As a result, a specimen with a non-
uniform density can be examined with the help of TEM. The part that is transmitted is
projected onto a fluorescent screen, which gives rise to a “shadow image” of the
specimen with its different parts displayed in varied darkness according to their density.
The image can be processed on a layer of photographic film, or detected by a sensor such as a CCD camera.

**Instrumentation:** A TEM has four main parts: electron source, electromagnetic lens system, sample holder, and imaging system. The electron source comprises of a cathode and an anode. The cathode is a tungsten filament which emits electrons when it is heated. A negative cap

![Figure 2-3: A schematic diagram of a Transmission Electron Microscope modified from [80]](image)

confine electrons in a loosely focused beam. The beam is accelerated in the direction of the specimen by the positive anode. Electrons at the rim of the beam will fall onto the
anode while the others at the center will pass through the small hole of the anode. The electron source works like a cathode ray tube. After leaving the electron source, electromagnetic lenses are used to accelerate and focus the electrons into a very thin beam by varying the magnetic field of electromagnetic lenses. The imaging system consists of another electromagnetic lens system and a screen. The other electromagnetic lens system is for enlarging the image and projecting it onto the screen. The screen has a phosphorescent plate which glows on being hit by electrons. The image forms in a similar manner to that of photography.

**Sample preparation:** The specimen must be of such a low density that it allows electrons to travel through the tissue. There are different ways to prepare the specimen for study. Very thin slices can be cut of the specimen from a piece of the material either by fixing it in plastic or working with it as frozen material. Another way to prepare the specimen is to isolate it and study a solution (e.g. viruses or molecules) in the TEM. Materials such as powders or nanotubes, can be quickly prepared by the deposition of a dilute sample containing the specimen onto support grids. One can stain the specimen in different ways and use markers to locate specific features in the sample [83]. It can be stained using negative staining material including heavy metals such as uranium and lead, which scatters electrons well and improves the image contrast in the microscope [84].

**Application:** TEM is used to characterize a variety of material in life sciences, nanotechnology, medical, biological and material research, forensic analysis, gemology and metallurgy [85]. The images allow the samples to be viewed on a molecular level, making it possible to analyze the structure and texture [86]. TEMs can also be used in semiconductor analysis and the manufacturing of computer and silicon chips [80].
2.4 Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) is a thermo-analytical technique used to obtain quantitative and qualitative information about physical and chemical changes that involve energy, or changes in heat capacity. It involves the measurement of a difference in the heat flow rate to the sample and an inert reference placed in sample crucibles while being exposed to a thermal regime via a temperature controlled program [87].

Principle: When the sample undergoes any physical or chemical transformation involving absorption or release of energy, it will require more (or less) heat flow to it when compared to the reference to maintain both at the same temperature. Depending upon whether the process requires more or less energy, they can be endothermic or exothermic. When the sample absorbs energy in the form of heat, the enthalpy change is said to be endothermic. Examples of endothermic processes are melting and vaporization. When the energy is released by the sample, the process is said to be exothermic. Examples of exothermic processes are crystallization and oxidation [87].

Instrumentation: The instrument consists of a thermally insulated furnace that can hold the samples for analysis. There are two sample positions in the furnace, one for the sample crucible (containing the material under investigation) and the other for the reference crucible (empty or containing the inert material). The sample crucibles are made of materials such as aluminum, platinum, stainless steel, nickel, etc that possess high thermal conductivity and compatibility with the sample material. The crucibles may be open, covered or sealed with a lid that is intact or pin holed depending upon the nature of sample.
Differential scanning calorimetry data can be obtained by two types of methods: (1) Power compensated DSC (2) Heat flux DSC

In power compensated DSC, there are two independent furnaces for heating the sample and the reference. Both the furnaces are embedded in a large temperature-controlled heat sink. They are heated in such a way that their temperatures are kept equal while these temperatures are increased or decreased linearly [88].

![Diagram of power compensated DSC sample holder and furnaces](image)

**Figure 2-4:** Schematic of Power compensated DSC sample holder and furnaces modified from [89]

In heat flux DSC, heat flows into the sample and the reference pan through an electrically heated constantan thermoelectric disc as shown in Fig. 2-5. Chromel/Constantan area thermocouples placed below the pans are used to measure the differential heat-flow to the sample and reference [88].
Application: DSC can be used to study pharmaceutical hydrates, polymorphs, glassy systems, protein denaturation, oxidation and other chemical reactions [90].

2.5 UV/Visible Spectroscopy

Ultraviolet/Visible molecular absorption spectroscopy is based upon electromagnetic radiation in the wavelength region of 160 to 780 nm (UV region: approximately 200-400 nm, visible region: approximately 400-800 nm) [88]. Absorption measurements based upon UV/Vis radiation find widespread application in quantitative and qualitative analysis of a substance, structure elucidation of organic compounds and detection of impurities.

