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Research Article

Investigation of Formulation Variables and Excipient Interaction on the Production of Niosomes

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Abstract. The aim of this study was to investigate the effects of formulation and process variables on the properties of niosomes formed from Span 40 as nonionic surfactant. A variety of formulations encapsulating Paclitaxel, a hydrophobic model drug, were prepared using different dicetyl phosphate (DCP) and Span 40-cholesterol (1:1) amounts. Formulations were optimized by multiple regression analysis to evaluate the changes on niosome characteristics such as entrapment efficiency, particle size, polydispersity index, zeta potential and *in vitro* drug release. Multiple regression analysis revealed that as Span 40-cholesterol amounts in the formulations were increased, zeta potential and percent of drug released at 24th hour were decreased. Besides, DCP was found to be effective on increasing niosome size. As a process variable, the effect of sonication was observed and findings revealed an irreversible size reduction on Span 40 niosomes after probe sonication. Monodisperse small sized (133±6.01 nm) Span 40 niosomes entrapping 98.2% of Paclitaxel with a weight percentage of 3.64% were successfully prepared. The drug–excipient interactions in niosomes were observed by differential scanning calorimetry and X-ray powder diffraction analysis. Both techniques suggest the conversion of PCTs' crystal structure to amorphous form. The thermal analyses demonstrate the high interaction between drug and surfactant that explains high entrapment efficiency. After 3-month storage, niosomes preserved their stability in terms of drug amount and particle size. Overall, this study showed that Span 40 niosomes with desired properties can be prepared by changing the content and production variables.

KEY WORDS: drug delivery systems; drug release; multiple regression; niosomes; paclitaxel.

INTRODUCTION

An ideal drug delivery system should carry the active agent to site of action and adequately release it during the medical therapy. There are several ways to achieve this goal and provide the desired biodistribution. Researchers have focused on drug carrier systems, changing the molecular structure of the active substance or optimizing the administration of the active agents to the body. In order to overcome the difficulties encountered during therapies with conventional dosage forms, to increase drug efficiency by reducing side effects and to increase patient compliance, a variety of drug delivery systems has been developed. Particularly, vesicular systems are drawing attention in this area, such as liposomes, niosomes, transferosomes, pharmacosomes, and provesicular systems, like proliposomes and proniosomes (1,2).

Several advantages make vesicular systems unique. Both the hydrophilic and lipophilic active agents are loaded in these systems. Loaded drug is released in a sustained manner and increased the bioavailability of hydrophobic drugs. Elimination of rapidly metabolized drugs can be delayed. Drug stability is enhanced, and rapid degradation can be prevented by vesicular systems. Among these drug carrier systems, niosomes have gained considerable importance. Niosomes (nonionic surfactant based vesicles) are bilayered vesicular systems formed by nonionic amphiphiles (surfactants) in aqueous solution (3). Niosomes are formed in uni- or multilamellar forms. They are analogs of liposomes with similar *in vivo* behaviors, but their chemical stability is better (4). The disadvantages of liposomes such as necessity of an inert atmosphere during production, changeable phospholipid purity, and high production cost can be eliminated with niosomes (5).

It is known that approximately 40% of the identified combinatory chemistry compounds are poorly water soluble, and this is a challenging problem in their formulation (6). Usage of drug delivery systems is a well-known approach to improve the solubility and retain the pharmacological activity of such drugs. In this study, a poorly water-soluble anticancer agent Paclitaxel (PCT) was chosen as a model drug to be encapsulated in niosomes to examine the effects of niosome components on niosome properties. PCT was selected because of its hydrophobic character and by considering the encountered problems of its marketed forms related to low solubility (7–10).

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The present study was designed (1) to prepare PCT-loaded Span 40 niosome formulations using different concentrations of dicetyl phosphate (DCP), Span 40, and cholesterol; (2) to evaluate the effects of formulation and process variables on characterization parameters (entrapment efficiency, particle size, zeta potential, *in vitro* drug release) of niosomes using multiple regression analysis; (3) to investigate the drug–excipient interaction in niosomes by differential scanning calorimetry (DSC) and X-ray powder diffraction analysis (XRPD); and (4) to evaluate the stability of niosomes.

MATERIALS AND METHODS

Materials

Paclitaxel (PCT), cholesterol, DCP, dialysis membrane (MWCO, 12,400) were purchased from Sigma-Aldrich, Inc. Wisconsin, USA. Span 40, was bought from Fluka, Switzerland. All other chemicals and components for buffer solutions were of analytical grade.

Preparation of PCT-Loaded Span 40 Niosomes

PCT-loaded Span 40 niosomes were formed by thin film method (11). Briefly, Span 40, DCP, and cholesterol were dissolved in chloroform and mixed with PCT solution in acetonitrile to obtain the molarities in Table I. A clear solution was obtained without any phase separation. Chloroform was removed under vacuum by a rotary evaporator (Buchi 200, BÜCHI Labortechnik AG, Switzerland) to obtain lipid film. Residue of the organic solvent was removed by vacuum application. Lipid film was hydrated with 10 ml of ultrapure water (Milipore Mili-Q-Gradient A10, USA) at 60°C by 15 min of extensive vortex mixing and 45 min of sonication in ultrasonic bath (Ultrasonic LC 30, Germany). Free PCT was separated by ultracentrifuge (Beckman Optima XL-100K, Germany) at 150,000×*g* for 1.5 h at 4°C. The pellet containing PCT-loaded niosomes was redispersed in ultrapure water to obtain a volume of 10 ml. Formulations were kept at 4°C for further studies.

