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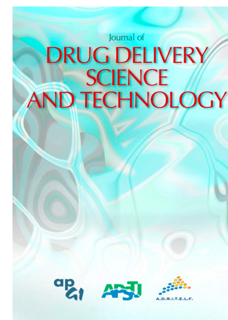

Formulation optimization and *in vitro* characterization of rifampicin and ceftriaxone dual drug loaded niosomes with high energy probe sonication technique

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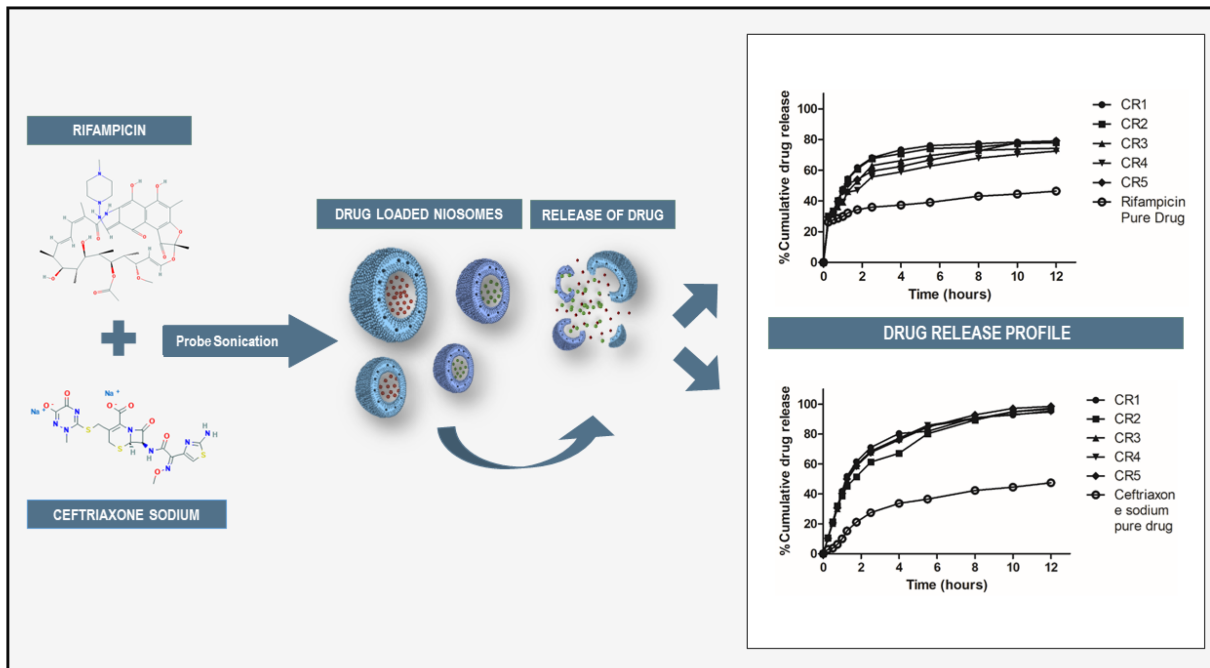

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Formulation optimization and *in vitro* characterization of rifampicin and ceftriaxone dual drug loaded niosomes with high energy probe sonication technique

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Abstract

The aim of the present study was to prepare niosomal formulations for dual drug therapy of ceftriaxone sodium and poorly water-soluble rifampicin 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. 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 165 nm to 893 nm. During the four weeks stability testing, the particle sizes of the empty niosomes were reduced, while the particle sizes of the drug loaded niosomes were increased very slightly. The optimized formulations resulted in stable niosomes with high drug entrapment efficiencies: entrapment efficiency was 99% for rifampicin and 96% for ceftriaxone. All the niosomal formulations showed faster *in vitro* drug release rates as compared to bulk drug formulations. In conclusion, ceftriaxone and rifampicin loaded niosomes prepared with Pluronic L121 and Span 60 resulted in stable, small sized niosomes with high drug entrapment efficiencies and improved drug release profiles.

Keywords: Ceftriaxone sodium; Design of experiment (DoE); Ecological probe sonication; Niosomes; Poor solubility; Rifampicin

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1. Introduction

In dual drug therapy, two active pharmaceutical agents (API) with synergistic drug effect are administered concurrently. For example, in cancer therapy, simultaneous administration of doxorubicin and paclitaxel has shown to be beneficial: paclitaxel causes depolymerization of microtubules, leading to mitotic arrest, and doxorubicin intercalates into the duplex preventing biosynthesis of nucleic acids, resulting cell apoptosis [1].

Tuberculosis is a global health problem that causes worldwide approximately 1.5 million deaths every year. Treatment of drug-susceptible tuberculosis requires combination anti-microbial therapy with a minimum of four antimicrobial agents applied over the course of 6 months time [2]. In antimicrobial therapy, the increasing number of infections caused by antimicrobial-resistant organisms, in particular the methicillin-resistant *Staphylococcus aureus* (MRSA), has led to high interest towards antimicrobial combination therapies [3,4]. The antibacterial drug combinations have been recommended extensively in clinical practice owing to enhanced bactericidal activity, reduced toxicity and selection pressure, and, most importantly, suppressed possibility of resistance [5]. Rifampicin and cephalosporins, such as ceftriaxone, combination therapy has been shown to be especially beneficial in cases where there is a low organism burden, *e.g.* in resistant biofilm infections [6]. Therefore, loading rifampicin along with ceftriaxone within the advanced drug delivery system such as niosomes is needed.

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Niosomes are vesicles made from self-organizing non-ionic surfactant systems, which encapsulate aqueous volume of API(s) with or without the addition of cholesterol and other lipid constituents [7,8]. Niosomes are able to encapsulate both hydrophilic and hydrophobic drugs [9], and they are good alternatives to liposomes due to their benefits of lower price, higher stability and better biodegradation [10]. By fabricating niosomes, the therapeutic efficacy of drugs has been increased, while reducing at the same time side effects [11].

More than 50 different types of drugs have been encapsulated in niosomes, and administered *via* inhalation, nasal, oral or parenteral routes [12]. The characteristics of drug material, membrane additives and method of preparation influence the structure and properties of niosomes [13-15]. Numerous non-ionic surfactants have been used for the

manufacturing of niosomes, *i.e.*, polysorbates, alkyl esters, alkyl ethers and alkyl amides [16-19]; mixtures of non-ionic surfactants have resulted in more stable, monodisperse and smaller niosomes [16]. Poloxamers, common pharmaceutical solubility enhancing agents [20,21], and permeation enhancers [22], have been extensively used as pharmaceutical excipients, though so far they have been less frequently utilized in niosomal formulations.