Principle [91]: Absorption of radiation in the ultraviolet region cause electronic as well as associated vibrational and rotational changes. A chromophore is a group of atoms that gives rise to absorption in the near-ultraviolet (400-190 nm). Most unsaturated groups and heteroatoms carrying lone pair electrons such as alkenes, aromatics, conjugated dienes, trienes, etc are potential chromophores. Valence electrons are the electrons of an
atom which are not participating in chemical bonding in molecules and are referred to as nonbonding or n electrons [92]. “n” electrons are located mainly in the atomic orbitals of nitrogen, oxygen, sulfur, and halogens in organic molecules. Electrons in n, σ, or π orbitals absorb ultraviolet or visible radiation and result in their promotion to some higher energy antibonding orbital (excited state). Fig 2-9 indicates the order of energy levels of various molecular orbitals. Complete resolution of the electronic and closely spaced vibrational and rotational bands is not possible and hence the overall shape of the absorption band is broad [92].

When a beam of radiation strikes any object it can be either absorbed, scattered, reflected, transmitted or excite fluorescence. Absorption and transmission are the two processes of concern in absorption spectrometry. According to the Beer-Lambert law, the concentration of a substance in solution is directly proportional to the “absorbance”, A, of the solution.

\[ A = \log \left( \frac{I}{I_0} \right) = \log (T) = \varepsilon c L \] ……………..Equation 2.4

Where L is the pathlength of the radiation through the sample, c is the concentration of absorbing species in that path, and \( \varepsilon \) is the extinction coefficient – a constant dependent only on the nature of the molecule and the wavelength of the radiation.
**Figure 2-6**: Hypothetical energy diagram modified from [91]

**Instrumentation:** A typical UV-Visible Spectrophotometer consists of light sources, wavelength selectors, sample containers, radiation transducers, and signal processors and readout devices. Tungsten filament lamp (covers 330-700 nm) and deuterium lamp (covers 200-300 nm) is commonly employed as a source of light. A monochromator (grating or prism) is used to split the beam into its component wavelengths. A Wavelength selector helps in the selection of a particular wavelength that exits through the monochromator. The detector measures the difference between the transmitted light through the sample and the incident light and sends it to the recorder. There are four types of spectrophotometers namely single-beam, double-beam in space, double-beam in time, and multichannel. Cells or cuvettes that hold the samples are made of high quality fused silica, quartz glass or plastic. The solvents used should not only be transparent but also have negligible absorbance in the UV region. Some of the commonly used solvents are water, methanol, ethanol, cyclohexane, benzene, diethyl ether, acetone, n-Hexane, etc [88].
**Figure 2-7:** A schematic of a single-beam UV-Visible spectrophotometer instrument [93]

**Application:** UV/Visible spectroscopy is used for the quantitative determination of analytes such as transition metal ions (i.e. copper sulfate), conjugated organic compounds (e.g. DNA, RNA, protein) [94], and biological macromolecules. It is also used as a detector for High-performance liquid chromatography (HPLC). The Woodward Fischer rules that predict the wavelength of the absorption maximum ($\lambda_{\text{max}}$) in an ultraviolet–visible spectrum of a given compound may be used for structural characterization of organic compounds containing conjugated systems [95].
Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Glyceryl monostearate

Figure 3-1: Chemical structure of Glyceryl Monostearate

Source: Fisher Scientific, Lot # XF0448, CAS 31566-31-1

Other Names: Emrest 2400 Aldo, Arlacel, Tegin, Unitolate, Witconol, Glycerin Monostearate, Glycerol Monostearate, Monostearin

Chemical name: Octadecanoic acid, monoester with 1,2,3-propane-triol

Molecular Formula: C_{21}H_{42}O_4

Molecular weight: 358.6 gram/mole

Specific gravity: 0.92

Physical state: White to cream-colored, wax-like solid in the form of flakes
Solubility: Soluble in hot ethanol, ether, chloroform, hot acetone, mineral oil, and fixed oils, practically insoluble in water, but it may be dispersed in water with the help of a small amount of soap or surfactant.

Glyceryl Monostearate is an aliphatic carboxylic acid ester with an HLB value of 3.8. It is used as a nonionic emulsifier, emollient, stabilizer, and plasticizer in foods, cosmetics, and oral and topical pharmaceutical formulations and is generally regarded as a nontoxic and nonirritant material[96]. Also used as a dispersing agent for pigments in oils or solids in fats, or a solvent for phospholipids such as lecithin. It is used as a lubricant for tablet manufacturing and may be used to form sustained-release matrices for solid dosage forms[97]. It has also been used as a matrix ingredient for implantable, biodegradable, controlled release dosage forms[98]. It is included in the FDA Inactive Ingredients Guide. It should be stored in a light resistant, tightly closed container in a cool and dry place.

