SEM Imaging Predicts Quality of Niosomes from Maltodextrin-Based Proniosomes

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Purpose. The limits to surfactant loading of proniosomes were determined and a rationale developed for the observed relationship between the composition of proniosomes and the quality of reconstituted niosome suspension.

Methods. A novel method for producing proniosomes with a maltodextrin carrier was recently developed, which provides for rapid reconstitution of niosomes with minimal residual carrier. A slurry of maltodextrin and surfactant was dried to form a free-flowing powder which could be rehydrated by addition of warm water. This method provided facile production of a wide range of proniosome compositions, and thus, allowed us to examine rehydration behavior for similar concentrations of surfactant over a wide range of film thickness. SEM images of proniosomes with various degrees of surfactant loading and images of pure surfactant were compared. Direct observation and particle size measurements by laser light scattering provided characterization of the final niosome preparations.

Results. Successful rehydration of surfactant to produce niosomes from dried film requires that the film be as thin as possible to avoid the clumping and precipitation that occurs when pure, granular surfactant is hydrated directly. The appearance of a coarse, broken surface on the proniosomes correlates with inefficient rehydration and occurrence of aggregation and precipitate in the final niosome suspension.

Conclusions. These observations provide an indication of the requirements for dry proniosomes to yield niosome suspensions of high quality.

KEY WORDS: niosomes; proniosomes; scanning electron microscopy; maltodextrin; hydration efficiency.

INTRODUCTION

Niosomes are vesicle systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs (1–2). Niosomes have been investigated as delivery vehicles for a wide range of systems and have recently been the subject of several reviews (3–4). In niosomes, the vesicle forming amphiphile is a nonionic surfactant such as Span 60 which is usually stabilized by addition of cholesterol and small amounts of an ionic surfactant such as dicetylphosphate.

Simple addition of aqueous phase to a dry powder of the nonionic surfactant is an inefficient and irreproducible method of making hydrated niosomes. The material tends to clump together and extensive agitation or other treatment is required to disperse the aggregates into a niosome suspension. Conventional production of niosomes (1–4), derived from the traditional method of liposome preparation (5), involves dissolving surfactant in organic solvent and then shell-drying the solution to form a thin film of surfactant on the inner surface of a flask. The film can then be rehydrated by adding aqueous phase at a temperature above the main phase transition temperature (T_m) and agitating for an extended period of time. Evidence suggests that the rehydration process involves swelling of exposed layers of surfactant which are then sheared off by agitation to form multilamellar vesicles (6).

Another method of producing niosomes is to coat a water-soluble carrier such as sorbitol with surfactant (7). The result of the coating process is a dry formulation (Fig. 1) in which each water-soluble particle is covered with a thin film of dry surfactant. This preparation is termed "proniosomes". The niosomes are reconstituted by the addition of aqueous phase at $T > T_m$ and brief agitation. For typical quantities of carrier and standard glassware, the surface area of the carrier is significantly greater than that of the macroscopic container. One would therefore assume that the dried film would be thinner on the carrier powder than in a container, and may hydrate more easily.

An alternative explanation for efficient niosome formation from proniosomes is based on the dissolution of the carrier to facilitate hydration of the surfactant. A published description of the hydration process of similarly prepared proliposomes (8) shows budding of liposomes from the proliposome surface (9). However, the study did not specifically address the question of whether efficient vesicle formation results because of the enhanced surface area producing very thin films or because the carrier dissolves from beneath the surfactant layer.

A proniosome formulation based on maltodextrin was recently developed that has potential applications in delivery of hydrophobic or amphiphilic drugs (10). The better of these formulations used a hollow particle with exceptionally high surface area. The principal advantage with this formulation was the amount of carrier required to support the surfactant could be easily adjusted, and proniosomes with very high mass ratios of surfactant to carrier could be prepared. Because of the ease of production of proniosomes using the maltodextrin-based slurry method, hydration of surfactant from proniosomes of a wide range of compositions can be studied. In the work reported here, scanning electron microscopy was used to examine the surface characteristics of the maltodextrin-based proniosomes at a variety of surfactant loadings, and related to the quality of the hydrated niosome preparations.

