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FORMULATION, OPTIMIZATION AND CHARACTERIZATION *TUMOR TARGETING SOLID LIPID NANOPARTICLES OF PACLITAXEL*

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ABSTRACT

Tumors are characterized by poorly differentiated, highly chaotic arrangement of vessels which have unsealed endothelial cell–cell junctions and discontinuous basement membrane. These are characterized by interstitial hypertension, low extracellular pH, hypoxia, angiogenesis, tumor– stromal cell interaction, cancer stem cells, and abnormal lymphatics and key microenvironmental features of solid tumors greatly impact the extravasation of polymeric nanomedicines like solid lipid nanoparticles (SLNs). Solid Lipid Nanoparticles (SLNs) are rapidly developing field of nanotechnology is one of the approaches to improve bioavailability by targeting the antineoplastic drug Paclitaxel (PTX) to the tumor. The present research work is to formulate and optimize the Paclitaxel SLNs by simple solvent injection method. Solid Lipid Nanoparticles (SLNs) of Paclitaxel were formulated by taking Tristearin and Stearylamine as lipid matrix, Tween 80 as surfactant and soya lecithin as co emulsifier. Sonication and stirring were important process to optimize the size of Solid Lipid Nanoparticles. The optimization was carried out of various variables on the basis of particle size, shape, and polydispersity index and drug entrapment efficiency. Solid Lipid Nanoparticles (SLNs) of Paclitaxel optimized with Tristearin and Stearylamine ratio (1.5:1.0), drug lipid ratio(1:10) with 1% of Tween80, stirring at speed about 3000 for 60

minutes and 2 minutes of sonication for minimum size. The optimized Formulation of SLN showed a % cumulative drug release of 79.94±1.88% up to 48 hours in PBS (pH 7.4).

KEYWORDS: Tumor, Solid Lipid Nanoparticles (SLNs), Paclitaxel (PTX), Tristearin, Stearylamine.

INTRODUCTION

Tumors are characterized by poorly differentiated, highly chaotic arrangement of vessels which have unsealed endothelial cell–cell junctions and discontinuous basement membrane. Due to their irregular organization, tumor microvessel walls are leaky and exhibit heterogeneous hyperpermeability compared to normal tissue.[1] Unlike normal tissues, tumors have functionally defective lymphatic vessels because cancer cells compress lymphatic vessels causing their collapse.[2] To develop potent therapeutic approaches for site-specific delivery of drugs to tumors using polymeric nanomedicines, it is imperative to understand the microenvironment of the tumor. Solid tumors are organ-like entities arising from stem cell populations and consist of cancer cells, non transformed stromal cells, blood vessels and the interstitium. Tumors shape their microenvironment and support the tumor and nonmalignant cells. Tumor microenvironments greatly influence its development and generate barriers that prevent therapeutic agents from accessing and killing cancerous cells in the tumor thereby limiting the efficacy of current chemotherapy. Interstitial hypertension, low extracellular pH, hypoxia, angiogenesis, tumor–stromal cell interaction, cancer stem cells, and abnormal lymphatics are key microenvironmental features of solid tumors greatly impact the extravasation of polymeric nanomedicines.[3]

The solid lipid nanoparticles (SLNs) are sub micron colloidal carriers (50-1000 nm), which are composed of physiological lipid dispersed in water or in an aqueous surfactant solution.[4] SLNs are colloidal carriers for drugs combine the advantage of polymeric nanoparticles, fat emulsions and liposomes simultaneously and avoiding some of their disadvantages.[5-6] These Nanoparticles made from solid lipids are attained major attraction as novel drug carrier for intravenous and nasal application as they have been proposed as an alternative particulate carrier system.

Paclitaxel extracted from the Pacific yew tree *Taxus brevifolia* with antineoplastic activity. Paclitaxel binds to tubulin and inhibits the disassembly of microtubules, thereby resulting in the inhibition of cell division. This agent also induces apoptosis by binding to and blocking

the function of the apoptosis inhibitor protein Bcl-2 (B-cell Leukemia 2).[7]

The present research work is to formulate and optimize the Paclitaxel SLNs by simple solvent injection method.

MATERIAL AND METHOD

Paclitaxel (PTX) obtains as a gift sample from Sun Pharmaceutical Industries Ltd, Gujarat (India). Soya Phosphatidyl Choline (SPC) was procured form Himedia. All other reagents and solvents were of analytical grade and were purchased from local suppliers. Deionised water was used throughout the study.

