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Effect of cholesterol and temperature on the elastic properties of niosomal membranes

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Abstract

The mechanical characteristics of non-ionic bilayer membranes composed of sorbitan monostearate, cholesterol and poly-24-oxyethylene cholesteryl were studied by measuring the modulus of surface elasticity (μ), a measure of membrane strength, as a function of cholesterol content and temperature. The modulus of surface elasticity increased slowly with increasing cholesterol concentration, with a sharp increase around 40 mol% cholesterol (on average an increment of 0.43×10^6 Nm^{-2} per molar percentage), and displayed a maximum of 6.5×10^6 Nm^{-2} around 47.5 mol% cholesterol. Further cholesterol resulted in a decrease in μ. Generally the interaction of cholesterol with the sorbitan monostearate should increase the rigidity of the membrane. However, the latter effect may be due to the formation of cholesterol clusters at high cholesterol content where excess amounts of cholesterol cannot interact with the sorbitan monostearate, and deposits on the bilayers compromising their uniformity, strength and permeability. This behaviour was evident when measurements were carried out above and below 25 °C.

Keywords: Niosomes; Cholesterol; Modulus of elasticity; Transition temperature

1. Introduction

The success achieved with liposomal systems has stimulated the search for other vesicle forming amphiphiles. Non-ionic surfactants were among the first alternative materials studied for drug delivery (Azmin et al., 1985). The self-assembly of synthetic surfactants and other non-phospholipids into vesicles was studied even earlier by cosmetic scientists when non-ionic surfactant vesicles or niosomes were reported (Vanlerberghe et al., 1972). Since this time a large body of research has sought to define these systems primarily as drug carriers but also as systems of interest to the colloid scientist. Synthetic surfactant vesicles, as the name implies, may also be fabricated from a vast array of amphiphiles, including a number of pharmaceutically acceptable materials. They may also be prepared in a variety of shapes and sizes and have a number of applications.

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The study of the effects of cholesterol on the physical and structural properties of vesicular bilayers is important for understanding the mechanism of interaction between cholesterol and non-ionic surfactants and phospholipids in niosome and liposome biomembranes respectively. It is particularly important from the biological standpoint since cholesterol is always present in biomembranes and influences a number of membrane properties such as ion permeability, enzymic activity, aggregation, fusion processes, elasticity, size and shape (Yeagle, 1985; Needham and Nunn, 1990; Uchegbu et al., 1992).

While studies of cholesterol in bilayers have usually focused on the intra-vesicle interactions, several interesting aspects related to mechanical properties, namely, the cohesion interaction between cholesterol and lipid, the mechanical stiffness of membranes, and their effect on membrane permeability to water, remain to be fully elucidated and quantified (Needham and Nunn, 1990). This also extends to studies on niosomal vesicles, where their membrane mechanical properties have not been completely studied.

The fluidity or intravesicle interactions of liposomes are also changed by cholesterol addition. It has been shown that phosphatidylcholine (PC) vesicles are more rigid and sustain more severe shear stress after introducing cholesterol (Liu et al., 2000). The maximum amount of cholesterol that can be incorporated into reconstituted bilayers is widely assumed to be about 50 mol% for liposomes, although the solubility limit for cholesterol in lipid shows a subtle dependence on lipid molecular structure (Needham and Nunn, 1990). Similarly, this has been found to be the case for niosomes (Uchegbu and Florence, 1995) where a 1:1 ratio between the cholesterol and non-ionic surfactants in the bilayers shows an optimum ratio for the production of physically stable niosomal vesicles.

Polyhedral niosomes prepared with no, or very low amounts of, cholesterol as described by Uchegbu and Florence (1995) tend to lose their viscoelasticity when exposed to large forces (during extrusion), and deform permanently after extrusion into tubular structures (Nasseri and Florence, 2003). On the other hand spherical niosomes or liposomes containing cholesterol recover their shape once the pressure is released, hence displaying an elastic memory. By the same token the deformability of erythrocytes is a critical determinant of blood flow in the microcirculation. High membrane elasticity was moreover suggested to be crucial for pushing a vesicle through a pore smaller than the average aggregate diameter (Gompper and Kroll, 1995; Cevc, 1995) as illustrated with a niosome inside a glass micropipette in Fig. 1. This has been argued to be of paramount importance for the success of the noninvasive, carrier-mediated material transport across the skin (Cevc, 1995, 1996).