3.1.2 Tween 80

![Chemical structure of Tween 80](image)

**Figure 3-2:** Chemical structure of Tween 80

Source: Spectrum Chemicals, Lot # C136790, CAS 156-27-1

Other names: Polyethylene oxide sorbitan mono-oleate, Polyoxyethylene sorbitan monooleate, sorbitan mono-9-octadecenoate, Soretytan (20) monooleate

Chemical name: Polyoxyethylene 20 sorbitanmonoleate

Molecular Formula: C_{64}H_{124}O_{26}
Molecular weight: 1310 gram/mole
Specific gravity: 1.08
Physical state: Yellow oily liquid
Soluble in water, alcohol, methanol, toluene, and ethylacetate. Insoluble in mineral oil

Tween 80 is a series of partial fatty acid esters of sorbitol and its anhydrides copolymerized with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides[99].

Tween 80 is a hydrophilic nonionic surfactant which is widely used as an excipient to stabilize suspensions and emulsions. They are also used as solubilizing agents and as wetting agents in nutritives, creams, ointments, lotions, and multiple medical preparations (e.g., vitamin oils, vaccines, oral and parenteral suspensions and anticancer agents) and as an additive in tablets [100]. It has been reported to assist in the delivery of certain drugs or chemotherapeutic agents across the blood brain barrier (BBB)[101].

3.1.3 Polyethylene Glycol 400

\[ \text{Chemical structure of Polyethylene Glycol 400} \]

\[
\text{Chemical name: } \alpha-\text{Hydroxy-}\omega-\text{hydroxy-poly(oxy-1,2-ethanediyl)}
\]

\[
\text{Chemical formula: } C_{2n}H_{4n+2}O_{n+1}
\]

Molecular weight: 380-420 g/mol
Physical state: Colorless liquid (viscous)

Specific gravity: 1.1254

Solubility: Soluble in water and aromatic hydrocarbons. Slightly soluble in aliphatic hydrocarbons

Storage: To be kept in a tightly closed container in a cool and well-ventilated area

Polyethylene glycols (PEG) have a broad range of application in pharmaceutical and cosmetic industry. It is used in cosmetics such as creams, jellies and lotions. It is easily absorbed by the skin acting as a solubilizer for the therapeutic agents added to the lotion.

It is used as a precipitant for the crystallization of RNA oligonucleotides in a form suitable for X-ray diffraction studies[102]. PEG 400 is nontoxic, not degraded by intestinal bacteria, not metabolized by tissues, rapidly excreted in the urine and hence used as a probe for the investigation of intestinal permeability [103]. They are known to cause hardening of the capsule shells in soft gelatin capsules by absorption of moisture from the gelatin in the shell [104].

3.1.4 5-Fluorouracil

![Chemical structure of 5-fluorouracil](image)

**Figure 3-4:** Chemical structure of 5-fluorouracil

Source: Spectrum chemicals, Lot # C137461, CAS 51-21-8

Chemical name: 2,4-Dihydroxy-5-fluoropyrimidine, 5-Fluoro-2,4(1H,3H)-pyrimidinedione

Chemical formula: C₅H₅FN₂O₂
Molecular weight: 130.08 g/mol

Physical state: White powder

Melting point: 282-286°C

Partition coefficient: log Pow: -0.677

Side effects: myelosuppression, mucositis, dermatitis, diarrhea, nausea, vomiting, sensitivity to light[105]

5-Fluorouracil (5-FU) is a chemotherapeutic agent known as anti-metabolites. It is an established form of chemotherapy for colorectal and pancreatic cancer[106]. It is also used for the treatment of inflammatory breast cancer. 5-FU is used in ophthalmic surgery due to its anti-scarring property at the traculectomy site. It is used topically in the form of a cream for the treatment of actinic (solar) keratoses and some basal cell carcinomas (of the skin)[107].

Any drug or supplement that interferes with blood clotting such as Vitamin E, non-steroidal anti-inflammatory drugs (NSAIDS), warfarin, ticlopidine, and clopidogrel can raise the risk of bleeding during treatment with 5-FU.

5-FU is an analogue of naturally occurring pyrimidine uracil and is metabolized via the same metabolic pathways as uracil [108]. As a pyrimidine analogue, it is transformed inside the cell into different cytotoxic metabolites. The latter is then incorporated into DNA and RNA, finally inducing cell cycle arrest and apoptosis by inhibiting the cell’s ability to synthesize DNA[107, 109].
3.2 Methods

3.2.1 Preparation of SLNs via microemulsion process

Preparation of solid lipid nanoparticles via microemulsion method was performed at a temperature above the melting point of the lipid. Formulations were prepared using Glyceryl Monostearate as the solid oil, Tween 80 as the surfactant, PEG 400 as the cosurfactant and deionized water as the dispersion medium. Appropriate quantities of oil, surfactant and cosurfactant were weighed and mixed at a temperature 10°C above the melting point of the lipid in a water bath. Water was heated to the same temperature as the lipid phase and added drop wise under mild stirring to the lipid melt. After each addition the sample was vortexed at 1000 RPM for 10 seconds and visualized for clarity. When turbidity that did not disappear after vortexing was observed the samples were sonicated for 5 min at a temperature above the melting point of the lipid. A transparent, thermodynamically stable system was formed when the compounds are mixed in the correct ratio for the microemulsion formation. This microemulsion was then dispersed in a cold aqueous medium (5-10°C) under mild mechanical mixing. The ratio of microemulsion to aqueous medium was 1:20.

