Ultrasonic Processing Technique as a Green Preparation Approach for Diacerein-Loaded Niosomes

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Abstract

In this study, the feasibility of ultrasonic processing (UP) technique as green preparation method for production of poorly soluble model drug substance, diacerein, loaded niosomes was demonstrated. Also, the effects of different surfactant systems on niosomes' characteristics were analyzed. Niosomes were prepared using both the green UP technique and traditional thin-film hydration (TFH) technique, which requires the use of environmentally hazardous organic solvents. The studied surfactant systems were Span 20, Pluronic L64, and their mixture (Span 20 and Pluronic L64). Both the production techniques produced well-defined spherical vesicles, but the UP technique produced smaller and more monodisperse niosomes than TFH. The entrapment efficiencies with the UP method were lower than with TFH, but still at a feasible level. All the niosomal formulations released diacerein faster than pure drug, and the drug release rates from the niosomes produced by the UP method were higher than those from the TFH-produced niosomes. With UP technique, the optimum process conditions for small niosomal products with low PDI values and high entrapment efficiencies were obtained when 70% amplitude and 45-min sonication time were used. The overall results demonstrated the potency of UP technique as an alternative fast, cost-effective, and green preparation approach for production of niosomes, which can be utilized as drug carrier systems for poorly soluble drug materials.

KEY WORDS

diacerein
mixed surfactant system
niosomes
thin-film hydration
ultrasonic processing technique

INTRODUCTION

Self-assembling nature of nonionic surfactants to form vesicular structures, niosomes, in aqueous environment is well known (1). Compared to liposomes, niosomes are different in chemical composition but similar in morphological structure, and hence, they are also termed as nonionic liposomes (2). Niosomes are gaining importance as drug delivery systems because of their intrinsic property being able to encapsulate both hydrophilic and lipophilic substances inside their structures. They have wide formulation versatility of being prepared by several nonionic surfactants, such as alkyl ethers, alkyl esters, and alkyl amides (3). They are nonionic, have low toxicity level, and can improve the therapeutic index of many drugs by site-specific targeting (4). The research interest in niosomes has been recently boosting because of disadvantages associated with liposomes, like high cost of raw materials, instability, and special handling and storage requirements (5). In addition, niosomal drug delivery is applicable for various drug delivery routes including intramuscular, intravenous, oral, ocular, pulmonary, and transdermal drug delivery (6).

During the last decades, a lot of research has been conducted to explore various physicochemical properties of niosomes including the choice of surfactant system, potential of niosomes to act as drug carriers for oral and transdermal delivery, effects of process-related variables, as well as the type and amount of formulation ingredients (7–12). In general, niosomes are prepared by conventional thin-film methods, which often require induction of organic solvents at one or more stages of the process. With these processes, residual solvents pose health risks to humans. Moreover, the multistep process with evaporation steps is

expensive and time consuming. To overcome these complications associated with thin-film hydration methods, a facile, ecofriendly, and cost-efficient synthesis approach is required for the development of niosomes without the use of organic solvents. Other methods studied or used for niosome preparation include, for example, reverse phase evaporation, ether injection, heating method, dehydration-rehydration, freeze and thaw, microfluidization, nitrogen bubbling, and proniosome technologies (13,14).

Sorbitan esters (Spans) and Pluronic class surfactants are widely employed for the development of niosomal drug delivery systems. These excipients are considered as safe in food as well as in pharmaceuticals (15). Pluronic surfactants are polyethylene oxide (PEO)-polypropylene oxide (PPO) block copolymers of varying molecular masses. The structural organization of Pluronic L64 shows interlinking of two hydrophilic PEO groups to one PPO group which is lipophilic in nature. This amphiphilic nature of Pluronic L64 makes it considerably attractive for using in diversified fields of drug delivery research (16). The presence of ethylene moieties in the Pluronic structure minimizes opsonization and clearance by reticuloendothelial macrophages, which improves pharmacokinetic properties of developed delivery system (17).

In the earlier studies, diacerein-loaded niosomes have been produced by thin-film hydration (TFH) method. Due to the environmental and economic concerns related to the technique, other methods need to be studied more systemically in order to utilize them more widely in production of drug-loaded niosomes. Accordingly, the aim of this work was to evaluate the feasibility of ultrasonic processing (UP) technique as facile, green, and cost-effective approach instead of the traditional TFH method for production of diacerein-loaded niosomes. Niosomes were produced with different surfactant systems including Span 20, Pluronic L64, and a mixed surfactant system (containing Span 20 and Pluronic L64). Diacerein is chondroprotective agent with disease-modifying effect for the treatment of osteoarthritis (18). Poor aqueous solubility of diacerein leads to variable bioavailability (19), which makes it a potential candidate for niosomal drug delivery.

