Journal of Drug Delivery Science and Technology 50 (2019) 27–33

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Process optimization of ecological probe sonication technique for production of rifampicin loaded niosomes

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ARTICLE INFO

Keywords:
Design of experiment (DoE)
Ecological green technology
Niosomes
Poor solubility
Probe sonication
Rifampicin

ABSTRACT

The aim of the present study was to develop an optimized niosome formulation for the encapsulation of a poorly water-soluble drug by the ecological probe sonication method. Pluronic L121 and Span 60 were used as surface active agents and the optimization of the composition was made with the aid of Design of Experiment (DoE) concept. Rifampicin was used as a model drug. Concentration levels of charge inducing agent, dicetylphosphate (DCP), and Pluronic L121 were studied as variables. Prepared niosomes with varying concentrations of DCP and Pluronic L121 resulted in small sized niosomes with sizes ranging from 190 nm to 893 nm. During the four weeks stability testing, the particle sizes were reduced slightly. The formulation containing 2 mg of DCP resulted in most stable niosomes with 75.37% entrapment efficiency. All the niosomal formulations showed higher *in vitro* drug release rates as compared to bulk drug formulation. As a conclusion, rifampicin loaded niosomes prepared with Pluronic L121 and Span 60 resulted in stable, small sized niosomes with improved drug release profile.

1. Introduction

Niosomes are self-organizing non-ionic surfactant vesicles, which encapsulate aqueous volume of drug(s) with or without the addition of cholesterol and other lipid contents [1,2]. Niosomes have the capability to encapsulate both lipophilic and hydrophilic drugs [3]. They are alternative to liposomes, and their main benefits as compared to liposomes are their lower price, higher stability and better biodegradability [4]. By making niosomes, the side effects of drugs have been reduced and the therapeutic efficacy has been increased [5]. More than 50 different drugs have been encapsulated into niosomes and administered via oral, nasal, ophthalmic, inhalation and parenteral routes [6].

The arrangement of non-ionic surfactants in bilayer or micellar form depends on the hydrophilic-lipophilic balance (HLB) of the surfactants. The properties of drug, other membrane additives, and the method of manufacturing also influence the structure and behavior of niosomes [7,8]. Hydrophilic drugs are surrounded by the bilayer of amphiphiles, while hydrophobic drugs are entrapped within the bilayer of vesicles [9]. A number of non-ionic surfactants are used for production of niosomes, such as polysorbates, alkyl ethers, alkyl amides and alkyl esters, but in many studies only a single surfactant is used [10–13]. However, if a mixture of two or more surfactants are used, stable, small and monodisperse niosomes can be reached [10]. Poloxamers are widely utilized pharmaceutical excipients, though they are less studied in formulating niosomes. They are well known to improve the solubility of poorly soluble drugs via solubilization effect [14,15] and they are also functioning as permeation enhancers [16], and hence, good candidates for excipients in niosomal formulations.

Niosomes are prepared by different methods, including thin film rehydration, reverse phase evaporation, and ether injection methods [17,18]. These methods require removal of organic solvents and they are expensive and time consuming. To overcome these problems, probe sonication method has been developed [19]. Probe sonication technique is an eco-friendly green technique with no addition of organic solvents. Besides, it is a simple and low cost technique. In this method, only aqueous phase of drug is mixed with surfactant, cholesterol and other surface additives, and subjected to ultra-sonication with a probe [19]. In an earlier study, where probe sonication and thin film hydration techniques were compared, both the production techniques produced spherical vesicles [19]. Further, niosomes produced by probe

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https://doi.org/10.1016/j.jddst.2019.01.012
Received 11 October 2018; Received in revised form 8 January 2019; Accepted 9 January 2019
Available online 11 January 2019

sonication technique were smaller with higher monodispersity having faster drug release rates as compared to niosomes produced by traditional technique.

As already mentioned, both hydrophilic and hydrophobic drugs can be encapsulated inside the niosomal structures, but poorly soluble hydrophobic drugs are mostly benefitting niosomal formulations. An example of group of drugs, which are good candidates for niosomal drug delivery, are drugs that benefit for targeted and sustained drug release, such as chemotherapeutic agents used for treatments of different type of cancers [20]. The challenge to deliver these kind of drugs in the body is their toxic side effects, poor water solubility and drug resistance [21]. To overcome these problems, niosomes are designed by optimum combination of non-ionic surfactants to increase the solubility, bioavailability, reduction in toxicity and increased residence time in blood circulation [22–24].