3.2.2 Preparation of SLNs via magnetic stirring process

This is a novel method for the preparation of solid lipid nanoparticles developed in our lab. A variety of surfactants in various compositions were evaluated for their potential to form SLNs. The oil: surfactant ratios that were tested ranged between 1:1 and 1:8. The solid lipid was heated to about 10 °C above the melting point of the lipid. Surfactant and cosurfactant are then added to the melted lipid. This was then vortexed to obtain a homogeneous mixture. The dispersion medium was heated to the same temperature as the
lipid phase and placed in an ice bath as shown in fig 3-5(a). The lipid and the surfactant mixture was then poured at a uniform rate into the heated dispersion medium and stirred on a magnetic stirrer at 700 RPM. The resulting SLN dispersion as shown in fig 3-5(b) was used for further analysis.

![Figure 3-5(a): Assembly](image1)  ![Figure 3-5(b): SLN dispersion](image2)

### 3.2.3 Preparation of SLN via a novel temperature modulated solidification process

#### 3.2.3.1 Blank SLNs

In this method glycerol monoesterate was used as a solid lipid, tween 80 as the surfactant, and deionized water as the dispersion medium. Appropriate quantities of lipid and surfactant were weighed and mixed at a temperature above the melting point of the lipid. This mixture was vortexed until a homogenous mixture was obtained. Deionized water was heated to the same temperature as the lipid melt. The lipid melt was then dispersed into the heated water placed in an ice bath and stirred at 4000 rpm for 45 min in a high shear mixer (model L5M-A, Silverson, USA). The resulting SLN dispersion was used for further analysis.
3.2.3.2 5-fluorouracil (5-FU) loaded SLNs

An appropriately weighed quantity of 5-FU was added in 1 g glyceryl monostearate and stirred for 48 h at 75°C and 1500 RPM. The required quantity of tween 80 was added to the molten mixture and mixed at a temperature above the melting point of the lipid. This mixture was vortexed until a homogenous mixture was obtained for about 1 h. The remaining procedure was identical to that reported earlier in the section on preparation of SLNs via a novel temperature modulated solidification process.

3.2.4 Lyophilization of SLN dispersion

The SLN dispersions were lyophilized (freeze dried) to obtain a dry powder. A particular SLN dispersion was placed in a 100 kDa Amicon Ultra-15 centrifugal filter unit and centrifuged at 7830 RPM for 30 min at 15°C temperature in a centrifuge 5430R (Eppendorf AG, Hamburg, Germany) using rotor F-35-6-30 to isolate the SLNs. The SLNs obtained on top of the filter were redispersed in appropriate amount of deionized water and the cryoprotectant anhydrous trehalose equivalent to the amount of glyceryl monostearate was added. The mixture was then placed in a fast freeze flask (Labconco, MO, USA) and then frozen quickly at -75°C. The frozen samples were lyophilized for 72 hours in the lyophilizer (FreeZone 2.5 liter benchtop freeze dry system, Labconco, MO, USA). The temperature was kept at about -49°C and vacuum maintained at 0.120mBar. The lyophilized samples were collected and stored in a dessicator for further characterization.

3.2.5 Droplet size determination of SLNs

Samples were prepared for particle size analysis by diluting 1 ml of the SLN dispersion with 10 ml deionized water. The droplet size of the resultant nanosuspension was
measured in a dynamic light scattering instrument (Nicomp 380 ZLS, Particle Sizing Systems, CA). The SLN dispersion samples were taken in disposable Durex borosilicate glass culture tubes (VWR Scientific products) and the particle size was determined by placing the sample in the path of a Helium Neon laser of wavelength 658 nm at a scattering angle of 90° and a temperature of 23°C.

3.2.6 Zeta potential determination of SLNs

Samples were prepared for zeta potential analysis by diluting 1 ml of the SLN dispersion with 10 ml deionized water. DLS instrument was used to measure Zeta Potential but in the electrophoretic light scattering mode (ELS). The SLN dispersion and redispersed lyophilized SLN samples were placed in a standard glass cuvette for measurement. The scattering angle was set at -14.06 and a temperature of 23°C.

