Maltodextrin-Based Proniosomes

Submitted: August 1, 2000; Accepted: December 20, 2000; Published: January 5, 2001.

Almira I. Blazek-Welsh and David G. Rhodes

Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, CT

ABSTRACT Niosomes are nonionic surfactant vesicles that have potential applications in the delivery of hydrophobic or amphiphilic drugs. Our lab developed proniosomes, a dry formulation using a sorbitol carrier coated with nonionic surfactant, which can be used to produce niosomes within minutes by the addition of hot water followed by agitation. The sorbitol carrier in the original proniosomes was soluble in the solvent used to deposit surfactant, so preparation was tedious and the dissolved sorbitol interfered with the encapsulation of one model drug. A novel method is reported here for rapid preparation of proniosomes with a wide range of surfactant loading. A slurry method has been developed to produce proniosomes using maltodextrin as the carrier. The time required to produce proniosomes by this simple method is independent of the ratio of surfactant solution to carrier material and appears to be scalable. The flexibility of the proniosome preparation method would allow for the optimization of drug encapsulation in the final formulation based on the type and amount of maltodextrin. This formulation of proniosomes is a practical and simple method of producing niosomes at the point of use for drug delivery.

Key Words: Niosomes, Proniosomes, Maltodextrin, Slurry method

INTRODUCTION

Many active compounds have limited aqueous solubility, so there is great need for delivery systems suitable for hydrophobic and amphiphilic drugs. One approach to this problem has been to use lipid-based vesicles as drug carriers. Multilamellar liposomes can be used for hydrophobic or lipophilic drugs that can partition into the lipid phase and unilamellar vesicles can be used to entrap water-soluble drugs in the interior aqueous space. Liposomes are currently approved or are in late-stage clinical trials for delivery of many drugs, including amphotericin B (AmBisome), doxorubicin (Doxil, Evacet),

daunorubicin (DaunoXome), anthracycline (Annamycin), nystatin (Nyotran), and cytarabine (DepoCyt). Niosomes are similar systems in which nonionic surfactants are used instead of phospholipids. The history, properties, and applications of niosomes have been recently reviewed (1, 2). Niosomes have been investigated for drug delivery through the most common routes of administration, such as intramuscular (3), intravenous (4, 5), subcutaneous (6, 7), ocular (8), oral (9, 10), and transdermal (11, 12, 13). Niosomes appear to be multilamellar surfactant structures (14), and are thus best suited for hydrophobic or amphiphilic drugs. Relatively insoluble compounds, such as the chemotherapeutics currently formulated in liposomes, could be delivered in these synthetic, nonionic surfactant vehicles. For example, recent studies showed a comparable efficacy between niosome-encapsulated doxorubicin and doxorubicin in solution at twice the dose (15), and that niosome-encapsulated cytarabine hydrochloride exhibited significantly delayed release compared to drug in solution (16).

The traditional method for producing niosomes or liposomes involves drying the lipid to a thin film from organic solvent, and then hydrating this film with the aqueous solvent of choice (17). The resulting multilamellar vesicles can be further processed by sonication, extrusion, or other treatments to optimize drug entrapment (18). Other methods, such as injection of lipids in water-miscible or water-immiscible solvents into an aqueous solution, detergent dialysis, or reverse-phase evaporation are complicated by the need to remove certain components following liposome formation (18). All of these methods are time consuming, and many involve specialized equipment. The thin film approach allows only for a predetermined lot size so material is often wasted if smaller quantities are required for a particular application or dose.

