PHARMACEUTICAL TECHNOLOGY

Characterization of Niosomes Prepared With Various Nonionic Surfactants for Paclitaxel Oral Delivery

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ABSTRACT: Nonionic surfactant based vesicles (niosomes) are novel drug delivery systems formed from the self-assembly of nonionic amphiphiles in aqueous media. In the present study niosomal formulations of Paclitaxel (PCT), an antineoplastic agent, were prepared using different surfactants (Tween 20, 60, Span 20, 40, 60, Brij 76, 78, 72) by film hydration method. PCT was successfully entrapped in all of the formulations with encapsulation efficiencies ranging between $12.1 \pm 1.36\%$ and $96.6 \pm 0.482\%$. *Z*-average sizes of the niosomes were between 229.3 and 588.2 nm. Depending on the addition of the negatively charged dicetyl phosphate to the formulations negative zeta potential values were obtained. High surface charges showed that niosomes can be suspended in water well and this is beneficial for their storage and administration. PCT released from niosomes by a diffusion controlled mechanism. The slow release observed from these formulations might be beneficial for reducing the toxic side effects of PCT. The niosome preparation method was found to be repeatable in terms of size distribution, zeta potential and % drug loading values. The efficiency of niosomes to protect PCT against gastrointestinal enzymes (trypsin, chymotrypsin, and pepsin) was also evaluated for PCT oral delivery. Among all formulations, gastrointestinal stability of PCT was well preserved with Span 40 niosomes. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:2049–2060, 2010

Keywords: paclitaxel; formulation; oral drug delivery; niosomes; surfactants; zeta potential; particle size; encapsulation efficiency; gastrointestinal enzymes; stability

INTRODUCTION

Paclitaxel (PCT) is an antineoplastic agent isolated from the bark of Taxus brevifolia (Fig. 1). PCT disrupts the dynamic equilibrium within the microtubules that are involved in various cellular functions including mitosis, maintenance of cell shape, cell motility and transport between organelles within the cell, thereby leading to cell death. Therefore, PCT demonstrates antitumor activity against a wide range of cancers such as breast cancer, ovarian cancer, colon cancer, lung cancer, prostate cancer, neck carcinoma, and AIDS-related Kaposi's sarcoma.[1–6] PCT has very low aqueous solubility. The reported solubility values for PCT are within the range of 0.3–30 μg/mL.[7,8] Due to poor solubility of the drug, the commercially available i.v. injection is formulated in a 1:1 combination of the solubilizing agent Cremophor® EL (polyethoxylated castor oil) and dehydrated etha-

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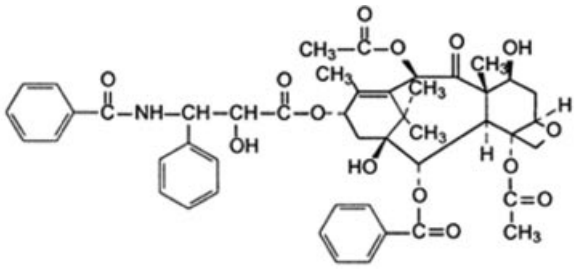

Figure 1. Structural formula of Paclitaxel.

nol.[1,9] However, some drawbacks exist in the clinical applications of this formulation such as severe hypersensitivity reactions, neurotoxicity, and nephrotoxicity observed after i.v. infusion of PCT resulting from the Cremophor® EL, rather than drug itself. Moreover, upon contact PCT formulation with polyvinyl chloride infusion sets, Cremophor® EL causes leaching of plasticizer diethylhexylphthalate. Another problem with the formulation is the precipitation of PCT after dilution in the infusion solutions.[5,10–12]

To circumvent the above drawbacks, alternative delivery systems have been investigated for i.v. and oral delivery of PCT. The systems for i.v. delivery of PCT include polymeric micro- and nanoparticles,[12] lipid nanoparticles,[2] polymeric micelles,[6,13] liposomes,[14] immunoliposomes,[8,14] PEGylated liposomes,[15] emulsions, microemulsions, and self-microemulsifying drug delivery systems.[4,5,16] These systems are designed to improve drug solubility, to provide a controlled drug release, and to facilitate drug targeting in tumor tissue. Implants and films have also been studied for local delivery of PCT to various solid tumor sites.[17,18]

Development of oral delivery systems of PCT as an anticancer drug has attracted considerable attention. Oral chemotherapy improves patient compliance and patients' quality of life. It eliminates the need for hospitalization, medical and nursing assistance. Oral chemotherapy may enable a sustained level of drug concentration in plasma preventing variable and low trough plasma drug concentrations and side effects can be reduced.[19] However, oral bioavailability of PCT is less than 10% due to its poor aqueous solubility and dissolution, high affinity for the intestinal and liver cytochrome P450 and the multidrug efflux pump P-glycoprotein (P-gp) in the gastrointestinal system.[1,10,20,21] To enhance bioavailability, one approach is the co-administration of the P-gp inhibitors such as cyclosporine and verapamil together with PCT oral solution containing solubilizing agent to dissolve the drug.[21] Although the oral bioavailability of PCT increases, the use of P-gp inhibitors is limited due to their pharmacological activity, especially for repeated administration.[10]