3.2.7 Morphology of SLNs

Transmission electron microscopy (TEM) helps to visualize the internal matrix and shape of individual nanoparticles. A drop of the suitably diluted sample was placed on a Formvar/Carbon 400 mesh copper grid (Ted Pella, CA) and allowed to dry for 15 minutes before removing the excess sample with a lint free wipe. The prepared grid was equilibrated overnight prior to processing via TEM. The images were procured in a Hitachi HD-2300 scanning transmission electron microscope in the phase contrast mode.

3.2.8 Thermal analysis of SLNs

A differential scanning calorimeter (PerkinElmer Diamond DSC, CT, USA) equipped with an intercooler 1P was used to analyze the thermal behavior of SLN samples and physical state of the drug in SLNs. Samples of pure 5-fluorouracil, pure glyceryl monostearate, blank lyophilized SLN, and lyophilized SLN containing 5-fluorouracil
were run on DSC. Samples (8-10 mg) were accurately weighed into 20 µl aluminium pans and then crimped. The thermograms were recorded over a temperature range of 10-350°C at a heating rate of 10°C/min under nitrogen purge gas maintained at a flow rate of 20 ml/min. The thermograms were analyzed using Pyris Manager (v 1.3) software.

3.2.9 Drug encapsulation efficiency studies of SLNs

A 12 ml sample of freshly prepared drug loaded SLN dispersion was centrifuged in a 100 kDa Amicon Ultra-15 Centrifugal Filter Unit at 7830 RPM for 30 min at 15°C temperature using centrifuge 5430R (Eppendorf AG, Hamburg, Germany). The amount of the unincorporated drug was measured by suitably diluting the filtrate and measuring its absorbance at 265 nm using single beam UV spectrophotometer (Agilent UV spectrophotometer 8453) against suitably diluted filtrate of blank SLN dispersion. Encapsulation efficiency was calculated by subtracting the amount of drug in the filtrate from the amount of drug originally added to the formulation.

3.2.10 In vitro drug release studies from SLNs

In vitro release profiles of 5-FU from the lyophilized SLNs were obtained by a dissolution test in phosphate buffer solution (USP phosphate buffer, release medium, 0.2 M, pH 7.4). Regenerated cellulose membrane (dialysis membrane with molecular weight cut off of 12-14 kDa, Fisherbrand® regenerated cellulose dialysis tubing, 44 mm diameter) was used. Lyophilized 5-FU loaded SLNs equivalent to 2 mg 5-FU and 10 ml phosphate buffer (pH 7.4) was placed into a dialysis bag that immersed into 100 ml phosphate buffer solution and the system was maintained at 37°C under mild agitation of 100 RPM/min in a reciprocal shaking bath. At predetermined time intervals, aliquots of
the release medium (4 ml) were withdrawn and assayed for drug release and replaced by 4 ml of fresh buffer. An assembly similar to the one described above was also prepared for the blank lyophilized SLN (to be used as blank for UV spectrophotometry). 5-FU in the release medium was quantified by UV spectrophotometry at 265 nm against the blank and cumulative release of 5-FU was calculated based on a pre-generated the calibration curve.
Chapter 4

Results and Discussion

4.1 Particle size analysis

Solid lipid nanoparticles are spherical lipid particles in the nanometer size range. Thus particle size is a critical parameter for evaluation during and after formulation of solid lipid nanoparticles. Table 4.1 represents the particle size data of SLN dispersions prepared by microemulsion and magnetic stirring process and the influence of various surfactants and surfactant-cosurfactant combination on the particle size. A few particles with higher particle size were sometime observed when hot microemulsion was dispersed in the cold aqueous medium and hence no further studies were performed using the microemulsion process.

<table>
<thead>
<tr>
<th>Formulation process</th>
<th>Type of surfactant</th>
<th>Particle size (nm) ± σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microemulsion</td>
<td>Tween 80</td>
<td>97.25 ± 45.02</td>
</tr>
<tr>
<td></td>
<td>Tween 80 + PEG 400</td>
<td>70.60 ± 12.34</td>
</tr>
<tr>
<td>Magnetic stirring</td>
<td>Tween 80</td>
<td>66.00 ± 6.23</td>
</tr>
<tr>
<td></td>
<td>Tween 80 + PEG 400</td>
<td>56.9 ± 9.68, 268.02 ± 128.64</td>
</tr>
</tbody>
</table>

Table 4.1: Particle size of SLN dispersion prepared by microemulsion and magnetic stirring method and the influence of various surfactants (σ is standard deviation, n=3)
SLN dispersions prepared by the magnetic stirring process and using tween 80 as the surfactant was further evaluated to check the influence of various preparation parameters on the particle size. An increase in the lipid concentration in the formulation lead to a concentration dependent increase in particle size, once the lipid concentration exceeded a threshold concentration. However the particle size decreased with an increase in surfactant concentration but plateaued off once a critical surfactant concentration was reached (Table 4.2). It has been established that the presence of surfactant reduces the surface tension between the lipid and water and facilitates solid particle formation during the cooling phase of SLN preparation [110]. Table 4.3 shows that the volume of the dispersion medium did not significantly affect particle size. Table 4.4 suggests that there is no significant change in the particle size with change in stirring time. Thus in the optimized formulation by the magnetic stirring process, 1:3 was used as the oil: surfactant ratio, 150 ml deionized water as the dispersion medium and the nanoemulsion obtained was stirred for 45 minutes.

