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Development, characterization and *in vitro* assessement of stearylamine-based lipid nanoparticles of paclitaxel

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The objective of the study was to design and evaluate a solid lipid nanoparticle (SLN) drug delivery system for delivery of paclitaxel. Components of the SLN were lipid (stearylamine) and surfactants (Pluronic F68 and Soya lecithin). The paclitaxel loaded nanoparticles were prepared by a modified solvent injection method. Experiments were carried out with excipients, where surfactants, lipid and drug molar ratios were varied to optimize the formulation characteristics. The *in vitro* drug release profile from the nanoparticles followed a diffusion controlled mechanism. The modified solvent injection method ensured high entrapment efficiency (∼ 75%), produced smaller, stable nanoparticles with a narrow size distribution and proved to be a reproducible and fast production method. The present study describes the feasibility and suitability of stearylamine based SLN produced using a mixture of surfactants to develop a clinically useful system with targeting potential for poorly soluble antineoplastic drugs.

1. Introduction

The poor water solubility of many anticancer drugs obstructs their clinical applications and complicates their oral administration. In recent decades, paclitaxel, one of the most exciting antineoplastic drugs found from nature (extracted from the bark of western *Taxus brevifolia*) has been found to be effective against a wide spectrum of cancers (Chang et al. 2001; Ishitobi et al. 2001; Spencer and Faulds 1994; Thigpen 2000). At present it is available as Taxol®, administered as an intravenous (i.v.) infusion, and Abraxane™, as an injectable albumin-based nanoparticle suspension. Several biodegradable and biocompatible polymers and lipids are being used for the controlled and targeted delivery of anticancer drugs to avoid the use of toxic adjuvants and to give the desired pharmacokinetics and cellular uptake by cancer cells. In order to overcome the poor solubility, low stability and toxic side effects associated with its present clinical application, much effort has been devoted to the development of delivery systems for paclitaxel, such as liposomes (Crosasso et al. 2000; Sharma and Straubinger 1994), nanoparticles (Fonseca et al. 2002; Kim et al. 2003; Mitra and Lin 2003: Mu and Feng 2002; Potineni et al. 2003; Suh et al 1998), parenteral emulsion (Kan et al. 1999; Lundberg 1997), water-soluble prodrugs and conjugates (Pendri et al. 1998; Rodrigues et al. 1995; Safavy et al. 2003), involving new aqueous-based formulations. Among such systems, lipid nanoparticles have drawn much attention owing to their submicron size, industrial scale up, use of physiological lipids and controlled release (Gasco 2007; Radomska-Soukharev 2007).
Encapsulation of paclitaxel into solid lipid nanoparticles (SLN) may offer new opportunities to minimize the difficulties encountered in its delivery. SLN are first generation lipid nanoparticles consisting of a lipid which is solid at room temperature (Gasco 2007). The use of lipid systems is important as the physiological and biodegradable lipids are hypothesized to enhance the oral absorption in the same way as the lipid components of normal food (Souto and Muller 2006). Previously, non-stealth and stealth SLN of bioacceptable lipids have been investigated as paclitaxel carriers (Cavalli et al. 2000) and recently sterically stabilized SLN of paclitaxel were prepared, characterized and examined for *in vitro* cytotoxicity (Lee et al. 2007). The submicron size and appropriate surface chemistry of SLN may contribute to their adhesion to and adsorption into cancer cells (Wong et al. 2007).
The purpose of this study was to formulate and assess paclitaxel loaded SLN (P-SLN) using a solvent injection method. Lecithin and poloxamer 188 were utilized as surfactants, and the formulation was optimized with respect to various process parameters and characterized in terms of surface morphology, particle size, zeta potential, surface chemistry, *in vitro* release profile and stability. The *in vitro* cytotoxicity of the system developed was also assessed using human hepatoma cell lines (HepG2).

2. Investigations, results and discussion

2.1. Optimization of SLN formulation

SLN were successfully synthesized by a modified solvent injection technique, using stearylamine as lipid and a mixture of surfactants - soya lecithin and poloxamer 188. Stearylamine possesses lipid and cationic properties and has been used mostly as a charge modifier in SLN (Venkateswarlu and Manjunath 2004).

The preparation and optimization of SLN involved several initial experiments to select the final optimal conditions with respect to the formulation composition and production conditions.

Influence of lecithin concentration: In initial experiments, the ether solution containing stearylamine and different amounts of lecithin was injected into the outer aqueous phase during the fabrication process. Table 1 shows the influence on the mean particle size and polydispersity index of SLN with or without lecithin in the presence of 1% w/v poloxamer. According to the data obtained from photon correlation spectroscopy (PCS), the results indicated that the absence of lecithin in the lipid phase produced SLN of large particle size, and, in the range of concentrations investigated, the size decreased as the lecithin concentration increased. Similar results were reported by Heiati et al. (1996), who showed that increasing phospholipid concentrations favoured the creation of additional water/oil interfaces and thus, the formation of smaller particles. Also, increasing amounts of lecithin resulted in higher polydispersity which could be related to the formation of multiple phospholipid layers. A concentration of 0.18 mmol (SLN4) was selected as optimal in order to achieve a compromise between size and polydispersity.

