RESEARCH ARTICLE

Effect of formulation and processing variables on the particle size of sorbitan monopalmitate niosomes

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In the last two decades, there was an extensive research focused on the study of synthetic amphiphilic vesicles, prepared by nonionic surfactants (niosomes). The particle size of these vesicles is critical for their intended therapeutic benefits. Formulation and processing factors affect greatly the physical characteristics of the resulted nanosystems. Therefore, the present work was adopted to investigate how proper manipulation of various formulations and processing factors on vesicular Z-average particle size. The selected variables were membrane additives, [including cholesterol (CHO), dicetylphosphate (DCP) and stearylamine (SA)], sonication time as well as drug loading factor (using mannitol and estradiol). Sorbitan monopalmitate (span 40) niosomes were prepared by the conventional thin film hydration method. Particle size, measured by Photon Correlation Spectroscopy, and polydispersity indices were measured and compared. The results indicated that CHO increased the vesicular size, with 2:1 and 1:1 (span 40:CHO) ratios showing the same size. Sonication reduced the vesicle size by 23, 35 and 42% after 10, 20 and 30 min, respectively. After 30 min, the effect of sonication was minor. The addition of charge inducing agents changed the zeta potential depending on the type of the additives. Surface charge increased the size by 24 and 11% when using DCP and SA, respectively. Drug incorporation increased the vesicle size to an extent based on its aqueous solubility. There were about 35 and 6.2% increase in vesicular size for estradiol and mannitol, respectively, supporting the partitioning of lipophilic drug within the fatty acyl side chains of the bilayer membrane.

Key words: *Cholesterol, niosomes, particle size, sonication, span 40*

INTRODUCTION

Drug delivery systems using colloidal particulate carriers such as liposomes or niosomes have distinct advantages over conventional dosage forms. Nonionic surfactant-based vesicles (niosomes) are formed from the self-assembly of nonionic amphiphiles in aqueous media resulting in closed bilayer structures. These structures are analogous to phospholipid vesicles (liposomes) and are able to encapsulate hydrophilic and lipophilic solutes and serve as drug carriers. The low cost, entrapping of more substances, ease of handling and storage and availability of prepared materials in pure form have led to the exploitation of these compounds as alternative to liposomes.[1-4] Niosomes, therefore, are promising drug carrier and have the potential to reduce the side effects of drugs and increased therapeutic effectiveness in various diseases. As of today more than 50 drugs are tried in niosome formulations by intravenous, per oral, transdermal, inhalation, ocular and nasal routes of administration. Synthetic surfactant vesicles, as the name implies, could be fabricated from a vast array of amphiphiles, including a number of pharmaceutically acceptable materials. They may also be prepared in a variety of shapes and sizes and have a number of applications.

Niosomes can be characterized by their physicochemical properties, such as particle size, lamellarity, surface charge and entrapment efficiency. Physicochemical properties of vesicles are very important as they influence their behavior both *in vitro* and *in vivo*.

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The average size of lipid/nonionic surfactant vesicles are important parameters with respect to physical properties and biological fate of them and their entrapped substances.[5,6] Particle size of vesicles has also been proven to have a considerable influence on therapeutic efficiency of vesicles, such as skin penetration.[7,8] Regarding toxicity, small-sized liposomes containing Amphotericin B were less toxic compared to larger ones.[9] In addition, vesicle size could also affect their phagocytic uptake[10] and drug targeting for solid tumors.[11] Vesicle size is also important for membrane permeability. Size also affect vesicles stability, where liposomes between 100 and 200 nm in diameter were found to be more stable and exhibited better drug retention compared to larger liposomes of the same composition.[12] All these reviews reflect the importance of vesicle characterization and the influence of some formulation factors are basic part of every research work dealing with these vesicles. However, this work aimed to investigate the potential effect of some formulation compositions and processing variables on the particle size of niosomes, in a trial to come out with a basic concept that could be used as reference in preparing vesicles of a desired size. Sorbitan monopalmitate (span 40) was selected as a model nonionic surfactant for the preparation of niosomes for transdermal delivery (unpublished data). Formulations composed of different concentrations of surfactant and cholesterol (CHO), charge-inducing agent, drugs of different aqueous solubility and sonication time were used to investigate their effect on the particle size of niosomes.