Corresponding Author: David G. Rhodes, Ph.D., Department of Pharmaceutical Sciences, School of Pharmacy, University of Connecticut, Storrs, CT 06269-2092; Telephone: 860-486-5413; Facsimile: 860-486-4998; E-mail: d.g.rhodes@uconn.edu

Proniosomes (19) (and proliposomes (20)) circumvent all of these complications. These are dry formulations of surfactant-coated carrier, which can be measured out as needed and rehydrated by brief agitation in hot water. Proniosomes (and proliposomes) are normally made by spraying surfactant in organic solvent onto sorbitol powder and then evaporating the solvent. Because the sorbitol carrier is soluble in the organic solvent, it is necessary to repeat the process until the desired surfactant loading has been achieved. The surfactant coating on the carrier is very thin and hydration of this coating allows multilamellar vesicles to form as the carrier dissolves (14, 17). The resulting niosomes are very similar to those produced by conventional methods and the size distribution is more uniform. It was suggested that this formulation could provide a suitable method for formulating hydrophobic drugs in a lipid suspension without concerns over instability of the suspension or susceptibility of the active ingredient to hydrolysis (19).

It was also observed, however, that certain solutes were affected by the presence of dissolved carrier. Although conventional niosome suspensions would include only the multilamellar surfactant particles in buffer, a typical formulation derived from sorbitol-based proniosomes would consist of multilamellar surfactant particles in buffer and dissolved sorbitol. The residual sorbitol concentration in the formulation reported previously (19) was 1.1 mol/L, and was observed to affect entrapment efficiency. Specifically, dissolved sorbitol could decrease entrapment efficiency to less than one half of that observed without sorbitol (19). Because the dissolved carrier can exert such an influence on entrapment behavior, one of the primary goals of subsequent research was to reduce the proportion of carrier to surfactant so that the amount of carrier in the final niosome suspension would be minimal.

Although not an issue in the process of making niosomes from proniosomes, it was difficult to coat sorbitol particles because sorbitol is soluble in chloroform and other organic solvents. If the surfactant solution was applied too quickly, the sorbitol particles would degrade and the sample became a viscous slurry. To avoid this constraint, several methods of making proniosomes were

attempted, but most proved to be time consuming and had narrowly constrained limits on acceptable production conditions. In this article, experiments are described in which maltodextrin was evaluated as an alternative to sorbitol as the carrier material in the proniosome preparations.

MATERIALS AND METHODS

Two morphological forms of maltodextrin, Maltrin QD M500 and Maltrin M700, were donated by Grain Processing Corporation (Muscatine, IA). Alprenolol hydrochloride, Span 60, and dicetylphosphate were purchased from Sigma (St Louis, MO). Cholesterol and chloroform (high-performance liquid chromatography [HPLC] grade) were purchased from Fisher (Pittsburgh, PA). All materials were used without further purification.

Proniosome Preparation

In previous work with proniosomes (16), optimized proportions of Span 60, cholesterol, and dicetylphosphate were determined (molar ratio 47.5:47.5:5, respectively), and these proportions were used in this work. A stock solution of surfactants in chloroform was prepared with 164 mmol/L Span 60, 164 mmol/L cholesterol, and 17.2 mmol/L dicetylphosphate. In the "1x" surfactant loading preparations (0.5 mmol/g), 1.45 mL of surfactant stock, containing 0.2375 mmol Span 60, 0.2375 mmol cholesterol and 0.025 mmol DCP, were added to 1 g of carrier material. Other surfactant loads were produced by the addition of proportional volumes of surfactant stock to the carrier material using the method described below. All water used in this work was distilled and then deionized using a Barnstead Nanopure filtration system (Dubuque, IA).

Proniosomes with maltodextrin as the carrier were prepared by a slurry method in contrast to the slow spray-coating method used with sorbitol (19). A rotary evaporator (Büchi Rotovapor-R, Flawil, Switzerland) was used to evaporate solvent with the rotation speed set at ~60 RPM. Vacuum was generated by a mechanical vacuum pump (Duo-Seal, Sargent-Welch Scientific Co, Skokie, IL), the proniosome sample temperature maintained at 25°C by a water bath, and chloroform collected in a liquid nitrogen solvent trap.

For the slurry method (1x), 10 g of maltodextrin powder was added to a 250-mL round-bottom flask

and the entire volume of surfactant solution (14.5 mL) was added directly to the flask. (Additional chloroform, ~30 mL, was added to preparations with low surfactant content to provide enough solvent to form a slurry.) The flask was attached to the rotary evaporator and vacuum was applied until the powder appeared to be dry and free flowing. The flask was removed from the evaporator and kept under vacuum overnight. Proniosome powder was stored in sealed containers at 4°C.