Influence of injected amount: SLN size and distribution gradually decreased with an increase in the volume of solvent injected up to 5.0 ml at constant lipid (0.12 mmol) and poloxamer concentrations (1% w/v) (Fig. 1a). Thereafter, further increase in the volume resulted in increased particle size and distribution. This suggested that injection of a volume of solvent of 5.0 ml was optimum, as the preparation process had a critical solvent/water ratio of 1:4.

Influence of homogenization rate: The homogenization process was performed at three different homogenization rates of 15000, 20000 and 25000 rpm, using a SilentCrusher M with dispersion tool 8F. Smaller size particles with a narrow size distribution were obtained with 20000 rpm, whereas an increase in homogenization rate to 25000 rpm increased the particle size and distribution, which was attributed to flocculation (Fig. 1b).

Influence of lipid concentration: The results revealed that as the molar fraction of lipids increased, SLN size increased and the particle size distribution remained practically unchanged (Fig. 1c). It was hypothesized that increased lipid concentration reduced the diffusion of lipid phase in the outer phase, and increased the viscosity of the lipid-solvent phase and thus the rate of droplet agglomeration, which was also observed in previous studies (Schubert and Müller-Goymann 2003; Siekmann and Westesen 1994).

Influence of poloxamer concentration: Stabilizing SLN by surfactant mixtures reduces the interfacial tension more than using a single surfactant (Cavalli 1998). The incorporation of poloxamer 188 in the aqueous phase resulted in more stable and smaller particle sizes than formulations with the same lipid and a single surfactant (Table 1). Poloxamer 188 would be expected to attach to the surface of the particles *via* its hydrophobic segment poly(propylene oxide) leading to adsorption layers of up to 7 nm in thickness (Muhlen 1996) and produce a protective hydrophilic coating *via* its poly(ethylene oxide) segment. A lack of stabilizing surfactant poloxamer 188 caused the particles to aggregate giving sizes in the micrometer range. Increasing poloxamer concentration from 0.1 to 1.5% w/v caused a highly significant particle size decrease from 240 to 70 nm with no significant increase in polydispersity index. The data confirmed the formation of a sterically stabilizing adsorbed poloxamer layer as is evident from the decrease in zeta potential values with increase in emulsifier concentration. However, increase of emulsifier concentration to 2.0%w/v in the formulation led to increased viscosity of the outer phase and flocculation, and thus reduced stabilization, resulting in a significant increase in particle size and polydispersity index.

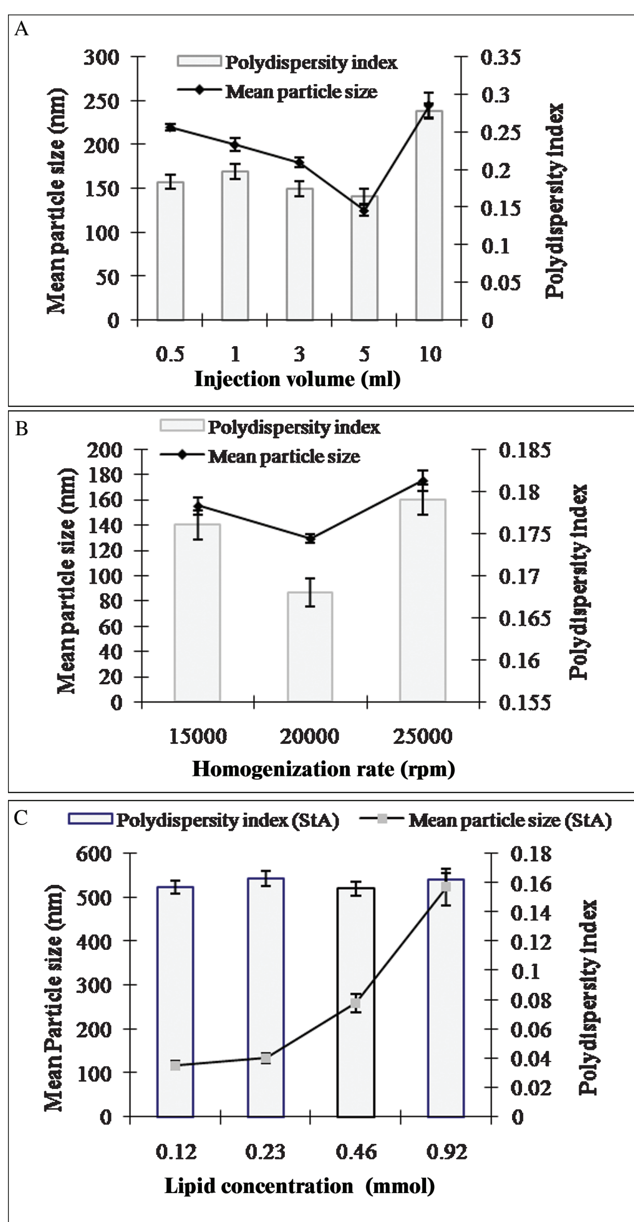


Fig. 1: Effect of different (a) injection volumes, (b) homogenization rates, and (c) concentrations of stearylamine on particle size and distribution of SLN (n = 3).

