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Sucrose stearate-based proniosome-derived niosomes for the nebulisable delivery of cromolyn sodium

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Abstract

A Novel approach was developed for the preparation of controlled release proniosome-derived niosomes, using sucrose stearates as non-ionic biocompatible surfactants for the nebulisable delivery of cromolyn sodium. Conventional niosomes were prepared by a reverse phase evaporation method followed by the preparation of proniosomes by spraying the optimized surfactant–lipid mixture of sucrose stearate, cholesterol and stearylamine in 7:3:0.3 molar ratio onto the surface of spray dried lactose powder. Proniosome-derived niosomes were obtained by hydrating proniosomes with 0.9% saline at 50 °C and mixing for approximately 2 min. All vesicles were evaluated for their particle size, morphological characteristics, entrapment efficiency, *in vitro* drug release, nebulisation efficiency and physical stability at 2–8 °C. In addition, coating carrier surface with the surfactant–lipid mixture, during preparation of proniosomes, resulted in smaller, free flowing, homogenous and smooth vesicles with high drug entrapment efficiency. Compared to a standard drug solution, a successful retardation of the drug release rate was achieved with the proniosome-derived niosomes, where the $t_{50\%}$ value of the release profile was 18.1 h compared to 1.8 h. Moreover, high nebulisation efficiency percentage and good physical stability were also achieved. The results are very encouraging and offer an alternative approach to minimize the problems associated with conventional niosomes like degradation, sedimentation, aggregation and fusion.

Keywords: Proniosomes; Cromolyn sodium; Nebulisation; Sucrose stearate; Physical stability

1. Introduction

Since the first description of liposomes in the early 1960s, it was encouraging to observe the plethora of both theoretical and practical applications, which this versatile drug delivery system has invoked. Drug delivery systems using colloidal particulate carriers such as liposomes have distinct advantages over conventional dosage forms because the particles can act as drug containing reservoirs and modification of the particle composition or surface can adjust the drug release rate and/or the affinity for the target site (Betageri and Habib, 1994). Aerosolized liposomes offer additional advantages of targeted drug delivery and amplified therapeutic effect (Weinstein and Leserman, 1984). Although the application of liposomes for improved aerosol drug delivery is encouraging, the nebulisation of liposomes exhibits some difficulties, including the instability and degradation by hydrolysis of aqueous dispersions on storage (Frfkjaer et al., 1984) and the leakage of the encapsulated drug on increase of the air flow pressure (Niven et al., 1992). Moreover, oxidation (Hunt and Tsang, 1981), sedimentation, aggregation (Wong and Thompson, 1982), high cost of synthetic phospholipids and variable purity of natural phospholipids have raised concerns over the adoption of liposomal drug delivery systems (Gregoriadis, 1984; Vora et al., 1998).

An alternative approach that overcomes several of these problems associated with liposomes involves the formation of niosomal dispersions (non-ionic surfactant based vesicles formed from the self-assembly of non-ionic amphiphiles in aqueous media resulting in closed bilayer structures) (Uchegbu et al., 1995; Uchegbu and Vyas, 1998). Preliminary in vivo studies indicate that niosomes behave like liposomes, prolonging the circulation of the entrapped drug and altering its organ distribution and metabolic stability (Rogerson et al., 1988).

Even though, the problems associated with the physical stability were not alleviated till Payne et al. (1986a) introduced

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proliposomes as a dry free-flowing granular product composed of water-soluble porous powder loaded with drug and phospholipids. These proliposomes could be hydrated immediately before use to form a multilamellar liposomal suspension suitable for administration.

In order to achieve high physical and chemical stability, Hu and Rhodes (2000) developed proniosomes that were loaded with non-ionic surfactants, instead of phospholipids commonly employed in proliposomes. These "solvent free solid state liposomes" may minimize the previously mentioned physical stability problems. The additional convenience of the transportation, distribution, storage and dosing would make these preparations promising industrial product.

Cromolyn sodium (CS) is a model anti-asthmatic and anti-allergic drug (Thompson, 1992). It is known that frequent inhalation of cromolyn sodium solution from nebulisers (4–6 times daily) is necessary because it is cleared rapidly with an elimination half-life of ≈80 min (Dollery, 1999). Many strategies were applied to improve the aerosolisation behaviour and the deposition of the drug in the lung (Chew et al., 2000, 2005; Steckel et al., 2003; Najafabadi et al., 2004; Abd Elbary et al., 2007; Heng et al., 2007). Therefore, In order to reduce number of doses and to achieve constant drug blood levels, controlled drug release niosomal and proniosome-derived niosomal formulations were developed using biodegradable sucrose esters.

To our knowledge, a limited number of studies were published considering the preparation and evaluation of proniosomes (Vora et al., 1998; Hu and Rhodes, 2000; Fang et al., 2001; Blazek-Welsh and Rhodes, 2001a,b; Alsarra et al., 2005). The majority of these publications were focused on the utilization of proniosomes in the transdermal drug delivery. In this study, a novel approach was developed that utilizes the prepared proniosome-derived niosomes in the nebulisable delivery of cromolyn sodium using sucrose esters.

Sucrose esters are non-ionic surfactants having a sugar substituent, sucrose, as the polar head group and fatty acids as non-polar groups. These esters contain different fatty acids (stearic, palmitic, myristic and lauric acid) in different ratios. The type of fatty acid and the degree of esterification determine the hydrophilic lipophilic balance (HLB) value and the melting point of these materials. They have HLB values from 1 to 16 and therefore, they can be applied in many areas of pharmaceutical and cosmetical technology as emulsifiers, solubilizing agents, lubricants, penetrating enhancers and pore forming agents (Csóka et al., 2007). Possible future utilization of these surfactants in more developed systems is expected because they have low toxicity, biocompatibility, and excellent biodegradability (Youan et al., 2003).

This work focused on optimization of the factors influencing the preparation of niosomes and proniosomes using the biodegradable sucrose esters surfactants. The influence of sucrose esters having different HLB values (11 and 16) on the in vitro drug release and the nebulisation behavior of the prepared conventional niosomes as well as proniosomes-derived niosomes was also investigated.

