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International Journal of Pharmaceutics 206 (2000) 109–122

international journal of pharmaceutics

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Erratum

Erratum to 'Proniosomes: A Novel Drug Carrier Preparation'☆ [Int. J. Pharm. 185 (1999) 23–35]

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☆ PII of original article: S0378-5173(99)00122-2.
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PII: S0378-5173(00)00513-5

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International Journal of Pharmaceutics 206 (2000) 110–122

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Proniosomes: A Novel Drug Carrier Preparation

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Received 11 July 1997; received in revised form 25 March 1999; accepted 30 March 1999

Abstract

A procedure is described for producing a dry product which may be hydrated immediately before use to yield aqueous niosome dispersions similar to those produced by more cumbersome conventional methods. These 'proniosomes' minimize problems of niosome physical stability such as aggregation, fusion and leaking, and provide additional convenience in transportation, distribution, storage, and dosing. This report describes the preparation of dispersions of proniosome-derived niosomes, comparison of these niosomes to conventional niosomes, and optimization of proniosome formulations. In addition, conventional and proniosome-derived niosomes are compared in terms of their morphology, particle size, particle size distribution, and drug release performance in synthetic gastric or intestinal fluid. In all comparisons, proniosome-derived niosomes are as good or better than conventional niosomes.

Keywords: Niosomes; Proniosomes; Drug release; Lipophilic

1. Introduction

Drug delivery systems using colloidal particulate carriers such as liposomes (Betageri et al., 1994) or niosomes (Schreier et al., 1994) 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. Although there are still some problems associated with the industrial production and clinical applications of colloidal particulate drug carrier systems, this class of drug carrier systems will likely play an increasingly important role in drug delivery.

Liposomes or niosomes in dispersion can carry hydrophilic drugs by encapsulation or hydrophobic drugs by partitioning of these drugs into hydrophobic domains. Liposomes are unilamellar or multilamellar spheroid structures composed of lipid molecules, often phospholipids, assembled into bilayers. Because of their ability to carry a variety of drugs, liposomes have been extensively investigated for their potential application in pharmaceutics; such as drug delivery (Couvreur et al., 1991; Gregoriadis et al., 1993; Kim et al.,

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0378-5173/00/$ - see front matter
PII: S0378-5173(99)00122-2

1993); for drug targeting (Booser et al., 1994); for controlled release (Barber et al., 1993); or for increasing solubility (Gregoriadis et al., 1993).

However, there remain significant problems in the general application of liposomes for drug delivery. In a dispersed aqueous system, liposomes have problems associated with degradation by hydrolysis (Frfkjaer et al., 1984) or oxidation (Hunt et al., 1981); and sedimentation, aggregation, or fusion of liposomes (Wong et al., 1982) during storage. Other problems associated with the clinical application of liposomes include difficulties in sterilization and large-scale production (Frfkjaer et al., 1984). It can be difficult to obtain large quantities of sterile product with defined and reproducible properties, which display adequate chemical and physical stability.

Problems with the physical stability of aqueous suspensions of liposomes have been addressed by Payne et al. (1986a,b) who introduced 'proliposomes', a dry free-flowing granular product which could be hydrated immediately before use. Proliposomes are composed of water-soluble porous powder as a carrier upon which one may load phospholipids and drugs dissolved in organic solvent. Proliposomes can be stored sterilized in a dry state and dispersed/dissolved to form an isotonic multilamellar liposomal suspension by addition of water as needed. It was reported that amphotericin B proliposomes could be stored for 9 months without significant changes in distribution of vesicle size, and for at least 6 months without loss of pharmacological activity (Payne et al., 1986a,b). Even though proliposome formulations are an improvement over conventional liposome dispersions in terms of the *physical* stability of the preparation, a vacuum or nitrogen atmosphere is still recommended during preparation and storage to prevent the oxidation of phospholipids (Payne et al., 1986a; Katare et al., 1990). To avoid technical difficulties associated with this requirement, alternatives to phospholipids should be of great interest.

One alternative involves formation of liposome-like vesicles from hydrated mixtures of cholesterol and nonionic surfactant such as monoalkyl or dialkyl polyoxyethylene ether non-ionic surfactants (Baillie et al., 1985; Uchegbu et al., 1995). These 'niosomes' can entrap solutes, are quite stable, and require no special conditions, such as low temperature or inert atmosphere for production or storage. The nonionic surfactants for this use are usually single-alkyl chain surfactant and/or sorbitan esters. Preliminary studies indicate that niosomes behave *in-vivo* like liposomes, prolonging the circulation of entrapped drug to alter its organ distribution and metabolic stability (Azmin et al., 1985; Rogerson et al., 1988), or to prolong the contact time of drug with the applied tissues in topical applications (Hofland et al., 1994).

Although the structure and properties of niosomes are similar to those of liposomes, the chemical stability of niosomes and the relatively low cost of the materials that form them makes niosomes more attractive than liposomes for industrial manufacturing. However, even though niosomes exhibit good *chemical* stability during storage, there may be problems of *physical* instability in niosome dispersions. Like liposomes, aqueous suspensions of niosomes may exhibit aggregation, fusion, leaking of entrapped drugs, or hydrolysis of encapsulated drugs, thus limiting the shelf life of the dispersion.