Assessment of PCT Entrapment Efficiency

Span 40 niosome suspension (10 µl) was disrupted by 90 µl of isopropyl alcohol. Obtained clear solution was diluted with methanol, and PCT concentration was estimated by previously validated high-performance liquid chromatography (HPLC) method (11). An HPLC (Agilent 1100 series, Avondale, Germany) system with a Waters Symmetry C18 reversed-phase column (150 mm×4.6 mm×5 µm) was used in isocratic mode at 25°C. The mobile phase was consisted of acetonitrile and water (60:40*v*/*v*). The UV detection was performed at a wavelength of 227 nm, and the flow rate was 1.0 ml/min. Samples of 20 µl were injected into the column for analysis. All solvents were degassed in ultrasonic bath prior to use. Between- and within-day reproducibility of the analytical method were 0.31 and 0.1%, respectively; the determination coefficient was 0.9999. Equations 1 and 2 were used

Table I. Composition of Niosome Formulations Prepared Using Different Amounts of Span 40, DCP, and Cholesterol

Formulation		FS1	FS2	FS3	FS4	FS5	FS6	FS7	FS8	FS9
Organic phase	Span 40	0.0475 M (191 mg)	0.0475 M (191 mg)	0.0475 M (191 mg)	0.0119 M (47.75 mg)	0.0119 M (47.75 mg)	0.0119 M (47.75 mg)	0.00297 M (27 mg)	0.00297 M (27 mg)	0.00297 M (27 mg)
	Cholesterol	0.0475 M (184 mg)	0.0475 M (184 mg)	0.0475 M (184 mg)	0.0119 M (46 mg)	0.0119 M (46 mg)	0.0119 M (46 mg)	0.00297 M (11.5 mg)	0.00297 M (11.5 mg)	0.00297 M (11.5 mg)
	DCP	–	0.0025 M (13.5 mg)	0.005 M (27 mg)	–	0.0025 M (13.5 mg)	0.005 M (27 mg)	–	0.0025 M (13.5 mg)	0.005 M (27 mg)
	PCT	0.234 mM (2 mg)	0.234 mM (2 mg)	0.234 mM (2 mg)	0.234 mM (2 mg)	0.234 mM (2 mg)	0.234 mM (2 mg)	0.234 mM (2 mg)	0.234 mM (2 mg)	0.234 mM (2 mg)
	Chloroform	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml
Aqueous phase	Ultrapure water	q.s.10 ml	q.s.10 ml	q.s.10 ml	q.s.10 ml	q.s.10 ml	q.s.10 ml	q.s.10 ml	q.s.10 ml	q.s.10 ml