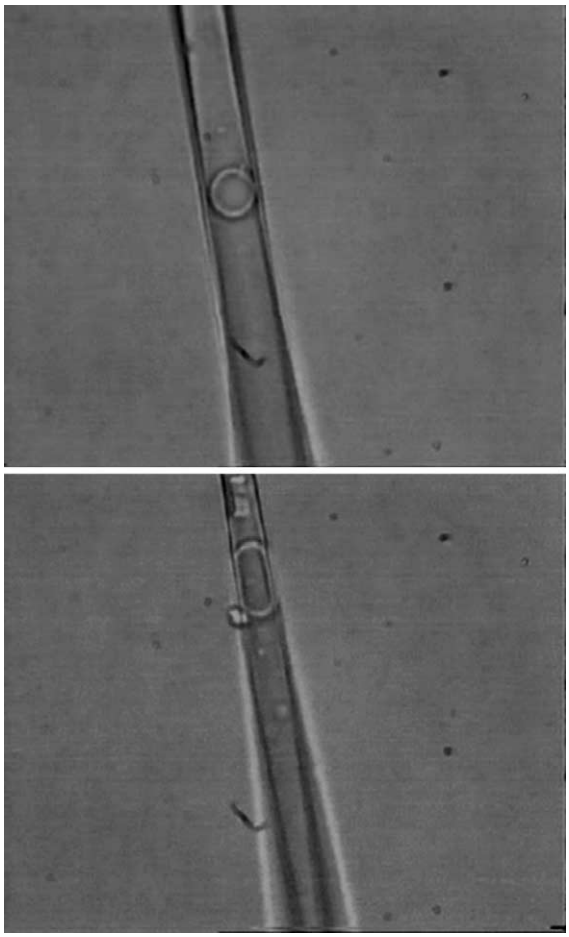

Fig. 1. Photomicrographs showing a niosome able to deform and squeeze through a glass micropipette channel smaller than its own diameter, owing to its elastic properties. This is perhaps a good in vitro analogy of a red blood cell squeezing through the narrow microcapillaries in vivo.

Results from differential scanning calorimetry (DSC) (Davis and Keough, 1983), have suggested that cholesterol-phospholipid interaction varies depending on the amount of cholesterol present, which may be an

indication of non-uniform lateral distribution of cholesterol in phospholipid bilayers existing in the gel state. At temperatures nearing the bilayer T_m it has been shown that there is a significant decrease of the mechanical properties of vesicles as well as cell membranes (Hianik and Haburčák, 1993) rendering them less elastic. In this paper we have investigated the effect of temperature and cholesterol composition on the modulus of surface elasticity of niosomes composed of sorbitan monostearate, cholesterol and poly-24-oxyethylene cholesteryl and observed the effect on the mechanical properties of the niosomal membranes of the interaction of the cholesterol with the sorbitan monostearate.

2. Materials and methods

2.1. Reagents and chemicals

Reagents and chemicals including sorbitan monostearate (Span 60) and cholesterol were obtained from Sigma (UK). Poly-24-oxyethylene cholesteryl (Solulan C24) was donated by Ellis and Everald (UK). All materials were used as obtained from suppliers without further purification and the water source was from an ultra high quality reverse osmosis water purifier (Elgastat UHQPS – Elga, UK). Borosilicate glass capillaries, with an inner diameter (i.d.) of 1.17 mm and outer diameter (o.d.) of 1.5 mm, were obtained from Harvard Apparatus, UK.

2.2. Preparation of spherical niosome filled micropipettes

Niosomes (60 mM) constituted from sorbitan monostearate, poly-24-oxyethylene cholesteryl and cholesterol (where the latter composition ranged from 10 to 70 mol%, and the poly-24-oxyethylene cholesteryl content was maintained at 5 mol% in all systems) were prepared by the hand-shaking method in water (Azmin et al., 1985; Baillie et al., 1985). To ensure a complete mixing of surfactant and cholesterol or lipid and cholesterol, the compositions dissolved in chloroform were probe-sonicated using a Soniprep 150 (Sanyo MSE UK; setting 5) at 30 s intervals for a total period of 5 min, allowing the solution to cool by submerging in ice. The solutions were then re-hydrated above their constituent transition temperature (60 °C). The resultant dispersions were centrifuged to obtain the largest fraction of vesicles residing in the pellet. Aliquots were diluted with water in order to obtain the minimum number of giant vesicles in a 0.1 ml volume of water to avoid blockage of the micropipette made from glass capillaries pulled into a parallel shape of approximately 5 μm diameter.