Influence of drug:lipid concentration: Drug entrapment and loading increased with increase in paclitaxel concentration from 0.05 to 0.25 mmol, while a paclitaxel concentration of 0.5 mmol exceeded the loading capacity of the system (Table 2). The components of the optimized formulation (P-SLN2) included stearyalmine and paclitaxel at 0.23 and 0.25 mmol, respectively. This formulation had a particle size of 96 ± 4.4 nm with entrapment efficiency and loading level of 75.42% and 31.5% respectively. Due to the absence of ionizable functional groups in the drug, loading had no effect on the zeta potential.

2.2. *Characterization of paclitaxel loaded SLN*

The mean particle size of all formulations obtained from PCS was between 70–130 nm and the polydispersity index was lower than 0.17 indicating a narrow size distribution. The particles had spherical shape as observed from TEM (Fig. 2). The optimized formulation had a zeta potential of 39.1 ± 0.8 mV. Solid-state characterization of the system was doneby X-ray diffraction. The diffraction pattern obtained for paclitaxel in SLN differed

Table 1: Effect of different lecithin and poloxamer 188 concentrations on particle size, polydispersity index and zeta potential of SLN (mean ± S.D., n = 3)

Formulation code	Stearylamine (mmol)	Lecithin (mmol)	Poloxamer (% w/v)	Mean particle size (nm)	PI	ZP (mV)
SLN0	0.12	0.00	1.0	715 ± 10.3	0.139 ± 0.05	
SLN1	0.12	0.02	1.0	560 ± 15.3	0.151 ± 0.07	
SLN2	0.12	0.04	1.0	395 ± 18.0	0.167 ± 0.01	
SLN3	0.12	0.09	1.0	312 ± 3.5	0.179 ± 0.06	
SLN4	***0.12***	***0.18***	***1.0***	***191 ± 6.4***	***0.202 ± 0.02***	
SLN5	0.12	0.35	1.0	205 ± 6.4	0.348 ± 0.09	
SLN6	0.23	0.18	0.1	244 ± 3.8	0.141 ± 0.07	45.0 ± 0.9
SLN7	0.23	0.18	0.5	175 ± 7.5	0.154 ± 0.09	43.7 ± 0.8
SLN8	0.23	0.18	1.0	111 ± 5.4	0.159 ± 0.05	41.9 ± 1.4
SLN9	***0.23***	***0.18***	***1.5***	***70 ± 11.6***	***0.165 ± 0.04***	***40.2 ± 1.5***
SLN10	0.23	0.18	2.0	131 ± 14.6	0.215 ± 0.06	39.7 ± 2.1

SLN: solid lipid nanoparticles, PI: Polydispersity index, ZP: Zeta potential.

Table 2: Effect of paclitaxel content on properties of paclitaxel loaded SLN

Formulation code	Stearylamine (mmol)	Paclitaxel (mmol)	Mean particle size (nm)	PI	ZP (mV)	EE (%)	Loading (%)
SLN9	0.23	0.0	70 ± 11.6	0.165 ± 0.04	40.2 ± 1.5	–	–
P-SLN1	0.23	0.05	89 ± 8.8	0.168 ± 0.03	38.2 ± 1.1	58.6 ± 4.2	12.0 ± 2.4
P-SLN2	***0.23***	***0.25***	***96 ± 4.4***	***0.162 ± 0.04***	***39.1 ± 0.8***	***75.42 ± 1.5***	***31.5 ± 2.1***
P-SLN3	0.23	0.5	129 ± 5.1	0.157 ± 0.07	38.0 ± 2.3	53.0 ± 2.3	18.12 ± 3.9

P-SLN: paclitaxel loaded SLN, PI: Polydispersity index, ZP: Zeta potential, EE: Entrapment efficiency.

to a considerable extent when compared with that of paclitaxel powder. The diffractogram of paclitaxel powder exhibited peaks at the following 2θ values: 5.7°, 9.0°, 10.2°, 11.3°, and 12.6° (Fig. 3a). Among these, the peak of highest intensity was located at 5.7° 2θ, and the peaks at 9.02° and 12.6° 2θ were broad. Several high-angle diffraction peaks were observed in paclitaxel loaded SLN at the following 2θ values: 5.3°, 10°, 11.1°, 12.7°, 13°, 13.8°, 14.2°, 16.9°, 19.3°, 20.5°, 21.3°, 21.8°, 23.1°, 23.5°, 25° and 26.5° (Figure 3b). The absence of sharp peaks in the diffraction spectra of paclitaxel loaded SLN as observed for paclitaxel powder confirmed that paclitaxel in SLN was in an amorphous state. To investigate the crystalline nature of incorporated paclitaxel, drug loaded SLN were also analyzed by DSC. DSC thermograms were recorded for paclitaxel, stearylamine, a physical mixture of the excipients and paclitaxel-loaded stearylamine SLN (Fig. 4). The DSC curve of paclitaxel showed a melting peak of pacliaxel around 219.59 °C (Fig. 4a). The melting peak was completely absent in the thermogram of drug loaded stearylamine SLN (Fig. 4d), which indicates that paclitaxel was dispersed in SLN either in amorphous form or molecularly dispersed.

