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Short communication

In vitro/in vivo characterisation of polyhedral niosomes☆

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Abstract

Non-ionic surfactant vesicles (niosomes) formed by a hexadecyl diglycerol ether ($C_{16}G_2$) and a series of polyoxyethylene alkyl ethers exhibit a variety of shapes dependant on their membrane composition. These surfactants form with an equimolar amount of cholesterol a mixture of largely spherical and tubular niosomes. In the absence of cholesterol, they form faceted polyhedral structures. The physicochemical and biological differences between polyhedral and spherical/tubular niosomes were studied. Polyhedral niosomes undergo a reversible shape transformation into spherical structures on heating above their phase transition temperature (T_m). The viscosity of polyhedral niosomes at room temperature is higher than their spherical counterparts due to their faceted and relatively rigid shape, and is more dependant on temperature due to shape transformation. At room temperature, polyhedral niosomes possess more rigid gel phase membranes and are less osmotically sensitive; however, they are more permeable because of a lack of or low levels of cholesterol in their membranes. Polyhedral niosomes loaded with luteinising hormone releasing hormone (LHRH), nonetheless, slow the release of drug compared to solution, albeit to a small extent. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Polyhedral niosomes; Hexadecyl diglycerol ether ($C_{16}G_2$)

1. Introduction

Niosomes are vesicular delivery systems which can be formed by aqueous dispersion of non-ionic surfactant films (Florence, 1993). They are known as analogues of liposomes, and have been used in cosmetic formulations and experimentally as drug carriers (Kerr et al., 1988; Florence and Baillie,

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1989; Uchegbu et al., 1995). Apart from conventional spherical vesicles, various structures of niosomes can be formed by varying the vesicle membrane compositions of certain mixed surfactant systems (Uchegbu and Florence, 1995; Uchegbu et al., 1996). For example, mixtures of hexadecyl diglycerol ether ($C_{16}G_2$): cholesterol: polyoxyethylene 24 cholesteryl ether (Solulan C24) were previously shown to form spherical, tubular, polyhedral and disk-like vesicles, depending on the molar ratio (Uchegbu et al., 1996). Polyhedral niosomes formed by $C_{16}G_2$ and Solulan C24, without cholesterol, possess an unconventional faceted structure which can encapsulate water soluble solutes. The purpose of the work described here was to gain more understanding into the behaviour of these niosomes with regard to their physicochemical and biological properties. The morphology, rheological and osmotic behaviour, and the ability of the polyhedral niosomes to deliver a model peptide as an IM depot were studied.

2. Materials and methods

Niosomes were prepared by hydrating a dry film mixtures of 300 μmol lipid/surfactants with water or 5 mM 5(6)-carboxyfluorescein (Sigma, UK) in phosphate buffered saline (pH 7.4) at 60°C for 1 h and then left to cool at room temperature. The morphology of niosomes formed by $C_{16}G_2$ (L'Oreal, France): Solulan C24 (Ellis & Everald, UK) at the molar ratio of 91:9 were found to be polyhedral in shape. Addition of an equimolar amount of cholesterol (Sigma, UK) to the main surfactant increases the curvature of the membranes and results in the formation of largely spherical vesicles and tubules, as in those formed by $C_{16}G_2$:cholesterol:Solulan C24 (49:49:2) or (45:45:10). The same events were also observed with niosomes formed by replacing $C_{16}G_2$ with any of the non-ionic surfactants from a series of polyoxyethylene alkyl ethers, namely a polyoxyethylene 2 cetyl ether ($C_{16}EO_2$, Brij 52), a polyoxyethylene 5 cetyl ether ($C_{16}EO_5$), a polyoxyethylene 2 stearyl ether ($C_{18}EO_2$, Brij 72), and a polyoxyethylene 5 stearyl ether ($C_{18}EO_5$; Sigma, UK). Formation of polyhedral vesicles in the absence of cholesterol was also found in the mixture of dipalmitoyl phosphatidyl choline (DPPC; Lipoid GmbH, Germany) and Solulan C24 (91:9) but not in the mixture of distearoyl phosphatidyl choline (DSPC; Lipoid GmbH, Germany) and Solulan C24 (91:9). This observation confirms