2.3. Deformation of vesicles within micropipettes

A micropipette filled with 0.1 ml of diluted vesicle dispersion was connected to a piezo-electric pump via non-expandable tubing (back-filled with water) and the pressure was measured to give a low speed of flow within a parallel micropipette. The contents of the micropipette were constantly observed at a point just before the narrow part of the pipette. Once a vesicle was in view, the pressure was turned off and the uncompressed vesicle image was captured using a video camera. The pressure was gradually raised and the vesicle was forced into the narrow part of micropipette (as shown in Fig. 1) where the images of deformed vesicle were captured and the pressure applied registered.

Evans (1973b) proposed the following equation to describe the two-dimensional hyper-elastic extension of an elemental strip of red cell membrane:

$$T_x = \mu(\lambda_x^2 - \lambda_x^{-2}) \quad (1)$$

where T_x is the normalised force (force divided by width) in the x direction and λ_x is the extension ratio (deformed length divided by original length) in the x direction. The constant μ characterises the hyper-elastic extension of the red cell membrane at constant surface area and is called “the shear modulus of surface elasticity”.

This procedure was carried out on a range of vesicles with various membrane compositions and at temperatures from 10 to 42 °C.

3. Results

The simple application of positive pressure to a giant vesicle flowing inside a micropipette narrower than its own diameter induces a well-defined deformation in the vesicle membrane and shape. The extension ratios measured from the change in length of the vesicle at

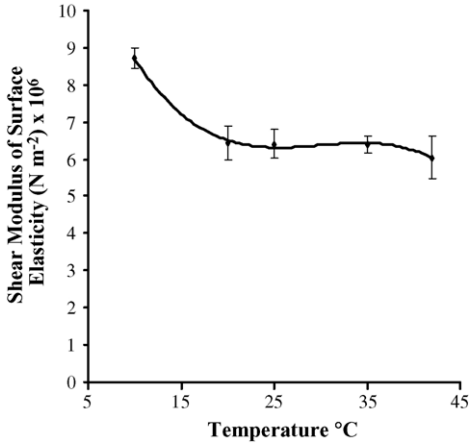

Fig. 2. Shear modulus of surface elasticity μ (Nm^{-2}) of niosomes with equimolar ratios of cholesterol and sorbitan monostearate as a function of temperature (°C) ($n = 10$).

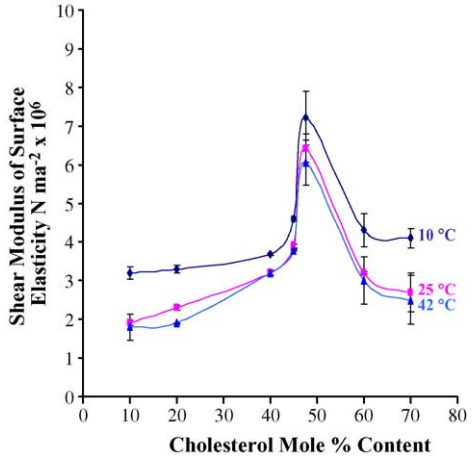

Fig. 3. Effect of cholesterol content on the shear modulus of surface elasticity μ (Nm^{-2}) of niosomes at the temperatures (°C) indicated. At all three temperatures the value for μ shows a peak at 47.5 mol% which may indicate a composition where all constituents interact to the highest degree providing the vesicle with a mechanically strong membrane ($n = 10$).

recorded pressures were substituted in Eq. (1) to calculate the shear modulus of surface elasticity (μ) of the vesicle membranes.

In order to quantify the elastic behaviour of the red cell membrane or vesicle using fluid shear force (Hochmuth and Mohandas, 1972) to deform the red cell within a narrow tube in which the surface area of the cell remains constant as the cell is elongated in the direction of flow, it was proposed (Evans, 1973a, b; Skalak et al., 1973) that the membrane be treated as a two-dimensional, incompressible material (that is, a material which deforms at constant surface area and constant membrane thickness) which is capable of large elastic deformations. Experiments indicate that μ remains constant over a wide range of deformations ($1.5 \leq \lambda_x \geq 4$). Extension ratios smaller than 1.5 are difficult to measure with the micropipette aspiration technique and in this study only deformations above a 1.5 ratio were used, as shown in Fig. 1.

