2015

Formulation, characterization and evaluation of paclitaxel loaded solid lipid nanoparticles prepared by temperature modulated solidification technique

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A Thesis

entitled

Formulation, Characterization and Evaluation of Paclitaxel loaded Solid Lipid Nanoparticles Prepared by Temperature Modulated Solidification Technique.

by

Ameya Abhay Deshpande

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

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August 2015

An Abstract of

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The aim of this research was to formulate, characterize, and evaluate the paclitaxel loaded solid lipid nanoparticles (SLNs) prepared by a temperature modulated solidification technique developed and optimized in our laboratory. The particle size analysis through dynamic light scattering (DLS) and transmission electron microscopy (TEM) revealed and confirmed the spherical shape and nanometer size range of the formulated nanoparticles. Zeta potential measurements confirmed the physical stability of the SLNs with a negative surface charge. Atomic force microscopy (AFM) studies were done to study the surface topography and particle size and shape. AFM data showed minimal aggregation and more or less spherical SLNs. Differential scanning calorimetry (DSC), powder X-ray diffraction (P-XRD) and Fourier transform infrared spectroscopy (ATR-FTIR) confirmed the conversion of bulk lipid into SLNs and high entrapment of paclitaxel into the lipid matrix. The optimized formulation had an entrapment efficiency of approximately 62%. The in-vitro drug release depicted a sustained release of paclitaxel from the SLNs over duration of one week. The drug release data was found to best fit and hence followed the Higuchi drug-release model.

Acknowledgements

I would like to express my special appreciation and thanks to my major advisor and committee chair Dr. Jerry Nesamony, you have been a tremendous mentor for me. I would like to thank you for encouraging my research and for allowing me to grow as a research scientist. Your advice on both research as well as on my career have been priceless. I would also like to thank my committee members, Dr. Sai Hanuman Sagar Boddu and Dr. Caren Steinmiller for serving as my committee members. I thank Dr. Kenneth Alexander and Dr. Gabriella Baki for all the support and valuable advice during these years. I also thank Dr. Zahoor Shah for agreeing to be graduate faculty representative. I thank Dr. Joseph Lawrence, for helping me with TEM studies. I thank Dr. Pannee Burckel for helping me with XRD. I would also like to thank Dr. Steven Gollmer and Dr. Elisha Injeti for their help in atomic force microscopy. I especially thank Dr. Wayne Hoss, for providing me financial support throughout the program.

A special thanks to my family. Words cannot express how grateful I am to my parents, and my brother for all of the sacrifices that you've made on my behalf. Your prayer for me is what has sustained me this far. I would also like to thank all of my friends who supported me during the research and writing, and incented me to strive towards my goal. At the end I would like to express appreciation to my friends and family here in Toledo for the precious memories over these years.

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GMS.....Glyceryl monostearate.

PCTL.....Paclitaxel.

S.D. .....Standard deviation.
S.E. .....Standard error.

Chapter 1

Solid Lipid Nanoparticles as a Drug Delivery System

1. Introduction

The *in-vivo* fate of a drug depends on the drug delivery system in which it is incorporated [1]. Lipid based formulations were first commercialized in the 1950s. Intralipid was introduced as a safe fat emulsion for parenteral nutrition followed by Diazemuls, Diazepam-Lipuro and Etomidate-Lipuro which were subsequently introduced into the market. The potential reason behind the popularity of lipid delivery systems is the reduced pain and inflammation at the site of injection [2]. A particular advantage offered by lipid colloidal carriers is the increase in bioavailability of poorly water-soluble drugs. Melt-emulsified nanoparticles based on lipids that are solid at room temperature offers several advantages when compared to nano-emulsions, nano-suspensions, mixed micelles, liposomes and polymeric nanoparticles. Even though polymeric nanoparticles have proven to be excellent drug carriers there are few associated limitations such as inclusion of residues from organic solvents used during formulation, toxicity of the polymer, and production scale up. In contrast, the solid lipid nanoparticles offer the following advantages.

1. Broad spectrum of route of administration (dermal, intravenous, etc.).

2. Good physical stability.
3. Protection from degradation of incorporated labile drugs.
4. Modulated (fast or sustained) release of the drug.
5. Targeted drug delivery.
6. No use of organic solvents during preparation.
7. Ease of scale up.
8. Excellent biocompatibility.
9. No need of special solvents.
10. Conventional emulsion production techniques can be used.
11. Raw materials same as used in the production of emulsions can be used.
12. Can be sterilized by commercial sterilization methods [3-5].

Solid lipid nanoparticles also have some disadvantages as follows:

1. Particle growth.
2. Gelation tendency.
3. Unexpected polymorphic transitions.
4. Possibility of metal contamination.
5. High polydispersity.
6. Thermal degradation of heat labile drugs.
7. Presence of super-cooled melts [5, 6].

Solid lipid nanoparticles have a mean diameter as measured by photon correlation spectroscopy (PCS) ranging from 50 to 1000 nm and are made of solid lipids. SLNs may be obtained from emulsions that are used for parenteral administration by replacing the

lipids in the liquid state by lipids in the solid state. SLNs are normally stabilized physically using surfactants. The major advantage that makes SLNs unique compared to polymeric nanoparticles, is that they can be produced/manufactured using high-pressure homogenization techniques used industrially for preparing emulsions. The emulsion production is generally equipped with temperature control units since elevated temperature sometimes favor emulsion production, which is equally applicable for producing SLNs by the hot homogenization technique [7]. The drug loading capacity of SLNs depends on certain parameters such as molecular weight of the drug, solubility of the drug in the lipid, hydrophobicity of the drug, the lipid matrix structure, stability of the drug and the polymorphic state of the lipid matrix [4, 8, 9]. Use of complex lipids results in better drug entrapment, as the incorporated drugs are located within the fatty acid chains, crystal imperfections and also in between lipid layers. Large amounts of drug cannot be accommodated by the highly ordered crystal lattices. Drug expulsion may result during transition to highly ordered lipid particles. Lipids crystalize with higher energy modifications (α and β) directly after the nanoparticles are formed. This leads to more imperfections in the crystal lattice of lipids thereby facilitating larger mounts of drug entrapment [10]. A triggered and controlled release of the drug is exhibited if the α-modification is preserved during storage and transformed (e.g. due to change in temperature). But, if the polymorphic transition leads to a β –modification, the drug will be discharged from the matrix of the lipid which may further lead to degradation of the drug and uncontrolled release of the drug [4].

1.1 Stability of Solid Lipid Nanoparticles:

1.1.1 Physical Stability:

Various parameters have been utilized to investigate the stability of SLNs such as particle size (using photon correlation spectroscopy, PCS; laser diffraction, LD), zeta potential and thermal analysis. Low viscosity of the dispersed liquid phase and high specific surface area of colloidal dispersions leads to rapid release of the drug from the droplets. The drug mobility is decreased in the solid physical state of the SLNs, which subsequently reduces drug diffusion out of the formulation thereby reducing the hydrolytic degradation. Destabilization of the colloid caused by the drug is reduced in SLNs due to drug immobilization in the dispersed solid lipid. Earlier, the melt-emulsification technique was used in an attempt to formulate saturated long-chain triglycerides with high melting temperatures into phospholipid stabilized aqueous suspensions but proved to be unsuccessful due to instability. Several aqueous SLN dispersions have been reported to be generally physically stable for more than one year [4, 11].

Various factors are found to affect the particle size of the SLNs. These factors are as follows; the excipient composition, the drug composition, the type of formulation process, temperature, sterilization process, the type of dispersing medium, freeze drying process, presence of stealth agent, storage conditions and the lipid composition. All these parameters are related to lipid crystallization processes that are ultimately responsible for particle growth [12].

Müller et al. demonstrated that the SLNs made from glyceryl palmitostearate or tribehenate remained stable for up to three years as determined by PCS with the average diameter of the major SLN population ranging from 160 to 220 nm [4].

The aqueous SLN dispersions are generally stable for up to three years, but some systems have shown an increase in particle size subsequently leading to gelation. Freitas and Müller conducted a study to investigate the factors responsible for the destabilization of the SLNs [13]. For this purpose they formulated a poloxamer 188 stabilized Compritol® SLN formulation. Its stability was tested as a function of three parameters viz. storage temperature, light exposure and packing material (untreated and siliconized vials of glass quality I). They found that the energy introduction lead to increase in the particle size and gelation. Also, there was a reduction in the zeta potential from approximately -25 mV to -15 mV. The packing material did not impose any significant effects. They came to a conclusion that by optimizing the storage conditions, a stability of three years could be achieved for the less stable aqueous Compritol® SLNs [13]. In another study, Freitas and Müller investigated the mechanism of gelation of aqueous dispersions of the SLNs [14]. They exposed Compritol® SLNs to different temperatures, packing materials and varying light exposure. Also, the SLNs were subjected to stress by shear forces for short-term tests and a long-term study of three years. Thermal analysis and particle size analysis were used as the tools of analyses. They found that after SLN production by hot homogenization of the melted lipid, the Compritol® SLNs crystallize in a mixture of stable β′ with unstable polymorphs (α, sub α). There was a significant increase in the recrystallization index due to light, temperature and shear forces because of transformation of the lipid to β’ modification leading to formation of a gel. SLNs that

have a mixture of polymorphic modifications are physically stable and an increase in their crystallinity index is slow when compared to SLNs containing single polymorph during storage [14].

Shahgaldian et al. investigated the stability of para-acyl-calix[4]arene based SLNs [15]. The concentrations of the organic solvent, surfactant, and the presence of a co-surfactant in the organic phase significantly affected the particle size of the SLNs produced by solvent displacement method. However, parameters such as the stirring speed, viscosity, pH of the aqueous phase, and hydrophobic chain length of the surfactant did not affect the particle size or formulation stability. They also found that the ionic strength of certain salts has a considerable effect on the stability of SLNs. Amongst the salts used to test the stability, sodium sulfate caused complete precipitation indicating destabilization of SLNs [15].

SLNs intended for intravenous administration have to meet stringent requirements. Particle of size larger than 5 μm may cause embolism subsequently leading to death. It is very important that there is no occurrence of aggregation and particle growth during storage. A potential solution to this issue is lyophilization. Lyophilized SLNs upon reconstitution demonstrated desirable qualities of intravenously injectable drugs. Lyophilization has also been proven to stabilize SLN formulations incorporated with hydrophilic drugs [16]. A slight increase in the SLN particle size has been reported after lyophilization using trehalose [17]. Trehalose is the most widely used cryoprotectant during lyophilization. The optimized ratios for its use are, trehalose to lipid ratio of 3:9 for neutral SLNs and a ratio of 2:6 for negatively charged SLNs. Trehalose was also

reported to be effective in preventing the expulsion of drug from SLNs upon reconstitution [4, 18].

Almeida et al. showed that certain peptides could be successfully incorporated in the lipid matrix of the SLNs [19]. They incorporated lysozyme in the SLNs through a cold homogenization technique. They monitored the influence of certain formulation parameters such as type of lipid, time of exposure to a range of temperatures, pressure and number of homogenization cycles on the integrity and activity of the lysozyme. It was found that these formulation parameters did not have a significant impact on the enzyme. The SLN incorporated lysozyme remained intact throughout the preparation process without loosing its activity, which was confirmed, by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method and the lysis rate of the *Micrococcus lysodeikticus*. They concluded that some proteins could sustain the stress during high-pressure homogenization thereby supporting the notion that SLNs can potentially be used as carriers of antigens for vaccine delivery [19].

In a SLNs suspension, the smaller particles can dissolve preferentially when compared to large particles in the suspension medium. The dissolved lipid then deposits on the larger particulate surfaces thereby resulting in an growth in the particle size of the larger particles at the expense of the smaller particles. This phenomenon is called Ostwald ripening and is also seen in emulsions causing an increase in the droplet size of the dispersed phase. SLNs are in general resistant to coalescence but are prone to creaming or gelling due to particle collision [12].

1.1.2 Chemical Stability:

Polymeric nanoparticles can cause chemical instability problems for incorporated drugs due to catalyst residues and molecular non-homogeneity. This, among other reasons, has limited commercialization of nanoparticulate systems. When compared to polymeric nanoparticles, SLNs protect the incorporated drugs from chemical degradation mediated through the liquid medium in which it is prepared [20]. Retinol is a drug, which is chemically unstable in aqueous media but demonstrates good stability in lipids. Volkhard et al. incorporated retinol in SLNs and found that the incorporated retinol demonstrated good chemical stability as the lipid matrix protected it against the surrounding aqueous environment [21].

Surfactants play an important role in the chemical stabilization of the incorporated drug that accumulates on the surface or outer shell of the SLNs. The surfactant choice and its concentration determines the chemical stability of the SLNs in water because it influences the solubility of the drug in aqueous phase and the surface characteristics of the SLNs.

Storage temperatures have a considerable influence on the chemical stability of the SLNs. Generally high temperatures promote degradation of the drug when compared to those stored at room temperature or low temperatures. This is because, at high temperatures there is reduced crystallization of the SLNs thereby increasing the mobility of the drug in the lipid matrix [20].

It has been proven that electrolytes have a profound effect on the stability of drug containing aqueous SLN dispersions. Electrolytes may lead to instability by reducing the zeta potential ultimately leading to gelation and promoting the formation of stable lipid

polymorph. Physically critical SLNs that are prone to instability may be stabilized by the controlled inhibition of the polymorphic modification [22].