Different methods have been used for preparation of niosomes, *i.e*,. the reverse phase evaporation technique, the ether-injection method and the extensively used thin film hydration method [23,24]. However, these methods are time consuming, ecotoxic, expensive, and they require removal of organic solvents. A more recent technique, called probe sonication method, is a simple, fast, eco-friendly and solvent free method, with low cost of production [25]. In our previous study, we have shown that spherical niosomes were obtained with both probe sonication and thin film hydration techniques, though niosomes prepared with probe sonication method were even smaller having faster drug release rates [25].

In niosomal structures, hydrophobic drugs can be encapsulated between the bilayer and hydrophilic drugs inside the bilayer structure of non-ionic surfactant systems. Accordingly, different types of drugs can be encapsulated into the niosomes, in which anticancer drugs are an example of class of drugs that have been formulated within niosomes for targeted and/or sustained delivery purposes [26]. The challenge is to achieve the combined therapy by loading multiple drugs into a single drug delivery system and delivering them to the site of action [27-29]. Although the loading of multiple APIs can be problematic due to the loading of APIs with different physicochemical characteristics [30], a carrier containing multiple drugs can promote the APIs' synergism and disease management [29].

The aim of the present study was to prepare niosomal formulations loaded with rifampicin and ceftriaxone as APIs for dual drug therapy purposes. In the production of niosomes, an environmentally friendly and cost-effective probe sonication method was used. Rifampicin is a Biopharmaceutics Classification System (BCS) class II drug having poor water solubility, and ceftriaxone sodium is a BCS class III drug, presenting low permeability [20-23,31,32]. These undesired characteristics of rifampicin and ceftriaxone make them good candidates for niosomal encapsulation.

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In order to improve the performance of the niosomes, a combination of non-ionic surfactants of Span 60 and Pluronic L121 was used for the construction of niosomes, as it has been shown in earlier studies that utilization of non-ionic surfactant mixtures have led more stable, monodisperse and smaller niosomes [16,25,33]. Pluronic L121 was selected due to its capability to improve the solubilization of poorly water-soluble drugs, like rifampicin in this study.

With the aid of factorial design, the exact composition of the niosome formulations was optimized. As variables in the factorial design, the amount of Pluronic L121 and charge imparting agent, dicetylphosphate, were altered in three different levels. Charge imparting agent was added to the composition in order to study the importance of the zeta-potential on drug loading and stability of niosomes.

2. Materials and methods

2.1.Materials

Rifampicin (Orion Pharma, Finland) and ceftriaxone sodium (Orion Pharma, Finland) were used as APIs in the formulations. Polyethylene oxide-polypropylene oxide-polyethylene oxide copolymer (PEO-PPO-PEO copolymer, Pluronic L121, Mn 4400, Sigma-Aldrich, USA) and Sorbitan monostearate (Span 60, Sigma-Aldrich, USA) were used as bilayer membrane formers. Dicetylphosphate (DCP, Sigma-Aldrich, USA) was used as charge imparting agent, and cholesterol (Sigma-Aldrich, USA) as membrane stabilizing agent. Sodium chloride, disodium hydrogen phosphate and potassium dihydrogen phosphate (all from Sigma-Aldrich, USA) were used for the buffer solution. Water used was Milli-Q water (Millipore, Merckmillipore, USA).

2.2.Preparation of niosomes

Niosomes were prepared by probe sonication method [25,33]. First, rifampicin and ceftriaxone sodium were mixed with 15 mL of water with the aid of magnetic stirrer, after which cholesterol, Span 60, Pluronic L121 and dicetylphosphate (DCP) were added. The mixtures were then subjected to probe sonication for 5 min time at 57 °C (probe temperature) in a pulsatile manner (50 sec sonication with 10 sec pause) at an amplitude of 30%. After probe sonication, niosome formulations were collected and stored at 4 ℃ for further physicochemical characterization. The amounts of Pluronic L121 and DCP

were the variables in the optimization of niosome formulations. The exact compositions of studied niosomal formulations are shown in Table 1.

Table 1. The exact compositions of the studied niosome formulations.

Formulations	Span 60 (mg)	Pluronic L121 (mg)	Cholesterol (mg)	DCP (mg)	Ceftriaxone sodium (mg)	Rifampicin (mg)	Water (mL)
E1	43	290	77.3	1	-	-	15
E2	43	290	77.3	2	-	-	15
E3	43	290	77.3	0	-	-	15
E4	43	246	77.3	1	-	-	15
E5	43	334	77.3	1	-	-	15
CR1	43	290	77.3	1	10	10	15
CR2	43	290	77.3	2	10	10	15
CR3	43	290	77.3	0	10	10	15
CR4	43	246	77.3	1	10	10	15
CR5	43	334	77.3	1	10	10	15

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2.3. Attenuated Total Reflectance–Fourier Transform Infrared (ATR–FTIR) spectroscopy

The interactions between the non-ionic surfactants, drugs, and other membrane additives were studied by ATR–FTIR spectroscopy. The ATR–FTIR analysis of all the individual constituents, physical mixture of the constituents, and one niosomal formulation (dried niosomes, dried in filter paper at room temperature) were performed. The spectra were collected by the FTIR spectrophotometer (Bruker Optics, Germany) with an ATR additional (horizontal) accessory (MIRacle, Pike Technology, Inc., Germany) in the wavenumber range of 400–4500 cm^{-1} and with a resolution of 4 cm^{-1}. The spectral data was analyzed by the OPUS 5.5 software with no pre-treatment of spectra. The measurements were performed at room temperature. All the measurements were performed in triplicate.

2.4. Thermal Analysis

The physical states of the rifampicin and ceftriaxone in the selected formulation were analyzed by using Differential Scanning Calorimetry (DSC 823e, Mettler Toledo, USA). The pure drugs (powder), individual constituents of the niosomes including Span 60,

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Pluronic L121 and cholesterol, physical mixture of the constituents and one niosomal formulation were weighed accurately in aluminum pans, which were further closed with cap having a tiny hole on it. The thermal scanning was conducted at a rate of 5 °C/min from 25 °C to 260 °C. The scans were recorded under the nitrogen gas flow at a rate of 50 mL/min. Indium was used as a reference standard for the equipment.

2.5.Drug entrapment efficiency

For the determination of drug entrapment efficiency, the formulations were ultra-centrifuged (Beckman Coulter, Optima LE-80K, USA) at 4 °C at a speed of 28 000 rpm for 1 h. The supernatant was collected, and the pellets were washed twice with water. The water was collected, and centrifugation was repeated. The drug concentration was measured in supernatants after washing steps. The percentage of entrapment efficiency (%EE) of drugs was calculated using the following equation (Equation 1):

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

where, Q_t is the amount of the drug used initially for the preparation of formulation and Q_r is the amount of the drug present in the supernatants. All the drug entrapment efficiency tests were repeated three times.