Fig. 2 presents the shear modulus of surface elasticity for niosomes prepared with 47.5 mol% cholesterol as a function of temperature. The values of μ for the niosome membranes reduce with rise in temperature from 10 to 20 °C, becoming relatively constant at higher temperatures.

Fig. 3 shows the values for μ of niosomes prepared with cholesterol contents ranging from 10 to 70 mol%. Each series was studied at three temperatures (except for the 47.5 mol% cholesterol system which was studied at five temperatures). From these results it is clear that niosomes also display sensitivity to cholesterol composition as well as temperature. The sensitivity to temperature is most pronounced when the temperature is reduced from 25 to 10 °C; an increase to 42 °C had minimal effect on the membrane properties. The maximum rigidity of the niosomal membranes at all three temperatures was observed at a cholesterol concentration of 47.5 mol% after which it decreased markedly. At 47.5 mol% of cholesterol there is also the same molar concentration of sorbitan monostearate (the remaining 5 mol% is the poly-24-oxyethylene cholesteryl).

4. Discussion

The coefficient μ is a shear modulus (also known as coefficient of elasticity or elasticity modulus). It is intrinsic to the membrane structure and for a given composition, the resulting bilayer structure and conformation will dictate the elastic behaviour of that membrane. The shear modulus represents the energy storage and static resistance to extensional deformations of the membrane surface. This is a property peculiar to solid or semi-solid materials; liquids have zero shear (elastic) moduli.

The measured value for the red cell membrane shear modulus is in the order of $10\,Nm^{-2}$ (Evans and Hochmuth, 1978). It has been shown (Waugh and Evans, 1978) that red cell membrane shear modulus decreases as temperature increases. Reported (Hianik and Haburčák, 1993) values for liposomes prepared from DMPC and cholesterol (50:50) lie around $12 \times 10^{-6}\,Nm^{-2}$ at room temperature, as determined using the aspiration technique. The much higher values of μ of red blood cells have been attributed (Needham and Nunn, 1990) to the relatively incompressible transmembrane proteins which confer enhanced compressibility on the composite structures of the red blood cell membranes. Similarly, the lower values of modulus of surface elasticity for niosomes could be due to a higher degree of interaction between the cholesterol and phospholipids in liposomal membranes than the interaction of cholesterol with sorbitan monostearate in niosomal membranes. There may also be a closer packing or a more compact fitting in the liposomal membranes than in niosomes.

Fig. 2 shows that the value of μ for niosomes with equimolar ratios of cholesterol and sorbitan monostearate is at its highest at 10 °C and becomes reasonably constant over the range 20–42 °C. A similar trend is also displayed for niosomes of variable cholesterol content as shown in Fig. 3. The highest values of μ are observed in the low temperature region. At 10 °C, the bilayers could be nearing the temperature at which the surfactant molecules transform into the solid phase from their gel phase, hence resulting in a greater resistance to deformation and a corresponding increased modulus of elasticity as the temperature is decreased. An appreciable decrease of μ might be expected at T_m due to the transformation from the gel to liquid state. For example, it has been noted (Hianik and Haburčák, 1993) that liposomes containing DMPC can have twice the modulus of elasticity when in their gel state than when in their liquid state. The T_m value of the niosomes of the composition used in the present study is approximately 60 °C and hence outside the range of temperatures considered. Consequently, the relatively constant value of shear modulus of surface elasticity for the vesicle membrane between 20 and 42 °C is expected.

The value of μ increases gradually with cholesterol content between 10 and 40 mol% suggesting that that the cholesterol content affects membrane elasticity, making the membrane more rigid. This effect is most pronounced between 40 and 47.5 mol% after which the effect is surprisingly reversed. Other investigations (Finean, 1990; Needham et al., 1988) using the X-ray diffraction studies have pointed out the non-uniform lateral distribution of cholesterol in phospholipid bilayers existing in the gel state, while in the liquid crystalline state the membranes behave like a homogenous phase. This finding may extend to niosomal membranes containing cholesterol, and may explain our data.

