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Effect of β-sitosterol concentration and high pressure homogenization on the chlorhexidine release from vesicular gels

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Abstract

Previous studies have confirmed that the phase transition of vesicular gels of hydrogenated phospholipids to the less ordered fluid vesicular state was induced by the increase of the β-sitosterol ratio in the whole gel system and consequently in the lipid bilayer. The purpose of the present study was to evaluate the influence of the β-sitosterol portion in the lipid bilayer and the effect of high pressure homogenization on the structural characteristics of the prepared gel systems. In addition the influence of β-sitosterol on the consequent chlorhexidine release from the obtained vesicles and liposomes was also examined. Lipid mixtures were prepared from different molar ratios of lecithin:sterol components (90:10–65:35 mol%). The obtained mixtures were hydrated with the aqueous solution of chlorhexidine digluconate in order to achieve a 30% (w/w) final concentration of the lipid mixtures and a 4% (w/w) concentration of the drug. One portion of the resultant multilamellar vesicles was homogenized by using high pressure. To characterize the homogenized and non-homogenized systems, transmission electron microscopy of the freeze-fractured samples and differential scanning calorimetry (DSC) were carried out. A vertical type diffusion cell was applied to determine the amount of released chlorhexidine digluconate. Along with the increase in β-sitosterol concentration, the fluidity of the membrane as well as its permeability also increased. The increased permeability—caused by the higher β-sitosterol concentration—and the high pressure homogenization, which increased the dispersity and therefore the surface area, enabled a higher amount of chlorhexidine to be released. The increase of drug release was more pronounced in the case of samples prepared with high pressure homogenization.

Keywords: Multilamellar vesicles (MLV); Vesicular phospholipid gel (VPG); High pressure homogenization; β-sitosterol; Chlorhexidine digluconate release; Transmission electron microscopy; Differential scanning calorimetry

1. Introduction

Liposomal encapsulation of drugs is a promising area in the development of antibacterial formulations for clinical use. Such vesicular carriers may allow increased drug concentration at infected sites but reduce drug toxicity (Pinto-Alphandary et al., 2000). Fluid and rigid liposomes have successfully been applied in antimicrobial therapy, while in the case of fluid liposomes with 'Fluidosomes' an enhanced effect could be observed against resistant strains and also with other drugs (Sachetelli et al., 2000; Beaulac et al., 1998).

Chlorhexidine is a water-soluble bisguanidine antiseptic agent, which possesses an effective broad-spectrum against a wide range of gram-positive and gram-negative microorganisms, yeast and fungi.

Nanoencapsulation of chlorhexidine resulted in increased drug delivery by mediating a more direct and prolonged contact between the carrier and bacteria, skin surface and skin follicles, and presented prolonged ex vivo topical antimicrobial activity against *Staphylococcus epidermidis* (Lboutounne et al., 2001). Sustained release of chlorhexidine was also achieved from a degradable polymeric matrix, which can be very useful also in

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the treatment of periodontitis (Yue et al., 2004). Different types of liposomes were found to have a potential application in the delivery of bactericides and antibiotics to a wide range of oral or skin-associated bacteria (Jones, 2005; DiTizio et al., 1998; Kaszuba et al., 1995).

The appropriate formulation of liposomes and vesicles for drug delivery systems can be developed by testing the physicochemical characteristics of the systems. A recent study has confirmed that drug entrapment, drug release, liposome morphology and liposome stability are all influenced by the lipid composition of multilamellar vesicles (MLVs) (Mohammed et al., 2004). The authors examined the effect of the amount of cholesterol, chain length and charge of the lipids on the characteristics of liposomes containing a poorly water-soluble drug.

The method of preparation can also have considerable impact on the properties of liposomes. High pressure homogenization can be applied for the preparation of different types of liposomes (Pupo et al., 2005; Barnadas-Rodríguez and Sabés, 2001). A semisolid gel produced by the high pressure homogenization method showed increased elasticity in comparison to the same composition of lipid dispersion produced by Ultraturrax. The change in viscoelastic properties could be attributed to the different particle–particle interactions since there are differences in particle size and particle size distributions. Decreasing the particle size is accompanied by a huge increase in surface area. Therefore, the number of contact points increases and so particle–particle interactions are more pronounced, leading to a three-dimensional network structure (Lippacher et al., 2002).