The aim of the present study was to improve the drug release profile of a poorly soluble drug by making a niosomal formulation with less utilized environmental friendly and cost efficient probe sonication method. Rifampicin was used as a model drug. Rifampicin is a Biopharmaceutics Classification System (BCS) class II antitubercular/antimicrobial drug and it inhibits the RNA polymerase enzyme [14–18]. Due to low solubility, polymorphism and degradation in gastric fluids, rifampicin is poorly bioavailable [19]. In order to improve the performance of the niosomes, a combination of Span 60 and Pluronic L121 was used for the production of niosomes. A mixture of two surfactants were selected to be used in this study, because the presence of two different surfactants has been shown to be able to improve the properties of the niosomes [10,19]. Pluronic L121 was selected based on its capability to increase solubilization and permeation of poorly soluble drugs. With the aid of factorial design, the composition of niosomal formulations was optimized.

2. Materials and methods

2.1. Materials

Rifampicin (Orion Pharma, Finland) was used as a model poorly water-soluble drug. Sorbitan monostearate (Span 60, Sigma-Aldrich, USA) and PEO-PPO-PEO copolymer (polyethylene oxide-polypropylene oxide-polyethylene oxide copolymer, Pluronic L121, Mn 4400, Sigma-Aldrich, USA) were used as membrane formers in niosomes. Cholesterol (Sigma-Aldrich, USA) was used as membrane stabilizer and dicetylphosphate (DCP, Sigma-Aldrich, USA) as charge tailoring agent. Disodium hydrogen phosphate, potassium dihydrogen phosphate and sodium chloride (all from Sigma-Aldrich, USA) were needed for buffer solutions. Dialysis membranes (Spectra/Por MWCO: 8–10 kD, Sigma-Aldrich, USA) were utilized for drug release testing. Water used in all the tests was Milli-Q water (Millipore, Merckmillipore, USA).

2.2. Methods

2.2.1. Preparation of rifampicin niosomes

For production of niosomes by probe sonication method [19], rifampicin was first mixed with 15 mL of water with the help of magnetic stirrer, after which cholesterol, Span 60, Pluronic L121 and DCP were added. The amount of Pluronic L121 and DCP were the variables in the optimization of the niosome production. Based on the preliminary studies, 290 mg of Pluronic L121 and 1 mg of DCP was selected as a central point for the central composite design. In the factorial design, three different levels of the variables was used: amount of Pluronic L121 was 246, 290 or 334 mg, and amount of DCP 0, 1 or 2 mg. The exact compositions of studied batches are presented in Table 1. The mixture was then subjected to probe sonication (Vibra Cell, Sonics & Materials, Inc., USA) for 5 min at 57 °C of probe temperature in a pulsatile manner (50 s sonication with 10 s pause) with 30% amplitude. After probe sonication, niosomes were collected and stored at 4 °C for physicochemical characterization.

2.2.2. Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy

The possible interactions between the drug, non-ionic surfactants and membrane additives were studied by Attenuated Total Reflectance - Fourier Transform Infrared (ATR-FTIR) spectroscopy. The ATR-FTIR analysis of all the individual components, physical mixture and one niosome formulation were performed. The spectra were collected by using FTIR spectrophotometer (Bruker Optics, Germany) with an additional horizontal accessory of ATR (MIRacle, Pike Technology, Inc., Germany). Spectral range from wavenumber (400-4500 cm^{-1}) was used with a 4 cm^{-1} resolution by using (OPUS 5.5) software with no spectral pretreatment at an ambient temperature [10,25]. The reproducibility of the technique was ensured by making triplicate separate measurements from one single sample.

2.2.3. Thermal analysis

The physical state of the rifampicin in the selected formulation was estimated by using Differential Scanning Calorimetry (DSC 823^e, Mettler Toledo, USA). Pure rifampicin, individual niosome constituents, physical mixture and selected formulation were accurately weighed (3–5 mg) in an aluminium pan and the pan was closed. The thermal scanning was carried out at 5 °C/min and heated from 25 °C to 260 °C. The scans were recorded under the flow of nitrogen gas at a rate of 50 mL/min. Indium was used as a reference standard for the DSC equipment.

2.2.4. Drug entrapment efficiency

For determining the drug entrapment efficiency, the formulations were ultracentrifuged at 28000 rpm for 1 h at 4 °C (Beckman Coulter, Optima LE-80K, USA). The supernatant was collected, the pellet at the bottom of the centrifuge tube was washed twice with water, water was collected and centrifugation was repeated. Drug concentration in the aqueous solution containing supernatants and water used for washing was determined. The percentage entrapment efficiency (EE%) of rifampicin was calculated by the following equation (Equation (1)) [26]:

$$EE\% = [(Q_t - Q_r)/Q_t] \times 100, \quad (1)$$

Where Q_t is the amount of drug initially used for the preparation of niosomes and Q_r is the amount of drug present in supernatant after centrifugation.

