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Note

Some properties of extruded non-ionic surfactant micro-tubes

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Abstract

Polyhedral non-ionic surfactant vesicles (niosomes) undergo complex shape transitions as a result of mechanical stress. When extruded under pressure from capillaries with exit diameters smaller than the diameter of the vesicles, a series of novel structures comprising mostly of tubules, vesicles inside tubules and concentric structures can be formed. The microtubules (up to 80 μm in length) form as a result of the pressure exerted on polyhedral niosomes, this leading to the fusion of many vesicles, the relative shear giving movement of the vesicles giving rise to the formation of three distinctive structures, namely tubules, vesicle within tubule and concentric ("whorl") morphologies. The entrapment efficiency of the tubules has been studied using a model solute 5(6)-carboxyfluorescein (CF), as has the effect of shear stress this and compaction pressure on the release of the entrapped solute. Deformation of the structures affects their ability to retain entrapped solute. Tubular structures heated above their transition temperatures reversibly transform into discrete vesicular structures.

Keywords: Microfabrication; Polyhedral niosomes; Microtubules; Non-ionic surfactants; Transition temperature; Entrapment efficacy

Water dispersible, poorly soluble amphiphiles, including non-ionic surfactants are known to self-assemble into spherical uni- or multi-lamellar vesicles (Azmin et al., 1985; Uchegbu and Florence, 1995). However, various tubular (Furhop and Helfrich, 1993; Chiruvolu et al., 1994; Uchegbu and Florence, 1995), disc-like (Walter et al., 1991; Lasic, 1992; Uchegbu et al., 1992) and toroidal (Isenberg, 1992; Lipowsky, 1995; Michalet and Bensimon, 1995) vesicle structures also can form as a result of amphiphile self-association. Highly structured 'geodesic' multi-vesicular structures arising from the association of many small non-ionic surfactant vesicles (niosomes) have also been described (Sternberg et al., 1995). The specific shapes obtained are governed by the type and amount of surfactant used, which in turn is governed by the geometric shape that the surfactants possess. External factors such as temperature, which affect the orientation of the components of the lamellar membrane, also play a part in the form and dimensions of the vesicles as shown in Fig. 1.

Extrusion of polyhedral vesicles through a micro-capillary (Arunothayanun et al., 1999a) shows that polyhedral niosomes (i.e. niosomes formed with low levels of cholesterol) do not retain their integrity (i.e. they lose their visco-elasticity) but, unlike spherical niosomes formed with higher levels of cholesterol, they extrude into elongated structures. We have shown (Nasseri and Florence, 2001) that by controlling the extrusion process, it is possible to obtain elongated structures of defined shapes forming narrow and hollow tubules up to 80 μm in length with a diameter approximately that of the capillary exit diameter (~1 μm). Such structures must arise as a result of the

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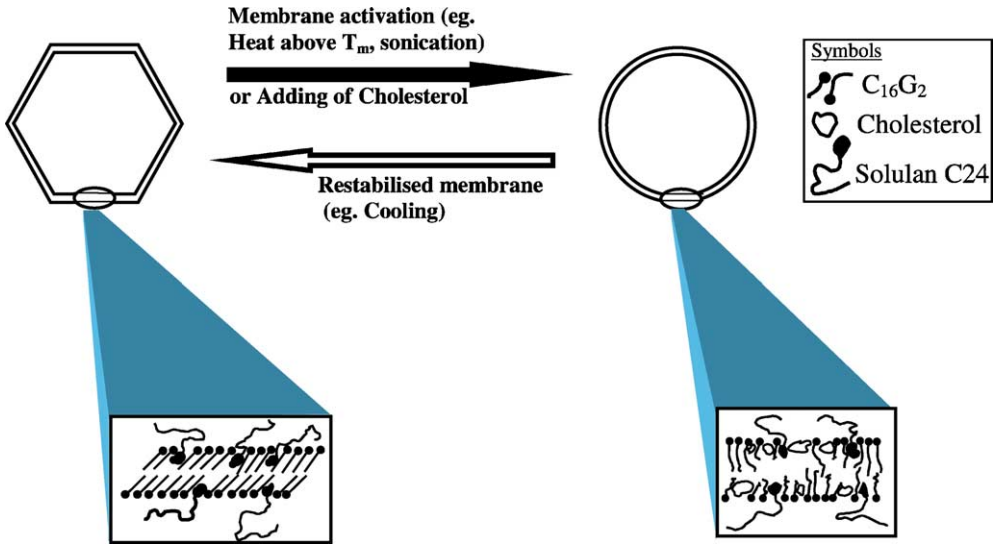

