J. Pharm. Pharmacol. 1997, 49: 606–610
Received January 27, 1997
Accepted February 28, 1997

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Polyhedral Non-ionic Surfactant Vesicles

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Abstract

Large polyhedral (2–10 μm) non-ionic surfactant vesicles (niosomes) formed from mixtures of a hexadecyl diglycerol ether ($C_{16}G_2$), a cholesteryl poly-24-oxyethylene ether (solulan C24) and a low level of cholesterol are being investigated as slow-release systems for ophthalmic, subcutaneous or intramuscular administration.

The phase-diagram of this three-component system has been constructed and these polyhedral vesicles are found to be in the gel (L_β) phase. Confocal laser-scanning microscopy was used to confirm the complex morphology of these vesicles. The thermo-responsive nature of release of entrapped carboxyfluorescein and nicotinamide adenine dinucleotide has been studied; release is increased with increase in temperature (37°C) even though the polyhedral vesicles still maintain their polyhedral shape at this temperature.

The results indicate that the thermo-responsive features of the niosomes are a result of reversible changes in bi-layer permeability caused by temperature-mediated alteration in the membrane-packing characteristics of the polyethoxylated cholesterol ether.

Water dispersible, poorly soluble amphiphiles, including non-ionic surfactants are known to self-assemble into spherical uni- or multi-lamellar vesicles (Uchegbu & Florence 1995). However, various tubular (Furhop & Helfrich 1993; Chiruvolu et al 1994; Uchegbu & Florence 1995), disc-like (Walter et al 1991; Lasic 1992; Uchegbu et al 1992) and toroidal (genus 2) (Isenberg 1992; Lipowsky 1995; Michalet & Bensimon 1995) vesicle structures also form as a result of amphiphile self-association. Highly structured 'geodesic' multivesicular structures arising from the association of many small non-ionic surfactant vesicles (niosomes) have been described by us (Sternberg et al 1995). The basic geometric parameters governing the self-assembly of amphiphiles into vesicles have been established, but the physicochemical parameters dictating the formation of the various forms of vesicles that arise from mixtures of surfactants still remain unclear. We describe here the formation of large polyhedral vesicles (niosomes) with sizes ranging from 2–10 μm in their largest dimension. They are prepared from a mixture of two non-ionic surfactants–a poorly soluble hexadecyl diglycerol ether ($C_{16}G_2$) (Florence et al 1990) and the water-soluble poly-24-oxyethylene cholesteryl ether (Solulan C24; Fig. 1) with and without dicetyl phosphate (DCP).

Materials and Methods

$C_{16}G_2$ was a gift from L'Oreal, France. Solulan C24 was obtained from DF Anstead, UK. Carboxyfluorescein (CF), nicotinamide adenine dinucleotide (NAD) and cholesterol were obtained from Sigma, UK. All organic solvents were of analytical grade and were obtained from Rathburn Chemicals, UK. Dialysis tubing was obtained from Medicell International, UK. Water was obtained from an Elgastat UHQ system.

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Polyhedral niosome preparation

Vesicles were prepared by the hydration of 60 mM $C_{16}G_2$–Solulan C24 (91:9) with 10 mL 1·5 mM CF or 10 mL NAD (10 mM) at 60°C, then exhaustive dialysis against Tris-buffered saline (TBS; pH 7·4) using Visking dialysis tubing (molecular weight cut off 12 000–14 000 Daltons) at 5°C.

Preparation of the $C_{16}G_2$–cholesterol–Solulan C24 ternary phase-diagram

Phases were examined after the hydration of thin films of surfactant–lipid mixtures (total concentration of surfactant and lipid, 60 mM) at 60°C with water or 1·5 mM CF. Vesicular structures were studied by optical microscopy and the presence of micelles was deduced by the formation of a clear isotropic