Preformulation Study

Preformulation studies are needed to ensure the development of a stable as well as therapeutically effective and safe dosage form and preformulation studies of the drug PTX include identification, physical appearance, melting point, solubility, standard curve and partition coefficient by authenticated methods described by various authors.

Determination of □Max

The PTX (10.0 mg) was accurately weighed and dissolved in approximately 5 ml of methanol. Volume was made up to 100 ml with 30:70 methanols: PBS (pH 7.4) to give a stock solution of 100 □g/ml. Then make 2 µg/ml aliquots and scanned between 200-400 nm absorption maxima on a UV/Visible spectrophotometer (Shimadzu 1800, Japan).

Drug Compatibility Studies with Selected Lipids

Drug compatibility with soya phosphatidyl choline (SPC) and Tristearin was studied. Solution of PTX (20□g/ml) was prepared in PBS (pH 7.4) using methanol as co solvent. Then, accurately weighed lipid (10 mg) transferred separately into 10 ml volumetric flasks containing drug solution. Absorbance was observed for each solution using UV Spectrophotometer against respective blank solution.

Preparation of Slns

The solid lipid nanoparticles were prepared by solvent injection method as reported by Hu *et al*., 2008.[8] Tristearin, soya lecithin, stearylamine and drug (10mg) were taken into different ratio and were dissolved in minimum quantity of absolute alcohol and heated about 70 ^{0}C in a beaker. Tween 80 (0.5% v/v) was dissolved in distilled water and heated at about 70 ^{0}C. Then the organic phase i.e. alcoholic solution containing lipid mixture and drug was added to

preheated aqueous solution at the same temperature (about 70^0C) at constant stirring. The preformed lipid suspension was then sonicated by using probe sonicator to form solid lipid nanoparticles (SLNs).

Optimization of Formulation and Process Variables

Various formulation variables i.e., lipid ratio, drug lipid ratio, lipid stearylamine ratio, emulsifier concentration and process variables i.e. stirring speed, stirring time and Sonication time which affect the preparation and properties of solid lipid nanoparticles were identified and studied. The optimization was carried out on the basis of particle size, shape, and polydispersity index and drug entrapment efficiency.

Optimization of Tristearin/Soya lecithin ratio

For optimization of lipids ratio, the SLNs formulations were prepared with varying ratio of two lipids i.e. tristearin and soya lecithin in the different ratios (viz. 1:0.5, 1:1, 1.5:1, 1:2 %w/w) keeping other parameters constant. Optimization was done on the basis of average particle size and poly dispersity index (PDI) of SLNs, which were determined using Zetasizer DTS ver.

Optimization of lipid stearylamine ratio

For optimization of lipid stearylamine ratio formulation was performed **in** various formulations containing different lipid stearylamine ratio (100:0.5, 100:1.0, 100:1.5 and 100:2.0 w/w) in selected batch containing Tristearin/Soya lecithin constant ratio. Optimization of lipid stearylamine ratio was done on the basis of two parameters average particle size and PDI.

Optimization of surfactant concentration

For optimization of concentration of Tween 80 was performed in previously optimized batch. Keeping the other parameters constant, SLN formulations were prepared using different concentration of Tween 80 in aqueous medium.

Optimization of stirring speed

For the optimization of stirring speed, was performed in optimized batch for Tristearin/Soya lecithin ratio, stearylamine and surfactant with different stirring speed (1000, 2000, 3000, 4000 rpm) and average particle size with their percent drug entrapment efficiency.

Optimization of stirring time

For the optimization of stirring time, previsouly selected batch with stirring speed to prepare solid lipid nanoparticles with different stirring time (30, 45, 60, 75 min.) and average particle size along with drug entrapment.