Fig. 1. The schematic represents the typical orientations of amphiphiles giving rise to specific shapes of polyhedral and spherical vesicles and the factors affecting shape and molecular conformation of polyhedral niosomes.

fusion of a large number of original vesicles prior to extrusion.

The purpose of the present work was to gain insight into the solute entrapment capability of these newly developed structures and to determine whether or not the extrusion process caused any loss of entrapped solutes, and to investigate the effect of temperature on their entrapment efficacy and shape.

Polyhedral vesicles were prepared from polyoxyethylene-5-cetyl ether ($C_{16}EO_5$) or polyoxethylene-5-stearyl ether ($C_{18}EO_5$) and poly-24-oxyethylene cholesteryl ether (Solulan C24) in molar ratios of 91:9 and 98:2, respectively, using the hand shaking method (Arunothayanun et al., 1999b). Surfactant mixtures were dissolved in 5 ml chloroform, and the solvent removed under reduced pressure at 30 °C and 60 rpm, in a 250 ml round bottom flask (Buchi Rotavapor-R). Residual organic solvent was removed by drying the surfactant film under a stream of nitrogen for 15 min. The dry surfactant film was then hydrated with 5 mM 5(6)-carboxyfluorescein (CF) incubated at 34 or 60 °C (depending on the preparation composition, slightly above the transition temperature of the surfactants) for 1 h with constant mild shaking on a mechanical shaker (Griffin flask shaker). Niosome dispersions with a final lipid/surfactant concentration of 60 mM were left to cool at room temperature and kept overnight at 4 °C before use.

A total of 1 ml of the CF loaded niosome dispersion was washed by dilution with 7 ml of PBS (pH 7.4) and ultracentrifugation at 200,000 × *g*, 4 °C, (Sorvall Combi Plus, UK) for 45 min. The supernatant was then discarded and the niosome pellets were re-suspended with PBS before washing again. The washed pellets were then re-suspended with 1 ml PBS. Preliminary studies indicated no change in size or entrapment on storage at room temperature for 24 h or at 4 °C for 2–3 days. In these studies, separation of unentrapped solute and assay were performed within 2–3 h of separation.

To quantify the entrapped CF, the procedure from Arunothayanun et al. (2000) was adopted. Encapsulation efficiency was calculated and expressed as a percentage of the available hydrating solute actually encapsulated.

Borosilicate glass capillaries (inner diameter of 1.17 mm; outer diameter of 1.5 mm) were obtained from Harvard Instruments, UK. The glass capillaries were pulled into circa 1–4 μm exit diameter tips using a pipette puller (Narishige model PC-10).

Micropipettes were filled with niosomes and connected to a piezo-electric pump, which controlled the