At all temperatures there is a peak in the value of μ at a cholesterol content of 47.5 mol% (corresponding to an equimolar mixture of cholesterol and sorbitan monostearate). In liposomes it has been suggested that a cholesterol to lipid molar ratio of 1:1represents a special situation in the membrane at which there is the most effective stabilisation of phospholipid–cholesterol complexes (Hianik and Haburčák, 1993). Finean (1990) has suggested that an interaction occurs between the glycerol oxygen at position 2 on the phospholipid head group and the β-OH group of cholesterol. The graph in Fig. 3 suggests that the same scenario occurs in the niosomal membrane of this preparation at an equimolar ratio of cholesterol to sorbitan monostearate.

Fig. 4 is a schematic showing the possible interaction between cholesterol and sorbitan monostearate within the bilayers of the niosome membrane. It is possible that the small hydrophilic 3 β-hydroxyl (β-OH) head group of the cholesterol in the bilayers is able to

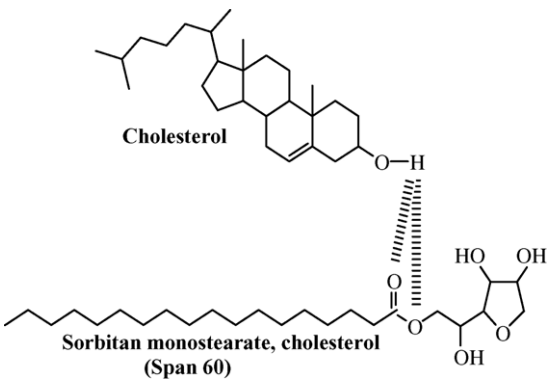

Fig. 4. Schematic showing the possible hydrogen bonding interaction between the β-OH group of the cholesterol and the oxygen primarily at the ketone group and also weaker interaction at the ester group.

position itself in the vicinity of the sorbitan monostearate ester group and the hydrophobic steroid ring orients itself parallel to the acyl chains of the non-ionic surfactant. This may in effect restrict the movement of the acyl chains of the bilayer. As shown in Fig. 4 the β-OH group of the cholesterol could form a hydrogen bond with the oxygen at the ester group of the sorbitan monostearate. However, it is also possible to form hydrogen bonds at the other oxygen functionalities of sorbitan monostearate, which enhance the stability of the bilayer. These interactions result in an increase in membrane cohesion, as shown by increase in the mechanical stiffness of the membranes. There is only one possible hydrogen bonding group on the cholesterol moiety and the results of this study suggest that the equimolar mixture represents the critical composition at which the two compounds can have extensive interaction at any of the mentioned sites. According to Finean (1990) excessive concentration of cholesterol can cause their cluster formation leading to non-uniform distribution along the bilayers affecting the integrity of the membrane. Our studies suggest that a similar scenario may also pertain in niosomal membranes.

It should be noted that the method adopted in this study is one of several ways of measuring the modulus of elasticity of niosomes. Since mechanical properties of membranes are characterised by considerable anisotropy (Passechnik and Hianik, 1991) it is important to measure the membrane deformation in different directions. Needham and Nunn (1990) for example, have demonstrated the monotonic growth of volume modulus compressibility of large liposomes made from stearolyloleoylphosphatidylcholine (SOPC) with increasing cholesterol concentration in lipid bilayers in the gel state.

5. Conclusion

Niosomal membranes prepared from sorbitan monostearate, cholesterol and Solulan C24, exhibit certain mechanical characteristics which differ from those of their liposomal counterparts with regards to their susceptibility to changes in cholesterol content and temperature. It is important, however, to note that the values of shear modulus of surface elasticity obtained in these experiments are apparent values and the experimental methods can have an influence on the results.

The information gained from the direct measurements made on a single large vesicle not only characterises the membrane and its intermembrane interactions from a fundamental materials science perspective, but also provides essential data on the mechanical properties required for the successful design and deployment of lipid and non-ionic vesicle capsules in applications such as drug delivery.

The hyperelasticity exhibited by flaccid red blood cells as well as phospholipid vesicles (liposomes) or non-ionic vesicles (niosomes), would ease their flow through small capillaries in the microcirculation and through other small apertures, for instance, in the spleen and bone marrow. The desired extent of content preservation of such vesicles and their overall integrity would also be militated by the membrane composition, hence, an in depth knowledge of the mechanical property of the vesicle membrane would be an appropriate indication of such integrity.

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