MATERIALS AND METHODS

Materials

Diacerein was a gift sample from Consolidated Chemical Laboratories (Pakistan). Sorbitan monolaurate (Span 20) was from Fluka Chemica (Germany), Pluronic L64 from Sigma-Aldrich (USA), cholesterol from Sigma-Aldrich (The Netherlands), dicetyl phosphate from Sigma-Aldrich (USA), methanol from Fluka (Switzerland), chloroform from Rathburn Chemicals Ltd Walkerburn (Scotland), potassium dihydrogen phosphate from VWR Prolab Chemicals (Belgium), disodium hydrogen phosphate from Sigma-Aldrich (USA), sodium chloride from Sigma-Aldrich (Germany), potassium chloride from Sigma-Aldrich (USA) and hydrochloric acid from VWR Prolab Chemicals (France). Water used was Milli-Q water (Merck Millipore, USA).
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Production of Niosomes

Niosomes were produced by two different techniques: TFH and UP techniques. Details of the both techniques are described in the following.

Niosome Production by Thin-Film Hydration Technique

Niosomes loaded with diacerein were prepared at 1:1 molar ratio of surfactant to cholesterol by adopted TFH technique (20). Three batches of niosome formulations were prepared from selected surfactants: Span 20 formulation, Pluronic L64 formulation, and third one from mixed system of both the surfactants (1:1) (Table I). The other materials included cholesterol (membrane stabilizer) and dicetyl phosphate (negatively charge inducing agent). Accurately weighed amounts of surfactants, cholesterol, and dicetyl phosphate were dissolved in organic mixture of chloroform and methanol (2:1, *v*/*v*). The organic solvents were evaporated in Rotavapor (Buchi, Switzerland) at 200 rpm under reduced pressure conditions until thin film of lipidic components was obtained. The film was dried overnight in vacuum desiccator in order to remove all the residual organic solvents. Diacerein was dissolved by magnetic stirring in phosphate-buffered saline (PBS, pH 7.4). The lipid film was hydrated for 2 h with PBS containing diacerein at 57°C. After preparation, the niosomal formulations were left aside at room temperature overnight to mature, after which they were stored at 4°C for further studies.

Table I

Compositions of Niosome Formulations

Materials	F_{SP20}	F_{PL64}	F_{Mixed}
Surfactant (mg)	69	580	324
Cholesterol (mg)	77	77	77
Dicetyl phosphate (mg)	5	5	5
Diacerein (mg)	73	73	73
Final volume of formulation (ml)	20	20	20
Niosomes were prepared with 1:1 molar ratio of surfactant to cholesterol			

Niosome Production by Ultrasonic Processing Technique

Niosomes with the same compositions as with the TFH method (Table I) were prepared by UP technique. Briefly, diacerein was mixed with 20 ml PBS (pH 7.4) by magnetic stirring. The drug suspension was then

added to the surfactant, cholesterol, and dicetyl phosphate mixture in glass vial. This system was probe-sonicated (Vibra Cell, Sonics & Materials, Inc., USA) in pulsed mode (50-s sonication and 10-s pause) at probe temperature of 57°C. For optimization of UP method, three different levels of sonication time and amplitude were studied.

Attenuated Total Reflectance Fourier Transform Infrared

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The possible interactions between the niosome components were studied by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. The ATR-FTIR spectra of pure raw materials, physical mixtures, and niosomes (F_{Mixed}, containing both surfactants) were taken using a Bruker VERTEX Series FTIR spectrometer (Bruker Optics, Germany) with a horizontal ATR accessory (MIRacle, Pike Technology, Inc., Germany) for sample placement. The ATR-FTIR spectra were recorded at the wavenumber range (4000–650 cm^{-1}) with a resolution of 4 cm^{-1} at ambient temperature using software (OPUS 5.5).

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Thermal Analysis

The physical state of diacerein in a selected formulation F_{Mixed} (containing both surfactants) was investigated by using differential scanning calorimetry (DSC) (DSC 823e, Mettler Toledo, USA). Pure raw materials, physical mixture, and formulation (F_{Mixed}) were accurately weighed in aluminum pans (3–5 mg) and covered with an aluminum cover. The thermograms were recorded at scan rate of 10°C min^{-1} by heating the samples from 10 to 250°C. These scans were recorded in an atmosphere of nitrogen at purging rate of 50 ml min^{-1}.

Drug Entrapment Studies

For drug entrapment studies, the niosomal formulations were subjected to ultracentrifugation process at 28000 rpm for 1 h at 4°C by using ultracentrifuge (Beckman Coulter, Optima LE-80K, USA). The isolated pellet obtained at the bottom of centrifuge tube was washed with PBS twice and centrifuged again for 1 h. The amount of drug in supernatant was determined after centrifugation at characteristics wavelength of the drug (258 nm) by UV spectrophotometer (VWR UV-1600PC, China). The percent entrapment efficiency (%EE) was determined according to the Eq. 1 (21):

$$\%EE = (Ct - Cr)/Ct \times 100 \quad (1)$$

where *Ct* is the total amount of diacerein and *Cr* is the amount of free drug present in supernatant. All the measurements were repeated three times.