MATERIALS AND METHODS

Two forms of maltodextrin, Maltrin QD M500 and Maltrin M700, were donated by Grain Processing Corporation (Muscatine, IA). Span 60 and dicetylphosphate were purchased from Sigma (St. Louis, MO). Cholesterol and chloroform (HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA). All materials were used as supplied, without further purification.

Proniosome Preparation

Proportions of Span 60, dicetylphosphate, and cholesterol were optimized in previous work with proniosomes (7).

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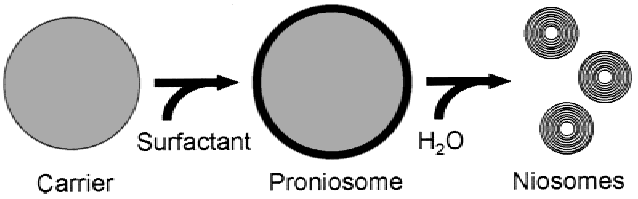

Fig. 1. Formation of niosomes from proniosomes. The proniosome consists of a water-soluble carrier (stippled) with a thin coating of surfactant. The interior of the carrier can be solid such as maltodextrin M500 or hollow such as maltodextrin M700.

These proportions (molar ratio 47.5 : 47.5 : 5, respectively) were used in this work. A stock solution of surfactants was prepared in chloroform with 164 mM Span 60, 164 mM cholesterol, and 17.2 mM dicetylphosphate. Surfactant loading of 0.5 mmol (surfactant) / g (carrier) was referred to as a "1×" preparation, and was made using 1.45 mL of surfactant stock (containing 0.2375 mmol Span 60, 0.2375 mmol cholesterol, and 0.025 mmol dicetylphosphate) 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 a slurry method, described previously (10). Briefly, a weighed sample of maltodextrin powder was added to a round bottom flask. A volume of surfactant solution required to attain the desired surfactant loading was added to the powder, and the flask was attached to a rotary evaporator with a liquid nitrogen cold trap. While rotating at ~60 RPM, vacuum was applied until the powder (proniosomes) appeared to be dry and free flowing. The sample was removed and kept under vacuum overnight to remove residual solvent. 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 using a Polaron E5100 (Au/Pd, Ar atmosphere, 180 mA, 1 min). SEM images were obtained using a Zeiss DSM 982 Gemini (2 kV).

Niosome Preparation and Characterization

The preparation of niosomes was similar to that described previously (10). Proniosome powder (prepared as described above) was weighed into screw cap vials. Deionized water at 80°C was added to yield a final surfactant concentration of 10 mM. The vials were agitated (four at a time) using a vortex mixer for the desired time, normally 30 s. Niosome suspensions in glass vials were photographed by illuminating horizontal vials from the bottom of the vial. A digital camera (Sony MVC-FD7) was used in "fine" resolution