DCP dicetyl phosphate, *PCT* Paclitaxel

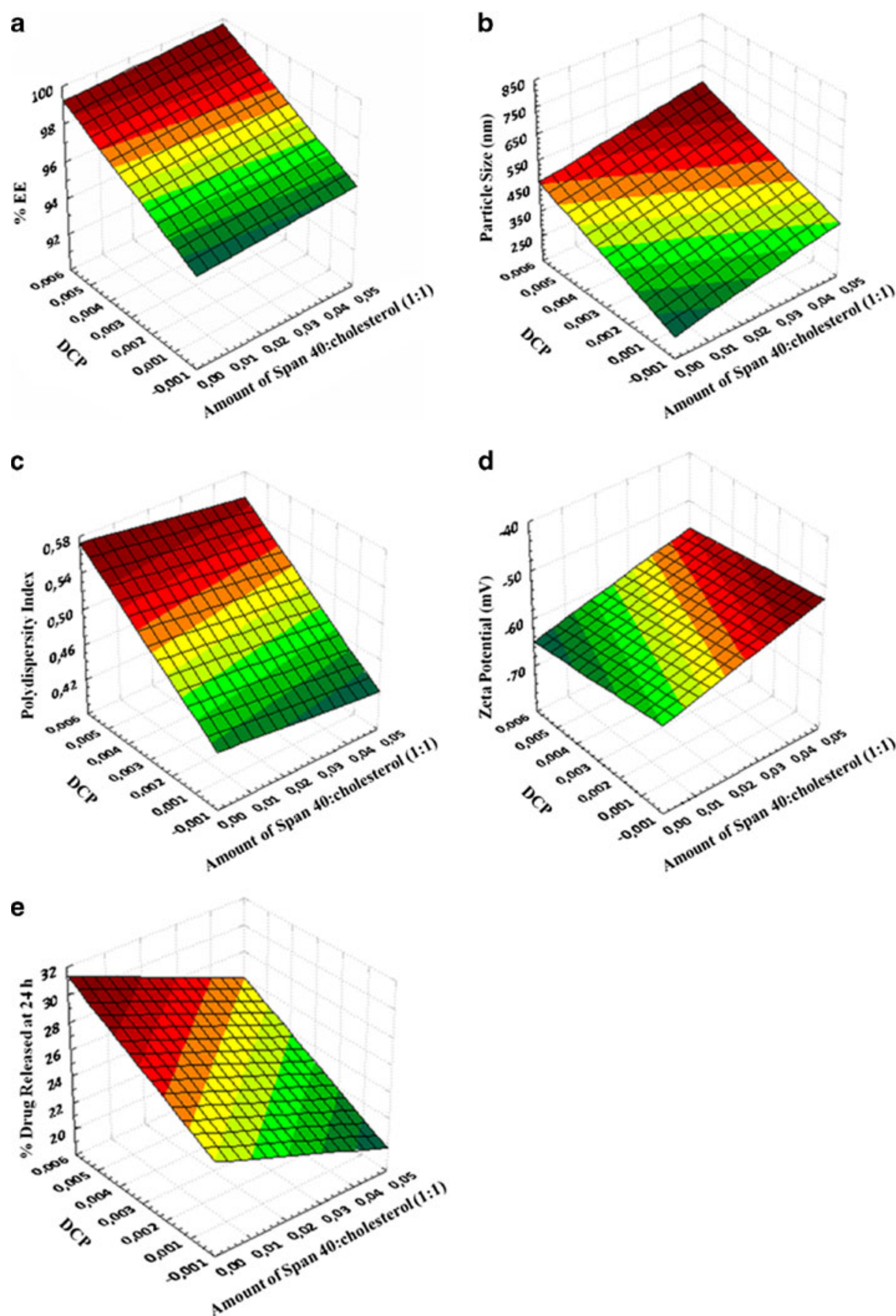

Fig. 1. Response surface plots showing the effects of different amounts of Span 40, DCP, and cholesterol on following niosome properties: **a** zeta potential, **b** encapsulation efficiency, **c** % drug release at 24 h, **d** particle size, and **e** polydispersity index (*DCP* dicetyl phosphate, *EE* encapsulation efficiency)

to calculate the entrapment efficiency (%) and drug weight in niosome (%)(12).

$$\%\text{Entrapment efficiency } (\%\text{EE}) = (a/b) \times 100 \quad (1)$$

$$\%\text{Drug weight in niosome} = a/(b + c) \times 100 \quad (2)$$

where a is the amount of drug loaded in niosomes (g), b is the amount of drug used in niosome preparation (g), and c is the amount of excipients used in niosome preparations (g).

Measurement of Particle Size and Distribution of the Niosomes

Particle size and distribution of niosomes were measured with a dynamic light scattering (DLS) method using Zetasizer (Malvern Zetasizer Nano ZS, Malvern Instruments, UK) (13).The analyses were performed with a He–Ne laser (633 nm) at a scattering angle of 175 ° at 25°C. Samples (40 µl) were diluted with 4 ml ultrapure water and filtered through Whatman no. 42 ashless filter paper. Size measurements were done in triplicate for each sample.

Determination of Zeta Potential

Zetasizer (Malvern Zetasizer Nano ZS, Malvern Instruments, UK) was used to measure the zeta potential of niosomes. Zeta potential was determined six times for each sample, and results were automatically calculated by the analyzer using the Smoluchowski equation (12).

Testing *In Vitro* Release of PCT from Niosome Formulations

The dialysis method was used to investigate PCT release from niosomal formulations (14).Span 40 niosomes containing 60 µg of PCT were placed into dialysis membrane bag (molecular weight cutoff, 12,000 Da) and sunk in the 50 ml of release medium, phosphate-buffered saline (PBS) (pH 7.4) containing 0.1% (*v*/*w*) Tween 80 to maintain the sink condition (15,16). The samples were placed in a water bath and shaken at 100 rpm 37°C. Samples of 1 ml were taken from the release medium at predetermined time intervals up to 24 h, and fresh medium was added to the dissolution medium ($n=3$). The collected samples were directly analyzed by previously validated HPLC method (11).Between- and within-day reproducibility of the analytical method were 0.74 and 0.15%, respectively; the determination coefficient was 0.9999.

Multiple Linear Regression Analysis

Multiple regression analysis was performed to investigate the factors affecting the final properties of niosomes. The amounts of Span 40-cholesterol (X_1) and DCP (X_2) were independent variables and entrapment efficiency, percent released drug, zeta potential, particle size, and polydispersity index (PDI) were dependent variables. A two-factor, three-level full factorial design (3^2) was formed to see the effects of independent factors on dependent factors as the measured characteristics. MINITAB statistical package program (USA) was used to perform the analysis; thus, statistical parameters and multiple regression equations were obtained.

Effect of Probe Sonication on Niosomes

Probe sonication is a known method to reduce the particle size of niosomes. In order to investigate irreversibility of this process, probe sonication was applied to selected niosome formulation (FS8) with optimum properties. FS8 niosomes were placed in an ice-water bath to prevent overheating, and sonication was applied by a probe sonicator at 42 W, 0.7 s mode for 0, 5, 10, 20, 30, and 60 min. Prepared formulations were coded as P1, P2, P3, P4, P5, and P6, respectively. Efficiency of probe sonication was determined by particle size measurements. The size measurements were repeated after 24 h to confirm the irreversibility of the size reduction process.