Scanning Electron Microscopy (SEM)

Proniosome powders were affixed to double-sided carbon tape, positioned on an aluminum stub, and excess powder removed. The stubs were stored under vacuum overnight. The samples were sputter-coated with Au/Pd under an argon atmosphere at 180 mA for 1 minute (Polaron E5100; VG MicroTech, West Sussex, UK). Electron micrographs were obtained using a field-emission SEM operating at 1 or 2 kV (Zeiss DSM 982 Gemini; LEO Electron Microscopy Ltd, Cambridge, UK).

Niosome Preparation and Encapsulation Measurement

Proniosome powder (prepared as described above) was weighed into screw cap vials. Water at 80°C was added and the vials capped. The vials were attached to a vortex mixer (Vortex Genie2, VWR Scientific, Bohemia, NY) and agitated at the maximum setting for 2 minutes. Niosome suspensions were prepared such that the total surfactant concentration was the same. This required hydration of different amounts of proniosomes depending on the surfactant loading as outlined in Table 1.

Table 1. Relative Proportions of Carrier and Surfactant Components Span 60 (SP60), Cholesterol (CH), and Dicetylphosphate (DCP), in Proniosomes with Different Surfactant Loading

Surfactant Loading	Carrier % w/w	SP60 % w/w	CH % w/w	DCP % w/w	Proniosome per mM surfactant, mg/mL	Carrier per mM surfactant, mg/mL
1×	82.8	8.5	7.6	1.1	2.41	2.0
4×	54.6	22.4	20.1	3.0	0.914	0.50
8×	37.5	30.8	27.6	4.1	0.665	0.25
16×	23.1	37.9	34.0	5.0	0.540	0.12
32×	13.1	42.8	38.4	5.7	0.478	0.063
64×	7.0	45.8	41.1	6.1	0.447	0.031
128×	3.6	47.5	42.6	6.3	0.431	0.016
surf	0	49.3	44.2	6.5	0.414	0

Measurement of partitioning (entrapment)

The fraction of drug associated with the niosomes was measured using a centrifugation assay. Niosomes with 100 mM surfactant were made, as described above, from proniosomes of the desired formulation by adding hot drug solution (alprenolol-HCl, 1 mM or 10 mM in pH 7.4 HEPES buffer, 80°C). The preparation was cooled to room temperature (20°C) and allowed to equilibrate for 30 minutes. Aliquots (4 × 1.5 mL) of the niosome suspension were centrifuged in polypropylene microcentrifuge tubes at 30,000 xg for 15 minutes, using a Beckman J2-21M centrifuge with a JA-18.1 rotor (Beckman-Coulter, Fullerton, CA). Depending on the surfactant loading, centrifugation produced either a floating cake, a soft pellet, or both. The supernatant was generally cloudy except for the 1x proniosome preparation. The supernatants for each niosome preparation and the stock solution were diluted 1:4 with buffer and centrifuged again to ensure complete removal of niosomes. The supernatants from the second centrifugation step were analyzed by HPLC using a Waters 717 plus autosampler, a Thermal Separation Products (TSP) Membrane Degasser, a TSP Constametric 4100 pump, and a HP Hypersil ODS column (5 μm, 4.0 × 25 mm). The mobile phase was 1:1 acetonitrile: 25 mmol/L potassium phosphate, pH 3.0. Drug concentration was measured by absorbance at 220 nm, using a TSP Spectromonitor 3200. Peak areas from 3 injections from 2 different samples were used to obtain averaged data. Drug concentrations measured in the supernatant were used directly as aqueous phase concentrations. Control samples without niosomes provided the total drug concentration, and the difference (control-aqueous) provided the amount of drug partitioned into niosomes. Standard deviation was determined by replicate measurements using samples from 2 different lots of proniosomes.