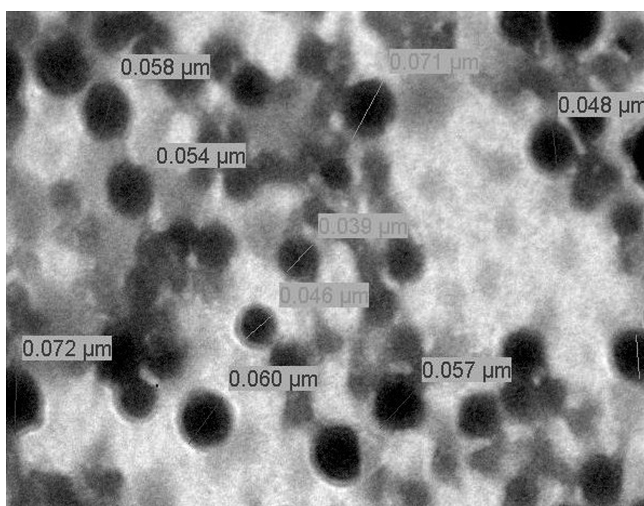
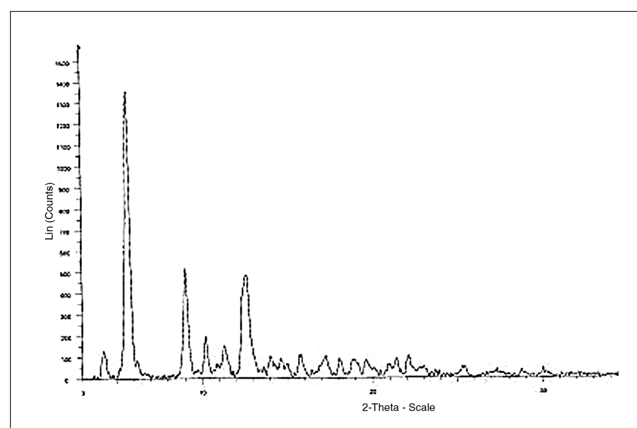
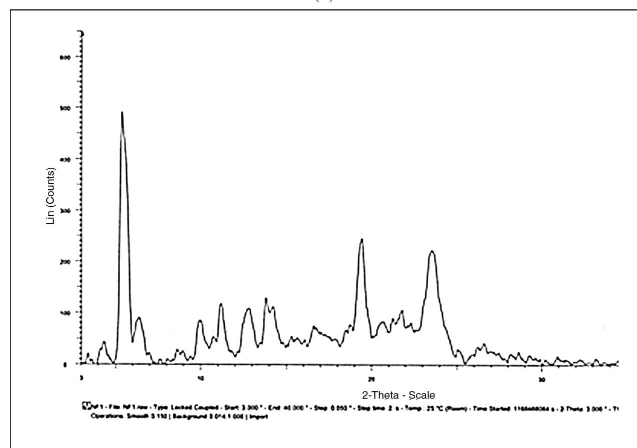


Fig. 2: TEM photomicrograph of paclitaxel loaded stearylamine SLN formulation.



(a)



(b)

Fig. 3: X-Ray diffractogram (a) paclitaxel and (b) paclitaxel-loaded stearylamine SLN.