Transmission electron Microscopy Analysis of Niosomes

The morphology of P6 niosomes was analyzed by transmission electron microscopy (TEM) using LEO 906 E TEM (Germany). A drop of niosome suspension was placed on a copper grid and allowed to penetrate for 1 min. Excess sample was removed by filter paper, and one drop of 1% phosphotungstic acid was added. The grid

Table II. Statistical Data Estimated by Multiple Linear Regression

	Dependent variables (Y)									
	Particle size		Polydispersity index (PDI)		% Drug release		Encapsulation efficiency (% EE)		Zeta potential	
Independent variables (X)	Coefficients	p value	Coefficients	p value	Coefficients	p value	Coefficients	p value	Coefficients	p value
Span40-cholesterol (1:1)[a] (X_1)	2,465	0.137	−0.445	0.528	−141	0.00900*	8.1	0.886	199	0.0210*
DCP[b] (X_2)	3,7842	0.0190*	21.2	0.00400*	599	0.213	685	0.204	−376	0.635
Constant	257	0.137	0.457	0.000*	28.2	0.000*	95.5	0.000*	−62.4	0.000*
R	0.510		0.560		0.540		0.260		0.320	

%EE $=8.1\ x_1+685\ x_2+95.5$

% Drug release $=-141X_1+599X_2+28.2$

Particle size $=2{,}465X_1+37{,}842X_2+257$

PDI $=0.445X_1+21.2X_2+0.457$

Zeta potential $=199x_1-376x_2-62.4$

$*p<0.05$

[a] Span 40-cholesterol (1:1) level: low level, 0.0029 M; medium level, 0.0119 M; high level, 0.0475 M.

[b] DCP: low level, 0 M; medium level, 0.0025 M; high level, 0.005 M

Table III. Characteristics of FS1–FS9 Niosomes (Results Are the Mean Values of n=3 for %EE, Drug Weight in Niosomes, Size, PDI, % drug release, and n=6 for zeta potential)

Code[a]	EE (%)±SE	Drug weight in niosome (%)±SE	Size (nm)±SE	PDI±SH	Zeta potential (mV)±SH	% Drug release at 24 h
FS1	95.7±5.75	0.51±0 .0305	210±3.98	0.401±0.0281	−46.7±2.19	25.160
FS2	99.9±1.10	0.51±0.0056	789±11.1	0.555±0.0558	−64.0±2.23	20.390
FS3	96.5±0.48	0.48±0.0024	377±3.04	0.504±0.0150	−50.1±1.00	23.250
FS4	94.2±2.57	1.97±0.0537	267±1.88	0.409±0.0026	−67.8±2.20	23.530
FS5	100±0.755	1.83±0.0138	416±6.30	0.565±0.0561	−41.3±1.07	30.880
FS6	100±3.66	1.63±0.0596	610±19.9	0.565±0.0189	−77.3±1.92	29.860
FS7	93.8±3.62	4.63±0.1788	322±12.6	0.474±0.0612	−72.0±1.83	19.360
FS8	98.2±2.22	3.64±0.0822	255±13.8	0.493±0.00491	−48.3±0.818	30.530
FS9	97.5±5.89	2.89±0.1745	379±13.4	0.535±0.0316	−64.8±0.351	30.740

EE encapsulation efficiency, *PDI* polydispersity index, *SE* standard error
[a] Refer to Table I for formulation details

was air-dried after removing the excess of solution with filter paper and viewed at 80 kV.

Investigation of Drug–Excipient Interactions in Niosomes

DSC and XRPD analysis of niosomes and excipients were performed to assess thermal and crystallinity properties. The physical mixtures of drug and excipients were used for comparison. The physical mixtures were prepared by mixing the powders in a glass mortar. The dry powder form of the niosomes was obtained by lyophilization. The weight ratios of the ingredients in niosomes without PCT and physical mixtures were kept the same with those of P6 niosomes in order to evaluate the diffractograms and thermograms, comparatively.

X-Ray Powder Diffraction (XRPD) Analysis

XRPD patterns were obtained by an X-ray powder diffractometer (Rigaku, Ultima I.V., Japan) with nickel-filtered Cu K_α radiation (wavelength, 0.154 nm) at 35 kV and 20 mA. The scans were recorded at a scanning speed of 5°–2θ/min (0.03° 2θ) in 2θ diffraction angle 5–40 or 5–30°. The codes and contents of the samples used in XRPD analysis were as follows: (a) physical mixture of DCP+cholesterol+Span 40, (b) PCT, (c) physical mixture of DCP+cholesterol+Span 40+ PCT, (d) P6 niosomes without PCT, and (e) P6 niosomes.

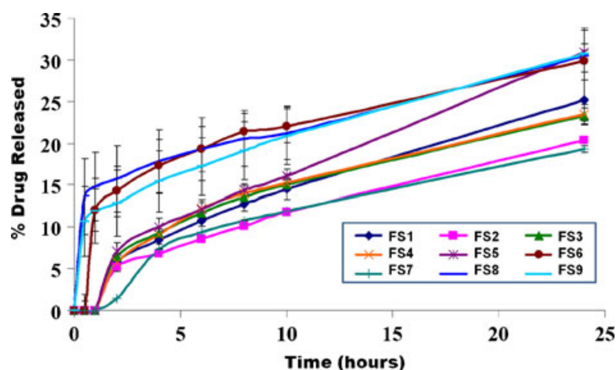

Fig. 2. PCT release from FS1–FS9 niosomes prepared with different dicetyl phosphate and Paclitaxel: (Span 40-cholesterol) amounts (refer to Table I for formulation details)

Differential Scanning Calorimetry Analysis

DSC was carried out with Shimadzu DSC 60 (Japan, Kyoto). The samples [(a) PCT, (b) DCP, (c) cholesterol, (d) Span 40, (e) physical mixture of DCP+cholesterol+Span 40+ PCT, and (f) P6 niosomes] were placed in aluminum pans and sealed. Thermograms were obtained by heating the samples from 20 to 260°C with a scan rate of 10°C/min.