Particle Sizing

Niosome suspensions were prepared using a variety of surfactant concentrations, as described above. The niosome suspension was mixed by repeated inversion before sampling to assure uniformity. A light-blockage particle sizer (Accusizer 770, Particle Sizing Systems, Santa Barbara, CA) was prepared by

flushing the system with Barnstead nanopure water until the particle count was below 100. A small volume of the suspension, up to 1 mL, was delivered to the 50-mL reservoir and counts accumulated for 30 seconds. Number-weighted particle size distributions were obtained.

RESULTS

Two types of maltodextrin carriers were used, Maltrin M500 and Maltrin M700. Both are very soluble in water but insoluble in a number of organic solvents including chloroform. Figure 1 shows SEM images for the uncoated particles, which were used to determine average particle sizes. Although chemically similar, the two maltodextrins have significantly different morphology. Maltrin M500 is composed of solid particles with average diameters of approximately 200 μm as determined from SEM. In contrast, Maltrin M700 is composed of hollow particles with average diameters from SEM of approximately 250 μm . The manufacturer's sieving measurements, averaged over many lots, indicate that the particle sizes of both M500 and M700 are primarily in the 300-800 μm range. Some particles of the M700 were broken in handling, and from these broken pieces the average thickness of the shells was estimated to be 2.1 μm ($\pm 1.2 \mu\text{m}$). (This average may not be representative of the mean thickness of intact M700 particles because thinner shells may be more fragile and bias the average.) Electron micrographs of proniosomes (1x) prepared from the maltodextrins are also shown in Figure 1. The M700 proniosomes appear very similar to the original material. However, the process of coating the M500 maltodextrin with surfactant leads to clumping and larger particles.

To evaluate the effects of minimizing the amount of carrier, a series of samples was made with different ratios of surfactant to maltodextrin carrier material. The smallest surfactant loading (referred to as 1x) included 0.5 mmoles of surfactant per gram of carrier. Ratios as high as 128x (64 mmoles surfactant per gram of carrier) were tested. The relative amounts of carrier and surfactant for each surfactant loading are given in Table 1. The relative weights of proniosomes and the concentration of carrier material in the final niosome suspension are also given. These amounts are the same for each type of carrier as the proniosomes were prepared by weight of carrier.

Figure 1. Electron micrographs of maltodextrins M500 (a) and M700 (b). The diameters of the uncoated particles are about 200 μm and 250 μm , respectively. Electron micrographs of 1 x surfactant-loaded proniosomes made with M500 (c) and M700 (d) show that properties of the proniosome particles are similar to those of the uncoated maltodextrin although the particles in (c) are significantly larger than in (a).

Figure 2. Electron micrographs of Maltrin M700 and M700-proniosomes prepared with different surfactant loading: (a) M700, (b) 1', (c) 4', (d) 8', (e) 16', (f) 32', (g) 128'. The hollow morphology of the original powder is maintained.

As the surfactant load increased (Figure 2), the observed thickness of the coating on the hollow M700 particles increased. The electron micrographs show that the hollow morphology of M700 was maintained during the preparation of proniosomes. At loads greater than 32x, the surface became quite rough. Under high loading conditions, the

rehydration process became less efficient and large (mm) pieces of aggregated surfactant could be seen in the vials (21). Electron micrographs of proniosomes made with M500 maltodextrin (not shown) showed that a similar increase in roughness of the coating occurred, but at lower surfactant loading due to the lower surface area of M500.

The mean diameter of niosomes produced from maltodextrin-based proniosomes was measured using a laser light scattering method similar to that described previously for sorbitol-based proniosomes (19). The diameter of niosomes from maltodextrin-based proniosomes was a distribution centered at 4-5 μm with a width at half maximum of 7-8 μm . Figure 3 shows that the particle size distributions varied little with carrier type, surfactant loading, or final concentration of surfactant in the niosome suspension. However, at loads greater than 32x, the number of niosomes in the suspension decreased because of inefficient hydration of the proniosomes (21).

