

EMULSOMES FOR LIPOPHILIC ANTICANCER DRUG DELIVERY: DEVELOPMENT, OPTIMIZATION AND *IN VITRO* DRUG RELEASE KINETIC STUDY

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ABSTRACT

Objective: The objective of the present study was to formulate and characterize paclitaxel (Ptx) loaded sterically stabilized emulsomes to provide non-toxic and biocompatible carriers with high Ptx loading efficiency.

Methods: Plain (P-Es) and sterically stabilized emulsomes (SS-Es) were prepared by a modified solvent evaporation method using tristearin as solid lipid and optimized for lipid to (DSPC+CHOL+DSPE-PEG)/ tristearin ratio, lipid/lipid-PEG (DSPC+CHOL/DSPE-PEG) molar ratio, solid lipid concentration, phospholipid concentration, organic to aqueous phase volume and homogenization time based on their effect particle size and entrapment efficiency. Optimized emulsomes were characterized for morphological features, *in vitro* drug release kinetics and protection from plasma protein.

Results: The emulsomes so formed were uniform in size with a mean particle diameter of 275 ± 5.52 and 195 ± 6.4 nm for P-Es and SS-Es respectively. All the formulations showed pH dependent drug release with a slow and sustained release profile. Slower drug release was observed from sterically stabilized emulsomes than the plain emulsomes. The drug release profile followed the Higuchi model with the Fickian diffusion pattern. The Pegylation of emulsomes significantly reduced the *in vitro* protein absorption.

Conclusion: The sterically stabilized emulsome can serve as a novel non-toxic platform with longer circulatory time for the delivery of Paclitaxel and other poorly water-soluble drugs as well.

Keywords: Emulsomes, Lipophilic, Paclitaxel, Pegylation, Drug release kinetics

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INTRODUCTION

Development of nanotechnology-based novel carriers has revolutionized the field of drug delivery. The nanocarrier offers ample opportunities to be modified and functionalized to suit the physicochemical requirements of individual drug entities thereby, allowing administration of almost all types of chemotherapeutics and biological molecules. In the recent decade, continuous efforts are being focused on nanocarrier based tumorspecific anticancer drug delivery to minimize systemic leakage. Most of the approved anticancer drugs are lipophilic which presents solubility problems in preparing pharmaceutically acceptable formulation with maximum efficacy and minimum toxicity [1, 2]. Co-solvents based techniques are most widely used to improve the solubility of poorly soluble drugs, which are reported to cause toxic effect and hypersensitive reactions upon administration [3]. Extensive research on nanoparticles has resulted in drug delivery systems capable of protecting bioactive, improve solubility, minimize leakage and avoid the use of toxic solubilizers. Most of these nanovehicles are comprised of polymer or lipid or polymer-lipid hybrid. Paclitaxel is the most widely used taxane for the treatment of a wide variety of tumors [4, 5]. Its intravenous formulation is marketed as Taxol® wherein Cremophor EL and ethanol (50:50 v/v) are used as a solubilizer. Cremophor is associated with several side effects rendering Taxol® clinically unacceptable [6]. To overcome this limitation emulsomes based formulation is proposed. Emulsomes have emerged to provide a biocompatible platform along with the potential to accommodate lipophilic drugs in large amount. They are comprised of two structural components, a lipidic core made from solidified fatty acid and phospholipid/s forming stabilizing envelope around the core, act as a stabilizer. The fatty core can hold hydrophobic drugs in a higher amount [7]. Pharmaceutically stable emulsomal formulation can be prepared without the need for additional surfactant or solubilizer. The lipophilic drug is well retained within the lipidic core of emulsome resulting in minimum leakage into systemic circulation thereby, safeguarding the healthy tissue from unwanted side effects. Furthermore, the surface

phospholipid of emulsomes can be easily pegylated to enhance stability and residence time in blood circulation. The conventional anticancer dosage forms present the issue of poor patient compliance because of repeated dosing requirements. Hence, nanocarriers are designed to provide controlled drug delivery at the tumor site with desired release kinetics for a prolonged period ranging from days to years. The formulation is intended to function as a biocompatible drug carrier providing prolonged drug release, minimum systemic leakage and reducing dosing frequency with better patient compliance. The Ptx release pattern from emulsomes will be analyzed by fitting *in vitro* drug release data into various mathematical kinetic models. Therefore, in the present study, Ptx loaded sterically stabilized emulsomes are envisaged as a potential drug delivery system for the anticancer drug with predictive drug release kinetics.

MATERIALS AND METHODS

Materials

Paclitaxel was obtained as a gift sample from Neon laboratories ltd. Mumbai, India. DSPC and Cholesterol and DSPE-PEG were obtained as a gift sample from Lipoid GmbH, Germany. Tristearin was purchased from Sigma Aldrich Chemie, GmbH. All other reagents and chemicals used were of analytical grade and purchased from a local chemical supplier.

