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Osmotic Behaviour of Polyhedral Non-ionic Surfactant Vesicles (Niosomes)

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Abstract

In addition to common spherical non-ionic surfactant vesicles (niosomes), disc-like, tubular, and polyhedral niosomes have also been reported. The permeability and osmotic activity of niosomes are important in determining their use as controlled-release drug-delivery systems. These properties have been compared for polyhedral niosomes prepared by hydrating a mixture of a hexadecyl diglycerol ether ($C_{16}G_2$), a poly(24)oxyethylene cholesteryl ether (Solulan C24), 91 : 9 or 98 : 2, and conventional spherical niosomes prepared from the same surfactants but with cholesterol.

When subjected to osmotic gradients, polyhedral niosomes, the membranes of which are in the gel phase, swell and shrink less than their spherical counterparts and they are more permeable to the hydrophilic solute 5(6)-carboxyfluorescein. In 2 M NaCl the rate of release of carboxyfluorescein from polyhedral niosomes (both containing 9% Solulan C24) into either a hypotonic (water) or an isotonic medium (2 M NaCl) was low. This contrasted with similarly loaded spherical niosomes and polyhedral niosomes containing 2% Solulan C24, from which release was high in hypotonic media (e.g. water) but less in an isotonic medium (2 M NaCl). For both polyhedral and spherical niosomes encapsulating carboxyfluorescein ($pK_a = 6{\cdot}4$), release rates were higher at pH 8 than at pH 5.

Polyhedral niosomes are thus, in general, less osmotically active than spherical niosomes because of their rigid but highly permeable membranes. The unusual polyhedral membrane impermeability to carboxyfluorescein co-entrapped with salt in hypotonic media is a function of Solulan C24 content, and is possibly a result of salting out of the polyoxyethylene chains; this is, therefore, a property that might be manipulated in the design of a drug-delivery system.

Cells have semi-permeable membranes and respond to osmotic gradients by means of diffusion or transporter proteins. Vesicles, formed either by phospholipids (liposomes) or non-ionic surfactants (niosomes), also have selectively permeable bilayer membranes which enable unequal diffusion of water across the membranes when the osmotic pressures of the solutions separated by the membranes are different. Osmotic stress can affect the properties of vesicles in many ways, inducing membrane undulation (Menger & Lee 1995), changing vesicle size (Sun et al 1986), shape (Berndl et al 1990) and membrane permeability (Iga et al 1989) — all crucial factors in the use of vesicles as drug-delivery systems.

We have previously reported the formation of non-spherical vesicles from non-ionic surfactants (Uchegbu et al 1992, 1996). Polyhedral niosomes, formed from mixtures of hexadecyl diglycerol ether ($C_{16}G_2$) and poly(24)oxyethylene cholesteryl ether (Solulan C24), have faceted structures which can encapsulate and release water-soluble solutes (Uchegbu et al 1997). Spherical vesicles can also be formed by $C_{16}G_2$ when equimolar amounts of cholesterol are present. It is therefore of interest to determine how vesicles with different shapes behave in the presence of osmotic stress. This study has further characterized $C_{16}G_2$ polyhedral

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niosomes and compared them with their spherical counterparts. Membrane permeability and osmotic properties have been examined as a function of niosome ultrastructure and composition by subjecting the vesicles to a range of osmotic gradients and measuring both average size changes and the release profiles of the water-soluble marker 5(6)-carboxyfluorescein. Both internal and external salt gradients were established.

Materials and Methods

Materials

Hexadecyl diglycerol ether ($C_{16}G_2$) was a gift from L'Oreal (France) and poly(24)oxyethylene cholesteryl ether (Solulan C24) was donated by Ellis & Everald (UK). Cholesterol, 5(6)-carboxyfluorescein and Trizma HCl were obtained from Sigma (UK). Isopropanol, hydrochloric acid, sodium hydroxide and sodium chloride were supplied by BDH Laboratory Supplies (UK). Chloroform (HPLC grade) was purchased from Rathburn Chemicals (UK). Water was from an ultra-high-quality reverse osmosis water purifier (Elgastat UHQPS, Elga, UK).