Differential Light Scattering Measurements for Particle Size, Polydispersity Index, and Zeta Potential

The hydrodynamic diameter (z-average), PDI, and zeta potential value for the niosomes were measured at 25°C using a Zetasizer Nano ZS instrument (Malvern Instruments Ltd., UK). Purified niosomal dispersions (50 µl) were diluted with Milli-Q water (10 ml) for the measurements. All runs were made in triplicate.

Transmission Electron Microscopy

The morphology and shape of niosomes was analyzed by transmission electron microscopy (TEM). The samples for TEM were prepared by dropping small amount of niosomal dispersion on carbon-coated 200-mesh copper grid, after which it was maintained horizontally for 1 min to let sample settle properly on the grid. The excess amount of sample was removed by filter paper and one drop of 2% uranyl acetate was added to the grid for staining. The samples were then imaged using TEM (Jeol JEM-1400, Jeol ltd, Japan) at an accelerated voltage input of 80 kV.

Stability Studies

Stability studies were performed at two different temperature conditions, 4 and 25°C, for a 3-month time period. For stability testing, the niosomal formulations were kept in sealed 20-ml glass vials. The size and PDI were measured as stability indicating variables at definite time intervals (0, 30, 60, and 90 days). Besides this, visual examination of formulations was also done to evaluate possible sedimentation, color changes, *etc*.

Drug Release Studies

Drug release studies from the niosomal formulations and plain diacerein suspension (3.33 mg ml^{-1}) were performed at two different pH values, at gastric pH 1.2 and intestinal pH 7.4, by a dialysis membrane method (22). All the drug release tests were performed under sink conditions. Dialysis membrane (Spectra/Por Canada, MWCO 8000–10,000 Da) was soaked and washed with distilled water for 24 h prior to use. One milliliter of each niosomal dispersion was enclosed in dialysis membrane and immersed in 350 ml of dissolution medium. The release studies were performed at 37°C with 100-rpm stirring speed. The aliquots of dialysate were sampled at predefined time intervals (0, 15, 30, 45, 60, 75, 105, 150, 240, 330, 480, 600, 720 min) for 12 h (relevant test time for oral sustained drug release) and replenished with equal amount of fresh buffer. The samples were analyzed spectrophotometrically with 258-nm wavelength.

RESULTS

Preparation of Niosomes

Diacerein-loaded niosomes were prepared successfully with both the techniques (TFH and UP) and with all the formulations listed in Table I. The sizes of the niosomal vesicles developed from TFH and UP

techniques were found to be in a range of 348–519 and 154–405 nm, respectively (Table II). The values of zeta potentials for the niosomes in the present study were from −33 to −63 mV.

Table II

Particle Size, Polydispersity Index, and Zeta Potential Values for the Niosomes ($n = 3$)

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Formulations	Size (nm)	PDI	Zeta potential (mV)
Thin-film hydration method			
F_{SP20}	519 ± 6	0.39 ± 0.03	−30.7 ± 3.6
F_{PL64}	517 ± 5	0.41 ± 0.04	−63.0 ± 3.5
F_{Mixed}	348 ± 4	0.42 ± 0.03	−44.3 ± 4.2
Ultrasonic processor			
F_{SP20}	405 ± 3	0.27 ± 0.01	−56.0 ± 5.0
F_{PL64}	154 ± 5	0.21 ± 0.05	−54.0 ± 2.5
F_{Mixed}	229 ± 7	0.19 ± 0.04	−50.0 ± 3.1
Type of surfactant and method of preparation significantly affected particle size, *i.e.*, $p < 0.05$			

Optimization of UP Method

After successful production of niosomes with UP technique, the process was optimized using Span 20 and cholesterol at 1:1 molar ratio (Table I, F_{SP20}) in order to get better %EE, smaller particle size, and narrow size distribution (low PDI value). The studied process variables were sonication time and amplitude, and three different levels with both the variables were studied (Table III). Based on the process variable studies, the most monodisperse formulation (PDI value 0.27) with small particle size (405 nm) and high entrapment efficiency (75.1%) were produced with 70% amplitude and 45-min sonication time. Accordingly, in the next step, where the effect of surfactant system was studied, these process variables were used.

Table III

Optimization of Process Variables with the UP Method: Effect of Sonication Time and Amplitude on %EE, Particle Size, and PDI

Amplitude (%)	Time (min)	15	30	45
50	%EE	n.d.	74.3 ± 2.4	75.2 ± 1.6
	Size (nm)	n.d.	283 ± 3	308 ± 3
	PDI	n.d.	0.40 ± 0.05	0.31 ± 0.02
70	%EE	80.6 ± 1.3	83.0 ± 2.8	75.1 ± 1.6
	Size (nm)	526 ± 3	446 ± 4	405 ± 3
	PDI	0.52 ± 0.01	0.43 ± 0.02	0.27 ± 0.01
90	%EE	n.d.	71.3 ± 2.4	72.7 ± 2.0
	Size (nm)	n.d.	283 ± 2	444 ± 5
	PDI	n.d.	0.39 ± 0.01	0.42 ± 0.01
Sonication time and amplitude significantly affected %EE and particle size, *i.e.*, $p < 0.05$				
n.d. not determined				