Methods

Formulation of plain emulsomes (P-Es) and sterically stabilized emulsomes (SS-Es)

Plain (P-Es) and sterically stabilized emulsomes (SS-Es) were synthesized from tristearin (solid lipid core), DSPC and cholesterol (outer stabilizing envelope) and DSPE-PEG (steric stabilizer) using a modified single emulsification-solvent evaporation method resulting in self-assembly [8, 9]. Weighed ratios of solid lipid were melted at 80 °C and then the drug previously dissolved in aqueous ethanolic solution (5 ml) was added to it under constant stirring (1000 rpm) on a magnetic stirrer while maintaining the temperature to 80°C.

The solution was stirred for 15 min at 1000 rpm to form homogeneous dispersion. Phospholipids and DSPE-PEG (for SSE) were dispersed in distilled water, heated to the same temperature and then stirred vigorously for 10 min at 80 °C at 5000 rpm. The organic phase was added dropwise (1 ml/min) into the aqueous phase while maintaining the temperature at 80 °C. After complete addition of the organic phase, the emulsion was stirred vigorously for 30 min at 10,000 rpm. The resulting emulsion was cooled to 4 °C on an ice bath resulting in the assemblage of phospholipids around the solid lipid core followed by the evaporation of the organic solvent. The emulsion was dialyzed using a dialyzing membrane with a molecular weight cut off of 10kDa to remove the remaining organic solvent and free molecules. The dispersion was sterilized by filtering through a filter unit with a 0.22 micrometer pore size. The emulsomes were then lyophilized using 5% sucrose as cryoprotectant and stored at 4 °C until use.

Optimization of emulsome formulations

Each of the emulsome formulations was optimized for phospholipid (PL: DSPC+CHOL+DSPE-PEG)/solid lipid (SL: tristearin ratio), lipid/lipid-PEG (DSPC+CHOL/DSPE-PEG) molar ratio, solid lipid concentration, phospholipid concentration, organic to aqueous phase volume and homogenization time based on their effect particle size (PS) and entrapment efficiency (EE).

Characterization of emulsome formulation

Vesicle size and size distribution

Maintaining constant size and size distribution for a prolonged period is an indication of stability of emulsome. Electron microscopy is widely used for the assessment of surface morphology, size and size distribution of emulsomes. Besides the routine laboratory techniques such as gel chromatography etc., techniques based on light scattering and electron microscopy are needed to be applied for the statistically significant analysis of size and size distribution of the carriers.

The average vesicle size and size distribution were determined by photon correlation spectroscopy using zetasizer (Nanoplus 5.01, particulate system). The sample of dispersion was diluted to 1:9 ratio with deionized water. The vesicle size distribution graph is represented by the average vesicle size (diameter) and polydispersity index.

Zeta potential

The zeta potential of a particle is the overall charge that the particle acquires in a particular medium. The knowledge of the zeta potential can help to predict the fate of the preparation *in vivo* and assess the stability of colloidal systems. Zeta potential of emulsome formulations was determined by photon correlation spectroscopy using Zetasizer Nanoseries (Nanoplus 5.01, particulate system) using a flow-through cell.

Shape and surface morphology

Shape and surface morphology of emulsome was determined by Transmission Electron Microscope (TEM) technique. The sample (10 µl) was placed on the grids and allowed to stand at room temperature for 90 s. Excess of the fluid was removed by touching the edge filter paper. All samples were examined under a transmission electron microscope (Tecnai G2, Hillsboro Oregon, USA) at an acceleration voltage of 100 kV and photomicrographs were taken at 1400X. The 3D view of the prepared emulsomes was photographed by Scanning Electron Microscopy, (SEM, NOVA NanoSEM 450).

Entrapment efficiency

Entrapment Efficiency of emulsome was determined using the method prescribed by Vyasa *et al.*, 2006 [10]. This method includes separation of untrapped drug using mini centrifuge column of Sephadex G-50 and then evaluate entrapment efficiency by disrupting the vesicle in a suitable solvent as given below.

Preparation of sephadex G-50 gel preparation

Sephadex G-50 (1.2 g) was allowed to swell in 20 ml of 0.9% NaCl solution for 5 h at room temperature with occasional shaking. The gel so formed was stored at 4 °C.

Preparation of mini-column

The hydrated gel was filled to the top in the barrel of 1 ml disposable syringe plugged with a Whatman filter pad. The barrels were placed in the centrifuge tubes, centrifuged at 2000 rpm for 3 min to remove the excess saline solution.

Entrapment efficiency determination

Eluted volume was removed from the centrifuged tubes and exactly 0.2 ml of emulsome suspension (undiluted) was applied dropwise on the gel bed in the center. Columns were again centrifuged at 2000 rpm for 3 min to expel and remove void volume containing emulsome into the centrifuge tubes. Elute was removed and 0.25 ml of saline was applied to each column and centrifuged as previously. The amount of drug entrapped in the vesicle was then determined by disrupting the vesicle using 1 ml of 0.1% v/v Triton-X 100, filtering it and the drug content was determined using UV-Vis spectroscopy at 229 nm. The percentage efficiency was determined by the following equation:

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Total amount of drug} - \text{amount of drug lost}}{\text{Total amount of drug}} \times 100$$

In vitro drug release profile

The *in vitro* Ptx release from the vesicle was investigated at a temperature of 37±1 C° using phosphate buffer pH (4.0) as dialysis media. 1 ml of drug-loaded vesicle suspension was placed in a dialysis membrane separately and dialyzed against 20 ml of phosphate buffer pH (4.0). The samples (1 ml each) were withdrawn periodically up to 24 hr and then the withdrawn volume was replaced immediately with fresh buffer media. The withdrawn sample was analyzed spectrophotometrically at 229 nm for Ptx.