Osmotic activity of polyhedral non-ionic surfactant vesicles

Polyhedral vesicles, prepared by hydrating a film of $C_{16}G_2$–Solulan C24, 91 : 9, with water at 60°C and storing for 24 h, were mixed with glucose or sodium chloride solutions, such that the external concentration was 0·93 M glucose or 0·154, 1·0 or 2·0 M NaCl, or with water as a control. Alterations in particle size in response to osmotic gradients were recorded by low-angle laser-light scattering (Mastersizer X, Malvern, UK).

Polyhedral vesicles loaded with 2 M NaCl were also prepared by using a hydrating solution of 2 M NaCl instead of water. These niosomes were diluted 10 times with either 2 M NaCl solution or water and the vesicles were then sized. Niosomes prepared from $C_{16}G_2$–cholesterol–Solulan C24, 49 : 49 : 2 and 45 : 45 : 10, formed spherical vesicles and served as control dispersions.

The release of carboxyfluorescein from polyhedral niosomes

The influence of the osmotic gradient. The effect of the external and ''internal'' osmotic gradients on release of carboxyfluorescein was studied. Preparation of niosomes with an external hypertonic solution involved hydration with carboxyfluorescein (5 mM) of appropriate surfactant–lipid mixtures ($C_{16}G_2$–Solulan C24, 91 : 9; $C_{16}G_2$–Solulan C24, 98 : 2; and $C_{16}G_2$–cholesterol–Solulan C24, 49 : 49 : 2). This was followed 24 h later by removal of unentrapped material by exhaustive dialysis at 4°C (Visking tubing, MW cut-off 12–14 kDa) and the amount of entrapped carboxyfluorescein was analysed as described elsewhere (Uchegbu et al 1992). These niosomes were then placed in dialysis tubing suspended in 0·154 M or 2 M NaCl and the fluorescence of the dialysate was periodically determined over a 5-h period at 25°C.

Niosomes containing a hypertonic salt solution were prepared as described above except that the surfactant–lipid mixtures were hydrated with a solution of carboxyfluorescein (5 mM) in 2 M NaCl. After 24 h unentrapped solute was removed by exhaustive dialysis against 2 M NaCl at 4°C. The release of carboxyfluorescein was then measured by placing these suspensions in a dialysis bag suspended in 2 M NaCl or water, as before.

The influence of pH. Polyhedral niosomes ($C_{16}G_2$–Solulan C24, 91 : 9) and spherical niosomes ($C_{16}G_2$–cholesterol–Solulan C24, 49 : 49 : 2) were prepared by hydrating surfactant–lipid films with carboxyfluorescein (5 mM) in Tris-buffered saline (TBS) 1/10 strength at pH 5 or 8. Dispersions were stored for 24 h and subsequently exhaustively dialysed against TBS 1/10 strength at the same pH and 4°C to remove unentrapped carboxyfluorescein. Entrapped carboxyfluorescein was estimated by disrupting the niosomes with isopropanol, diluting the resulting solution with TBS at the same pH, and measuring the fluorescence (excitation 486 nm, emission 514 nm). The release of carboxyfluorescein was estimated fluorimetrically at pH 5 and pH 8.

Results and Discussion

Osmotic activity of polyhedral non-ionic surfactant vesicles

It is well known that lipid vesicles are osmotically sensitive (Bangham et al 1967; De Gier 1995). Changes in vesicle size in response to osmotic stress have been used to monitor this property (Hantz et al 1986; Sun et al 1986; Rutkowski et al 1991; Hallett et al 1993). In this work, the osmotic behaviour of niosomes was investigated by following the change in mean size by use of low-angle laser-light scattering, which furnishes an

equivalent-sphere size distribution for non-spherical particles. It is difficult to describe the size of a polyhedral structure by use of one parameter because only a spherical particle can be characterized by its diameter (Allen 1992; Bernhardt 1994). However, by means of the spherical-equivalence principle we can obtain a diameter of an equivalent sphere which has the same measured property as our non-spherical particles, depending on the technique used, e.g. sedimentation velocity, surface area, or in this example, volume. Thus, a change in the size of the polyhedral structure leads to a change in the diffraction pattern, and the change in the size of the equivalent-sphere model can be followed.