2. Materials and methods

2.1. Materials

Cromolyn sodium was kindly provided by El-Kahira Drug Company, Cairo, Egypt. Stearylamine was purchased from Sigma Chemicals, St. Louis, MO, USA. Cholesterol was purchased from Dolder Ltd., Basle, Switzerland. Two different grades of sucrose esters; sucrose stearate D18-11 (HLB = 11), sucrose stearate D18-16 (HLB = 16), were purchased from Mitsubishi-Kagaku Foods Corporation, Japan. Spectra Por© molecular porous membrane tubing (M.W. cut off 12,000–14,000) was purchased from Spectrum Medical Industries, USA. Spray dried lactose was purchased from Meggle, Germany. Glucose monohydrate and Sorbitol were obtained from Fisons Scientific Equipment, Loughborough, UK, lactose monohydrate (Lactochem®) was purchased from Borculo Domo, Netherlands. Disodium hydrogen phosphate and Potassium dihydrogen phosphate were obtained from E. Merck, Darmstadt, Germany. Uranyl acetate was kindly provided by Riedel-deHäen, Germany. Diethyl ether was purchased from S.D. Fine-Chem. Ltd., Poicha, India. All other reagents were of analytical reagent grade and were obtained from EL-Nasr Company, Cairo, Egypt.

2.2. Methods

2.2.1. Preparation of niosomes

Drug containing niosomes were prepared by the reverse phase evaporation (RPEV) method commonly employed for the entrapment of hydrophilic drugs (Uchegbu and Vyas, 1998), using sucrose stearate as a non-ionic surfactant, cholesterol as an enhancer of niosomal membrane rigidity and stearylamine as a positive charge inducer. Various molar ratios of sucrose stearate D18-11:cholesterol:stearylamine were prepared and evaluated. Appropriate amounts of sucrose stearate, cholesterol and stearylamine (10 mg/ml) were dissolved in 1:1 chloroform:diethyl ether mixture in a 100 ml round bottom flask. The aqueous phase containing cromolyn sodium (20 mg/2 ml) was added to the organic phase so that the organic to aqueous phase ratio was 5:1. The flask was covered with Parafilm® to prevent evaporation of the organic phase. The mixture was then sonicated in an ultrasound bath (50 W) at 50 °C for 5 min (Labsonic L, Braun Diessel Biotech, Melsungen, Germany). A stable white emulsion was produced from which the organic solvents were slowly evaporated at 50 °C using a rotary vacuum evaporator (Büchi Rotavapor, Type R110, Switzerland) till a thin film was formed on the wall of the flask. The film was hydrated with 10 ml 0.9% saline to produce an aqueous niosomal suspension containing 100 mg cromolyn sodium/10 ml (equivalent to five doses). This suspension annealed at 50 °C in a thermostatically controlled water bath (Model W10, Medingen Sitz Freeital, Germany) for 1 h, then left to stand at room temperature over night to obtain drug-containing niosomes. The composition of different formulations is shown in Table 1.

Table 1
The composition of different niosomal formulations (N1–N6), S* is the aqueous drug solution

Formulae	Cromolyn sodium (mg)	Surfactant:cholesterol:stearylamine (molar ratios)
S*	20	–
N1	20	10:0:0
N2	20	7:3:0
N3	20	3:7:0
N4	20	7:3:0.1
N5	20	7:3:0.3
N6	20	7:3:0.5

2.2.2. Selection of an appropriate carrier for the preparation of proniosomes

In order to choose the appropriate carrier that could be used in the preparation of proniosomes in this study, the following carriers namely; glucose monohydrate, sorbitol, lactose monohydrate and spray dried lactose were tried. To minimize the effect of particle size on the carrier properties, all tested carriers were sieved for 15 min using a sieve shaker (Setaccio Di Prova, Milano, Italy) through a test sieve with an aperture width of 80 μm which was placed over a test sieve with an aperture width of 63 μm. The powder fraction that was retained on the 63 μm sieve was used. Carrier particles surface morphology was examined using image analysis optical microscopy consists of a colour video camera (JVC, Victor Company, Japan) connected to an IBM compatible computer adopted with an Image analysis software (Leica Imaging Systems, Cambridge, England). The morphology of different carriers was quantified by shape descriptors derived from the length (L), width (W), perimeter (P) and area (A) of the projected image. The elongation ratio E ($E = L/W$) and shape factor ($S_{cir} = 4\pi \times A/P^2$) defined as a factor which combines both geometric shape and surface smoothness (Zeng et al., 2000) were calculated. The flowability of these carriers was assessed using a tap density tester (Jolting Volumeter Model SVM 2/UZ, Erweka, Germany). A graduated cylinder was filled with approximately 100 g powder. The cylinder was first tapped 10 times and the volume occupied by the powder was determined (V_{10}). The cylinder was then tapped 500 times and the volume recorded (V_{500}). Hausner's ratio, given by the relation V_{10}/V_{500} was calculated.

2.2.3. Preparation of proniosomes

The proniosomes were prepared according to the method developed by Hu and Rhodes (2000), keeping the molar ratio of sucrose stearate:cholesterol:stearylamine constant at 7:3:0.3. Appropriate amounts of sucrose stearate, cholesterol, stearylamine and cromolyn sodium were dissolved in a 3:1:1 propylene glycol:water:95% ethanol mixture. The prepared solution (10 ml) was sequentially sprayed onto the surface of spray dried lactose powder in a 100 ml round bottom flask so that spray dried lactose:surfactant ratio was 10:1. During the spraying period (approximately 5 min), the rate of application was controlled at 2 ml/min so that the powder bed of spray dried lactose did not become overly wet such that slurry would form. The flask was attached to a rotary evaporator. The evaporator was then evacuated and the rotating flask was lowered into a thermostatically controlled water bath maintained at 50 °C. The flask was rotated in the water bath under vacuum until the powder appeared to be dry (15–20 min). Another aliquot of solution was then introduced. This process was repeated until all the solution was applied. After addition of the final aliquot, evaporation was continued for 1 h. The loaded powder was further dried in a desiccator (Desiccator Jar, H-4960, Humboldt Mfg. Co., Norridge, USA) at room temperature overnight (approximately 16 h). The absence of residual traces of water was verified by Karl-Fisher technique. Proniosome-derived niosomal dispersion (formula PN) was obtained by hydrating the resulting proniosomes with 0.9% saline to obtain a concentration of 20 mg/2 ml at 50 °C and vortex mixing (VSM-3 Variable Speed Vortex Mixer, PRO Scientific Inc., Oxford) at 1500 rpm for 2 min. Further characterizations of the prepared vesicles were carried out after separation of free drug

2.2.4. Separation of free (un-entrapped) drug

The non-encapsulated drug was separated from the niosomal dispersions by centrifugation of the dispersion at 15,000 rpm at 4 °C for 60 min using a cooling centrifuge (Megafuge 1.0 R, Heraeus, Germany). The supernatant was removed and the pellet (residue) was resuspended in 0.9% saline. This process was repeated twice to ensure that free drug was completely removed.

