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The effect of processing variables on the physical characteristics of non-ionic surfactant vesicles (niosomes) formed from a hexadecyl diglycerol ether

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Abstract

Niosomes are vesicles formed by self-assembly of non-ionic surfactants. In this investigation, the effects of processing variables, particularly temperature and sonication, on the physical characteristics and phase transitional behaviour of two niosomal systems based on a hexadecyl diglycerol ether ($C_{16}G_2$) have been studied. Systems containing $C_{16}G_2$, cholesterol and poly-24-oxyethylene cholesteryl ether (Solulan C24) in the molar ratios 91:0:9 and 49:49:2 were prepared by aqueous dispersion of films, followed by examination of 5(6)-carboxyfluorescein entrapment, particle size and morphology. The thermal behaviour was examined using high sensitivity differential scanning calorimetry (HSDSC) and hot stage microscopy, while the effects of sonication were studied in terms of size and morphology, both immediately after preparation and on storing for 1 h at room temperature and 60°C. Polyhedral niosomes were formed from systems containing $C_{16}G_2$ and Solulan C24 alone, while cholesterol-containing systems formed spherical vesicles mixed with tubular structures; the polyhedral systems were found to have a larger particle size and higher CF entrapment efficiency. HSDSC studies showed the polyhedral systems to exhibit an endotherm at 45.4°C and a corresponding exotherm at 39.1°C on cooling which were ascribed to a membrane phase transition; no equivalent transition was observed for the cholesterol containing systems. Hot stage microscopy showed the polyhedral vesicles to convert to spherical structures at $\sim$48°C, while on cooling the spherical vesicles split into smaller structures and reverted to the polyhedral shape at $\sim$49°C. Sonication resulted in the polyhedral vesicles forming spherical structures which underwent a particle size increase on storage at room temperature but not at 60°C. The study suggests that the polyhedral vesicles undergo a reversible transition to spherical vesicles on heating or sonication and that this morphological change may be associated with a membrane phase transition.

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1. Introduction

Niosomes are vesicular delivery systems which are formed via aqueous dispersion of non-ionic surfactant films (Vanlerberghe et al., 1973; Florence, 1993). These systems have been studied as potential delivery vehicles for a range of drugs (Florence and Baillie, 1989) including doxorubicin (adriamycin) (Uchegbu et al., 1995). In addition to conventional spherical vesicles, niosomes may exist in a range of morphological forms depending on membrane composition. In particular, combinations of hexadecyl diglycerol ether ($C_{16}G_2$), cholesterol and poly-24-oxyethylene cholesteryl ether (Solulan C24) have been shown to form spherical, tubular or polyhedral vesicles depending on the proportions of the constituents (Uchegbu et al., 1996). Such systems can entrap a number of solutes and have been studied as drug carriers (Arunothayanun et al., 1997; Uchegbu et al., 1997). However, there is to date little information available on the effects of processing conditions on the behaviour of these systems; such effects are of considerable importance in the development of these vesicles as viable delivery forms. In addition, given the clear similarities between niosomes and liposomes, information regarding the phase transitional behaviour of the former is scarce. Such information is recognised as being of fundamental importance in the liposome field (Taylor and Morris, 1995), primarily because liposome formation takes place at a temperature above the gel to liquid crystal transition (Bangham et al., 1965). In addition, the phase behaviour determines the temperature dependence of the drug release properties (Papahadjopoulos et al., 1973) and may give an indication of drug location within the lipid bilayers (Fildes and Oliver, 1978). In this investigation, the effects of two processing variables (temperature cycling and sonication) on vesicle size, entrapment efficiency and morphology have been studied using two membrane compositions. Furthermore, the novel use of differential scanning calorimetry (DSC) as a means of characterising the phase transitional behaviour of the vesicles is described. In this manner it is intended that the relationship between membrane composition, vesicle physical characteristics and phase transitional behaviour may be elucidated.