CHARACTERIZATION OF SLNs

Particle size determination

The average particle size and size distribution of the solid lipid nanoparticles were determined by photon correlation spectroscopy using a Zetasizer DTS ver. 4.10 (Malvern Instrument, UK). The samples of solid lipid nanoparticle dispersions were diluted to 1:9 v/v with deionized water. The particles size and size distribution were represented by average (diameter) of the Gaussion distribution function in the logarithmic axis mode.[9]

Surface charge measurement

The surface charge of solid lipid nanoparticle was determined by measurement of zeta potential (□) of the lipid nanoparticles calculated according to Helmholtz-Smoluchowsky from their electrophoretic mobility. For measurement of zeta potential, Zetasizer DTS ver 4.10 (Malvern Instrument, UK) was used. The field strength was 20 V/cm on a large bore measures cell. Samples were diluted with double distilled water adjusted to a conductivity of 50 □S/cm with a solution of 0.9% NaCl.[10]

Particle Morphology (TEM)

Transmission electron microscope was used as a visualizing aid for particle morphology. The sample (10µL) was placed on the grids and allowed to stand at room temperature for 90 sec. Excess fluid was removed by touching the edge with filter paper. All samples were examined under a transmission electron microscope (Philips Morgagni 268, Eindhoven, Netherlands) at an acceleration voltage of 100 kV, and photomicrographs were taken at suitable magnification (PM 4.11-4.12).

Entrapment Efficiency

Drug entrapment of the PTX in SLNs was determined by dispersing the known molar concentration of paclitaxel loaded SLNs in cellulose dialysis bag (MWCO 1000 Da, Sigma, Germany). This solution was dialyzed with help of magnetic stirring (50 rpm; Remi, Mumbai, India) in cellulose dialysis bag against PBS (pH 7.4) under sink condition for 10 min to remove any unentrapped drug from the formulation. One mL aliquot was withdrawn

and diluted ten times in a volumetric flask with 30:70 methanols: PBS (pH 7.4). Absorbance was measured spectrophotometrically (Schimadzu, 1800 Japan) at 237 nm to indirectly estimate the amount of drug entrapped within the system. The dialyzed formulation was then lyophilized and further characterized.[11,12]

RESULT AND DISCUSSION

Preparation of plain SLNs was carried out employing solvent injection method, which involves the rapid diffusion of solvent across the solvent-lipid phase into the aqueous phase. Tristearin, PC, stearylamine and drug were dissolved in ethanol, maintained at an elevated temperature of 70^0C with continuous stirring. This solution was injected into an aqueous solution of Tween 80 maintained at the same temperature given above with continuous stirring and sonicated under probe sonicator.

The prepared nanoparticulate formulation was optimized for various parameters like lipid lecithin ratio, drug-lipid ratio, surfactant ratio, stirring time, stirring speed and sonication time to obtain nanosized SLNs with maximum drug entrapment.

Lipid employed in the production of SLNs was first subjected to optimization by varying the ratio of Tristearin: PC from 1:0.5 to 1:2, keeping Tristearin quantity as constant. The prepared formulation was characterized on the basis of particle size and PDI. It was observed that upon increasing the concentration of tristearin, an increase in the size and PDI was observed. This may be due to the increase in the surfactant behavior of the tristearin on the formulation (Table 1).Optimized formulation L_3 with 1.5:1.0 tristearin/ lecithin ratio was selected for further optimization as it showed an optimum size i.e., 210.4±2.41 nm and PDI of 0.234 (Table 1 and figure 1).

Table 1: Optimization of lipid/lecithin ratio.

S. No.	Formulation Code	Tristearin/Soya Lecithin Ratio % w/w	Average Particle Size (nm)	Polydispersity Index (PDI)
1.	L_1	1.0 : 0.5	327.4 ± 3.15	0.412
2.	L_2	1.0 : 1.0	280.2 ± 3.49	0.341
3.	**L_3**	**1.5 : 1.0**	**210.4 ± 2.41**	**0.234**
4.	L_4	2.0 : 1.5	258.1 ± 2.39	0.355

S.D. ± Mean (n=3)