2.2.5. Differential light scattering measurement

The diameter of the niosomes (z-average), polydispersity index (PDI) and zeta potential were measured for all the formulations using Zetasizer Nano ZS (Malvern Instruments Ltd., USA). The niosomal dispersions (20 μL) were diluted with water (15 mL) before the measurement to avoid multi scattering phenomenon. All the measurements were performed in triplicates.

2.2.6. Transmission electron microscopy

The morphology of the niosomes was analyzed by the transmission electron microscopy (TEM, Jeol JEM-1400, Jeol Ltd, Japan) using an acceleration voltage of 80 kV. For the sample preparation, niosomal dispersions were negatively stained with freshly prepared 2% uranyl acetate solution, mounted on a carbon-coated copper grid, and air-dried before analysis.

2.2.7. Stability studies

The stability of niosome formulations was determined by storing the niosomal dispersions in sealed 20 mL glass vials at 4 °C in refrigerator. The size, PDI and zeta potential values of the stored formulations were evaluated at predefined time intervals (0, 1, 2, 3 and 4 weeks after preparation). Besides this, visual examination about the physical

Table 1
Composition of different niosome formulations.

Formulations	Span 60 (mg)	Pluronic L121 (mg)	Cholesterol (mg)	DCP (mg)	Drug (mg)	Milli-Q H_2O (ml)
RA1	43	290	77.3	1	–	15
RA2	43	290	77.3	1	10	15
RA3	43	246	77.3	1	–	15
RA4	43	246	77.3	1	10	15
RA5	43	334	77.3	1	–	15
RA6	43	334	77.3	1	10	15
RA7	43	290	77.3	2	–	15
RA8	43	290	77.3	2	10	15
RA9	43	290	77.3	0	–	15
RA10	43	290	77.3	0	10	15

changes was also done.

2.2.8. Drug release studies

The drug release studies were carried out at phosphate buffer pH 7.4 solution in a vessel using a magnetic stirrer. For the dissolution study, dialysis membrane was soaked in water for 24 h prior the testing. Just before the dissolution tests, the aqueous dispersions of the formulations (1 mL) were poured inside the dialysis membrane, clamped and put into the dissolution vessel. The study was carried out at 37 °C, the amount of the medium was 350 mL, and stirring speed was 100 rpm. The aliquots were sampled at predefined time intervals (0, 15 min, 30 min, 45 min, 60 min, 75 min, 105 min, 2.5 h, 4 h, 5.5 h, 8 h, 10 h, 12 h) and replenished with the same amount of fresh buffer. Samples withdrawn from the dissolution media were analyzed on the UV–Vis spectrophotometer (UV−1600PC, VWR Int. bvba, China) at a wavelength of 475 nm.

3. Results and discussion

3.1. Physical characterization of niosomes

Rifampicin loaded niosomes were prepared with the combination of surfactants Span 60 and Pluronic L121. All the formulations of niosomes were containing equal quantities of Span 60, cholesterol and drug. By changing the quantities of Pluronic L121 and DCP, the composition for optimized formulation was determined. Three different levels of these two parameters were studied in the central composite design. The optimization of the process was performed based on the critical quality attributes (CQAs). Selected CQAs for niosomal formulations were small size (size below 350 nm), low PDI (< 0.5) and negative zeta potential (< −25 mV). The value of PDI less than 0.5 is considered to have low polydispersity and low/no aggregation [9]. The zeta potential is related to the stability of the niosomes and it is due to the addition of DCP as a membrane additive. The absolute values of zeta potential close to 30 mV or higher are considered more stable due to the electrostatic repulsive forces [9].

As has been shown in previous studies, the addition of cholesterol gives rigid, stable and intact niosome structures with low PDI value, while, without cholesterol, formulations have gel like appearance [27]. Span 60 leads to stable and large sized niosomes with maximum entrapment efficiency, and the high HLB value (more than 11) hinders the formation of vesicles [28]. The poorly water-soluble drugs are entrapped efficiently by Pluronic L121 and it leads to low PDI value as well as more stable vesicular niosomes [29–31]. The membrane additive DCP is a negative imparting agent, which reduces the aggregation tendency due to significant interparticle electric repulsion and it helps to formulate monodisperse niosomes with low PDI value [32]. Accordingly, in this study the amount of Pluronic L121 and DCP were the variables for the factorial design in order to optimize the composition based on CQAs.