MATERIALS AND METHODS

Materials

Chemicals including span 40, mannitol, dicetylphosphate (DCP), stearylamine (SA) and estradiol (purity 98%), CHO and Sephadex G50 were obtained from Sigma (St. Louis, MO, USA). Estradiol (2, 4, 6, 7-^{3}H (N)) and ^{14}C-Mannitol were obtained from DuPont NEN Life Science Products. Scintillation cocktail (Optiphase 'HiSafe'3)was obtained from LKB Scintillation Product, UK. All other chemicals and reagents were of analytical grade and used as obtained from suppliers without further purification, and water source was from an ultra high-quality reverse osmosis water purifier.

Methods

Preparation of niosomes

The nonionic surfactant vesicles were prepared by the conventional thin film hydration method. Span 40 (SP40) and CHO, at different specified molar ratios (5:1, 4:1, 3:1, 2:1 and 1:1 SP40:CHO m.r.), were dissolved in chloroformmethanol mixture (2:1 V/V), to give a final niosomal dispersion concentration of 50 mg/ml. The lipid mixture was added to a 100-mL rounded bottom flask, and the solvent was evaporated under reduced pressure at a temperature of 60°C (above the transition temperature of Span 40 which is about 46°C) by a rotary evaporator (BÜCHI, HB-140, Flawil, Germany). The evaporation step continued until almost all organic solvent was evaporated and a thin lipid film was deposited on the wall of the flask. The excess, nonevaporated organic solvent was removed by keeping the flask in a desiccator under vacuum overnight. The lipid film was hydrated with 5 mL of double distilled water. The hydration was continued for 1 hr, while the flask was kept rotating at 60°C in the rotary evaporator. The niosomal suspension was further hydrated at room temperature for 2 hrs in order to complete the swelling process. The hydrated niosomes were sonicated for 10 mins using a B12 FTZ bath sonicator, and size analysis was then conducted immediately after preparation. In the subsequent studies, niosomes of 3:1 SP40:CHO m.r. was used as a model formulation.

Effect of sonication time on vesicle particle size

Niosome suspensions (3:1 SP40:CHO m.r.) were sonicated for 10, 20, 30, 40 and 60 mins using a B12 FTZ temperature-controlled bath sonicator (40 Hz), and particle size was determined at each time interval, using the unsonicated niosomes as control.

Effect of membrane charge on particle size

The effect of charge on particle size of niosomes was studied. DCP and SA were used to impart either negative or positive charge, respectively. Charge-inducing agent was incorporated at a concentration of 5mg/ml and dissolved in diethyl ether/chloroform mixture before the solvent evaporation step.

Effect of drug solubility on particle size

The effect of incorporation of drugs of different solubility on the particle size of niosomes was investigated. Mannitol and estradiol were selected as model hydrophilic and lipophilic drugs, respectively. Drug was added at a concentration sufficient to produce 2 mg/ml of the final niosome suspension. The incorporation of the drug during noisome preparation depended on its solubility characteristics. Mannitol, being water soluble, was dissolved in the hydration solution (double distilled water) during hydration of the deposited thin film. For lipophilic estradiol, it was dissolved together with lipid mixture in the organic solvent before evaporation step.

Effect of entrapment efficiency

Entrapment efficiency was determined using a mini-column centrifugation method.[13] Radioactive Mannitol (^{14}C-labelled) and Estradiol (^{3}H-labelled) were used in this experiment. Aqueous and ethanolic stock solutions of radiolabelled mannitol and estradiol, respectively, were prepared and used in the preparation of niosomes. Niosomes were prepared as mentioned above where the final drug concentration was 2 mg/ml. To prepare the mini-column, Whatman GF/B filter pads were inserted in the bottom of the barrel of a 2.5 cm^{3} syringe, which was then filled with Sephadex G50 gel. Excess water was removed from the gel by centrifugation at 2000 rpm for 3 min using a WIFUG Lab centrifuge (WIFUG, Bradford, UK). Niosomal suspension (200 μl) was added dropwise to the centre of