Reversed vesicles to be used as oral dosage forms were developed by Mollee et al., 2000. They studied the effect of incorporated cholesterol and water on physicochemical parameters of the vesicles and found that phase separation of certain compositions of bilayer have consequences for the application of those dosage forms. Microdomain structure formation of the polymeric carrier, which can be followed by analysing the thermodynamic parameters of phase transition (temperature, enthalpy, heat capacity and width of the transition), plays an important role in controlling the drug release process (Alvarez-Lorenzoa et al., 2005). The phase behaviour of the phospholipid bilayer has been extensively studied by differential scanning calorimetry until now. The effect of sterols was observed on the domain properties of the bilayer and it was found that the interaction between the sterol and the phospholipid is a complex process which can be influenced by the type and concentration of sterol or the structure of the phospholipid molecule (Halling and Slotte, 2004; McMullen et al., 1993). The main component of the soybean sterols is β-sitosterol, which was found to be more effective in rearranging of the acyl chains of soybean lecithin bilayers than the cholesterol. (Schuler et al., 1990).

Vesicular phospholipid gels (VPG) produced by high pressure homogenization were examined in previous studies (Brandl et al., 1991; Bender et al., 2002; Farkas et al., 2004). Thermodynamical, microscopic and rheological tests were carried out in order to examine the structure of the resulting systems. Due to the increased sterol concentration in the vesicles, phase transition could be observed which influenced drug release from the systems (Farkas et al., 2004). The studied multilamellar and multivesicular systems are semisolid matrices, which can incorporate hydrophilic compounds in the aqueous compartments and can be used as sustained release therapeutic systems, e.g. for oral application of chlorhexidine.

The aim of the present study was to evaluate the influence of the β-sitosterol concentration in the lipid bilayer and high pressure homogenization on the structure of the prepared gel systems, and their influence on the subsequent chlorhexidine release from the obtained vesicles and liposomes.

2. Materials and methods

2.1. Materials

Hydrogenated soy lecithin, Lipoid S PC-3 (generous gift from Lipoid GmbH, Germany), β-sitosterol, chlorhexidine gluconate, 20% aqueous solution (Sigma–Aldrich Chemie GmbH, Germany) and bi-distilled water were used for the liposome preparation. Other ingredients were of analytical grade.

2.2. Preparation of vesicular gels

The film method was used to prepare multilamellar vesicles (MLV). The required amount of S PC-3 and β-sitosterol were dissolved in absolute ethanol. The solvent was removed under vacuum by rotary evaporation (Buechi, Germany) and high vacuum was employed for drying the film overnight. The molar ratio of the lecithin:sterol components varied in the range of 90:10–65:35 (mol%). The mixture was hydrated with an aqueous solution of chlorhexidine digluconate giving a 30% (w/w) final concentration of the lipid mixtures and 4% (w/w) of the drug in each MLV sample. The MLV dispersion was separated into two parts. One of them was then homogenized by the high pressure homogenizer (Gaulin Micron Lab 40, Germany) for 10 times at a pressure of 70 MPa in order to obtain VPG samples.

The MLVs were white gels of a semisolid, creamy consistence and VPGs were viscoelastic, semisolid, somewhat transparent gels.

2.3. Transmission electron microscopy of a freeze-fractured replica

Drops of the preincubated sample were placed on the gold specimen holder, which was then immediately plunged into partially solidified freon and then placed and stored in liquid nitrogen. The samples were fractured at −100 °C in a Balzers BAF 301 freeze-etch device and then shadowed at −110 °C with Pt/C (2 nm) at 45° elevation and with C (25 nm) at 90° elevation. The Pt/C layer, which was obtained was then cleaned with distilled water and mounted on 200 mesh copper grids. Transmission electron microscope (CM10; Philips) was used for the visualization of the pretreated samples.

2.4. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (PL-DSC Model 12000, PL Thermal Sciences Ltd., UK-Epsom) equipped with a liquid

nitrogen cooling accessory was used to determine the transition temperatures and enthalpies of the gel-to-liquid crystalline phase transition of lipid bilayers. The heating rate was 5 K min^{-1}. A sample volume of 5–10 mg was weighed and sealed in aluminium pans. At least 3 separate samples from each VPGs were measured. The instrument was calibrated with indium. Calibration was performed every 24 h after burning out the oven up to 500 °C. For calculating the phase transition point both the onset and the peak maxima were observed. The parameters were determined either manually or automatically by the software. The onset is less dependent on sample parameters like mass or homogeneity, which may be an advantage in some cases.

2.5. Redispersion of VPG-s

VPGs prepared in Section 2.2. were redispersed by their 10-fold dilution with distilled water under magnetic stirring for 10 min. The redispersed samples were then examined in the drug release experiments.

2.6. Drug release study

A vertical type diffusion cell was used for the drug release measurements. The liposome dispersion was filled into the donor phase of the apparatus. The acceptor medium was distilled water, which was stirred with a magnetic bar. The donor and acceptor phases were separated by a dialysis membrane (Dianorm GmbH, Germany). The amount of the permeated chlorhexidine digluconate was measured spectrophotometrically at 255 nm over a period of 2 h.