The chemical stability of the lipid carriers, particularly oxidation, depends on the structure and composition of the lipids. Antioxidants can be added to prevent the oxidation of the lipids. Examples of antioxidants include α-tocopherol and butyl hydroxy toluene.

Chemical stability of lipids is also dependent on the pH of the dispersion medium. Lipids are observed to have maximum stability and minimum hydrolysis rate at the pH of 6.5 [12, 23].

The simplest and the most important way to minimize hydrolysis is the elimination of the water from the formulation. Either spray drying or lyophilization may achieve the elimination of water from the SLN formulation. The obtained solid powder may then be used by reconstitution having the same particle size distribution as the original [24].

It is observed that the exposure to light reduces the zeta potential of the SLNs, ultimately leading to gelation. Freitas and Müller found that a SLN dispersion gelled when it was exposed to artificial light when stored in white glass leading to the conclusion that light has a destabilizing effect on the SLNs [13]. It has also been proven that the high-energy radiations (UV, short wavelength radiations, etc.) further decrease the stability of the SLN suspension [13].

Optimizing the process parameters such as freezing velocity, the re-dispersion medium used, etc. can minimize particle size changes caused by the lyophilization process. Increase in the drying time does not have considerable beneficial effects on the formulation. It has also been found that the cryo-protector has a stabilizing effect on the

freeze-dried SLNs, when re-dispersed. Removal of protonated species before lyophilization from the dispersion medium has proved to be beneficial in terms of stability [16, 24, 25].

As an alternative to lyophilization, spray-drying may be used to convert the SLN dispersion into a dried product having long-term stability. However, destabilization may occur due to processing parameters such as shear forces and temperature. Also, it is very important to optimize the re-dispersion properties. Freitas and Müller investigated these parameters on few SLN dispersions [26]. They found that, on addition of carbohydrates, the sugar formed a layer around the particles thereby preventing the coalescence of the molten lipid droplets and also protected the emulsifier film against removing off from the surface thereby reducing the effect of shear forces [26].

1.2 Drug Incorporation and Loading Capacity of Solid Lipid Nanoparticles:

Various drugs have been investigated as potential agents for incorporation into SLNs. The examples include timolol [27], deoxycorticosterone [28], doxorubicin [29], idarubicin [29], pilocarpine [30], thymopentin [31], diazepam [32], paclitaxel [25], retinol [33], acyclovir [34, 35], tetracaine [35], etomidate [36], cyclosporin [37], azido thymidine palmitate [38], oxazepam [11], diazepam [11], cortisone betamethasone valerate [11], camptothecin [39] and piribedil [40].

Drug loading capacity is an important parameter to investigate the suitability of a drug carrier system. The drug loading capacity is related to the nature of the lipid and is generally expressed in terms of percentage. The drug loading capacity varies depending on the drug incorporated in the SLNs. Drug loading capacities ranging from 1% to 50%

have been reported. 5- Florouracil was found to be encapsulated 46% in SLNs prepared by novel temperature modulated solidification technique [6].

Factors that determine the drug loading capacity of SLNs are as follows:

1. Drug solubility in the lipid melt.
2. Drug melt and lipid melt miscibility.
3. Structure of the lipid matrix,
4. Polymorphic state of the lipid [7].

However, it has been observed that the drug loading capacities of lipid nano-carriers are comparatively lower due to low solubilization capacities of the molten lipids for the hydrophobic drug entities. Super-cooled melts have found to have higher drug-loading capacities when compared to crystallized nanoparticles. Hard fats have higher drug-loading capacities due to their crystalline nature when compared to pure monoacid triglycerides[11].

The drug solubility reduces when the lipid melt is cooled down. Hence, the solubility of the drug in the lipid should be higher than required. The drug solubility is enhanced in the presence of mono- or di-glycerides and also by addition of solubilizers. A good drug loading capacity is obtained when polydisperse lipids that are generally used in cosmetics, are used in SLNs.

The chemical nature of the lipid is a key-determining factor of drug loading capacity in SLNs. Determination of crystallinity of the lipids and other excipients may prove to be beneficial for predicting the drug entrapment. Optimized drug incorporation and physical characterization of the lipids and other excipients using analytical techniques such as

nuclear magnetic resonance, powder X-ray diffraction, differential scanning calorimetry, fourier transform infrared spectroscopy, etc. are required [7].

The polymorphic form of the lipid is a major determinant of the drug loading capacity. The polymorphic form in which a lipid is present differs depending on whether it is in the bulk form or if it is in the form of nanoparticles. It has been found that the lipid, when present as nanoparticles, recrystallizes at least partially if not fully into α-form. In-contrast, the bulk lipid is found to recrystallize into β-form. The formation of the β-form results in expulsion of the drug from the lipid matrix. This transformation is slower for long-chain triglycerides than short chain triglycerides. Dispersed lipids are found to recrystallize in α-form whereas the bulk lipids are found to recrystallize in β′-modification followed by transformation into the β-form if heated above the bulk melting temperature and then cooling under controlled conditions.

SLNs may be optimized in a way to produce and maintain some fraction of α-form to achieve better entrapment and controlled release of the drug[7, 41-44].

1.3 Drug Release from Solid Lipid Nanoparticles:

The release of the entrapped drug from the SLNs is governed by the following principles,

1. An inverse relationship exists between the release of the drug and the partition coefficient of the drug.
2. Smaller particle size promotes higher surface area thereby leading to higher drug release.
3. Homogeneous dispersion of the drug in the lipid matrix causes slow release of the drug.

4. Lipid crystallinity and high drug mobility leads to rapid release of the drug from the SLNs [45].

Numerous studies have been carried out on the effect of formulation parameters and process conditions on the release of drug from the SLNs.

SLNs incorporated with tetracaine and etomidate demonstrated burst release with 100% release of the drug in less than 1 minute. This release pattern was attributed to the large surface area of nanoparticles and higher percentage of drug in the outer layer of the nanoparticles. However, a prolonged drug release pattern was observed for lipid soluble prednisolone loaded SLNs. Hence, it was concluded that SLNs incorporated with lipophilic drugs follows prolonged release patterns [46].

In another investigation, Jenning et al. compared the drug release pattern of the SLNs with the drug release pattern of nanoemulsions [47]. Retinol was incorporated into the SLNs and nanoemulsions. Retinol is highly unstable in water and degrades within few days at room temperature. As the vitamin A has very low aqueous solubility even in the presence of surfactants, it was assumed that the detected amount of vitamin A was present in the lipid phase of the SLNs or Nano-emulsion. They found two different types of drug release profiles for SLNs and nanoemulsions. For the initial 6 h only one-third fraction of the drug was released from the SLNs compared to that released from nanoemulsion. However, for the fractions collected after 18 and 24 h, the drug release rate of SLNs increased and even exceeded the release rate of nanoemulsion which was attributed to the evaporation of water from SLNs during the experiment and subsequent formation of gel leading to faster release of drug [47].

The parameters that affect the release of drug from SLNs are temperature, amount of drug incorporated, lipid structure, drug structure, duration of production, processing equipment, lyophilization process, sterilization process [45]

Among these, the two major parameters that influence the release of drug from the SLNs are temperature and presence of a surfactant.

1.3.1 Influence of Temperature on Release of Drug from SLNs:

The release profile of SLNs generally follows a biphasic pattern. A burst release is observed initially followed by a prolonged release. Drug release investigations have proven that highest burst release is observed at highest temperatures of production and also if hot homogenization is used as the method of production. Also, burst release is found to decrease with decreasing production temperature and is negligible with cold homogenization technique. The use of high temperature facilitates solubility of drug in the aqueous phase. Hence, use of lower production temperatures may eliminate burst release of the drug [45, 46].

1.3.2 Influence of Surfactants on Release of Drug from SLNs:

The amount of surfactant or surfactant mixture in the formulation also influences the burst release of the drug from SLNs. It has been found that at high surfactant concentrations the burst release of the drug is higher, while at lower concentrations of surfactants or surfactant mixtures the burst release is lower. This phenomenon is supported by the hot homogenization process in which redistribution of the drug occurs between the lipid and the aqueous phase during the heating process followed by a subsequent cooling process. As the dispersion of lipid and water is heated, the drug travels from melted lipid droplet to the aqueous phase. Then as the oil/water emulsion is

being cooled, the solubility of the drug in the water continuously decreases with a decrease in the temperature leading to repartitioning of the drug into the lipid phase. A formation of the solid lipid core including the drug starts at the recrystallization temperature of the lipid. As the temperature further decreases the pressure on the drug increases to repartition into the lipid phase because of the decrease in the solubility of the drug in water. But, as the lipid core is crystallized, the drug cannot incorporate into the lipid core [7]. This leads to supersaturation of the aqueous phase with the drug and results in formation of outer liquid layer of SLN enriched with the drug. Therefore, hydrophilic drugs are observed to have higher burst release [6, 46]. Use of surfactants facilitates dissolution of drug in the aqueous phase. Hence, SLNs having low surfactant concentration or no surfactant may eliminate burst release [45, 46].

1.3.3 Controlled Release of Actives from SLNs:

It has been found that there is a reduction in burst release with an increase in the particle size of the SLNs. Also, with a little larger particle sizes, prolonged release could be obtained (i.e. micro-particles). Homogenization process has a considerable influence on the release of drug. The drug release profiles may be altered depending on the production technique (i.e. hot/cold homogenization) of SLNs and type of lipid used. The sustained release of drug from SLNs may be explained based on the concept of molecular distribution of the drug in the lipid according to the solid solution model. The elimination of burst release with cold-homogenization process may be attributed to the homogeneous molecular distribution of the drug in the solid lipid matrix which results in the formation of a solid dispersion prior to homogenization and particle formation process. Because the

SLNs are solids at room temperature, the mobility of drug is highly reduced thereby leading to controlled release of drug [20].

1.4 Sterilization of Solid Lipid Nanoparticles.

All drug loaded nano-particulate formulations share the requirement of the solvent being sterile, non-pyrogenic, safe, non-toxic and non-irritating for both in-vivo and in-vitro uses [9]. Sterility refers to the absence of all viable microorganisms including viruses. Currently, a sterility assurance level (SAL) of 10^{-6} is acceptable in pharmaceuticals [48]. The final product quality of a pharmaceutical is dependent upon the microbiological attributes of the pharmaceutical ingredients. The presence of microorganisms in pharmaceutical products may reduce their efficacy. A high level of sterility is maintained and controlled for the products intended for parenteral and ophthalmic use. Various other products to be applied on mucus surfaces or deep open wounds, which are liable to microbial contaminations, also have sterility requirements [49]. Raw materials, process equipment, facility, personnel, etc. are the potential sources of microbial contamination in pharmaceutical products. SLNs are primarily used in parenteral delivery due to the nanometer size range. Various sterilization techniques that are currently available may have adverse effects on the physicochemical characteristics of the SLNs leading to toxicity and efficacy concerns for SLNs. The different methodologies that potentially could be utilized for the sterilization of SLNs are discussed in the subsequent sections [9].

1.4.1 Autoclaving:

Autoclaving is a technique in which, high temperature (121°C) at high pressure in the form of steam is utilized for a duration of 15-20 minutes to kill the microbes [9].

Autoclaving is the most common technique used for sterilization. This technique holds very well for heat stable drugs but cannot be used for heat labile drugs that degrade due to heat [50]. Schwarz et al. investigated the effects of autoclaving on the SLNs containing sterically stabilizing poloxamer [51]. They found that, it is not possible to sterilize the nanoparticles because of the reduced steric stabilization at the high temperatures of autoclaving [51]. Cavalli et al. tested the effects of autoclaving on the size and polydispersity of the nanoparticles [32]. They prepared the SLNs from three oil in water microemulsions. The internal phase constituted of different lipid matrices. The lipids used were 7.53% w/w of stearic acid, behenic acid or Acidan N12. They concluded that, the SLNs were stable during the sterilization process and they remained in a spherical shape even after the autoclaving. They found that there was a slight change in the size of the blank and drug loaded nanoparticles. The change in polydispersity values was very slight. Hence, they concluded that SLNs maintained the narrow size distribution through out the investigation period [32].

SLNs are more stable during autoclaving when compared to metallic and polymeric nanoparticles [9]. No toxicity was observed on rabbit corneal epithelial cells with cyclosporine A loaded SLNs when autoclaved at 110°C whereas there was a significant cell viability drop when autoclaved at 120°C [52]. The possible reason for this phenomenon was attributed to the release of Tween 80 from formulations at a high temperature of 120°C as similar effects were observed when the cells were treated with Tween 80 alone. The autoclaving was found to have a positive effect on the physical stability of SLNs as it increased the zeta potential thereby preventing particle-particle aggregation [52].

In conclusion, most of the SLNs are found to increase in size after autoclaving possibly due to coalescence of the lipid particles at high temperature but the biological effects remain unaltered.[9, 52]

1.4.2 Filtration:

Filtration is an alternative for heat labile drugs that cannot sustain sterilization by autoclaving. Sterile filtration can be used only if the mean particle size of the nanoparticles to be sterilized is below 200 nm. If the majority of the particles are of size 200 nm or more, it may clog the filter quickly making it impossible to filter any more dispersion [51]. Nylon66 UTTIPORR 0.45mm membrane having critical water surface tension value >80dynes/cm may be preferentially used for SLNs. Such membranes can be manufactured by treating nylon66 membrane with acrylates possessing –OH groups in their chemical structure [53]. As sterile filtration does not involve use of any chemicals and as there is no use of any harsh condition such as high temperature or pressure, the use of this technique may be extended for solid lipid nanoparticles provided their particle diameter range is below 200 nm [51, 53, 54].