First, formulations without the drug were produced, in order to find the central point for the composition design (RA1), and when the formulation filled the CQA criteria, model drug was added to the composition, and the central composite design model was formed (Table 1). Based on the preliminary screening studies, niosomes containing 290 mg of Pluronic L121 and 1 mg of DCP fulfilled all the CQA criteria, and hence this composition was selected as a central point for the factorial design (RA1).

The particle size information, PDI values and zeta potentials of different studied formulation are shown in Table 2. The particle size in all the formulations ranged between 193 nm and 893 nm and PDI values were from 0.38 to 0.72. All the drug containing formulations fulfilled the particle size requirement of CQAs: the particle sizes ranged from 205 nm to 350 nm. The largest particle size was reached with the composition having lowest amount of DCP (RA10), and the smallest particles were formed with the lowest amount of Pluronic L121. The center point with the drug (RA2) showed PDI value a little above the CQA value, but all the other drug containing compositions showed acceptable PDI values. Differences in size and PDI between the unloaded and drug loaded niosomes were small, except with the highest Pluronic L121 concentration (RA5 vs. RA6).

TEM image of the optimized formulation is shown in Fig. 1. Based on the TEM image, niosomes have well defined spherical shape with a definite wall enclosing aqueous core. Moreover, the mean niosome size from TEM analysis was in a good agreement with that obtained by DLS experiment.

The stability of the niosomal formulations at 4 °C was studied by monitoring the changes in the particle size, PDI and zeta potential values. After one week of storage, size and PDI values were slightly lowered. The formulations showed zeta potential values between −27 mV and −39 mV (Table 2). High absolute zeta potential values in all the formulations indicated good stability. During the storage time of one month at the 4 °C, only minor changes in zeta potential values as well as in particle size and PDI values were observed, indicating good stability of all the studied niosomal formulations.

Table 2
Size, PDI, zeta-potential and entrapment efficiency values of different niosomal formulations.

Formulations	Size average (nm)	PDI	Charge (mV)	EE%
RA1	195.6 ± 12.8	0.492 ± 0.047	−27.5 ± 0.9	–
RA2	314.8 ± 23.9	0.548 ± 0.087	−32.0 ± 0.1	73.38%
RA3	300.5 ± 36.6	0.448 ± 0.034	−38.8 ± 0.3	–
RA4	205.6 ± 10.0	0.482 ± 0.034	−35.6 ± 1.7	71.13%
RA5	893.6 ± 135.5	0.725 ± 0.117	−39.9 ± 5.2	–
RA6	297.8 ± 2.4	0.387 ± 0.005	−36.0 ± 0.5	65.64%
RA7	236.3 ± 36.0	0.391 ± 0.105	−27.5 ± 0.9	–
RA8	255.3 ± 24.6	0.381 ± 0.053	−37.6 ± 1.0	75.37%
RA9	443.5 ± 86.7	0.469 ± 0.037	−34.9 ± 3.4	–
RA10	350.8 ± 62.3	0.481 ± 0.072	−35.5 ± 0.7	80.11%

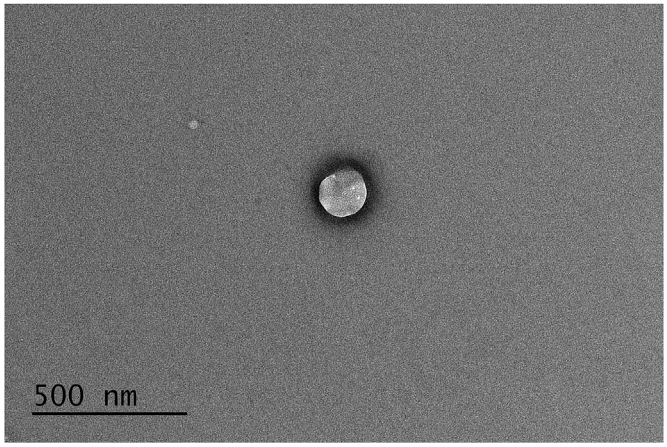

Fig. 1. TEM image of the optimized niosomal formulation.

3.2. Drug entrapment efficiency (EE%)

Entrapment efficiency is an important factor that depicts the formulation characteristics of niosomes. In this study, the entrapment efficiency of the formulations was increased from 71.13% to 73.38% as the amount of Pluronic L121 was increased (from 246 to 290 mg), but the difference was very small. And, at the same time, particle size was increased from ca. 205 nm–315 nm. Accordingly, it is difficult to say if the amount of Pluronic L121 or the change in particle size has more impact on the entrapment efficiency. With small particle systems it is typical that the increase in particle size also increases the entrapment efficiency. When the Pluronic L121 amount was further increased (to 334 mg) the entrapment efficiency was decreased (65.64%), but the particle size was kept quite constant. The critical micelle concentration (CMC) of Pluronic L121 is 0.0004 (wt%), so its concentration in all the formulations were above the CMC [33]. However, when the Pluronic concentration was the highest, the increased solubilization tendency of Pluronic L121 resulted that more rifampicin was soluble in aqueous media, which was reflected by the lowered entrapment efficiency [9,29].