A dry product which could be hydrated immediately before use would avoid many of the problems associated with aqueous niosome dispersions and problems of physical stability (aggregation, fusion, leaking) could be minimized. The additional convenience of the transportation, distribution, storage, and dosing would make 'dry niosomes' a promising industrial product. In the work reported here, we describe the preparation of dry niosomes, 'proniosomes.' This is a dry, free-flowing, granular product which, upon addition of water, disperses or dissolves to form a multilamellar niosome suspension suitable for administration by oral or other routes.

2. Materials and methods

2.1. Materials

The nonionic surfactant used was NF grade sorbitan monostearate, Span 60 (kindly donated

by ICI Americas). Cholesterol (CH), 5(6)-carboxyfluorescein (CF), and sorbitol (NF grade) were purchased from Fisher Scientific. Dicetyl phosphate (DCP) was purchased from Aldrich Chemicals. Ibuprofen was USP XXII grade (Spectrum Chemical). Chloroform (ethanol stabilized HPLC grade) was purchased from Malinckrodt Specialty Chemicals. Ethanol and 2-amino-2-(hydroxy-methyl)-1,3-propanediol (practical) were purchased from EM Science. Sephadex G-25 (medium) was purchased from Sigma Chemicals (St Louis, MO 63178). Triton X-100 was kindly donated by Union Carbide, and used as received. Spectra Por dialysis membrane tubing (MW cut-off 6000–8000, diameter 14.6 mm) was purchased from Spectrum Medical Industries, and was soaked and washed with distilled water before use. Ultrapure water, used for all aqueous solutions, was from a Milli-Q filtration system (Millipore). All chemicals were used without further purification.

Simulated gastric fluid was prepared according to USP XXII, using a solution of NaCl (2.0 g/l, pH 1.2 by addition of HCl). Because pepsin interfered with the determination of released drugs, it was not included in this preparation. Because there is no substrate for pepsin in the release system, the lack of pepsin in this simulated gastric fluid should have no effect on drug release rate. Simulated intestinal fluid was also prepared according to USP XXII. Monobasic potassium phosphate (6.8 g) was dissolved in 250 ml of distilled water, and 190 ml of 0.2 N NaOH and 400 ml of water were added. This solution was adjusted to a pH of 7.5 with 0.2 N NaOH and diluted to 1000 ml. To avoid interference with drug assay, pancreatin was omitted from the formulation, but this should not perturb the release rate.

2.2. Methods

2.2.1. Preparation of niosomes

Conventional niosomes were prepared based on published reports (Yoshioka et al., 1994), using Span 60, cholesterol, and dicetyl phosphate. Sonication of the niosome preparation, an optional procedure reported by others was not used. Stock solutions of surfactant components (and drug, if necessary) were used to provide flexible formulation of various surfactant mixtures. Using a 500 mM solution of Span 60 (in chloroform-ethanol, 5:1, V/V), a 500 mM solution of cholesterol (in chloroform), and a 50 mM dicetyl phosphate solution (in chloroform), the desired volumes were added to a 100 ml round-bottom flask. The flask was attached to a rotary evaporator (Buchi Rotavapor RE120), lowered into a 60°C water bath, and the organic solvents were evaporated under reduced pressure to form a thin, dry film on the wall of the flask. Any excess organic solvents were removed by leaving the flask in a desiccator under vacuum overnight. The dried lipid film was hydrated when required with buffer (or other aqueous solution, Section 2.2.2), followed by vigorous shaking in an incubator (Double Metabolic Shaking Incubator, Precision Scientific Group) at 60°C for about 1 h. The resulting niosome dispersion was then left to cool, and was used as a control to which niosome dispersions prepared from proniosomes could be compared. Conventional, drug-containing niosomes were prepared by adding drug (250 mM ibuprofen in ethanol) to the surfactant mixture prior to evaporating the organic solvent, or by addition of drug (1 mM CF) to the aqueous solution used to rehydrate the surfactant.

2.2.2. Preparation of proniosomes

A 100 ml round-bottom flask containing 1 g of sorbitol was attached to the rotary evaporator. A surfactant mixture was prepared from stock solutions as described above to produce the desired ratio of Span 60, cholesterol, and dicetyl phosphate with a total concentration of 100 mM. The surfactant solution was introduced into the round-bottom flask on the rotary evaporator by sequential spraying of aliquots onto the surface of sorbitol powder. During the spraying period, the rate of application was controlled so that the powder bed of sorbitol did not become overly wet (such that a slurry would form). The evaporator was then evacuated and the rotating flask was lowered into a water bath at 65–70°C. The flask was rotated in the water bath under vacuum for 15–20 min or until sorbitol appeared to be dry,

and another aliquot of surfactant solution was introduced. This process was repeated until all of the surfactant solution had been applied. After addition of the final aliquot, evaporation was continued until the powder was completely dry (about 20–30 min). The material was further dried in a desiccator under vacuum at room temperature overnight. This dry preparation is referred to as 'proniosomes' and was used for preparations and for further study on powder properties.