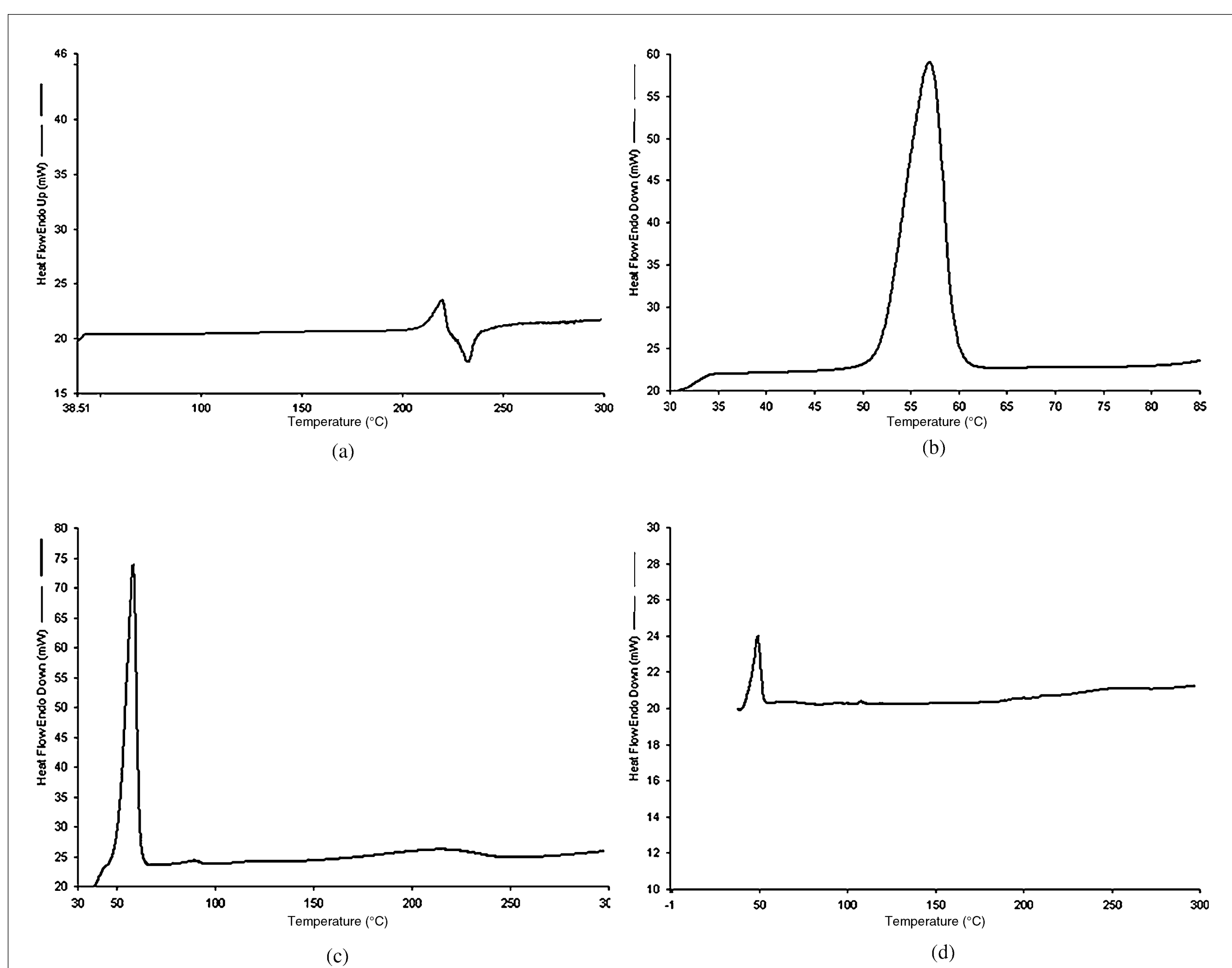


Fig. 4: DSC thermogram of (a) paclitaxel, (b) stearylamine, (c) physical mixture, and (d) paclitaxel-loaded stearylamine SLN.

2.3. *In vitro release studies*

The *in vitro* release kinetics of paclitaxel from SLN followed square root or Higuchi's kinetics, i.e. a matrix diffusion controlled mechanism. The slopes obtained from the Higuchi plot were proportional to an apparent diffusion coefficient (Table 3). The low initial burst was due to the smaller amount of drug adsorbed on the hydrophilic surface generated by the poly(ethylene oxide) part of poloxamer, and the subsequent slow and sustained release was due to diffusion of the molecularly dispersed drug from the inner lipid matrix (Fig. 5).

2.4. *Stability in simulated gastric fluid*

The present work aimed at oral administration of paclitaxel using SLN as delivery system, so the SLN were evaluated for their stability in simulated gastric medium. No aggregation was observed for SLN with a protective coating of poloxamer 188, whereas SLN in the absence of poloxamer 188 displayed immediate and substantial aggregation after incubation in gastric medium. The particle size of poloxamer-coated SLN remained unchanged, while HPLC analysis of the supernatant obtained after centrifugation showed a negligible amount of drug, suggestive of negligible lipid degradation in gastric medium at the time points studied. The sterically stabilizing properties of poloxamer 188 were not affected by the low pH.and provided a protective layer around the nanoparticles, preserving the structural integrity of the SLN.

Table 3: *In vitro* release kinetics of paclitaxel loaded SLN with different paclitaxel contents

Formulations	Higuchi Kinetics	
	r^2	slope
P-SLN1	0.9856	1.86
P-SLN2	0.9844	3.61
P-SLN3	0.9561	4.59

2.5. *Stability studies*

The stability studies showed that all the SLN formulations prepared had good stability over a period of at least three months with respect to particle size, shape, zeta potential and drug content, and did not show any lipid peroxidation, when stored at 4 °C (Fig. 6). In the present formulation, α-tocopherol was used as an anti-oxidant in the lipid phase and 15% w/v aqueous trehalose solution was used as a cryoprotectant during the lyophilisation process. The results revealed that more than 99.99% of the drug was retained for 90 days in all the SLN formulations during storage at 4 °C. The stability of the present SLN formulations was also observed at 25 °C/60% relative humidity [RH] and 40 °C/75% RH over 90 days. With increasng storage time, the zeta potential dropped at 40 °C/75% RH, as the outer hydrophilic poloxamer coating dissolved at high temperature and relative humidity leading to reduced steric stability of the system. This resulted in a significant increase in the particle size

Table 4: CC_{50} values of free paclitaxel and paclitaxel loaded SLN in HepG2 cell lines by MTT assay (mean ± S.D.)