2.6.Differential light scattering analysis

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

2.7.Transmission electron microscopy

Transmission electron microscope (TEM, Jeol JEM-1400, Jeol Ltd, Japan) was used for the morphological analysis of the niosomes. An acceleration voltage of 80 kV was used and the sample was negatively stained using 2% of uranyl acetate solution. For TEM analysis, niosome suspensions were diluted in order to be able to avoid aggregated samples and to study separated niosome particles. Samples were mounted on carbon coated copper mesh and dried in room temperature before analysis.

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2.8.Stability studies

The stability of all the formulations was determined by storing them at 4 °C in a sealed 20 mL glass vial. The size, PDI and zeta-potential values were recorded at predefined time intervals (fresh preparation, 1, 2, 3 and 4 weeks after manufacturing and storage). All the measurements were repeated three times.

2.9.Dissolution studies

The dissolution studies of all the niosomal formulations were carried out in phosphate buffer saline pH 7.4 at 37 °C under continuous stirring in a glass vessel with an established method utilized in earlier studies [9,25,33]. For the dissolution, the dialysis membrane (Spectra/Por MWCO: 8–10 kD, Sigma-Aldrich, USA) was soaked in water for 24 h time prior the study. Then, 1 mL of niosomal dispersion was added inside the dialysis membrane, membrane ends was clamped, and the membrane was put in 350 mL of dissolution medium, under stirring at 100 rpm. The aliquots were sampled and replenished with the same volume of fresh buffer 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). The withdrawn samples were analyzed for rifampicin and ceftriaxone sodium concentrations with UV-Vis spectrophotometer (UV-1600PC, VWR Int. bvba, China) at wavelengths of 475 nm and 241 nm, respectively. The sampling and concentration analysis were performed in triplicates.

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2.10. Design of Experiment (DoE) and Data analysis

In factorial design set up for optimization of niosomal formulation, central composite design for two factors with axial design points were utilized in DoE. The amounts of Pluronic L121 and DCP were the variables in the optimization of niosome formulations. If not otherwise stated, all the results are given as an average value and standard deviation of three separate measurements.

3. Results and discussion

3.1.Characterization of niosomes

Rifampicin and ceftriaxone sodium were co-loaded into niosomes, prepared with Pluronic L121 and DCP as formulation variables. Both empty and drug loaded formulations were produced with the same factorial design. Fixed concentrations of Span 60, cholesterol and

drugs were used. The optimization of the formulations containing both the drugs was performed containing 290 mg of Pluronic L121 and 1 mg of DCP as a central point in the factorial design. The exact compositions of the different formulations are presented in Table 1.

The physicochemical characteristics of niosomes, such as average size (< 350 nm), PDI (< 0.5) and zeta-potential (< −30 mV) values were considered as critical quality attributes (CQAs). Here, PDI values lower than 0.5 indicates low level of aggregated niosomes. Similarly, a zeta-potential value below −30 mV indicates the presence of electrostatic repulsive forces, which result in a higher stability of the system [34]. DCP was added for adjusting the zeta-potential value. The previous study suggested that the presence of cholesterol resulted in more stable, rigid and intact niosomes, without gel formation, and for that reason, cholesterol was added to the composition [35].

Typically, non-ionic surfactants presenting a high hydrophilic-lipophilic balance (HLB) value hinder the formation of bilayer structure. Here, we used Span 60 to promote the formation of stable, rigid, intact and large niosomes, with the capability of high entrapment efficiency [36]. Additionally, Pluronic L121 encapsulates hydrophobic drugs more efficiently, and it has solubilization properties, which is important for efficient dissolution of poorly water soluble drugs [37-39].

In this study, the average sizes of the produced niosomes ranged between 165 nm and 893 nm, with PDI values from 0.333 to 0.725 (Table 2). The drug-loaded niosomes were smaller than corresponding empty niosomes, with sizes varying between 165 nm and 206 nm. All the drug-loaded niosomes have PDI values below 0.5, and zeta-potential values ranging from −25.9 mV to −39.9 mV, meaning acceptable quality.

The morphology of the niosomes was studied by TEM (Figure 1). Before TEM analysis niosome suspensions were diluted in order to be able to monitor the structure and form of single niosomes. Though the size of the niosomes based on TEM figures seemed to be in good agreement with DLS determinations, it is important to notice that DLS measurements are much more reliable for particle sizing due to the large amount of particles measured for the analysis in that technique.

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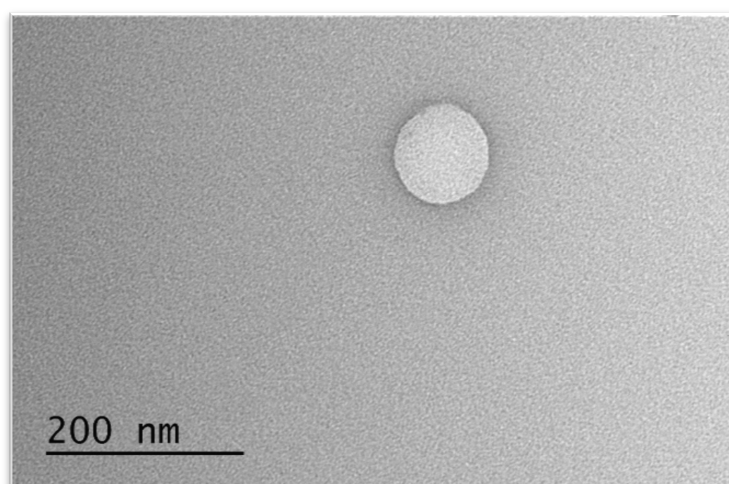

Figure 1. Example of one TEM image of a single niosome showing its morphology and shape. Image is taken from niosomal formulation from batch CR1.

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3.2. Drug entrapment efficiency

The percentages of entrapment efficiency (%EE) values of all the niosome formulations containing rifampicin and ceftriaxone were high, and the differences in the values between different batches were very small (Table 2). The formulations CR3, CR4 and CR5 had highest entrapment efficiency values. The formulation CR3 was without DCP. The formulation CR4 was prepared with the lowest amount of Pluronic L121 (246 mg), and formulation CR5 contained the highest amount of Pluronic L121 (336 mg). Accordingly, the quantities of Pluronic and DCP affected on CQAs and %EE, but the exact relations are not clear. The %EE of hydrophobic rifampicin was higher in all the batches as compared to hydrophilic ceftriaxone sodium. Part of the hydrophilic ceftriaxone might have escaped to outer aqueous phase during the preparation, while hydrophobic rifampicin preferred the hydrophobic environment inside the niosomes.

Table 2. Physical characteristics and %EE values for all the prepared niosomal formulations.