2. Experimental

2.1. 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 and Everald (UK). Cholesterol and 5(6)-carboxyfluorescein (CF) were obtained from Sigma (UK). Chloroform (HPLC grade) was purchased from Rathburn (UK). The water source was an ultra high quality reverse osmosis water purifier (Elgastat UHQPS, Elga, UK).

2.2. Preparation of niosomes

Niosomes containing molar ratios of 91:9 $C_{16}G_2$: Solulan C24 and 49:49:2 $C_{16}G_2$: cholesterol: Solulan C24 were prepared. Dried films were prepared by dissolving the lipid and surfactant in chloroform followed by evaporation under vacuum at 60°C. Vesicles were prepared by hydrating a dried film mixture of 300 μmol lipid/surfactants with 5 ml of water (or, where stated, 5 mM CF prepared in phosphate buffered saline (PBS) pH 7.4) at 60°C for 1 h under gentle agitation, resulting in suspensions containing 60 mM lipid/surfactant. The dispersions were then left to cool to room temperature prior to experiments. The unsonicated niosomes were sized by laser diffraction (MasterSizer X, Malvern, UK).

2.3. Separation of unencapsulated material and determination of encapsulation efficiency

First 1 ml of the CF loaded niosome dispersion was washed by dilution with 7 ml of PBS (pH 7.4) and ultracentrifuged at $200\,000 \times g$ (Sorvall

Combi Plus, Sorvall, UK) at 4°C for 45 min. The supernatant was then discarded and the niosome pellets were resuspended with PBS before washing again. The washed pellets were then resuspended 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 and assay were performed within 2–3 h of preparation.

To quantify the amount of entrapped CF, 0.1 ml of the dispersions was added into the 10-ml volumetric flask. To the flask was added 1 ml of isopropanol to disrupt the niosomes. The volume was then made up with PBS. Further dilutions may be prepared in the case of the solution being too concentrated. A calibration plot was produced by diluting stock solutions of CF with PBS whilst ensuring that final dilutions contained 10% v/v isopropanol. The fluorescence of these solutions was measured on a Perkin Elmer LS-3 fluorescence spectrometer (excitation 486 nm, emission 514 nm). Encapsulation efficiency was calculated and expressed as a percentage of the available hydrating solute actually encapsulated.

2.4. Investigation into the phase transitional behaviour using HSDSC

Studies were conducted using a high sensitivity differential scanning calorimeter (HSDSC) (MicroDSC, Setaram, UK). First, ~ 300 mg of each niosomal dispersion prepared in water was introduced into the measurement vessel whilst an equivalent mass of water was introduced in the reference vessel. The difference in heat flow between both vessels was then measured as a function of temperature. Samples were scanned at a rate of 1 K/min from 10 to 70°C, followed by cooling to 10°C. Studies were conducted in duplicate with excellent reproducibility between runs.

2.5. Morphological studies

Changes in morphology of unsonicated niosomes loaded with CF were observed using a LINKAM system (BCS196) with temperature control fitted to a Nikon Microphot FXA light microscope. Transmission electron microscopy was also performed in freshly sonicated and unsonicated niosomes with a Philips 201 TEM using a voltage of 100 kV; the vesicles were stained with 1% aqueous phosphotungstic acid prior to viewing.

2.6. Size stability of sonicated niosomes

Polyhedral and spherical/tubular niosome dispersions formed by $C_{16}G_2$: cholesterol: Solulan C24 were probe sonicated for 4 min using an MSE PG100 150-W probe sonicator with the instrument set at 15% of its maximum power output followed by storage at either room temperature or 60°C. Size stability after sonication of submicron niosomes was followed by using the photon correlation spectroscopy (PCS) on a Malvern AutoSizer 2C (UK) at 25°C by diluting the dispersion to 4 ml in filtered (0.22-μm pore size) water. Results are expressed as the *z*-average mean diameter.