Other approaches for the improvement of the oral bioavailability of PCT include colloidal systems such as lipid nanocapsules,[10] polymeric micelles,[11] poli (lactid-*co*-glycolid) nanoparticles,[20,22] and PEGylated PCT prodrug.[3] Particle uptake by the gastrointestinal system is an alternative pathway of absorption. Particle size, surface modification, and zeta potential are critical factors influencing particle uptake. The colloidal carriers may enable transcellular, paracellular transport and/or nonspecific uptake mechanisms (by M-cells and intestinal epithelial cells).[23] Therefore, colloidal systems of PCT may have potential to circumvent efflux by the P-gp pumps and improve bioavailability.

Nonionic surfactant based vesicles (niosomes) are formed from the self-assembly of nonionic amphiphiles in aqueous media resulting in closed bilayer structures. Niosomes have been prepared from different classes of nonionic surfactants, for example, polyoxyethylene alkyl ethers (Brij™),[24] sorbitan monoesters (Span 20, 40, 60, and 80),[25,26] polyoxyethlene sorbitan monoesters (Tween 20. 60, 61, 80).[27,28] Cholesterol is generally added to the nonionic surfactant. This gives rigidity and orientational order to the niosomal bilayer.[29] Often niosomes are needed to be stabilized by the addition of a charged molecule to the bilayer such as dicetyl phosphate (DCP) in order to prevent the aggregation of niosomes.[30] These vesicles are able to encapsulate both lipophilic and hydrophilic drugs and protect them against acidic and enzymatic degradation in the gastrointestinal tract.

The aim of this study is to investigate the potential of niosomes on increasing the oral bioavailability of PCT. PCT loaded niosomes were prepared using different surfactants (Tween 20, Tween 60, Span 20, Span 40, Span 60, Brij 76, Brij 78, Brij 72) with varying hydrophilic-lipophilic balance (HLB) values by film hydration method. The physicochemical properties of niosomes, such as percent drug loading, particle size, zeta potential, *in vitro* drug release profiles, vesicle stability and protection against gastrointestinal enzymes were researched. Thus, the appropriate surfactant type to form the niosomes for oral delivery of PCT was determined.

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MATERIALS AND METHODS

Materials

Paclitaxel (PCT), cholesterol, dicetyl phosphate (DCP), Span 60, pepsin (from porcine stomach musoca, 3200–4500 U/mg protein), chymotrypsin (from bovine pancreas, 40–60 U/mg protein), trypsin (from bovine pancreas, 10,000 BAEE U/mg protein), dialysis membrane (MWCO: 12,400) were purchased from Sigma–Aldrich, Inc. (Milwaukee, WI). Trifluoroacetic acid Span 20, Span 40, Tween 20, Brij 76, Brij 78 and Brij 72 were bought from Fluka (Buchs, Switzerland). Tween 60 and Tween 80 were purchased from Merck (Darmstadt, Germany). All other chemicals and components for buffer solutions were of analytical grade preparations.

Methods

Niosome Formation

Film hydration method was used to prepare niosomes. Before encapsulating PCT in niosomes a preformulation study was done without active agent to observe the vesicle formation and to choose appropriate parameters to use in formulation preparation. Span 40, DCP and cholesterol dissolved in chloroform to obtain the molarities in Table 1. Organic solvent was removed under vacuum by rotary evaporator (Buchi 200, BÜCHI Labortechnik AG, Flawil, Switzerland) and a film was obtained. Extra vacuum was applied to remove residual organic solvents. This film was hydrated with 10 mL of ultrapure water at 60°C by (a) 10 min of extensive vortex mixing or (b) 20 min of extensive vortex mixing or (c) 30 min of sonication in ultrasonic bath (Ultrasonic LC 30, Singen, Germany) or (d) 15 min of extensive vortex mixing and 45 min of sonication in ultrasonic bath. Formulations were kept in 4°C for further studies and the shape of vesicles was observed by using an optical microscope (Leica DM 4000B, Wetzlar, Germany). By this way the method of agitation during film hydratation step was chosen.

Preparation of Drug-Loaded Niosomes

In the present study, the molar ratio of surfactant–cholesterol–DCP was 47.5:47.5:5. PCT loaded niosomes of Span, Tween, and Brij (Tab. 2) were prepared at the same total lipid concentration and the influence of surfactant structure on physicochemical properties of niosomes was investigated.