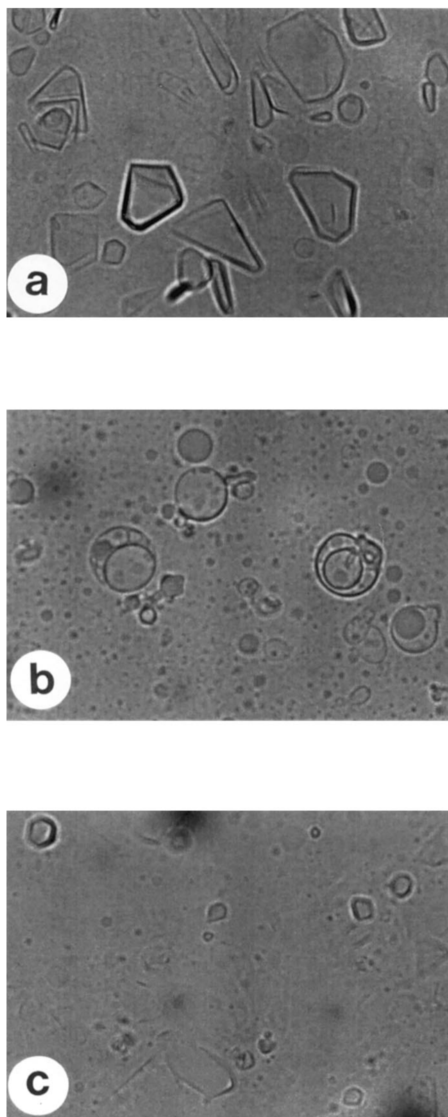

Fig. 1. Photomicrographs of (a) polyhedral niosomes formed by $C_{16}EO_5$:Solulan C24 (91:9) in water at room temperature which undergo a reversible shape transformation into (b) spherical niosomes on heating to 35°C, and then return to (c) polyhedral structures on cooling to 30°C.

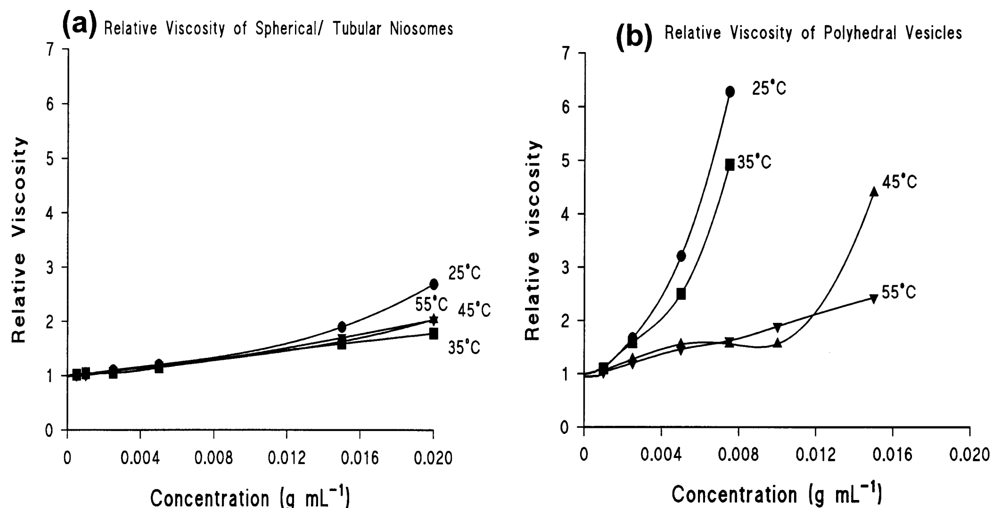

Fig. 2. Relative viscosity of (a) spherical/tubular niosomes formed by $C_{16}G_2$:cholesterol:Solulan C24 (45:45:10) and (b) polyhedral niosomes formed by $C_{16}G_2$:Solulan C24 (91:9) as a function of temperature and lipid/surfactant concentration (from Florence et al., 1999).

that the molecular properties of amphiphiles influence properties of the membranes including their ultrastructures.