Proniosome-derived niosome dispersions were obtained by hydrating the proniosome preparation with 80°C distilled water and vortex mixing for 2 min. The resulting niosome dispersion was used for the determination of the entrapment efficiency, particle size analysis, and morphological studies. The entrapment efficiency of ibuprofen in niosomes was used as a criterion for the evaluation of the tested formulations. Drug-containing proniosome-derived niosomes were prepared in a manner analogous to that used for the conventional niosomes, by adding drug (250 mM ibuprofen in ethanol) to the surfactant mixture prior to spraying the solution onto the sorbitol, or by addition of drug (1 mM CF) to the aqueous solution used to dissolve the sorbitol.

2.2.3. Scanning electron microscopy

Proniosomes, prepared as described above, were sprinkled on double-sided conductive carbon tape on an aluminum stub. Excess sample was blown off with compressed air. The specimen was then coated with Au/Pd (60/40) using a Ladd Sputter Coater at 2.5 KV and 20 mA for 45 s. The coated specimen was observed using a Philips 515 Scanning Electron Microscope at 50 KV and recorded on Polaroid PIN 55 film.

2.2.4. Transmission electron microscopy

The morphology of hydrated niosome dispersions prepared by conventional methods and from proniosomes was determined using transmission electron microscopy. A drop of niosome dispersion was diluted 10-fold using deionized water. A drop of diluted niosome dispersion was applied to a carbon-coated 300 mesh copper grid and left for 1 min to allow some of the niosomes to adhere to the carbon substrate. The remaining dispersion was removed by absorbing the drop with the corner of a piece of filter paper. After twice rinsing the grid (deionized water for 3–5 s) a drop of 2% aqueous solution of uranyl acetate was applied for 1 s. The remaining solution was removed by absorbing the liquid with the tip of a piece of filter paper and the sample was air dried. The sample was observed with a JEOL 100 CX transmission electron microscope at 80 KV.

2.2.5. Measurement of angles of repose

The angle of repose of dry proniosome powder was measured by a funnel method (Lieberman et al., 1990). Briefly, the sorbitol powder or proniosome powder was poured into a funnel which was fixed at a position so that the 13 mm outlet orifice of the funnel is 10 cm above a level black surface. The powder flowed down from the funnel to form a cone on the surface, and the angle of repose was then calculated by measuring the height of the cone and the diameter of its base.

2.2.6. Particle size and particle size distribution

A small aliquot (100 μl) of niosome dispersion was dispersed in 50 ml of distilled water and measured immediately with a Sald-1100 Laser Diffraction particle size analyzer. The particle size range of was set to 0.1–45 μm and the refractive index range was set to 0.2–1.7μm. The particle size distribution of niosomes was calculated internally.

2.2.7. Entrapment efficiency

Free ibuprofen was separated from niosome-entrapped ibuprofen by centrifugation. When a 1 ml aliquot of niosomes was centrifuged at 180 000 × *g*, a stiff, floating fraction containing the niosomes formed at the top of the tube, and a clear, niosome-free solvent fraction remained at the bottom. The clear fraction was used for determination of the free drug. A 0.2 ml aliquot of the clear fraction was diluted to 3 ml with ethanol and the diluted solutions used to obtain first order derivative absorbance spectra between 200 and 300 nm, using a Hewlett Packard 8452A Diode Array Spectrophotometer. (The derivative spectrum was more reliable than the OD_{265} read-

ings called for in the standard USP assay because it was less sensitive to scattering from residual niosome particles, which could cause significant scattering.) The derivative spectrum from ibuprofen exhibited positive and negative peaks at 260 and 276 nm, respectively. Standard curves made by measuring spectra for known concentrations of ibuprofen in 93% ethanol demonstrated that the absolute difference between these peaks was proportional to the concentration of ibuprofen. Standard curves were linear, with a slope of 6.5 mg/ml per OD. Thus, the concentration of ibuprofen was determined by measuring $d'A_{260/276}$, the difference between the first order derivative absorbance at 260 and at 276 nm, and comparing this value to $d'A_{260/276}$ data from known concentrations of ibuprofen using a standard curve. The drug concentration in the niosome fraction was determined in a similar manner, diluting a 0.2 ml aliquot of the niosome fraction to 3 ml with ethanol. The entrapment efficiency of the drug was defined as the ratio of the mass of niosome-associated drug to the total mass of drug.

2.2.8. Release of ibuprofen from niosomes

Dialysis tubes containing 2.5 ml of ibuprofen-containing niosomes and 2.5 ml of simulated gastric fluid or simulated intestinal fluid were placed into a flask containing 125 ml of simulated gastric fluid or simulated intestinal fluid at 37°C. These flasks were placed in a Lab-Line Environ-Shaker, and shaken at 100 rpm at a temperature of 37°C. At timed intervals, 1.0 ml aliquots were taken from the release medium (dialysate). At the end of the timed experiment, the dialysis tubes were cut open and allowed to leak into the release medium. A 1.0 ml aliquot of this solution was sampled to determine the concentration corresponding to 100% release. The final sample was clarified by adding 1 drop of Triton X-100. The released fraction of ibuprofen at a specified time was determined by comparing $d'A_{260/276}$ for the sample to $d'A_{260/276}$ measured for the 100% release samples.