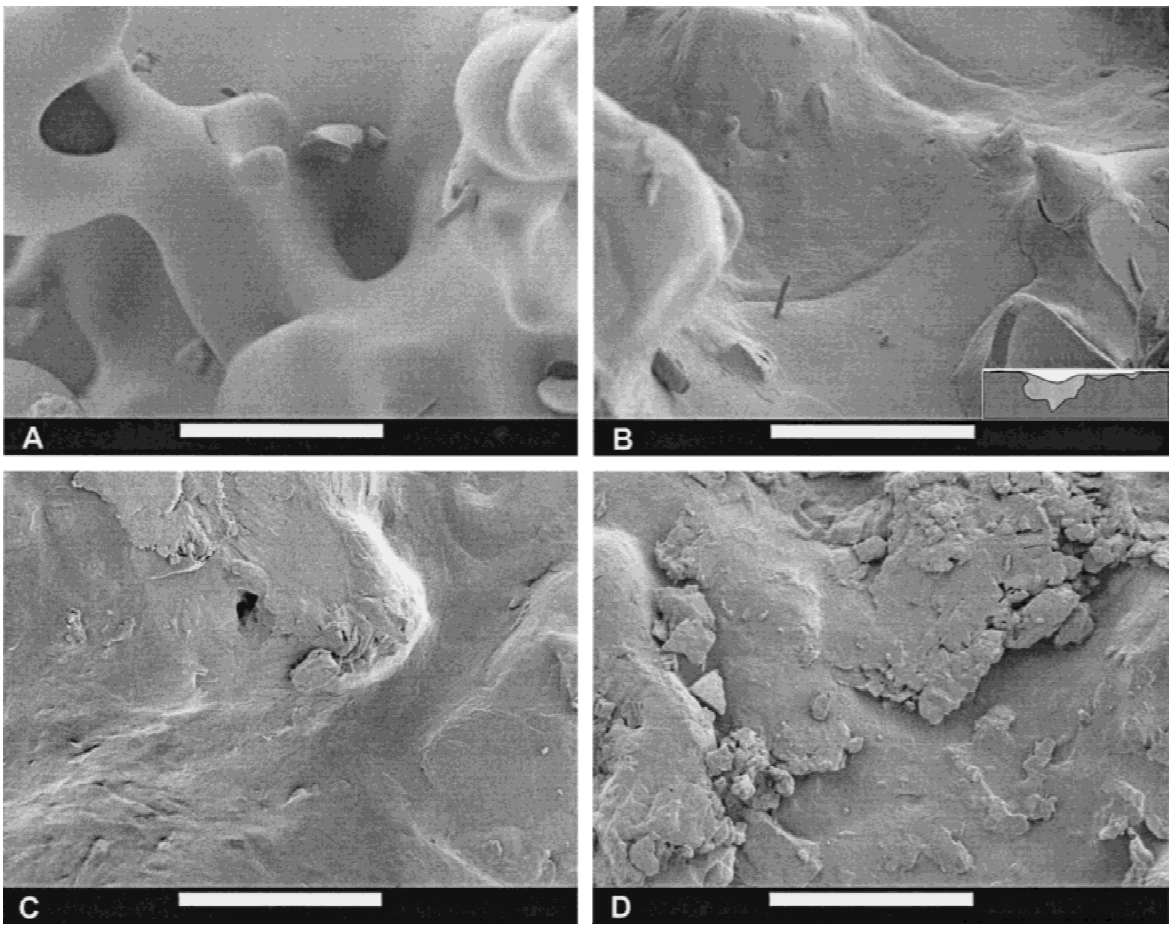

Fig. 2. Surface structure of maltodextrin M500 (a) and M500-based proniosomes (b) made at 1× surfactant loading. The inset shows a possible explanation for the apparent change in surface texture, as described in the text. Surface micrographs are also shown for 3× (c) and 8× (d). Scale bar represents 20 μm.

mode. Photographs were taken approximately 5 min following niosome preparation. Niosomes were characterized by particle size measurement using an Accusizer, model 770 (Particle Sizing Systems, Santa Barbara, CA), within 1 h of preparation. The 50 ml mixing reservoir was filled with water, and 50 μl of niosome suspension added using a wide orifice pipettor tip so as not to exclude large particles. The particle sizer has a size range of 0.5 to 400 μm. Data were smoothed with a Savitzky-Golay algorithm (5.5%) using Peakfit 4.0 (SPSS, Chicago, IL).