As expected, the dispersion of various niosome formulations in environments of different osmotic strength led to changes in vesicle diameter as shifts in the size-distribution were observed. The size-distribution given by laser diffraction correlates well with results from optical microscopy. The size change was dependent on the actual nature of the vesicles and on the concentration of salt (Figure 1) or glucose solution (Figure 2). In general, polyhedral vesicles responded less to a salt osmotic gradient than did spherical formulations (Figure 1 and Table 1). Although it has previously been shown that the extent of the size change resulting from osmotic gradients depends on the initial diameter of vesicles (Sun et al 1986), our results suggest that the composition of the vesicle membranes is a more important factor. With spherical niosomes the sensitivity to osmotic stress was not dependent on the level of Solulan C24 in the vesicles. When the osmotic stress across these spherical niosome membranes arises from internal

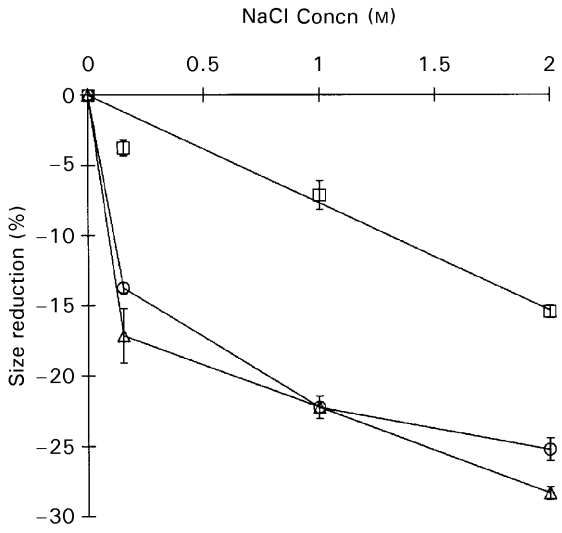

Figure 1. The reduction in the mean diameter of niosomes encapsulating water 5 h after dispersion in NaCl solutions. □ Polyhedral niosomes, $C_{16}G_2$–Solulan C24, 91 : 9, initial diameter 10·65 μm; ○ spherical niosomes, $C_{16}G_2$–cholesterol–Solulan C24, 49 : 49 : 2, initial diameter 9·96 μm; △ spherical niosomes, $C_{16}G_2$–cholesterol–Solulan C24, 45 : 45 : 10, initial diameter 8·80 μm.

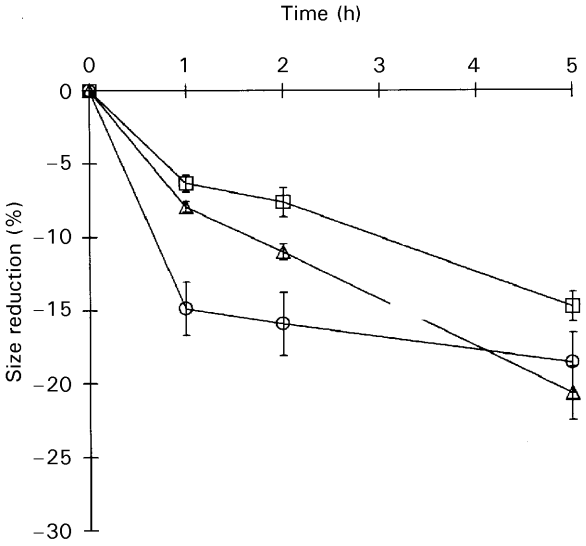

Figure 2. The reduction in the mean diameter of niosomes encapsulating water as a function of time after dispersion in glucose solution. □ Polyhedral niosomes, $C_{16}G_2$–Solulan C24, 91 : 9, initial diameter 11·76 μm; ○ spherical niosomes, $C_{16}G_2$–cholesterol–Solulan C24, 49 : 49 : 2, initial diameter 9·11 μm; △ spherical niosomes, $C_{16}G_2$–cholesterol–Solulan C24, 45 : 45 : 10, initial diameter 14·14 μm.