3. Results and discussion

3.1. The effect of β-sitosterol concentrations on the morphology of multilamellar vesicles

Fig. 1 illustrates the morphology of freeze-fractured samples of MLVs containing 15% (w/w) β-sitosterol both before (a) and after (b) homogenization, while Fig. 2 represents the same samples of 30% (w/w) β-sitosterol concentrations before (a) and after (b) homogenization.

Fracture of the sample preferably occurs along the hydrophobic layer with weak H-bonds. An increased amount of β-sitosterol produces more continuous flexible sheets of bilayer.

The more fluid sheets of MLVs could be clearly distinguished in the case of samples containing 30% (w/w) β-sitosterol concentrations. MLVs of lower β-sitosterol concentrations are rather concentric multilamellar units. The homogenization process disrupts the multilamellar structure and results in more dispersed systems.

3.2. The effect of β-sitosterol concentrations on the thermal behaviour of homogenized and non-homogenized MLV samples

Fig. 3a and b shows the thermograms of various MLV samples before and after homogenization. Preceding homog-

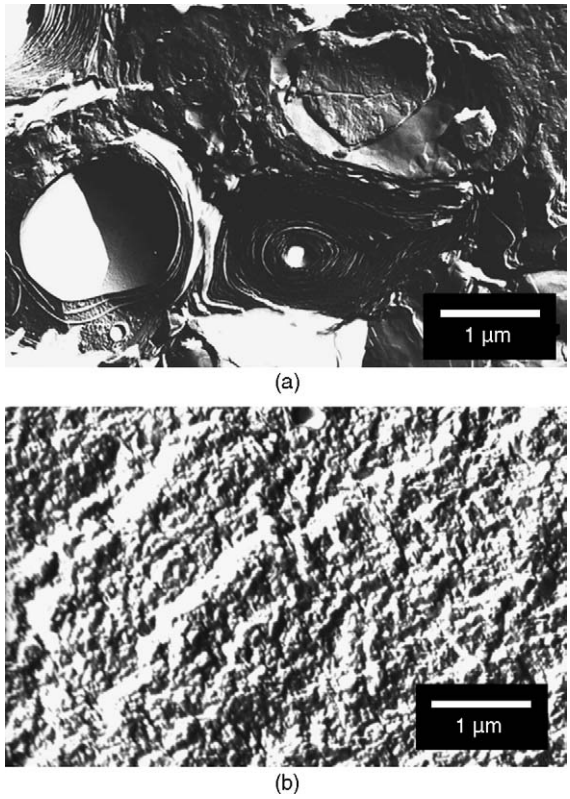

Fig. 1. Transmission electron microscopic images of vesicular gels containing 15% β-sitosterol (a) preceding homogenization and (b) after homogenization.

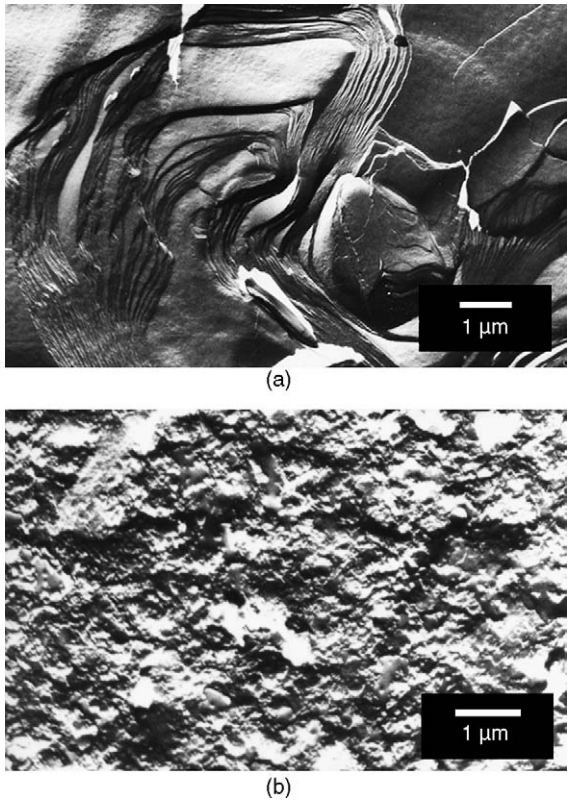

Fig. 2. Transmission electron microscopic images of vesicular gels containing 30% β-sitosterol (a) preceding homogenization and (b) after homogenization.