Optical Microscopy

Niosome suspensions were made from proniosomes of various surfactant loadings or from granular surfactant, as described above. A drop of the suspension was placed on a glass microscope slide, and the slide positioned on an Olympus IMT2 inverted-stage microscope. Photographs were taken using an Olympus OM1 and Kodak Ektachrome film (200 ASA). To assure random selection of areas to be photographed, the slide was positioned by viewing the top of the

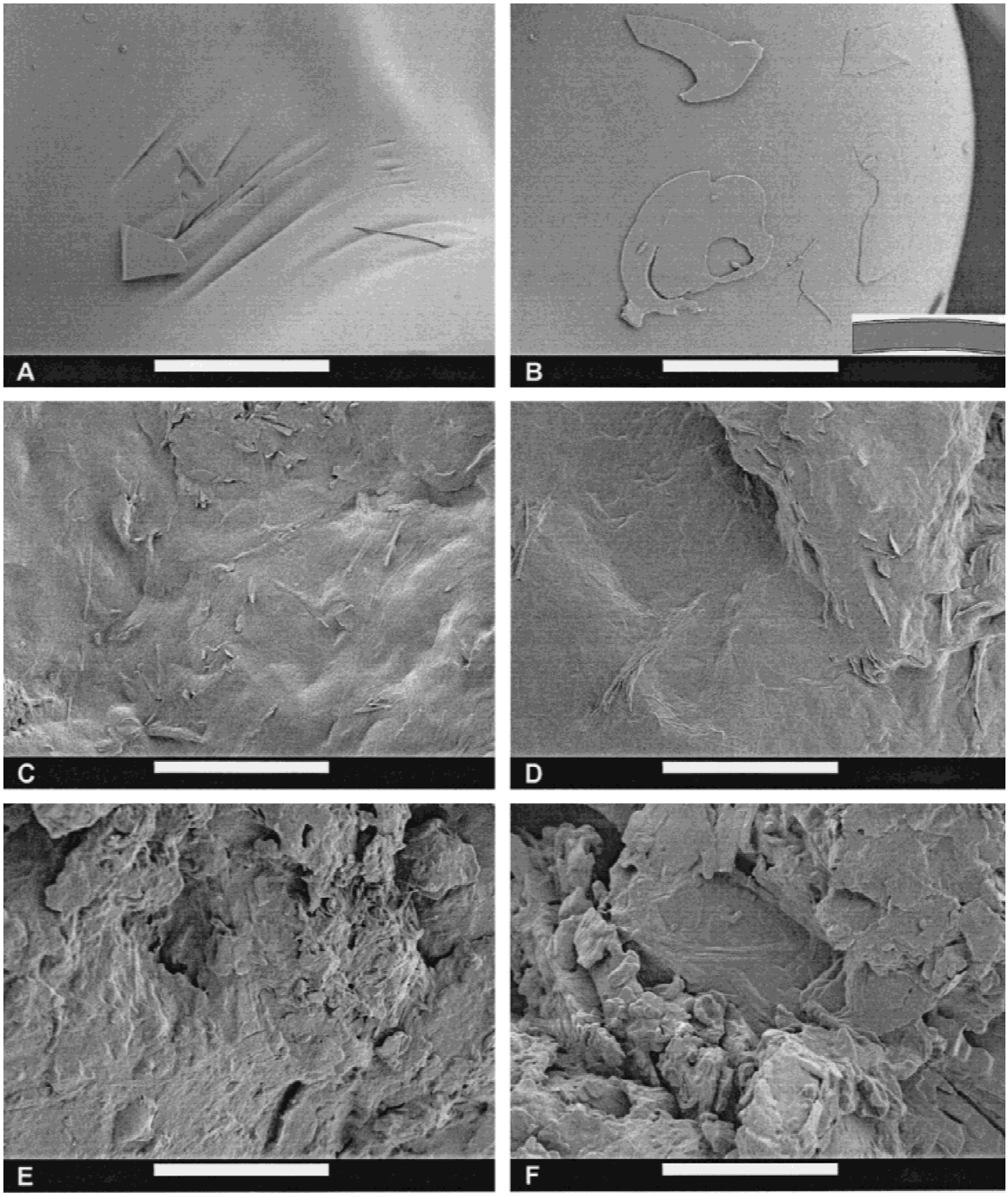

Fig. 3. Surface structure of maltodextrin M700 (a) and M700-based proniosomes with surfactant loadings of 1× (b), 8× (c), 16× (d), 32× (e), and 128× (f). As shown in the inset in panel (b), the surfactant coating on a smooth surface may be thinner and more uniform. Scale bar represents 20 μm.