2.2.9. Release of CF from niosomes

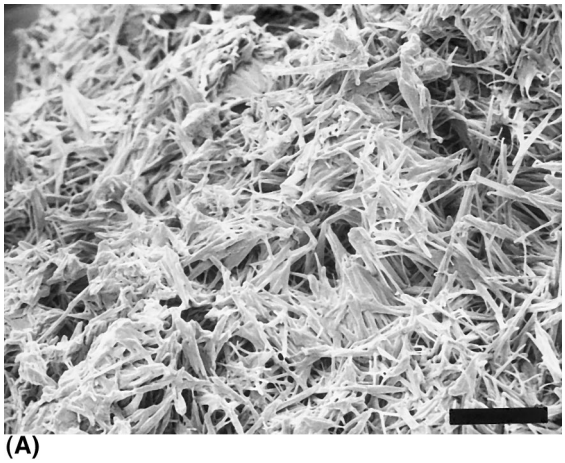
Free CF was separated from niosome-associated CF by gel exclusion chromatography. A 2.0 ml aliquot of CF-containing niosome preparation was loaded onto a Sephadex G-25 column (3×55 cm) and eluted using 10 mM Tris buffer, pH 7.4. Fractions containing the niosomes were pooled to a total volume of approximately 30 ml. A portion of the pooled niosome fractions (14 ml) was sealed in a dialysis tube (14.6 mm) and the sealed tube was placed into a flask containing 100 ml of simulated gastric fluid or simulated intestinal fluid at 37°C. The flask was shaken at 100 rpm and $T = 37$°C. At specified time intervals, 2 ml aliquots were taken from the release medium (dialysate) and 2 ml of fresh simulated fluid were added to the flask. At the end of the timed experiment, the dialysis tubes were cut open and the contents allowed to leak into the release medium. An aliquot (2 ml) of this solution was sampled to determine the concentration corresponding to 100% release. To measure fluorescence, the 2 ml aliquots were made basic by adding three drops of 4 N NaOH and clarified by adding one drop of Triton X-100 to disrupt the niosomes. The fluorescence intensity of the samples was measured at an excitation wavelength of $\lambda_{ex} = 490$ nm and emission wavelength of $\lambda_{em} = 519$ nm, using a Hitachi F-2000 fluorescence spectrophotometer. The fraction of CF released was determined by comparison to the fluorescence intensity measured in the 100% release samples.

3. Results

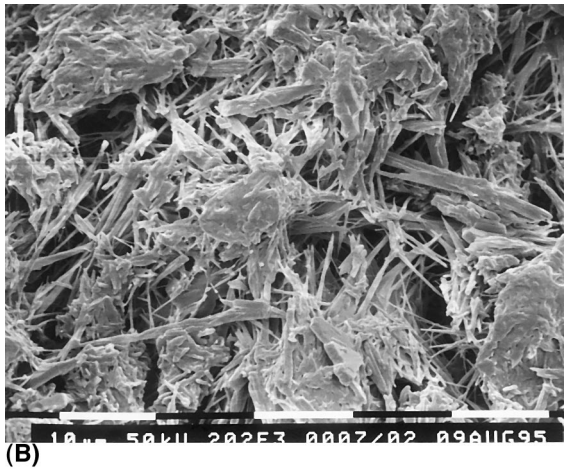
3.1. Morphology of dry proniosome powder

Scanning electron microscopy of uncoated sorbitol (Fig. 1A) and dry proniosome powder (Fig. 1B) reveals that there appears to be a slight difference in the appearance of the surfaces. The powder in (Fig. 1B) appears to be smoother and to have fewer 'fine features' such as whiskers and sharp corners. This surface change is probably caused by some brief dissolution of surface molecules of sorbitol (particularly, thin or sharp features) in the organic solvent used as a carrier for surfactants sprayed onto the surface of the sorbitol. Once the organic solvents were evaporated, the dissolved sorbitol may have recrystallized as surfactants which were deposited at the

new surface. As expected, these effects apparently removed some of the fine crystalline structures on the surface of the pure sorbitol powder, making the surface of the proniosome powder appear smoother than that of pure sorbitol. This explanation is supported by observations of samples in which the sorbitol powder was allowed to become overly wet during spraying. In these samples (not shown), there was a significant deterioration of the surface fine structure of the proniosome powder.



(A)



(B)

Fig. 1. Scanning electron micrographs of proniosomes. (A) Commercial sorbitol powder exhibits crystals with sharp edges and fine structure. (B) Proniosomes, prepared from the same batch of sorbitol used in (A), are generally similar, but have somewhat less well defined features. Scale bar indicates 10 μm.

Table 1
Angles of repose of uncoated sorbitol and proniosomes

Preparation	Angle of repose
Sorbitol	43.19 ± 0.75
Proniosomes	34.66 ± 2.10
Proniosomes ($2 \times$ [a])	37.65 ± 1.07

[a] The mass of sorbitol was doubled, but the mass of surfactant was kept constant.

3.2. Angle of repose

Results of measurements of the angle of repose of proniosome powder and pure sorbitol are summarized in Table 1, and indicate that the angle of repose of dry proniosome powder is smaller than that of pure sorbitol. This is consistent with the scanning electron microscopic observation of proniosome powder, in which it was observed that the proniosome surface was smoother. If the proportion of sorbitol to surfactants in the formulation is increased, the angle of repose of dry proniosome powder increases slightly, more closely approaching the angle measured for pure sorbitol.

