Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 278 (2004) 63–70
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Effect of β-sitosterol on the characteristics of vesicular gels containing chlorhexidine

E. Farkas [a], R. Schubert [b], R. Zelkó [c,*]

[a] *Pharmaceutical Institute, Semmelweis University, Hőgyes E. Street 7, Budapest 1092, Hungary*
[b] *Department of Pharmaceutical Technology, Albert-Ludwigs-University of Freiburg, Hermann-Herder-Street 9, Freiburg D-79104, Germany*
[c] *University Pharmacy Institute of Pharmacy Administration, Semmelweis University, Hőgyes E. Street 7-9, Budapest 1092, Hungary*

Received 16 June 2003; received in revised form 3 February 2004; accepted 18 February 2004

Available online 22 April 2004

Abstract

Previous studies confirm that β-sitosterol is very effective in altering the molecular packing of soybean lecithin bilayers even more than the cholesterol. The primary aim of the present study was to evaluate the influence of the β-sitosterol portion in the lipid bilayer on the physical–chemical characteristics of the prepared gel systems, and its influence on the consequent drug release from the liposomes obtained from vesicular phospholipid gels (VPG-s) by redispersion. VPG-s were prepared of different molar ratios of lecithin:sterol components (10:90–35:65 mol%). The mixture was hydrated with the aqueous solution of chlorhexidin digluconate in order to achieve 30% (w/w) final concentration of the lipid mixtures and 4% (w/w) concentration of the drug in each homogenized VPG sample. To characterize the obtained VPG systems optical microscopic examinations using polarized light, differential scanning calorimetry (DSC), photon correlation spectroscopy (PCS), and dynamic surface tension measurements were carried out. Vertical type diffusion cell was applied to determine the amount of released chlorhexidine digluconate. As a result of the surface tension-decreasing effect of β-sitosterol, the membrane deformability and the dispersity of the system increased. The increased dispersity and fluidity significantly increased the extent of released chlorhexidine from the vesicles.

Keywords: Vesicular phospholipid gels (VPG); β-Sitosterol; Chlorhexidine digluconate release; Dynamic surface tension; Dispersity

1. Introduction

Vesicular systems were investigated as new carriers for various therapeutical applications. The topical use of these systems (Touitou et al., 1994; Meisner and Mezei, 1995; Maitani et al., 2000; Bouwstra and Honeywell-Nguyen, 2002) has many advantages, for example, the biocompatibility or the good solubilizing efficacy of the membrane constituents.

Brandl et al. (1990) prepared vesicular phospholipid gels (VPG-s) by high pressure homogenization, which allowed the large scale production of the liposomes. The developed single-step liposome preparation method resulted in small unilamellar vesicles with narrow particle size distribution. The highly concentrated liposomes form semi-solid gel and when the gel is redispersed, diluted liposome dispersion can be obtained.

* Corresponding author. Tel.: +36-1-476-3600/3048; fax: +36-1-217-0927.
E-mail address: zelrom@hogyes.sote.hu (R. Zelkó).

0378-5173/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.02.022

By virtue of the in vitro drug release and the entrapment investigations of VPG-s (Kaiser et al., 2003) containing 5-Fluorouracil, good applicability of the gel is expected topically as implants or intravenously, as redispersed liposome. Prolonged drug release with matrix-controlled diffusion kinetics can be achieved by using VPG formulations, where the drug release appears to be controlled by the size and the structure of liposome membrane. Liposomes containing the antibacterial agent have been found more effective against typical oral bacteria than when being used as a free drug (Catuogno and Jones, 2003).

Several studies support that the physical state of vesicles has a great impact on the drug release and on the penetration of vesicles through biological barriers. Liquid state vesicles were found to be more effective in enhancing the drug transport than the gel state vesicles. Deformability of liposomes has an influence on the dermal penetration. Elastic liposomes that contain a surfactant beyond phosphatidylcholin are able to reach the deeper region of the stratum corneum more rapidly than the rigid vesicles (Bouwstra and Honeywell-Nguyen, 2002).

Animal cells mainly contain cholesterol while plant cells contain more complex mixtures of sterols. Like cholesterol, phytosterols are also able to modify the structural and functional properties of the membrane lipid bilayers (Mora et al., 1999). 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. The changes of the membrane fluidity at the presence of different sterols were studied by steady-state fluorescence anisotropy (Schuler et al., 1990). Mixtures of long-chain saturated phosphatidylcholines compose well arranged lipid layers below the phase transition. Calorimetric studies confirm that added cholesterol will reduce the interaction forces in the lipid bilayer and will act as liquefier. Cholesterol enhanced the rate of permeability of liposomes deriving from saturated lecithins below their transition temperature (Demel and De Kruyff, 1976).