Formulations	Size (nm)	PDI	Zeta-potential (mV)	%EE Rifampicin	%EE Ceftriaxone sodium

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E1	195.6±12.8	0.492±0.047	-27.5 ±0.9	-	-
E2	236.3±36.0	0.391±0.105	-27.5± 0.9	-	-
E3	443.5±86.7	0.469±0.037	-34.9± 3.4	-	-
E4	300.5±36.6	0.448±0.034	-38.8±0.3	-	-
E5	893.6±135.5	0.725±0.117	-39.9±5.2	-	-
CR1	187.2±3.5	0.421±0.018	-25.9± 0.7	98.71	95.73
CR2	164.8±6.1	0.333±0.039	-29.1± 0.2	98.86	95.88
CR3	192.5±13.7	0.455±0.087	- 7.2± 1.2	99.59	96.84
CR4	195.4±16.6	0.499±0.036	-28.6± 1.2	99.30	96.41
CR5	205.7±18.9	0.473±0.095	-29.6± 0.3	99.49	96.67

3.3.Interaction studies

ATR–FTIR spectroscopy gives information related to compatibility of all the ingredients present in formulations. The ATR–FTIR spectra of rifampicin, ceftriaxone sodium, all the excipients, physical mixture of the niosomal formulation and corresponding niosomal formulation CR1, are shown in Figure 2.

Rifampicin showed the bands for acetyl group and furanone (C=O) at 1713 cm^{-1} and 1733 cm^{-1}, respectively. Vibrations at broad band area (3565 cm^{-1} -3150 cm^{-1}) were due to –OH group. Due to amide group, C=O peak at position 1566 cm^{-1}, and due to N-CH_3, peak at 2883 cm^{-1}, were seen, as reported earlier [40].

Ceftriaxone sodium showed a broad band at 3530–3570 cm^{-1} due to the amide group. In β-lactam ring, 6-H and 7-H stretching are shown at 2948 cm^{-1}. At 1772 cm^{-1} and 1670 cm^{-1} stretching of C=O and of β-lactam and amide bond were observed. The stretching of oxime (C=N) was detected at 1592 cm^{-1}, and a vibration band on a broad band area at 1515–1570 cm^{-1} was due to acrylic amide. The stretchings of C-O and N-O were observed at 1060 cm^{-1} and 1025 cm^{-1}, respectively, as reported elsewhere [41].

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Span 60 showed the peaks at 2916 cm^{-1} and 2849 cm^{-1} due to –OH stretching. The peak for the 5-membered cyclic ring was seen at 1734 cm^{-1}. The small broad band peaks ranging from 1000 cm^{-1} to 1200 cm^{-1} can be ascribed to the aliphatic groups, which are also reported in previous studies [42].

Pluronic L121 showed peak stretch of asymmetrical methyl C–H at 2990 cm^{-1}. The scissoring bondage of C-H group at 1480 cm^{-1}, symmetrical C–H bond at 1387 cm^{-1} and ether linkage of C–O–C at 1120 cm^{-1} were observed, as previously reported [43]. Cholesterol showed ATR-FTIR peak of acetyl group at 2931 cm^{-1}, $-CH_3$ (symmetric) at 2866 cm^{-1}, vinyl group at 1770 cm^{-1}, and R–O group at 1055 cm^{-1}, as observed in earlier findings [44].

The spectra of physical mixture and corresponding niosomal formulation CR1 were similar, and the peaks were diffused, which is due to interaction between the glycerol group in Span and β-OH group in cholesterol [25,45]. The characteristic spectral peaks of pure drugs were not observed in the spectrum of the niosome formulation, as observed also in the previous study [16]. The diffusion of the spectra of physical mixture and niosomal formulation indicated drug excipient interactions.

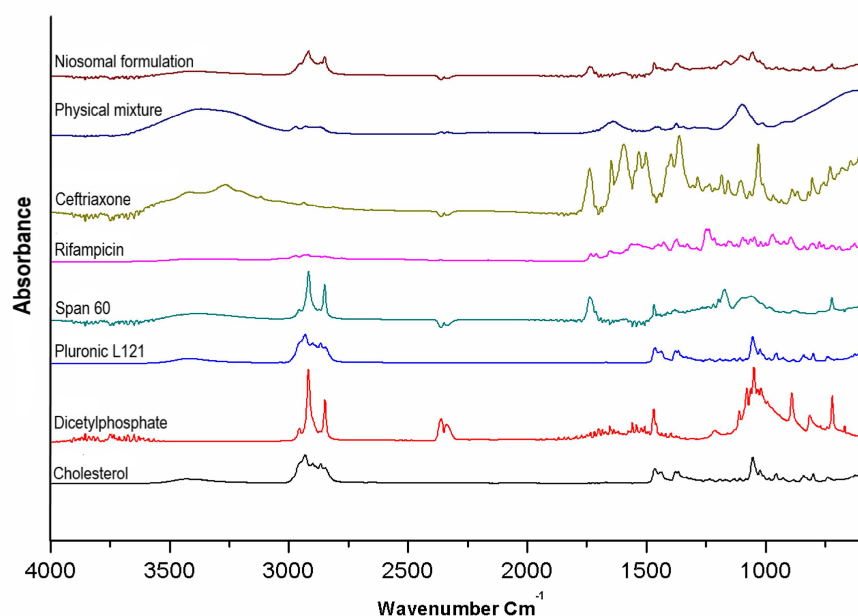

Figure 2. ATR−FTIR spectra of all the pure raw materials, physical mixture of niosome composition (CR1) and corresponding niosomal formulation (CR1) containing ceftriaxone sodium and rifampicin.

The thermal DSC analysis showed characteristic melting endotherms of Span 60, cholesterol, DCP, rifampicin and ceftriaxone sodium at 54 °C, 150 °C, 78 °C, 184 °C and 162 °C, respectively (Figure 3). Additionally, a small peak of ceftriaxone was detected at 47 °C. The physical mixture of optimized formulation showed a slightly broader peak at 59 °C, which is the indication of interaction of Span 60 and cholesterol, as already described with ATR−FTIR results part, and as reported in previous observations [16].

Studied niosomal formulation CR1 showed endothermic events between 79–122 °C, but no clear drug melting peaks were observed. The relative drug amount in the formulations were small, which could cause the lack of the characteristic melting peaks. The presence of drugs inside the vesicles is not detectable and with low total drug quantity it is expected that sharp or prominent melting peaks are not shown. It is also possible that the drug is dispersed in molecular level to the excipients, as was suggested by the ATR−FTIR results, or that the drug is in amorphous form, but it is not possible to confirm these conclusions based on the DSC results alone.