Surfactant, DCP and cholesterol dissolved in chloroform to obtain the molarities in Table 1. To this solution 2 mg PCT in acetonitrile was added. Organic solvent was removed under vacuum by rotary evaporator and a film was obtained. Extra vacuum was applied to remove residual organic solvents. This film was hydrated with 10 mL of ultrapure water by 15 min of extensive vortexing and 45 min of sonication in ultrasonic bath at 60°C. Nonincorporated PCT was separated by ultracentrifuge at 150,000*g* for 1.5 h at 4°C.[31] The PCT loaded niosomes in the precipitate were redispersed in ultrapure water to obtain a volume

Table 1. Compositions of the Niosome Formulations

Formula Code	F1	F2	F3	F4	F5	F6	F7	F8
Organic phase								
Span 40 (M)	0.0475	—	—	—	—	—	—	—
Span 20 (M)	—	0.0475	—	—	—	—	—	—
Span 60 (M)	—	—	0.0475	—	—	—	—	—
Tween 20 (M)	—	—	—	0.0475	—	—	—	—
Tween 60 (M)	—	—	—	—	0.0475	—	—	—
Brij 76 (M)	—	—	—	—	—	0.0475	—	—
Brij 78 (M)	—	—	—	—	—	—	0.0475	—
Brij 72(M)	—	—	—	—	—	—	—	0.0475
Cholesterol (M)	0.0475	0.0475	0.0475	0.0475	0.0475	0.0475	0.0475	0.0475
DCP (M)	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
PCT (mM)	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234
Chloroform (mL)	10	10	10	10	10	10	10	10
Water phase								
Ultrapure water (mL)	q.s.10	q.s.10	q.s.10	q.s.10	q.s. 10	q.s.10	q.s.10	q.s.10

Table 2. Chemical Names and HLB Values of Nonionic Surfactants in the Niosome Formulations, PCT Encapsulation Efficiency of Niosomes and % PCT Released in 24 h

Code	Type of Surfactant	Chemical Name	HLB Value	Entrapment Efficiency (% ± SE)	% PCT Released in 24 h
F1	Span 40	Sorbitan monopalmitate	6.70	96.6 ± 0.482	22.2 ± 2.342
F2	Span 20	Sorbitan monolaurate	8.60	91.5 ± 1.93	28.6 ± 1.937
F3	Span 60	Sorbitan monostearate	4.70	86.4 ± 2.04	19.9 ± 0.870
F4	Tween 20	Polyoxyethylene (20) sorbitan monolaurate	16.7	20.3 ± 0.620	32.1 ± 1.39
F5	Tween 60	Polyoxyethylene (20) sorbitan monostearate	15.0	42.5 ± 0.353	33.1 ± 0.283
F6	Brij 76	Polyoxyethylene (10) stearyl ether	12.4	79.5 ± 1.32	19.7 ± 0.359
F7	Brij 78	Polyoxyethylene (20) stearyl ether	15.3	12.1 ± 1.36	27.6 ± 1.52
F8	Brij 72	Polyoxyethylene (2) stearyl ether	4.90	81.1 ± 0.124	23.8 ± 3.17

of 10 mL. Formulations were kept in 4°C for further studies.

PCT Encapsulation Efficiency

The PCT concentration in the niosomes was estimated by HPLC after disrupting the niosomes with isopropyl alcohol and dissolving PCT in methanol.[32] The incorporated drug (%) was calculated using the following equation[33]

$$\text{Drug incorporated}\,(\%) = \frac{a}{b} \times 100$$

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

HPLC Analysis

Paclitaxel concentrations were measured by HPLC (Agilent 1100 series, Waldbronn, Germany).[13] 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 a mixture of acetonitrile and water (60:40, v/v). The UV detection was performed at a wavelength of 227 nm, the flow rate was 1.0 mL/min and the injection volume was 20 μL. Before using all solvents were degassed in ultrasonic bath. The linearity range of the method used was 0.5–20 μg/mL with an r (correlation coefficient) value of 0.9999. Within-day precision was 0.10% and between-day precision was 0.31%. Detection and quantification limits of the method were 0.0737 and 0.223 μg/mL, respectively.[34]

Determination of Niosome Size and Shape

The size of niosomes was measured using dynamic light scattering (DLS) with a zetasizer (Malvern Zetasizer Nano ZS, Malvern Instruments, Worcestershire, UK).[35,36] The analyses was performed with He–Ne Laser (633 nm) at 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. Atomic force microscope (Nanomagnetics Instruments, Oxford, UK) was used to image the formulations. A drop of diluted niosomes was applied in a mica slide and completely dried under nitrogen. The analysis was performed in tapping mode.

Determination of Zeta Potential

Zeta potential was measured using PALS (phase analysis light scattering) with a zetasizer (Malvern Zetasizer Nano ZS, Malvern Instruments). Measurements were performed on the same samples prepared for size analysis. Zeta potential was determined six times for each sample. Results were automatically calculated by the analyzer using the following Smoluchowski equation:

$$m = \frac{ez}{h}$$

where z is the zeta potential, m is the mobility, e is the dielectric constant, and h is the absolute viscosity of electrolyte solution.