1.4.3 Irradiation:

Presently, radiation is possibly the best and most convenient method to sterilize solid lipid nanoparticles [55, 56]. Various radiations may be used for the sterilization purposes such as gamma irradiation (γ), electron beam, X-rays, UV light irradiation or use of subatomic particles. Gamma rays are generated by the self-disintegration of Cobalt-60 or Cesium-137 and are most widely used for sterilization because of their high penetration ability. Irradiation is inert to chemicals and heat, and does not leave any residue and hence is considered to be the best sterilization technique [9]. In an experiment, stavudine

loaded bare and surface modified SLNs were subjected to terminal sterilization by gamma irradiation at increasing levels of 5, 15, and 25 kGy [55]. The impact of irradiation was studied on various parameters of SLNs. It was found that the drug content of the SLNs was not affected by irradiation at any level. No significant change in the particle size of the lyophilized SLNs was observed, even at the high radiation doses. There was no significant change in color and odor of the surface modified SLNs either [55]. Unfortunately, studies on radiation induced chemical changes have not been performed by most investigators. We know that degradation is not always characterized by change in particle size but may involve formation of lysophosphatides or fatty acids which preserve the particle size but might result in toxicity such as hemolysis. Hence, it is important to investigate the chemical aspects of irradiation sterilization on SLNs [57].

1.4.4 Other Methods:

Other methods of sterilization such as treatment with formaldehyde and ethylene oxide have been investigated for sterilization of SLNs [9]. Formaldehyde sterilization may be used for the treatment of formulations containing heat labile active ingredients. But the method has the limitations of toxicity and carcinogenicity and hence is rarely used [58]. Medical devices are routinely sterilized by treatment with ethylene oxide. Being a strong alkylating agent, ethylene oxide has a strong denaturing effect on the nucleic acids and functional groups of micro-organisms and hence acts as a strong sterilizing agent [59]. However, ethylene oxide is flammable, explosive and also toxic and carcinogenic [58, 59]. Because of the above-mentioned disadvantages, these methods have not been possibly investigated intensively for the sterilization of lipid nanoparticles [9].

1.5 Routes of Administration for SLNs.

1.5.1 Oral Administration:

SLNs may be administered orally after transformation into a traditional oral dosage form such as tablets, pellets, capsules or powders. SLN dispersion can be used in place of a granulation fluid during wet granulation processes. SLNs can be directly tableted if the dispersion is transformed to powder form by spray drying or lyophilization. Dry SLN powder may be filled into hard gelatin capsules or SLNs may be directly produced in liquid polyethylene glycol 600 and filled into soft gelatin capsule. Also, SLNs may be directly commercialized as dry powders in sachet after spray drying or lyophilization since spray drying was found to be a more cost effective method [7].

Various researchers have investigated SLNs for oral delivery of a variety of active compounds [60-64]. Cho et al. formulated SLNs incorporated with docetaxel which has poor oral bioavailability [60]. SLNs were surface modified with tween 80 or D-alpha-tocopheryl poly(ethylene glycol 1000) succinate. The results indicated an increase in the oral bioavailability of docetaxel and thus SLNs may serve as an efficient oral drug delivery system for docetaxel [60].

SLNs containing insulin were prepared and evaluated by Sarmento et al for oral delivery [61]. The SLNs were based on cetyl palmitate and were produced by a modified solvent emulsification-evaporation technique based on water/oil/water double emulsion. Oral administration of the formulated insulin loaded SLNs to diabetic rats provided a considerable hypoglycemic effect during 24 hours indicating SLNs to be potential oral delivery system for insulin [61].

1.5.2 Parenteral administration:

Drugs in the form of proteins and peptides are commercially available in parenteral dosage forms in the market. These are prone to enzymatic degradation and hence are not administered orally. To avoid frequent administration and to increase patient adherence, development of a controlled release parenteral formulation based on SLNs can provide an effective therapy [45]. The application of SLNs in parenteral delivery ranges from intra-articular delivery route to intravenous administration [7]. Various researchers have performed investigations on intravenous performance of SLNs [65-68]. Zara et al. investigated the effect of stealth and non-stealth SLNs incorporated with doxorubicin following intravenous administration [65]. The pharmacokinetics and tissue distribution of doxorubicin incorporated in SLNs were compared with that of the commercial doxorubicin solution. The formulations were administered intravenously to conscious rabbits. Brain distribution of doxorubicin was obtained only with SLNs. The use of stealth agent facilitated the distribution of doxorubicin to the brain. SLNs were found to significantly reduce the heart and liver distributions of doxorubicin [65].

Manjunath et al. assessed the bioavailability of nitrendipine loaded SLNs following intravenous and intraduodenal administration to rats [67]. Pharmacokinetic studies were performed following intravenous and intraduodenal administration of nitrendipine loaded SLNs to conscious male Wistar rats while the tissue distribution studies were carried out in Swiss albino mice after intravenous administration of nitrendipine loaded SLNs and was compared to nitrendipine suspension. An increase in AUC and decrease in clearance was observed with nitrendipine loaded SLNs when compared to the nitrendipine suspension. The effective bioavailability of the nitrendipine SLN was found to be higher

after intraduodenal administration when compared to nitrendipine suspension. Hence, they found SLNs to be a suitable carrier system for improvement of bioavailability of nitrendipine [67].

1.5.3 Topical Application:

SLN dispersions with low lipid content (5% and less) are found to have small particle sizes. Higher viscosity and lipid concentrations are beneficial for dermal application. Hence, in most of the cases, it is necessary to incorporate the SLNs in an ointment or gel to obtain a formulation that could be applied to the skin. High lipid concentrations in SLNs may result in a semisolid form during SLN preparation that may be further utilized as a topical formulation. However, this approach can lead to an increase in particle size [1]. Numerous studies have been performed on topical application of SLNs [69-73]. Lippacher et al. produced semisolid SLN dispersion by high-pressure homogenization technique in a one step process [69]. They were successful in obtaining SLNs in colloidal size range even with high volume concentration of dispersed lipids. The obtained formulation had viscoelastic properties similar to standard dermal formulations, which was proven by elastic responses obtained from oscillatory rheology experiments. The formulation demonstrated good particle size stability and gel structure stability [69].
In another investigation, Khalil et al. formulated SLNs incorporated with meloxicam [72]. High shear homogenization and ultrasonication technique were used for the production of SLNs. They investigated the influence of different formulation compositions i.e. lipid composition, concentration of lipid type and their types along with surfactant concentration on drug release and physicochemical properties of SLNs. The

formulated SLNs were found to have spherical shape, high entrapment efficiency, good stability and sustained drug release up to 48 hours [72].

1.5.4 Pulmonary Administration:

Various researchers have investigated the utility of SLNs in pulmonary delivery [74-76]. Varshosaz et al. studied the biodistribution of amikacin loaded SLNs following pulmonary delivery. This investigation was attempted to increase the concentration of amikacin for the treatment of cystic fibrosis lung infections. Drug loaded cholesterol SLNs and free drug were administered to male rats through pulmonary and intravenous delivery routes respectively. From the obtained results, it was found that the pulmonary delivery reduced the drug side effects in kidneys and also prolonged the drug dosing interval since the drug was released in a sustained manner thereby improving patient adherence [74].

Sildenafil loaded SLNs were developed for the treatment of pulmonary arterial hypertension by Paranjpe et al [76]. They used phospholipids and triglycerides as the lipids and microchannel homogenization technique for production of SLNs. From the wide angle X-ray diffraction and differential scanning calorimetric studies it was found that polymorphic transitions occurred during SLN preparation thereby converting intermediate β' to stable β form. The particle size analyses revealed that the blank SLNs maintained consistent particle sizes over a period of 6 months when compared to sildenafil loaded SLNs. The drug loaded SLNs showed increase in particle size after manufacturing and further increase within weeks of storage. Particle size was found to change as a function of emulsifier concentration after nebulization and redispersion of the SLNs. Particle sizes were smaller than 1 μm throughout [76].

1.5.5 Rectal Administration:

Rectal administration is frequently used for pediatric patients. Parenteral and rectal routes of administration are preferred when quick therapeutic response is required. Rectally administered drugs are found to achieve better plasma levels and therapeutic effectiveness when compared to orally or intramuscularly administered drugs of similar dose [77].

Sznitowska et al. investigated the rectal administration of three different formulations of diazepam which includes organic-aqueous diazepam rectal solution (containing ethanol, benzyl alcohol and propylene glycol), submicron emulsion, and SLNs [78]. The submicron emulsion was prepared with 20% w/w MCT oil, egg lecithin and poloxamer while the SLNs were prepared with 10% w/v cetyl palmitate and Plantacare 2000 (alkyl glucoside) was used as a non-ionic surfactant. 4 mg/ml of diazepam was incorporated in all the formulations and 2 mg/kg of dose was administered to the rabbits. The submicron emulsion was found to have similar pharmacokinetics as the solution. However, the relative bioavailability of SLNs was found to be as low as 47% and hence they concluded that SLNs are not a potential carrier system for the rectal administration of diazepam [78]

1.5.6 Ocular Administration:

SLNs have shown to improve interaction with ocular mucosa due their biocompatible and mucoadhesive properties. Hence, SLNs prolong the drug duration in the cornea [20, 45]. Cavalli et al. evaluated SLNs as drug delivery system for topical ocular administration of the drug tobramycin [79]. The formulated SLNs had average diameter below 100 nm and a polydispersity index below 0.2. Longer retention times on the corneal surface and in the conjunctival sac were obtained with drug free, fluorescent SLNs compared to an aqueous

fluorescent solution. Tobramycin SLNs were found to produce significantly higher tobramycin bioavailability in the aqueous humor of rabbits that were topically administered with tobramycin SLNs containing 0.3% tobramycin when compared to an equal dose of tobramycin administered by standard commercial eye drops. Hence, it was concluded that SLNs are a promising drug delivery system for the ocular administration of tobramycin [79].

1.6 Solid Lipid Nanoparticles as Potential Carriers of Anticancer Agents.

Lipid nanoparticles have been used to incorporate various anticancer agents. These have been evaluated adequately for their *in-vitro* and *in-vivo* efficacies. SLNs have demonstrated to have lesser side effects with an increase in the efficacy and residence time of cytotoxic drugs [80]. Use of chemotherapy for the treatment of solid tumors has faced challenges and the outcomes mostly remain unsatisfactory. The response rates of chemotherapy for pancreatic cancer, esophageal cancer and ovarian cancer have found to be as low as 20% [81]. Presently, more than 85% of human cancers are solid tumors [82]. Cytotoxic drugs that are administered conventionally are highly unpredictable as they are found to bind extensively to body tissues and serum proteins [81]. Delivery of the active ingredient specifically to tumor cells is very important for appropriate therapeutic activity. If the delivery of drugs to the tumor were inadequate, it would lead to regrowth of the tumor cells and may even cause development of resistant cells. Cytotoxic drugs pose toxicity even to the non-target cells, especially, rapidly dividing cells of bone marrow and gastrointestinal tract [81, 83]. Use of chemotherapeutic agents results in many side effects due their poor specificity and the side effects may be acute or chronic. They often lead to inconvenience and discomfort and in some cases may even cause

death. The frequent side effects caused by the chemotherapy are alopecia, vomiting, depression, anemia, mouth sores, nausea and vomiting, thrombocytopenia, neutropenia, etc [83, 84].

A significant portion of the new cancer cases found every year are drug resistant. Drug resistance occurs because either the cancers are inherently untreatable or are resistant to the broad spectrum of anticancer agents and their combinations. If particular tumor cells exhibit simultaneous resistance to a number of chemically and functionally different chemotherapeutic agents they are said to be multi drug resistant (MDR) [85]. Solid tumor cells are found to have more drug permeation barriers which prevents the drug to attain adequate concentration inside the tumor cells and hence cancer cells prove to be more resistant to chemotherapy than normal cells [81].

Newer agents such as monoclonal antibodies, cytokines, viral/non-viral gene vectors, genetically engineered cells, etc. have been developed for the delivery of anticancer drugs to the tumors. These agents being large in size pose a challenge to deliver to the tumor cells [82].

1.6.1 Significance of SLNs as Anticancer Carriers:

SLNs are found to have "enhanced permeation and retention" (EPR) effect. Tumor tissues have pathophysiological and anatomical imperfections that are absent in the normal cells. Nanoparticles, proteins, polymer drug conjugates and other macromolecules accumulate selectively in these solid tumor cells. This phenomenon is used to target the tumor cells and is called the EPR effect [83, 86]. The vasculature of the tumor cells varies from that of the normal tissue cells. The tumor cells have irregular shaped, dilated, leaky and defective blood vessels. Also, they feature disorganized endothelial cells with large

fenestrations with the absence of or abnormality in the perivascular cells and the basement membrane or smooth-muscle layer. A wide lumen and poor lymphatic drainage are the representative features of tumor tissues. These characteristics enable extensive passage of blood plasma components like macromolecules, nanoparticles and lipid particles into the tumor tissue. These macromolecules are retained in the tumor because of the slow venous return in the tumor tissue and its poor lymphatic clearance leading to EPR [87].