<table>
<thead>
<tr>
<th>Oil : surfactant</th>
<th>Mean volume diameter (nm) ± σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>70.27 ± 2.81, 1074.2 ± 196.73</td>
</tr>
<tr>
<td>1:3</td>
<td>66.00 ± 6.23</td>
</tr>
<tr>
<td>1:4</td>
<td>55.75 ± 6.43</td>
</tr>
<tr>
<td>1:5</td>
<td>57.35 ± 6.57</td>
</tr>
</tbody>
</table>

**Table 4.2:** Influence of the surfactant concentration on the particle size of the SLN dispersion prepared via the magnetic stirring process (σ is standard deviation, n=3)
<table>
<thead>
<tr>
<th>Dispersion medium (ml)</th>
<th>Mean volume diameter (nm) ± σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>61.38 ± 4.63</td>
</tr>
<tr>
<td>100</td>
<td>65.80 ± 0.42</td>
</tr>
<tr>
<td>150</td>
<td>65.53 ± 4.95</td>
</tr>
<tr>
<td>200</td>
<td>64.36 ± 0.21</td>
</tr>
<tr>
<td>300</td>
<td>66.00 ± 6.23</td>
</tr>
</tbody>
</table>

Table 4.3: Influence of the amount of dispersion medium on the particle size of the SLN dispersion prepared via the magnetic stirring process (σ is standard deviation, n=3)

<table>
<thead>
<tr>
<th>Time of stirring (min)</th>
<th>Mean volume diameter (nm) ± σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>66.00 ± 6.23</td>
</tr>
<tr>
<td>60</td>
<td>57.43 ± 2.93</td>
</tr>
</tbody>
</table>

Table 4.4: Influence of the time of stirring on the particle size of the SLN dispersion prepared via the magnetic stirring process (σ is standard deviation, n=3)

Similar process characterization studies were performed on the temperature modulated solidification process to evaluate the effect of surfactant on the particle size. Based on comparison of visual clarity and the Tyndall effect (bluish tinge when observed in light) shown in fig. 4-1 and fig. 4-2, an oil: surfactant ratio of 1:2 was found sufficient to prepare SLNs by the temperature modulated solidification process but produced large particles with the magnetic stirring process (fig. 4-3). Excess surfactant may potentially be present in the formulation in various forms such as monomer, micelles or liposomes and drug can also be entrapped in micelles [8]. Thus in the optimized formulation prepared by the temperature modulated process, oil: surfactant ratio of 1:2 was used, 150
51 ml deionized water used as the dispersion medium and the nanoemulsion obtained was stirred for 45 minutes.

**Figure 4-1:** SLN dispersion prepared via the magnetic stirring process with oil: surfactant ratio of 1:2

**Figure 4-2:** SLN dispersion prepared via the temperature modulated process with an oil: surfactant ratio of 1:2
5-fluorouracil was incorporated in the SLNs prepared via the temperature modulated solidification process and all the further studies were performed on the same. Table 4.5 demonstrates that drug incorporation did not significantly affect the particle size of the formulation. Lyophilization of the SLN dispersion increased the particle size but it remained in the nanometer size range. The instrument output of DLS experiments are represented in figures 4-31 to 4-7. Tween 80 micelles can be seen in the particle size distribution (size 6-10 nm) obtained by dynamic light scattering and this effect has been reported by other research groups as well [111].

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Particle size (nm) ± σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank SLN dispersion</td>
<td>59.47 ± 12.74</td>
</tr>
<tr>
<td>Blank lyophilized SLNs</td>
<td>429.5 ± 132.64</td>
</tr>
<tr>
<td>SLN dispersion with drug</td>
<td>64.4 ± 6.79</td>
</tr>
<tr>
<td>Lyophilized SLNs with drug</td>
<td>407.53 ± 155.33</td>
</tr>
</tbody>
</table>

Table 4.5: Particle size of SLN dispersions prepared by temperature modulated solidification process (σ is standard deviation, n=3)
Figure 4-4: Representative particle size distribution of blank SLN dispersion via the magnetic stirring process prepared with an oil: surfactant ratio of 1:2

Figure 4-5: Representative particle size distribution of blank SLN dispersion prepared via temperature modulated solidification process
Figure 4-6: Representative particle size distribution of blank redispersed lyophilized SLNs prepared via temperature modulated solidification process

Figure 4-7: Representative particle size distribution of 5-FU loaded SLN dispersion prepared via temperature modulated solidification process
Figure 4-8: Representative particle size distribution of 5-FU loaded redispersed lyophilized SLNs prepared via temperature modulated solidification process

4.2 Zeta potential

The magnitude of the zeta potential is an important factor that determines the stability of SLN dispersions. The zeta potential was measured for the SLN dispersion and lyophilized SLNs.