Formulation	CC50 (μg/ml) Incubation time (h) 48	72
Paclitaxel	0.55 ± 0.05	0.26 ± 0.03
P-SLN2	0.021 ± 0.004	0.011 ± 0.002

n = 3; $p > 0.05$.

of the nanoparticles to the micrometer range due to aggregation. Approximately 50% and 10% of the initial drug content were retained in SLN after 90 days of storage at 25 °C/60% and 30 days of storage at 40 °C/75% RH, respectively. The lipid degradation was also confirmed by the formation of a red colored complex in the TBARS assay (Ohkawa et al. 1979).

2.6. *In vitro cytotoxicity assay*

The cellular uptake of the nanoparticles varied considerably depending on their size, chemical composition, hydrophobicity and charge. *In vitro* cytotoxicity studies by MTT assay measured the cytotoxic concentrations at 50% (CC_{50}) cell inhibiton and showed that the positively charged stearylamine lipid nanoparticles had a higher affinity to intestinal epithelia. Stearylamine based SLN exhibited low CC_{50} values at 48 and 72 h. The outermost coating of poloxamer 188 contributed to the cellular interaction (Wu et al. 2005) synergistically with the improved interaction of positively charged nanoparticles with the negatively charged cell surface. The drug loading of P-SLN2 was found to be ∼ 32%. Encapsulation of paclitaxel in SLN lowered cell viability and gave higher cytotoxicity compared with paclitaxel alone (Table 4). The CC_{50} of paclitaxel decreased from 0.55 μg/ml for free paclitaxel to 0.021 μg/ml for P-SLN2 and from 0.26 μg/ml for free paclitaxel to 0.011 μg/ml for P-SLN2, after 48 and 72 h of incubation, respectively. The cytotoxicity achieved with stearylamine based paclitaxel loaded SLN (positively charged) was much higher compared with a stearic acid based paclitaxel loaded SLN (negatively charged) prepared previously in our lab (Pandita et al. 2009), confirming the additive effect of nanoparticle composition and charge. Also, the stearylamine lipid matrix was well tolerated as no cytotoxicity of the unloaded SLN was observed on HepG2 cell line at the concentrations used. Thus, the stearylamine based paclitaxel loaded SLN system could be utilized more effectively for targeting the intestinal mucosa for uptake by Peyers' patches followed by cellular absorption. The present lipid-based system is proposed as a means of bypassing some of the chemical and physical barriers associated with paclitaxel.

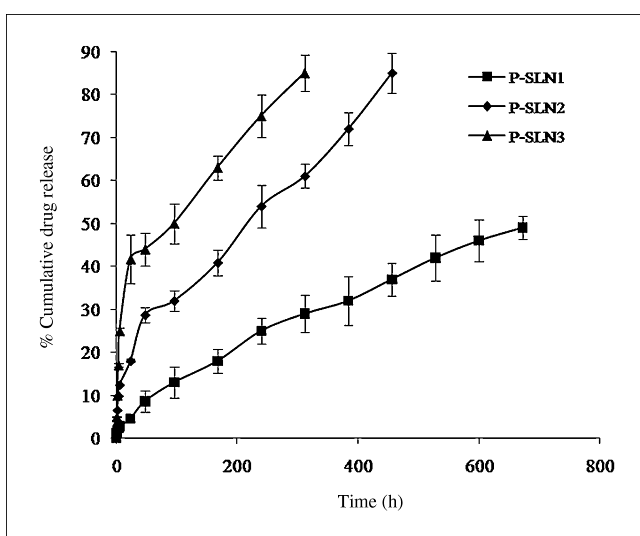
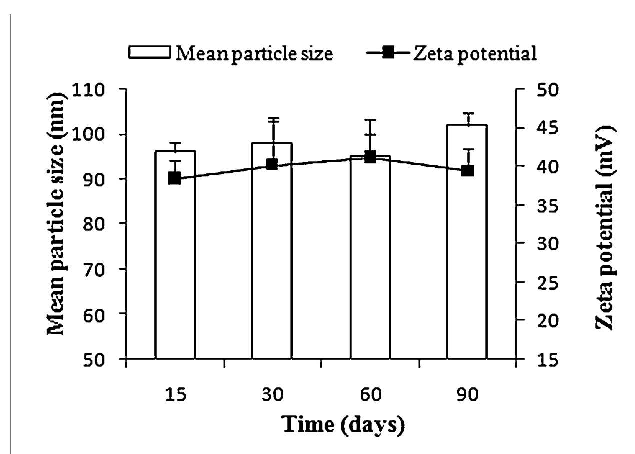
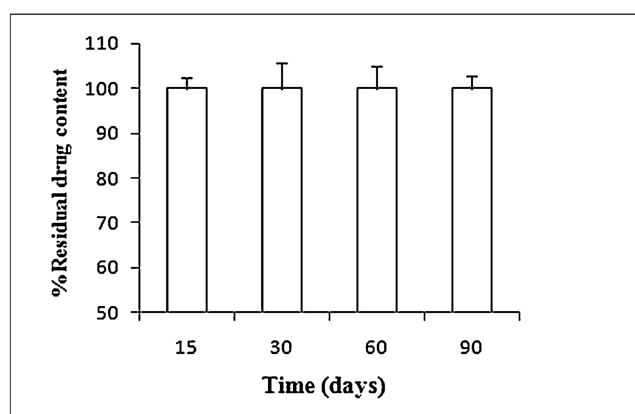


Fig. 5: *In vitro* drug release from P-SLN1, P-SLN2 and P-SLN3 with 0.05, 0.25, 0.5 mmol paclitaxel loading, respectively (mean ± S.D., n = 3).