sample, and assuring (from scattered light) that it was being illuminated. Using this location, the image was focused and photographs obtained. Overall magnification was confirmed by photographing 5 μm (4.988 ± 0.035) size standards (Duke Scientific, Palo Alto, CA).

RESULTS

Maltodextrin-based proniosomes were made with both solid (Maltrin M500) and hollow (Maltrin M700) particle morphologies. The surface texture of the uncoated solid particles is highly convoluted and irregular (Fig. 2a), but the hollow particles have balloon-like forms with a smooth surface (Fig. 3a). Most of the M700 particles had holes (10), which may have resulted from the manufacturing process. Some of the particles were broken, presumably due to handling (data not shown). Based on measurements of the shell thickness (2.1 μm) (10) and the density of maltodextrin (1.41 g/cm^3) (11), the specific surface area of M700 particles is estimated to be approximately 0.85 m^2/g. Previous work (10) demonstrated that coating maltodextrin M700 particles using a slurry method did not alter the gross morphology of the particles. Thus, the surface area is maintained during the preparation of proniosomes.

Proniosomes were made with different surfactant loadings, from 0.5 mmol surfactant per gram of maltodextrin (1×) to 64 mmol surfactant per gram of maltodextrin (128×). Figures 2 and 3 show that the surface characteristics of proniosomes made with these surfactant loadings differed. Comparison of M500 and 1× M500 proniosomes (Figs. 2 a and b) showed that much of the fine structure on the surface of M500 particles had been filled by surfactant in the proniosome, even at the low loading (1×). The inset drawing in Fig. 2b illustrates the filling effect of surfactant on an irregular surface. The surfactant coating would not be uniform, with thicker layers deposited at points of deeper invagination.

The surface texture of the proniosomes based on hollow M700 maltodextrin particles, shown in Figs. 3b–f, was smooth up to a relatively high loading, 16×, but above this loading (≥32×), the surface was rough and cracked. In contrast, proniosomes based on solid particle M500 maltodextrin developed a rough, cracked appearance for loadings as low as 8× (Fig. 3d). The surfaces of high load proniosomes were quite thick and coarse in texture, similar in appearance to particles of granular surfactant (Fig. 4). In some of the scanning electron micrographs from 64× or 128× samples (data not shown), it appeared that the adhesion of the surfactant to the maltodextrin particle was inadequate to support the thick layers formed. Some of these fragments appeared to have fractured from the rounded surface of a M700 maltodextrin particle. In other cases, the maltodextrin surface was visible on proniosome particles that appeared to have lost fragments of the thick surfactant coat. The surfactant layer thickness was approximately 10 and 50 μm for 8× M700 and 128× M700 proniosomes, respectively, consistent with the estimated carrier particle surface area, the density of the surfactant, and the relative amounts of carrier and surfactant. The thickness was estimated from SEMs with an edge-on view of the surfactant layer (data not shown).