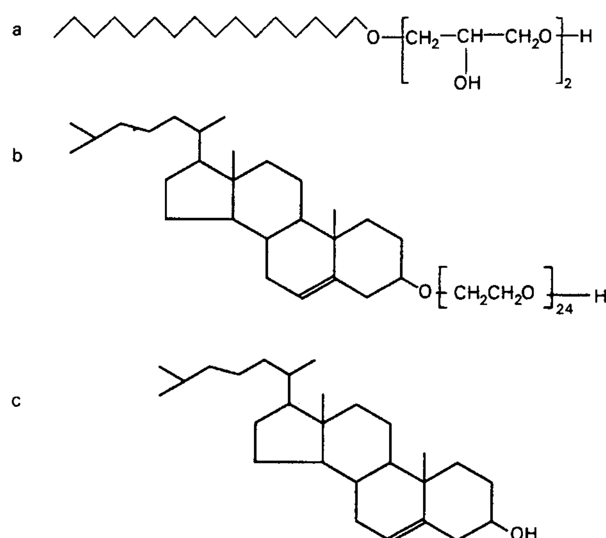


FIG. 1. The structures of hexadecyl diglycerol ether (a), $C_{16}G_2$, cholesteryl poly (24) oxyethylene cholesteryl ether (Solulan C24) (b) and cholesterol (c).

liquid when the surfactant(s) were dissolved in water. Vesicles were sized by photon correlation spectroscopy and laser diffraction-both from Malvern Instruments, UK.

The release of CF from niosomes

Polyhedral niosomes prepared as described above were assayed for entrapment of CF by disruption of the niosome membrane with isopropanol (0·1 mL of the vesicular dispersion to 1 mL of isopropanol). The released CF was then estimated by fluorimetry (excitation 386 nm, emission 418 nm). Polyhedral niosomes were also assayed in a similar manner for entrapment of NAD except that NAD was estimated by ultraviolet (UV) spectrophotometry at 259 nm.

The release of CF and NAD from the niosomes was assessed by suspension of the CF-containing niosomes in Visking dialysis tubing within a thermostatted medium (TBS; pH 7·4). The concentration of CF or NAD in the dialysate was estimated by periodic sampling of the dialysate then fluorimetry as described above.

Confocal laser-scanning microscopy (CLSM)

For the observation of CF fluorescence the 488 nm line of the argon laser was attenuated by a factor of 10^2–10^3 and the dichromatic beam-splitter (> 510 nm) and band pass filter (510–521 nm) were set to maximize selective detection of emitted light. The pixel resolution (128 × 128) and spatial sampling were adjusted to prevent any distortions in the three-dimensional reconstruction (Software packages IKD (International Data Language) 3·9).

Conventional fluorescence microscopy

A Nikon Microphot FXA equipped with a fluorescent light source was also used for further characterization.

Vesicle phase behaviour

The membrane-phase transition behaviour of these polyhedral niosomes was studied by differential scanning calorimetry (Perkin-Elmer 7 series thermal-analysis system, heating rate 5° min^{-1}). The phase behaviour of various surfactant mixtures was also studied by time-resolved X-ray diffraction (Caffrey & Hing 1987; Caffrey 1989).

Results

Thin films of vesicle suspensions were observed by confocal laser-scanning microscopy and images rapidly collected (scan time ~ 1 s $frame^{-1}$) to minimize artefacts arising from vesicle motion. Although the possibility of gross translational movement of the vesicles can be discounted, minor membrane fluctuations cannot. Images produced from the three-dimensional reconstruction of confocal data show clearly the non-spherical nature of the structures (Fig. 2). The vesicles are seen to have between 4 and 12 straight edges of similar length. The stable polyhedral vesicles described here have been found not only to have pronounced straight edges but also to have a flattened contour so that, in cross section, they resemble erythrocytes (Fig. 2). This is the first report of polyhedral self-assembled structures. Above the phase-transition temperature the more typical rounded morphology is observed.

The membrane phase-transition behaviour of these polyhedral niosomes revealed a single phase transition endotherm centred at 44°C. Time-resolved X-ray diffraction measurements on various surfactant mixtures also confirmed the polyhedral niosomes to be in the gel (L_β) state at the temperature of observation and in the fluid (L_α) state above the phase-transition temperature (Table 1).

These large polyhedral structures, previously not reported in surfactant systems, are stable for at least 36 days (Fig. 2c) and can encapsulate and slowly release aqueous markers, such as CF (Fig. 3), and nucleotides, such as NAD (Fig. 4). This polyhedral shape is also observed in $C_{16}G_2$ niosomes prepared in the absence of Solulan C24 (Fig. 5). A small amount of dicetyl phosphate was added to prevent vesicle aggregation. Polyhedral vesicular structures exist in a defined region of the ternary $C_{16}G_2$–Solulan C24–cholesterol phase-diagram, characterized and drawn in Fig. 6, after the hydration of mixtures in water, confirming structures obtained by fluorescence microscopy. Heating the discome structures above 35°C also furnished a clear liquid.