3. Results and discussion

Changes in the morphology of polyhedral niosomes are temperature-dependent as well as being sensitive to the presence of cholesterol. Hot stage microscopy (LINKAM system BCS196 with temperature control fitted to a Nikon Microphot FXA light microscope), revealed that polyhedral niosomes undergo a reversible shape transformation into spherical structures on heating above their phase transition temperature (Fig. 1). Phase behaviour was also investigated using high sensitivity differential scanning calorimeter (HSDSC; MicroDSC, Setaram, UK). Approximately 300 mg of each niosome dispersion (60 mM) prepared in water was introduced into the measurement vessel whilst an equivalent mass of water was introduced in the reference vessel. Samples were scanned at a rate of 1 K/min from 10 to 70°C, followed by cooling to 10°C. The results indicate that the membranes of polyhedral niosomes are in the gel state at room temperature, with the shape transformation into spherical niosomes occuring on heating above their phase transition temperature (T_m) in which their membranes are in the liquid-crystalline state.

The rheological properties of polyhedral niosomes were studied using an Ostwald U-tube (size M2, Phillip & Harris, UK) at 25°C ($\pm$0.1°C). Samples were diluted with water to the required concentrations and left to equilibrate for 1 h. Relative viscosity (η_{rel}) was calculated by comparing efflux time with that of water. Fig. 2 compares the relative viscosity of suspensions of polyhedral

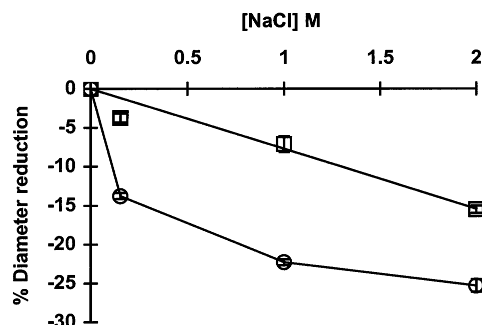

Fig. 3. The reduction in niosome mean diameter (niosomes encapsulating water) 5 h after dispersion in NaCl solutions of (□) polyhedral niosomes—$C_{16}G_2$:Solulan C24 (91:9) and (○) spherical niosomes—$C_{16}G_2$:cholesterol:Solulan C24 (49:49:2) (from Florence et al., 1999).

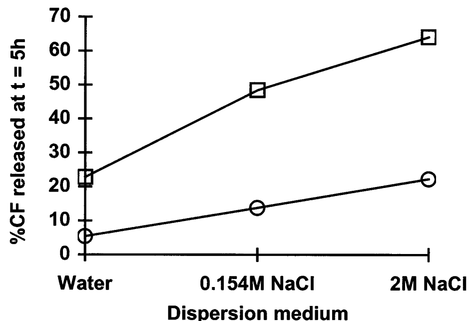

Fig. 4. The cumulative CF released from (□) CF loaded polyhedral niosomes—$C_{16}G_2$:Solulan C24 (91:9) and (○) CF loaded spherical niosomes—$C_{16}G_2$:cholesterol:Solulan C24 (49:49:2) prepared in water 5 h after dispersion in NaCl solutions.

niosomes formed by $C_{16}G_2$:Solulan C24 (91:9) and that of spherical niosomes formed by $C_{16}G_2$:cholesterol:Solulan C24 (45:45:10). At 25 and 37°C, the viscosity of polyhedral niosomes is much higher than that of spherical niosomes due to their faceted structures which resist flow. On increasing the temperature above the transition temperature (T_m of $C_{16}G_2$:Solulan C24 (91:9) is 45°C), polyhedral niosomes undergo transformation into spherical structures, while cholesterol-rich spherical niosomes remain intact. There are thus different temperature effects on the flow pattern of the systems. On increasing the temperature, the viscosity of both decreases due to reduced vesicle-solvent interactions, but the less dramatic decrease of spherical niosomes (Fig. 2), highlights the overriding importance of shape.