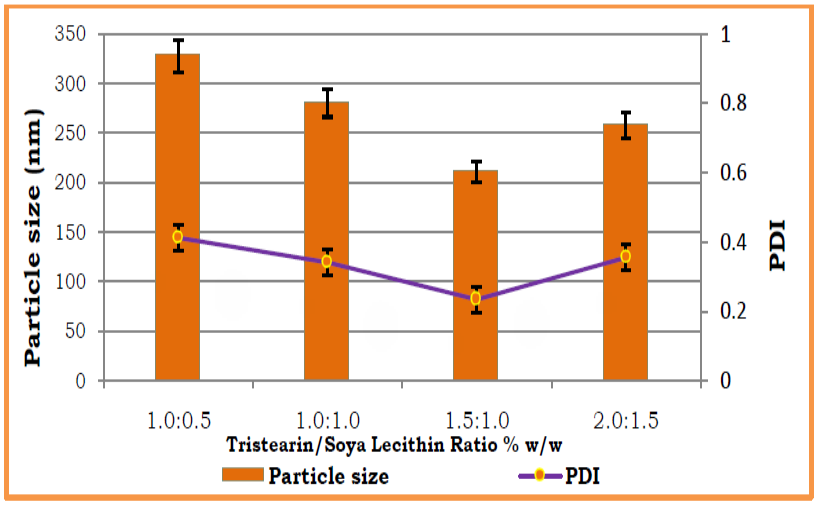

Figure 1: Optization of lipid/lecithin ratio.

Stearylamine (SA) ratio was optimized keeping lipid ratio constant and it was observed that particle size tends to increase from 209.6±4.32 to 291.2±7.85 with an increase in the amount of SA. Stearylamine provides positive charge to the SLN surfaces. Thus positively charged groups on the surface of nanoparticles repel each other and hence the particles obtained were of bigger size. L_3A_2 with particle size 237.6±6.14 and PDI 0.228 were selected for optimization of other variables (Table 2 and Figure 2).

Table 2: Optimization of Lipid/Stearylamine ratio.

S. No.	Formulation Code	Lipid/SA Ratio (mg)	Average Particle Size (nm)	PDI
1.	L_3A_1	100: 0.5	209.6 ± 4.32	0.371
2.	**L_3A_2**	**100:1.0**	**237.6 ±6.14**	**0.228**
3.	L_3A_3	100:1.5	259.7 ± 0.96	0.257
4.	L_3A_4	100:2.0	291.2 ± 7.85	0.284

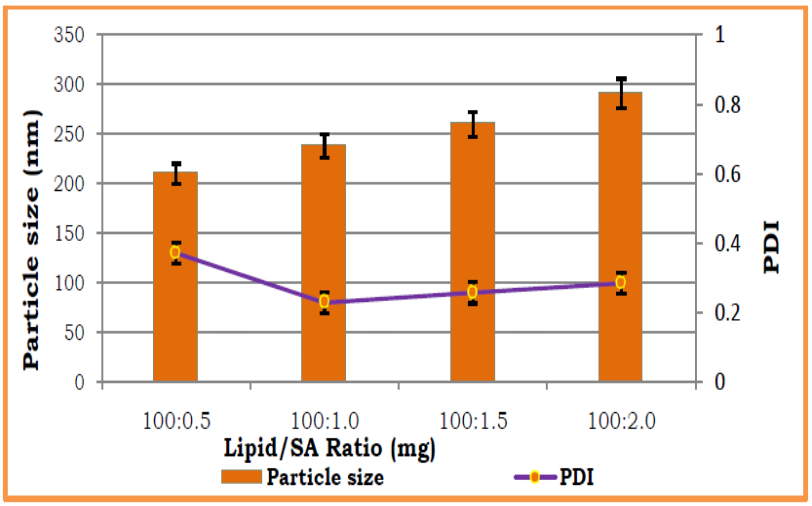

Figure 2: Optimization of Lipid/Stearylamine ratio.

The effect of tween 80 was also observed on particle size and PDI. The particle size was found to be decreased upon increasing the concentration of tween 80. This might be due to the decrease in the surface tension between organic phase and aqueous phase, which ultimately seem to allow formation of nano range particles. Particles of optimum size 231.6±2.6 nm with PDI 0.250 were obtained at 1% surfactant concentration. However on further increasing surfactant concentration, the particle size increases because of formation of aggregates. Hence $L_3A_2S_2$ was selected for further optimization of the formulation (Table 3 and Figure 3).

Table 3: Optimization of Surfactant Concentration.

S. No.	Formulation Code	Tween 80 Concentration (% w/v)	Average Particle Size (nm)	PDI
1.	$L_3A_2S_1$	0.5	315.5 ± 2.7	0.315
2.	**$L_3A_2S_2$**	**1.0**	**237.6 ± 2.6**	**0.250**
3.	$L_3A_2S_3$	1.5	247.1 ± 3.2	0.273
4.	$L_3A_2S_4$	2.0	274.5 ± 2.9	0.281

S.D. ± Mean (n=3)