Drug "entrapment" was measured by a centrifugation assay, in which the untrapped drug was assayed by HPLC and the "entrapped" drug determined by difference. For 1 mmol/L alprenolol and 100 mmol/L surfactant, $89\% \pm 2\%$ of the drug was associated with the niosomes for all proniosome surfactant loadings except 1x. For niosomes derived from 1x proniosomes, only $72\% \pm 4\%$ of the drug was associated with the niosomes. At higher drug concentrations, a lower proportion of the drug was associated with the niosomes. For 10 mmol/L alprenolol, only $54\% \pm 5\%$ of the drug was niosome-associated.

DISCUSSION

The objective in developing proniosomes was to devise a method of producing a nonionic surfactant-based dosage at the point of use to avoid problems of physical and chemical stability found in storage of some surfactant-based dosage forms. By creating a dry formulation, issues related to hydrolysis of the active ingredient or surfactants are avoided; by forming the suspension as needed, precipitation and aggregation are avoided. Although the sorbitol-based proniosomes (19) accomplished these objectives, the effect of the carrier on entrapment efficiency remained problematic. Significant concentrations of

Figure 3 Size distributions of niosome suspensions obtained from the hydration of the indicated proniosomes. The average size of niosomes derived from 1x proniosomes (a) was similar for both M500 and M700 proniosomes at a wide range of surfactant concentration. The particle size distribution showed little variation with surfactant loading (b).

sorbitol altered the distribution of one model compound (19). However, making proniosomes with a reduced amount of sorbitol was a tedious process and began to compromise the advantages of proniosomes related to minimizing film thickness. The use of maltodextrin as the carrier in the proniosome preparation permitted flexibility in the amounts of surfactant and other components, which greatly enhances the potential application of proniosomes in a scaled-up production environment.

Although maltodextrin is a polysaccharide, it has minimal solubility in organic solvents tested here. Thus, it was possible to coat the maltodextrin particles by simply adding surfactant in organic solvent to dry maltodextrin and evaporating the solvent. An analogous process with sorbitol results in a solid, surfactant/sorbitol cake. Because the

maltodextrin particle morphology is preserved (Figure 2), hollow blown maltodextrin particles can be used for a significant gain in surface area. The higher surface area results in a thinner surfactant coating, which makes the rehydration process more efficient (17). For drugs where maltodextrin is found to affect encapsulation efficiency, the maltodextrin can be minimized by producing proniosomes with greater surfactant loading. The maximum loading that still permits efficient hydration of the surfactant coating is 3.3 grams surfactant per gram of carrier or 8 mmol/L surfactant per gram of carrier (21). Nevertheless, efficient encapsulation of amphiphilic drug into the niosomes was achieved for a wide range of surfactant loadings (4x to 128x). This result suggests that this approach provides considerable latitude in developing specific formulations where other considerations may be important.

For amphiphilic or hydrophobic drugs, in liposome or niosome formulations, it is more appropriate to describe association of drug with the lipid microparticles as "partitioning" rather than "entrapment" of the drug. For unilamellar vesicles with large aqueous interior volumes, water-soluble drugs can be entrapped if their permeability across the bilayer constituting the vesicle wall is limited. For multilamellar structures like the niosomes in this study, most of the hydrated microparticle is surfactant. In these structures, hydrophobic drugs can partition to the hydrophobic interior of a bilayer and amphiphilic molecules can partition to the interfacial environment on either the inner or outer leaflet of the bilayer.

The extent to which drugs partition to such interfaces is not necessarily correlated to their bulk-phase partitioning behavior (22). Because the drugs are not homogeneously distributed throughout the membrane volume, partitioning between aqueous phases and vesicles should not be expressed in terms of volume-based units such as molarity. Moreover, for vesicles of heterogeneous composition, units standardized to moles of surfactant (e.g., mole fraction) unreasonably equate the contributions of all constituents, and the molar composition of natural membranes may not be well known. Thus, a mass-based measure of drug lipophilicity was adopted (23), defining a membrane-based partition coefficient ($K_{p(mem)}$). By analogy, a

surfactant-based partition coefficient ($K_{p(s)}$) can now be defined as:

$$K_{p(s)} = \frac{g(\text{drug}) / g(\text{surfactant})}{g(\text{drug}) / g(\text{water})}.$$