the column, followed by centrifugation as before. Then 250 μl of water was added and centrifugation was repeated. The nonentrapped drug remained bound to the gel, while vesicles traversed the gel and were collected from the first and second stage of centrifugation. After separation of the nonentrapped material, five aliquots of 10 μl each of niosome suspensions were separately added to five scintillation vials each contained 5 ml of scintillation cocktail and 1 ml of distilled water. The vials were vortex mixed and counted using scintillation counter (Tri-Carb liquid scintillation analyzer, Packard, USA). The total counts of noisome suspensions before separation of the free drug was also determined by the same procedure. The entrapment efficiency was expressed as the percent of drug captured[14] and determined using equation 1:

$$\%\text{Entrapment efficiency} = CPM(S)\ X\ Df\ /\ CPM\ (T).100 \qquad \text{Eq.1}$$

Where CPM(S) is the counts per minutes of 10 μl of niosomes after separation of the free drug, Df the dilution factor and CPM(T) is the counts per minutes of 10 μl of niosomes before separation of the free drug.

Determination of particle size

The diameter of niosomes was determined using a Zetamaster S particle electrophoresis and particle size analyzer (Malvern Instruments Ltd., Malvern, UK). The instrument was used to measure both particle size (Photon Correlation Spectroscopy – PCS) and zeta potential.

PCS measures particles in the size range of 10 nm to 3 μm in diameter. The system uses a 4 mW laser diode operating at 670 nm that illuminates a dilute sample held in a transparent cuvette placed inside a metal holder. Light scattered at a fixed angle of 90° to the incident beam is detected by photon counting multiplier. PCS is the analysis of the time dependence of intensity fluctuation in scattered laser light due to Brownian motion of particle in suspension. As small particles diffuse more rapidly than larger ones, the rate of fluctuation of scattered light intensity varies accordingly. So, the translational diffusion coefficient (D) can be measured and used to determine the mean hydrodynamic radius of the particle using Stockes-Einstein equation:[15]

$$D = KT/6\eta R_h \qquad \text{Eq. 2}$$

Where, K is Boltzman's constant, T absolute temperature, η is solvent velocity and R_h is the mean hydrodynamic radius.

The refractive index and viscosity values of the external phase (water) were used during particle size measurements. To avoid interference from particulate matter in the dispersion medium, samples were diluted with deionized double distilled water passed through a 200 nm filter.

The polydispersity index (PDI), which is the width of the particle size distribution curve, was determined as a measure of the homogeneity. Small values of PDI < 0.1 indicate a homogeneous population, while a PDI > 0.3 indicates its high heterogeneity.[7]

Determination of zeta potential

The particle charge was qualified as zeta potential. The electrophoretic mobility was determined by Laser Doppler anemometry using a Zetamaster S particle electrophoresis and particle size analyzer (Malvern Instruments, UK). Measurements were performed at field strength of 20 V/cm in distilled water and based on measuring the electrophoretic mobility of charged particles. Electrophoresis describes the motion of a charged particles submerged in a liquid under the influence of an applied electric field. When an electric field is applied across an electrolyte, charged particles are attracted to the electrode of opposite charge. Viscous forces acting on the particles tend to oppose this movement. When equilibrium is reached between these two opposing forces, the particles move with constant velocity. The velocity is dependent on the strength of electric field, the dielectric constant, the viscosity of the medium and the zeta potential. The velocity of a particle in a unite electric field is referred to as its electrophoretic mobility. Electrophoretic mobility is related to zeta potential by Henery equation:[15]

$$U_E = \varepsilon\ \zeta\ f(Ka)/6\pi\eta \qquad \text{Eq. 3}$$

Where, U_E, ε, ζ and η are electrophoretic mobility, dielectric constant of the liquid, zeta potential and viscosity, respectively. Where *f(Ka)* is a factor including double electric layer thickness and particle diameter (equals 1.0 for nonpolar media or 1.5 for polar media). Electrophoretic determination of zeta potential are most commonly made in aqueous media and at moderate electrolyte concentration, *f(Ka)* in this case is 1.5, and this is the value used in Smoluchuchowski approximation:

$$U_E = \varepsilon\ \zeta/4\ \pi\eta \qquad \text{Eq. 4}$$

From equations 3 and 4, and for aqueous medium at 25°C, the relationship between mobility and zeta potential is 12.5 mV per mobility unite as follow:

$$\zeta = 12.85\ U_E \qquad \text{mV} \qquad \text{Eq. 5}$$

All studies were conducted at least in triplicates. Student *t*-test was used for statistical analysis.