<table>
<thead>
<tr>
<th>Type of the sample</th>
<th>Zeta potential (mV) ± σ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank SLN dispersion</td>
<td>-12.84 ± 1.89</td>
</tr>
<tr>
<td>Blank lyophilized SLNs</td>
<td>-18.12 ± 4.8</td>
</tr>
<tr>
<td>SLN dispersion with drug</td>
<td>2.66 ± 2.23</td>
</tr>
<tr>
<td>Lyophilized SLNs with drug</td>
<td>3.07 ± .26</td>
</tr>
</tbody>
</table>

Table 4.6: Zeta potential of blank and 5-Fluorouracil loaded solid lipid nanoparticles (σ is standard deviation, n=3)
The zeta potential value for dispersion and lyophilized blank lipid nanoparticles was approximately -15 mV. Glyceryl monostearate is a fatty acid ester that imparts a negative surface charge on the lipid particles and thus the SLN formulation possesses a negative zeta potential. The zeta potential values for the dispersion and lyophilized lipid nanoparticles loaded with 5-fluorouracil was approximately 3.0 mV. The drug molecules covering and coating the surface of the drug incorporated SLNs is likely contributing to the reversal of surface charge.

**Figure 4-9**: Zeta potential distribution of blank SLN dispersion prepared via temperature modulated solidification process
Figure 4-10: Zeta potential distribution of 5-FU loaded SLN dispersion prepared via temperature modulated solidification process

Figure 4-11: Zeta potential distribution of blank redispersed lyophilized SLNs prepared via temperature modulated solidification process
Figure 4-12: Zeta potential distribution of 5-FU loaded redispersed lyophilized SLNs prepared via temperature modulated solidification process

4.3 TEM of SLNs

The shape and surface morphology of blank and 5-FU loaded dispersion and redispersed lyophilized SLNs prepared via the temperature modulated solidification method were studied using TEM. In the TEM study, the size of the lipid nanoparticles was found to be in agreement with the dynamic light scattering data for all the samples. All the particles were found to be roughly spherical in shape with a well defined periphery. TEM image of 5-FU loaded SLNs also provides the structural information of the SLN[112]. The lipid nanoparticles appear to be less dense in the core with a well defined shell. No obvious aggregation of the lipid nanoparticles was observed in the TEM images.
**Figure 4-13:** TEM image of Blank SLN dispersion

**Figure 4-14:** TEM image of 5-FU loaded SLN dispersion
**Figure 4-15:** TEM image of Blank redispersed lyophilized SLNs

**Figure 4-16:** TEM image of 5-FU loaded redispersed lyophilized SLNs
4.4 DSC analysis

Differential scanning calorimetry was used to investigate the crystallization behavior of pure glyceryl monostearate, pure 5-fluorouracil, lyophilized blank SLN and lyophilized 5-FU loaded SLN prepared via low temperature solidification process. The pure glyceryl monostearate exhibits an endothermic thermal event peaking at 71 ºC. Lyophilized blank and drug loaded SLN samples exhibits a glyceryl monostearate peak at 61 ºC. The onset temperatures of pure glyceryl monostearate, lyophilized blank SLN and lyophilized 5-FU loaded SLN are 65 ºC, 51ºC and 51ºC respectively. The decrease in the peak and the onset temperatures can be attributed to the reduction in particle size and increase in the surface area leading to a decrease in melting enthalpy when compared to heat flow through larger particulates, which require more energy to overcome lattice forces [113]. Therefore, the lower melting enthalpy values for the lyophilized samples suggest lower ordered lattice arrangement. As a result, it can be concluded that the lipid within the nanoparticles must be in a less ordered arrangement compared to the pure glyceryl monostearate [113]. The thermogram for pure 5-FU shows a sharp endothermic peak at 285 ºC [23]. The thermogram of 5-FU loaded lyophilized SLN shows a small endothermic peak for the 5-FU at 289 ºC which can be attributed to a small fraction of drug which may be present in the crystalline form. Endothermic peak of trehalose used as cryoprotectant was observed at 100 ºC, 111 ºC, 121 ºC and 206 ºC[114]. DSC runs for the lyophilized SLN samples shows an endothermic peak (endothermic overshoot) at around 350 ºC due to the physical aging phenomena near the glass transition temperature for trehalose [115].
Figure 4-17: DSC thermograms of pure Glyceryl Monostearate (A), pure 5-fluorouracil (5-FU) (B), lyophilized blank SLN (C) and lyophilized 5-FU loaded SLN (D)