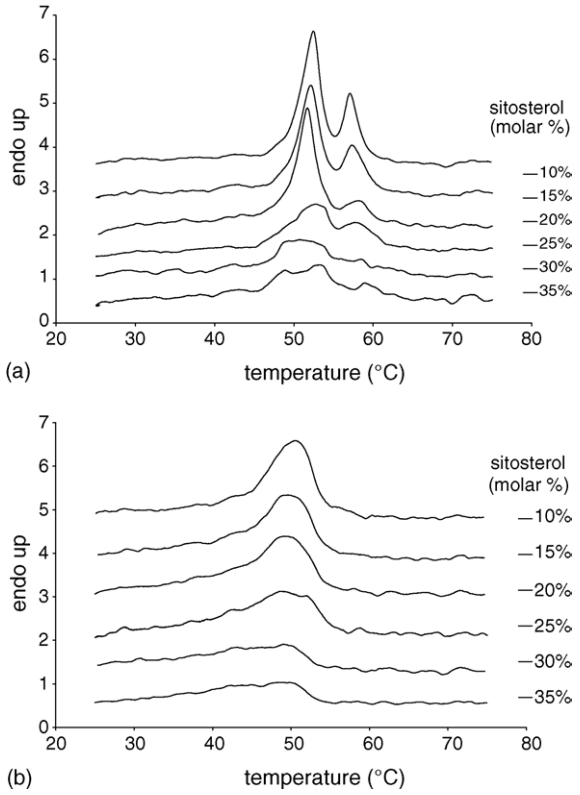

Fig. 3. Thermograms of (a) MLVs preceding homogenization and (b) VPGs after homogenization.

enization two endothermic peaks can be found at 10–25 mol% β-sitosterol concentrations with regards to the presence of individual membrane components. The higher β-sitosterol concentrations decreased the enthalpy of the peaks, thus indicating the more fluid, less ordered membrane structure. This phenomenon was studied more extensively by other authors observing the interaction between the sterol and phospholipid components (Schuler et al., 1990; Halling and Slotte, 2004).

As a result of the homogenization, one of the peaks disappeared indicating an energy-induced alteration of binding forces between the membrane constituents, thus decreasing the onset temperature of the peak. Table 1 summarizes the onset temperature and enthalpy values of MLV and VPG samples at the main transition. The temperature width at half maximum of the characteristic peaks became bigger after homogenization thus indicating a less ordered structure of lower co-operativity.

Table 1
Onset temperature and enthalpy values of the gel–liquid crystalline transition

β-sitosterol amount (mol%)	Onset temperature of main transition (°C)		Enthalpy change (J/g)	
	MLV	VPG	MLV	VPG
10	49.4; 55.8	45.76	16.61	15.19
15	49.6; 55.9	46.36	15.94	14.62
20	49.0; 56.2	46.1	13.24	13.20
25	46.2; 55.9	39.9	10.52	10.18
30	46.6	36.1	9.04	8.57
35	45.6	33.9	7.85	7.85

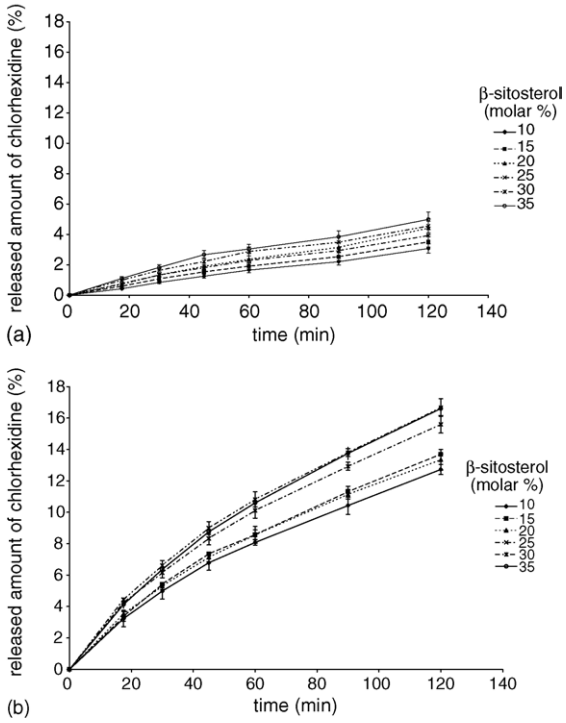

Fig. 4. Chlorhexidine release (a) from MLVs preceding homogenization and (b) from VPGs after homogenization. Average values of 3 parallels ± S.D.

3.3. The effect of β-sitosterol concentration on the chlorhexidine gluconate release from homogenized and non-homogenized MLV samples

Along with the increase of β-sitosterol, the amount of chlorhexidine released also increased. This tendency was independent of the homogenization (Fig. 4a and b). A possible explanation of this phenomenon could be the more fluid and permeable membrane structure created by the incorporated β-sitosterol. The homogenization resulted in more dispersed samples of higher surface area (Lippacher et al., 2002). The effect of homogenization on the dispersity of the samples was more dominant compared to the effect of β-sitosterol concentration as can be seen in Fig. 1a and b, respectively.

4. Conclusions

Four times more chlorhexidine was released from the homogenized MLV samples compared to the non-homogenized samples of the same composition. The effect of β-sitosterol concentrations on the samples was less pronounced from the point of the extent of drug release than that of the high pressure homogenization.

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