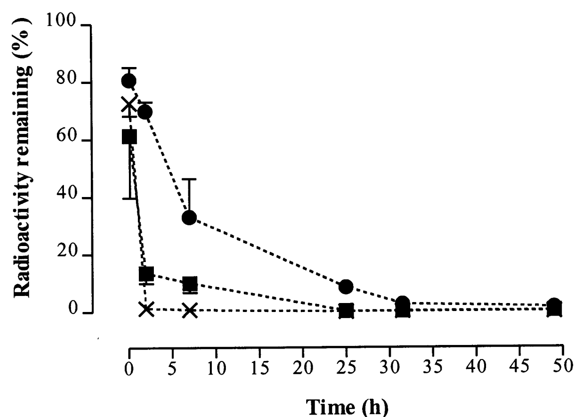

Fig. 5. ^{125}I-LHRH as % remaining at the site of intramuscular injection in rats. ^{125}I-LHRH prepared in PBS at pH 7.4 (X), encapsulated in polyhedral niosomes (■), and in spherical/tubular niosomes (●) (from Arunothayanun et al., 1999).

Osmotic behaviour of gel state polyhedral niosomes formed by $C_{16}G_2$:Solulan C24 (91:9), in comparison to their spherical counterparts formed by $C_{16}G_2$:cholesterol:Solulan C24 (49:49:2), was investigated by challenging the vesicles to a range of osmotic gradients. These were performed by preparing niosomes in water and then dispersing into 0.154, 1.0, or 2.0 M sodium chloride (BDH, UK) solutions, 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). It was found that polyhedral vesicles showed smaller responses to a salt osmotic gradient compared to spherical formulations (Fig. 3). When niosomes were prepared encapsulating CF in water and dispersed into water, 0.154, or 2 M NaCl, the amounts of CF released from the vesicles were measured fluorimetrically (Perkin Elmer LS-3 fluorescence spectrometer; excitation wavelength 486 nm, emission wavelength 514 nm) and were found to be dependent on the salt gradients as a coupled outflux with water (Fig. 4). The higher release of CF from the polyhedral niosomes is due to the lack of membrane cholesterol. This higher permeability of polyhedral niosomes and their faceted structure are likely to be responsible for the lower response to osmotic stress.

Polyhedral and spherical niosomes, prepared from $C_{16}G_2$:cholesterol:Solulan C24 (91:0:9) and (49:49:2), respectively, were studied in vivo using luteinising hormone releasing hormone (LHRH) as a model peptide. ^{125}I-LHRH (Sigma, UK) prepared in PBS (pH 7.4), encapsulated in polyhedral niosomes, or in spherical niosomes were injected intramuscularly at the posterior hind leg of male Wistar rats (Bantin and Kingman Universal Ltd., UK; weight of 190–230 g). Rats were sacrificed at 10 min, 2, 7, 25, 32 and 49 h after injection and the remaining of ^{125}I-LHRH at the site of injections was monitored using a gamma counter (1275 Minigamma Gamma Counter, LKB Wallac, Turku, Finland). It was found that both niosomes are able to protect LHRH from being cleared immediately from the injection site. Fig. 5

shows that 99% ^{125}I-LHRH prepared in PBS was cleared from the site of injection in the first 2 h, while 14 and 70% of injected ^{125}I-LHRH prepared in polyhedral and spherical niosomes, respectively, was still present at the injection site at this time. All the ^{125}I-LHRH in polyhedral niosomes was cleared from the injection site by 25 h, while 8.5% of the radioactivity in spherical niosomes could still be detected. Spherical niosomes possessing the more stable membranes as previously observed, were able to prolong the circulation of ^{125}I-LHRH in the blood.

Insights gained from these studies provide more understanding into various aspects of polyhedral niosomes, whose properties differ from their spherical counterparts. Whether or not polyhedral systems will find a use in drug delivery will depend on whether shape factors can be found to be of greater importance than membrane composition in determining fate in vivo.

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