Drug Entrapment Efficiency

Entrapment of diacerein in the developed formulations by TFH and UP methods was determined, and the values are presented in Table IV. The drug entrapment efficiency with Span 20 as a surfactant was considerably higher with UP technique (75.1 *vs.* 51.2%), while with Pluronic L64 or mixed surfactant systems, the TFH method produced higher entrapment efficiency values. However, the %EE values with mixed surfactant system and Pluronic L64 were considerably higher with both the techniques (from 75.1 to 90.5%).

Table IV

Drug Entrapment Efficiency of Niosomal Formulations ($n = 3$)

Method	F_{SP20}	F_{PL64}	F_{Mixed}
TFH technique	51.2 ± 2.0	89.9 ± 1.6	90.5 ± 3.4
UP technique	75.1 ± 3.1	82.6 ± 2.1	75.5 ± 3.3
Type of surfactant significantly affected %EE in both methods as $p < 0.05$			
Nature of method significantly affected %EE for each surfactant system, *i.e.*, $p < 0.05$			

Interaction Studies

Possible interactions between the drug and niosomal components were studied with ATR-FTIR spectroscopy (Fig. 1) and DSC (Fig. 2) by analyzing pure raw materials, their physical mixtures and one niosomal formulation "~~F_{Mixed}~~." "F_{Mixed}". The ATR-FTIR spectra of active diacerein showed a characteristic broad –OH stretching band of –COOH at 3300 cm^{-1}, stretch aliphatic symmetric group at 2937 cm^{-1}, and two carbonyl (C=O) stretching peaks appeared as strong bands at 1768.63 cm^{-1} (ester group), 1595.04 cm^{-1} (C=C stretch aromatic), 761.84 cm^{-1} (m-substituted benzene), and 705.90 cm^{-1} (benzene) (Fig. 1). The accurate spectrum of diacerein indicated purity of the samples (23). Span 20 showed major characteristic peaks at 3000 cm^{-1} (stretch aliphatic symmetric), 1745 cm^{-1} (carbonyl group), and 1143 cm^{-1} (C–O stretch) (24). Pluronic L64 showed peaks at 2881.50 cm^{-1} (C–H group) and 1105 cm^{-1} (C–O ester) (25). The IR spectrum of cholesterol showed major peaks at 2931.41 cm^{-1} (acetyl groups), 2866.83 cm^{-1} (symmetric $–CH_3$), 1770.20 cm^{-1} (vinyl group), and 1055.17 cm^{-1} (R–O strong) (26).

Fig. 1

ATR-FTIR spectra of pure materials, physical mixture, and niosome formulation with mixed surfactant system (F_{Mixed})

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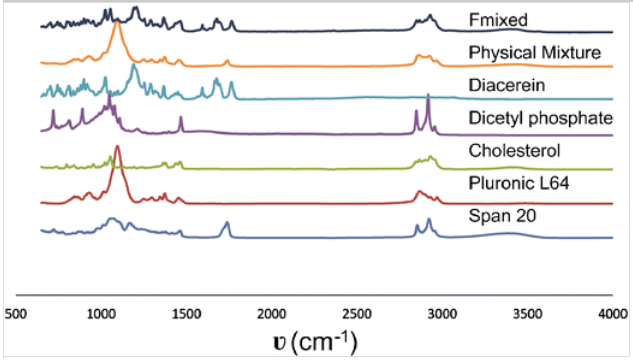
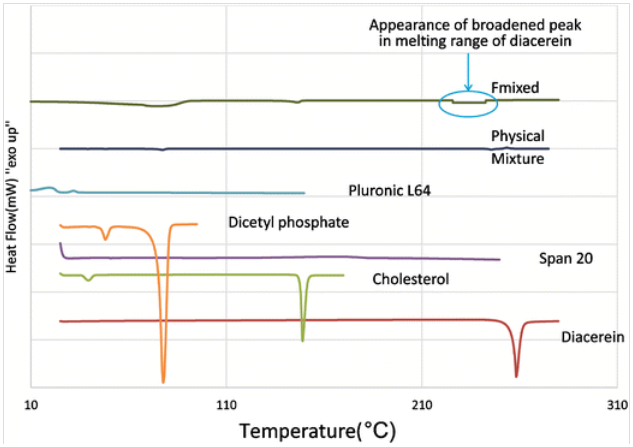


Fig. 2

DSC thermograms of pure materials, physical mixture, and niosome formulation with mixed surfactant system (F_{Mixed})