Cytotoxic anticancer agents are found to be heterogeneous. As there are different classes of compounds that act as anticancer agents, there is diversity in their molecular structure and physicochemical properties. Polymeric material may not bind to this diverse group of anticancer actives. However, SLNs are versatile and have the ability to incorporate these cytotoxic agents. The newer variations of SLNs such as polymer-lipid hybrid nanoparticles, and lipid-drug conjugate nanoparticles are capable of incorporating even hydrophilic drugs. A wide variety of lipids and hard fats can be utilized to prepare SLNs. Also, most of the emulsifiers approved by the drug regulatory agencies can be used to formulate SLNs [81].

Yang et al. performed a study to investigate the specific targeting of an anticancer drug camptothecin after incorporation into SLNs administered via intravenous administration and compared it with camptothecin solution [39]. They used high-pressure homogenization technique to formulate the SLNs. They were successful in producing SLNs with a mean diameter of 196.8 nm and a zeta potential of - 69.3 mV with prolonged in-vitro drug release of up to one week. Area under the curve and mean residence time of the camptothecin SLNs in the tested organs such as brain, heart and

reticuloendothelial cells containing organs were found to be much higher than the camptothecin solution. This date demonstrates SLNs potential as a sustained drug release and targeting drug delivery system [39].

Yu et al. used stearyl chloride to acylate 5-fluorouracil and produced N1-stearyl-5-fluorouracil to enhance liver targeting and reduce the side effects [88]. It was then incorporated into the SLNs prepared by the physical agglomeration method. The SLNs were found to have a diameter of 240.19 nm and drug loading of 20.53 %. A distribution study in mice showed that N1-stearyl-5-fluorouracil could double the 5-fluorouracil concentration in liver demonstrating good targeting properties [88].

Chen et al investigated two types of long-circulating SLNs as colloidal carriers of paclitaxel formulated with Brij78 and Poloxamer F68 respectively [89]. Pharmacokinetics of these was compared with the injection of Paclitaxel formulated in Cremophor EL in Kun Ming (KM) mice. It was found that the paclitaxel loaded SLNs had longer half lives compared to paclitaxel injection [89].

Koziara et al. used paclitaxel against gliomas and other brain metastases [90]. Paclitaxel being poorly permeable through blood brain barrier and due to serious side effects of the paclitaxel solvent Cremophor ELR, it was incorporated into cetyl alcohol/polysorbate nanoparticles. They used in situ rat brain perfusion model to evaluate the brain uptake of the nanoparticles. The drug brain uptake was found to increase significantly following entrapment of paclitaxel in nanoparticles [90]. Lee et al. developed a formulation of paclitaxel loaded SLNs intended for parenteral administration [91]. Hot homogenization technique was used as the method of production with trimyristin as a solid lipid core and egg phosphatidylcholine and PEGylated phospholipid as stabilizers. The particles were

around 200 nm in size and had a zeta potential of 38 mV suggesting its applicability for parenteral use. Cytotoxicities were tested on MCF-7 breast cancer cell line and OVCAR-3 human ovarian cancer cell line and was found to be comparable to those of a commercially available Cremophor EL-based paclitaxel formulation [91].

Tamoxifen loaded SLNs were investigated by Fontana et al against breast cancer [92]. The SLNs were prepared by microemulsion and precipitation techniques. The formulated SLNs were found to have dimensions suitable for parenteral administration. The SLNs in the intact form demonstrated a prolonged release of drug following in-vitro plasmatic drug release studies. Also, the in-vitro antiproliferative studies carried out on MCF-7, human breast cancer cell line, indicated an antitumoral activity comparable to the free drug. The results indicated SLNs to be a potential carrier system for the prolonged delivery of drug via intravenous administration [92]. SLNs can be used to actively target tumors at a specific site such as brain tumors by modifying the physicochemical properties of the surface thereby modifying the bio-distribution of the lipid nanoparticles [83, 93].

1.6.2 Incorporation of Hydrophilic Anticancer Actives in SLNs:

It is obvious that since lipids are the main constituent of SLNs, that lipophilic drugs are more efficiently incorporated into SLNs. This can be supported by the fact that irrespective of the technique used for the production of the SLNs, the drug needs to partition between the molten lipid and aqueous phase at some point of the production process to achieve a good entrapment efficiency. But, as mentioned earlier, there are quite a few anticancer drugs that are hydrophilic and ionic in nature. The examples of water-soluble anticancer drugs include 5-fluorouracil and mitomycin-C. Also, there are a

few lipophilic anticancer agents that are better utilized in their salt form as the they allow diluting and administering with the aqueous vehicles. [1, 81]. Bhandari et al. investigated the effect of use of different lipids and lipid combinations to enhance the entrapment efficiency of a hydrophilic drug isoniazid [94]. They were successful in proving that after the use of appropriate lipid combination in an appropriate ratio, the entrapment efficiency of a hydrophilic drug like isoniazid could be significantly increased. They achieved an entrapment efficiency of approximately 84% which is considerably higher than other reported data [94]. Pandey et al. have previously reported entrapment efficiency for Isoniazid of 45% [95]. In a similar study, Shah et al. investigated the entrapment efficiency of a water-soluble drug, ciprofloxacin hydrochloride [96]. It was found that, SLNs could be potentially utilized as hydrophilic drug carriers with improved entrapment efficiency and controlled release of the drug [96]. Liu et al. used phospholipid complexes technology to incorporate hydrophilic drug diclofenac sodium in SLNs [97]. This technology was used to improve the solubility of diclofenac sodium in the lipid. They prepared SLNs by modified emulsion/solvent evaporation method. They were successful in formulating SLNs with small particle size of approximately 200 nm and a high entrapment efficiency of around 75% incorporating the given hydrophilic drug diclofenac sodium [97]. In another study, SLNs was investigated as a drug delivery system to target the delivery of a water-soluble drug gabapentin to the brain [98]. Gabapentin was successfully incorporated in the SLNs prepared by solvent injection technique. The formulated SLNs were spherical in shape with particle size was in the nanometer range [98]. Wang et al. synthesized a derivative of highly water soluble and potent drug 5-fluoro-20-deoxyuridine [99]. The investigation aimed at targeting the drug to the brain.

Hence, 30,50 -dioctanoyl-5-fluoro-20 –deoxyuridine was synthesized as the derivative and incorporated in SLNs. The SLNs had an entrapment efficiency of 96.62% and had good brain targeting efficiency. Hence, SLNs proved to be a potential drug targeting carrier system for the treatment of central nervous system disorders [99]. Hydrophilic drugs can be successfully incorporated into solid lipid nanoparticles by appropriatly selecting the lipids and other formulation ingredients and optimization of the process parameters that ultimately lead to entrapment of effective amount of drug into SLNs [94, 96-99].

1.7 Conclusions:

SLNs have emerged as a promising drug delivery system in recent years as its potential is gradually and eventually being realized. Many drugs having therapeutic activity are highly lipophilic in nature. Incorporation and delivery of such lipophilic active ingredients at the drug delivery site/target site has always been a concern. SLNs have been found to have several desirable characteristics required for the delivery of such lipophilic agents to the target site of action. SLNs not only efficiently deliver lipophilic drugs but also carry and deliver hydrophilic drugs. When compared to other colloidal carrier systems, SLNs are found to be highly stable being present in the solid form. SLNs share the advantages of both polymeric nanoparticles and nanoemulsions. SLNs being composed of biocompatible lipids have shown negligible toxicity after administration or application. Production scale up of SLNs is easy and economical when compared to that of other colloidal systems. Site-specific delivery and sustained drug delivery are two typical advantages of SLNs. Ability to apply sterilization to the final formulation (terminal sterilization) is a major advantage of the SLNs. SLNs can be administered via

various routes of administration such as oral, parenteral, topical, pulmonary, etc. thereby demonstrating its application flexibility.

SLNs have been extensively investigated recently as antitumor drug carriers. They have been proven to be a potential carrier system for tumor targeting. Cytotoxic drugs are particularly more reactive, unstable, toxic and structurally and physico-chemically diverse when compared to other drug classes. SLNs can accommodate all these different classes of compounds into its solid core.

It is expected that in near future, the modified forms of the SLNs such as nanoparticulate lipid carriers (NLC), stealth SLNs and targeted SLNs with combination drugs will be optimized thereby reducing side effects and increasing the efficacy to serve as potential carriers of antitumor drugs [81].

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Chapter 2

Significance of Thesis Research

1Paclitaxel is an antineoplastic drug used as a chemotherapeutic agent. Paclitaxel has been extensively investigated over the past few decades because of its significant anticancer activity against various cancers. Currently, paclitaxel is prescribed to treat most aggressive forms of ovarian, lung and breast cancer and also, AIDS-related Kaposi's Sarcoma [91]. Preparing a viable paclitaxel formulation is a challenge because of its poor water solubility. Taxol® was the first clinically available formulation of Paclitaxel consisting of 1:1 combination of Cremophor EL and ethanol as solvent in which paclitaxel was dissolved. However, side effects such as severe hypersensitivity, nephrotoxicity and neurotoxicity were associated with the use of Cremophor EL. This has encouraged many researchers to develop paclitaxel formulations free of Cremophor EL [100]. Hence, the goal of our research was to develop a formulation incorporated with paclitaxel using formulation ingredients that are biocompatible in the human body thereby minimizing the side effects. Also, our aim was to provide a sustained release of the paclitaxel to reduce the frequency of drug administration thereby improving the patient compliance with a targeted delivery at the site of action. To achieve this, paclitaxel was incorporated in solid lipid nanoparticles and was characterized for various

parameters. The temperature modulated solidification technique utilized for the production of SLNs does not require the use of any organic solvent and is cost effective with good a scale-up potential [6, 101].

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Chapter 3

Formulation, Characterization and Evaluation of Paclitaxel loaded Solid Lipid Nanoparticles Prepared by Temperature Modulated Solidification Technique.

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KEYWORDS: P-XRD, ATR-FTIR, DSC, nanometer, colloidal, sustained release.

Figures: 11.

Tables: 4.

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3.1. Abstract

The aim of this research was to formulate, characterize, and evaluate the paclitaxel loaded solid lipid nanoparticles (SLNs) prepared by a temperature modulated solidification technique developed and optimized in our laboratory. The particle size analysis through dynamic light scattering (DLS) and transmission electron microscopy (TEM) revealed and confirmed the spherical shape and nanometer size range of the formulated nanoparticles. Zeta potential measurements confirmed the physical stability of the SLNs with a negative surface charge. Atomic force microscopy (AFM) studies were done to study the surface topography and particle size and shape. AFM data showed minimal aggregation and more or less spherical SLNs. Differential scanning calorimetry (DSC), powder X-ray diffraction (P-XRD) and Fourier transform infrared spectroscopy (ATR-FTIR) confirmed the conversion of bulk lipid into SLNs and high entrapment of paclitaxel into the lipid matrix. The optimized formulation had an entrapment efficiency of approximately 62%. The in-vitro drug release depicted a sustained release of paclitaxel from the SLNs over duration of one week. The drug release data was found to best fit and hence followed the Higuchi drug-release model.

3.2. Introduction

Paclitaxel (PCTL) has broad activity against various tumors and is prescribed to treat aggressive malignancies such as ovarian cancer, breast cancer, lung cancer, head and neck, esophagus, bladder, endometrium, hematological and pediatric malignancies [102]. PCTL is isolated from the bark of *Taxus brevifolia.* PCTL acts by targeting

tubulin. It has been observed that the cells treated with PCTL have difficulty with spindle assembly, cell division and chromosome segregation. PCTL stabilizes and protects microtubules against disassembly [103]. PCTL has poor water solubility and hence poses a challenge in developing viable pharmaceutical formulations. To improve the solubility of PCTL, the first clinically available formulation used a blend of Chremophor EL and ethanol as a solvent to solubilize the PCTL. Cremophor EL has been observed to have side effects such as severe hypersensitivity, nephrotoxicity and neurotoxicity thereby encouraging many researchers to develop a formulation containing PCTL that is free of Cremophor EL [100].

SLNs have emerged as a potential colloidal drug delivery systems which combines the advantages of various traditional colloidal drug delivery systems and at the same time avoid major shortcomings [7]. SLNs are formulated using solid lipids which are comparatively inexpensive and also physiologically safe as they fall under the generally recognized as safe (GRAS) category. Also, they possess better physical stability, good reproducibility and can be produced at comparatively lower costs [1, 101].

In the present study we formulated solid lipid nanoparticles to successfully carry and deliver paclitaxel to the desired site with a sustained delivery. Paclitaxel was incorporated in the SLNs formulated using the lipid, glyceryl monostearate (GMS) and tween 80 as an emulsifier. The feasibility of SLNs as a potential drug delivery system for paclitaxel was demonstrated through extensive characterization of various properties such as particle size, particle shape, drug entrapment efficiency, solid-state characteristics and drug release [91].

3.3. Materials and Methods.

3.3.1. Materials.

Glyceryl Monostearate (GMS) was obtained from PCCA (Houston, TX). Tween 80, Acetonitrile (HPLC grade), sterilized Phosphate buffer solution (PBS) was purchased from Fischer Scientific (Fair Lawn, NJ). Paclitaxel was obtained from TSZ CHEM. Anhydrous D-trehalose was obtained from Arcos Organics (Fair Lawn, NJ).