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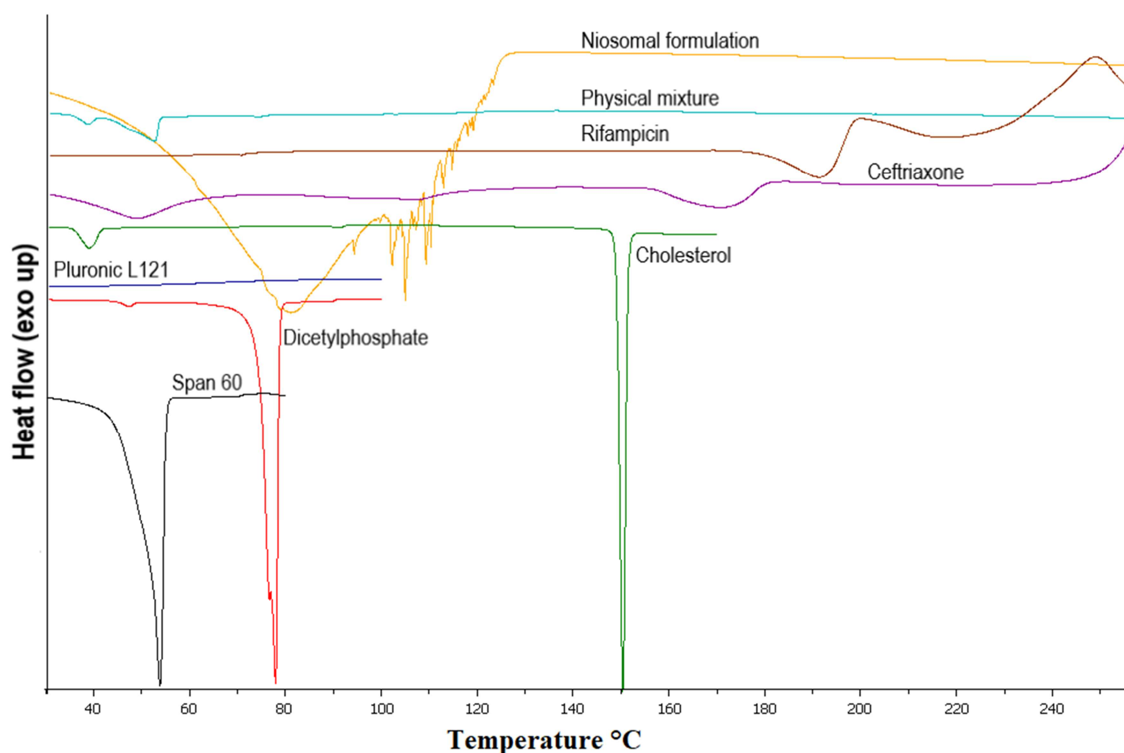

Figure 3. DSC thermograms of pure materials, physical mixture of niosomal composition (CR1) and niosomal formulation (CR1) containing ceftriaxone sodium and rifampicin.

3.4.Stability Studies

The stability study was carried out at 4 °C for all the niosomal formulations, and the results are shown in Table 3. After one week of storage, the formulations without drug loading (E1-E5) decreased in size. The formulations loaded with drugs showed slight increase in size (CR1-CR5), except formulation CR2, which had the highest concentration of DCP and lowest PDI value.

Formulations E1, E2, CR1 and CR2 showed stable particle sizes with only minor variations during the storage time of one month. The sizes of the rest of the drug loaded formulations increased very slightly, but remained below the determined CQA value for particle size. Particle sizes of the empty niosomes decreased during the storage. The PDI value of the formulation E5 was 0.725 and remained high during storage. The formulation E5 had the highest amount of Pluronic L121, and its PDI value was above 0.5 even after 1

week of storage time. All the drug-loaded niosomes remained stable with PDI values below 0.5.

The zeta-potential values of all the formulations remained close to or below −30 mV, which indicates stable niosomes. The formulations E1, E2, CR1, and CR2 were the most stable niosome formulations with the smallest and most stable particle sizes and PDI values.

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Table 3. Particle size information, PDI values and zeta-potentials of the empty and drug loaded niosomal formulations stored at 4 C for four weeks time (n=3).

Time	Parameters	E1	E2	E3	E4	E5	CR1	CR2	CR3	CR4	CR5
Fresh sample	Size (nm)	195.6±12.8	236.3±36.0	443.5±86.7	300.5±36.6	893.6±135.5	187.2±3.5	164.8±6.1	192.5±13.7	195.4±16.6	205.7±18.9
	PDI	0.492±0.047	0.391±0.105	0.469±0.037	0.448±0.034	0.725±0.117	0.421±0.018	0.333±0.039	0.455±0.087	0.499±0.036	0.473±0.095
	Zeta-Potential	-27.5 ±0.9	-27.5± 0.9	-34.9± 3.4	-38.8±0.3	-39.9±5.2	-25.9± 0.7	-29.1± 0.2	-37.2± 1.2	-28.6± 1.2	-29.6± 0.3
1 week	Size (nm)	223.4±11.4	175.8±5.8	294.4±8.0	199.3±5.4	244.8±7.6	253.2±4.2	175.8±71.4	248.4±28.2	251.8±18.1	255.8±25.3
	PDI	0.340±0.046	0.198±0.040	0.362±0.038	0.282±0.014	0.535±0.020	0.256±0.003	0.365±0.106	0.445±0.126	0.366±0.082	0.318±0.106
	Zeta-Potential	-23.8±0.9	-26.9± 0.9	-28.2± 1.0	-28.5 ±0.6	-30.4±0.5	-30.1±1.3	-28.2±1.8	-29.3±1.1	-33.0±2.1	-29.2±1.6
2 weeks	Size (nm)	191.9±3.3	178.7±3.6	347.4±9.0	212.7±6.1	245.2±5.5	190.7±2.3	163.2±6.1	253.2±4.7	218.0±7.3	265.5±11.1
	PDI	0.168±0.039	0.169±0.028	0.375±0.030	0.299±0.022	0.543±0.015	0.212±0.012	0.270±0.030	0.343±0.023	0.267±0.034	0.257±0.070
	Zeta-Potential	-23.8±0.9	-28.1± 2.2	-29.5±2.5	-28.2 ±3.0	-26.3±0.5	-28.0±0.3	-28.7±3.1	-28.6±0.5	-24.4±1.3	-24.1±1.2
3 weeks	Size (nm)	199.4±4.9	172.2±1.7	296.3±12.6	208.7±4.6	291.6±8.3	189.3±2.3	169.2±8.9	269.2±4.7	228.0±7.3	269.5±11.1
	PDI	0.213±0.011	0.151±0.033	0.320±0.051	0.324±0.020	0.532±0.051	0.246±0.003	0.271±0.106	0.421±0.127	0.467±0.041	0.357±0.070
	Zeta-Potential	-27.2 ±0.4	-28.9± 0.5	-28.6±1.5	-31.6 ±2.2	-28.9±0.2	-29.3±0.2	-29.7±3.1	-32.6±0.5	-29.4±1.3	-28.3±3.2
4 weeks	Size (nm)	186.8±1.6	182.0±2.7	311.8±8.1	190.0±2.5	255.8±34.8	191.7±4.3	172.1±4.5	279.6±7.1	237.0±2.5	274.8±9.1
	PDI	0.138±0.025	0.226±0.016	0.364±0.042	0.208±0.037	0.534±0.078	0.210±0.106	0.231±0.003	0.443±0.103	0.453±0.034	0.412±0.125
	Zeta-Potential	-25.5 ±1.3	-27.3± 1.5	-26.7±0.2	-27.8 ±1.0	-29.8±1.1	-30.1±4.2	-28.9±2.1	-31.3±1.5	-31.2±2.1	-32.4±7.2

3.5.Dissolution Studies

The drug release studies of all the ceftriaxone sodium and rifampicin loaded niosome formulations and controls (pure ceftriaxone sodium and pure rifampicin) were carried out in phosphate buffer saline at pH 7.4 (Figures 4 and 5). A burst release of drugs from all the niosome formulations was observed in the beginning of the dissolution testing, which was due to the presence of Pluronic L121, as concluded in previous findings [46]. The burst release of rifampicin was higher as compared to ceftriaxone.