2.2.5. Characterization of the prepared niosomes and proniosomes

2.2.5.1. Determination of drug entrapment efficiency percentage. The concentration of the entrapped drug was determined after lysis of niosomal dispersions with 1:1 absolute alcohol–propylene glycol mixture and sonication. 0.1 ml of the drug suspension was diluted to 10 ml with absolute alcohol–propylene glycol mixture in a test tube. The tube was then covered with a Parafilm® and sonicated for 5 min. A clear solution was obtained, from which 1 ml was taken and suitably diluted with the solvent mixture. The concentration of the drug was determined spectrophotometrically (Shimadzu UV–1601 PC Double Beam, Kyoto, Japan) at λ_{max} 325 nm (Moffat et al., 1986) against the samples withdrawn from empty niosomal dispersion treated in a similar manner. The method was validated, the accuracy, repeatability (intra day and intermediate precision (inter day) and reliability were ensured. The recovery% were >98%. The entrapment efficiency was determined relative to the original drug concentration according to the following equation (Hao et al., 2002)

$$EE\% = \left[\frac{ED}{TD}\right] \times 100$$

where EE% is the entrapment efficiency percent, ED is the entrapped drug concentration and TD is the theoretical drug concentration (20 mg/2 ml).

2.2.5.2. Particle size determination. The vesicle size of each niosomal (formulae N1–N6) and proniosome-derived niosomal formulations (formulae PN(D18-11 and D18-16) was determined using Mastersizer S laser diffracometer (Malvern

Instruments, Malvern, Worcestershire, UK) at 25 ± 0.5 °C. For size measurements, the preparation was appropriately diluted with purified water and measured using a lens (with a laser range of 300 mm that measure particle size range from 0.5 to 900 μm, and a beam length of 2.4 mm was attached to a measuring cell). The obscuration level was kept at 10% at a stable count rate. Three replicates were taken for each sample and polystyrene beads was used as a standard to check instrument performance. The polydispersity index (PI) was determined as a measure of homogeneity. Small values of PI (<0.1) indicate a homogeneous population, while PI values >0.3 indicate high heterogeneity (Sentjurc et al., 1999).

2.2.5.3. Scanning electron microscopy (SEM). Scanning electron micrographs were taken to illustrate the morphological differences between the prepared proniosome-derived niosomes (formula PN D18-11) and the original spray dried lactose powder. The samples were sprinkled and fixed on a SEM holder with double sided adhesive tape and coated with a layer of gold of 150 Å for 2 min using a Sputter coater (Edwards, S-150A, England) working in a vacuum of (3×10^{-1} atm) of Argon gas. The samples were examined using a scanning electron microscope (Jeol, JSM T20, Tokyo, Japan).

2.2.5.4. Transmission electron microscopy (TEM). The morphological characteristics of the conventionally prepared niosomes (formula N5) as well as proniosome-derived niosomes (formula PN D18-11) were examined using transmission electron microscopy. A sample drop was diluted 10-fold using de-ionized water and a drop of this diluted dispersion was applied to a collodion-coated 300 mesh copper grid and left for 5 min to allow some of the niosomes to adhere to collodion (pure grade that meets analytical specification of Ph Helv VI and DAB6, Fluka Chemie GmbH, Buchs SG, Schweiz). The remaining dispersion was removed by adsorbing the drop with the corner of a piece of filter paper (Whatman International Ltd., Maidstone, England). A drop of 2% aqueous solution of uranyl acetate was applied for 1 min. The remaining solution was then removed and the sample was air dried and examined with a transmission electron microscope (Jeol, 1200 EXII, Tokyo, Japan).

2.2.5.5. In vitro drug release. This study was carried out using a USP dissolution tester (Apparatus I) (Hanson SR6, CA, USA). The drug loaded niosomal dispersions, containing the equivalent of 20 mg cromolyn sodium, were placed in glass cylindrical tubes (2.5 cm in diameter and 6 cm in length). Each tube is tightly covered with a Spectra por© molecular porous membrane tubing from one end and attached to the shafts of the USP Dissolution tester apparatus, instead of the baskets, from the other end. The shafts were then lowered to the vessels of the dissolution apparatus containing 50 ml of Sorensen's phosphate buffer (pH 7.4) so that the dissolution medium outside and the vesicles preparation inside were adjusted at the same level. The release study was carried out at 37 ± 0.5 °C, and the stirring shafts were rotated at a speed of 50 rpm. Five milliliter samples were withdrawn periodically at predetermined time intervals of 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 24, 36 and finally at 48 h. Every withdrawal was followed by replacement with fresh medium to maintain a constant volume. The samples were analyzed spectrophotometrically at 325 nm against the samples withdrawn at respective time interval from empty niosomal dispersion treated in a similar manner and the results were the mean values of three runs each representing one batch. In order to understand the barrier presented by the dialysis membrane, the in vitro release study of plain drug solution (formula S) of the same concentration (20 mg/2 ml) was carried out in a similar manner. The obtained release data were subjected to kinetic treatment according to zero, first and Higuchi diffusion models (Higuchi, 1963). The correlation coefficient (r), the order of release pattern and $t_{50\%}$ value was determined in each case. The obtained $t_{50\%}$ values were subjected to statistical evaluation using ANOVA at a 5% level of significance.