3.3.2. Methods.

3.3.2.1. Preparation of Paclitaxel loaded solid lipid nanoparticles.

Preparation of solid lipid nanoparticles was carried out using temperature-modulated solidification technique [6, 101]. An appropriate amount of paclitaxel was added to 1g of GMS in a small test tube and was subjected to shaking in a temperature-controlled shaker (Multi-therm shaker H5000-H, Benchmark Scientific Inc., NJ, USA) at a temperature of 75ºC and a speed of 1500 rpm for a duration of 48 hours. After that, 2g of Tween 80 were added to it. The molten mixture of paclitaxel, Tween 80 and GMS was continuously heated in a water bath (150ml) at a temperature not exceeding 90ºC with intermittent vortexing of the mixture every 5 minutes of heating for 20 seconds. Simultaneously, 150ml of deionized water was heated for an hour to 80ºC. The molten mixture was then added to the heated deionized water and the dispersion was placed in an ice bath and homogenized at a speed of 4000 rpm 45 minutes. The homogenized dispersion was placed on a bench top and kept undisturbed for 30 minutes. The dispersion then was filtered using Amicon® Ultra Centrifugal filters (Ultracel®-100K) by subjecting it to centrifugation for 20 minutes. The obtained residue was re-suspended using 40 ml of deionized water. The amount

of dispersion obtained then was divided into two equal parts. One of the parts was directly subjected to freezing at -80ºC after addition of suitable amount of D-trehalose while the other part was subjected to dialysis (using deionized water as dialysis medium) for duration of 2 hours and then subjected to refrigeration at -80ºC after addition of suitable amount of D-trehalose. Both of the individual parts were then subjected for lyophilization for duration of 72 hours. The obtained lyophilized products were weighed individually and used for further analysis.

3.3.2.2. Particle Size Analysis and Optimization Studies for Extracting the Surfactant from SLNs.

Removal of surfactant from the final formulation is necessary as it can cause hazardous effects on cells. These studies were performed on blank SLNs. As mentioned earlier in the formulation procedure, half of the non-lyophilized SLN dispersion was subjected to dialysis using a dialysis bag membrane (Fisherbrand® regenerated cellulose Dialysis tubing, MWCO: 12000-14000) for 2 hours. Deionized water was used as a medium for dialysis. Particle size analysis was performed after each of the following stages of the formulation procedure, which included homogenization, Amicon filtration, dialysis and lyophilization. The water used as the dialysis medium was subjected to particle size analysis. The particle size was determined by using dynamic light scattering (Nicomp 380 ZLS, CA, USA) equipped with a 100mW He-Ne laser of wavelength 658 nm and a photodiode array detector. All samples were transferred to disposable Durex® borosilicate glass culture tubes (Kimble Chase, Vineland, NJ) and measured at 23°C and at a scattering angle 90°.

The particle size was expressed as a volume-weighted diameter. Nicomp software was used for data acquisition and analysis.

3.3.2.3. Zeta Potential Analysis.

The zeta potential of the blank and paclitaxel loaded SLNs were determined using dynamic light scattering (Nicomp 380 ZLS, CA, USA) equipped with a 100mW He-Ne laser of wavelength 658 nm and a photodiode array detector. 1mg of the lyophilized samples were re-dispersed in 10ml of deionized water and vortexed (Fischer scientific, digital vortex mixer) at 3000rpm for 5mins prior to measurement. The samples were then transferred to standard plastic cuvettes and analyzed at 23°C and at an angle of 14.06° in the electrophoretic light scattering (ELS) mode. Nicomp software was used for data acquisition and analysis.

3.3.2.4. Imaging by Transmission Electron Microscopy (TEM).

5mg of the lyophilized blank and lyophilized PCTL-loaded SLNs were re-dispersed in 10ml of deionized water, vortexed at 3000rpm for 5 minutes. One drop of the prepared sample was pipetted onto a Formvar/Carbon 400 mesh copper grid (Ted Pella, CA) and was allowed to air dry for 24 hours at room temperature prior to imaging using a transmission electron microscope (Hitachi HD-2300A, Hitachi High Technologies America, IL, USA) operated at an acceleration voltage of 200kV.

3.3.2.5. Atomic Force Microscopy (AFM).

Imaging by Atomic force microscopy was carried out on a Nanosurf Easyscan 2 AFM instrument equipped with a cantilever and camera affixed on the top of the Easyscan 2 head. The instrument was operated in a dynamic force mode with a speed of 0.1 millimeters per second and a scan range of 25 micrometers. The results were

translated by the Easyscan 2 controller and recorded by Easyscan 2 software. The samples after various formulation steps i.e. homogenization, amicon filtration, dialysis were collected and were diluted with deionized water in 1:10 v/v proportion and one drop of each was placed separately on a mica disk and allowed to dry for 24 hours. The blank lyophilized and PCTL-loaded lyophilized samples were resuspended in deionized water in 1:10 w/v proportion and one drop of each was placed on a mica disk separately and allowed to dry for 24 hours. All the dried samples were then subjected to AFM analysis.

3.3.2.6. Differential Scanning Calorimetry (DSC).

Pure GMS, pure PCTL, pure D+(-) trehalose, lyophilized blank and lyophilized PCTL-loaded SLNs were studied for the thermal behavior using a Mettler Toledo DSC822^{e} star system. 5-8 mg of samples were weighed in 100 µl aluminum pans and crimped. An empty pan served as a reference. A heating rate of 10°C/min and a scanning range of 25 to 350° C were employed under nitrogen while recording the thermograms. The thermograms were analyzed using Star-e software.

3.3.2.7. Powder X-ray Diffraction (pXRD).

X-ray scattering measurements were performed on pure GMS, pure paclitaxel base, and lyophilized blank and PCTL-loaded SLNs, with a PANanlytical's X-ray diffractometer (PANanlytical's X'pert Pro Tokyo, Japan) equipped with X'Celerator high speed detector and CuKα source with a voltage of 45 kV, and a current of 40 mA. The samples were crushed, placed in an aluminum sample holder, and packed smoothly using a glass slide. The instrument was operated in the continuous scanning

speed of 4°/min over a 2θ range of 5° to 40° and the results were evaluated using the X-Pert data collector version 2.1 software.

3.3.2.8. Fourier Transform Infrared Spectroscopy (FTIR).

The spectra for pure GMS, pure PCTL base, and lyophilized blank and PCTL-loaded SLNs, were recorded with a Thermo Scientific NICOLET iS5 Fourier Transform Infrared Spectrometer (iD3 ATR) equipped with a Zinc-Selenium crystal on which the samples were directly placed and 16 scans collected. The spectra were obtained at a resolution of 4 cm^{-1}.

3.3.2.9. Drug Entrapment Efficiency.

Approximately 5 mg of lyophilized PCTL-loaded SLNs, formulated by incorporating 50 mg,75 mg and 100 mg of PCTL, respectively, were solubilized in 10 ml of acetonitrile by vortexing at 3000 rpm for 2 minutes. The solution was then filtered through 0.45 μm non-sterile, solvent-resistant polytetrafluoroethylene (PTFE) syringe filter (Fisherbrand, USA). 10μl of the filtrate was used as the injection volume for analysis by reversed-phase high performance liquid chromatography (RP-HPLC) using Waters HPLC e2695 separation module equipped with a photo diode array detector (Waters 2998). A symmetry® C18 column (Waters, USA, 3.5 μm, 4.6 x 75 mm), was used as the stationary phase. Acetonitrile and water in a ratio of 52:48 was used as the mobile phase. An isocratic flow rate of 0.5 ml per minute with a column temperature of 30°C was employed during the analysis. A wavelength of 227nm was used for the detection of PCTL. Empower 3.0 software was used for data analysis. A calibration curve of pure PCTL in acetonitrile was used for quantification. The drug entrapment efficiency was calculated using the following formula:

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Practical PCTL content}}{\text{Theoretical PCTL content}} \times 100$$

3.3.2.10. In-vitro Drug Release Studies.

100mg of lyophilized PCTL loaded SLNs were accurately weighed and dispersed in 1ml of phosphate buffer solution (PBS, pH 7.4) in a sealed dialysis membrane (Spectra/Por® Dialysis Membrane MWCO:3500) with a molecular size cut off of 3500 Daltons. The dialysis membrane was placed in a beaker containing 500ml of 30% ethanol solution and maintained at 37°C + 0.5°C in a water bath, covered with parafilm, and under magnetic stirring of 125 rpm. 1ml of samples were collected at regular time intervals i.e. after 15 minutes, 30 minutes, 1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 42, 48, 60, 72, 96, 120, 144 and 168 hours and replaced with fresh 30% ethanol solution. The amount of PCTL released was quantified by the HPLC method described earlier [89].

3.3.2.11. Drug Release Data Modeling.

To determine the best fit of the release profile of the formulated SLNs, the release data was plotted according to the four different kinetic models i.e. zero order drug release model, first order drug release model, Higuchi drug release model and Korsemeyer-Peppas drug release model. The regression coefficient (R^2) was used as an indicator of the best-fit. The release rate constant values were determined for each model [104, 105].

3.3. Results and Discussions:

3.3.1. Preparation of Paclitaxel Loaded Solid Lipid Nanoparticles.

The SLNs were prepared using a temperature modulated solidification technique. This technique involves regulating the temperature of the surrounding environment with concurrent solidification and formation of lipid nanodispersion. Glyceryl monostearate and Tween 80 were used as the lipid and surfactant respectively in the formulation procedure. Shear forces applied by the high-speed homogenization followed by sudden cooling lead to formation of the SLNs. Tween 80, the surfactant, lead to a reduction in an interfacial tension between the molten lipid and the aqueous phase as the temperature was reduced with simultaneous homogenization process thereby leading to formation of SLNs. The formulation was optimized by a regular and uniform variation of the formulation parameters such as drug/lipid ratio, surfactant concentration and the emulsification time. Increases in the lipid concentration were found to increase the particle diameter. This signifies lipid concentration dependent increases in particle size after reaching the threshold value [106]. This process is simple and free of any organic solvent hence, has a high potential of scale-up. To limit the surfactant-based toxicity in cell cultures and animal tissues, a minimum possible amount of surfactant was used in the optimized formulation with a lipid/surfactant ratio of 1:2. Heydenreich et al investigated and compared the effect of three different purification techniques viz., ultrafiltration, ultracentrifugation and dialysis on cellular toxicity and physical stability of the SLNs [107]. In our formulation technique, ultracentrifugation was used as a method of purification and a means of improving the physical stability of SLNs. Aqueous

dispersions/suspensions have poor stability making it necessary to utilize special handling and storage conditions. Lyophilization is good solution to transform aqueous dispersions into solid forms. Lyophilized powders have high stability [108]. Freeze drying involves stresses that destabilize the nanoparticulate dispersions. It causes aggregation and sometimes irreversible fusion of nanoparticles leading to destabilization. As a solution, cryoprotectants are added before freezing. Cryoprotectants protects the formulation from freezing stress [109]. Trehalose was chosen as the cryoprotectant in the optimized formulation after uniformly varying sucrose, mannitol and trehalose in the formulation and subsequently evaluating the particle diameters of the lyophilized SLNs. Optimized formulation contained 1:1 ratio of lipid to trehalose [6, 101].

3.3.2. Particle Size Analysis and Optimization Studies for Extracting the Surfactant from SLNs:

The particle size of blank SLNs was analyzed at each step of formulation procedure i.e. after homogenization, amicon filtration, dialysis and lyophilization. An increase in average particle diameter was observed after amicon filtration and lyophilization processes (Table 1 and Fig.1). Particle size increase after amicon filtration may be attributed to partial elimination of surfactant (Tween 80) micelles from the formulation causing particle coalescence thereby shifting the average particle diameter to the higher range. Despite use of cryoprotectants, an increase in particle diameter has been observed after lyophilization [110]. Particle diameter increases after lyophilization may be attributed to the use of lesser volume of redispersion media compared to that used during preparation leading to an increase in SLN

concentration and interparticulate interaction favoring aggregation to form larger structures. In addition, cryoprotectant may deposit on the surface of the SLNs further increasing the overall particle diameter [101]. The average particle diameter of paclitaxel loaded SLNs was measured separately and was found to be 419.5 ± 14.84 nm.