4.5 Drug encapsulation efficiency

An indirect method was used to determine the encapsulation efficiency of SLNs prepared via low temperature solidification process[116]. The SLN dispersion was centrifuged in a 100 kDa Amicon Ultra-15 Centrifugal Filter Unit and the free drug present in the filtrate was analyzed by UV-Visible spectrophotometer. As shown in fig. 4-14, the calibration curve was constructed by measuring the absorbance at 265 nm of solutions of five different concentrations of drug in water. This method is suitable for determining encapsulation efficiency when the concentration of the drug is high in the filtrate [117]. Encapsulation efficiency studies were performed by loading 30 mg, 35 mg and 40 mg 5-fluorouracil in 1 g GMS. Encapsulation efficiency was observed to be around 30.00 % for all the three concentrations. No significant increase in the encapsulation efficiency was observed by increasing the concentration of drug and thus 30 mg 5-FU was used for
further studies. The low encapsulation efficiency may be attributed to the relatively lower solubility of 5-FU in glyceryl monostearate when compared to deionized water [118]. Due to the water soluble nature of the drug it is easily expelled to the external aqueous phase during the process of lipid re-crystallization [116]. The saturation solubility of the 5-Fu in the aqueous phase also increases with an increase in the temperature of the aqueous phase and surfactant concentration [2].

![Figure 4-18: Calibration curve of 5-fluorouracil in water (absorbance taken at wavelength 265 nm, n=3)]
4.6 *In vitro* drug release studies

*In vitro* drug release from redispersed lyophilized SLNs was performed in phosphate buffer pH 7.4 by using a dialysis bag. As shown in fig. 4-16, the calibration curve of 5-fluorouracil was constructed by measuring the absorbance at 265 nm of solutions of five different concentrations of the drug in 0.2 M phosphate buffer, pH 7.4. The *in vitro* drug release profile obtained from the dialysis bag experiment is shown in fig. 4-17. Drug release was found to be 17% after 2 h. The release profile was characterized by an initial and variable rapid release followed by a continuous release[119]. The initial burst release may be due to the drug located on or near the surface of the SLNs and the large surface to volume ratio of the nanoparticles geometry owing to their size [119]. After the initial burst release, the release profile displayed a plateau, resulting from the diffusion of the drug dispersed in the lipid matrix [119].
Figure 4-20: Calibration curve of 5-fluorouracil in 0.2 M phosphate buffer pH 7.4 (absorbance taken at wavelength 265 nm, n=3)

\[ y = 0.0501x - 0.0006 \]
\[ r^2 = 0.9888 \]

Figure 4-21: *In vitro* drug release from redispersed 5-fluorouracil lyophilized SLNs in 0.2M phosphate buffer pH 7.4 at 37 °C (n=3)
Water soluble drugs exhibit the tendency to migrate to the aqueous phase, hence concentrating at the surface of the particles and exhibiting the burst effect. The low drug release from the SLNs could be due to the structural integrity of the SLN matrix which may impede drug diffusion [120, 121].
Conclusion

In this research solid lipid nanoparticles were successfully prepared by three preparation methods namely microemulsion technique, magnetic stirring technique and temperature modulated solidification technique of which the latter two were developed in our laboratory. 5-fluorouracil was incorporated in the SLNs prepared via the temperature modulated solidification process. The developed techniques were simple, reproducible, prepared nanoparticles without the need of organic solvents or any sophisticated instruments and have the potential to easily scale up for large scale production. The particle sizes of SLN dispersion prepared via the three techniques was approximately 66 nm and that of redispersed lyophilized solid lipid nanoparticles was below 500 nm. SLN dispersions were stable for approximately 1 week after which the nanoparticles aggregated to form larger particles. The SLN dispersions were lyophilized to stabilize the solid lipid nanoparticles and the lyophilizates exhibited good redispersibility upon ultrasonication for 2 min. Morphological studies using TEM images showed spherical to oval particles with well defined periphery. The SLNs appeared to be less dense in the core with a well defined shell and the particle size was in concordance with particle size analysis data obtained from dynamic light scattering. A decrease in the enthalpy and onset temperature for the melting point of glyceryl monostearate in the DSC thermograms confirmed the reduction in the particle size. The drug encapsulation
efficiency was found to be approximately 30% which may be due to the water soluble nature of 5-FU leading to rapid partitioning into the aqueous phase and hence decreased encapsulation into the SLNs. *In vitro* drug release studies from redispersed lyophilized SLNs showed that 17% of the encapsulated drug was released within 2 h. An initial burst release of 5-FU indicates its adsorption onto or near the surface of SLNs. The low drug release from the SLNs may be due to the structural integrity of the lipid matrix leading to establishing a barrier preventing drug diffusion. Future work in this research project includes investigating the effect of pH on *in vitro* drug release of 5-FU from SLNs, drug incorporation in the SLNs prepared via microemulsion process and magnetic stirring process, *in vitro* cell toxicity studies, and formulation testing in animal models.
References


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