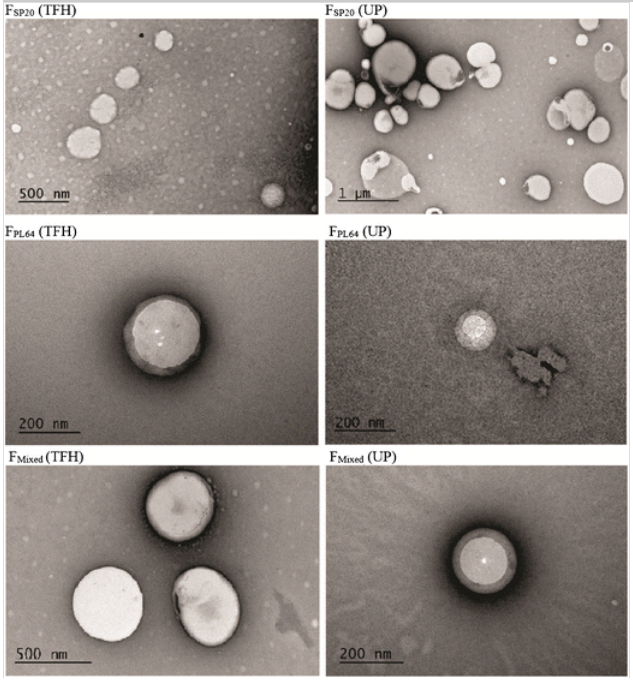
The DSC thermograms for pure raw materials, their physical mixture, and niosomal formulation "F_{Mixed}" are shown in Fig. 2. DSC thermograms of pure diacerein and cholesterol showed the sharp endothermic melting peaks at 258 and 150°C, respectively, confirming their crystalline nature (1,24). Dicetyl phosphate (charge imparting agent) showed a characteristic endothermic melting peak at 77°C.

Morphology

Morphology of all the niosomal formulations was determined using transmission electron microscope (TEM). TEM images confirmed the formation of almost spheroidal vesicles by both the TFH and UP methods (Fig. 3). Niosomes were found to be in nice intact form and images predicted clearly the integrity of vesicles. The particle sizes seen in TEM images for the formulations (F_{SP20}, F_{PL64}, and F_{Mixed}) were in good agreement with the differential light scattering (DLS) size measurements.

Fig. 3

TEM images of niosomal formulations with different surfactant systems produced by thin-film hydration (*left column*) and ultrasonic processor technique (*right column*)

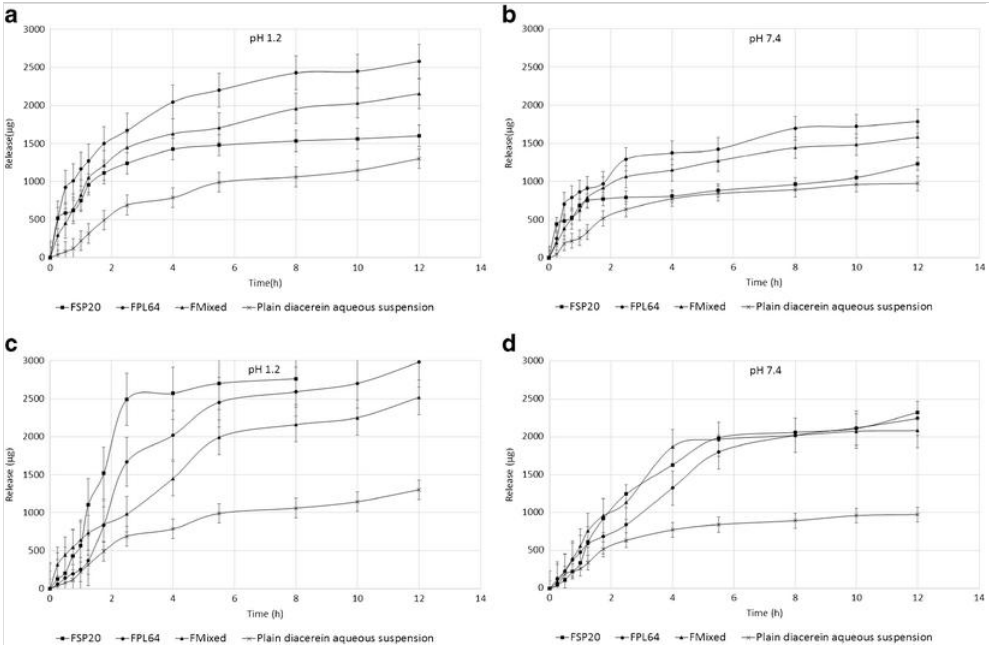


Drug Release Studies

The *in vitro* drug release profiles from the plain diacerein suspension and six niosomal formulations at gastric pH (1.2) and intestinal pH (7.4) are presented in Fig. 4. The release rates of all niosomal formulations were higher than that of plain drug suspension. At pH 1.2, TFH formulations F_{SP20}, F_{PL64}, and F_{Mixed} showed 85, 78, and 65% drug release, respectively, whereas UP formulations gave drug release of 100, 98, and 91% , during 12-h time. In comparison, plain drug suspension released only 38% of the drug at this pH at the same time span. At pH 7.4, TFH formulations F_{SP20}, F_{PL64}, and F_{Mixed} showed 65, 54, and 48% release, respectively, whereas UP formulations resulted in drug release of 85, 78, and 75% during 12-h time. Again, plain drug suspension released only 29% of diacerein at pH 7.4 within the same time period. Overall, at both the pH values, there was a burst in drug release during the first 2 h, which was followed by sustained drug release.