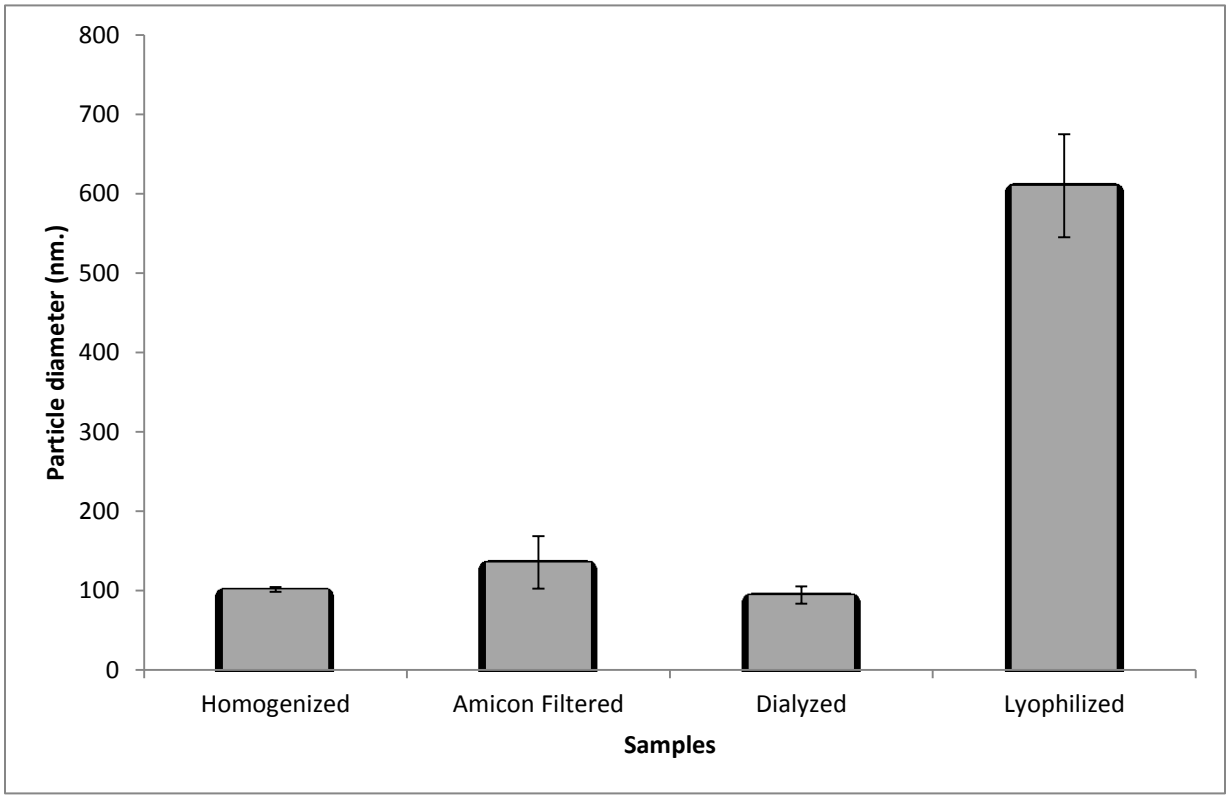


Figure 1: Particle size analysis of blank SLNs. The data represent the mean values (n=3) ± S.E.

Process samples	**Average particle diameter ± standard deviation (nm.)**
Homogenized	101.46 ± 3.11
Amicon filtered	135.45 ± 33.16
Dialyzed	94.45 ± 10.90

Lyophilized	609.92 ± 64.88

Table 1: Particle sizes of different process samples. The data represent the mean values (n=3) ± S.E.

As mentioned earlier, surfactants have proven toxic effects on cells and human tissues [107]. Hence, an additional step of dialysis was introduced in the formulation procedure to limit the surfactant to minimum amount. The media used for dialysis step during the formulation step was analyzed for particle size by DLS using the nicomp analysis mode to actually observe the particle size of the majority of the particle population. The observed particle sizes for high majority population were in the range of 10 to 20nm (Table 2) denoting the presence of micelles of surfactant, tween 80 in the dialysis media and hence confirming the extraction of surfactant from the SLN dispersion.

	Peak-1		***Peak-2***		***Peak-3***	
	Particle diameter (nm.)	*Percentage*	*Particle diameter (nm.)*	*Percentage*	*Particle diameter (nm.)*	*Percentage*
Average ± Standard deviation	14.73 ± 3.46	88 ± 2.64	137.53 ± 80.40	6.03 ± 6.43	1791.5 ± 1519.5	6.23 ± 3.72

Table 2: Dialysis media particle size analysis. The data represent the mean values (n=3) ± S.D.

3.3.3. Zeta Potential:

Zeta potential measurements were performed for lyophilized blank and lyophilized PCTL loaded SLNs (Table 3). All the obtained zeta potential values were negative in charge because of the presence of glyceryl monostearate which is a fatty acid ester [111]. Zeta potential is a major factor to evaluate the stability of any colloidal dispersion [112]. Stable dispersion of particles is possible when the absolute value of zeta potential is above 30 mV (both negative and positive) because of the repulsions produced by the surface electric charge of the particles in the dispersion [7, 112, 113]. Lower zeta potential values may lead to interparticulate interaction resulting in flocculation and coagulation. As the obtained zeta potential values for PCTL-loaded SLNs were over negative 30 mV, the SLNs were found to be physically stable.

	Lyophilized blank SLNs (mV)	**Lyophilized PCTL-loaded SLNs (mV)**
Zeta potential	-24.74 $\pm$ 0.17	-32.85 $\pm$ 0.33

Table 3: Zeta potential measurement results. The data represent the mean values (n=3) $\pm$ S.E.

3.3.4. TEM:

TEM was used to visualize the morphology of SLNs. Various characteristics such as particle size, shape and internal structure of the nanoparticulate carrier system can be efficiently evaluated by using TEM [114]. The TEM images of lyophilized blank and lyophilized PCTL loaded SLNs were captured (Figure 2). TEM images revealed the spherical shape of the particles and confirmed particle diameter in nanometer range

similar to that obtained from DLS measurements. A core shell model with drug-enriched core was observed in TEM image of the PCTL loaded SLNs.

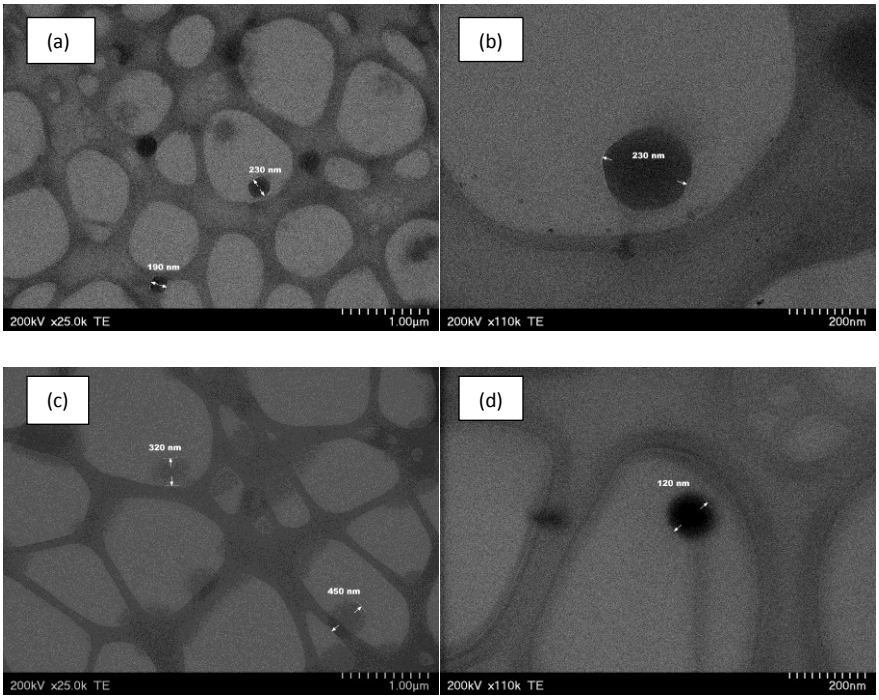


Figure 2: TEM images of (a) and (b) Lyophilized blank SLNs; (c) and (d) Lyophilized drug Paclitaxel loaded SLNs.

3.3.5. AFM:

AFM studies were done to study the morphology of the SLNs at various formulation steps and also their particle aggregation. Researchers have reported clustering of the SLNs thereby increasing the particle size after imaging by AFM. This clustering of the SLNs was attributed to the sample preparation method for AFM imaging in which the samples may not be completely dry which means that the samples are still hydrated leading to particle aggregation [115]. We observed similar outcomes. The particle aggregation and hence the particle sizes for lyophilized blank and lyophilized PCTL-loaded SLNs was higher than that for the other process samples (Figure 3). This supports the particle size data obtained with DLS and TEM.

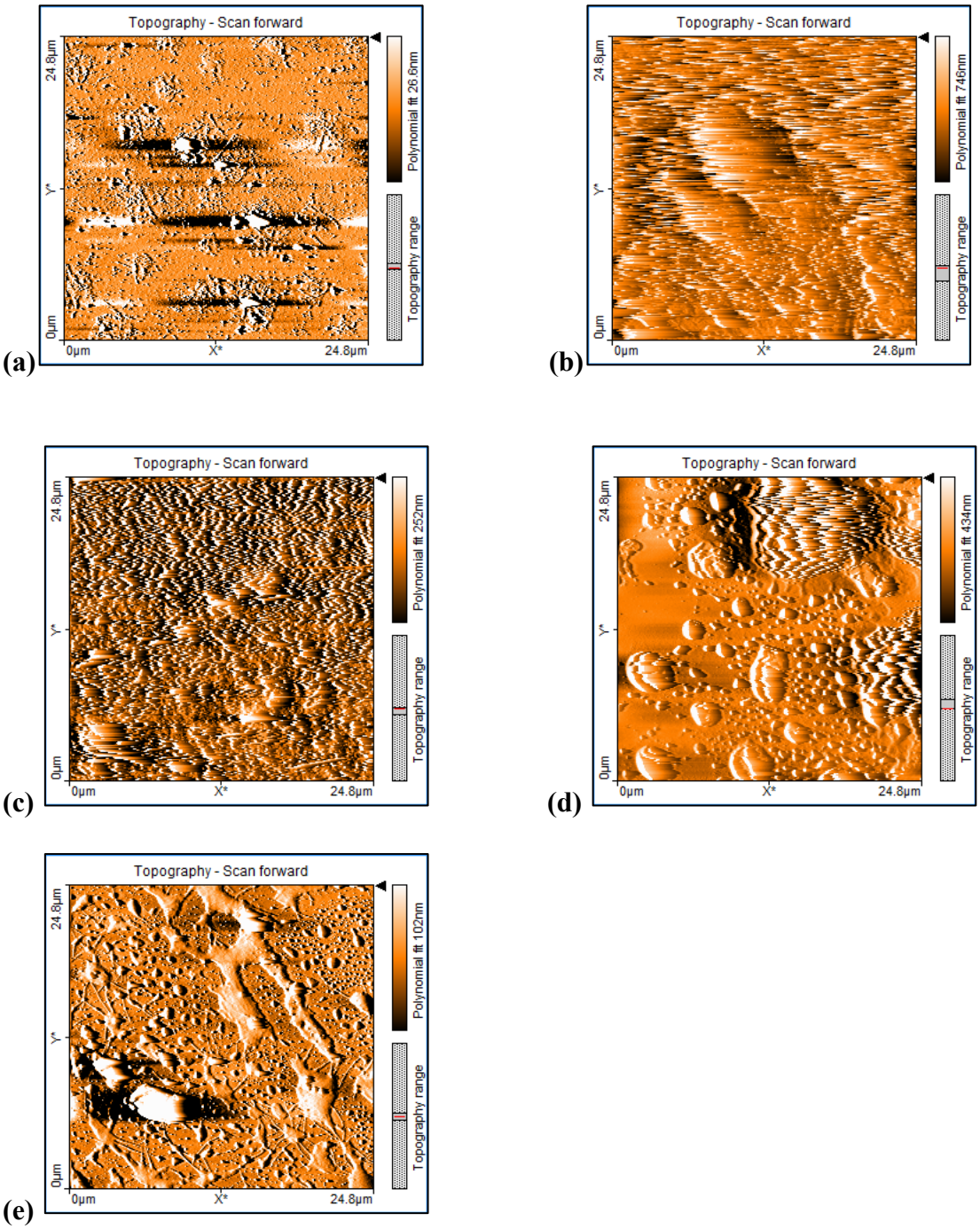


Figure 3: Shaded line-fit projection of AFM data for (a) blank homogenized sample, (b) blank amicon filtered sample (c) blank dialyzed sample, (d) blank lyophilized sample and (e) PCTL-loaded lyophilized sample of SLNs.

3.3.6. DSC:

In the technique that we used (temperature modulated solidification technique), the lipid initially melts and later solidifies by cooling resulting in the recrystallization of the lipid. The GMS exhibited an endothermic peak with a peak temperature of 71° C. The formulated SLNs melted at comparable temperature and exhibited melting peaks approximately 8 degrees lower than that of the pure lipid (Figure 4). This indicates a successful transformation of the lipid bulk into nanoparticles through polymorphic transition of lipid crystallinity from β'- modification to α-modification [91, 116, 117]. The degree of crystallinity is reduced and also the melting enthalpy is decreased with the formation of nanoparticles. An endothermic peak was observed in the DSC thermogram of pure PCTL with an onset at 216.26° C, and a peak temperature of 223.68° C [118]. An exothermic peak was observed immediately after the melting endothermic peak of PCTL indicating degradation of PCTL [119] . The DSC thermogram of PCTL-loaded SLNs did not exhibit a peak at or around 223.68° C (Figure 4) indicating either the successful conversion of crystalline PCTL into amorphous form in the SLNs or drug being successfully dispersed into the melted matrix of GMS [32, 120].