2.2.5.6. Nebulisation of the prepared formulations. The nebulisation of the niosomal formulations as well as the plain drug solution was carried out using a compressor nebuliser system (Medel® AS3, Class II b, Italy), supplied with an air jet nebuliser (Medejet® Basic, Italy). The compressor air flow was 10 l/min and the average nebulisation rate was 0.38 ml/min. The generated aerosols were drawn through a Laser diffraction particle size analyzer (Malvern Mastersizer-S, Malvern Instruments, UK). The nebeulizer was clamped in a vertical position such that the mouth piece was 2.5 cm from the center of the Malvern beam. This distance was consistent for all experiment and equated to the anatomical distance from mouth to back of the throat, thereby correlating well with clinical condition. The aerosol was diverted through the beam approximately 5 mm in front of the 300 mm Fourier transform lens and drawn away by extraction into a suction pump. The mean droplet size of each aerosol was determined in triplicates.

The aerodynamic behavior of the generated aerosols was evaluated using the twin-stage impinger (TSI) (Copley Instruments, Nottingham, UK) following the stated procedure in British Pharmacopoeia (2004). Each suspension was filled into the nebuliser. The mouthpiece of the nebuliser was attached to the TSI by means of a suitable adaptor. A 7 and 30 ml of 1:1 absolute ethyl alcohol and propylene glycol mixture were introduced into the upper and lower impingement chambers, respectively. The vacuum pump of the impinger was switched on and the flow rate was adjusted using a flow meter attached to the pump at 60 l/min. After 10 s the nebuliser was switched on till complete nebulisation of the suspensions then, the nebuliser was switched off. After 5 s, the pump of the impinger was switched off and the nebuliser was disconnected and the impinger was dismantled the washings were collected in graduated flasks in addition to the remaining drug fraction The inner surfaces of the impingement chambers (stages 1 and 2) were washed separately with absolute ethyl alcohol and propylene glycol mixture 1:1 as well as the dead volume which was the volume of solution that remained in the nebuliser cup at the end of the nebulisation (non-emitted fractions) were determined for their drug fractions spectrophotometrically (Hess et al., 1996) at λ_{max} 325 nm for each formula (Shimadzu UV–1601 PC Double Beam, Kyoto, Japan). The neb-

Table 2
Characterization parameters of the prepared formulae (mean ± S.D., $n = 3$)

Formulae	Surfactant:cholesterol: stearylamine molar ratio	Drug entrapment efficiency (%)	Niosome particle size (μm)	Polydispersity index	$t_{50\%}$ of release profiles (h)	Aerosol droplet size (μm)	Nebulisation efficiency (%)
S	–	–	–	–	1.53 ± 0.43	2.15 ± 0.58	62.45 ± 2.31
N1	10:0:0	20.14 ± 1.51	2.70 ± 0.51	0.25 ± 0.12	5.88 ± 0.56	2.91 ± 0.44	56.57 ± 2.22
N2	7:3:0	30.05 ± 2.10	3.21 ± 0.25	0.28 ± 0.08	14.03 ± 0.78	3.35 ± 0.33	51.19 ± 1.42
N3	3:7:0	37.92 ± 1.22	4.22 ± 0.11	0.35 ± 0.11	24.98 ± 0.97	5.66 ± 0.27	35.60 ± 2.11
N4	7:3:0.1	36.18 ± 1.75	2.95 ± 0.16	0.24 ± 0.13	14.99 ± 1.02	3.10 ± 0.43	56.55 ± 1.17
N5	7:3:0.3	42.15 ± 2.19	2.50 ± 0.51	0.20 ± 0.07	21.12 ± 0.95	2.65 ± 0.10	61.25 ± 1.88
N6	7:3:0.5	43.78 ± 1.28	2.45 ± 0.23	0.21 ± 0.09	23.04 ± 1.12	2.58 ± 0.11	63.55 ± 2.17
PN (D18-11)	7:3:0.3	49.96 ± 2.36	1.75 ± 0.12	0.13 ± 0.05	18.97 ± 1.31	2.12 ± 0.33	67.95 ± 1.58
PN (D18-16)	7:3:0.3	41.87 ± 3.28	1.23 ± 0.18	0.11 ± 0.4	4.16 ± 0.31	1.76 ± 0.35	77.19 ± 3.27

ulisation efficiency percentage could be calculated as follows (Desai and Finlay, 2002):

$$\text{Nebulisation efficiency (\%)} = \frac{\text{Drug fraction collected in stage 2 of the TSI}}{\text{Theoretical drug concentration submitted to nebulisation (20 mg/2 ml)}} \times 100$$

2.2.6. Physical stability of the niosomal dispersions

Samples of the conventional niosomal dispersions were sealed in 30 ml clear glass vials and stored at refrigeration temperature (2–8 °C) for 90 days. In a parallel line, samples of proniosomes (D18-11) were treated similarly and the hydration of these samples was carried out after the same period. The entrapment efficiency as well as the mass median diameter (MMD) were determined and compared to the freshly prepared conventional and proniosome-derived niosomal dispersions, respectively.

2.2.7. Statistical analysis

The data obtained from different formulations were analyzed for statistical significance by one-way analysis of variance (ANOVA) adopting SPSS statistics program (version 14, SPSS Inc., Chicago, USA) followed by post hoc multiple comparisons using the least square difference (LSD). Differences between series were considered to be significant at $P \leq 0.05$.

3. Results and discussion

3.1. Preliminary investigations

3.1.1. Effect of the surfactant:cholesterol molar ratio on niosomal formulations

As shown in Table 2 and graphically illustrated in Figs. 1 and 2, it was clear that, formula N3 containing the highest cholesterol molar ratio had resulted in significant ($P < 0.05$) increase in the mean niosome particle size, drug entrapment efficiency percentage and $t_{50\%}$ value of the release profiles than other formulae N1, N2 containing lower cholesterol molar ratio. These results are in accordance with Guinedi et al. (2005) and Namdeo and Jain (1999) and who found that the incorporation of cholesterol into niosomes delayed drug release in vitro. A possible explanation of these findings is related to the ability of cholesterol to abolish the gel to liquid phase transition of niosomal systems and thus improves the encapsulation of hydrophilic drugs. Moreover, it enhances the membrane rigidity by condensing the packing of surfactants in the bilayer membranes. Therefore, it induces a limited drug release through the membrane out of the vesicles (Youan et al., 2003; Socacin et al., 2000; López et al., 2005). Moreover, it was clear, from the same table, that increasing cholesterol molar ratio led to an increase in the mean droplet size of the aerosolized niosomes with marked reductions in the nebulisation efficiency percentages.