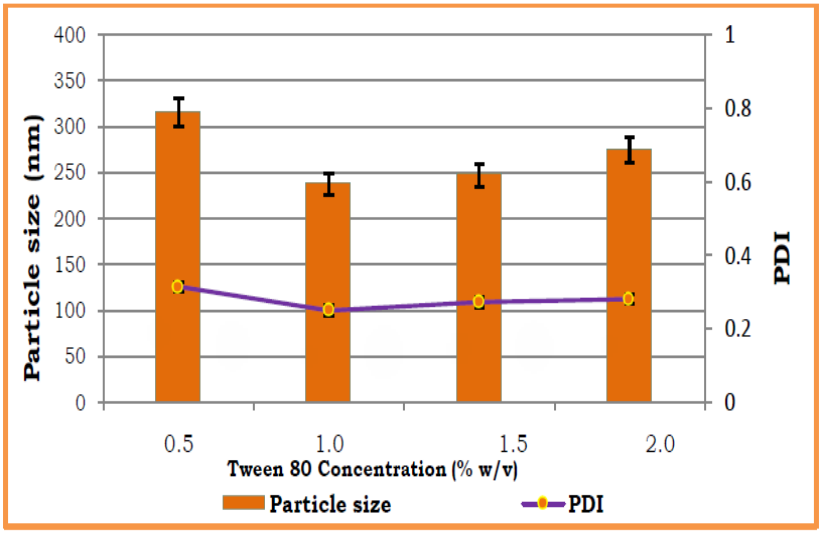

Figure 3: Optimization of Surfactant Concentration.

Drug-lipid ratio was also optimized on the basis of particle size and percent drug entrapment. It was observed that on increasing the amount of drug, the entrapment efficiency increased up to drug: lipid ratio of 10:100 while on further increasing drug concentration in the lipid, the entrapment efficiency was found to be constant (Table 4 and Figure 4). This could be due to the saturation of lipid bilayers with the drug. Same effect was observed on the particle size also. So $L_3A_2S_2D_2$ was selected for further optimization of process variables.

Table 4: Optimization of Drug/ Lipid ratio.

S. No.	Formulation Code	Drug/Lipid Ratio % w/w	Average Particle Size (nm)	% Drug Entrapment
1.	$L_3A_2S_2D_1$	5.0 : 100	207.7 ± 2.80	69.4 ± 0.5
2.	$L_3A_2S_2D_2$	10.0 : 100	233.1±1.97	83.7 ± 0.6
3.	$L_3A_2S_2D_3$	15.0 : 100	260.6 ± 3.12	83.4 ± 0.4
4.	$L_3A_2S_2D_4$	20.0 : 100	261.3 ± 2.94	83.6 ± 0.2

S.D. ± Mean (n=3)

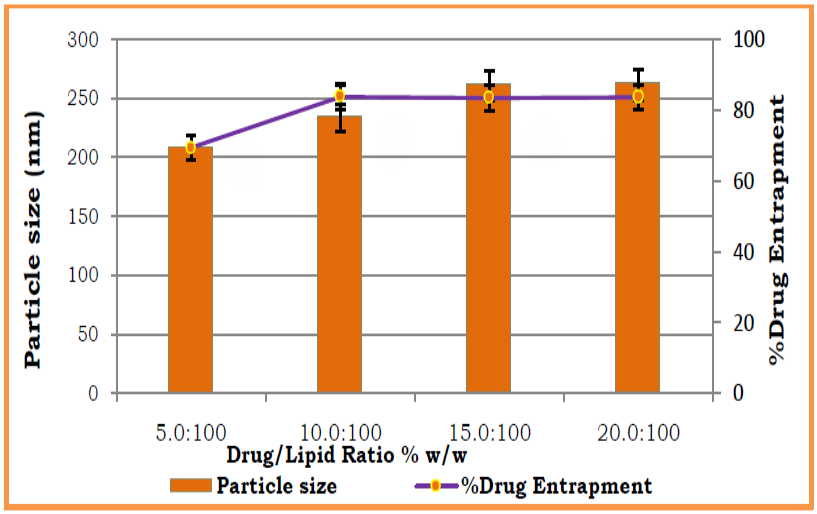

Figure 4: Optimization of Drug/ Lipid ratio.