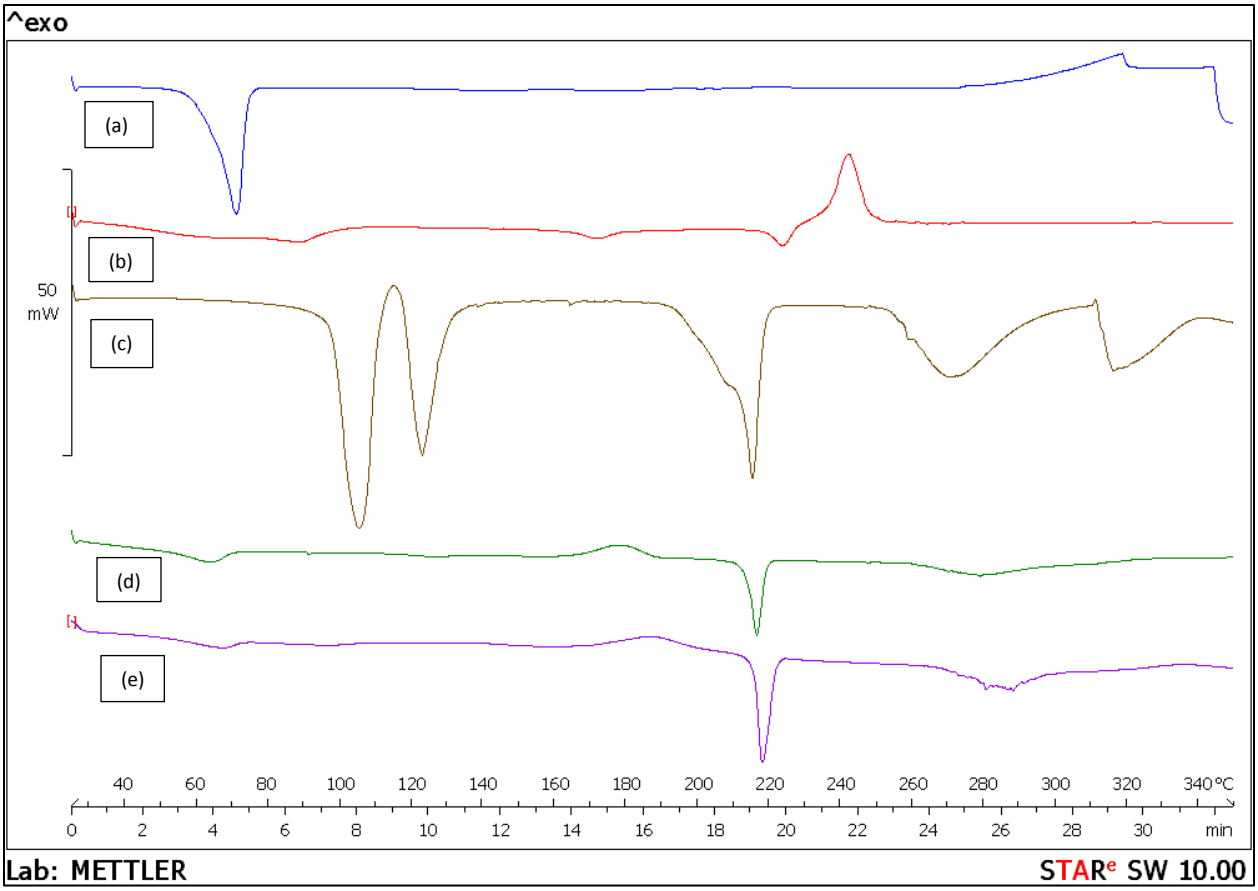


Figure 4: DSC thermograms for (a) pure GMS (b) pure PCTL (c) pure D (+) - trehalose (d) blank lyophilized SLNs (e) PCTL-loaded lyophilized SLNs.

3.3.7. pXRD:

Powder X-ray diffraction patterns were analyzed to study the solid-state nature of the SLNs. The diffraction patterns of the lyophilized PCTL-loaded SLNs and blank lyophilized SLNs were compared with that of the pure PCTL and pure GMS respectively (Figure 5). Paclitaxel diffractogram exhibited sharp and high intensity peaks at 5.09, 5.14, 5.19, 9.60, 12.48, 37.71 and 43.94°. These peaks were either of very low intensity, less sharp, or completely absent in the diffractogram of PCTL-loaded SLNs. This indicates that paclitaxel is present in its amorphous form in the SLNs. It also indicates the high drug loading capacity of the SLNs [121]. Broad and

high intensity peaks were displayed at 2θ equals 19.54 and 23.46° by pure GMS. These two characteristic peaks of GMS were observed in the XRD patterns of blank as well as PCTL-loaded SLNs in lower intensities indicating decreased crystallinity of GMS in SLNs [122]. No differences were observed in XRD patterns of blank and PCTL-loaded SLNs. The XRD results support data obtained from DSC.

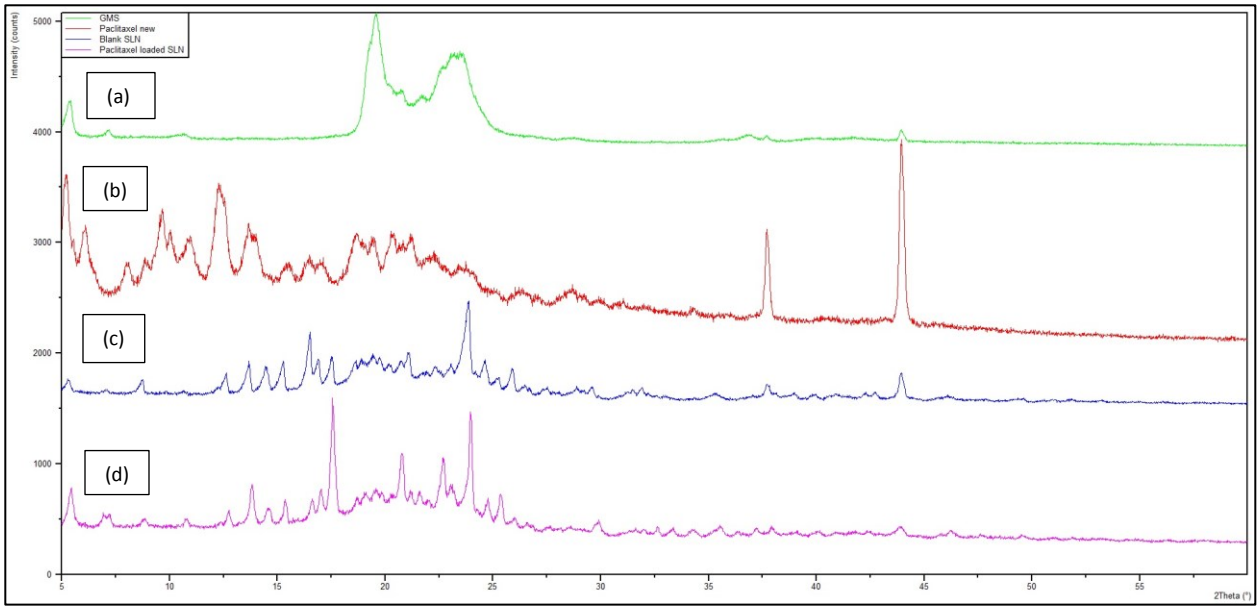


Figure 5: P-XRD patterns of (a) pure GMS (b) pure Paclitaxel (c) lyophilized blank SLNs (d) lyophilized paclitaxel loaded SLNs.

3.3.8. ATR-FTIR:

FTIR analysis was performed to study the structural composition of the SLNs. The spectrum obtained for blank SLNs was found to be corresponding to that of pure GMS (Figures 6a and 6c). These spectra distinctly showed absorption bands for OH stretching at 3495 cm^{-1}, C-H stretching at 2913 cm^{-1}, C=O stretching at 1729 cm^{-1}, and C-H bending at 1470 cm^{-1} and 1177 cm^{-1}. PCTL-loaded SLNs displayed the characteristic absorption bands of PCTL assigned to N-H stretching at around 3495-3300 cm^{-1}, C=O stretching from ester group at 1731 cm^{-1}, amide bond at 1645 cm^{-1},

ester bond stretching at 1243 cm^{-1} and C-N stretching at 1316 cm^{-1} and at 1645 cm^{-1}, 1073 cm^{-1}, 966 cm^{-1} and 708 cm^{-1} to aromatic bonds [123], in addition to those of GMS (Figure 6d). Hence, the paclitaxel loaded SLNs spectrum confirms the entrapment of PCTL in the lipid matrix of SLNs. The absorbance peak in the region of 1200 to 1350 cm^{-1}were specifically analyzed to assess the nature of solid lipids as the peak in this region provides vital information about conformation of CH_2 groups [124]. It was observed that pure GMS displayed sharp and clear peaks in the region 1200-1350 cm^{-1} indicating highly the ordered and crystalline structure of GMS (Figure 6a). However, broad peaks were observed for PCTL-loaded SLNs in the same region suggesting a reduction in the degree of crystallinity of GMS (Figure 6d). This supports the data obtained from DSC and P-XRD.

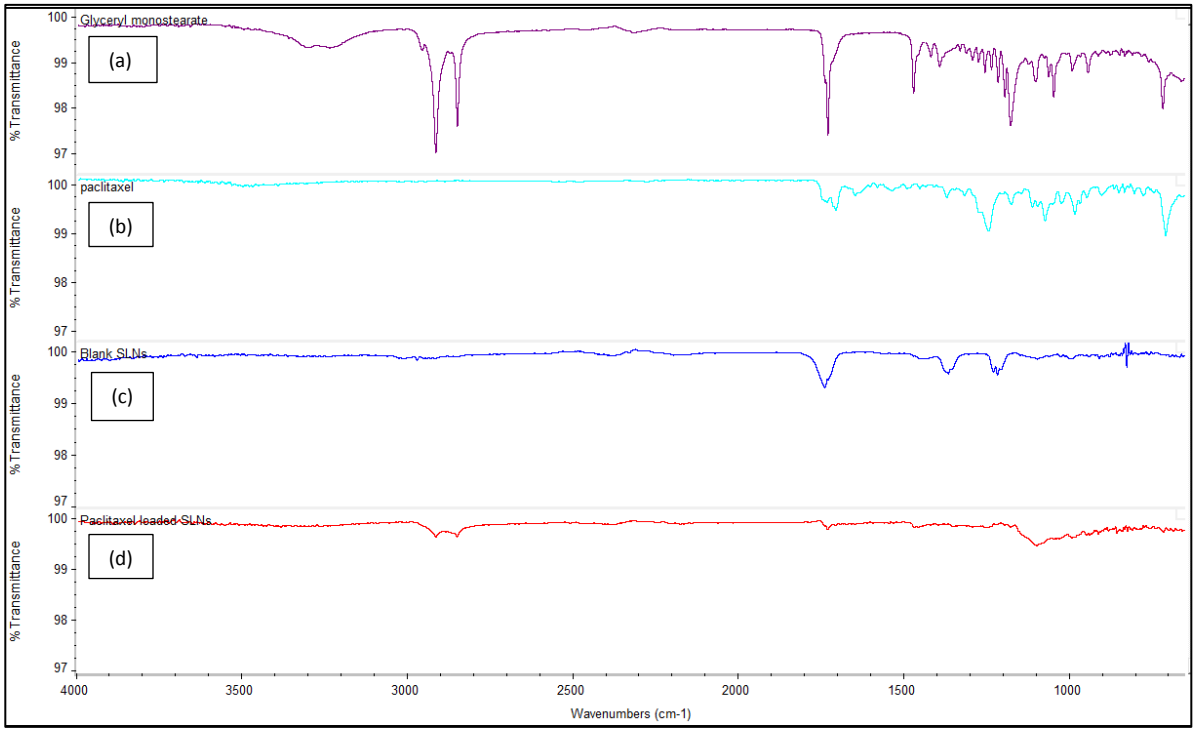


Figure 6: FTIR spectra of (a) pure GMS (b) pure PCTL (c) lyophilized blank SLNs, and (d) lyophilized PCTL-loaded SLNs.

3.3.9. Drug Entrapment Efficiency and Amount of Drug per mg of SLNs:

To determine the drug entrapment efficiency, lyophilized batches of both non-dialyzed and dialyzed SLNs containing 50 mg (50-N, 50-D); 75 mg (75-N, 75-D) and 100 mg (100-N, 100-D) of PCTL per mg of GMS respectively, were used. Relatively higher drug entrapment was obtained for the non-dialyzed batches of SLNs compared to the dialyzed batches of SLNs except for 50 mg concentration (Figure 7). This suggests that during the dialysis step, there is a loss of certain amount of drug along with the surfactant. Hence, it was confirmed that the dialysis step is not beneficial to the entrapment of drug into the SLNs. Among the non-dialyzed batches, 50-N showed lower entrapment compared to 75-N and 100-N (Figure 7). The entrapment efficiencies of 75-N and 100-N batches were similar and were approximately 62% (Figure 7). The amount of PCTL per mg of SLNs was calculated and was found to be highest for 100-N batch (Figure 8). Hence, 100-N was selected to be the optimized formulation for the further studies. GMS was the lipid used in this formulation. GMS is a monoglyceride that creates an ordered solid lipid matrix leading to reduced number of imperfections in the SLN structure. This leads to reduced space to accommodate drug molecules in the crystal lattice [101].