The above results can be substantiated by the increase in the viscosity of the nebulised niosomal dispersions containing higher cholesterol molar ratios (N1, N2 and N3 showed viscosity values of 4 ± 0.25, 5.5 ± 0.37 and 7.6 ± 0.31 cp, respectively) which in turn were expected to produce larger droplets and/or the increase in the number of particles in a droplet with the increase in cholesterol concentration that would consequently expected to increase the aerodynamic diameter and hence decreased the drug nebulisation efficiency percentage (Gonda, 1985).

Bridges and Taylor (2000) and Bridges et al. (1995) studied the factors influencing the droplet formation of the aerosolized niosomal dispersions in air jet nebulisers and showed that the higher viscosity of the nebulised fluid may adversely alter the

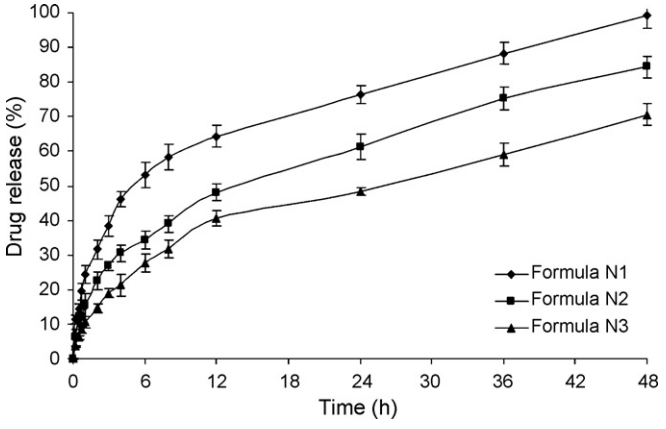

Fig. 1. In vitro drug release from niosomal formulations having different surfactant:cholesterol molar ratios in Sorensen's phosphate buffer (pH 7.4) at 37 ± 0.5 °C (mean ± S.D., $n = 3$).

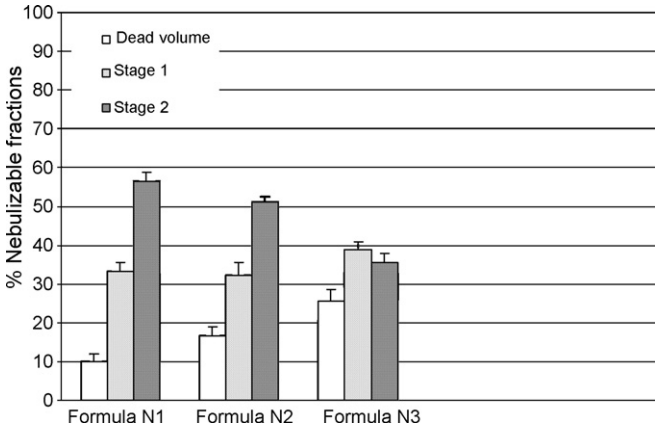

Fig. 2. Nebulisable drug fraction percentages aerosolized in a twin-stage impinger at 60 l/min from niosomes having different surfactant:cholesterol molar ratios (mean ± S.D., $n = 3$).

properties of the generated aerosols by increasing aerosol droplet size and extending the time required for nebulisation. This consequently minimizes their nebulisation efficiency. Of the prepared niosomes, those having a surfactant:cholesterol molar ratio of 7:3 achieved a good balance between the nebulisation efficiency and the drug release rate and was chosen for further studies.

3.1.2. *Effect of stearylamine molar ratio on niosomal formulations*

Table 2 and Figs. 3 and 4 show the results of the preliminary investigated niosomes prepared using different stearylamine molar ratios namely 7:3:0.1, 7:3:0.3 and 7:3:0.5 surfactant:cholesterol:stearylamine. It was clear that, the niosomal incorporation of stearylamine has led to more improvements in drug entrapment efficiency percentages, marked retardations in drug release rates and increases in the nebulisation efficiency percentages than the neutral niosomes (those prepared without stearylamine). This could be attributed to:

(a) The strong electrostatic forces involved in the interaction of the negatively charged drug with the positively charge inducer stearylamine.

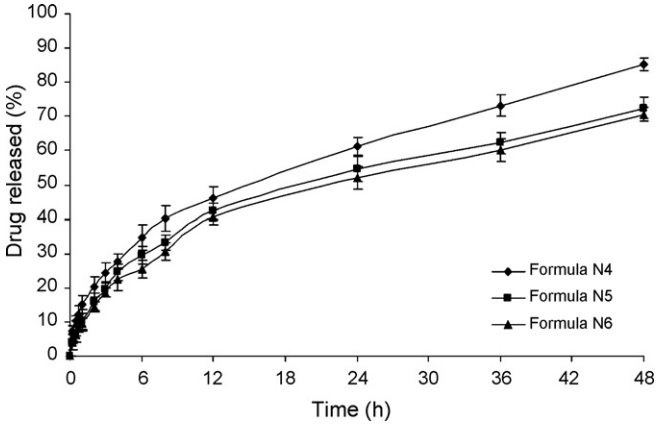

Fig. 3. In vitro drug release from niosomal formulations having different surfactant:cholesterol:stearylamine molar ratios in Sorensen's phosphate buffer (pH 7.4) at 37 ± 0.5 °C (mean ± S.D., $n = 3$).

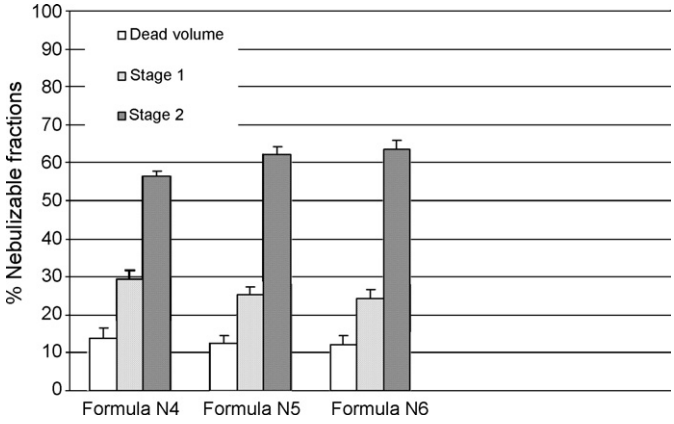

Fig. 4. Nebulisable drug fraction percentages aerosolized in a twin-stage impinger at 60 l/min from niosomes having different surfactant:cholesterol:stearylamine molar ratios (mean ± S.D., $n = 3$).