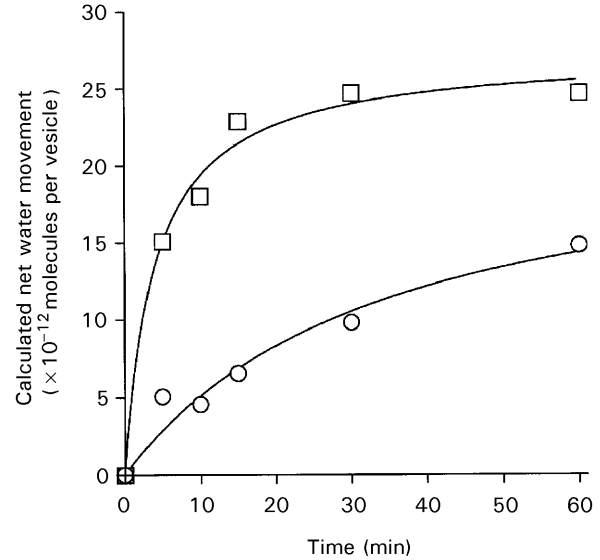

Figure 3. Calculated number of water molecules diffusing from spherical ($C_{16}G_2$–cholesterol–Solulan C24, 49 : 49 : 2) vesicles, obtained by assuming that the reduction in volume is caused by water efflux from the vesicles in 0·15 M NaCl (○) and 2 M NaCl (□). The estimated initial net water flux values for niosomes dispersed in 0·15 M and 2 M NaCl are $1{\cdot}2 \times 10^7$ molecules $m^{-2}\,s^{-1}$ (or 0·12 L $cm^{-2}\,h^{-1}$) and $4{\cdot}6 \times 10^7$ molecules $m^{-2}\,s^{-1}$ (or 0·49 L $cm^{-2}\,h^{-1}$), respectively; those in animal epithelial tissues are in the range 0·4–75·0 L $cm^{-2}\,h^{-1}$ (House 1974b).

Table 1. The increase in the mean diameter of niosomes encapsulating 2 M NaCl after being dispersed in water for 5 h.

Niosome composition	Size increase (%)
Polyhedral ($C_{16}G_2$–Solulan C24, 91 : 9)	10
Spherical ($C_{16}G_2$–cholesterol–Solulan C24, 45 : 45 : 10)	23
Spherical ($C_{16}G_2$–cholesterol–Solulan C24, 49 : 49 : 2)	60

Table 2. The percentage release of carboxyfluorescein from niosomes 5 h after dispersion in NaCl solutions.

Dispersion medium	Polyhedral niosomes	Spherical niosomes
Water	22·8 ± 0·4	5·4 ± 0·2
0·154 M NaCl	48·4 ± 0·5	13·8 ± 0·5
2·0 M NaCl	64·2 ± 0·7	22·3 ± 0·5

Data are means ± s.d. (n = 3). Polyhedral and spherical niosomes were prepared from $C_{16}G_2$, cholesterol and Solulan C24 in the molar ratios 91 : 0 : 9 and 49 : 49 : 2, respectively.

salt concentration, low levels of Solulan C24 (2%) increased the reaction of the niosomes to osmotic stress. A 60% increase in size was observed for NaCl-containing niosomes when dispersed in water (Table 1) compared with a 25% decrease in diameter after 5 h for niosomes containing water dispersed in 2 M NaCl (Figure 1). This observation compares favourably with studies on erythrocytes, which have more room for swelling than for shrinkage (House 1974a). The response of these niosomes to osmotic stress was fairly rapid, as can be deduced from the estimated water flux across the membrane (Figure 3).

On being dispersed in glucose solution the mean size of all niosomes also decreased (Figure 2) although the extent of shrinkage was less for the polyhedral niosomes.

Selective permeability is obviously the key to an osmotic stress reaction. The observation that polyhedral niosome membranes are less likely to change in size than those of vesicles formed with the inclusion of cholesterol agrees well with previous observations (Baillie et al 1985). The incorporation of cholesterol into liposomal membranes reduces the permeability of the membrane to solutes (De Gier et al 1968; Baillie et al 1985). This reduced solute permeability will enable selective membrane permeability to water and, in turn, produce the type of measurable reaction to osmotic stress observed here.

It is possible that several factors are involved. The permeability of polyhedral niosomes to entrapped carboxyfluorescein was greater than that of spherical niosomes (Table 2) and it is likely that this greater permeability is in part responsible for the minimal change in size in response to osmotic stress. When glucose solutions were used to produce osmotic gradients the osmotic stress reaction of these polyhedral niosomes was greater than that for salt gradients (Figures 1 and 2). This is probably because the permeability of the polyhedral niosomes to glucose is lower than to electrolytes.