Fig. 4

Release profiles of niosomal formulations with different surfactant systems produced by thin-film hydration (~~*left column*~~*up*, **a** and **b**) and ultrasonic processor technique (~~*right column*~~*down*, **c** and **d**)



Stability Studies

The physical stability of the vesicular structures in liquid state is the main challenge with this type of drug delivery systems, and particle size is the most important indicator of the stability. Two batches of formulations prepared by TFH and UP techniques were studied for the stability determination at two different temperatures, namely 4 and 25°C, for a 3-month period. The observed changes in the vesicle size and PDI values are summarized in Table V. At both the temperatures, the particle size and PDI showed time-dependent changes. The formulations prepared by the UP technique showed greater increase in particle size and PDI compared to the TFH, though the UP niosomes were more monodisperse after production. The size of all niosomal formulations stored at 25°C was increased markedly as compared to samples stored at 4°C.

Table V

Effect of Storage Conditions and Time on Vesicle Size and PDI

Formulation		Start		One month		Two months		Three months	
Method	Batch	Size (nm)	PDI	Size (nm)	PDI	Size (nm)	PDI	Size (nm)	PDI
When stored at 4°C									
TFH	F_{SP20}	519 ± 6	0.47 ± 0.03	319 ± 3	0.39 ± 0.02	545 ± 4	0.49 ± 0.06	584 ± 5	0.76 ± 0.05
	F_{PL64}	517 ± 5	0.49 ± 0.04	388 ± 4	0.41 ± 0.03	912 ± 8	0.55 ± 0.04	1233 ± 10	0.58 ± 0.07
	F_{Mixed}	348 ± 4	0.42 ± 0.03	353 ± 2	0.46 ± 0.02	635 ± 11	0.54 ± 0.03	972 ± 12	0.53 ± 0.04

Formulation		Start		One month		Two months		Three months	
Method	Batch	Size (nm)	PDI	Size (nm)	PDI	Size (nm)	PDI	Size (nm)	PDI
UP	F_{SP20}	405 ± 3	0.27 ± 0.01	863 ± 5	0.68 ± 0.06	911 ± 8	0.70 ± 0.06	1145 ± 7	0.76 ± 0.08
	F_{PL64}	154 ± 5	0.21 ± 0.05	810 ± 6	0.79 ± 0.03	922 ± 5	0.82 ± 0.03	1237 ± 12	0.88 ± 0.05
	F_{Mixed}	229 ± 7	0.19 ± 0.04	458 ± 8	0.44 ± 0.07	608 ± 4	0.57 ± 0.04	997 ± 15	0.61 ± 0.03
When stored at 25°C									
TFH	F_{SP20}	519 ± 6	0.47 ± 0.03	529 ± 8	0.42 ± 0.04	845 ± 7	0.59 ± 0.03	956 ± 11	0.67 ± 0.08
	F_{PL64}	517 ± 5	0.49 ± 0.04	663 ± 13	0.68 ± 0.06	983 ± 10	0.63 ± 0.05	1595 ± 9	0.66 ± 0.06
	F_{Mixed}	348 ± 4	0.42 ± 0.03	468 ± 5	0.46 ± 0.02	1178 ± 8	0.79 ± 0.06	1304 ± 7	0.82 ± 0.04
UP	F_{SP20}	405 ± 3	0.27 ± 0.01	890 ± 6	0.47 ± 0.08	1257 ± 6	0.54 ± 0.06	1487 ± 3	0.68 ± 0.08
	F_{PL64}	154 ± 5	0.21 ± 0.05	902 ± 8	0.36 ± 0.05	1129 ± 3	0.56 ± 0.04	1645 ± 8	0.70 ± 0.06
	F_{Mixed}	229 ± 7	0.19 ± 0.04	436 ± 3	0.47 ± 0.04	1255 ± 2	0.69 ± 0.04	1301 ± 11	0.77 ± 0.05

DISCUSSION

In this work, diacerein-loaded niosomes were successfully prepared by both utilized techniques. In TFH, the method involved hydration of thin lipid film with PBS (pH 7.4) containing diacerein. The temperature was kept above gel to liquid transition temperature of the surfactants during the evaporation and hydration steps because this is required for the production of niosomes (27). As compared to the niosomes produced by TFH technique, UP technique produced smaller niosomes with lower PDI values indicating more monodisperse, and hence more stable samples (28). This indicates good stability for the systems, because colloidal particulate systems with zeta potential eigenvalues higher than 30 mV are considered as stable when the system is electrostatically stabilized (29). The negative surface charge of niosomes is due to the addition of dicetyl phosphate (2.5% M) in the formulation enabling to resist agglomeration. The higher values of zeta potential were adequate for electrostatic stabilization. This indicated that niosomes can be easily suspended in water, and this is very important for their administration and storage (30). In addition to this, dicetyl phosphate was dissociated sufficiently at basic pH 7.4 due to its higher pKa (4.5) leading to higher values of zeta potential of niosomes (9) in between −30 to −63 mV in TFH method and −50 to −56 mV in case of UP technique.