Niosome Stability

PCT-loaded Span 40 niosomes were evaluated for storage stability. Stability test was consisted of visual observation, analytical measurement of drug content, particle size, and zeta potential analysis. For this purpose, PCT-loaded niosomes in 10-ml colored glass vials were placed in a refrigerator at 5±3°C for 3 months. Samples were analyzed at the end of first and third months. The results were statistically compared with each other using one-way ANOVA (SPSS 9.0, USA).

RESULTS AND DISCUSSION

Effect of Excipients on Niosome Formulations and their Optimization

The results of the multiple linear regression (MLR) analysis (coefficients and R values) and analysis of variance (p values) are shown in Fig. 1 and Table II including MLR equations. Negative signs of the coefficients indicate negative quantitative (antagonistic) effect of the factor on the measured response just as positive signs indicate positive

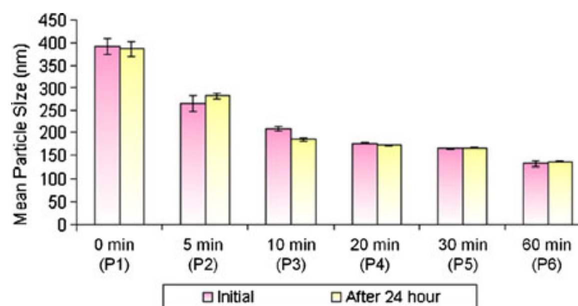

Fig. 3. Effect of the sonication time on particle size of niosomes and changes on niosome sizes 24 h after probe sonication

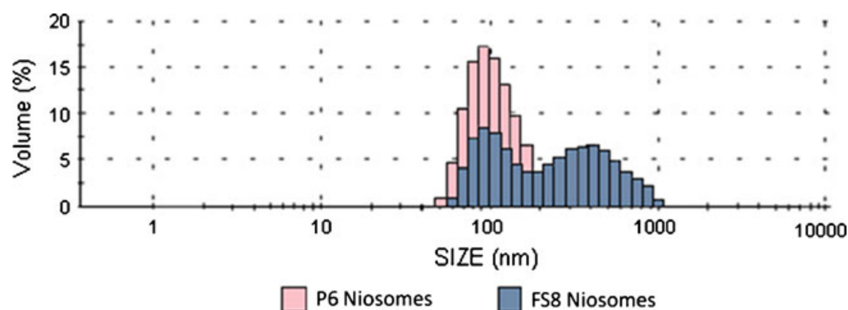

Fig. 4. Particle size distribution of FS8 niosomes after probe sonication

quantitative (synergistic) effect. Contribution of the factors with insignificant p values ($p>0.05$) on measured responses is not important, and these factors can be considered as negligible.

PCT Entrapment Efficiency

Entrapment efficiency is an important parameter for niosomes to provide their usage as drug carriers. The entrapment efficiencies (% EE) of PCT in FS1–FS9 formulations were very high in all formulations and varied between 93.8 and 100% (Table III). The decreasing Span 40-cholesterol amounts did not have significant effect on entrapment efficiency ($p>0.05$). If encapsulation was evaluated as the PCT amount per weight of carrier, it was shown that within the used Span 40-cholesterol levels, higher amounts of PCT could have been loaded in niosomes. This is an advantage in terms of obtaining niosomes with low total mass and encapsulating high dosed active agents.

As shown in Fig. 1a, %EE was seemed to increase with DCP addition, but this change was not significant ($p>0.05$). Ruckmani and Sankar (17) stated that the effect of charge on niosome bilayers is a matter of debate. However, they reported that inclusion of DCP alters the entrapment, but that it also depends upon the alkyl side-chain of the surfactant. For the drug, zidovudin in Span 40 and 60 formulations, DCP decreased entrapment, whereas in Span 20 and 80 formulations, entrapment was increased. DCP may possibly interact electrostatically with positively charged drugs and control the %EE (18). In the present case, DCP was not expected to be effective on PCT encapsulation because PCT is not electrostatically charged (19).

Particle Size, PDI, and Zeta Potential Analysis

The size and PDI of the niosomes were between 209.5 and 788.6 nm and 0.40 and 0.56 (Table III). As seen on Fig. 1b and c, the increase in DCP amount was found to be significantly efficient on increasing the niosome size and PDI ($p<0.05$). DCP is an anionic surfactant in hydrophilic nature. Incorporation of a charge inducing agent such as DCP into the niosome membrane leads water efflux into the bilayer and increases separation between bilayers (20). Thus, membrane thickness and particle size also increase. Especially in FS1–FS3 niosomes, which contain high amounts of Span 40-cholesterol, the size-increasing effect of DCP was clearly observed. The increase in PDI in these formulations showed the wide distribution of the niosome size due to DCP. These results suggest that usage of different DCP amounts is appropriate for controlling the size of niosomes.