Drug release kinetics study

The kinetic profile of paclitaxel release from emulsomes was determined by applying the data obtained from *in vitro* drug release study into various mathematical kinetic models namely zero order, first release, Higuchi model and Korsmeyer-Peppas model. The parameters like regression coefficients and drug release rate constants were compared to understand the release mechanism from emulsomes. 'n' value calculated from Korsmeyer-Peppas model suggests the type of diffusion pattern followed by the drug.

In vitro plasma protein absorption study

To study the influence of PEG as a stealth layer, *in vitro* plasma protein absorption study was performed. Emulsomal formulations were incubated in 5% bovine serum albumin and observed for change in particle size and size distribution due to protein adsorption using zetasizer.

Data analysis

All the results from the studies are average of triplicate experiments (n=3) at a given time point. The observations were tested statistically using one-way analysis of variance ANOVA followed by Turkey-Kramer test using graph pad prism software (version 3.00, Graph pad Software, San Diego, California, USA). Results were expressed as mean±standard deviation (SD). A difference of p<0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Modified solvent evaporation was used to prepare the emulsomes in which tristearin was incorporated to form hydrophobic solid core; polyethylene glycol (PEG) which is covalently conjugated to 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) forms the stealth shell around emulsomes for avoiding particle agglomeration; and DSPC and cholesterol which formed a stabilizing envelope around the hydrophobic core. Specifically, solid lipid precipitates to form the lipidic core which entraps the poorly-water soluble drug, Paclitaxel; DSPC, Chol and DSPE-PEG assemble around the core to form a lipid monolayer/multilayer offering sufficient stability to the emulsomes avoiding the need to incorporate surfactant to form stabilized formulation. Table 1 describes the composition of both

types of emulsome formulations. The method of preparation resulted in uniform emulsome formulation with narrow size

distribution as indicated by a significant p-value of <0.01 for polydispersity index (table 2).

Table 1: Composition of optimized formulations

Formulation code	PL: DSPE-PEG (w/w)	PL: SL (w/w)	Organic: aqueous phase volume (v/v)	SL concentration (%)	Homogenization time (min)
P-Es	-	1:1	1:10	10	15
SS-Es	1:3	1:1	1:10	10	15

P-Es: Plain emulsomes, SS-Es: Sterically stabilized emulsomes, PL: phospholipid, DSPE-PEG: 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-Poly(ethyleneglycol), SL: Solid lipid

Table 2: Physicochemical properties of optimized formulations

Formulation code	Size (nm)	PI	Zeta potential (mV)	%EE
P-Es	275±5.52*	0.112±0.011**	-30.5±2.0*	72.54±3.41*
SS-Es	195±6.4**	0.124±0.012**	-35.8±2.1*	75.56±3.25**

Data represents mean±SD (n=3), *Statistically significant difference at *p<0.05,** Statistically significant difference at p<0.01

Formulation variables for the optimization of emulsomes

The formulations were optimized based on the effect of lipid/lipid-PEG (DSPC+Chol/DSPE-PEG) molar ratio, total lipid to (DSPC+Chol+DSPE-PEG)/solid lipid (tristearin) mass ratio, the effect of solid lipid concentration (tristearin), the effect of phospholipid concentration, the effect of organic to aqueous phase volume ratio on the particle size (PS) and entrapment efficiency (%EE). The formulation variables which resulted in the small size of particles with higher drug entrapment were taken as optimized formulations.

Effect of PL/DSPE-PEG molar ratio on particle size (PS) and entrapment efficiency (EE%)

DSPE-PEG is known and used for providing steric stabilization to the nanocarrier systems. The study conducted revealed that the size of the emulsomes was reduced with the addition of DSPE-PEG. The size of the plain emulsomes without DSPE-PEG was found to be 320.5±5.8 nm (fig. 1). The particle size of SS-Es was found to decrease from 320.5±5.8 nm to 190.5±4.77 nm when the PL to DSPE-PEG molar ratio was changed from 1:0 to 1:5. The size of emulsomes at 1:3 molar ratio was found to be significantly lower (p<0.01) as compared to plain emulsomes with a mean particle diameter of 195.5±5.6 nm. No significant difference in the size of emulsomes was found on increasing the ratios from 1:3 to 1:5. Hence, 1:3 was taken as an optimized ratio. The decrease in size was owing to the presence of the PEG on the surface of the emulsomes which could prevent the aggregation of the nanoparticles as the pegylated hydrophilic end of the DSPE-PEG provides steric hindrance to the particles [11]. The percentage entrapment efficiency (%EE) was found to be 78.20±2.8 at 1:3 for SS-Es (fig. 1 A). The sterically stabilized emulsomes so formed had a relatively low polydispersity index indicating narrow size distribution. The zeta potential value of the formulation was sufficiently (-32 mV) high (p<0.01) signifying a thermodynamically stable formulation with minimum agglomeration. The higher zeta potential infers highly charged particles that remain separated due to electric repulsion, preventing the formation of coagulate upon storage. The zeta potential of formulations was found in accordance with the reported literature which recommends the ZP value of approximately -30 mV is considered optimum for attaining stable nanoformulation [12].