In Vitro Release of PCT from Niosome Formulations

The dialysis method was used to investigate PCT release from niosomal formulations.[15] Niosomal suspensions containing 60 μg of PCT was placed into dialysis membrane bag (molecular weight cut off 12,000 Da). The bags were sunk in the 50 mL of release medium, PBS (pH 7.4) containing 0.1% (v/w) Tween 80 to maintain the sink condition.[13,14,37] The samples were placed in a water bath and shaken at 100 rpm 37°C. 1 mL of samples was 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 HPLC. The mobile phase was a mixture of acetonitrile and water (60:40, v/v). Waters Symmetry C18 column (150 mm × 4.6 mm × 5 μm) was used at 25°C. The UV detection was performed at a wavelength of 217 nm. The flow rate was 1.0 mL/min and the injection volume was 50 μL. The linearity range of the method used was 0.05–6.0 μg/mL with an r (correlation coefficient) value of 0.9999. Within-day precision was 0.15% and between-day precision was 0.74%. Detection and quantification limits of the method were 0.0154 and 0.0468 μg/mL, respectively.

Cumulative percentages of the drug released from the niosomes were calculated. The mathematical models, zero order, first-order exponential function, Hixson-Crowell cubic root law, Higuchi square root of time equation and Weibull distribution were fitted to individual dissolution data with the linear regression module of SPSS 9.0 for Windows (SPSS, Chicago, IL) (Tab. 3).[38] The model parameters, their standard errors and descriptive statistics of regression for each model were calculated. The best fitted mathematical model that describes the dissolution profiles was determined.

Table 3. The Applied Mathematical Models in the *In Vitro* Drug Release Study

Function	Equation
Zero order	$C = C_0 - kt$
First order	$\ln C = \ln C_0 - kt$
Hixson-Crowell	$C_0^{1/3} - C^{1/3} = kt$
Higuchi	$Q = kt^{1/2}$
Weibull	$\log[\ln(1/(1-Q))] = \beta \log \tau - \beta \log \tau_d$

C, amount of remaining drug in the formulation at time t; C_0, initial amount of drug in the formulation; k, dissolution rate constant; Q, amount of drug released at time t; β, shape parameter; τ_d, time at which 63.2% of the material is release; τ, time.

Stability of Niosomes in Gastrointestinal Enzymes

The stability of formulations in GI enzymes trypsin, α-chymotrypsin and pepsin was investigated. Five hundred microliter of niosome containing 30 μg/mL PCT or free PCT in PBS pH 7.4 was mixed with 500 μL of 5 IU trypsin solution in phosphate buffer pH 7.8 or 16 IU α-chymotrypsin solution in phosphate buffer pH 7.8 or 2640 U pepsin solution in simulated gastric fluid (SGF) pH 1.2. The mixtures were incubated at 37°C, 3 h for trypsin and α-chymotrypsin and 1 h for pepsin.[24,26] At the end of the incubation time 100 μL trifluoroacetic acid (TFA) was added to 100 μL of sample to stop the enzymatic reaction. Samples were diluted with methanol and PCT content was measured by HPLC as given for PCT encapsulation efficiency ($n = 3$).

Method Repeatability

To assess the repeatability of film hydration method F1 formulation was prepared for three times and size distribution, zeta potential and % drug loading values of each batches were measured.

RESULTS AND DISCUSSION

Niosome Formation

In our study nonionic surfactants were preferred because of their low toxicities. Cholesterol is added to the formulations as a membrane stabilizing agent. Although interactions between cholesterol and surfactant bilayer depend on both the structure of the lipid chain and the hydrophilic head group, incorporation of cholesterol at increasing amounts causes to broaden and eventually disappear the gel-to-liquid phase transition of lipid bilayers. Cholesterol alters the fluidity of chains in bilayers and increases the degree of orientational order leading decreased permeability. A 1:1 molar ratio of cholesterol to surfactant is generally included in most formulations for the formation of physically stable niosomes.[39] DCP is a charged molecule and used to prevent niosome aggregation and increase the stability of colloidal

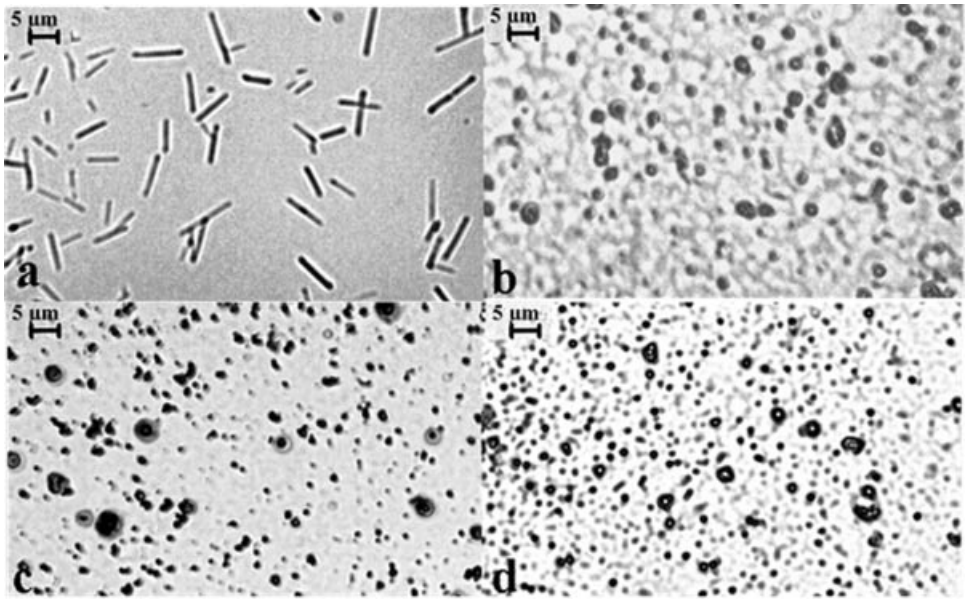

Figure 2. Micrographs of Span 40 niosomes prepared after (a) 10 min of vortex mixing, (b) 20 min of vortex mixing, (c) 30 min of bath sonication, and (d) 15 min of vortex mixing + 45 min of bath sonication.