Niosomes were negatively charged with zeta potentials between −41.3 and −77.3 mV. The charges of the niosomes were expected to depend on the amount of DCP, which is commonly used as a negative charge inducer. Interestingly, the MLR results have shown that Span 40-cholesterol amount was significantly effective on the vesicles charge (Table II) ($p<0.05$). The zeta potential of niosomes was decreased with increasing Span 40-cholesterol amount (Fig. 1d) possibly depending on the decreasing molecular DCP distribution onto the bilayer surfactant structure.

In Vitro Release of PCT from Niosome Formulations

The PCT release profiles from FS1–FS9 formulations in PBS-Tw (pH 7.4) medium are given in Fig. 2. In Table III, the

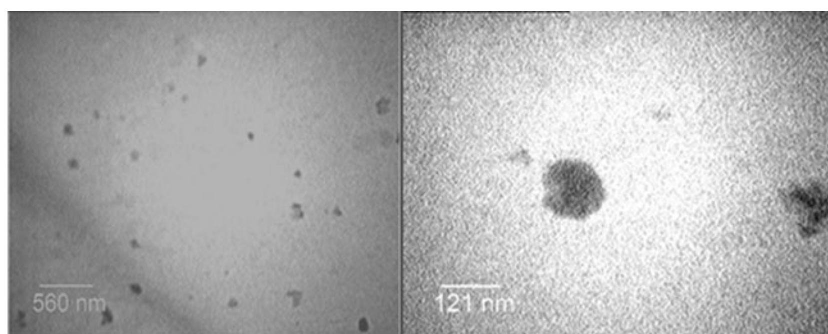

Fig. 5. Transmission electron microscopy (TEM) micrographs of P6 niosomes

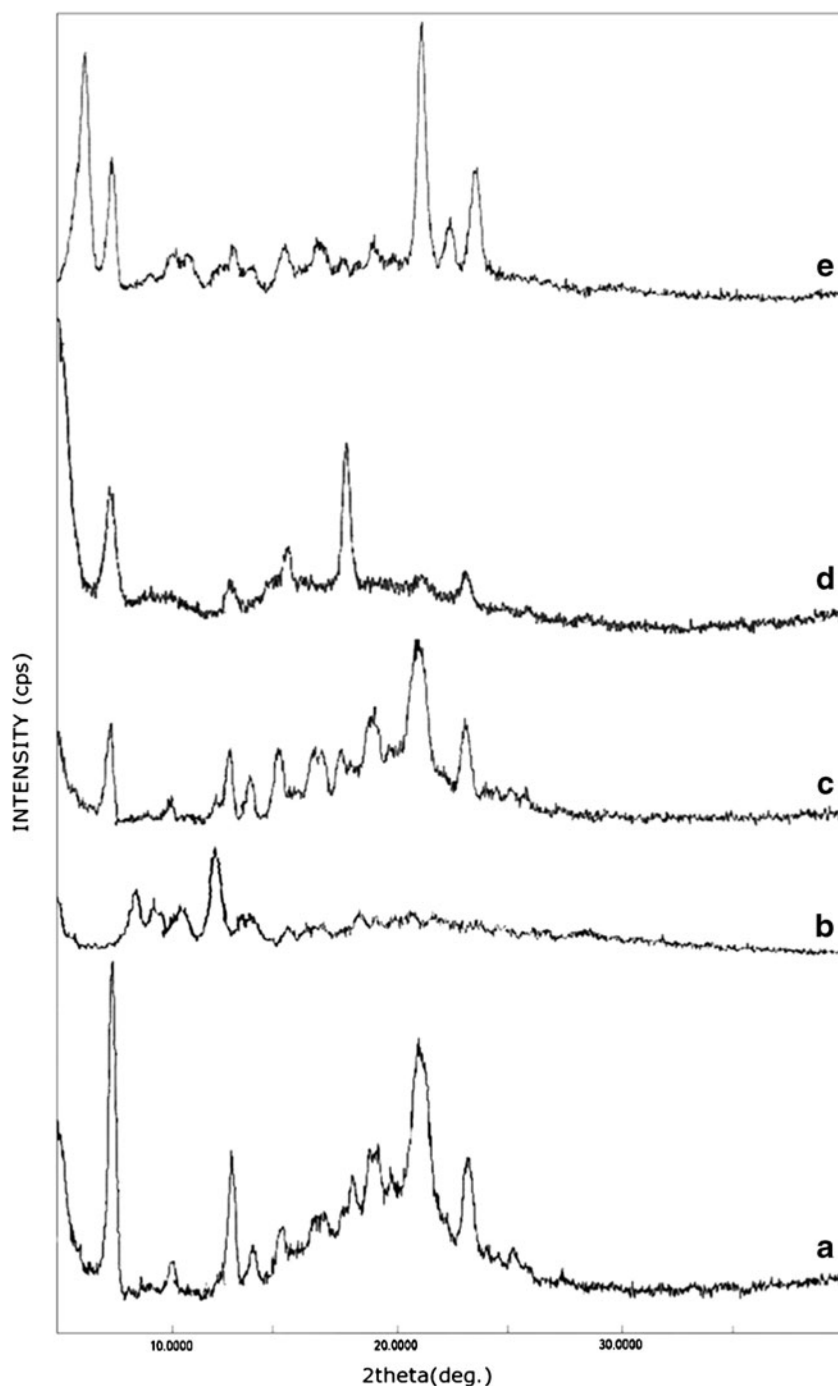

Fig. 6. X-ray powder diffraction (XRPD) diffractograms of **a** physical mixture of DCP+ cholesterol+Span 40, **b** PCT, **c** physical mixture of DCP+cholesterol+Span 40+PCT, **d** P6 niosomes without PCT, **e** P6 niosomes (*DCP* dicetyl phosphate, *PCT* Paclitaxel)