Effect of organic to aqueous phase volume ratio on particle size and entrapment efficiency

The aqueous phase volume was varied from 5 to 20 ml whereas the organic phase was kept constant. Increasing the volume of the aqueous phase first resulted in a decrease in particle size followed by an increase in size later. The decrease in particle size could be due to larger external space for the dispersal of microdroplets avoiding collision and consequent aggregation [13]. The decrease in particle size resulted in increased entrapment efficiency because of the larger

particle surface area. Further increment in aqueous volume would reduce the net shear applied on the emulsomes under constant external energy input leading to increased particle size later.

The entrapment efficiency was found to decrease with the increasing volume of the aqueous phase which occurred because the amount of drug partitioning in the organic phase reduced during emulsification while the drug loss increased with the evaporation of the aqueous phase [14].

The size of P-Es and SS-Es reduced from 270.5±2.5 nm to 230.2±3.5 nm and 256.5±2.6 nm to 195.5±3.28 nm with the change in ratio from 1:2 to 1:10 (fig. 2). On further increasing the aqueous phase volume the size was increased and hence, ratio 1:10 was selected as the optimum ratio. The entrapment efficiency at optimum ratio was found to be 70.25±4.4 and 75.50±2.5 for PE and SSE respectively.

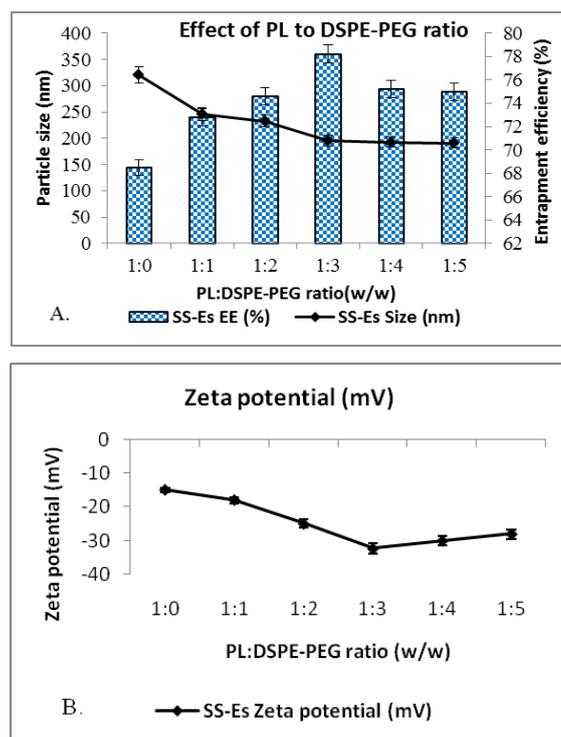


Fig. 1: A. Graphs showing the effect of PL to DSPE-PEG ratio on size and entrapment efficiency of emulsomes. B. Plot showing the change in zeta potential of emulsomes on the addition of DSPE-PEG to PL. Data represents mean±SD (n=3)

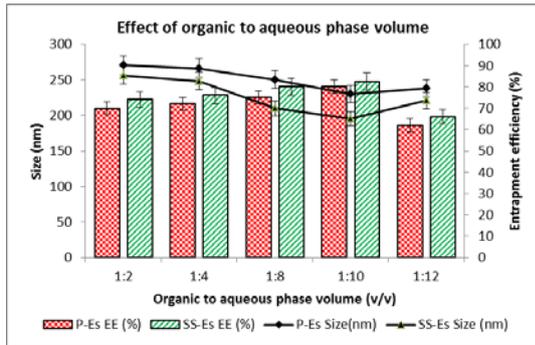


Fig. 2: Graph showing the effect of organic to aqueous phase volume on size and entrapment efficiency of emulsomes. Data represents mean±SD (n=3)

Effect of PL to SL ratio on particle the size and entrapment efficiency EE%

The size of the emulsomes was found to decrease from 650.5±1.5 nm to 310.2±2.5 nm and 556.4±3.5 to 235.5±3.6 nm for P-Es and SS-Es respectively when the ratio of PL: SL was varied from 0.25:1 to 1.5:1. At 0:1 ratio of PL/SL, the formulation was pure lipid nanoparticles which aggregated immediately to form larger particles of about 840.5±3.7 and 650.8±4.5 nm (fig. 3 A). The addition of PL in higher concentration allowed the formation of stable and nanosized emulsomes wherein the solid lipid formed the core and the phospholipids formed a stabilizing envelope around it. The ratio of