The polyhedral niosomes formulated with Solulan C24 were found to have thermo-responsive solute-release characteristics at a temperature below the phase-transition temperature (Tm, 44°C) of the vesicles (Fig. 3).

Discussion

Electron microscopy has been used to study the faceted microstructure of certain much smaller phospholipid assemblies (Anderson et al 1995), but in the current study, in addi-

Table 1. Phase transitions of the system hexadecyl diglycerol ether ($C_{16}G_2$)-poly-24-oxyethylene cholesteryl ether (Solulan 24) and water over the range 15 to 55°C.

$C_{16}G_2$–Solulan C24	Transitions	
	[Water] = 55%	[Water] = 80%
100:0	Gel (L_β)→fluid (L_α) state at 42·5°C	Gel (L_β)→fluid (L_α) state at 43°C
90:10	Gel (L_β)→fluid (L α) state at 41°C	Gel (L_α)→fluid (L_α) state at 43°C
80:20	Gel (L_β)→fluid (L_α) state at 39°C	Gel (L_β + diffuse band*→diffuse band at 42°C
70:30	Gel (L_β) →fluid (L_α) state at 38°C	Gel (L_β) + diffuse band at 40°C
60:40	Gel (L_β)→fluid (L_α) state from 28 to 38°C	Gel (L_β) + diffuse band at 40°C
50:50	Gel (L_β)→fluid (L_α) state from 28 to 37·5°C	Gel (L_β) + diffuse band at 40°C
40:60	Gel (L_β)→fluid (L_α) state from 31 to 36·5°C	Gel (L_β) + diffuse band at 39°C
30:70	Gel (L_β)→hexagonal phase at 35°C	Phase separation
20:80	Gel (L_β)→hexagonal phase at 35°C	Phase separation
10:90	Hexagonal phase	Isotropic liquid
0:100	Cubic phase	Isotropic liquid

*Probably related to the presence of the L_α phase.