3.3. Preparation of niosomes

Preparation of niosomes by standard methods results in a heterogeneous dispersion, prone to sedimentation or aggregation. Of greater concern is the difficulty of completely hydrating the lipid film on the wall of the round-bottom flask. Even after 1 h of hydration at 60°C with vigorous shaking, there is sometimes surfactant residue remaining on the wall of the flasks, and additional time is required. Although the surfactant film is thin, it has finite thickness and hydration initially occurs at the surface. This layer becomes quite viscous, and tends to remain adhered to the surface. As a result, full hydration of surfactant film is difficult, and the loss of some lipids in the preparation process is possible if one is not careful to agitate the flask for an adequate time.

Compared with conventional niosomes, the preparation of niosome dispersions from these proniosomes is much more convenient. Apparently due to the great surface area of the lipid film

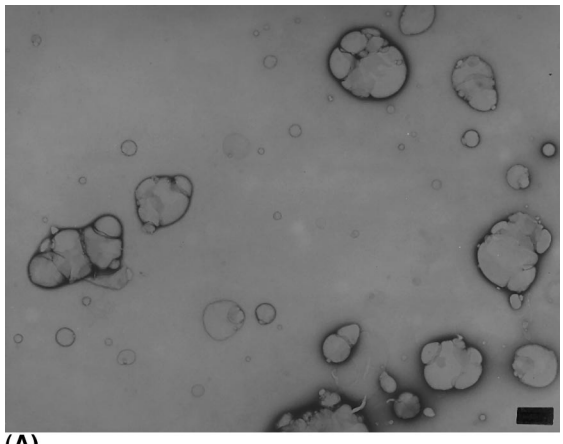


Fig. 2. Electron micrograph of niosomes. (A) Conventional niosomes (SP/CH/DCP = 60:35:5). (B) Proniosome-derived (SP/CH/DCP = 60:35:5). Scale bar indicates 10 μm.

that forms on the surface of sorbitol, the hydration of the proniosomes and the formation of the niosome dispersion is very easy. Because the surfactant is all coated on the soluble sorbitol, there is little risk of material loss.

3.4. Morphology of niosomes

Transmission electron microscopy was used to compare proniosome-derived niosome dispersions to those prepared by conventional hydration of dried film. (Fig. 2A) shows that niosomes formed by direct hydration are very heterogeneous, but (Fig. 2B) suggests that niosomes prepared from proniosomes are more uniform.

Particle size analysis of niosome preparations (Fig. 3) shows that the conventionally prepared niosomes are larger and slightly more heterogeneous than those derived from proniosomes. Although the size distributions are approximately the same, the average particle size of proniosome-derived niosomes is approximately 6 μm while that of conventional niosomes is about 14 μm. Altering the proportion of surfactant to sorbitol appears to have a minor effect over the limited range tested.

3.5. Effects of formulation on ibuprofen entrapment efficiency

With the total concentration of surfactants (Span 60, CH, DCP) kept constant, the ratio of Span 60 to cholesterol was changed to investigate the effect of this ratio on ibuprofen entrapment efficiency. With 5 mM DCP, ratios of Span 60:CH from 65:30 to 30:65 were tested, (Table 2) but there was little variation in entrapment efficiency over this range. At lower concentrations of DCP, 2.5 mM and 0 mM (data not shown, see footnote to Table 2), the entrapment efficiency is slightly lower, but the ratio of Span 60 to CH still has little effect. In all cases, the entrapment efficiencies are in the range of 85–97%. The high entrapment efficiency is probably due to the lipophilic character of ibuprofen.

With the concentration of Span 60 kept at a constant 60 mM, the molar ratio of cholesterol to DCP was changed (over a relatively narrow range, 40:0, 37.5:2.5, 35:5) as shown in Table 3. (The concentration of ibuprofen was kept at 10 mM.) Only a very small concentration range of DCP was tested because high DCP concentrations can inhibit formation of niosomes. At 55 mM Span 60 (data not shown) the entrapment efficiency of ibuprofen is not significantly affected over a comparable CH/DCP range. The entrapment efficiencies are about 85–96% under all of these conditions.

In one selected formulation (Span 60/CH/DCP = 47.5/47.5/5) the total concentration of surfactants was kept constant at 100 mM but the concentration of ibuprofen was changed to verify that the drug was not significantly altering the

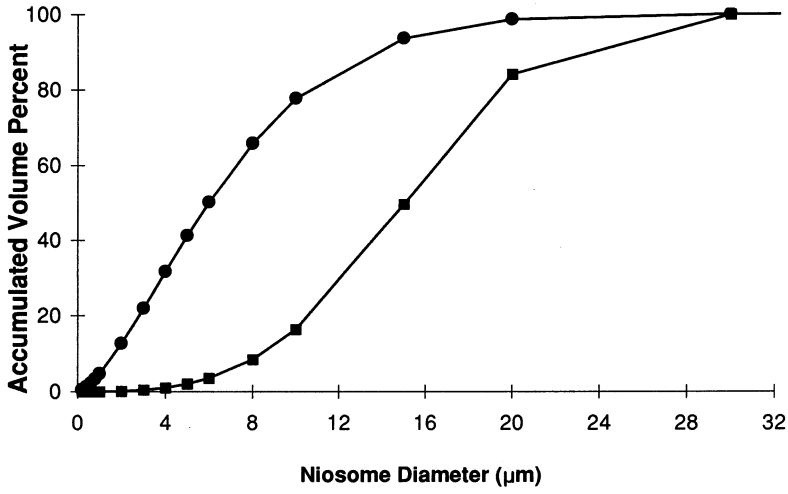


Fig. 3. Particle size analysis of niosomes: Particle size distribution of conventional niosomes (–●–) and proniosome-derived niosomes (–■–), presented as the accumulated volume percentage.