(b) The repulsion between likely charged niosomes which would minimize niosomal aggregations and hence, improving the nebulisation efficiency percentages.

Statistical analysis to demonstrate the significance of stearylamine addition using ANOVA revealed that, formulae N5 and N6 were significantly ($P < 0.01$) higher than N2, N4 with respect to the above parameters.

Unexpectedly, there was no significant ($P > 0.05$) difference between formulae N5 and N6. This means that the significance of the influences of stearylamine incorporation was concentration dependent. Therefore, formula (N5) containing a lower surfactant:cholesterol:stearylamine molar ratio was chosen for the preparation of proniosomes.

3.1.3. *The selection of an appropriate carrier for the preparation of proniosomes*

Desirable characteristics of the selected carrier that could be used in the preparation of proniosomes were addressed by Payne et al. (1986a,b). These include; safety and non-toxicity, free flowability, poor solubility in the loaded mixture solution and good water solubility for ease of hydration. Fig. 5 revealed that sorbitol and spray dried lactose had nearly spherical shape. However, glucose monohydrate and lactose monohydrate particles were orthorhombic and pyramidal in shape, respectively. The flowability of sorbitol and spray dried lactose was compared and Hausner's ratio was determined to be 1.21 ± 0.03 and 1.13 ± 0.02, respectively. The lower Hausner's ratio, the better the flowability of the powder. Moreover, it was difficult to coat sorbitol, glucose monohydrate and lactose monohydrate with the loading mixture solution due to their solubility in this solution and upon application, the samples became viscous slurries. Therefore, spray dried lactose which achieved the desired balance of excellent flowability, poor solubility in the loaded mixture solution and good solubility in water as well was chosen for the preparation of proniosomes.

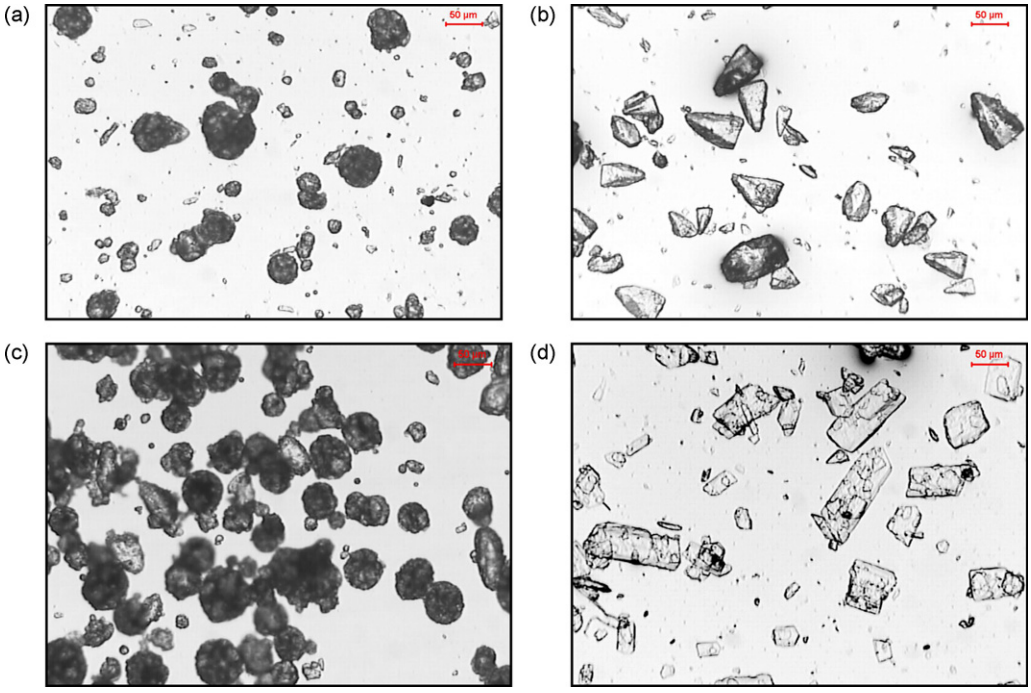

Fig. 5. Image analysis optical micrographs of (a) sorbitol, (b) α-lactose monohydrate, (c) spray dried lactose, and (d) glucose monohydrate.

3.1.4. The selection of an appropriate carrier–surfactant ratio

Three different spray dried lactose:surfactant ratios were preliminary tried namely, 5:1, 10:1 and 20:1. The preparation of proniosomes was so tedious when the ratio was 5:1 because the loading solution can be introduced and sprayed onto a small amount of spray dried lactose particles each time and thus, the spraying–evaporating process became very time consuming. The opposite held true for higher carrier:surfactant ratios of 10:1 and 20:1 but unexpectedly, no marked improvements in dry proniosome properties were observed upon using 20:1 over 10:1 ratio. Therefore, a ratio of 10:1 was chosen for preparation of proniosomes in an aim to minimize amount of carrier in the final niosome suspension.

3.2. Characterization of niosomes and proniosome-derived niosomes

3.2.1. Particle size and entrapment efficiency

The mean particle size as well as the entrapment efficiency of the selected conventional niosomes and the proniosome-derived niosomes are shown in Table 2. It was clear that, conventionally prepared niosomes were significantly ($P < 0.05$) larger (2.45–4.22 μm) and more heterogeneous than those derived from proniosomes-derived niosomes, PN (D18-16) and PN (D18-11) (1.23 and 1.75 μm, respectively). This may be attributed to the great surface area of the formed film on the surface of the carrier and the ease of proniosomes hydration (Hu and Rhodes, 2000).

The difficulty of the complete hydration of the films formed on the walls of the round bottom flasks during the preparation of niosomes was responsible for their lower drug entrapment efficiency compared to PN (D18-11) proniosome-derived niosomes ($49.96 \pm 2.36\%$). In niosomes, the quite inner viscous layers of the films tend to remain adhered to the flask surface even after 1 h of hydration at 50 °C with vigorous shaking. A risk of material loss with reduction in drug entrapment efficiency was possible.

Indeed, coating the carrier surface with the surfactant–lipid mixture during the preparation of proniosomes favored the complete hydration of the surfactant–lipid film.