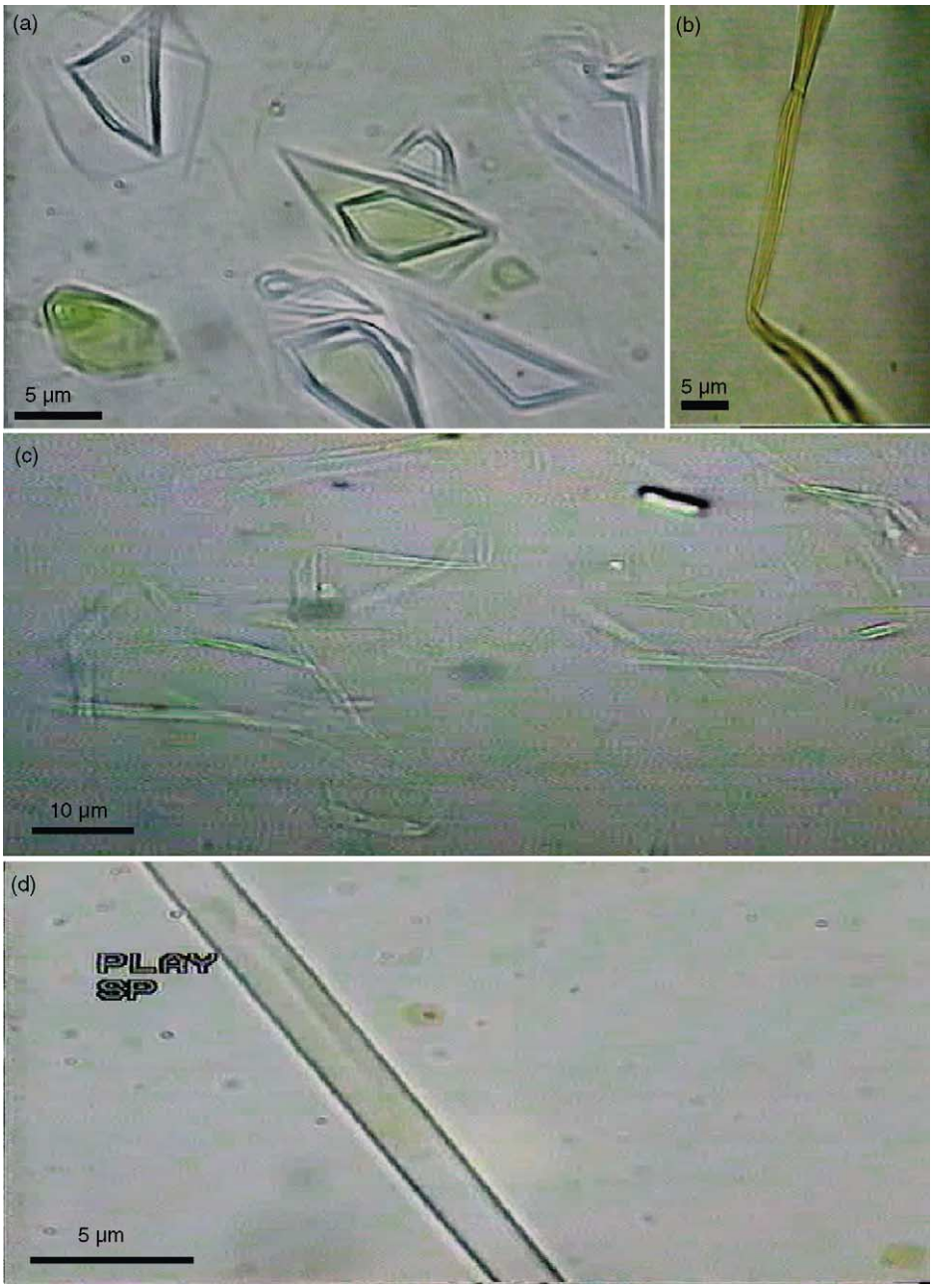

Fig. 2. Videomicrographs of polyhedral niosomes entrapping CF, (a) before extrusion, (b) as an extruded microtubule during the process of extrusion, (c) a collection of extruded microtubules, and (d) a typical microtubule, all in deionised water.

frequency and duration of the nitrogen pulse (controllable from 6.89 to 2.07×10^4 N m^{-2}) used to drive the vesicles from the micropipettes into a Petri dish with the chosen medium. A minimum pressure of 3.45×10^3 N m^{-2} was required for fusion of the polyhedral vesicles to occur to form tubule structures. Events were followed using a video camera attached to the Nikon-Microphot-FXA light microscope utilising a distant

lens with Nikon UV light (model HB-10101AF). Individual frames were captured on computer using Hauppage capturing software (Win TV model No. 404).