When considering the impact of DCP, formulation without DCP showed maximum entrapment efficiency (80.11%). As the DCP concentration was increased (1 mg) the entrapment efficiency decreased, but further increase in the concentration of DCP (2 mg) increased the entrapment efficiency as indicated in Table 2. Again, the highest entrapment efficiency was reached with largest particle size (ca. 350 nm), which might have been the main reason behind this behavior instead of the changes in the amount of DCP. Accordingly, the amount of DCP alone is not directly responsible for the behavior and these parameters need to be looked in combination.

3.3. Interaction studies

The ATR-FTIR study has a major role in pharmaceutical compatibility assessment of all the ingredients used in the formulation development. The technique is used as a pre-formulation study for the development of products. The ATR-FTIR spectra of rifampicin and all the excipients individually, the physical mixture of the optimized formulation and corresponding optimized niosomal formulation are shown in Fig. 2.

Rifampicin as a pure drug showed the characteristic band peak at 1713 cm^{-1} due to acetyl group and 1733 cm^{-1} due to furanone C=O. Vibration on a broad band 3565-3150 cm^{-1} due to −OH, 1566 cm^{-1} due to amide C=O and 2883 cm^{-1} due to N−CH_3 groups [34]. Span 60 showed the peaks at 2916.75 cm^{-1} (−OH stretch, broad), 2849.58 cm^{-1} (−OH stretch, broad), a cyclic 5-membered ring peak at 1734.65 cm^{-1} and small peaks from 1000 to 1200 cm^{-1} due to

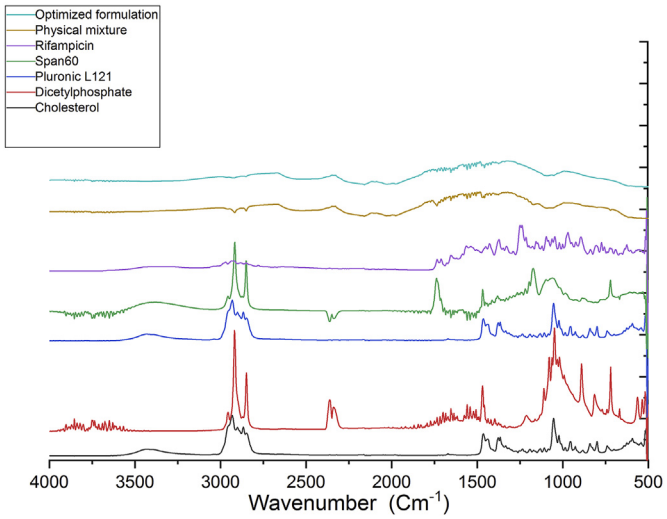

Fig. 2. ATR-FTIR spectra of pure materials, physical mixtures and optimized niosomes.

aliphatic groups [35]. Pluronic L121 showed the peak stretch at 2990 cm^{-1} of as asymmetrical methyl C−H, scissoring of C−H bondage at 1480 cm^{-1}, symmetrical C−H bond at 1387 cm^{-1} and at 1120 cm^{-1} an ether linkage (C−O−C) [36]. Cholesterol showed the ATR-FTIR peaks at 2931.41 cm^{-1}, 2866.83 cm^{-1}, 1770.20 cm^{-1}, and 1055.17 cm^{-1} due to (acetyl group, symmetric −CH_3, vinyl group and R−O group) respectively [25].

The ATR-FTIR spectra of physical mixture of the optimized formulation and niosomes of the same formulation were evaluated for identifying any changes in peaks or peak shifts indicating interactions in the formulation (Fig. 2). The spectra of physical mixture of the formulation and optimized niosomes were similar and they both showed diffusion of the peaks. In earlier studies with Spans and cholesterol, it has been found out that membrane stabilization in niosomes is based on interactions between the glycerol oxygen in Spans and β-OH group in cholesterol, which was causing changes in the spectra [19,37]. Characteristic spectral features of the drug were not seen in the spectra of physical mixture or niosomes. The relative drug amount in these samples were very low, and it is possible that the concentration in the mixture is below the detection limit, and/or overlapping events are disturbing the analysis.