3.2.2. Scanning and transmission electron microscopy

The surface characteristics of spray dried lactose and dry proniosome powders are illustrated in scanning electron micrographs shown in Fig. 6a and b. Uncoated spray dried lactose powder appears to have many irregularities, fine structures as whiskers and sharp corners. On the contrary, dry proniosome powder appears to be smoother than the carrier particles. The influence of the coating process on the surface characteristics of carrier particles was studied by Blazek-Welsh and Rhodes (2001b), where they suggested the smoothness of the proniosome surface to be due to:

(a) The filling effect of surfactant on the fine structures located on the surface of the carrier, where thicker layers of surfactant were expected to be deposited at points of deeper invaginations.
(b) Brief dissolution of surface molecules of the carrier (particularly, thin or sharp features) in the solvent mixture sprayed onto the carrier surface. Once the solvent was evaporated, the dissolved carrier surface molecules as well as, other components in the solvent mixture, were recrystallized onto the new surface. As expected, these effects apparently removed some of the fine crystalline structures of the carrier

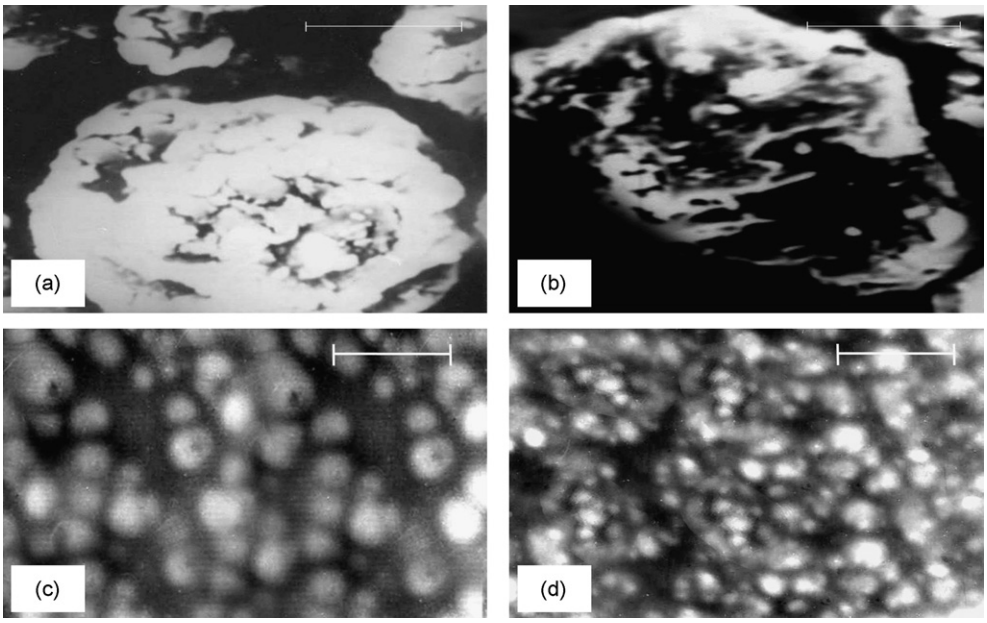

Fig. 6. Scanning electron micrographs of spray dried lactose powder (a) and proniosome-derived niosomes (b) (scale bar indicates 25 μm) and transmission electron micrographs of niosomes (c) and proniosome-derived niosomes (d) (scale bar indicates 5 μm).

powder, making the surface of proniosome powder appear smoother.

The transmission electron micrographs revealed that conventionally prepared niosomes are heterogeneous with more large vesicles (Fig. 6c) while more uniform suspensions with fewer large vesicles were achieved with proniosome-derived niosomes (Fig. 5d).

3.2.3. *In vitro release of the drug*

Fig. 7 showed the in vitro drug release from the different formulations. The kinetic analysis of the drug release data followed diffusion controlled mechanism from niosomes and proniosome-derived niosomes as well. The general features of the release profile of the proniosome-derived niosomes, prepared using D18-11 were similar to that of conventionally prepared niosomes. It was clear that 10.38 ± 1.11 and $12.34 \pm 1.26\%$ of the drug were released from formulae N5 and PN (D18-11) after 1 h, respectively. This similarity was maintained throughout the release profiles where nearly similar drug percentages (72.39 ± 2.67 and $76.1 \pm 2.14\%$) were released after 48 h with non-significant difference ($P > 0.05$) in the calculated $t_{50\%}$ values; $21.12 \pm 0.95$and 18.97 ± 1.31 h in both formulae, respectively. These results were in accordance with the work done by Hu and Rhodes (2000) who found that overall, gastric or intestinal fluid release profiles of ibuprofen from proniosome-derived niosomal dispersions or niosomes prepared by conventional methods showed little difference.

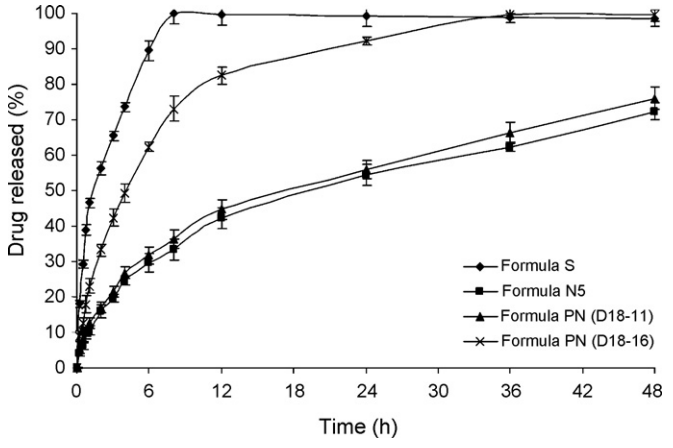

Fig. 7. In vitro drug release from the selected niosomes, proniosome-derived niosomes and plain solution in Sorensen's phosphate buffer (pH 7.4) at 37 ± 0.5 °C (mean ± S.D., $n = 3$).

The incorporation of sucrose stearate (D18-16) having a higher HLB value (16) modified the drug liberation profile. Significant increases ($P < 0.01$) in the percentage drug released (23.25 ± 1.98 and 99.75 ± 1.56) were achieved after 1 and 48 h, respectively. The initial quick release of the drug could be explained by drug diffusion from the core of niosomes, while after this the drug liberation was regulated by diffusion throughout the swollen niosomal membranes.