In each experiment a defined volume of 0.1 ml of the CF containing polyhedral niosomes was placed into the capillary micropipettes and all extruded under defined pressure ($1.9 \times 10^4\,N\,m^{-2}$) into 1 ml PBS contained in a small Petri dish. After complete extrusion of 0.1 ml niosomal suspension, the inside of micropipette was washed free from any expelled and adsorbed CF twice with 0.1 ml of PBS. The released CF was then washed off by centrifugation at 10,000 rpm for 5 min and the supernatant was separated from the pellet. The pellet was washed with defined volumes of PBS and the wash added to the supernatant. This was repeated three times. The supernatant was diluted accordingly with PBS and analysed fluorimetrically to obtain the extent of CF release.

Shape transformation was observed by attachment of a video camera to the hot stage microscope and the relevant videos at designated temperatures captured.

Results and discussion

Video photomicrographs seen in Fig. 2 show polyhedral niosomes (a) before (b), (c) and (d) after extrusion under pressure through a capillary of exit diameter of 1–2 μm. The behaviour of these polyhedral niosomes under pressure inside the capillaries may shed light on their behaviour in the capillary blood supply or in their movement through complex organs. We first investigated the effect of shear stress on vesicle integrity in retaining their entrapped solute and, by narrowing or widening the aperture size of micropipettes; the extent of shear could be controlled. The initial release of CF as a result of shear and attrition is shown in Fig. 3 for the two sets of preparations. The initial amount of CF recovered from the medium increases as the size of the micropipette aperture becomes smaller, as shear forces rise.

The physico-chemical properties of the bilayer membranes can be diverse depending on the composition of the membranes. Bilayers undergo changes in physical state with temperature. Above the phase transition temperature (T_m) the bilayer exists as a two-dimensional liquid. Below this temperature the bilayer

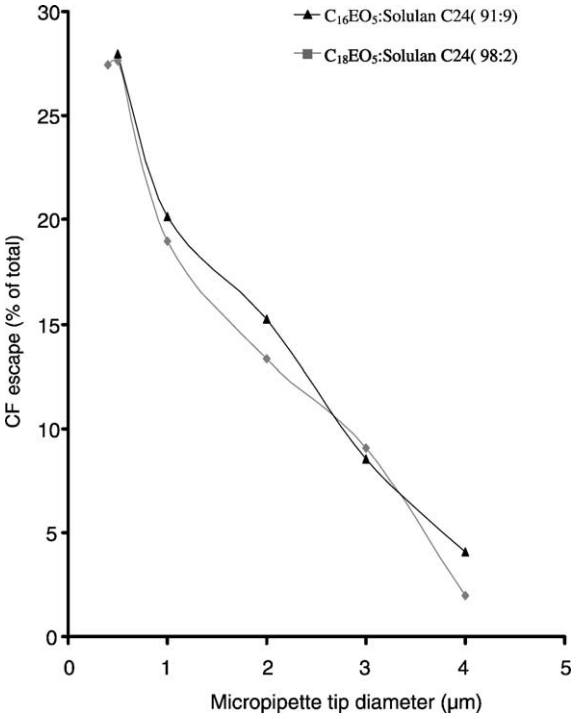

Fig. 3. CF release as a result of extrusion of polyhedral niosomes via differing micropipette aperture diameters showing the percentage release of total entrapped CF at time zero after extrusion for the two preparations.

is a two-dimensional solid and the lipid molecules are tightly packed in a crystal lattice (Gruner, 1987).

The value of the T_m varies with the composition of membranes. Lipids with longer hydrophobic chains form membranes which have higher phase transition temperatures and which are less permeable to entrapped solute compared to those formed by lipids with shorter chain lengths (Buckton et al., 1992). The mechano-chemical properties of membranes will be different depending on their polymorphic state (Evans and Kwok, 1982; Needham and Zhelev, 1996).