0.25:1 to 0.5:1 resulted in particles with a larger size and a small zeta potential value (fig. 3 B). This might occur because the former ratio might not be covering the core completely resulting in unstable emulsomes with lower zeta potential values at which the particle formed aggregates. At the smaller value of zeta potential van der Waals attractive forces dominated the repulsive force leading to flocculation and particle aggregation [15]. As the ratio was increased above 0.5:1 to 1:1, the emulsomes formed were small and stable with the size range of 310.2±2.5 nm (zeta potential value of -32±0.12 mV) and 235.5±3.6 nm (zeta potential -35±0.15 mV) for P-Es and SS-Es with and respectively. It is reported that nanoparticles with a large negative or positive value of zeta potential are less prone to aggregation or increase in particle size indicating good physical stability [16, 17]. Hence, a higher zeta potential value of emulsome ascertains the thermodynamic stability of Ptx loaded emulsomal system. Thus at the 1:1 ratio of PL: SL, the concentration of PL reached the value at which the core was completely surrounded by the phospholipid envelope reducing the interfacial tension between lipid core and aqueous phase consequently favoring the formation of emulsomes with smaller size and sufficient electrostatic repulsion resulting in a relatively stable formulation [18]. On the other hand, all PL do not get incorporated in the particles when 30% lipid is employed resulting in the formation of vesicles in addition to emulsomes. Thus, the optimal PL amount of PL that yields stable and desired nanoparticles are with 20% PL: SL. As the ratio of PL was increased to a higher range, the excess lipids content may reach the value above CMC of (~ 0.4 mg/ml) resulting in the assembly of liposomes. This result in an overall increase in the size of the emulsomes and lowered the zeta potential value. Conversely, when the PL to SL ratio is too low the paucity of lipid leaves the core uncovered resulting in a higher zeta potential value.

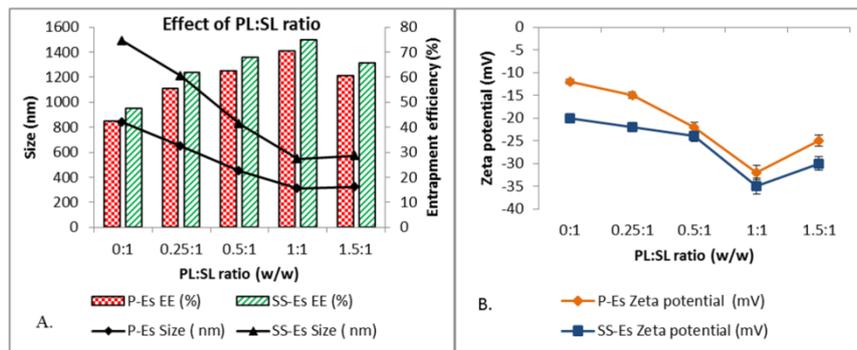


Fig. 3: A. Graphs showing the effect of PL to SL ratio on size and entrapment efficiency of emulsomes. B. Plot showing the change in the zeta potential of emulsomes with PL: SL ratio. Data represents mean±SD (n=3)

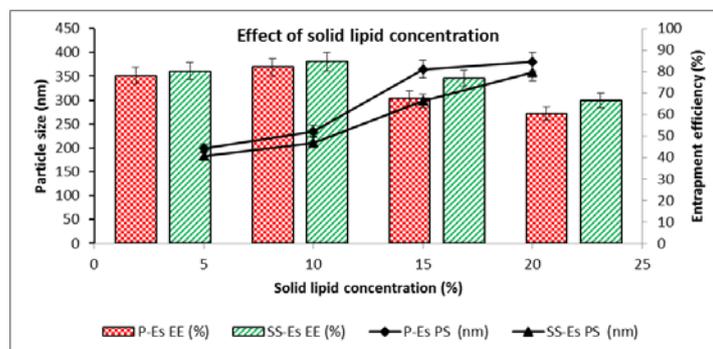


Fig. 4: Graphs showing the effect of SL concentration on particle size and entrapment efficiency of emulsomes. Data represents mean±SD (n=3)

Effect of solid lipid concentration on particle size and entrapment efficiency

As the concentration of the solid lipid was increased from 5% to 20%w/w the size of emulsomes was found to increase from 198.5±4.5 nm to 380.4±2.4 nm and 182.3±3.5 nm to 358.5±1.5 nm

for P-Es and SS-Es formulations respectively (fig. 4). This increase in size can be attributed to the increase in the viscosity of the organic phase and reduction in spontaneous diffusion of lipid in the aqueous phase as the concentration of solid lipid was increased. As the concentration of the core lipid was increased beyond 10%w/w, the PL becomes insufficient to completely cover the core resulting in the

coalescence of the lipid and instability with an increase in the diameter of the particles decreased entrapment efficiency.