3. Results and discussion

3.1. Effects of membrane composition on vesicle shape, size, and entrapment efficacy

Fig. 1a shows that vesicles formed by $C_{16}G_2$: Solulan C24 (91:9) posses faceted membranes and can entrap a hydrophilic solute, CF, inside the vesicles, as reported by Uchegbu et al. (1997). We have also found that $C_{16}G_2$ alone can form polyhedral niosomes, as the addition of low amounts of Solulan C24 does not affect the shape of the vesicles, but provides a steric barrier on the vesicle surface which can prevent aggregation. In contrast, vesicles formed by $C_{16}G_2$: cholesterol: Solulan C24 (49:49:2) appear to be largely spherical in shape with some tubules in evidence (Fig. 1b). The mean sizes of the polyhedral and spherical/tubular niosomes formed by $C_{16}G_2$: cholesterol: Solulan C24 in the ratios 91:0:9 and 49:49:2 were 8.0 ± 0.3 and 6.6 ± 0.2 μm, respectively (Fig. 2), indicating that the polyhedral vesicles were slightly larger. The entrapment of CF inside the polyhedral niosomes was found to be higher than in the spherical/tubular niosomes (Fig. 2); this

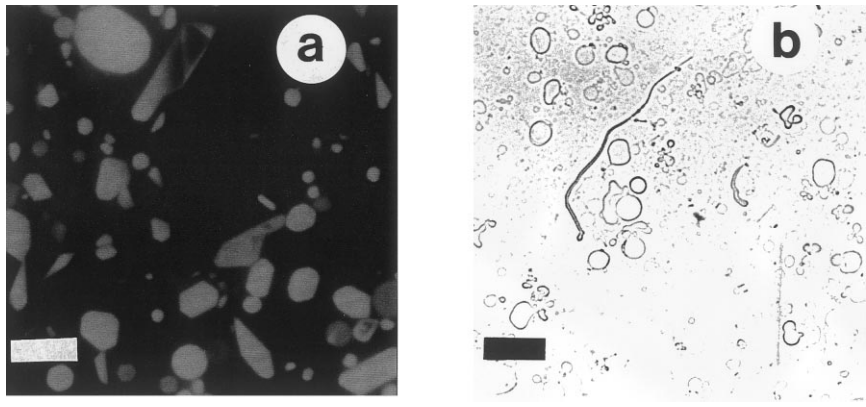

Fig. 1. (a) A fluorescence photomicrograph of CF loaded polyhedral niosomes formed by $C_{16}G_2$: Solulan C24 (91:9). (b) Niosomes formed by $C_{16}G_2$: cholesterol: Solulan C24 (49:49:2) are mixtures of largely spherical vesicles and some tubules. Bar = 20 μm.

may be a function of the size and shape of the polyhedral vesicles, as well as differences in membrane flexibility during formation. In addition, most of the tubules cannot be observed under fluorescent light, implying that they do not entrap CF to any appreciable extent, leading to a reduction in the overall entrapment within the spherical/tubular systems.

3.2. Phase transition behaviour of polyhedral and spherical/tubular niosomes

The DSC traces of polyhedral niosomes formed by $C_{16}G_2$: Solulan C24 (91:9) generally show a main endothermic transition on heating and a main exothermic transition (Fig. 3a) with a small second exothermic transition on cooling (Fig. 3b). The temperature and the energy associated with the transitions are shown in Table 1. It is well established that such thermal events represent transitions from the gel to the liquid crystalline state in liposomes. While it is not clear at this stage whether the transitions observed here have the same origin, it is reasonable to suggest that the events represent phase changes in the vesicle bilayers.