In thermal analysis, Span 60, DCP, cholesterol and rifampicin showed their characteristic melting endotherm at 54 °C, 78 °C, 150 °C, and 182 °C, respectively, as shown in Fig. 3. The physical mixture showed a slightly broader peak at 57 °C indicating the slight interaction between the Span 60 and cholesterol [37]. The niosome formulation indicated extra peaks between the 79 °C- 107 °C indicating material interactions. These interactions increased the stability and rigidity of the vesicular bilayer in niosomes, which was seen also in considerably high entrapment efficiency values as well as in enhanced storage stability.

No rifampicin melting peak or shift in the baseline due to T_g of amorphous drug was seen in the physical mixture nor in the prepared niosomes. The drug amount in the composition was low, which could be one reason for that these events are not seen in the thermograms, or drug might have formed molecular level mixtures with excipient(s). However, based on the results, it is impossible to confirm which was the case. Molecular level mixtures can increase the entrapment efficiency values, and physical form of the drug inside the niosomes is important because it can influence the *in-vitro* and *in-vivo* release patterns of the drug [38].

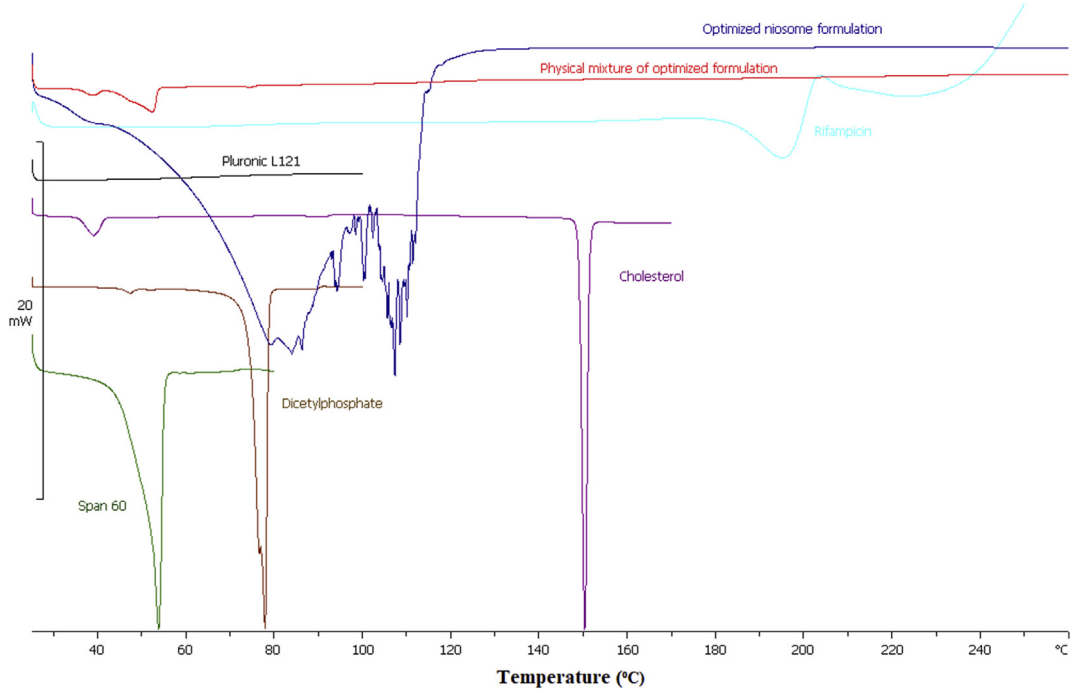

Fig. 3. DSC thermograms of pure materials, physical mixtures and optimized niosome formulation.

3.4. Stability studies

The storage stability at 4 °C of all the niosome formulations with or without drug was studied for a period of 1 month (Table 3). After one week of storage time, formulations RA6 containing highest amount of Pluronic L121 and RA10 with highest amount of DCP resulted in slightly reduced niosome size. All the other drug loaded niosomes increased in size during the first week of storage. This kind of behavior is typical for niosomes. When the sonication stress is over, e.g. after one week of storage, niosomes are relaxed and they become fully matured due to which particle size reaches the stable final value [10].

After the first week, all the formulations tended to reduce in size. The formulation RA7 of empty niosomes and corresponding drug loaded formulation RA8 showed the best results, small stable particle size with low PDI value throughout the 1-month storage period. The formulations RA7 without drug and RA8 with rifampicin contained high concentration of DCP (2 mg, Table 3). With these niosomes (RA7 *vs* RA8), addition of the drug to the composition clearly increased the particle size, same kind of behavior was also seen with the central point (RA1 *vs* RA2) composition.