niosome system dispersions providing surface charge to the vesicles.[40–43]

The vesicle forming ability of Span 40 was investigated after vortex mixing and/or bath sonication application. The size of particles formed could not be detected by zetasizer due to the "poor result quality" notice of the Software. So these systems were studied using an optic microscope. The studies with the microscope gave a qualitative idea about the size and shape of the vesicles. The micrographs in Figure 2 confirm the formation of vesicular structures from Span 40 by classic film method. Influence of agitation on shape was clearly demonstrated. Niosomes appeared as large vesicles with tubular shape (Fig. 2a) in case of 10 min vortex mixing. As we extent vortexing time and/or add ultrasound sonication, this led niosomes breakdown into spherical ones (Fig. 2b–d). The most homogenous and smallest particle sizes were obtained after 15 min vortexing and 45 min ultrasound sonication. The particle size of these last vesicles was also successfully measured with zetasizer (Tab. 4, F1 formulation). This hydration time and method of agitation were chosen to be used in other formulations.

Table 4. Particle Size and Zeta Potential of Niosomes

Formula Code	*Z*-Average ± SE (nm)	PI ± SE	Peaks (nm) ± SE (% Area of the Peak Indicating the Volume of Particle Population)			Zeta Potential (mV ± SE)
			1st peak	2nd peak	3rd peak	
F1	377.3 ± 3.04	0.50 ± 0.02	74.7 ± 1.7 (16)	389.7 ± 15.3 (75)	5515 ± 25 (9)	−52.7 ± 0.8
F2	229.3 ± 1.49	0.38 ± 0.01	99.8 ± 8.9 (25)	305.7 ± 9.04 (37)	958.9 ± 89.4 (30)	−58.4 ± 1.1
F3	512.0 ± 20.40	0.59 ± 0.01	129.6 ± 5.8 (82)	566.0 ± 26.5 (15)	5506 ± 24 (4)	−44.4 ± 1.9
F4	267.3 ± 1.08	0.26 ± 0.00	53.9 ± 8.3 (17)	401.0 ± 8.14 (78)	5054 ± 103 (5)	−41.7 ± 2.7
F5	229.8 ± 0.63	0.40 ± 0.01	94.8 ± 2.4 (32)	608.6 ± 64.5 (65)	5374 ± 100 (3)	−43.5 ± 1.9
F6	409.6 ± 5.13	0.62 ± 0.01	99.2 ± 2.3 (17)	620.5 ± 26.7 (78)	5493 ± 2 (5)	−50.0 ± 1.2
F7	235.1 ± 1.48	0.41 ± 0.00	81.2 ± 2.6 (36)	422.4 ± 25.3 (52)	5259 ± 170 (11)	−43.0 ± 2.2
F8	588.2 ± 11.6	0.55 ± 0.02	75.7 ± 8.8 (8)	767.0 ± 77.3 (88)	5461 ± 8 (4)	−53.2 ± 2.0

SE, standard error; PI, polydispersity index.

Particle Size and Zeta Potential of Niosomes

Dynamic light scattering size measurement results of the prepared niosomes are given in Table 4. The particle size distribution of niosomes was trimodal. The polydispersity indexes (PI) were between 0.26 and 0.62 and generally, decreased with the increasing HLB values. The PI value, an estimate of the width of the distribution, indicates the size heterogeneity. Therefore, the mean particle size distributions, the area of the peak indicating the volume of a certain population of particles, are also shown as volume distribution (Tab. 4). *Z*-average is defined as the mean diameter based upon the intensity of scattered light. *Z*-average sizes of the niosomes were in the range of 229.3–588.2 nm. The smallest *Z*-average values were observed for the Tweens (F4 and F5 formulations), Brij 78 (F7 formulation), and Span 20 (F2 formulation) niosomes. Formation of bigger vesicles was resulted from the smaller head groups and increasing length of alkyl chains in the structure of surfactants.[27,32,35] Regarding particle size distribution, 2nd peaks showed the highest contribution to the total scattering intensity. According to the 2nd peaks, as the HLB values increased, average volume sizes of the niosomes decreased. This result agrees with the above finding (Tabs. 2 and 4).

AFM image of F1 formulation is given in Figure 3. Niosomes presented a spherical shape and a large variation in vesicle size. This finding is consistent with DLS results. The variation in vesicle size may be due to niosome fusion which may well result from interactions between the mica substrate and niosome surfaces.