In contrast to the polyhedral systems, no equivalent transition was observed in the spherical/tubular niosomes formed by $C_{16}G_2$: cholesterol: Solulan C24 in the ratio 49:49:2, the DSC trace showing only a flat baseline over the temperature range studied. Increasing the amount of cholesterol into the membranes of liposomes has been found to increase membrane fluidity to the extent where the phase transition is abolished (Chapman, 1968; Taylor and Morris, 1995), hence an analogous process may be taking place here. It should also be appreciated, however, that temperature-induced changes in the distribution of vesicle components is a possibility that may not be fully excluded at this early stage of development of these systems.

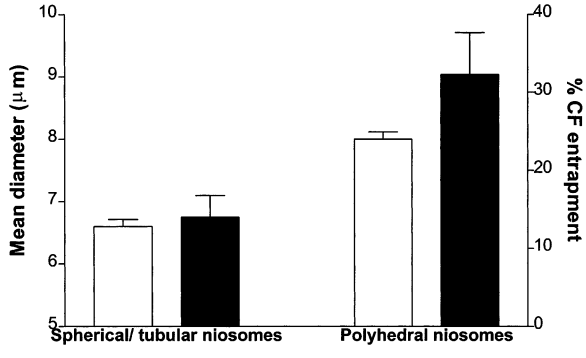

Fig. 2. The diameter (white bars) and % entrapment (solid bars) of CF loaded unsonicated spherical/tubular niosomes ($C_{16}G_2$: cholesterol: Solulan C24, 49:49:2) and polyhedral niosomes ($C_{16}G_2$: Solulan C24, 91:9). Data is presented as mean and S.D. ($n = 3$).

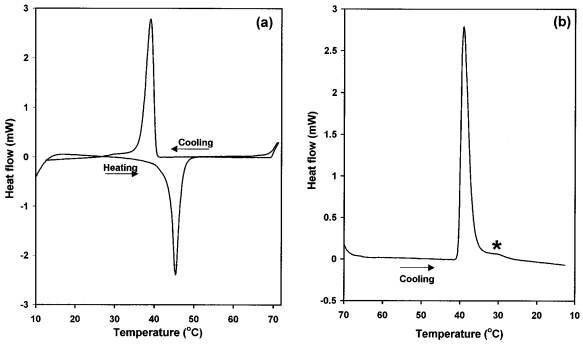

Fig. 3. (a) A DSC trace of polyhedral niosomes ($C_{16}G_2$: Solulan C24, 91:9) which shows a main endothermic transformation at 45°C on heating from 10 to 70°C, with an exothermic transformation detected at 39°C on cooling. (b) A second transition was also detected at 30°C (*) after the main exothermic peak.

Table 1
Phase transition parameters collected from the DSC traces of polyhedral niosomes formed by $C_{16}G_2$: Solulan C24 (91:9) using HSDSC[a]

Temperature scanning cycle	Peak temperature (°C)	Enthalpy (J/g)
Endothermic transition (heating from 10 to 70°C)	45.38	1.5195
Exothermic transition (cooling from 10 to 70°C)	39.08	−1.5599

[a] Data are presented as mean ($n = 2$).

3.3. Temperature-induced vesicle shape transformation

Initial observations of the freshly prepared polyhedral niosomes revealed that these systems are first formed as spherical vesicles at 60°C which, on cooling to room temperature, transform into polyhedral structures. The temperature dependence of the vesicle morphology was therefore studied using the hot stage microscopy. Fig. 4a shows a polyhedral vesicle formed by $C_{16}G_2$: Solulan C24 (91:9) at 25°C which, on heating, transformed into a spherical vesicle at 48°C (Fig. 4b). On cooling from 55°C, the vesicle produced a cluster of smaller spherical niosomes at 49°C (Fig. 4c) before reverting to the polyhedral structures at 35°C (Fig. 4d). It was found, therefore, that the temperature at which the membranes of polyhedral niosomes underwent a phase change (T_c) observed by HSDSC (45.4°C) corresponds reasonably well to the temperature in which the change of the polyhedral structure into the spherical shape is observed by hot stage microscopy (48°C). The data presented here suggests that the phase change observed on increasing the temperature results in a change in vesicle ultrastructure, presumably due to the alteration in membrane flexibility above this temperature. In contrast, no shape transformation was found on heating spherical/tubular niosomes formed by $C_{16}G_2$: cholesterol: Solulan C24 (49:49:2) from room temperature to 70°C as their membranes are already in the liquid state, as shown by HSDSC. HSDSC therefore allows precise quantification of the temperature and energy associated with this transition.