PDI values of all the freshly prepared formulations were less than 0.5 except formulation RA5 (0.725). After one week, the PDIs of all the formulations were reduced, though PDI of RA5 was still slightly higher than 0.5. The formulations RA7 (without rifampicin) and RA8 (with rifampicin) showed most stable and low PDI values during the storage indicating good stability.

If the zeta-potential of niosomes are close to or below −30 mV, particles are quite stable due to interparticle repulsive forces, which are able to prevent particle aggregation. All the formulations in this study showed negative zeta-potentials close to or below −30 mV, and the differences in zeta-potential values were small. Accordingly, based on zeta-potential values there were no big differences between the different formulations.

3.5. Drug release studies

The dissolution studies of all the rifampicin loaded formulations and control (pure rifampicin drug) was carried out in phosphate buffer saline pH 7.4 (Fig. 4). A burst release of drug from all the niosome formulations was seen in the beginning of the dissolution testing, which was caused by the presence of Pluronic L121 [39]. It has been reported previously that Pluronic L121 has good solubilization behavior due to which the dissolution rate of the niosomal formulations increased when compared with pure drug [35]. The drug release profiles from all the niosomal formulations were similar during the first 2 h of drug release testing. After the first 2 h, the drug release increased following the order: RA6 < RA10 < RA4 < RA8 < RA2 with the percentages 61.69%, 62.75%, 66.84%, 70.78% and 75.90%, respectively, drug released within 12 h. The pure drug released 32.43% at the same time period.

While the presence of Pluronic L121 increased the drug release in all the niosomal formulations, effect of its relative amount on the drug release was not that clear: with the highest amount of Pluronic L121 (334 mg), the formulation RA6 showed least drug released, and the formulation (RA4) with lowest amount of Pluronic L121 (246 mg) showed comparatively more drug release. Highest drug release value (75.90%) was reached with the formulation RA2, which contained 290 mg Pluronic L121. Formulation RA10 without DCP and RA8 with highest amount of DCP (2 mg) released 62.75% and 70.78%, respectively.

Particle sizes of the different drug loaded niosomal formulations were from 205 to 350 nm; it is well known from literature that particle size is also affecting the drug release behavior, as well as the drug loading level in the formulations. The high concentration of cholesterol results in more rigid niosomes and DCP as a charge tailoring agent is changing the electrical properties of niosomes, which can have impacted on the interactions as well as drug release behavior.

Formulations releasing highest amount of drug after 12 h drug release testing, RA2 and RA8, were both containing 290 mg of Pluronic L121. The amount of DCP was 1 mg in RA2 and 2 mg in RA8. Their particle sizes (315 and 255 nm) and entrapment efficiencies (73 and 75%) had intermediate-level values. Accordingly, as discussed before, the faster dissolution rate was not caused by a single factor, but it was more combined effect.

Table 3
Particle size, PDI and zeta-potential values of different niosomal formulations after 0, 1, 2, 3 and 4 weeks storage at 4 °C.