$L_3A_2S_2D_2$ showed varied effects on varying stirring speed and time. Particle size and percent drug entrapment decreases from 290.5±4.3 to 207.3±5.8 and from 83.1±0.5 to 79.4±0.4 respectively, on increasing the stirring speed from 1000 to 4000 rpm (Table 5.5). On varying stirring time from 30 minutes to 75 minutes the size decreased from 305.4±4.1 to 205.5±3.0 and percent drug entrapment decreases from 82.9±0.4 to 79.4±0.7 (Table 5). This may be due to breaking of upper surface of the particles because of high speed and duration of stirring. On the basis of decreased particle size with maximum drug entrapment formulation $L_3A_2S_2P_3T_3D_2$ was further study for optimization of sonication time (Table 5 and figure 5).

Table 5: Optimization of Stirring Speed.

S. No.	Formulation Code	Stirring Speed (rpm)	Average Particle Size (nm)	%Drug Entrapment
1.	$L_3A_2S_2D_2P_1$	~1000	290.5 ± 4.3	83.1 ± 0.5
2.	$L^3A_2S_2D_2P_2$	~2000	245.8 ± 2.1	82.5 ± 0.9
3.	$L_3A_2S_2D_2P_3$	~3000	215.4 ± 3.9	81.8 ± 0.7
4.	$L_3A_2S_2D_2P_4$	~4000	207.3 ± 5.8	79.4 ± 0.4

S.D. ± Mean (n=3)

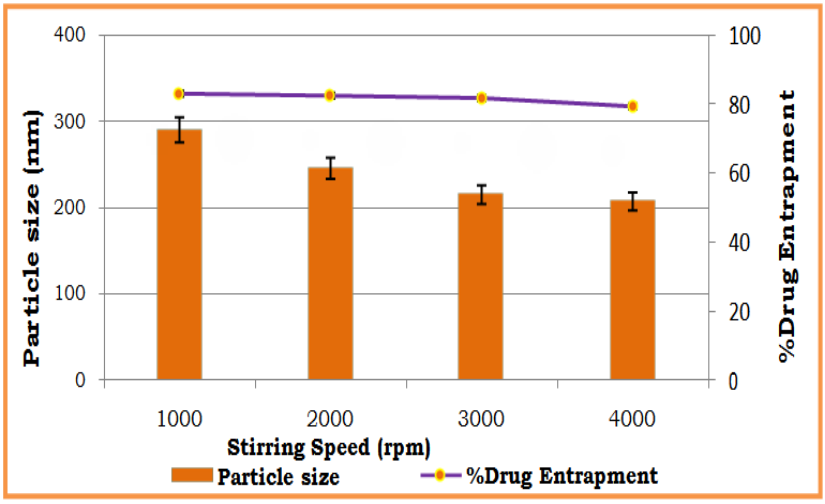

Figure 5: Optimization of Stirring Speed.

For the optimization of sonication time, formulation $L_3A_2S_2D_2P_3T_3$ was selected to prepare solid lipid nanoparticles with different sonication time (1, 2, 3, 4 min.). Sonication time 2.0 minutes was found to be optimum for the formulation of small sized particles (201.1±3.7nm) with maximum drug entrapment efficiency (79.3±0.5 %).

Table 6: Optimization of Sonication time.

S. No.	Formulation Code	Sonication Time (min)	Average Particle Size (nm)	%Drug Entrapment
1.	$L_3A_2S_2\ D_2P_3T_3$ I	1.0	281.2 ± 2.1	83.5 ± 0.7
2.	**$L_3A_2S_2D_2P_3T_3$ J**	**2.0**	**201.1 ± 3.7**	**79.3 ± 0.5**
3.	$L_3A_2S_2D_2P_3T_3$ K	3.0	198.7 ± 1.9	77.6 ± 0.6
4.	$L_3A_2S_2D_2P_3T_3$ L	4.0	175.4 ± 2.5	72.3 ± 0.5

S.D. ± Mean (n=3)