Freshly prepared polyhedral niosomes are first formed as spherical vesicles which, on cooling, transform into polyhedral structures (Arunothayanun et al., 1999b). We, therefore, studied the morphological changes of microtubules formed from polyhedral systems using hot stage microscopy. When the microtubules were exposed to heat a transformation is observed (Fig. 4). On increasing the temperature,

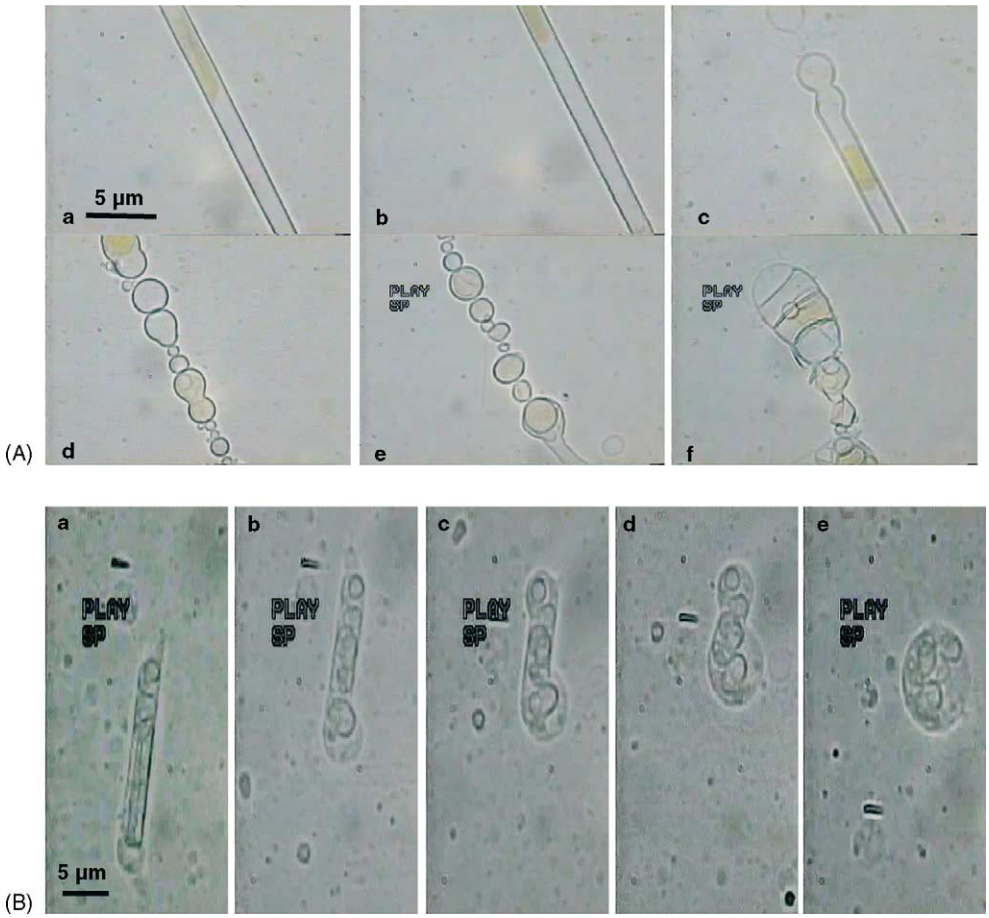

Fig. 4. Shape transformation of (A) a microtubule fabricated from $C_{16}EO_5$ at (a) 25 °C splitting to (b, c, d and e) a chain of spherical structures at 35 °C. On cooling multifaceted structures started forming below 35 °C as seen in (f). (B) A sequence showing a micro-tubule fabricated from $C_{18}EO_5$ on heating to 55 °C.

the microtubules, like their polyhedral counterparts, undergo a reversible shape transformation into chain of spherical structures and similarly develop into polyhedral structures on cooling.

It is clear that novel structures can be fabricated from polyhedral niosomes. They possess the same constituents but have different morphologies. We have demonstrated the effect of shear stress (which may occur in vivo) and which ultimately affects carrier integrity. The controlled process of compaction leading to the tubular structures can alter their physico-mechanical characteristics, resulting in large microtubules, perhaps a form suitable for depot drug delivery systems with potential uses in ophthalmic, dermal, subcutaneous or intramuscular administration, where their shape makes them relatively viscous and perhaps maintains their residence time. The presence of low transitional temperatures nearing the body temperature where these structures display a shape transformation similar to their polyhedral counterparts may have a potential use in temperature induced release.

Acknowledgements

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