(a)



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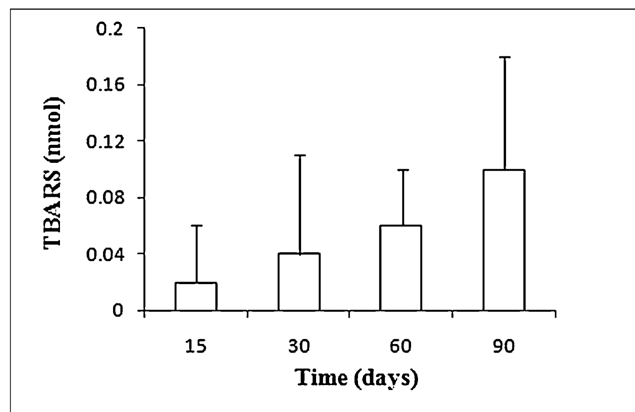

(c)

Fig. 6: Effect of storage on (a) mean particle size and zeta potential, (b) drug content, and (c) TBARS formation of paclitaxel loaded SLN formulations at 4 °C for different time intervals for three months (mean ± S.D., n = 3).

2.7. *Conclusions*

In the present study, stearylamine based SLN were prepared and optimized for use as a drug delivery platform for paclitaxel. The solvent injection method, a faster method for producing SLN, was optimized with respect to process parameters, i.e., amount injected and homogenization rate, and formulation, i.e., molar

ratios of lecithin, poloxamer 188, stearylamine and paclitaxel. Paclitaxel loaded SLN were characterized using PCS, TEM, XRD and DSC, and were found to exhibit good stability with ~ 75% entrapment efficiency. *In vitro* studies showed biphasic slow and sustained release following Higuchi's kinetics. The *in vitro* cytotoxicity of paclitaxel was significantly increased when encapsulated in SLN. Finally, the present SLN formulation offers the potential to provide improved formulations of poorly water-soluble drugs with improved stability and is suitable for developing a clinically useful system with targeting potential. Further studies are necessary to document their performance under *in vivo* conditions.

3. Experimental

3.1. Materials

Paclitaxel (MW 853.9) (99.87% w/w) was donated by Dabur Pharma Ltd. (India), Stearylamine (StA) was purchased from Sigma (USA), soya lecithin was obtained from BDH Laboratory (England). Poloxamer 188 (Pluronic® F-68) was obtained from BASF Corp., Sigma (USA). Dialysis tubing cellulose membrane, D9277 (avg. flat width 10 mm; 0.4 in.), which retains most proteins of molecular weight 12,000 or greater) was purchased from Sigma, USA. All other materials and reagents were obtained from Sigma unless otherwise stated and used without further purification.

3.2. Preparation of solid lipid nanoparticles

The method used for the preparation of SLN was based on a modified solvent injection technique (Schubert and Müller-Goymann 2003). Briefly, 5 ml of ether solution containing stearylamine, soya lecithin and α-tocopherol was injected through an injection needle into poloxamer solution maintained at $40 \pm 2\,^{\circ}C$ under continual stirring. The mixed system was homogenized for 1 h using a SilentCrusher M with dispersion tool 8F (Heidolph Instruments GmbH & Co. KG, Germany). The resultant dispersion was lyophilized in the presence of trehalose. The freeze dried SLNs were resuspended in distilled water prior to evaluation.

3.3. Physicochemical characterization of SLN

3.3.1. Measurement of particle diameter and zeta potential

Measurement of average diameter, polydispersity index and zeta potential was performed using photon correlation spectroscopy (PCS) (Zetasizer Nano ZS90, Malvern Instruments, UK). SLN dispersions were diluted 1:20 with filtered distilled water prior to analysis. The values given are an average of three measurements at 25 °C.