Measured $K_{p(s)}$ for alprenolol (1 mmol/L) partitioning into niosomes from maltodextrin-based proniosomes was 202 so it appears that these niosomes can be used as a carrier for amphiphilic drugs. At higher concentrations of alprenolol (10 mmol/L), the measured $K_{p(s)}$ was 30.6 so this concentration has begun to approach the capacity limit for the surfactant bilayers. Bulk phase measurements of alprenolol partitioning in octanol/buffer (HEPES pH 7.4) were comparable to the previously reported $K_{o/w}$ of 12.0 (24). It has been reported that retention times from HPLC using "immobilized artificial membrane" (IAM) columns provide a better indication of membrane-partitioning behavior than bulk phase measurements or reverse phase (C18) retention times. Although these measurements should better mimic the interfacial environment than would a bulk solvent phase, IAM data for alprenolol indicated $K_P = 31.6$ (25). This value is comparable to that obtained for 10 mmol/L alprenolol, but significantly smaller than that obtained for 1 mmol/L alprenolol.

The ability of a lamellar surfactant phase to accommodate an amphiphilic ligand depends on the structure of the surfactant phase. Drugs partition less easily into highly ordered systems such as phospholipid membranes with added cholesterol (22). Similarly, the presence of drug in the lamellar surfactant phase can affect its structure and dynamics, and thus its ability to accommodate more drug. In other words, the capacity of a lamellar surfactant phase is limited and nonlinear. These effects may well explain the measured differences in alprenolol partitioning into niosomes. Because of the amphipathic structure of alprenolol, it should partition more easily into an interfacial phase than a bulk phase of either high or low polarity. Because the IAM column is composed of saturated phosphocholine residues, it may be expected to have more order than the surfactant mixture in our niosomes. Most relevant to this work is the fact that the niosomes from maltodextrin-based proniosomes can act as effective carriers for amphiphilic drugs.

CONCLUSIONS

Maltodextrin-based proniosomes are a potentially scalable method for producing niosomes for delivery of hydrophobic or amphiphilic drugs. The method is simple and overcomes several problems encountered in previous studies. The niosomes produced using this method are effective carriers for amphiphilic drugs.

ACKNOWLEDGEMENTS

The authors thank Dr. Marie Cantino and Jim Romanow, University of Connecticut, for their assistance with the scanning electron microscopy and Dr. Diane Burgess, University of Connecticut, for the use of the particle-sizing instrument. This work was supported by NIH grant GM55973 (DGR).