behavior of the niosome preparation. The entrapment efficiency of ibuprofen showed no significant difference, remaining at about 95%. Although the entrapment efficiency did not depend on the ibuprofen concentration, observations of niosome suspensions under an optical microscope showed that crystalline drug existed in the preparation when the concentration of ibuprofen was over 12 mM. Therefore, lower ibuprofen concentrations (10 mM) were used in the optimal formulation.

The concentration of sorbitol has no measurable effect on entrapment efficiency of ibuprofen, based on comparison of surfactant:sorbitol ratios from 1:11 to 1:22. The preparation of proniosomes is difficult when the concentration of sorbitol is lower than 1.1 M. Because only a very small volume of the solution of membrane-forming components can be introduced and sprayed onto the limited amount of sorbitol each time, the spraying-evaporating process becomes very time consuming. Because a higher sorbitol concentration did not improve the formulation, 1.1 M sorbitol was used for the formulation of proniosomes.

3.6. Release of ibuprofen from niosomes

The release of ibuprofen from niosomes in gastric fluid is much slower than from ibuprofen

Table 2
Effect of Span 60/CH ratio on ibuprofen entrapment efficiency[a]

Molar ratio span 60/CH	Entrapment efficiency (%)
65.0:30.0	93.9 ± 3.3
60.0:35.0	92.3 ± 3.0
55.0:40.0	92.9 ± 4.7
47.5:47.5	93.8 ± 3.5
45.0:50.0	93.9 ± 3.4
40.0:55.0	92.4 ± 7.0
30.0:65.0	90.8 ± 4.5

[a] Data obtained with 5% DCP. With 2.5% DCP the average entrapment efficiency was 93.2 ± 3.7, and with 0% DCP the average entrapment efficiency was 89.0 ± 5.0.

Table 3
Effect of CH/DCP ratio on ibuprofen entrapment efficiency[a]

Molar ratio CH/DCP	Entrapment efficiency (%)
40.0:0.0	91.9 ± 7.1
37.5:2.5	94.2 ± 1.5
35.0:5.0	92.3 ± 3.0
32.5:7.5	89.4 ± 7.5
30.0:10.0	91.3 ± 6.5

[a] All data were obtained with 60% Span 60. At 55% Span 60, the average entrapment efficiency was 90.6 ± 4.4.

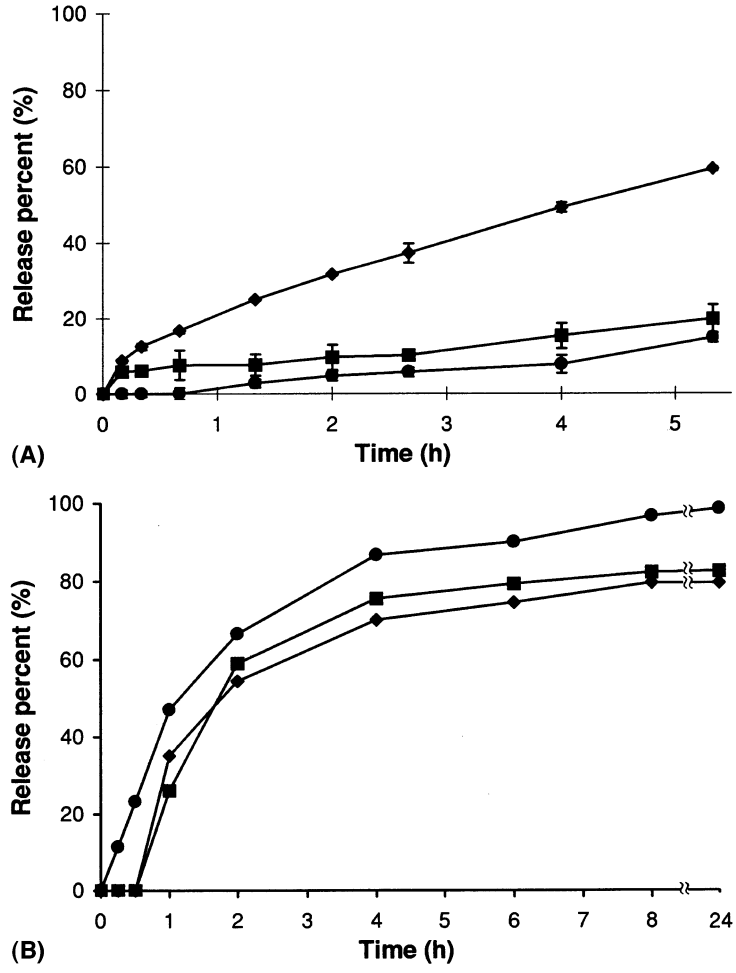


Fig. 4. Release of ibuprofen in simulated gastric fluid (A) and in simulated intestinal fluid (B) for ibuprofen solution (–◆–), conventional niosomes (–■–), proniosome-derived niosomes (–●–). All formulations used the surfactant composition SP/CH/DCP = 47.5/47.5/5.

solution (Fig. 4A). The ibuprofen solution represents equilibration of the dialysis system, and showed release of about 60% within 5 h. After 5 h, ibuprofen release from niosomes had only reached about 10–15% (although conventional niosomes with Span 60/CH/DCP/ibuprofen = 47.5/47.5/5/10 reached 20%). Overall, gastric fluid release profiles of ibuprofen from niosome dispersions prepared from proniosomes or by conventional methods show little difference.