3.3.2. Drug entrapment efficiency (%EE) study

The %EE of the paclitaxel in SLN was evaluated using a modification of the procedure used by Dong et al. (Dong and Feng 2004). Approximately 3 mg of lyophilized SLN was subjected to DCM extraction. The DCM layer was evaporated and the residue was reconstituted with 50/50 (v/v) acetonitrile/water mixture and vortexed for 90 s. Quantitative analysis of paclitaxel in this extract was performed on 20 μl aliquots of the reconstituted sample by high performance liquid chromatography (HPLC) using a Thermo Finnigan LC surveyor system-photo diode array detector equipped with a reverse-phase LiChroCART® RP 18 analytical column (250 × 4 mm i.d., pore size 5 μm, Merck, LiChrospher® 100) and LiChroCART® RP 18 pre-column (4 × 4 mm i.d., pore size 5 μm, Merck, LiChrospher® 100). The mobile phase consisted of acetonitrile and purified water (70:30), with a flow rate of 1 ml/min and detection was at 227 nm with a photo diode array detector. The %EE and drug loading were calculated by applying Eqs. (1) and (2), respectively.

$$EE\,(\%) = (\text{amount of drug in } SLN_{(mg)}/\text{initial amount of } drug_{(mg)}) \times 100 \quad (1)$$

$$\text{Loading}\,(\%) = (\text{amount of drug in } SLN_{(mg)}/\text{amount of } SLN_{(mg)}) \times 100 \quad (2)$$

3.3.3. Transmission electron microscopy

A FEI Philips Morgagni 268D transmission electron microscope (TEM) (Philips, USA) was used to visualize the particles and evaluate the surface morphology of the particles. Samples were sprayed on copper grids after staining with 2% sodium phosphotungstic acid solution for contrast enhancement.

3.3.4. X-ray powder diffraction

X-ray diffraction pattern of paclitaxel powder and paclitaxel loaded SLN were recorded on a Bruker D8 ADVANCE X-ray diffractometer at room temperature with Cu as source of radiation at 30 kV and 40 mA. The scan range of 2θ was 3–40° at a rate of 0.050° with a counting time of 2 s for each step.

3.3.5. Thermal analysis of freeze dried SLNs

Differential scanning calorimetry (DSC) analysis was performed using a differential scanning calorimeter (Perkin Elmer Pyris 6 DSC, USA) at a heating rate of 10 °C/min over a temperature range of 35–300 °C under an inert nitrogen atmosphere at a flow rate of 50 ml/min. A sample of about 10 mg was taken for analysis in standard aluminium DSC pans.

3.4. In vitro release study

The *in vitro* experiment was performed using a diffusion technique to determine the amount of paclitaxel released from each formulation. A dialysis membrane with a molecular weight cut-off of 12 KDa containing 1 ml of SLN dispersion (3.1 mg/ml) with a paclitaxel concentration of 1 mg/ml was used as the donor compartment. The receptor medium was 15 ml phosphate buffer saline (PBS), pH 7.4, containing 0.1% (v/v) Tween 80 to maintain a sink condition, stirred by magnetic bar at 120 rpm and maintained at 37 °C. At fixed time intervals, the complete receptor medium was replaced by freshly prepared receptor medium. The samples were analysed in triplicate by HPLC as described previously. All the experiments were done in triplicate.

3.5. Stability study in simulated gastric fluid

SLN formulations were incubated at 37 °C in simulated gastric medium (USP XXIV, pH 1.2, pepsin 0.32% w/v). Samples were collected at times 0, 1 and 3 h and centrifuged for 5 min at 20000 rpm to precipitate particle aggregates and enzymes. The supernatant was subjected to an extraction procedure for paclitaxel and analyzed by HPLC to test for the leak/presence of paclitaxel and thus lipid degradation. The stability of SLN was evaluated in terms of particle aggregation, and drug content. Size of the particles in the dispersion was measured by PCS.

3.6. Stability studies of paclitaxel-loaded SLN

The stability of each SLN formulation was evaluated in terms of particle size, morphology, zeta potential, drug content and TBARS assay after storage at 4 °C followed over 15, 30, 60 and 90 days.

3.7. Cell cytotoxicity study

HepG2 human hepatoma cell line (ATCC No.HB-8065) was cultured in minimum essential medium (Gibco, USA) supplemented with Earl's salt (1.5 g/L), fetal calf serum (10%), L-glutamine (2 mM), sodium pyruvate (1%), streptomycin (100 mg/mL), penicillin (100 IU/mL) and amphotericin B (0.25 mg/mL). The cells were grown at 37 °C in a humidified atmosphere containing 5% CO_2. The cells were seeded at a density of 1×10^5 cells/ml in serum containing medium in 96-well plates. When the culture reached confluency (typically 24 h after plating), the medium was replaced with fresh medium and all the test formulations at varying concentrations were added and incubated for 72 h. 4 h prior to completion of incubation, 20 μl of 5 mg/ml 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) was added to each well and incubated further for the remaining time. Then the medium was removed and 100 μl of dimethyl sulphoxide was added. Plates were incubated for 30min at 37 °C and absorbance was measured at 550 nm using a microplate reader (PowerWave X, BIO-TEK Instruments Inc.). The 50% cell cytotoxic concentration (CC_{50}) of paclitaxel and the paclitaxel loaded SLN formulations against the HepG2 cell line at 48 h and 72 h was determined.

3.8. Statistical analysis

Values were expressed as mean±SD. Statistical significance of differences was examined using one-way analysis of variance (ANOVA) followed by LSD post-hoc test. A probability value (p) of less than 0.05 was considered to be statistically significant.

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