The physical background of TFH and UP techniques is quite different when considering formation of niosomes. Ultrasonic processor technique is so-called high energy technique, in which high energy input to the system during a short time period produces niosomal structures. The ultrasonic processing technique induces high cavitation forces (31), resulting in efficient mixing of the system and yielding smaller sized niosomes in comparison to TFH method. On the other hand, TFH is a multistep process, where changes are taken place much slower. The relative large size of niosomes in case of TFH is due to the reason that in TFH process local chemical and mechanical environment is not well controlled, which results more heterogeneous niosomes (1). Due to this different physical backgrounds of these two techniques, it is clear that for example during the formation of niosomes the organization of the polymeric chains as well as the localization of the charge inducing agent are different, which causes the differences in the particle sizes and zeta potential values.

The effect of sonication time and amplitude on %EE, particle size, and PDI value were studied in order to optimize process variables for production of niosomes by UP technique. The most monodispersed formulation (PDI value 0.27) with small particle size (405 nm) and high %EE (75.1%) was produced with 70% amplitude and 45-min sonication time. When the sonication time was increased from 15 to 30 min, the %EE was also increased, whereas it showed a decline when the sonication was further continued until 45-min exposure (Table III). Too long sonication time caused larger vesicles to be reformulated, which led to drug leakage from niosomes and, accordingly, lower entrapment efficiencies. The inverse relationship was observed for particle size and PDI *vs.* sonication time, as smaller niosomes with low PDI were obtained after the 45-min sonication (Table III). Similar results were observed by Pando *et al.*, where the size of resveratrol niosomes was reduced after a prolonged sonication time (32). The smaller particle size itself could cause the lower entrapment efficiency values, because the drug entrapment efficiencies are typically lower with smaller particle sizes (2,33). The amplitude parameter behaved in the same way as the process time (Table III): when the amplitude was increased from 50 to 70%, the %EE was increased, whereas it showed a decline when going toward 90% amplitude. The reason for this was again the higher amplitude that was damaging the vesicles, which led to a leakage of the drug from the niosomes. As the amplitude was increased from 70 to 90%, the particle size and PDI were increased (Table III). The possible reason for this behavior was increased energy output of probe leading to excessive heat generation, which caused the increased particle size and more polydisperse samples.

One of the desirable properties of a successful drug delivery systems is high %EE (34). The drug entrapment efficiency with Span 20 as a surfactant was considerably higher with UP technique (75.1 *vs.* 51.2%), while with Pluronic L64 or mixed surfactant systems, the TFH method produced higher entrapment efficiency values. However, the %EE values with mixed surfactant system and Pluronic L64 were considerably higher with both the techniques (from 75.1 to 90.5%). The low %EE values with Span 20 is possibly due to short alkyl chain with an HLB value of 8.6. This leads to the formation of less dense monolayer structures with hydrophobic environments of alkyl chains in niosome bilayers, which are able to accommodate lower amounts of the hydrophobic drug (35,36). With both the techniques, Pluronic L64 either alone or mixed with Span 20 niosomes yielded high %EE values (Table IV). The structural configuration and amphipathic nature of Pluronic L64 contributed for the higher diacerein entrapment (37). Moreover, F_{Mixed} system comprising Span 20 and Pluronic L64 yielded promising entrapment results of diacerein (90.5%) with TFH. The Pluronic L64 combined with Span 20 produced good mixed systems with better interactions

to entrap diacerein, which suggested a need for further experimentation to explore the mixed surfactant system for a variety of other drugs possessing different pharmacological properties. In this study, the TFH produced a little higher %EE values compared to the UP technique, although the differences were quite small, with the exception of Span 20, where the %EE was increased with the UP method. Span 20 is liquid at room temperature, and its liquid nature might be responsible for easy dispersion by the probe of UP technique forming niosomes with considerably high %EE (38).

Drug-polymer interaction studies were probed using FTIR spectroscopy and DSC analysis. While comparing the spectrum of diacerein with physical mixture (containing both the surfactants) and with the niosome formulation (F_{Mixed}), the sharpening of peak at 2937 cm^{-1} (−COOH) in physical mixture and niosomes (F_{Mixed}) were detected. The pattern of interactions observed in this study were similar as found in an earlier study (39), in which the formulation components of niosomes, Span 60 and cholesterol in a molar ratio of 1:1, presented the special situation of bilayer membrane stabilization by the interactions between glycerol oxygen at position 2 in Span 60 molecule and β-OH group in cholesterol molecule. In the present investigation, this interaction between the glycerol oxygen of Span 20 and β-OH group of cholesterol provided strong stabilization for the bilayer structure, which could be seen as sharpening of the peaks in ATR-FTIR spectrum of the F_{Mixed} in the range of 2900–3000 cm^{-1} (Fig. 1).