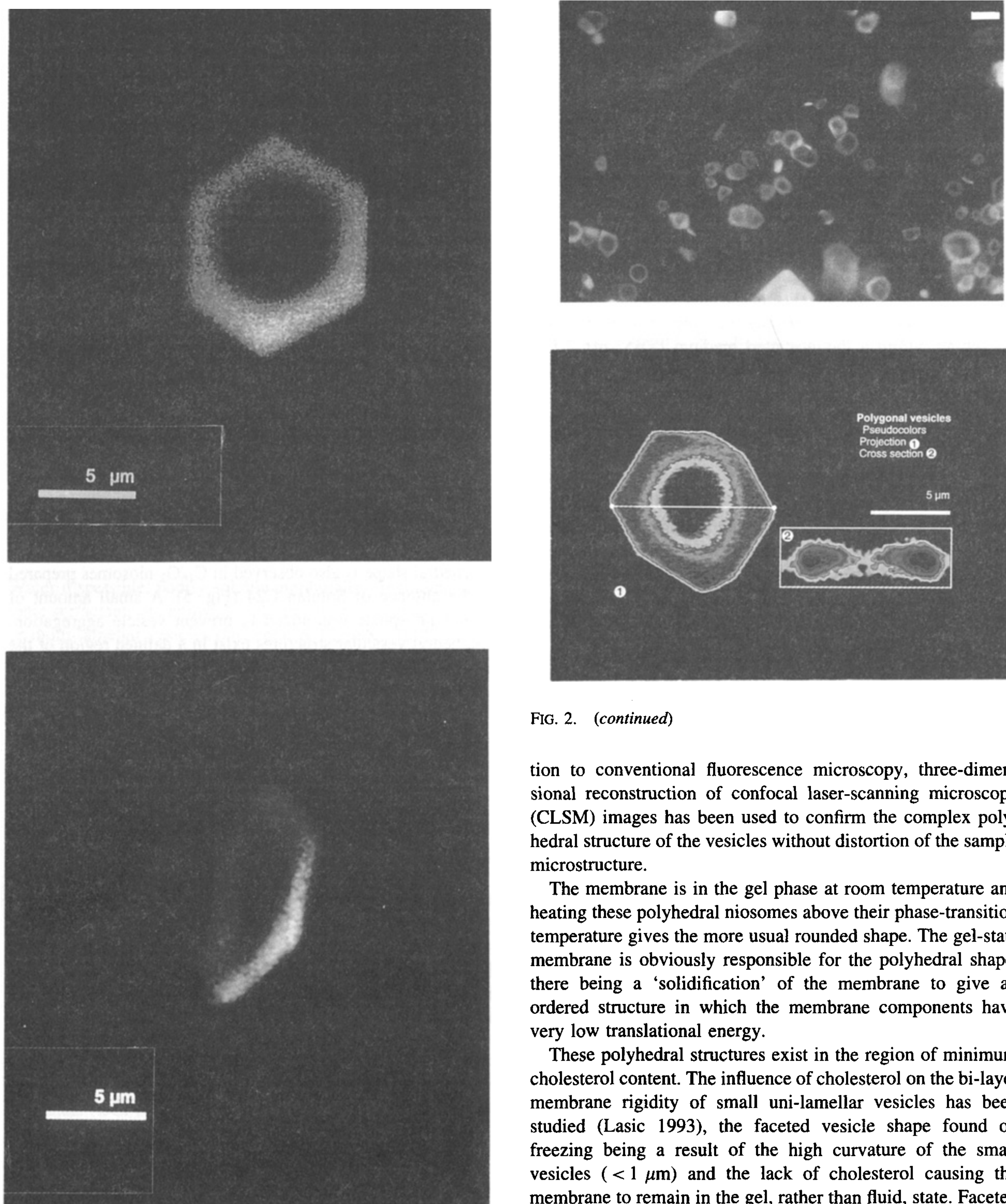


FIG. 2. The three-dimensional reconstruction from a series of confocal laser-scanning micrographs of polyhedral vesicles prepared from a mixture of $C_{16}G_2$ and solulan C24 (91:9). a. Vesicles viewed over their wide axis, b. viewed over a narrower axis (×4400), c. fluorescence micrograph of polyhedral vesicles, prepared as described in a, after storage for 36 days (×1000; bar = 10 μm; note the three associated polyhedral vesicles which appear fused and a further three associated vesicles which appear not to be fused), d. Seudocolour images of polyhedral vesicles, prepared as described above, viewed over the wide axis (1) and over the narrower axis (2) (× 6000). The variation in vesicular volume is shown by the varying fluorescence intensity.