After 12 h of release testing, the amount of ceftriaxone released from niosome formulations was over 94.8 % in all the batches (Figure 4). The niosome formulation CR4 containing the lowest amount of Pluronic L121 (246 mg) showed the slowest release rate of ceftriaxone (94.8% in 12 h), while the formulation containing the highest amount of Pluronic L121 exhibited the fastest release of ceftriaxone (98.5% in 12 h).

The amount of rifampicin released from the niosomes in 12 h ranged from 72.4 to 79.1% (Figure 5). Again, the formulation CR4 containing the lowest amount of Pluronic L121 (246 mg) exhibited the slowest release of rifampicin (72.4% in 12 h). The center point formulation CR1, and formulation CR5 containing the highest amount of Pluronic L121 (334 mg), showed the fastest release of rifampicin (in both the batches, 79.1% after 12 h).

Pluronic L121 is acting as a solubilizer and, hence, the higher the amount, the faster the drug release [42]. This behavior was observed with both the studied drugs. From the release profile of niosome formulations it was clear that an increased concentration of Pluronic L121 improved the drug release profiles, while the least quantity led to a slower drug release.

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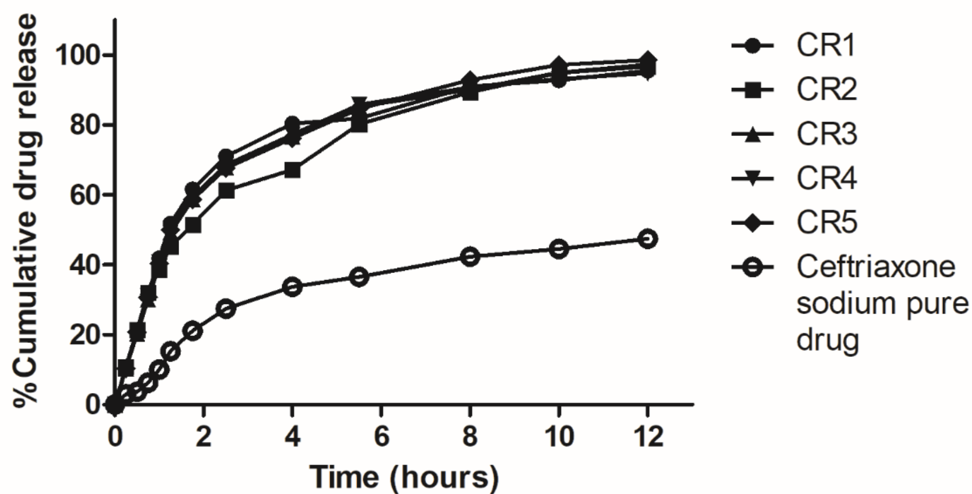

Figure 4. Ceftriaxone sodium release profiles from niosome formulations at pH 7.4.

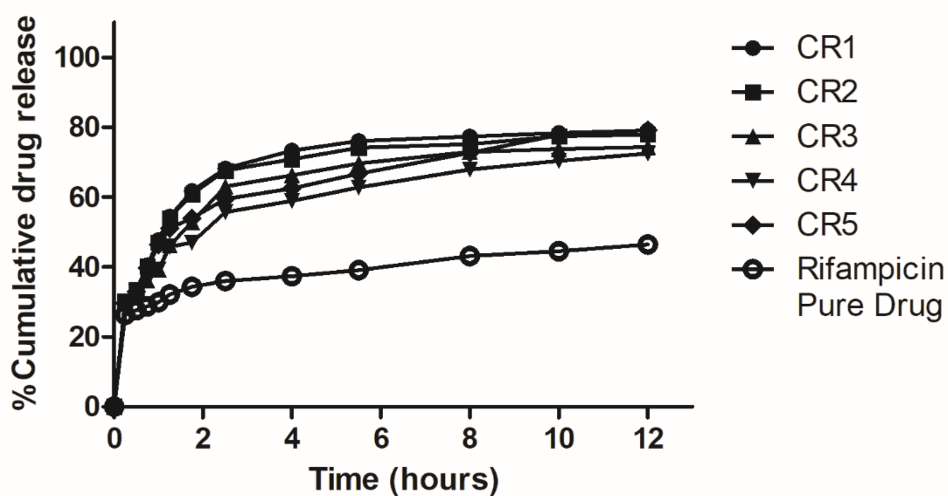

Figure 5. Rifampicin release profiles from niosome formulations at pH 7.4.

Conclusion

In this study, dual drug niosomal formulations were developed, containing both hydrophobic rifampicin and hydrophilic ceftriaxone sodium. The formulated niosomes showed small average sizes (165±6 nm) with low PDI values (0.3±0.0). Drug entrapment

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efficiencies of both the drugs were very high, with values over 96%. Four weeks stability studies at 4 °C showed good colloidal stability. The drug release profiles of both the drugs were improved when compared to the pure drugs, and presence of Pluronic L121 improved the drug release due to the solubilization effect. The formulations showed controlled drug release over 12 h time. Accordingly, formulated ceftriaxone and rifampicin loaded niosomes were stable and small in size having high drug entrapment efficiencies as well as improved drug release profiles.

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References

1. C. Feng, H. Zhang, J. Chen, S. Wang, Y. Xin, Y. Qu, Q. Zhang, W. Ji, F. Yamashita, M. Rui, X. Xu, Ratiometric co-encapsulation and co-delivery of doxorubicin and paclitaxel by tumor-targeted lipodisks for combination therapy of breast cancer, Int. J. Pharm. 560 (2019) 191-204. Doi: 10.1016/j.ijpharm.2019.02.009.

2. C.A Kerantzas and W.R Jacobs, Origins of combination therapy for tuberculosis: lessons for future antimicrobial development and application, mBIO 8 (2017), 1586-1516. DOI: 10.1128/mBio.01586-16

3. L.A. Barbee, O.O. Soge, K.K. Holmes, M.R. Golden, In vitro synergy testing of novel antimicrobial combination therapies against Neisseria gonorrhoeae, J. Antimicrob. Chemother. 69 (2014) 1572-1578. Doi: 10.1093/jac/dkt540.