As the zeta potential increases the charged particles repel one another and they become more stable against aggregation. Zeta potential measurements indicated negative zeta potential values ranging between −41.7 and −58.4 mV for the formulations depending on the addition of the negatively charged dicetyl phosphate. These values are sufficiently high for electrostatic stabilization. This shows that niosomes can be suspended in water well and this is very important for their storage and administration.[44,45] Zeta potential could not be related with the type or HLB values of the surfactants but both surfactant type or encapsulation efficiencies might affect the zeta potential values (Tab. 4).

PCT Encapsulation Efficiency

PCT was successfully entrapped in all of the formulations prepared with various nonionic surfactants. The encapsulation efficiency (EE) of PCT in the niosomes varied between 12.1% and 96.6%. The higher values of % EE (79.51–96.61) were observed for the niosomes made with the surfactants having lower HLB values (F1–F3, F6, and F8 formulations) (Tab. 2). A negative linear relationship ($y = -5.876x + 125.67$, $r^2 = 0.7338$) existed between the HLB values of the used surfactants and EE of niosomes (Fig. 4). PCT possesses a high molecular weight (MW: 854 Da) and lipophilicity ($K_{o/w} = 311$).[10,46] The enhanced entrapment of the PCT could be attributed to the increased capacity of the lipophile environment in the surfactant bilayer resulting from the increas-

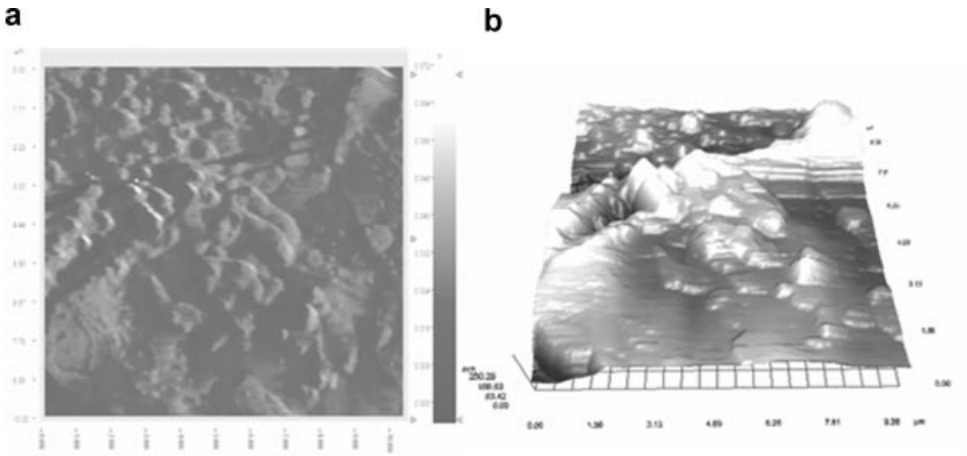

Figure 3. (a) AFM image of F1 formulation in amplitude mode and (b) AFM 3D image of topography of F1 formulation.

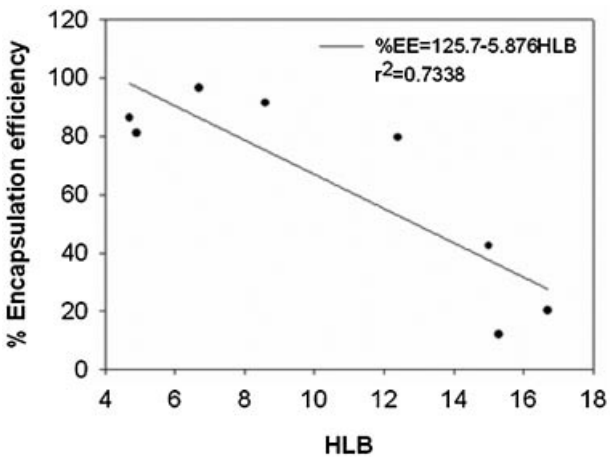

Figure 4. Relation between % encapsulation efficiency of drug and HLB values of nonionic surfactants used for preparation of niosomes.

ing alkyl chain length.[47] PCT encapsulation efficiencies of Tween 20, Tween 60, and Brij 78 (F4, F5, and F7 formulations) niosomes which have bigger hydrophilic head groups were between 12.06% and 42.47%.

In Vitro Release of PCT from Niosome Formulations

The dialysis method was used to investigate the *in vitro* PCT release from niosome formulations. In order to maintain sink conditions 0.1% (v/w) Tween 80 was added to the release medium.[20,23] The release profiles of PCT are shown in Figure 5 and the percent release of PCT from the formulations at the end of 24 h was given in Table 2. The highest percentages of drug released (32.1% and 33.1%) were obtained with F4 and F5 formulations which were prepared with Tweens with high HLB values. A similar result was observed for Brij niosomes in which Brij 78 niosomes with the highest HLB released the highest drug percent (27.6%) in 24 h. As it is mentioned drug entrapment efficiency is low in niosomes prepared with surfactants having an HLB value of 15 and above. So there is a negative relation between encapsulation efficiency and release. This result can be related to weaker drug-lipid chain interrelations of lypophilic PCT in the lipid layers of niosomes prepared from surfactants with hydrophilic character.[13,41] The PCT release from Span niosomes (F1–F3) is increased from 19.9% to 28.6% as the HLB of the surfactant increased from 4,70 to 8.60. A decrease in the alkyl chain length leads an increase on drug release.