The entrapment efficiency of the drug was found to increase with the increase in solid lipid content because with the increased viscosity of the medium the drug diffusional resistance into the aqueous phase increased resulting in higher drug entrapment in the hydrophobic core. The higher drug loading efficiency with the increase in solid core content suggests a high affinity between Ptx and tristearin [19].

Effect of homogenization time on particle size and entrapment efficiency

The addition of energy is an important factor that governs the emulsification process. To study the influence of this factor on particle size and size distribution homogenization time was varied from 5 to 15 min. An increase in homogenization time resulted in a progressive decrease in the mean diameter of emulsomes and narrow size distribution (fig. 5). The increase in energy input with time caused the rapid formation of nanosized core lipid droplets with a narrow polydispersity index. However, on further increasing sonication time particle size was found to increase. This could be ascribed to turbulent flow which increases the velocity of the medium leading to collision of the particles, formation of agglomerates and decreased entrapment efficiency [20]. The size of P-Es and SS-Es at optimized sonication time was found to be 250.5±2.5 nm and 215.6±2.3 respectively and entrapment efficiency at optimized concentration was found to be 76.4±3.2 and 80.2±2.8 for P-Es and SS-Es respectively.

Physicochemical properties of emulsomes

The composition and process variables at which optimized formulation was obtained are shown in table 1. The optimized formulations were characterized for various parameters like shape, size and zeta potential. The various physicochemical parameters are summarized in table 2. TEM and SEM micrograph shows the spherical geometry of emulsomes (fig. 6 A and B). Emulsomes formed were uniform in size as suggested by lower values of polydispersity index. The mean particle diameter of P-Es and SS-Es were in the range of 275±5.52 and 195±6.4 nm (fig. 7 A and B). The zeta potential of P-Es and SS-Es was found to be -30.5±2.0 and -35.8±2.1 respectively (fig. 7 C and D).

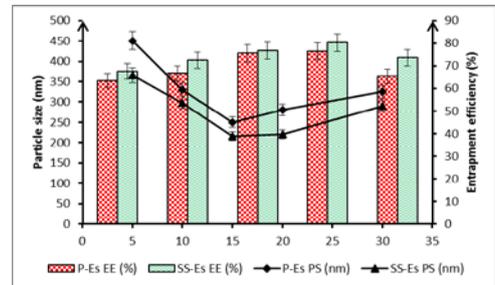


Fig. 5: Graphs showing the effect of homogenization time on the particle size of emulsomes, data represents mean±SD (n=3)

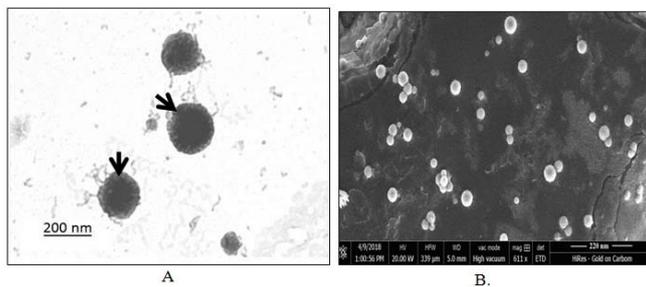
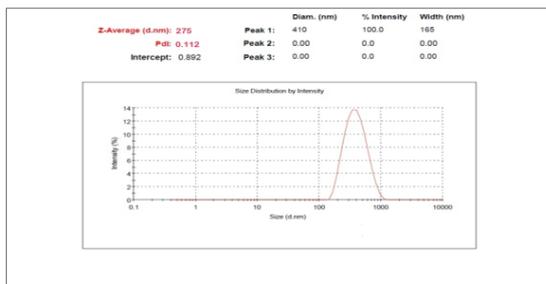
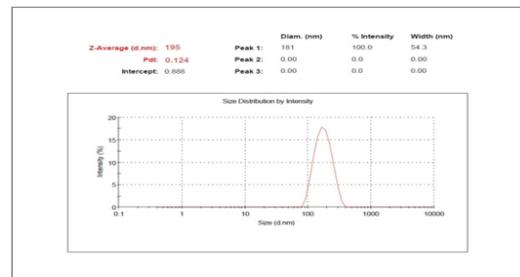


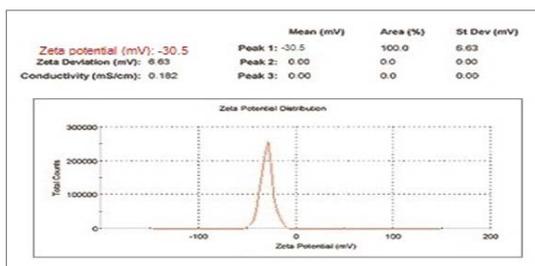
Fig. 6: A. Tem micrograph of SS-Es. The outer light periphery indicated by the arrow shows the presence of PEG on the surface of emulsome. B. SEM micrograph of SS-Es



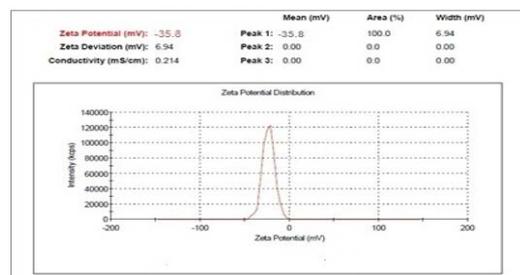
A. P-Es



B. SS-Es



C. P-Es



D. SS-Es

Fig. 7: Plots showing the size distribution of A. P-Es; B. SS-Es and zeta potential value of C. P-Es; D. SS-Es.