3.4. Size stability of sonicated polyhedral niosomes

Fig. 5 shows the initial sizes after sonication and the size stability on storage at room temperature or 60°C for the two niosomal systems. Transmission electron micrographs (TEM) of unsonicated polyhedral niosomes revealed that the nanometer range fraction of the vesicle population were polyhedral in shape (Fig. 6a). However, the freshly sonicated polyhedral niosomes were seen to consist of spherical vesicles in the nanometer range (Fig. 6b), with no large polyhedral vesicles seen using light microscopy.

When the size of $C_{16}G_2$ niosome dispersions was reduced by sonication and the dispersions were stored at room temperature, a difference in the stability was observed between the formulations (Fig. 5). Cholesterol- rich spherical/tubular $C_{16}G_2$ niosomes did not show an alteration in size on storage at room temperature, under the experimental conditions used, whereas sonicated polyhedral niosomes were found to be stable when stored at high temperature (60°C) but not at room temperature.

On storing sonicated $C_{16}G_2$: Solulan C24 (91:9) systems at room temperature for 1 h, large polyhedral vesicles were again visible by light microscopy. Taken together with the observations outlined above, these data indicate that in addition to size reduction, sonication leads to the formation of spherical as opposed to polyhedral vesicles. On storage above the transition temperature, the vesicles remain stable due to the membrane existing in the $> T_c$ state, for which the spherical ultrastructure is the thermodynamically favoured form. However, storage below T_c leads to both an increase in particle size and the formation of polyhedral vesicles. Consequently, the apparently counterintuitive observation that the sonicated systems are more stable at elevated temperatures may be interpreted in terms of the conformational state of the vesicle membrane.

4. Conclusions

The study has indicated that $C_{16}G_2$: Solulan C24 (91:9) films form polyhedral vesicles on hydration which may be converted to a spherical ultrastructure on exciting the membrane, either by heating above the transition temperature (identified using HSDSC) or by sonication. The effect of these processes on vesicle shape and associated membrane conformation is summarised in Fig. 7. It is suggested that in both cases such excitation leads to an increase in membrane fluidity which