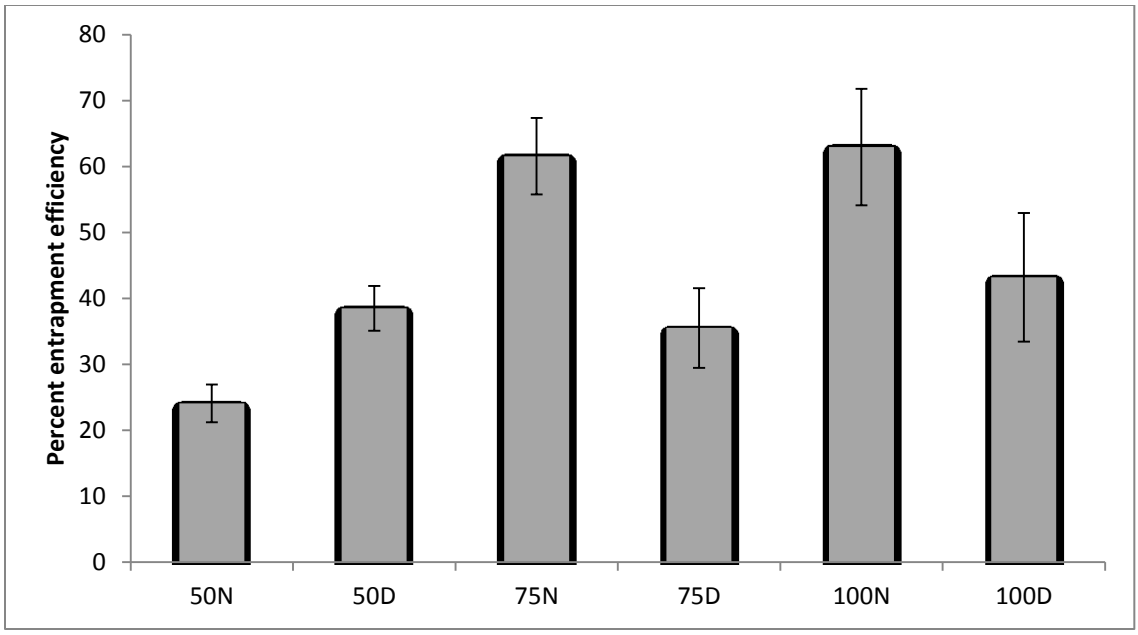


Figure 7: The entrapment efficiency of PCTL in SLNs. The data represent the mean values (n=3) ± S.E.

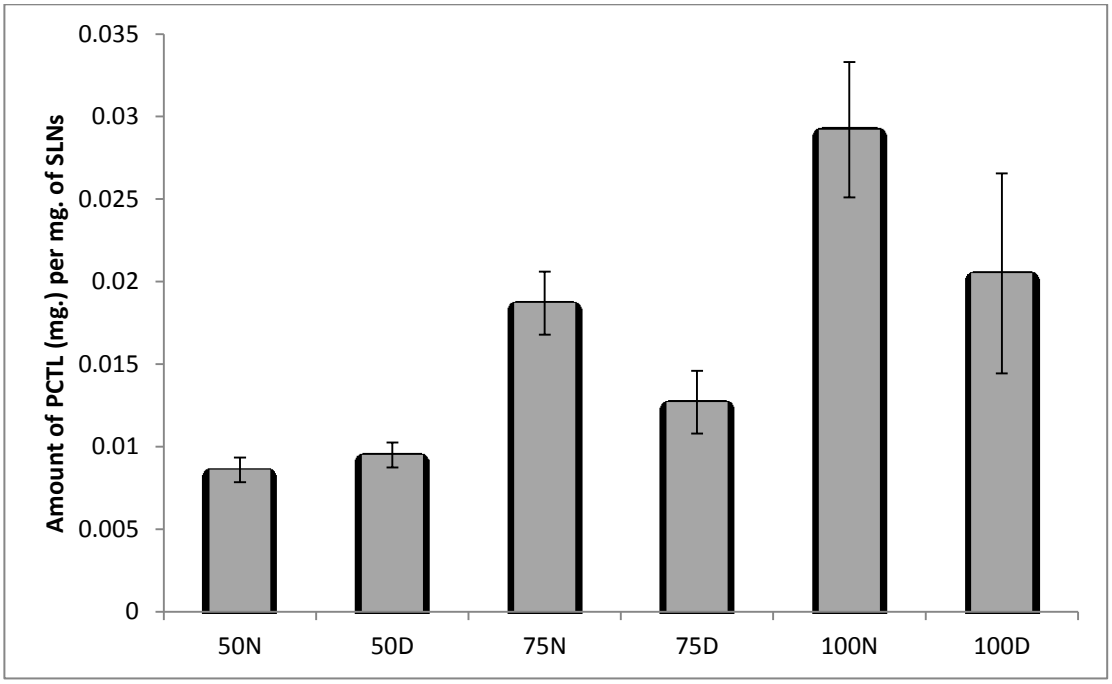


Figure 8: Amount of PCTL (mg) per mg of SLNs. The data represent the mean values (n=3) ± S.E.

3.3.10. In-vitro Drug Release Studies and Kinetic Modeling of the Drug Release Data:

The in-vitro drug release profile of PCTL loaded SLNs is shown in (Figure 9). The SLNs showed a biphasic release pattern with an initial burst release in the first 15 minutes releasing approximately 13% of drug followed by subsequent sustained release up to a week. A maximum of approximately 48 % of drug release was obtained from the SLNs. Several other researchers have reported similar biphasic drug release profiles for SLNs [125-127]. This release profile may be explained based on the partition effects of the drug between molten lipid and water phase during the homogenization step in the formulation procedure. During this step, hot water is used which is gradually cooled in an ice bath with simultaneous homogenization. As the temperature of the water gradually drops, the solubility of drug in water decreases which leads to re-partitioning of the drug into the lipid phase. As the recrystallization temperature of the lipid is reached, a solid core starts forming including the drug present in lipid phase at this temperature. Further decreases in the temperature imposes more pressure on the drug to re-partition into the lipid phase as its aqueous solubility further decreases. However, the already formed lipid core cannot take up anymore drug consequently leading to increased concentration of drug in the outer shell or surface of the SLNs [7]. The sustained release of PCTL can be utilized to prolong the release of PCTL subsequently reducing the frequency of drug administration. The *in-vitro* release data was fitted into various kinetic models and was found to best fit the Higuchi drug release model (Table 4 and Figure 10c).

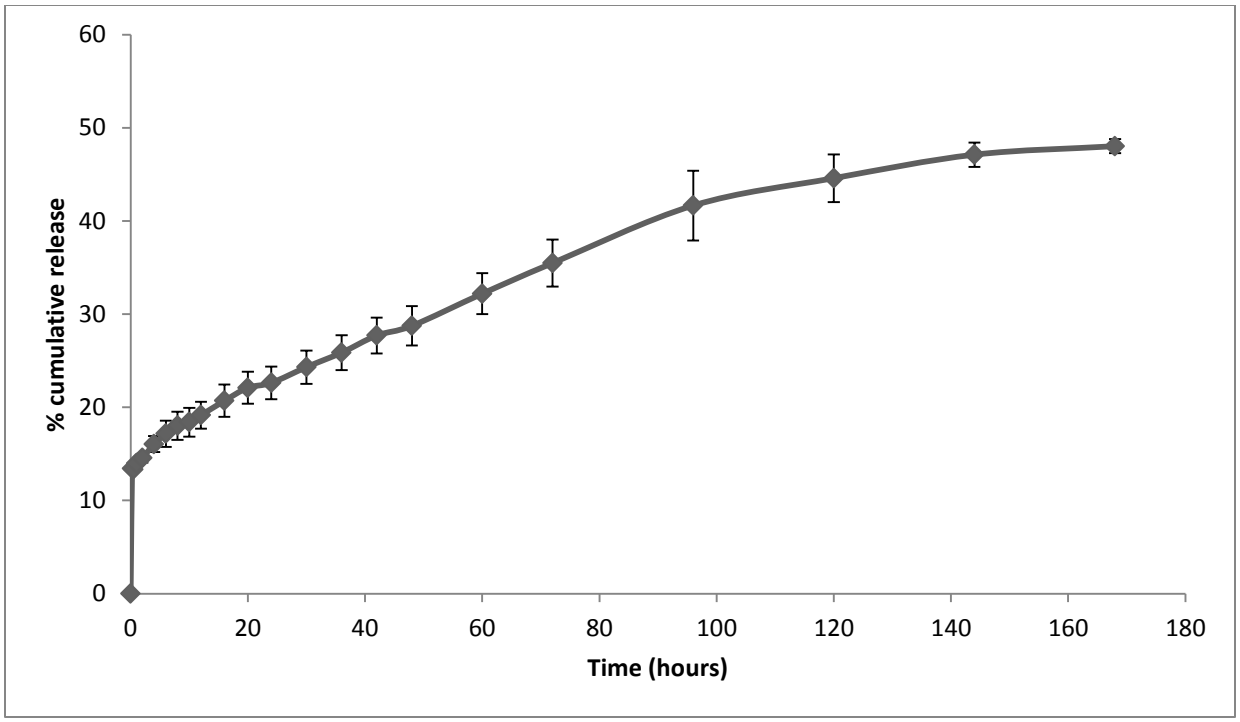
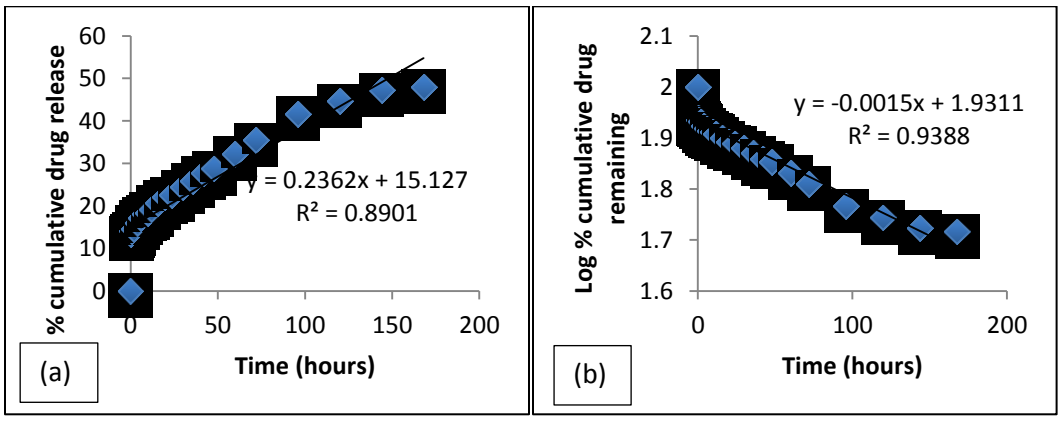


Figure 9: The *in-vitro* drug release profile of PCTL loaded SLNs. The data represent the mean values (n=3) ± S.E.



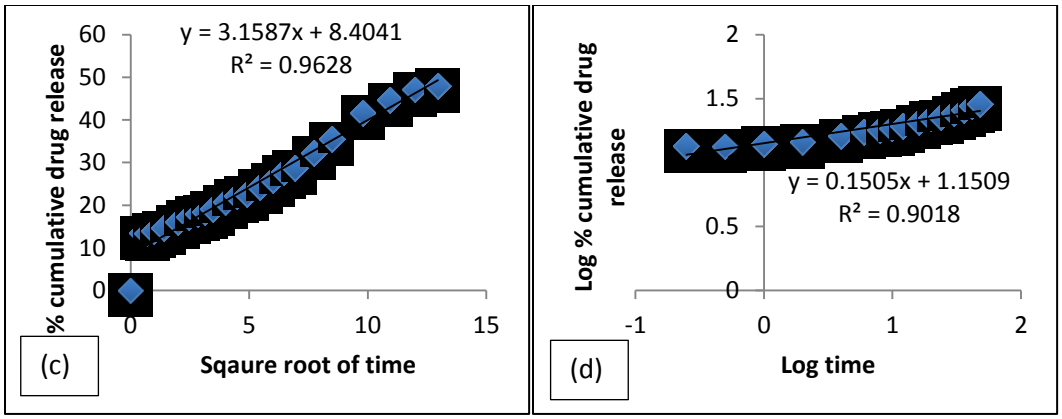


Figure 10: Kinetic model plots for (a) Zero order (b) First order (c) Higuchi and (d) Korsemeyer-Peppas model.

Kinetic models	R^2-value	k-value (hour^{-1})
Zero-order	0.89009	0.2362
First-order	0.93878	0.0006
Higuchi	0.96281	3.1587
Korsemeyer-Peppas	0.90181	14.1547

Table 4: Kinetic parameters of drug release data.

Based on data from the drug release models, drug release was found to best fit the Higuchi drug release model based on the highest R^2 value (Figure 10 and Table 4). Hence, the release of drug from the SLNs follows Fickian diffusion as the drug diffuses out from the lipid matrix.

3.4. Conclusions:

In this study, paclitaxel loaded SLNs were formulated successfully by temperature modulated solidification technique. The particle size analysis by DLS and imaging by TEM confirmed the spherical shape of the formulated nanoparticles in the nanometer

size range. The AFM studies further confirmed the spherical shape and nanometer size of SLNs. Results obtained from DSC, P-XRD and FTIR provided an insight on the solid-state characteristics and also confirmed the entrapment of paclitaxel inside the matrix of the lipid. Our preparation technique entrapped approximately 62% of paclitaxel within the nanoparticles. The *in-vitro* release studies showed sustained release of paclitaxel over duration of one week. This suggests that the formulated nanoparticles may be used as a potential medication for anticancer therapy.

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