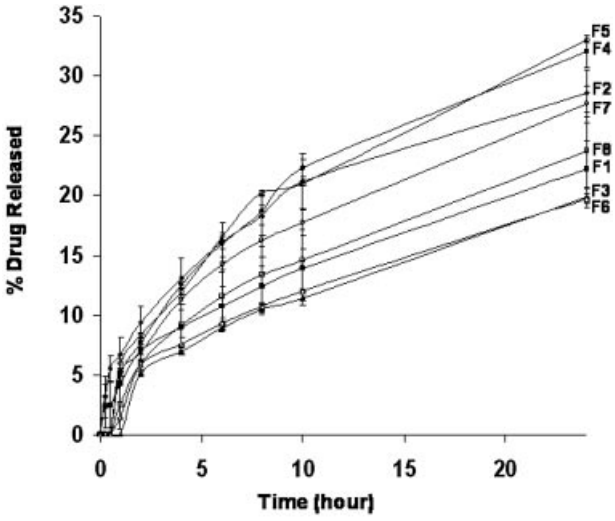

Figure 5. PCT release from the niosome formulations made with different nonionic surfactants. F1 (■), F2 (◆), F3 (▲), F4 (●), F5 (◇), F6 (Δ), F7 (○), and F8 (□).

When we consider all formulations, drug release from Tween niosomes seems faster (Fig. 5). This result can be attributed to structural differences on niosome assembly. The bilayer structure upon hydration of the film would be looser and more flexible due to high aqueous solubility of Tweens, leading an increased permeability to solutes. It has been stated that more hydrophobic Span surfactants form more compact niosomes when hydrated in presence of cholesterol.[29] The molecular weight of the drug and its interaction between niosome membrane also play an important role in release profile. The slow release from the formulations might be beneficial for reducing the toxic side effects of PCT in *in vivo*.

Mathematical models are commonly used to predict release mechanisms and compare the release profiles. The parameters of the mathematical models employed for the release data of PCT from niosomes are given in Table 5. Considering the determination coefficients Higuchi model fit best to the release data among other models. This result demonstrated that PCT molecules were dispersed in the niosomes matrix and there is not any possible interaction between drug and niosome material. Hixson–Crovell model is the secondary kinetic that fit to release data. This model explains the dissolution behaviors of particulate systems. It shows that particulate systems preserve their spherical shape during drug release period. Therefore it was concluded

Table 5. Parameters of the Mathematical Models and Descriptive Statistics of Regression for *In Vitro* Release Data

Model	Statistics	F1	F2	F3	F4	F5	F6	F7	F8
Zero order	r^2	0.858	0.843	0.902	0.870	0.875	0.887	0.867	0.889
	k_0	1.02	1.11	0.856	1.379	1.404	0.837	1.17	1.01
	SE	0.146	0.170	0.100	0.189	0.188	0.106	0.161	0.126
	RMS	10.4	14.1	4.85	17.4	17.2	5.45	12.7	7.78
First order	r^2	0.865	0.871	0.907	0.897	0.910	0.901	0.896	0.909
	k	1.18×10^{-2}	1.33×10^{-2}	9.88×10^{-3}	1.67×10^{-2}	1.73×10^{-2}	9.31×10^{-3}	1.38×10^{-2}	1.18×10^{-2}
	SE	0.00141	0.00181	0.00112	0.00200	0.00192	0.00109	0.00166	0.00132
	RMS	1.15×10^{-3}	1.60×10^{-3}	6.10×10^{-4}	1.95×10^{-2}	1.79×10^{-3}	5.78×10^{-4}	1.35×10^{-3}	8.52×10^{-4}
Hixson-Crowell	r^2	0.916	0.867	0.944	0.924	0.926	0.931	0.914	0.927
	k	1.95×10^{-2}	2.58×10^{-2}	1.57×10^{-2}	2.80×10^{-2}	5.83×10^{-3}	1.60×10^{-2}	2.35×10^{-2}	1.97×10^{-2}
	SE	0.00198	0.00336	0.00128	0.00267	0.00270	0.00145	0.00240	0.00184
	RMS	3.10×10^{-3}	9.00×10^{-3}	1.30×10^{-3}	5.70×10^{-3}	2.87×10^{-2}	1.68×10^{-3}	4.58×10^{-3}	2.71×10^{-3}
Higuchi	r^2	0.965	0.996	0.965	0.983	0.984	0.979	0.986	0.982
	k	4.60	6.28	3.70	6.47	6.60	3.81	5.55	4.63
	SE	0.277	0.138	0.233	0.280	0.279	0.188	0.224	0.209
	RMS	4.27	1.06	3.04	4.37	4.35	1.97	2.80	2.43
Weibull	r^2	0.894	0.661	0.901	0.880	0.867	0.909	0.871	0.915
	τ_d	2.24	17.6	2.15	3.14	3.25	2.42	3.22	2.72
	SE	0.0806	0.104	0.0673	0.0839	0.0887	0.0631	0.0824	0.0636
	β	0.881	0.570	0.795	0.890	0.886	0.782	0.838	0.818
	SE	0.112	0.145	0.0931	0.116	0.123	0.0873	0.114	0.0881
	RMS	3.37×10^{-2}	7.46×10^{-2}	3.10×10^{-2}	4.81×10^{-2}	5.38×10^{-2}	2.72×10^{-2}	4.65×10^{-2}	2.77×10^{-2}