RESULTS AND DISCUSSION

Effect of cholesterol concentration on particle size

CHO is the most common membrane additives found in vesicular systems. Similar to liposomes, the addition of CHO (a rigid steroid molecule) to the surfactant was required to form a stable nonionic surfactant-based vesicles. It is mainly

important to study the effect of CHO on physical properties of vesicles since CHO almost always present in lipid vesicles as well as biomembranes and influences a number of membrane properties such as ion permeability, aggregation, fusion processes, elasticity, size and shape.[16,17] Inclusion of CHO in membrane increases the rigidity of the bilayer and reduces the leakage of water soluble substances through membranes.

The effect of CHO concentration on Z-average size of niosomes was studied using different SP40:CHO m.r. The Z-average size and PDI of different niosomal formulations are shown in Table 1. The results indicated that niosomal size increased ($P<0.05$) linearly with increasing CHO concentration in agreement with previous findings.[18-21] Such size increment was in a consistent manner, with PDI values reduced in a similar trend, reflecting improvement in the homogeneity of niosome populations with increasing CHO contents.

It was also noticed that although particle size of niosome formulation of 1:1 was higher compared to that of 2:1 m.r., nevertheless it was found to be statistically insignificant ($P>0.05$). However, PDI was less in 1:1 m.r. indicating a more homogenous vesicle population. It is widely accepted that CHO should be incorporated in liposomes or niosome in about 50 mol% concentration. From the obtained data, it could be suggested that vesicle size could be more or less the same when CHO is incorporated in the bilayer at 30-50 molar ratio, though a more uniform size distribution could be obtained using the higher ratio.

To explain the increased particle size of niosomes with increasing CHO content, it is important to understand the mechanism by which CHO is incorporated in the bilayer membrane. Being amphipathic, CHO can insert itself into the bilayer membrane with its hydrophilic head oriented towards the aqueous surface and aliphatic chain line up parallel to the hydrocarbon chains in the center of the bilayer. It is known that CHO increases the chain order of the liquid-state bilayer and strengthen the nonpolar tail of the nonionic surfactant.[22] At low CHO concentration, it is feasible to expect that CHO would had resulted in close packing of surfactant monomers with increasing curvature and reducing size. However, increasing CHO content, with its known lipophilic nature (log P of 7.02), and consequently reducing nonionic surfactant content, would have resulted in increased hydrophobicity of the bilayer membrane and may had imparted disturbance in the vesicular membrane, thus, increasing vesicle radius in a way to establish a more thermodynamic stable form.

Table 1: Effect of cholesterol concentration on niosomes Z-average size and polydispersity indices (PDI)

Formulation (SP40:CHO m. r.)	Particle size (μm)	PDI
5:1	0.38 (±0.082, 3)	0.27 (±0.04, 3)
4:1	0.47 (±0.093, 3)	0.22 (±0.05, 3)
3:1	0.61 (±0.073, 4)	0.18 (±0.018, 4)
2:1	0.87 (±0.126, 3)	0.15 (±0.07, 3)
1:1	0.90 (±0.194, 4)	0.11 (±0.05, 4)

-Values between brackets are standard deviation and number of replicates, respectively.

The obtained results could also be explained based on the membrane rigidity resulted from CHO inclusion. It is well accepted that incorporation of CHO imparts rigidity to the bilayer membrane, thus improve the physical stability for many niosomal systems.[23] Additionally, CHO can stabilize the bilayer structure by eliminating the phase transition temperature peak of the vesicles, thereby strengthening the bilayer structures and diminishes bilayer micro fluidity,[24] a situation that would interfere with the size reduction during sonication step. At low concentration of CHO, the vesicular membranes are more flexible and more liable to the effect of ultrasound waves, resulting in smaller size. With increasing CHO concentration, the hardness of the membranes increased with increased resistance to sonication, thus producing vesicles with bigger size.