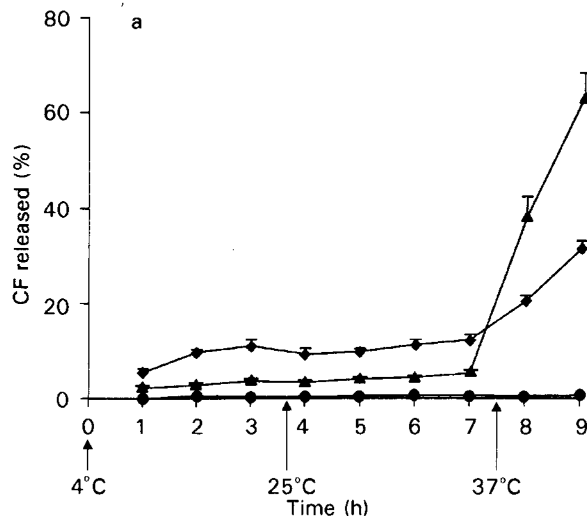
FIG. 2. *(continued)*

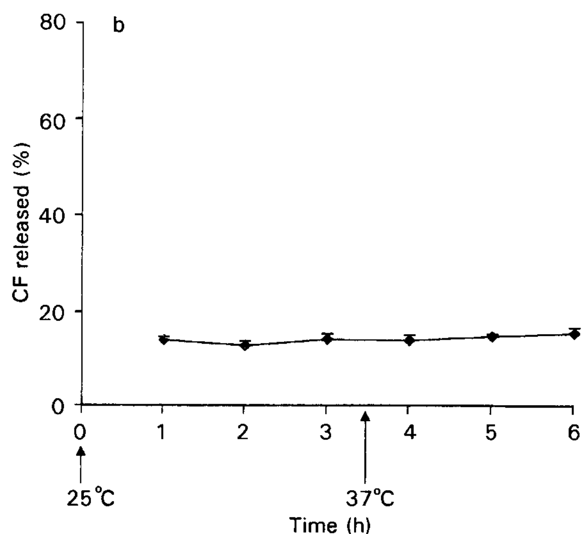
tion to conventional fluorescence microscopy, three-dimensional reconstruction of confocal laser-scanning microscopy (CLSM) images has been used to confirm the complex polyhedral structure of the vesicles without distortion of the sample microstructure.

The membrane is in the gel phase at room temperature and heating these polyhedral niosomes above their phase-transition temperature gives the more usual rounded shape. The gel-state membrane is obviously responsible for the polyhedral shape, there being a 'solidification' of the membrane to give an ordered structure in which the membrane components have very low translational energy.

These polyhedral structures exist in the region of minimum cholesterol content. The influence of cholesterol on the bi-layer membrane rigidity of small uni-lamellar vesicles has been studied (Lasic 1993), the faceted vesicle shape found on freezing being a result of the high curvature of the small vesicles (< 1 μm) and the lack of cholesterol causing the membrane to remain in the gel, rather than fluid, state. Faceted phospholipid vesicles have also been studied by electron microscopy by use of rapid freezing techniques (Anderson et al 1995). No freezing or cooling below ambient temperature occurred in the current study.

Faceted vesicles have also been found during the characterization of non-ionic surfactant vesicles as drug delivery vectors using the surfactants $C_{16}G_3$ (a hexadecyl triglycerol ether) (Rogerson 1986) and $C_{16}EO_5$ (a hexadecyl poly-5-oxyethylene ether) (van Hal 1994), although the three-dimensional structures of these vesicles were not reported. The





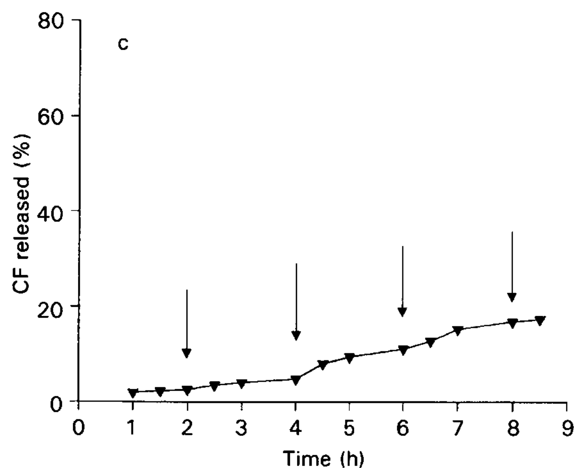


FIG. 3. The release of CF from exhaustively dialysed CF niosomes. The temperature of the surrounding medium was altered as shown by the arrows. All data points represent the mean ± s.e.m. of at least three measurements. a. Polyhedral niosomes prepared from $C_{16}G_2$–Solulan C24 (91:9) (◆) and $C_{16}G_2$–Solulan C24 (95:5) (▲) and spherical/tubular niosomes prepared from $C_{16}G_2$–cholesterol–Solulan C24 (45:45:10) (●), b. polyhedral niosomes prepared from $C_{16}G_2$–DCP (98:2), c. polyhedral niosomes prepared from $C_{16}G_2$–Solulan C24S–DCP (88·5:6·5:5); arrows indicate points at which the medium was heated to 37°C for 10 min.

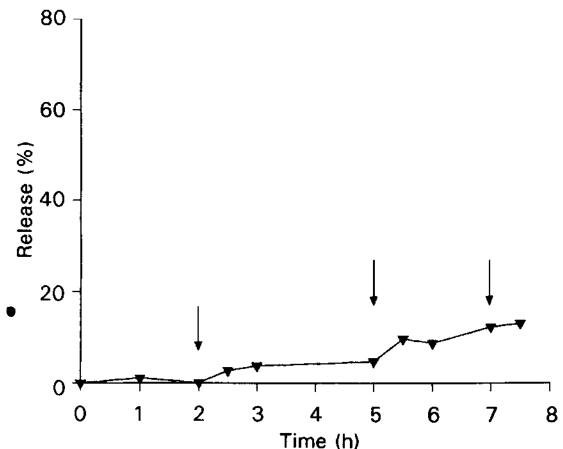


FIG. 4. The release of NAD from polyhedral niosomes prepared from $C_{16}G_2$–Solulan C24 (91:9).