In simulated intestinal fluid, the release profiles of ibuprofen from niosomes (Fig. 4B) are markedly different from those in gastric fluid. Within approximately 8 h, the ibuprofen release from various preparations reached equilibrium at levels of about 80–90% of the levels attained from aqueous ibuprofen solution. One proniosome preparation (Span 60/CH/DCP/ibuprofen = 60/35/5/10), appeared to exhibit slightly faster release, but most preparations, prepared from proniosomes or by conventional methods, showed little difference in the ibuprofen release profile in intestinal fluid.

Table 4
Effect of SP:CH ratio on CF entrapment efficiency[a]

Molar ratio SP/CH	Entrapment efficiency (%)
70:25	4.3 ± 0.4
60:35	4.9 ± 0.4
50:45	5.5 ± 1.1
47.5:47.5	7.0 ± 1.2
40:55	6.2 ± 2.3
30:65	5.1 ± 0.7

[a] All data were obtained at 5% DCP.

Table 5
Effect of DCP on CF entrapment efficiency

Molar ratio SP/CH/DCP	Entrapment efficiency %
50:50:0	4.3 ± 0.5
48.75:48.75:2.5	5.3 ± 0.5
47.50:47.50:5.00	7.0 ± 1.2
46.25:46.25:7.50	6.3 ± 0.9
45.00:45.00:10.00	6.4 ± 1.1

3.7. Effects of formulation on CF entrapment efficiency

For formulations in which the concentrations of other surfactants were kept constant, the molar ratio of Span 60 to cholesterol has a significant effect on the entrapment efficiency of CF. The results (Table 4) show that the ratio of surfactant to cholesterol is optimal when Span 60 and cholesterol are present in equimolar amounts. This is consistent with the results reported by other investigators working with conventional niosomes (Yoshioka et al., 1994). This molar ratio of Span 60 to cholesterol was used in all further studies.

Increasing the proportion of dicetyl phosphate in the 100 mM surfactant mixture, (Table 5) slightly increased the entrapment efficiency of CF over the concentration range of 0–5 mM, but had little effect on the entrapment efficiency of CF at levels over 5 mM. In proniosome formulations used for further studies, 5 mM dicetyl phosphate was used.

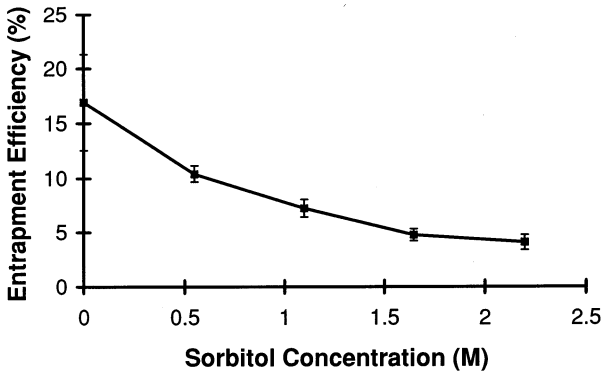


Fig. 5. Effect of sorbitol concentration on CF entrapment efficiency. The point at 0 represents entrapment in conventional niosome preparations. All formulations used the surfactant composition Span 60/CH/DCP = 47.5/47.5/5.

The entrapment efficiency of CF appeared to be lower in proniosome-derived niosomes than in conventional niosomes. This is apparently due to the effect of the sorbitol on the CF distribution. Fig. 5 shows that CF entrapment decreased with increasing proportions of sorbitol, approaching a value of 4% at 2M sorbitol. The origin of this effect is still uncertain, but conventional niosomes made with sorbitol-containing buffer had CF entrapment efficiency equivalent to that obtained with proniosome-derived niosomes. Efforts to use smaller amounts of sorbitol could increase the CF entrapment, but the preparation is more tedious because the surfactants must be added slowly. Compromising the considerations of the CF entrapment efficiency and the convenience of proniosomes preparation, a sorbitol concentration of 1.1 M was used for the studies reported here.

Using Span 60/CH/DCP = 47.5/47.5/5, CF-containing niosomes were prepared by conventional dry film methods and from proniosomes, such that the final concentrations of all surfactant components were identical in each formulation. The entrapment efficiency in niosomes formed by conventional methods (16.9 ± 4.4%) is much higher than that of proniosome-derived niosomes (7.2 ± 0.8%). This was apparently due only to the effect of the high sorbitol concentration, because if conventional niosomes were produced by rehydrating a film with CF in 20% sorbitol, the entrapment efficiency (6.8 ± 1.1%) is approximately the same as that determined for proniosome-derived niosomes.