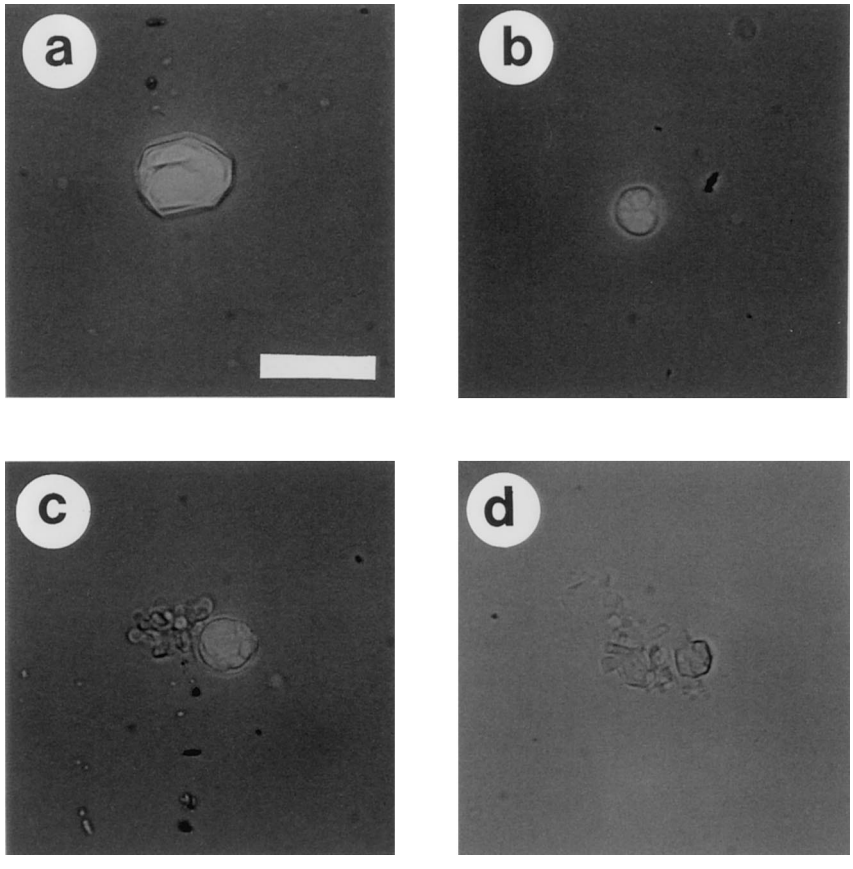

Fig. 4. Shape transformation of a large polyhedral vesicle formed by $C_{16}G_2$: Solulan C24 (91:9) at (a) 25°C to (b) spherical structure at 48°C was observed on heating. On cooling from 55°C, the spherical vesicle split into (c) a group of small spherical vesicles at 49°C and then again formed (d) polyhedral vesicles at 35°C. Bar = 20 μm.

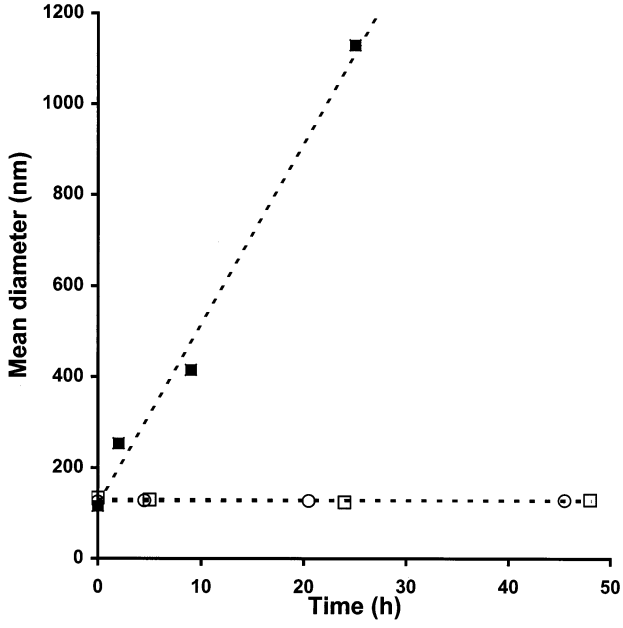

Fig. 5. Diameter as a function of time and temperature of sonicated polyhedral niosomes ($C_{16}G_2$: Solulan C24, 91:9) stored at (■) 25°C and (□) 60°C, and (○) sonicated spherical/tubular niosomes ($C_{16}G_2$: cholesterol: Solulan C24, 49:49:2) stored at 25°C.

favours a spherical vesicle conformation. Storage of the sonicated vesicles below the transition temperature results in both an increase in particle size and conversion to the polyhedral shape which is consistent with the above hypothesis. Addition of cholesterol to the membrane leads to spherical vesicles which were stable on sonication under the study conditions. The presence of cholesterol was found to abolish the phase transition in a manner which may be analogous to that observed for liposomes. It is further suggested that the accompanying increase in membrane fluidity on addition of cholesterol may favour the formation of spherical vesicles, leading to their increased storage stability after sonication.