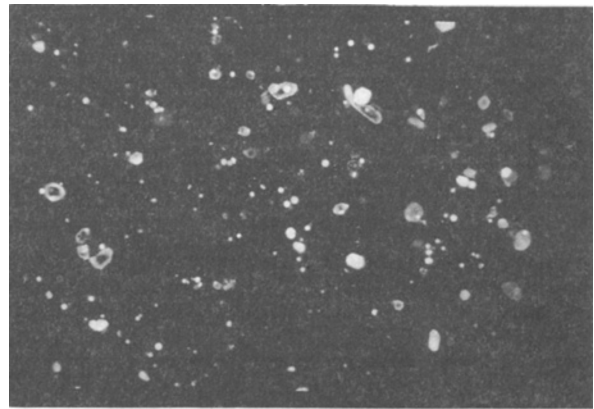


FIG. 5. × 500 Fluorescence micrograph of polyhedral vesicles prepared from a mixture of $C_{16}G_2$ and DCP (98:2).

release of CF from gel-phase polyhedral niosomes and fluid-phase spherical or tubular niosomes (Fig. 3a) revealed that release of CF or NAD from polyhedral niosomes containing solulan C24 increased when the temperature of the dispersion was raised from 25°C to 37°C. However when solulan C24 was omitted from the polyhedral niosome formulation (Fig. 3b) elevation of the temperature had no effect on the rate of release of CF. These results indicate that the thermo-responsive features observed with these polyhedral solulan C24-containing niosomes are a result of the changes in bi-layer permeability caused by temperature-mediated alteration in the membrane-packing characteristics of the polyethoxylated cholesterol ether. The phenomenon was reversible (Fig. 3c, Fig. 4); a reversible vesicular thermo-responsive systemhas previously been observed only for thermo-responsive liposomes containing the thermo-responsive polymer *N*-isopropylacrylamide (Kono et al 1994).

These polyhedral systems are being investigated as slow-release depot systems for ophthalmic, subcutaneous or intramuscular administration.

Acknowledgements

We acknowledge the co-operation of the Institut für Pathologie, Gesellschaft für Strahlen und Umweltforshung, Neuherberg, Germany, for providing access to the confocal laser-scanning microscope.

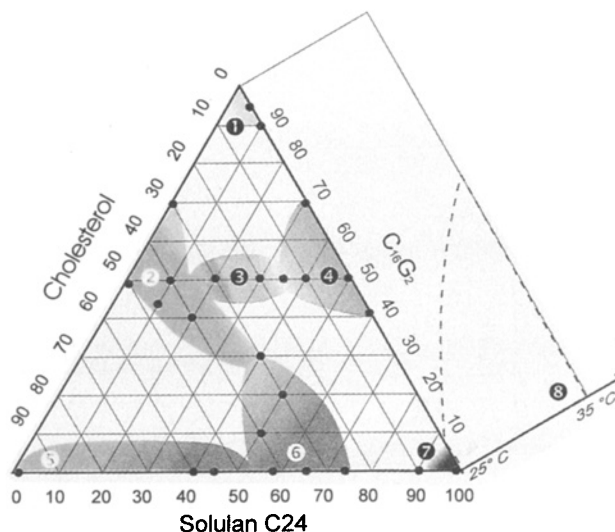


FIG. 6. The $C_{16}G_2$–cholesterol–Solulan C24 ternary phase–diagram. Region 1, polyhedral vesicles (2–10 μm); region 2, spherical, helical and tubular vesicles (0·5–10 μm); region 3, discomes (10–30 μm), large vesicles (40 μm) and small spherical and helical vesicles (0·5–10 μm); region 4, discomes (12–60 μm) and possibly Solulan C24 micelles; region 5, cholesterol crystals; region 6, spherical vesicles (0·5–10 μm); region 7, a clear liquid (Solulan C24 micelles); region 8, mixed micelles formed at elevated room temperature.

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