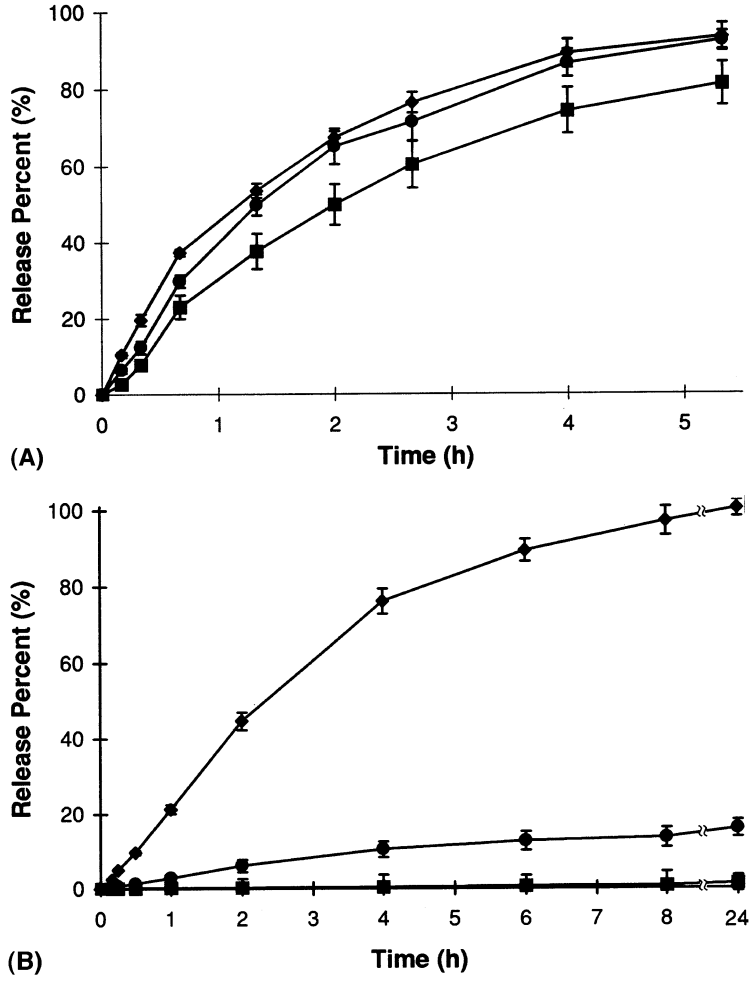


Fig. 6. Release of CF in simulated gastric fluid (A) and in simulated intestinal fluid (B) for CF solution (–◆–), conventional niosomes (–■–), proniosome-derived niosomes (–●–). All formulations used the surfactant composition SP/CH/DCP = 47.5/47.5/5.

3.8. Release of CF from niosomes

The release rate of CF from niosome preparations in simulated gastric fluid (Fig. 6A) is comparable to the control 'release rate' from free solution. After 5 h, about 80–95% of the CF is released from niosomes in simulated gastric fluid. Two possible explanations for the rapid release of CF from niosomes in gastric fluid include (a) the possibility that the unionized fraction of CF is higher at the pH of gastric fluid such that the penetration of CF through the niosomes is facilitated or (b) the structural integrity of the niosome is compromised at low pH in gastric fluid. In some experiments in simulated gastric fluid, aggregation of niosomes has been observed. 'Leakage' of hydrophobic or amphiphilic molecules would be less significant since this association is more likely to be partitioning rather than entrapment.

The release rate of CF from niosome preparations in intestinal fluid is significantly lower than that of the control solution (Fig. 6B). A negligible amount of CF is released from the conventional niosome preparation, and only about 15% of CF in the proniosome-based preparations is released,

but an apparent equilibrium is reached within 8–10 h.

4. Discussion

This project was designed to investigate the possibility of manufacturing proniosomes and using proniosome-based niosomes as drug carriers. The results reported here indicate that proniosomes are very promising as drug carriers. Compared to liposomes of natural or synthetic phospholipids, niosomes have the advantage that chemical degradation problems, such as oxidation and hydrolysis, may be largely alleviated. Compared to liposome or niosome suspensions, proniosomes represent a significant improvement by eliminating physical stability problems, such as aggregation or fusion of vesicles and leaking of entrapped drugs during long-term storage. Compared to niosomes prepared by conventional means, proniosome-derived niosomes are superior in their convenience of storage, transport, and dosing. The release data indicate that proniosome-derived niosomes are at least as effective as conventional niosomes in their release characteristics, and may therefore offer improved bioavailability of some drugs with poor solubility, controlled release formulations, or reduced adverse effects of some drugs.

Because proniosomes are a dry powder, further processing is possible. To provide convenient unit dosing, the proniosome powder may be processed to make beads, tablets, or capsules. Angle of repose measurements indicated that the fluidity of proniosome dry powder is equal to or better than that of sorbitol powder, so further processing of proniosome powder should be straightforward.

Although the original intent of our proniosome development was to provide an alternative drug delivery vehicle to liposomes, one of the greatest advances offered by proniosomes is their ease of use. The hydration of proniosome powder is much easier than the long shaking process required to hydrate surfactants in the conventional dry film method and can be implemented in a 'point-of-use' application. Proniosome derived niosome suspensions appear to be as good or better than conventional niosome preparations, and may be an appropriate preparation to use as a hydrophobic drug carrier.

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