amounts released at the end of 24 h are shown. A biphasic PCT release profile was obtained with formulations. The burst effect in the first phase is beneficial for generating the initial drug dose. The percent of drug released at the end of 24 h was decreased significantly with increasing Span 40-cholesterol amounts ($p < 0.05$) as clearly seen in Fig. 1e. This result is compatible with the literatures emphasizing that addition of increasing amounts of cholesterol enhances membrane rigidity, thus decreasing the efflux of the drugs from the vesicles (21). It is concluded that niosomes with desired release rate of drug can be prepared by changing cholesterol–surfactant amounts. Besides this, the physicochemical properties (solubility, pK_a, crystallinity, etc.) of the active agent have an important role on drug release rate. PCT is highly lipophilic and does not ionize in physiological pH range; thus, the partitioning of PCT molecules to the bilayer lipidic membranes of niosomes might have caused the slow release of the drug (18–21). The presence of DCP in niosomes seems to increase drug release but not at statistically significant level. This can be explained by the enhanced separation on bilayered lipid layers caused by DCP.

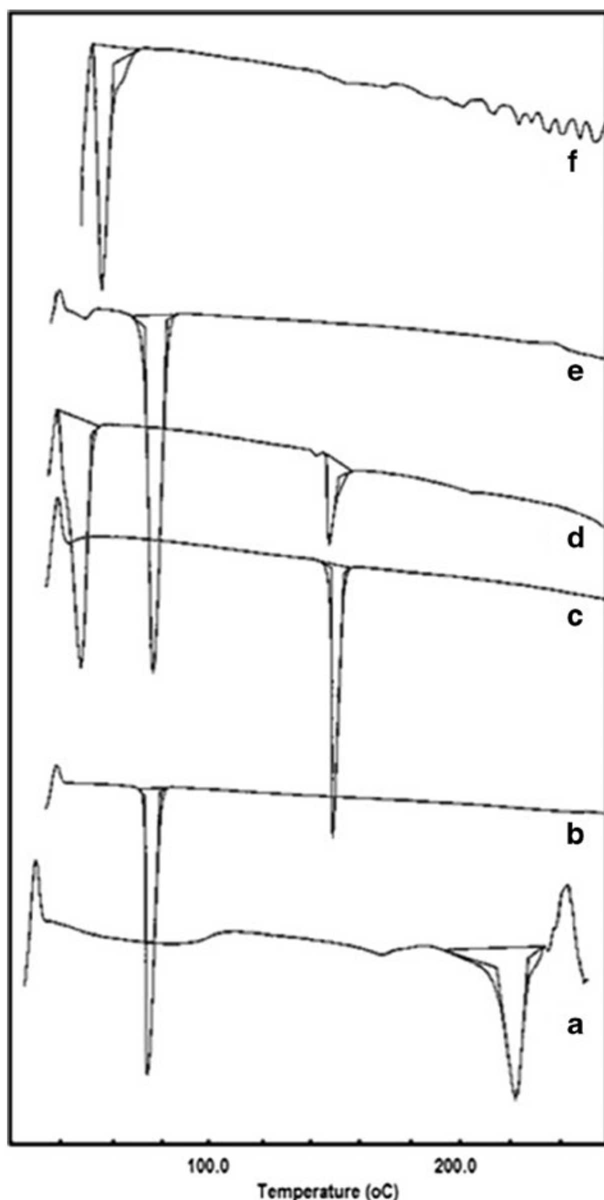

Fig. 7. Differential scanning calorimetry (DSC) thermograms of **a** PCT, **b** DCP, **c** cholesterol, **d** Span 40, **e** physical mixture of DCP+ cholesterol+Span 40+PCT, **f** P6 niosomes (*DCP* dicetyl phosphate, *PCT* Paclitaxel)

The characterization studies on FS1–FS9 niosomes have demonstrated that FS8 niosomes prepared with low level of Span 40-cholesterol and medium level of DCP had high PCT entrapment efficiency, low particle size, and entrapped higher amounts of PCT per unit weight.

Effect of Probe Sonication on Niosomes

The particle size and particle size distribution of niosomes have an important role on their biodistribution. A narrow particle size distribution is desired for drug carriers. The effect of probe sonication was evaluated as an important process variable on niosome properties. The mean particle size was decreased by prolonging sonication time (Fig. 3). The particle size measurements performed 24 h after sonication has revealed that this size reduction was irreversible and the small vesicle size was preserved (Fig. 3). The optimum sonication time for obtaining smallest niosomes was 60 min (P6 niosomes). When the size distribution of P6 niosomes was compared with that of untreated FS8 niosomes; the change of widespread and bimodal particle size distribution of FS8 niosomes into monomodal distribution of P6-coded niosomes is seen in Fig. 4. This result is compatible with literatures (22).