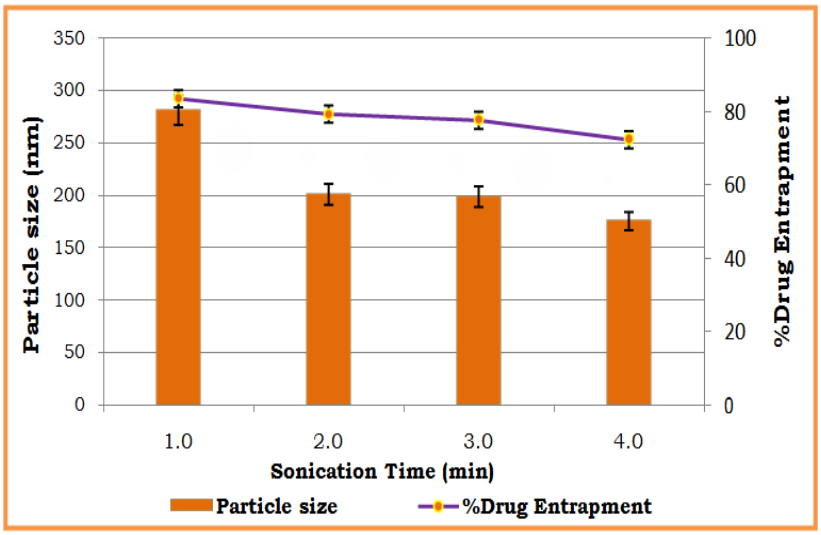

Figure 6: Optimization of Sonication time.

Drug entrapment was determined by dialysis using a dialysis membrane and was found to be 79.3±0.5%. Keeping all optimization parameters under consideration, entrapment was found to be optimum in the selected formulation (Table 7).

Table 7: Optimized Parameter of Optimized Formulation ($L_3A_2S_2D_2P_3T_3J$).

S.No.	Parameter	Optimized Value
1.	Tristearin: Soya Lecithin ratio	1.5 : 1.0
2.	Drug: Lipid ratio	10:100 mg
3.	Surfactant concentration	1% w/v
4.	Stirring Time	60 min
5.	Stirring Speed	~3000 rpm
6.	Sonication Time	2 min

$L_3D_2A_2S_2P_3T_3$ J, where,

L = Lipid /Lecithin ratio; A = Lipid Stearylamine ratio; S = Concentration of tween 80; D = Drug

P = RPM (stirring speed); T = Stirring time; J = Sonication time

In vitro drug release study of formulations was carried out using dialysis tube. Formulation SLN showed a % cumulative drug release of 79.94±1.88% up to 48 hours in PBS (pH 7.4). (Table 8).

Table 8: Particle Size, PDI, % Drug Entrapment.

Formulation Code	Particle Size (nm)	Polydispersity Index (PDI)	% Drug Entrapped	% cumulative drug release
($L_3D_2A_2S_2P_3T_3$ J)	201.1 ± 3.7	0.234	31.09±0.71	79.94±1.88%

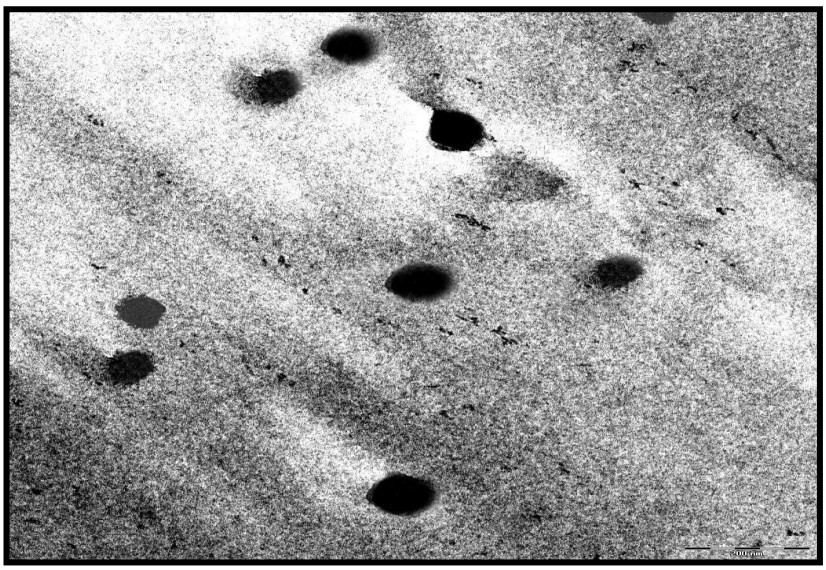

Figure 5.9: TEM image of plain SLNs

CONCLUSION

The Solid lipid nanoparticles were successfully formulated, optimized and characterized of Paclitaxel. SLNs were prepared employing solvent injection method, which involves the rapid diffusion of solvent across the solvent-lipid phase into the aqueous phase. Physicochemical characterization including particle size, particle size distribution, Zeta potential, scanning electron microscopy, and in-vitro release profile were carried out.

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