Based on light scattering measurements (Fig. 5a), the yield of niosomes in the 0.5 to 100 μm size range was lower for proniosomes with high surfactant load although the number average particle size appeared to be similar for all samples. This may be due to the fact that the instrument used to measure the particle size distribution could only measure to a maximum size of 400 μm. Figure 5b shows the particle count

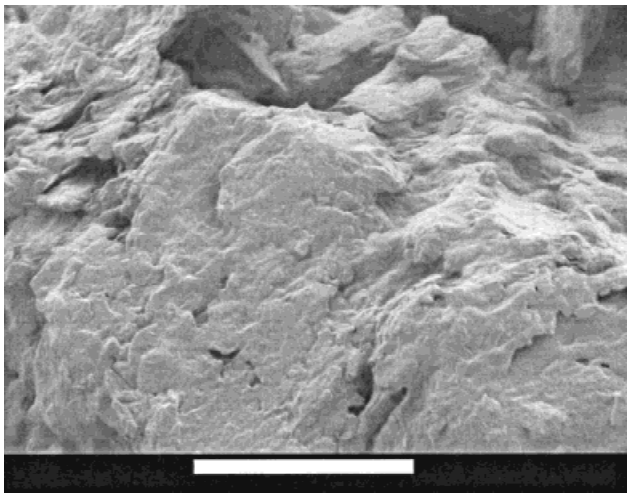

Fig. 4. Surface structure of granular surfactant. The composition is the same as that used to coat maltodextrin particles to make proniosomes. Scale bar represents 20 μm.

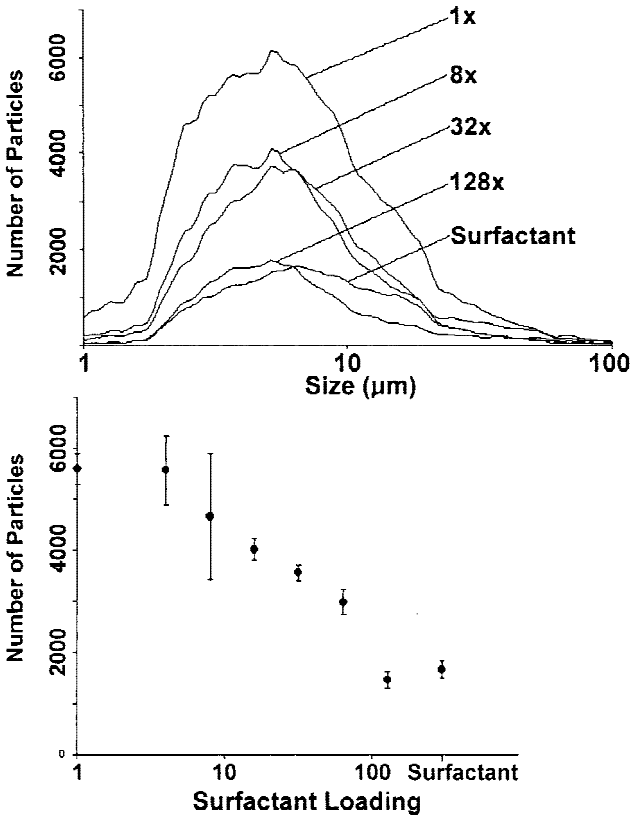

Fig. 5. Particle size distributions based on laser light scattering measurements(a). The data were not normalized. The same volume of sample was added to the dilution reservoir, so the height of the distribution approximates the actual count of particles of a specific size in the suspension. Surfactant loadings for each trace are indicated. The number of particles of 5 μm diameter (4.95–5.05 μm) is plotted as a function of surfactant load (b). Each point represents a mean (± S.D.).