REFERENCES

1. Uchegbu IF, Vyas SP. Non-ionic surfactant based vesicles (niosomes) in drug delivery. *Int J Pharm.* 1998;172:33-70.
2. Uchegbu IF, ed. Synthetic Surfactant Vesicles: Niosomes and Other Non-Phospholipid Vesicular Systems, Drug Targeting and Delivery, Volume 11. Amsterdam: Harwood Academic Publishers; 2000.
3. Arunothayanun P, Turton JA, Uchegbu IF, Florence AT. Preparation and *in vitro/in vivo* evaluation of luteinizing hormone releasing hormone (LHRH)-loaded polyhedral and spherical/tubular niosomes. *J Pharm Sci.* 1999;88(1):34-38.
4. Pillai GK, Salim MLD. Enhanced inhibition of platelet aggregation *in-vitro* by niosome-encapsulated indomethacin. *Int J Pharm.* 1999;193:123-127.
5. Namdeo A, Jain NK. Niosomal delivery of 5-fluorouracil. *J Microencapsulation.* 1999;16(6):731-740.
6. Oommen E, Tiwari SB, Udupa N, Kamath R, Devi PU. Niosome entrapped B-cyclodextrin methotrexate complex as a drug delivery system. *Ind J Pharmacol.* 1999;31:279-284.
7. Medda S, Mukhopadhyay S, Basu MK. Evaluation of the *in-vivo* activity and toxicity of amarogentin, an antileishmanial agent, in both liposomal and niosomal forms. *J Antimicrob Chemo.* 1999;44:791-794.
8. Saettone MF, Perini G, Carafa M, Santucci E, Alhaique F. Non-ionic surfactant vesicles as ophthalmic carriers for cyclopentolate: a preliminary evaluation. *STP Pharma Sciences.* 1996;6(1):94-98.
9. Rentel C-O, Bouwstra JA, Naisbett B, Junginger HE. Niosomes as a novel peroral vaccine delivery system. *Int J Pharm.* 1999;186:161-167.
10. Sihorkar V, Vyas SP. Polysaccharide coated niosomes for oral drug delivery: formulation and *in vitro* stability studies. *Pharmazie.* 2000;55(2):107-113.
11. Schreier H, Bouwstra J. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. *J Controlled Release.* 1994;30:1-15.
12. Waranuch N, Ramachandran C, Weiner ND. Effect of lipid composition on topical delivery of cyclosporin-A from nonionic liposomal formulations: an *in vitro* study with hairless mouse skin. *J Liposome Res.* 1997;7(2&3):261-271.
13. Sentjurs M, Vrhovnik K, Kristl J. Liposomes as a topical delivery system: the role of size on transport studied by the EPR imaging method. *J Controlled Release.* 1999;59:87-97.
14. Yoshioka T, Sternberg B, Florence AT. Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60, and 80) and a sorbitan triester (Span 85). *Int J Pharm.* 1994;105:1-6.
15. Uchegbu IF, Double JA, Turton JA, Florence AT. Distribution, metabolism and tumoricidal activity of doxorubicin administered in sorbitan monostearate (Span 60) niosomes in the mouse. *Pharm Res.* 1995;12(7):1019-1024.
16. Ruckmani K, Jayakar B, Ghosal SK. Nonionic surfactant vesicles (niosomes) of cytarabine hydrochloride for effective treatment of leukemias: encapsulation, storage, and *in vitro* release. *Drug Dev Ind Pharm.* 2000;26(2):217-222.
17. Bangham AD, Standish MM, Watkins JC. Diffusion of univalent ions across the lamellae of swollen phospholipids. *J Mol Biol.* 1965;13:238-252.
18. Van Winden ECA, Zuidam NJ, Crommelin DJA. Strategies for large scale production and optimized stability of pharmaceutical liposomes developed for parenteral use. In: Lasic DD, Papahadjopoulos D, eds. Medical Applications of Liposomes. Amsterdam: Elsevier; 1998:567-604.
19. Hu C, Rhodes DG. Proniosomes: a novel drug carrier preparation. *Int J Pharm.* 1999;185(1):23-35.
20. Payne NI, Timmins P, Ambrose CV, Ward MD, Ridgway F. Proliposomes: A Novel solution to an old problem. *J Pharm Sci.* 1986;75(4):325-329.
21. Blazek-Welsh AI, Rhodes DG. SEM imaging predicts quality of niosomes from maltodextrin-based proniosomes. Submitted to *Pharmaceutical Research*.
22. Mason RP, Rhodes DG, Herbet LG. Reevaluating equilibrium and kinetic binding parameters for lipophilic drugs based on a structural model for drug interaction with biological membranes. *J Med Chem.* 1991;34:869-877.
23. Rhodes DG, Newton R, Butler R, Herbet L. Equilibrium and kinetic studies of the interactions of salmeterol with membrane bilayers. *Molecular Pharmacology.* 1992;42: 596-602.
24. Barbato F, Caliendo G, La Rotonda MI, Morrica P, Silipo C, Vittoria A. Relationships between octanol-water partition data, chromatographic indices and their dependence on pH in a set of beta-adrenoceptor blocking agents. *II Farmaco.* 1990;45(6):647-663.
25. Pidgeon C, Ong S, Liu H, Qiu X, Pidgeon M, Dantzig AH, Munroe J, Hornback WJ, Kasher JS, Glunz L, Szczerba T. IAM Chromatography: an *in vitro* screen for predicting drug membrane permeability. *J Med Chem.* 1995;38(4):590-594.