Physical mixture and niosomal formulation (F_{Mixed}) containing both amphiphiles, *i.e.*, Span 20 and Pluronic L64, showed relatively similar behavior with heat changes indicating broad transitions, which are generally characteristics of lipid mixtures containing cholesterol. This suggested possible interactions between the components of developing bilayered vesicles (40). However, the DSC thermograms of physical mixture and niosome formulation showed also the melting range of diacerein (245–260°C), a small broadened peak indicating the presence of crystalline drug.

Broadening of the endothermic peak was referred to relatively small amount of drug (15%) in niosomal formulation F_{Mixed} compared to surfactants and cholesterol. Physical state of drug in drug delivery systems is of utmost importance as it affects not only the *in vitro* and *in vivo* drug release but also the stability of the system (41). Moreover, the DSC results (broadened peaks of niosomes F_{Mixed}) highlighted the effect of cholesterol in abolishing the gel-liquid transition temperature of the surfactants employed in the present study (33) and F_{Mixed} (1:1 ratio of surfactants to cholesterol) showed broader peaks in niosomes that further confirmed the formation of rigid vesicles with improved entrapment of drug (42). The present study also revealed that the broadened peak of diacerein in the niosome formulation depicted interactions between diacerein and niosome components justifying the higher entrapment of diacerein in the developed niosomal vesicles (40).

TEM revealed that niosomes exist in nice intact form, and images predicted clearly the integrity of vesicles. The particle sizes seen in TEM images for the formulations (F_{SP20}, F_{PL64}, and F_{Mixed}) were in good agreement with the DLS measurements. From TEM images, the structure of the niosomes could be seen: the outer core of vesicle including the drug material inside the surfactant/lipid bilayer was darker compared to the inner core (7).

The release rates of all niosomal formulations were higher than that of plain drug suspension. This indicated the solubilization effect of diacerein in the surfactant vesicles, which in turn increased the drug release (43). At pH 1.2, TFH formulations F_{SP20}, F_{PL64}, and F_{Mixed} showed 85, 78, and 65% drug release, respectively, whereas UP formulations gave drug release of 100, 98, and 91% during 12 h. In comparison, plain drug suspension released only 38% of drug at this pH at the same time span. At pH 7.4, TFH formulations F_{SP20}, F_{PL64}, and $F_{\sout{MIX}Mixed}$ showed 65, 54, and 48% release, respectively, whereas UP formulations resulted in drug release of 85, 78, and 75% during 12 h. Again, plain drug suspension released only 29% of diacerein at pH 7.4 within the same time period. Overall, at both the pH values, there was a burst in drug release during the first 2 h, which was followed by sustained drug release. The difference in drug release profiles between the formulations was probably due to the differences in vesicle sizes, as was also noticed in an earlier study (44). It was documented that an inverse relationship exists between the particle size and release rate (45), which was also seen in this study. Since the UP formulations had smaller vesicle sizes than the TFH formulations, faster drug release rates of diacerein were found out from the UP formulations. The F_{Mixed} system exhibited the slowest release rate compared to the formulations containing the individual components, which could be due to the greater affinity of drug for the combined niosome matrix, which has also been reported in an earlier study (37). However, with all the niosomal formulations, the release rate was increased remarkably as compared to the plain drug suspension (Fig. 4). The burst release during the first 2 h is beneficial as initial loading dose followed by a sustained maintenance dose are good parameters in many drug delivery conditions (46).

At both the temperatures, the particle size and PDI showed time-dependent changes. The formulations prepared by the UP technique showed greater increase in particle size and PDI compared to the TFH; that was probably due to the smaller original vesicle size, as has been reported earlier (47). It is also worth noting that the UP niosomes were more monodisperse in the beginning (lower PDI values). The small-sized niosomes possess greater surface energy and more tendencies to aggregate in order to lower the excess free energy (48). The size of all niosomal formulations stored at 25°C was increased markedly compared to 4°C. This result implies that the stability of niosomes is primarily affected by temperature. Thus, it is pertinent to suggest that the niosomes should be stored in refrigerated conditions (4°C) to maintain their stability during this period.

CONCLUSION

The study showed that ultrasonic processing technique has potential to act as an eco-friendly alternative to TFH method for the production of diacerein-loaded niosomes. Niosomes were successfully prepared with Span 20, Pluronic L64, and mixed surfactant system at equimolar ratio of cholesterol by using TFH and UP techniques, but UP technique was able to produce smaller-sized niosomes with more homogeneity than the TFH method. The entrapment efficiencies and the release profiles were found comparable for both types of formulations.

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