Effect of sonication time on particle size

Sonication is one of the most popular methods used for producing a population of lipid vesicles of known size. Although sonication has been long used to produce nano-size of many lipid systems, little is known about the exact mechanism by which the average vesicular size decreases with exposure to ultrasound.[25] The principal effect of sonication is cavitation (bubble formation), which has been shown to be responsible for many physical effects of ultrasound on lipid membranes. It is well known that ultrasound mechanical waves generate cavitation bubbles in liquids. Bubbles whose size is near the resonant size for the applied frequency begins to oscillate nonlinearly and eventually collapse. As a result of such collapse, a violent implosion occurs that produces extremely high temperatures, high pressures, and shock waves.[26] In work conducted using liposomes, it has been postulated that such ultrasonic high energy randomly and uniformly shatters large liposomes into smaller discoid sections called bilayer phospholipid fragments. These fragments fold up into thermodynamically stable liposomes. Conversely, tiny unstable vesicles, formed during sonication, may fuse together to form slightly larger, stable vesicles.[27,28]

In this study, niosomal formulations (all at 3:1 m.r. SP40:CHO) were exposed to ultrasound wave for different time intervals, untreated vesicles were used as control. The obtained results are shown in Figure 1.

The figure shows that the efficiency of reducing the mean hydrodynamic radius of niosomes clearly increases with increasing the exposure time for ultrasound waves up to 30 min, after which the extent of size reduction is minor.[29] Sonication resulted in decreased vesicular size by 23±2.3%, 35±1.2% and 42±2.5% after 10, 20 and 30 min, respectively,

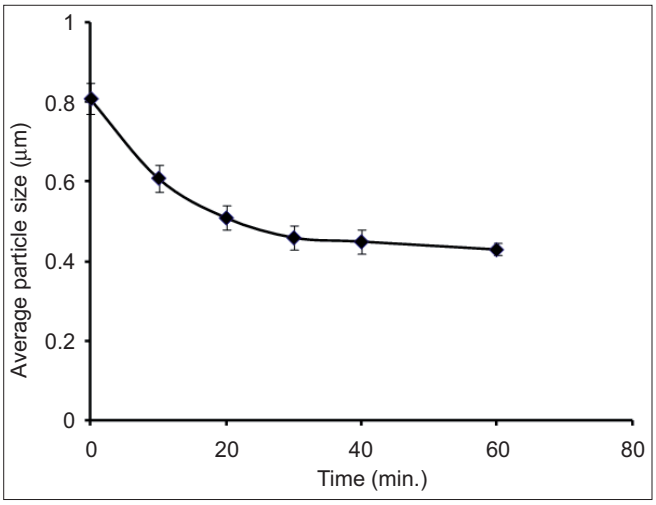

Figure 1: Effect of sonication time on SP40:CHO (3:1 m.r.) niosome particle size

compared to control untreated vesicles. The overall reduction in size by the end of the 60 min was about 51±3.6%. It seems that the effect of the ultrasound waves on vesicles size declines by time. The maximum magnitude of reduction obtained during the first 10 min of exposure as evidenced by the highest percentage reduction in size. After 20 min, there was only 12% size reduction compared to that at 10 min. The extent of size reducing capability became even less after 30 min as shown by only 7% decrease compared to that of 20 min. After 30 min, the impact of sonication seems to be almost negligible as there was insignificant ($P>0.05$) decrease in size till the end of the experimental time. From the obtained results, it could be concluded that the influence of sonicaion reduces by time, 30 min exposure to ultrasound is suggested to be enough to obtain the minimum size. As size reduction is largely dependent on the degree of cavitations, so it could be proposed that the ability of ultrasound waves to create cavitations bubbles in the niosome suspension medium reduced by time.