There seems to be evidence from these studies of coupled flux of water and carboxyfluorescein. It might have been expected that the release of carboxyfluorescein into NaCl or glucose solutions would be enhanced as water moved in the same direction. The release of carboxyfluorescein from both polyhedral and spherical niosomes into external salt solutions (0·15 and 2 M NaCl) was, however, only slightly greater than its release from these niosomes into water (Table 2). The extent to which osmotic gradient affects both types of niosome seems similar, although the polyhedral niosomes are intrinsically more permeable to carboxyfluorescein (Table 2 and Figure 4). This suggests that the lower sensitivity of the polyhedral niosomes to osmotic stress is, in addition to being a function of the increased permeability of the membranes, also a function of the reduced elasticity of the membranes, which are less likely to be able to accommodate changes in vesicle size (Sun et al 1986). These polyhedral niosome membranes are in the gel phase at room temperature (Uchegbu et al 1997) and are thus more rigid than niosome membranes in which the gel-to-liquid-phase transition is abolished, as it is with cholesterol-containing membranes (Chapman 1968; Taylor & Morris 1995).

The effect of co-entrapped NaCl on carboxyfluorescein release

The effect on carboxyfluorescein release of water flux into the vesicles was studied as a test of coupled flux. The co-entrapment of carboxyfluorescein with 2 M NaCl changed the release profile of carboxyfluorescein from both polyhedral and spherical niosomes (Figure 5). For spherical niosomes the rate of release into a hypotonic medium (water) was higher than into an isotonic (2 M NaCl) medium, whereas one might have expected the opposite. A similar response was reported for liposomes (Yoshikawa et al 1983). For polyhedral niosomes containing 9% Solulan C24, however, there was no difference between release into a hypotonic medium (Figure 5) and that into 2 M NaCl. When polyhedral niosomes contained only 2% Solulan C24, the release of carboxyfluorescein into the

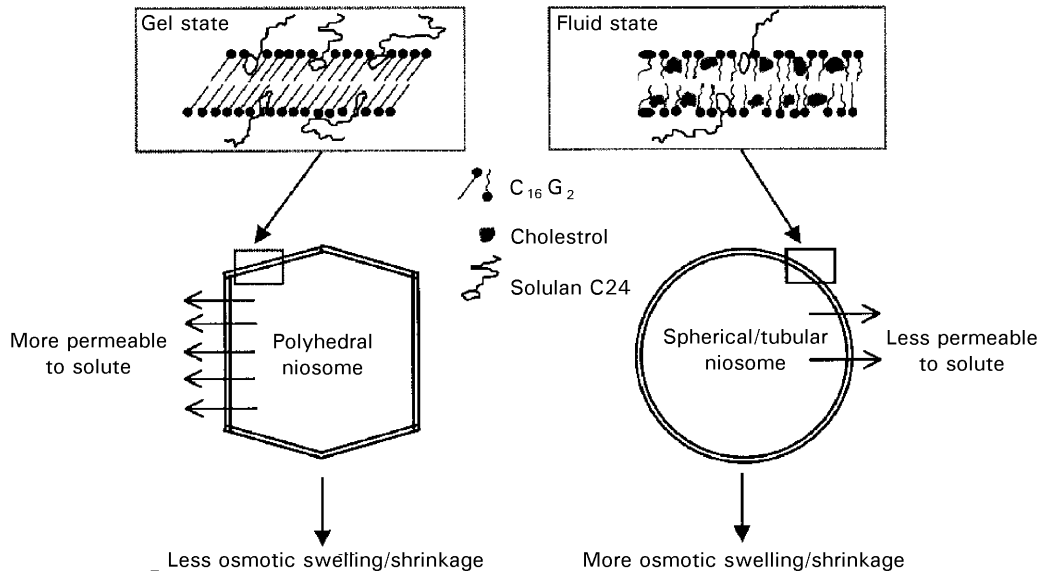

Figure 4. Schematic representation of niosomal membrane structures and osmotic behaviour at room temperature.

hypotonic medium was greater (Figure 6). Figure 7A shows the carboxyfluorescein-loaded polyhedral niosomes of $C_{16}G_2$–Solulan C24 prepared in NaCl and left to dialyse in the isotonic NaCl solution. The ultrastructure of the niosomes was found to be unchanged, with the high fluorescence content being observed inside the vesicles after 3 days of dialysis at room temperature. Figure 7B shows that these niosomes had become swollen when left to dialyse in water for 3 days at room temperature. It is noted that niosomes formed with 9% Solulan C24 still contain a large amount of carboxyfluorescein inside the vesicles (Figure 7b) whereas those formed with 2% Solulan C24 can not be observed under fluorescent light, implying the presence of a small amount of carboxyfluorescein inside the vesicles. NaCl, which can salt out polyoxyethylene chains (Graham 1992), might cause membranes containing high levels of polyoxyethylated compounds (Solulan C24) to become more hydrophobic, resulting in lower permeability to carboxyfluorescein. Small changes in the level of