In vitro drug release

The most desirable feature of an anticancer formulation is the ability to remain stable at physiological pH while releasing their payloads at the tumor site (acidic pH) [21]. Hence, *in vitro* drug release studies were performed at physiological pH (7.4) and tumor pH (4.0).

The various formulations followed similar drug release profile at physiological and acidic pH which suggests that lipids and DSPE-PEG do not alter the drug release kinetics. The studies revealed that the drug release from P-Es and SS-Es was found to be 40.5±2.7% and 32.5±1.5% respectively in PBS pH 7.4 on the first day (24 h) (fig. 8A). The initial burst release could be attributed to the rapid release of phospholipid associated Ptx. The cumulative drug release then followed a sustained and slow release pattern for 7 d. The cumulative drug release during the next 6 d was recorded to be 75.50±4.5% and 66.80±3.5% from P-Es and SS-Es respectively.

The slower release from SS-Es as compared to P-Es was ascribed to be due to the presence of pegylated surface layer on the emulsomes which resisted the structural change and remain rigid preventing the entry of solvent molecules to the lipidic core. The drug release was faster at acidic pH (pH 4.0) from both the formulations with values of 52.5±1.5% and 48.6±2.5% respectively during 24 h. This faster release as compared to pH 7.4 suggests pH-dependent behavior of the system. The Ptx is released slowly at 7.4 indicating most of the molecules remain within the emulsomes, thereby reducing systemic toxicity. The initial higher drug release from these formulations over 24 h as compared at acidic pH was because of disassociation of the phospholipid bilayer in the acidic medium providing larger space for the drug to diffuse out easily and also allows entry of larger number of solvent molecules. The entry of water into the system results in hydrolysis of core, erosion and faster drug release over time.

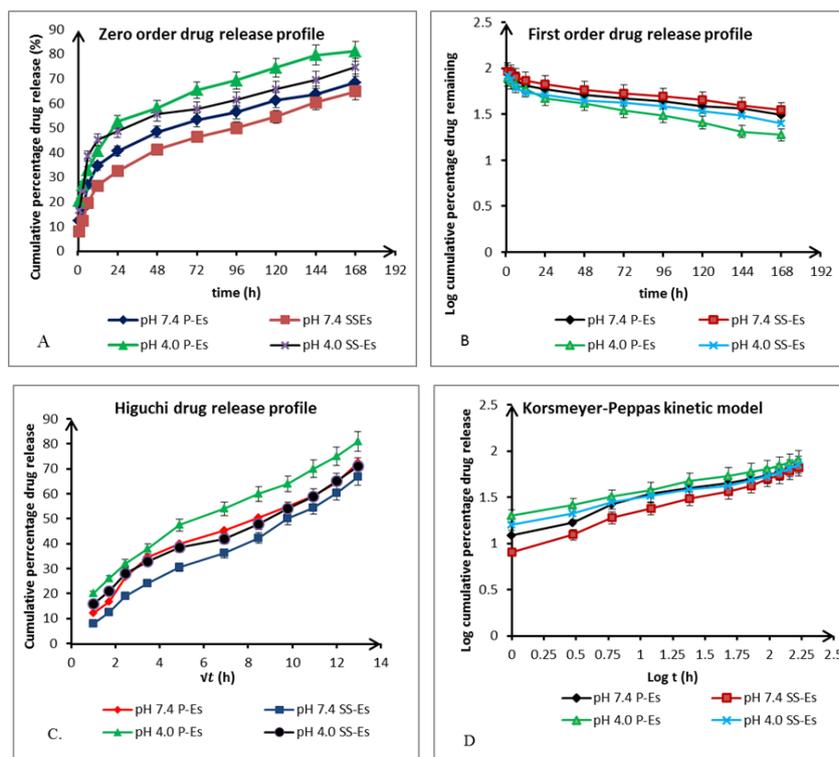


Fig. 8: Plot showing the *in vitro* drug release kinetics as per the various mathematical models A. Zero order drug release model. B. First order drug release model. C. Higuchi model D. Korsmeier-peppas model. Data represents mean±SD (n=3)

Drug release kinetics study

The *in vitro* cumulative percentage drug release data were analyzed using mathematical kinetic models for studying the release pattern of Ptx. Various graphs representing a particular kinetic model were plotted to assess the mechanism and kinetics of drug release (fig. 8). The graphs were plotted between cumulative percentage drug release (Q) vs time (t), log cumulative percentage drug remaining vs t, cumulative percentage drug release (Q) vs \sqrt{t} and log cumulative percentage drug release vs log t for zero order, first order, Higuchi

model and Korsmeier-Peppas model respectively. The regression coefficients obtained in each case were compared to find out the model which was best suited the drug release pattern from emulsomes (table 3). The highest value of regression coefficient 'R' from the plot of Q vs \sqrt{t} reveals drug release followed Higuchi kinetic model which implies diffusion controlled drug release from emulsome. Korsmeier-Peppas model was applied to state the type of diffusion, which depends on the value of diffusion coefficient 'n' (slope). Then n value was found to be less than 0.5 suggest Fickian transport of the drug [22].