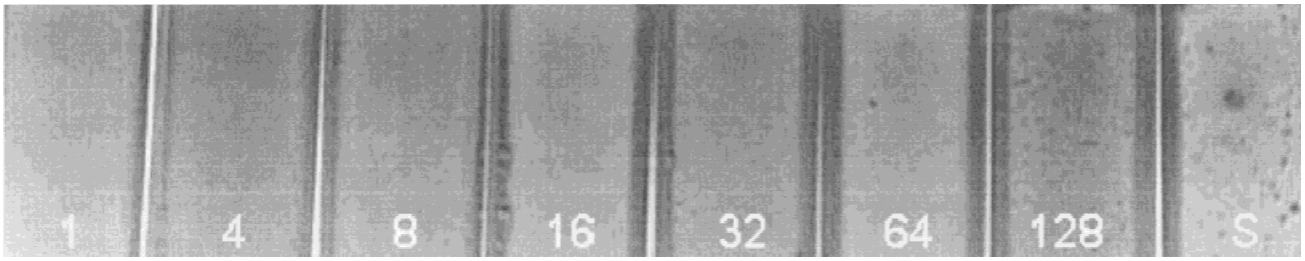

Fig. 6. Niosome suspensions made from 1×, 4×, 8×, 16×, 32×, 64×, and 128× M700-based proniosomes, and from dried granular surfactant (S). Photo was taken within 5 min of agitating the samples. In the images, particulates appear as dark spots. The vial diameter is 2.2 cm.

for one size population (4.95–5.05 μm) near the mean particle size. The number of 5 μm particles decreased with increased surfactant load and the particle count for the 128× sample was similar to that obtained with granular surfactant. Visual examination revealed the presence of large particulate material in the suspensions prepared from the high load proniosomes (Fig. 6). Proniosomes with lower loads appeared to produce more uniform suspensions with fewer large particles.

Observations of the niosome suspensions with a light microscope confirmed the results of the particle sizing measurements. The optical micrographs showed that the number of small diameter vesicles was greater in samples made from low load proniosomes, and that the number of large vesicles was greater in the samples from high load proniosomes (Fig. 7). The niosome suspensions from 1× and 16× proniosomes were very similar (Figs. 7a and 7b), but the suspensions from 128× proniosomes had fewer 5 μm niosomes and relatively more vesicles in the 30–50 μm size range (Fig. 7c). Images from 4× and 8× proniosomes were similar to 1× and 16×, while those of 32× and 64× and surfactant samples were similar to those observed for 128×.

DISCUSSION

For many years, one of the most common methods for producing niosomes has been by hydration of a film shell-dried in a round bottom flask. The hydration of dried films is thought to involve a process of swelling of "blebs" from the surface and shearing of these protrusions to produce multilamellar niosomes (6). The principal benefit of this method is its simplicity, but there remain some disadvantages. Once the dried film has been prepared, it is not possible to make up any less than the entire sample. In addition, it is usually necessary to agitate the sample for a prolonged period, up to one hour at elevated temperature, to obtain a uniform preparation. A method of producing niosomes within minutes from a dry preparation based on maltodextrin was recently developed (10). The method was simple and appeared to be flexible and scalable.

Minimizing the amount of carrier in the proniosome preparation has been a key objective of this work. It was not known how much surfactant could be supported by the carrier material without compromising the quality of the final product. Previously, it was demonstrated that proniosomes could be prepared with as high as 64 mmol of surfactant per gram carrier and still have high levels of drug entrapment (10). However, at such high surfactant loads, the suspensions had large particulates that generally made these samples unacceptable.

The data presented here demonstrate that the appearance of these partially hydrated surfactant particles in a niosome preparation is correlated with coarse, broken structures on the surface of the proniosomes. There is apparently a compromise between minimizing the amount of carrier and the quality of the niosomes formed, such that the maximum useful surfactant loading is approximately 8 mmol of surfactant per gram of M700 carrier. Because the coating of M500 particles is much thicker than that on M700 particles at any given surfactant loading, M500 proniosomes are a less efficient system for niosome formation. Particulates form at a lower level of surfactant loading. The differences between the proniosome properties can be attributed to differences in surface area between the solid M500 and the hollow M700 particles.