Statistical analysis of the results shown in Fig. 7 revealed that formula PN (D18-11) would provide a better control of the drug release rates than formula PN (D18-16) due to its significant ($P < 0.01$) higher drug entrapment efficiency and significant ($P < 0.01$) longer $t_{50\%}$ of the drug release profile as well. Therefore, based on the previous characteristics formula PN (D18-11) was chosen to progress to physical stability testing. On the other hand, the drug release from the solution was quite different, where $46.63 \pm 1.14\%$ of the drug was detected in dissolution medium in the first hour and was completely released after 8 h only. The great difference between the calculated $t_{50\%}$ values of release profiles of the proniosomal formulations and the drug solution could indicate that the former formulations are capable of controlling the rate of drug release.

Table 3
Change percentages of the vesicle size and drug entrapment efficiency of formulae N5 and PN (D18-11) after storage for 90 days at 2–8 °C (mean ± S.D., $n = 3$)

Storage period	Vesicle size (μm)		Drug entrapment efficiency (%)	
	Formula N5	Formula PN (D18-11)	Formula N5	Formula PN (D18-11)
Freshly prepared	2.50 ± 0.51	1.75 ± 0.12	42.15 ± 2.19	49.96 ± 2.36
90 days	2.87 ± 0.48	1.87 ± 0.36	37.59 ± 2.45	47.66 ± 2.21
Change%	↑14.84 ± 0.63	↑6.85 ± 1.08	↓10.81 ± 2.07	↓4.59 ± 1.53

3.2.4. Nebulisation behavior

As shown in Table 2 and Fig. 8, the droplet size of the aerosolized conventional niosomes was larger (2.58–5.66 μm) than those of proniosome-derived niosomal dispersions (1.76–2.12 μm). There was an inverse proportion relationship between the droplet size and the nebulisation efficiency percentage. The larger mean vesicle size, the larger the dead volume and hence, the lower the ability of niosomal formulations to reach stage 2 of the TSI. This result could be substantiated by upon nebulisation, large droplets baffled, returned and remained in the nebuliser cup. During this process, evaporation occurred and an increase in the concentration in the nebuliser cup (dead volume) took place (Hess et al., 1996). According to Gonda, 1985, increasing this concentration would consequently increase the aerodynamic diameter and hence decreased the drug nebulisation efficiency percentage. Accordingly, the nebulisation efficiency percentages of proniosome-derived niosomal dispersions were significantly higher ($P < 0.05$) than that of conventional large size niosomes.

Interestingly, the droplet size of the aerosolized plain drug solution (2.15 ± 0.58 μm) is close to that obtained upon aerosolization of the formula PN (D18-11). However, the nebulisation efficiency percentage of the former was lower (62.45 ± 2.31%). This could be explained with respect to the ability of sucrose stearate, as a non-ionic surfactant, to reduce surface tension and minimize the aggregates in proniosomal dispersions and consequently decreases their aerosol droplet size (Bower et al., 1996). The significant ($P < 0.05$) improve in drug nebulisation efficiency percentage (stage 2) of formula PN (D18-16) over that of PN (D18-11) with a lower drug fraction percentages in stage 1 as well as lower dead volume could be related to its higher HLB value.

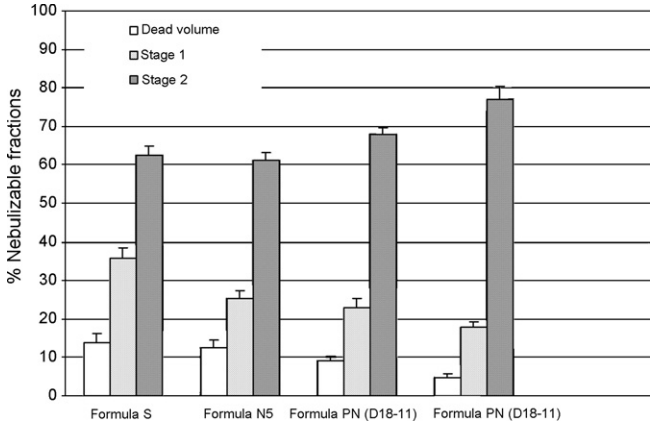

Fig. 8. Nebulisable drug fraction percentages aerosolized in a twin-stage impinger at 60 l/min from the selected niosomes, proniosome-derived niosomes and plain solution (mean ± S.D., $n = 3$).

3.3. Physical stability

The data presented in Table 3 revealed that, after storage for 90 days the mean vesicle size of conventional niosomes (formula N5) and proniosome-derived niosomes (formula PN (D18-11) was not significantly different ($P > 0.05$) from the same formulae when freshly prepared. However, the drug entrapment efficiency percentage of conventional niosomes was significantly lower ($P < 0.05$) following storage than the freshly prepared formulations (%decreased = 10.81 ± 2.07%).

Therefore, the mean particle size and the drug entrapment efficiency percentages of the proniosome-derived niosomes showed little, if any changes upon storage at 2–8 °C for 90 days. This suggests that proniosomes offered a more stable system that could minimize the problems associated with conventionally prepared niosomes like degradation by hydrolysis, or oxidation, sedimentation, aggregation and fusion during storage.

4. Conclusion

The study has indicated the possibility of manufacturing prolonged release proniosome-derived niosomes as promising drug carriers for the nebulisable delivery of cromolyn sodium. Sucrose stearates was successfully used as non-ionic surfactants in the preparation of niosomal dispersion when particular combinations (sucrose stearates D18-11 + cholesterol + stearylamine in 7:3:0.3 molar ratios) were used. The HLB values of the sucrose stearates had a pronounced effect on the drug liberation and the nebulisation efficiency. Proniosome-derived niosomes with high drug entrapment (50%) could be achieved by sequential spraying the niosomal dispersion onto the surface of spray dried lactose. This formula could be nebulised efficiently (68%) with good keeping properties when stored at 2–8 °C for 90 days in aerosol droplets of appropriate size (2 μm) for inhalation, thus fulfilled the main objectives of the present work. This finding opened the door for future use of sugar esters as low toxic, biodegradable and biocompatible surfactants for the preparation of convenient, stable, easily hydrated free flowing dry powder in the inhalation therapy

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