Time	Parameters	RA1	RA2	RA3	RA4	RA5	RA6	RA7	RA8	RA9	RA10
0 week	Size (nm)	195.6 ± 12.8	314.8 ± 23.9	300.5 ± 36.6	205.6 ± 10.0	893.6 ± 135.5	297.8 ± 2.4	236.3 ± 36.0	255.3 ± 24.6	443.5 ± 86.7	350.8 ± 62.3
	PDI	0.492 ± 0.047	0.548 ± 0.087	0.448 ± 0.034	0.482 ± 0.034	0.725 ± 0.117	0.387 ± 0.005	0.391 ± 0.105	0.381 ± 0.053	0.469 ± 0.037	0.481 ± 0.072
	Charge (mV)	−27.5. ± 0.9	−32.0 ± 0.1	−38.8. ± 0.3	−35.6 ± 1.7	−39.9. ± 5.2	−36.0 ± 0.5	−27.5 ± 0.9	−37.6 ± 1.0	−34.9 ± 3.4	−35.5 ± 0.7
1 week	Size (nm)	223.4 ± 11.4	436.1 ± 16.4	199.3 ± 5.4	257.5 ± 19.0	244.8 ± 7.7	242.0 ± 7.3	175.8 ± 5.8	337.9 ± 12.8	294.4 ± 8.0	211.7 ± 14.1
	PDI	0.340 ± 0.046	0.458 ± 0.030	0.282 ± 0.014	0.410 ± 0.007	0.535 ± 0.020	0.381 ± 0.020	0.198 ± 0.040	0.240 ± 0.022	0.362 ± 0.038	0.258 ± 0.030
	Charge (mV)	- 23.8. ± 1.0	- 31.4 ± 1.1	- 28.5 ± 0.6	- 26.2 ± 0.4	- 30.4 ± 0.5	- 22.4 ± 0.7	- 26.9 ± 0.9	- 28.2 ± 1.0	- 28.2 ± 1.0	- 29.3 ± 2.1
2 weeks	Size (nm)	191.9 ± 3.3	300.1 ± 18.9	212.7 ± 6.1	284.4 ± 32.5	245.2 ± 5.5	253.8 ± 20.1	178.7 ± 3.6	253.5 ± 25.3	347.4 ± 8.9	327.8 ± 49.8
	PDI	0.168 ± 0.039	0.287 ± 0.023	0.299 ± 0.022	0.450 ± 0.008	0.543 ± 0.015	0.358 ± 0.015	0.169 ± 0.028	0.272 ± 0.045	0.375 ± 0.030	0.424 ± 0.002
	Charge (mV)	- 23.8. ± 1.0	- 24.2 ± 0.9	- 28.2 ± 3.0	- 21.2 ± 2.2	- 26.3 ± 0.5	- 28.0 ± 1.3	- 28.1 ± 2.2	- 23.7 ± 0.6	- 29.5 ± 2.6	- 23.8 ± 0.6
3weeks	Size (nm)	199.4 ± 4.9	325.9 ± 12.8	208.7 ± 4.6	258.9 ± 27.1	291.6 ± 8.3	173.0 ± 12.2	172.2 ± 1.7	240.4 ± 2.4	296.3 ± 12.6	251.2 ± 22.7
	PDI	0.213 ± 0.011	0.310 ± 0.031	0.324 ± 0.020	0.359 ± 0.026	0.532 ± 0.051	0.362 ± 0.054	0.151 ± 0.033	0.232 ± 0.019	0.320 ± 0.051	0.335 ± 0.036
	Charge (mV)	- 27.2 ± 0.4	- 24.1 ± 0.2	- 31.6 ± 2.2	- 22.2 ± 0.6	- 28.9 ± 0.2	- 22.3 ± 2.7	- 28.9 ± 0.5	- 23.9 ± 0.6	- 28.6 ± 1.5	- 24.9 ± 1.4
4 weeks	Size (nm)	186.8 ± 1.6	328.3 ± 14.4	190.0 ± 2.5	211.4 ± 12.8	255.8 ± 34.8	160.8 ± 20.6	182.0 ± 2.7	250.9 ± 15.9	311.8 ± 8.1	311.7 ± 30.5
	PDI	0.138 ± 0.025	0.509 ± 0.077	0.208 ± 0.037	0.373 ± 0.011	0.534 ± 0.078	0.297 ± 0.088	0.226 ± 0.016	0.285 ± 0.035	0.364 ± 0.042	0.416 ± 0.013
	Charge (mV)	- 25.5 ± 1.3	- 29.6 ± 0.3	- 27.8 ± 0.9	- 21.4 ± 1.0	- 29.8 ± 1.1	- 21.7 ± 1.5	- 27.3 ± 1.5	- 22.4 ± 0.2	- 26.7 ± 0.2	- 23.6 ± 0.3

Fig. 4. Drug release profiles of niosome formulations containing rifampicin at pH 7.4.

4. Conclusion

In the present study, the poorly water-soluble drug, rifampicin as a model drug, was encapsulated inside the niosomes. The core of niosomes was produced with Span 60 and varying concentrations of Pluronic L121 and dicetylphosphate (DCP). Both two variables, the amounts of Pluronic L121 and DCP, had some impact on the properties of the formed niosomes, but all the prepared drug loaded formulations still showed reasonable drug entrapment efficiency, acceptable size, good stability and higher dissolution profile as compared to bulk drug formulation. Based on the study, the use of Span 60 and Pluronic L121 in combination for the preparation of niosomes are a promising potential carrier system for the encapsulation of poorly soluble drugs for improved drug release profiles.

Declarations of interest

None.

Acknowledgements

The International Research Support Initiative Program of Higher Education Commission of Pakistan is acknowledged for the travel grant awarded to Mr. Daulat Haleem Khan for the research visit in University of Helsinki, Finland. Prof. Hélder A. Santos acknowledges financial support from the Sigrid Jusélius Foundation (Decision No. 4704580), the European Research Council under the European Union's Seventh Framework Programme (FP/2007–2013, Grant No. 310892) and the HiLIFE Research Funds.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2019.01.012.

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