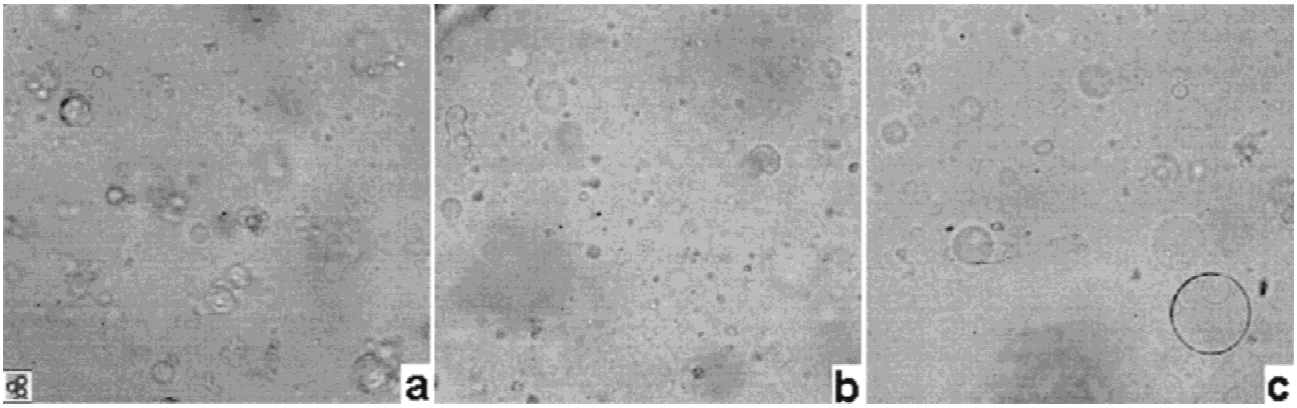

Fig. 7. Optical micrographs of niosome suspensions prepared from M700 proniosomes with different surfactant loadings. Specific samples are the same as in Fig. 6: from proniosomes 1× (a), 16× (b), and 128× (c). Images were obtained approximately 30 min after agitation of the samples. The inset in panel (a) shows 5 μm size standard beads.

When the same amount of surfactant is deposited evenly, the film thickness will be greater for the carrier with the lower surface area.

Based on the surfactant load at which aggregates formed, on a per weight basis, M700 can carry 3–4 times as much surfactant as M500 particles. Assuming that once the surfactant layer thickness reaches 15–20 μm, pieces of surfactant break off from the carrier and large particles are generated in the niosome suspension, the surfactant load that would result in this thickness can be estimated. The specific surface area of M500 maltodextrin has been measured to be 0.54 m^2/g (11) and M700 has been estimated to be 0.85 m^2/g. In large part, the high surface area of M500 comes from the tortuosity of the M500 particles. If the M500 particles were assumed to be smooth spheres of the average particle size (500 μm), the specific surface area would be only 0.0085 m^2/g, 1.6% of the actual value. If M500 surface roughness were such that the convoluted surface of M500 could be preserved as the surfactant accumulated, the capacity of M700 would be only 1.57 greater than that of M500. Experimentally, the loading difference is about four times better for M700. The loading of M500 is apparently a process in which initially, the surface gaps and holes are filled to form a more regular surface with a smaller effective area. Then, as more surfactant is added, the surfactant thickness increases rapidly.

At higher surfactant loading, it appears that the coating can no longer be characterized as a "thin film" and the behavior more closely resembles that of granular surfactant. It is likely that the hydration and shearing required to produce uniform multilamellar vesicles are only rapid enough for smooth films. Particulates formed in samples with high surfactant loadings may result from fragmentation of the thick film and hydration of these large (~50 μm) particles of unsupported surfactant. To hydrate granular surfactant directly results in a similar difficulty as the surfactant particles themselves hydrate incompletely. Although these partially hydrated particles would probably disappear after additional heating and extended agitation, this added processing detracts from the ease of generating niosomes from proniosomes.

CONCLUSIONS

These proniosomes allow one to prepare a colloidal suspension of drug/surfactant niosomes suitable for delivery. For effective proniosomes, it is essential that the surfactant coating be smooth and uniform to allow for rapid and consistent hydration. Under these conditions, proniosomes provide flexible dosing, minimal carrier, and simple preparation.

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