TEM analysis has demonstrated the presence of individual P6 niosomes in spherical shape. The images in Fig. 5 confirmed the niosome formation. TEM results were also compatible with DLS particle size measurements.

Investigations of Drug–Excipient Interaction in Niosomes

The peaks in XRPD diffractogram of the physical mixture of DCP, cholesterol, and Span 40 indicated the crystal structure of these excipients (Fig. 6a). The diffractograms of PCT and physical mixture of DCP, cholesterol, Span 40 plus PCT are given in Fig. 6b and c. When the diffractograms of the physical mixtures in Fig. 6a and c were compared, any characteristic peak for PCT could not be observed. The number and intensity of diffraction peaks were decreased in diffractogram of P6 niosomes without drug (Fig. 6d) compared to those of physical mixtures (Fig. 6a, c). This indicated that, during niosome preparation process, the excipients were partially changed into amorphous nature. Examined XRPD diffractogram of P6 niosomes containing PCT (Fig. 6e), an extra scattering peak at about θ=5–7.5° appeared unlike the peaks obtained from drug free niosomes. Two scattering peaks at θ=15–20° in XRPD patterns of empty niosomes (Fig. 6d) disappeared; new small peaks between θ=10 and 20° and three sharp peaks between θ=20 and 25° were exhibited in XRPD patterns of PCT-loaded niosomes (Fig. 6e). These peaks are not superimposed with that of PCT. These results suggest that PCT was not in crystalline form inside niosomes. DSC thermograms given in Fig. 7a–d demonstrated

Table IV. Stability Test Results of P6 Niosomes Kept at 5±3°C

	P6 niosomes[a]		
	Initial	1 month	3 month
PCT concentration (μg/ml)±SE	187±3.04	182±1.61	179±1.10
Size (nm)±SE	133±6.01	147±2.25	140±2.40
PDI±SE	0.294±0.00600	0.332±0.0150	0.202±0.0120*
Zeta potential (mV)±SE	−64.3±2.26	−58.8±0.75	−20.3±0.260*

PCT Paclitaxel, *PDI* polydispersity index, *SE* standard error
*$p<0.05$, investigated parameters are significantly different from initial measurements
[a] P6 niosomes are FS8 with 60s of sonication process

that PCT, DCP, cholesterol, and Span 40 were in crystal structure exhibiting sharp melting peaks at 222.7, 77.3, 148.6, and51.12, respectively. The second peak at 145.1°C in Fig. 7d was the flash point of Span 40. The melting points of each sample were compatible with literature data (23–26). The physical mixture composed of PCT, DCP, cholesterol, and span 40 showed an endothermic peak of DCP at 77.57°C demonstrating that DCPs' crystal structure was protected (Fig. 7e). At 51.17°C, there was a wide peak of Span 40 showing that it can be partially decomposed due to applied temperature. The absence of PCT peak on this thermogram has been taken to represent the only evidence of PCT amorphization. The physical state of the drug inside the carrier system is important because it can affect the *in vitro* and *in vivo* drug release (27). The initial burst effect in *in vitro* release profiles can be explained by the amorphous form of PCT, which is more soluble than the crystalline form of the drug. In the thermogram of P6 niosomes, there was only an endothermic peak of Span 40 at 54.77°C (Fig. 7f). This showed that Span 40 preserved its crystal structure while forming an ordered bilayer structure of niosomes with other excipients. The PCT peak was absent in the thermogram of P6 niosomes. This situation shows that PCT was in amorphous structure and molecularly dispersed in niosomes as emphasized by Dong and Feng (28). The amorphous PCT may be favorable due to the enhanced solubility of active agent (29). It was stated by Nasr *et al*. (30) that absence of drug's crystalline melting peak after niosomal encapsulation shows the high interaction between drug and surfactant bilayers of niosomes. This also explains the high entrapment of PCT into niosomes. In accordance with this, the entrapment of active agent in niosomes was high in prepared formulations. Regarding the chemical structures of the materials, the hydroxyl groups in PCT and Span 40 can be expected to easily form hydrogen bonds. These bonds prevent transitions between the amorphous and crystal forms during storage, therefore enhancing the stability of the system (31).

Niosome Stability

According to the visual evaluations on P6 niosomes kept at 5±3°C in the first and third months, the color of the niosomes did not change. Niosomes did not precipitate 1 month after preparation, but after 3 months, an easily dispersible precipitate was formed. Particle size measurements did not show any significant size change, and PCT amount remained unchanged (Table IV). The zeta potential and PDI were decreased after 3-month storage. This decrease in niosome charge and also PDI during storage might result from the aggregation of smaller niosomes present in the distribution at third month (20). To prevent this, as an alternative, niosomes can be stored after lyophilization.

CONCLUSION

This study indicated that niosomes can be tailored to achieve desired properties using different surfactant, cholesterol, and charge inducer compositions. Besides this, probe sonication was found to be effective on reducing and stabilizing the size of niosomes. By changing the excipients (surfactant, DCP, and cholesterol amounts) and process variables (probe sonication), spherical niosomes that entrap high amounts of PCT per unit weight and have small particle size with a monodisperse distribution were prepared. This system was found to be a good drug carrier candidate for PCT delivery. The XRPD and DSC analysis adequately indicated the drug–excipient interactions and amorphous conversion of the drug leading increased solubility.

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