We believe that these observations may have important implications for the development of polyhedral vesicles as drug delivery systems. Knowledge of the phase transition behaviour is clearly essential in order to optimise product performance and HSDSC appears to provide an excellent means of identifying the temperature and energetics of this event. Such information allows prediction of vesicle conformation and stability and opens up several possibilities for further refinement. For example, the study of the effects of drug incorporation on T_c may allow identification of the location of that drug within the vesicle. Alternatively, manipulation of the transition temperature may facilitate the development of thermally responsive delivery systems.

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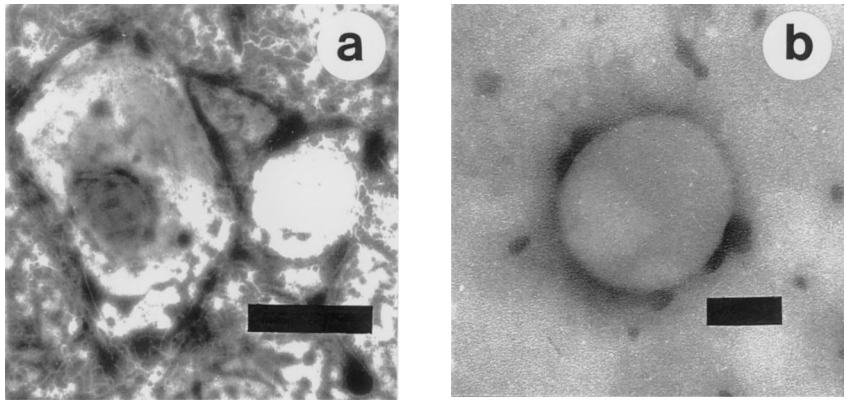

Fig. 6. Transmission electron micrographs of freshly prepared polyhedral niosomes formed by $C_{16}G_2$: Solulan C24 (91:9) (a) unsonicated (bar = 2 μm), and (b) sonicated (bar = 0.4 μm).

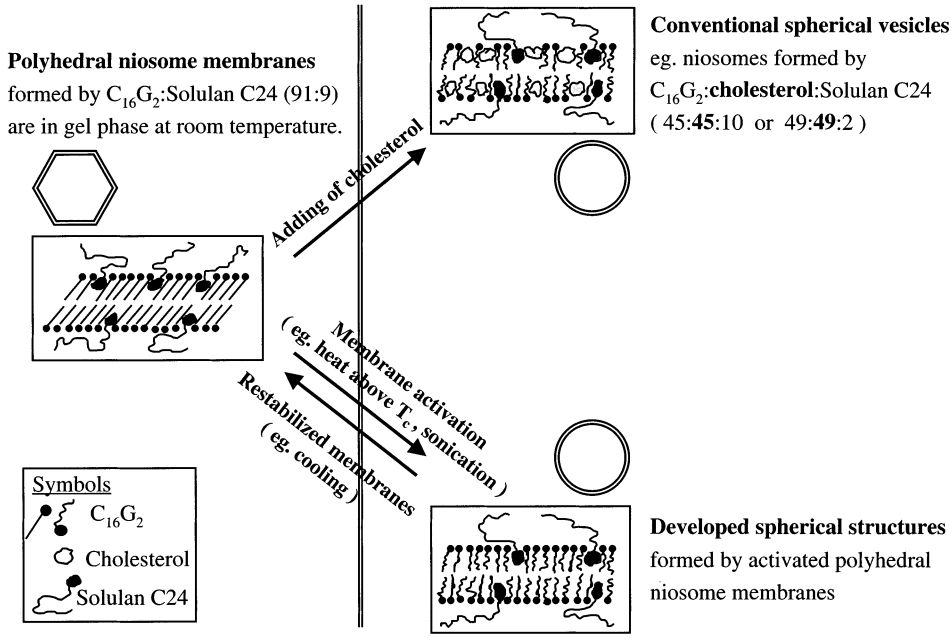

Fig. 7. The schematic represents factors affecting shape and molecular conformation of polyhedral niosomes.

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