Effect of charge type on particle size

Theoretically, noisome formation requires the presence of a particular class of amphiphile to prevent vesicle aggregation. Therefore, charged molecules are one membrane additives which are often included in the vesicular bilayer structure with the aim of improving stability via electrostatic means. Using a charge-inducing agent, each vesicle will carry the same electrical charge which produces electrostatic repulsion between adjacent vesicles, thus remain discrete and prevent vesicles flocculation, aggregation and fusion. Moreover, preparing charged vesicles was sometimes aimed to improve the therapeutic efficiency of vesicles, as charged liposomes were found to be selectively taken by certain tissues in the body. For example, cationic liposomes have been shown to preferentially target the angiogenic endothelium of tumors[30] and enhanced pulmonary absorption of insulin compared to neutral one.[31]

Both DCP and SA are commonly used as charge-inducing agents to impart negative or positive charge to vesicular surface, respectively. At 3:1 SP40:CHO m.r., the effect of charge type on the particle size of niosomes was studied using uncharged vesicles as control. Vesicle surface charge was estimated by measuring particle electrophoretic mobility and expressed as zeta potential (ζ) value. The results are shown in Table 2.

In general, control niosomes should not posses any charge. On the contrary, it showed a negative charge with a zeta potential value of -11.6±2.4 mV. This might be attributed to the preferential adsorption of hydroxyl ions at vesicle surface. The contribution of CHO to this charge is not understood as much less is known on its effect on the surface charge. The inclusion of charge-inducing agents largely changed the zeta potential values.

Regarding the effect of inclusion of charged molecule into bilayers on the Z-average particle size, addition of charge inducing agent increased vesicles' size in agreement with previous studies,[19,32,33] and opposing others who reported reduced vesicle hydrodynamic radius.[34,35]

For DCP, the vesicles showed a zeta potential value of -50.7±4.2 mV which is significantly higher than the control vesicles ($P< 0.05$) with a percentage size enlargement of 24±2.1%. This negative charge could be due to ionization of the acidic (-HPO_4) group of DCP. For the cationic SA, positive charges dominate on the vesicular surface with a average zeta potential of 19.3±2.8 mV (percentage enlargement of 11±1.6%), due to protonation of the basic –NH_2 group.[36] Both charged vesicles showed close PDI values [Table 2], indicating improved vesicles homogeneity and stability with respect to control.

Generally speaking, the inclusion of charged molecules into bilayers would increase the volume of the aqueous compartment due to interaction between charged moiety and the surfactant head groups. Such interaction will develop the charge that creates mutual repulsion between nonionic surfactant bilayers and hence increases particle size. The incorporation of the charge inducing molecules within the vesicles usually occurs spontaneously during the thin film hydration process. The nature and molecular structure of the ionized species will affect the extent of size enlargement.

Comparing the results obtained from DCP and SA, anionic vesicles showed larger size compared to cationic ones ($P < 0.05$). The bulkiness of DCP ($C_{32}H_{67}O_4P$) molecule with its two cetyl chains might have resulted in less tightly packed bilayer membranes which would, therefore, reduce the ability of the nonionic surfactant membranes to curve and split resulting in larger particle size,[19,33] compared to the less bulky SA ($C_8H_{15}NO_8$), molecules. Other possible explanation could be based on the zeta potential values and charge density of

the bilayer membranes. The zeta potential value of anionic vesicles [Table 2] reflects a high charge density that would amplify the electrostatic repulsion force between the DCP, SP40 and CHO head groups having a similar charge. The effect of SA on vesicle size was moderate relative to DCP, though the overall result is the increased size compared to control vesicles.

Effect of drug solubility on entrapment efficiency and niosome particle size

Entrapment efficiency is expressed as the percentage of the total amount of drug used initially. The physicochemical properties of the drug, especially partition coefficient, are important determinant of the extent of its incorporation in vesicles.[37] It is well accepted that lipophilic drugs are preferentially up taken by niosomes compared to hydrophilic ones. The results of entrapment efficiencies are presented in Table 3.