Table 3: Parameters for kinetic models applied to *in vitro* drug release data from emulsome formulations

Formulation	Zero order		First order		Higuchi		Korsmeier-peppas	
	R ²	K	R ²	K	R ²	K	R ²	n
P-Es (pH 7.4)	0.8875	0.3011	0.9512	0.0025	0.9947	4.4170	0.9766	0.3252
SS-Es (pH 7.4)	0.9470	0.3166	0.9755	0.0024	0.9912	4.5573	0.9925	0.3942
P-Es (pH 4.0)	0.9128	0.3255	0.9766	0.0033	0.9958	5.5072	0.9968	0.2659
SS-Es (pH 4.0)	0.9383	0.2860	0.9755	0.0022	0.9997	4.3476	0.9858	0.2730

R: regression coefficient. K: release constant, n: diffusion coefficient

Plasma protein absorption study

The major obstacle in achieving long circulatory nanocarriers is their recognition and clearance by the Reticulo-endothelial system (RES). The protein opsonin gets absorbed on the surface of nanoparticles which facilitates identification by the macrophages followed by phagocytosis. DSPE-PEG incorporated in the PL envelope of emulsomes confers stealth property, safeguarding them from RES uptake and prolonging circulatory time. Analysis of the stealth property was performed by incubating emulsome formulation in 5% bovine serum albumin and distilled water as a control for a definite period of time and observing the change in their particle size and particle size distribution. The mean diameter of both the formulation remained unchanged after 24 h in distilled water. After 12 h of incubation in BSA the particle size of P-Es and SS-Es remained unchanged. However, 24 h later a prominent change in the size of P-Es was observed with the size of 575 ± 2.5 nm signifying protein absorption on the emulsomes while the size of SS-Es was slightly changed indicating smaller protein absorption (fig. 9). The data indicate pegylation limits the protein absorption tendency, hence acts as a stealth layer protecting the nanosystem from RES uptake while increasing the circulatory time and enhancing efficacy.

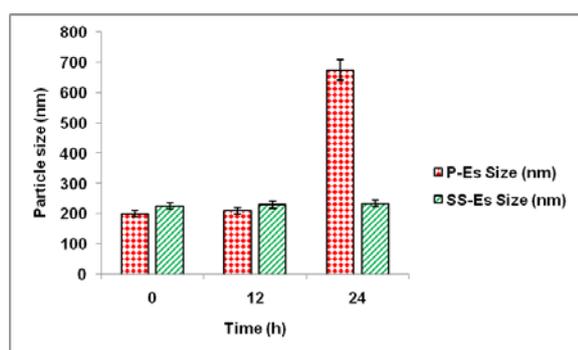


Fig. 9: Change in size of emulsomes on incubation with 5% BSA, data represents mean \pm SD (n=3)

CONCLUSION

Formulation of clinically acceptable Ptx formulation is challenging owing to its poor aqueous solubility and lower bioavailability. Compritol based emulsomes were studied as an alternative to cremophore based formulation to provide a biocompatible and safe nanocarrier for delivery of Ptx. The drug can be easily entrapped within the solid lipid core without the use of any additional solubilizer/surfactant or co-solvent. The outstanding feature of the system lies in its ability to form stable nanoformulation without the need for surfactant. The phospholipids envelope surrounding the solid lipid core acts as a natural stabilizer. The emulsomes were successfully synthesized by the modified solvent evaporation method. The method used was found to be reproducible for loading lipophilic drug within emulsomes. Ptx loaded emulsomes were optimized successfully to attain the desirable physicochemical properties in terms of size, shape, surface charge, drug entrapment efficiency and showed pH dependent drug release. The formulation was stable at physiological pH with minimum leakage of the drug. Hence, emulsomes are envisaged to protect the healthy tissue from the toxic effect of anticancer agent while in the systemic circulation. The faster release at acidic pH highlights their potential to deliver drugs specifically to the tumor site. The surface of emulsomes can be easily pegylated to formulate long circulatory nanocarriers and circumvent RES uptake. Moreover, phospholipid envelope can be tailored with a variety of ligand which widens the applicability of emulsomes in the field of targeted drug delivery to numerous receptors for the delivery of drug and biological molecules against several infections and disorders. The present preliminary study provides insight for the potential of sterically stabilized emulsome

as a novel and non-toxic platform for the delivery of Paclitaxel and other poorly water-soluble drugs as well.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

All authors have contributed equally. The authors have no conflict of interest.

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