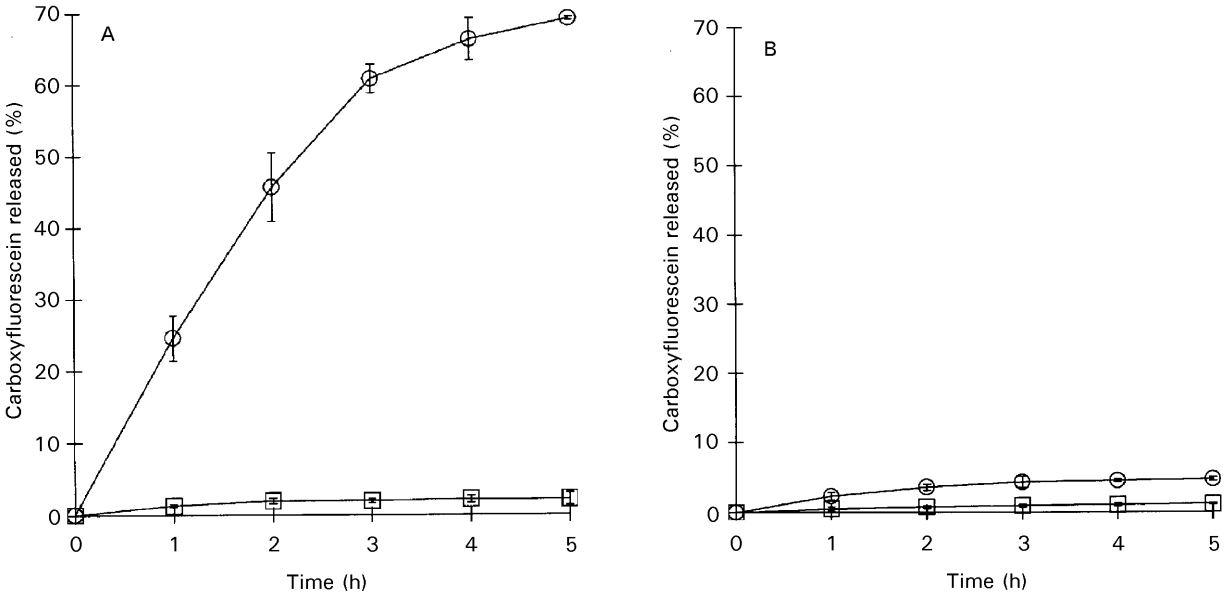

Figure 5. The release of carboxyfluorescein from niosomes encapsulating carboxyfluorescein + 2 M NaCl into hypotonic media (water) (A) and into isotonic media (2 M NaCl) (B). □ Polyhedral niosomes, $C_{16}G_2$–Solulan C24, 91 : 9; ○ spherical niosomes, $C_{16}G_2$–cholesterol–Solulan C24, 49 : 49 : 2.

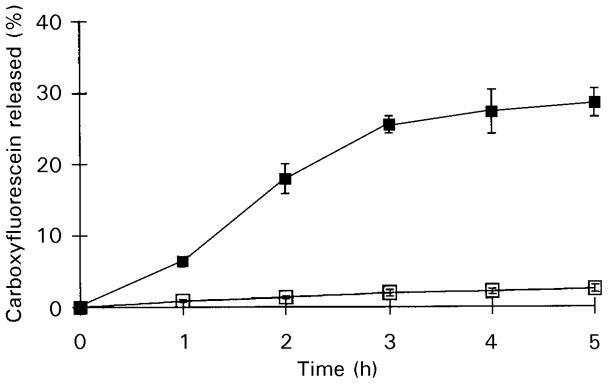

Figure 6. The release of carboxyfluorescein from polyhedral niosomes encapsulating carboxyfluorescein and 2 M NaCl into hypotonic media (water). □ $C_{16}G_2$–Solulan C24, 91 : 9; ■ $C_{16}G_2$–Solulan C24, 98 : 2.

hydration of the membrane-forming polyoxyethylated surfactants are crucial to membrane ultrastructure because the complete formation of the membrane depends on the concentration of water (Usselmann & Müller-Goymann 1984). It is also worth noting that the swollen polyhedral niosomes in Figure 7b are still faceted in appearance; this indicates that such structures can resist changes in size.