For estradiol, the calculated average percentage entrapment efficiency was 96.04±2.1%, meaning that about 39.5 μg of estradiol was entrapped per 1.0 mg lipid. Such relatively high drug loading was expected considering drug lipophilicity (log P of 2.3), resulting in the preferential partitioning of drug to the lipid phase of the vesicles. For mannitol, the entrapment efficiency was 11.2±0.52%, meaning that about 4.5 μg of the drug was entrapped per 1.0 mg of lipid, which is accepted regarding drug hydrophilicity (log P of -2.47) and being small molecule that can leak easily from the vesicles. The relatively large size of the prepared vesicles may be responsible for the obtained relatively high entrapment of such highly polar drug, due to increased aqueous content of the vesicle available for entrapping a large volume of drug solution.[38]

The encapsulation of drug in lipid/nonionic surfactant vesicles usually increases particle size, probably by interaction of drug with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayers, thereby increasing vesicle size.[39] Other factor is the relative volume of water and lipid phases. The effect of drug aqueous solubility on niosome particle size is presented in Table 3. There was about 35 and 8.6% increase in the average size of niosomes containing estradiol and mannitol, respectively, relative to control unloaded vesicles. The table also shows that drug incorporation into vesicle decreased homogeneity of the particle size as shown by increased PDI relative to control vesicles.

For mannitol, a hydrophilic drug, the drug is expected to be passively entrapped in the core. It is thus suppose to have no effect on the vesicle size. This was confirmed by the nonsignificant increase ($P > 0.05$) in vesicle size in presence of mannitol. For lipophilic estradiol, it is expected to be located between the fatty acyl side chains of the bilayer membrane as the hydrocarbon chains provide a good solubilizing environment for the drug molecules. This may increase the hydrodynamic diameter of the vesicles. additionally, it was stated that drug lipophilicity is not the only factor affecting the solubility of drug molecules in the surfactant bilayers, geometric constrains governs the distribution of the lipophilic drug into vesicles.[II][37] Therefore, the relatively bulky estradiol molecules (M.W. 272.38) may have occupied a large space within the bilayer membranes contributing further to the increased size.

Table 2: Effect of charge-inducing agent on zeta potential (ζ), particle size and polydispersity indices (PDI) of niosomes

Formulation	ζ (mV)	Particle size (μm)	PDI
SP40:CHO	-11.6 (±1.4, 4)	0.61 (±0.073, 4)	0.19 (±0.0253, 4)
SP40:CHO:DCP	-50.7 (±3.7, 3)	0.76 (±0.057, 3)	0.14 (±0.0183, 3)
SP40:CHO:SA	+19.3 (±2.8, 4)	0.68 (±0.042, 4)	0.11 (±0.0204, 4)

-Values between brackets are standard deviation and number of replicates, respectively

Table 3: The percentage entrapment efficiency, particle size and polydispersity indices (PDI) of niosomal systems containing β-estradiol (SP40:CHO:Estradiol) and mannitol (SP40:CHO:Mannitol) relative to control (SP40:CHO)

Formulation	%Entrapment efficiency	Particle size (μm)	PDI
SP40:CHO	–	0.611 (±0.073, 4)	0.19 (±0.0253, 4)
SP40:CHO: Estradiol	96.04 (±2.1, 4)	0.823 (±0.078, 4)	0.26 (±0.02, 4)
SP40:CHO: Mannitol	11.2 (±0.52, 3)	0.664 (±0.054, 3)	0.22 (±0.03, 3)

-Values between brackets are standard deviation and number of replicates, respectively

CONCLUSIONS

Synthetic amphiphilic vesicles, prepared by nonionic surfactants, can be optimized so as to control the distribution of the drug for pharmacological and clinical benefits. Among the characteristics of these vesicles that is crucial for their intended therapeutic effect is particle size. Controlling the size of these vesicles is a possibility by controlling the vesicle constituents and processing variables. Going through this work, one can have a thought on how to obtain a vesicular dispersion with a particular size, either big or small. Commonly used membrane additives, such as cholesterol and charge-inducing agents, can extremely affect the vesicle size. The physicochemical properties of encapsulated drug, either hydrophilic or lipophilic, can also have a considerable impact on vesicle size. Processing variables such as application of ultrasound waves, have observable effect on size only at the early time of application. Generally speaking, it could be concluded that we can approach a particular particle size of lipid vesicles by the proper manipulation of formulation compositions.

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