4. O. Soren, K. Sidelmann Brinch, D. Patel, Y. Liu, A. Liu, A. Coates, Y. Hu, Antimicrobial peptide novicidin synergizes with rifampin, ceftriaxone, and ceftazidime against antibiotic-resistant enterobacteriaceae in vitro, Antimicrob. Agents Chemother. 59 (2015) 6233-6240. Doi: 10.1128/AAC.01245-15.

5. K. Skinner, J.A.T. Sandoe, R. Rajendran, G. Ramage, S. Lang, Efficacy of rifampicin combination therapy for the treatment of enterococcal infections assessed in vivo using a Galleria mellonella infection model, Int. J. Antimicrob. Agents 49 (2017), 507-511. https://doi.org/10.1016/j.ijantimicag.2016.12.006. s

6. G.N. Forrest, K. Tamura, Rifampin combination therapy for nonmycobacterial infections, Clin. Microbiol. Rev. 23 (2010) 14-34. Doi: 10.1128/CMR.00034-09.

7. S. Kumar, D. Bhargava, A. Thakkar, S. Arora, Drug Carrier Systems for Solubility Enhancement of BCS Class II Drugs: A Critical Review, Crit. Rev. Ther. Drug Carrier Syst. 30 (2013) 217–256. doi:10.1615/CritRevTherDrugCarrierSyst.2013005964.

8. C.T. Lo, A. Jahn, L.E. Locascio, W.N. Vreeland, Controlled Self-Assembly of Monodisperse Niosomes by Microfluidic Hydrodynamic Focusing, Langmuir. 26 (2010) 8559–8566. doi:10.1021/la904616s.

9. D.H. Khan, S. Bashir, A. Correia, M.I. Khan, P. Figueiredo, H.A, Santos, L Peltonen, Utilization of green formulation technique and efficacy estimation on cell line studies for

dual anticancer drug therapy with niosomes, Int. J. Pharm. 527 (2019) 118764. https://doi.org/10.1016/j.ijpharm.2019.118764.

10. K. Kuotsu, K. Karim, A. Mandal, N. Biswas, A. Guha, S. Chatterjee, M. Behera, Niosome: A future of targeted drug delivery systems, J. Adv. Pharm. Technol. Res. 1 (2010) 374. doi:10.4103/0110-5558.76435.

11. P.L. Yeo, C.L. Lim, S.M. Chye, A.P. Kiong Ling, R.Y. Koh, Niosomes: a review of their structure, properties, methods of preparation, and medical applications, Asian Biomed. 11 (2018) 301–314. doi:10.1515/abm-2018-0002.

12. E. Essa, Effect of formulation and processing variables on the particle size of sorbitan monopalmitate niosomes, Asian J. Pharm. 4 (2010) 227. doi:10.4103/0973-8398.76752.

13. G. Kumar, P.R. B, Nonionic surfactant vesicular systems for effective drug delivery—an overview, Acta Pharm. Sin. B. 1 (2011) 208–219. https://doi.org/10.1016/j.apsb.2011.09.002

14. I.F. Uchegbu, S.P. Vyas, Non-ionic surfactant based vesicles (niosomes) in drug delivery, Int. J. Pharm. 172 (1998) 33–70. doi:10.1016/S0378-5173(98)00169-0.

15. Z. Sadeghi Ghadi, R. Dinarvand, N. Asemi, F. Talebpour Amiri, P. Ebrahimnejad, Preparation, characterization and in vivo evaluation of novel hyaluronan containing niosomes tailored by Box-Behnken design to co-encapsulate curcumin and quercetin, Eur. J. Pharm. Sci. 130 (2019) 234–246. doi:10.1016/j.ejps.2019.01.035.

16. M.I. Khan, A. Madni, L. Peltonen, Development and in-vitro characterization of sorbitan monolaurate and poloxamer 184 based niosomes for oral delivery of diacerein, Eur. J. Pharm. Sci. 95 (2016) 88–95. doi:10.1016/j.ejps.2016.09.002.

17. I. Escudero, R.M. Geanta, M.O. Ruiz, J.M. Benito, Formulation and characterization of Tween 80/cholestherol niosomes modified with tri-n-octylmethylammonium chloride (TOMAC) for carboxylic acids entrapment, Colloids Surfaces A Physicochem. Eng. Asp. 461 (2014) 167–177. doi:10.1016/J.COLSURFA.2014.07.042.

18. L. Di Marzio, C. Marianecci, M. Petrone, F. Rinaldi, M. Carafa, Novel pH-sensitive non-ionic surfactant vesicles: comparison between Tween 21 and Tween 20, Colloids Surfaces B Biointerfaces. 82 (2011) 18–24. doi:10.1016/j.colsurfb.2010.08.004.

Journal Pre-proof

Journal Pre-proof

19. S. Moghassemi, A. Hadjizadeh, Nano-niosomes as nanoscale drug delivery systems: An illustrated review, J. Control. Release. 185 (2014) 22–36. doi:10.1016/j.jconrel.2014.04.015.

20. R. Basak, R. Bandyopadhyay, The encapsulation of hydrophobic drugs in Pluronic F127 micelles: the effects of drug hydrophobicity, solution temperature and pH, Langmuir. 29 (2014) 4350–4356. https://doi.org/10.1021/la304836e

21. C.-F. Lee, H.-W. Tseng, P. Bahadur, L.-J. Chen, C.-F. Lee, H.-W. Tseng, P. Bahadur, L.-J. Chen, Synergistic Effect of Binary Mixed-Pluronic Systems on Temperature Dependent Self-assembly Process and Drug Solubility, Polymers (Basel). 10 (2018) 105. doi:10.3390/polym10010105.

22. E. V Batrakova, S. V Vinogradov, S.M. Robinson, M.L. Niehoff, W.A. Banks, A. V Kabanov, Polypeptide Point Modifications with Fatty Acid and Amphiphilic Block Copolymers for Enhanced Brain Delivery, Bioconjug Chem. 16 (2005) 793–802. doi:10.1021/bc049730c.

23. V. Ravalika, A.K. Sailaja, Formulation and Evaluation of Etoricoxib Niosomes by Thin Film Hydration Technique and Ether Injection Method, Nano Biomed. Eng. 9 (2017) 242–248. doi:10.5101/nbe.v9i3.p242-248.

24. L. Kanaani, M.M. Tabrizi, A.A. Khiyavi, I. Javadi, Improvement the Efficacy of Cisplatin by Niosome Nanoparticles Against human breast cancer cell line BT-20 : an in vitro study, Asian Pacific J. Cancer Biol. 2 (2017) 25–26. doi:10.22034/APJCB.2017.2.2.25.