r^2, determination coefficient; RMS, residual mean square; SE, standard error of model parameter.

that the drug was released from the niosomes by a diffusion controlled mechanism. This result was in good consistency with experimental results observed by Guinedi.[48]

Stability of Niosomes in Gastrointestinal Enzymes

Oral administration is the most preferred route for delivering a therapeutically active substance. Unfortunately, acids and digestive enzymes in the stomach and small intestine can degrade some active substances. The amount of the substance reaching systemic circulation and in turn, its bioavailability decreases. To evaluate the stability of PCT in GI media the amount of unchanged PCT was measured after incubating drug and drug loaded niosomes in pepsin, trypsin and chymotrypsin (Fig. 6).

Plain drug preserved 96.1% of its structure without degradation in SGF containing pepsin, while this percent decreased to 75.8% and 27.8% in trypsin containing phosphate buffer pH 7.8 and chymotrypsin containing phosphate buffer pH 7.8, respectively.

Trypsin and chymotrypsin are intestinal endopeptidases. Endopeptidases hydrolyze the bond internal to the terminal bonds of the peptide chain.[49] Chymotrypsin cleaves peptides at the carboxyl side of tyrosine, tryptophan, and phenylalanine because these three amino acids contain aromatic rings, which fit into a "hydrophobic pocket" in the enzyme. Thus, chymotrypsin may hydrolysis the "benzoylamino" part of PCT molecule. In general niosomal encapsulation has protected PCT from chymotrypsin degradation.

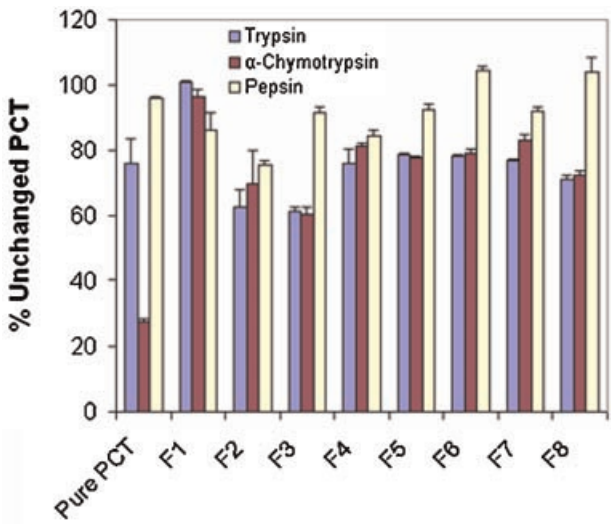

Figure 6. Stability of PCT and PCT loaded niosomes against gastrointestinal enzymes.

Table 6. Repeatability of the Production Method

Production Number Initial	*Z*-Average ± SE (nm)	Zeta Potential (mV ± SE)	EE (% ± SE)
F1-1	412 ± 26.01	−58.1 ± 1.10	93.6 ± 0.612
F1-2	423 ± 3.53	−53.0 ± 0.872	98.6 ± 1.22
F1-3	448 ± 1.69	−54.2 ± 0.976	94.4 ± 2.51
RSD	4.31%	4.84%	2.81%

SE, standard error; RSD, relative standard deviation.

Trypsin predominantly cleaves peptide chains at the carboxyl side of the amino acids lysine and arginine but it is not as specific as chymotrypsin to cleave peptides at the carboxyl side of aromatic rings. In PCT case protective effects of different vesicles were variable. F1 niosomes showed the best protection against Trypsin degradation. When we consider all formulations the GI stability of PCT seems to be well preserved with F1 formulation.

Method Repeatability

The size distribution, zeta potential and % drug loading values of F1 formulation prepared in three different parallel batches were measured (Tab. 6). The method repeatability was evaluated through the relative standard deviation (RSD) of these characterization parameters, and it was found to be 4.31%, 4.84%, and 2.81%, respectively. These low RSD values showed the robustness of the preparation method in terms of size distribution, zeta potential, and % drug loading values.

CONCLUSION

PCT was successfully loaded in niosomes. Relationships between surfactant type and characterization parameters of niosomes were established. *In vitro* studies showed that F1 formulation might be more beneficial for oral drug delivery among other formulations. F1 formulation will be further evaluated for its *in vivo* behaviors.

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