The effect of pH on carboxyfluorescein release

Because carboxyfluorescein is an organic carboxylic acid with a pK_a of 6·4, at pH 5 it is undissociated whereas at pH 8 it is predominantly ionised. Table 3 shows that release of carboxyfluorescein was greatest from polyhedral niosomes irrespective of pH. At pH 8, when the fully dissociated molecule predominates, the release rates were generally higher than at pH 5 (Table 3). Formation of the insoluble form of drugs inside the vesicles can exert a retaining effect on drugs (Vemuri & Rhodes 1994; Lasic et al 1995). At pH 5, carboxyfluorescein (5·0 mM) precipitates from solution overnight. From these studies it is concluded that these two different types of niosome differentiated between ionised and un-ionised species, preferentially retaining the more insoluble species that might have precipitated within the vesicle interior.

Table 3. The percentage release of carboxyfluorescein from niosomes after dispersion in pH 5 and pH 8 buffer.

Time (h)	Polyhedral niosomes		Spherical niosomes	
	pH 5	pH 8	pH 5	pH 8
1	11·6 ± 2·8	13·2 ± 3·2	1·8 ± 0·3	8·3 ± 0·1
2	18·5 ± 2·7	20·5 ± 2·8	2·7 ± 0·4	13·3 ± 0·3
3	23·1 ± 2·3	25·7 ± 2·9	3·7 ± 0·5	16·5 ± 1·4
4	26·4 ± 2·6	30·4 ± 3·3	4·4 ± 0·5	18·8 ± 0·1
5	28·0 ± 3·1	33·8 ± 2·2	4·9 ± 0·5	20·0 ± 0·3

Data are means ± s.d. (n = 3).

Conclusion

Polyhedral niosomes found in cholesterol-poor regions of the $C_{16}G_2$–cholesterol–Solulan C24 ternary phase diagram have less tendency than spherical niosomes to swell and shrink when subjected to osmotic gradients. Spherical niosomes are found in regions where $C_{16}G_2$ and cholesterol are in a 1 : 1 molar ratio. This behaviour is because of a combination of a high membrane permeability to solutes and a more rigid and less deformable membrane structure. High levels (9%) of the polyoxyethylene cholesteryl ether Solulan C24 in polyhedral niosomes reduced the rate of release of carboxyfluorescein when co-entrapped with NaCl. This might be because of salting out of the hydrophilic polyoxyethylene chains, thus reducing the permeability of the membrane to hydrophilic

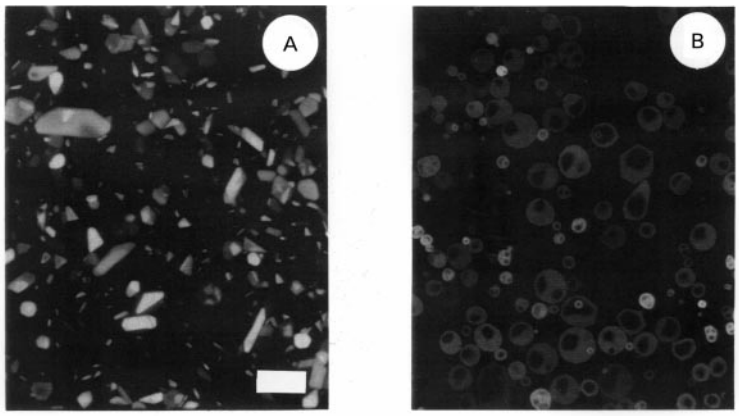

Figure 7. Photomicrographs of polyhedral niosomes ($C_{16}G_2$–Solulan C24, 91 : 9) loaded with carboxyfluorescein in 2 M NaCl after dialysis against 2 M NaCl (A) or water (B) for 3 days at room temperature (bar = 25 μm).

carboxyfluorescein. A study of carboxyfluorescein release at pH 5 and pH 8 revealed that the more soluble form of this aqueous volume marker was released to a greater extent than the insoluble form, both from conventional cholesterol-containing spherical niosomes and from polyhedral niosomes.

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