25. M.I. Khan, A. Madni, J. Hirvonen, L. Peltonen, Ultrasonic Processing Technique as a Green Preparation Approach for Diacerein-Loaded Niosomes, AAPS PharmSciTech. 18 (2017) 1554–1563. doi:10.1208/s12249-016-0622-z.

26. B. Amiri, H. Ahmadvand, A. Farhadi, A. Najmafshar, M. Chiani, D. Norouzian, Delivery of vinblastine-containing niosomes results in potent in vitro / in vivo cytotoxicity on tumor cells, Drug Dev. Ind. Pharm. 44 (2018) 1371–1376. doi:10.1080/03639045.2018.1451880.

27. Q. Liu, J. Zhang, W. Sun, Q.R. Xie, W. Xia, H. Gu, Delivering hydrophilic and hydrophobic chemotherapeutics simultaneously by magnetic mesoporous silica

Journal Pre-proof

nanoparticles to inhibit cancer cells, Int. J. Nanomedicine. 7 (2012) 999–1013. doi:10.2147/IJN.S28088.

28. F. Kratz, A. Warnecke, Finding the optimal balance: Challenges of improving conventional cancer chemotherapy using suitable combinations with nano-sized drug delivery systems, J. Control. Release. 164 (2012) 221–235. doi:10.1016/j.jconrel.2012.05.045.

29. H. Zhang, G. Wang, H. Yang, Drug delivery systems for differential release in combination therapy, Expert Opin. Drug Deliv. 8 (2011) 171–190. doi:10.1517/17425247.2011.547470.

30. D. Liu, L.M. Bimbo, E. Mäkilä, F. Villanova, M. Kaasalainen, B. Herranz-Blanco, C.M. Caramella, V.P. Lehto, J. Salonen, K.H. Herzig, J. Hirvonen, H.A. Santos, Co-delivery of a hydrophobic small molecule and a hydrophilic peptide by porous silicon nanoparticles, J. Control. Release. 170 (2013) 268–278. doi:10.1016/j.jconrel.2013.05.036.

31. L. Kanaani, M.M. Tabrizi, A.A. Khiyavi, I. Javadi, Improvement the Efficacy of Cisplatin by Niosome Nanoparticles Against human breast cancer cell line BT-20 : an in vitro study, Asian Pacific J. Cancer Biol. 2 (2017) 25–26. Doi:10.22034/APJCB.2017.2.2.25.

32. O.C. Jeon, S.R. Hwang, T.A. Al-Hilal, J.W. Park, H.T. Moon, S. Lee, J.H. Park, Y. Byun, Oral delivery of ionic complex of ceftriaxone with bile acid derivative in non-human primates, Pharm. Res. 30 (2013) 959–967. doi:10.1007/s11095-012-0932-0.

33. D.H. Khan, S. Bashir, P. Figueiredo, H.A. Santos, M.I. Khan, L. Peltonen, Process optimization of ecological probe sonication technique for production of rifampicin loaded niosomes, J. Drug Deliv. Sci. Technol. 50 (2019) 27–33. doi:10.1016/j.jddst.2019.01.012.

34. N.B. Mahale, P.D. Thakkar, R.G. Mali, D.R. Walunj, S.R. Chaudhari, Niosomes: Novel sustained release nonionic stable vesicular systems - An overview, Adv. Colloid Interface Sci. 183–184 (2012) 46–54. doi:10.1016/j.cis.2012.08.002.

35. S. Somjid, S. Krongsuk, J.R. Johns, Cholesterol concentration effect on the bilayer properties and phase formation of niosome bilayers: A molecular dynamics simulation study, J. Mol. Liq. 256 (2018) 591–598. doi:10.1016/j.molliq.2018.02.077.

Journal Pre-proof

36. L. Basiri, G. Rajabzadeh, A. Bostan, Physicochemical properties and release behavior of Span 60/Tween 60 niosomes as vehicle for α-Tocopherol delivery, LWT. 84 (2017) 471–478. doi:10.1016/j.lwt.2017.06.009.

37. E.S. Lee, K. Na, Y.H. Bae, Super pH-Sensitive Multifunctional Polymeric Micelle, Nano Lett. 5 (2005) 325–329. doi:10.1021/nl0479987.

38. E.S. Lee, Y.T. Oh, Y. Seok Youn, M. Nam, B. Park, J. Yun, J.H. Kim, H.-T. Song, K.T. Oh, Binary mixing of micelles using Pluronics for a nano-sized drug delivery system, Colloids Surfaces B Biointerfaces. 82 (2011) 190–195. doi:10.1016/j.colsurfb.2010.08.033.

39. G.A. Abdelbary, M.I. Tadros, Brain targeting of olanzapine via intranasal delivery of core–shell difunctional block copolymer mixed nanomicellar carriers: In vitro characterization, ex vivo estimation of nasal toxicity and in vivo biodistribution studies, Int. J. Pharm. 452 (2013) 300–310. doi:10.1016/j.ijpharm.2013.04.084.

40. C. Vora, R. Patadia, K. Mittal, R. Mashru, Risk based approach for design and optimization of stomach specific delivery of rifampicin, Int. J. Pharm. 455 (2013) 169–181. doi:10.1016/j.ijpharm.2013.07.043.

41. H. M. Owens, A. K. Dash, Ceftriaxone Sodium: Comprehensive Profile, in: Profiles Drug Subst. Excipients Relat. Methodol., Elsevier, 2003: pp. 21–57. doi:10.1016/S0099-5428(03)30002-4.

42. F.-T. Li, D.-S. Zhao, Q.-Z. Luo, R.H. Liu, R. Yin, Research on surface-modification of Nano-TiO2 by span 60, J. Ceram. Process. Res. 9 (2008) 398–400.

43. M.J. Newman, M. Balusubramanian, C.W. Todd, Development of adjuvant-active nonionic block copolymers, Adv. Drug Deliv. Rev. 32 (1998) 199–223. doi:10.1016/S0169-409X(98)00011-8.

44. M.I. Khan, A. Madni, S. Ahmad, M.A. Mahmood, M. Rehman, M. Ashfaq, Formulation design and characterization of a non-ionic surfactant based vesicular system for the sustained delivery of a new chondroprotective agent, Brazilian J. Pharm. Sci. 51 (2015) 607–616. doi:10.1590/S1984-82502015000300012.

Journal Pre-proof

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45. B. Nasseri, Effect of cholesterol and temperature on the elastic properties of niosomal membranes, Int. J. Phar. 300 (2005) 95–101. doi:10.1016/j.ijpharm.2005.05.009.

46. G.A. Abdelbary, M.M. Amin, M.Y. Zakaria, Ocular ketoconazole-loaded proniosomal gels: formulation, ex vivo corneal permeation and in vivo studies, Drug Deliv. 24 (2017) 309–319. doi:10.1080/10717544.2016.1247928.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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