The purpose of the present study was to evaluate the influence of the β-sitosterol portion in the lipid bilayer on the physical–chemical characteristics of the prepared gel systems, and its influence on the consequent drug release through the liposomes.

2. Materials and methods

2.1. Materials

Hydrogenated soy lecithin, Lipoid S PC-3 (Lipoid GmbH, Germany), β-sitosterol, chlorhexidine gluconate (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 VPG-s

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 (Buchi, Germany) and high vacuum was employed for drying the film overnight. The molar ratio of the lecithin:sterol components varied in the range of 10:90–35:65 (mol%). The mixture was hydrated with the aqueous solution of chlorhexidin digluconate obtaining 30% (w/w) final concentration of the lipid mixtures and 4% (w/w) of the drug in each MLV sample.

The MLV dispersion was then homogenized by the high pressure homogenisator (Gaulin MICRON LAB 40, Germany) 10 times at pressures of 70 MPa to obtain VPG samples. The VPG-s were viscoelastic semisolid white opaque gels.

2.3. Polarizing microscopy

Formation of multilamellar vesicles was observed under optical microscope (Leitz Dialux 20, Leitz Wetzlar Germany, equipped with Mettler FP 82 hot stage) using polarized light. 100–320× magnification was applied to record the texture elements of the samples.

2.4. Differential scanning calorimetry (DSC)

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 between 5 and 10 mg was weighted and sealed in aluminium pans. At least three separate samples of each VPG were measured. The instru-

ment 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 maximum were observed. The parameters were determined 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. Dynamic surface tension measurements

The dynamic surface tension of different liposomal dispersions was determined by the Wilhelmy plate method using a computer-controlled and programmable tensiometer (KSV Sigma 70, RBM-R. Braumann GmbH, Germany) after an equilibration at 25 °C for 1 h. Measuring parameters were as follows: minimum number of cycles: 5; minimum measuring time: 5 min; speed up: 20 mm/min. The Wilhelmy plate method involves a repeated sinking of the plate in the dispersion and, as expected on the basis of statistical thermodynamics, not always the same molecules contact with the plate in the repeated measurements. This is exactly the cause of repetition, that is, the resulted "averaging" eliminates most of the effects of random fluctuations. In discontinuous materials these fluctuations are naturally larger, so, the standard deviation of the measured surface tension has to be definitely higher than in "homogeneous" dispersions.

2.6. Redispersion of VPG-s

VPG-s prepared in Section 2.2 were redispersed by their 10× dilution with distilled water under magnetic stirring for 10 min. The redispersed samples were applied for the PCS and drug release experiments.

2.7. Photon correlation spectroscopy (PCS)

The mean droplet size and size distribution were determined with a Malvern Zetasizer (Malvern Instruments, UK). Each redispersed VPG sample (liposome) was diluted with filtered water in order to reach a count rate of 100–150 kCps. The average vesicle size distribution was determined by number-based non-gaussian fit to raw data which had been collected over 5 min at 25 °C at an angle of 90°.

2.8. Drug release study

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

3. Results and discussion

3.1. The effect of β-sitosterol concentrations on the characteristics of multilamellar vesicles (MLV)

Fig. 1a–f illustrates the MLV-s of different β-sitosterol concentrations preceding the homogenization of the system. Along with the increase of the β-sitosterol ratio (10–35 mol%) in the lipid bilayers of MLV, the number of spherical vesicles decreases. While Fig. 1a and b represent the dominant presence of Maltese crosses referring to the formation of spherulits in the MLV samples, in Fig. 1c vesicles of elongated shapes appear and those became dominant in Fig. 1d–f. Bilayers, sliding into cylindrical myelinic figures, can be seen at 20% β-sitosterol ratio (Fig. 1c), and they characterize the texture of the systems at higher β-sitosterol contents (Fig. 1d–f). As a result of spherulit to cylindrical myelinic texture transition of the supramolecular structure, the physical characteristics of the system were greatly modified.

3.2. The effect of β-sitosterol concentrations on the characteristics of homogenized MLV samples (VPG-s and redispersed VPG-s)

Fig. 2a–f show the size distribution of redispersed VPG-s of different β-sitosterol portions. Along with the increase of β-sitosterol in the lipid bilayer, the intensities of the particle size distribution curves of different samples were shifted to smaller diameters. The latter phenomenon also indicates the β-sitosterol induced phase transition of vesicles to less ordered more fluid supramolecular elements confirmed by visual observations with light microscope. The enthalpy changes of different VPG samples determined by DSC

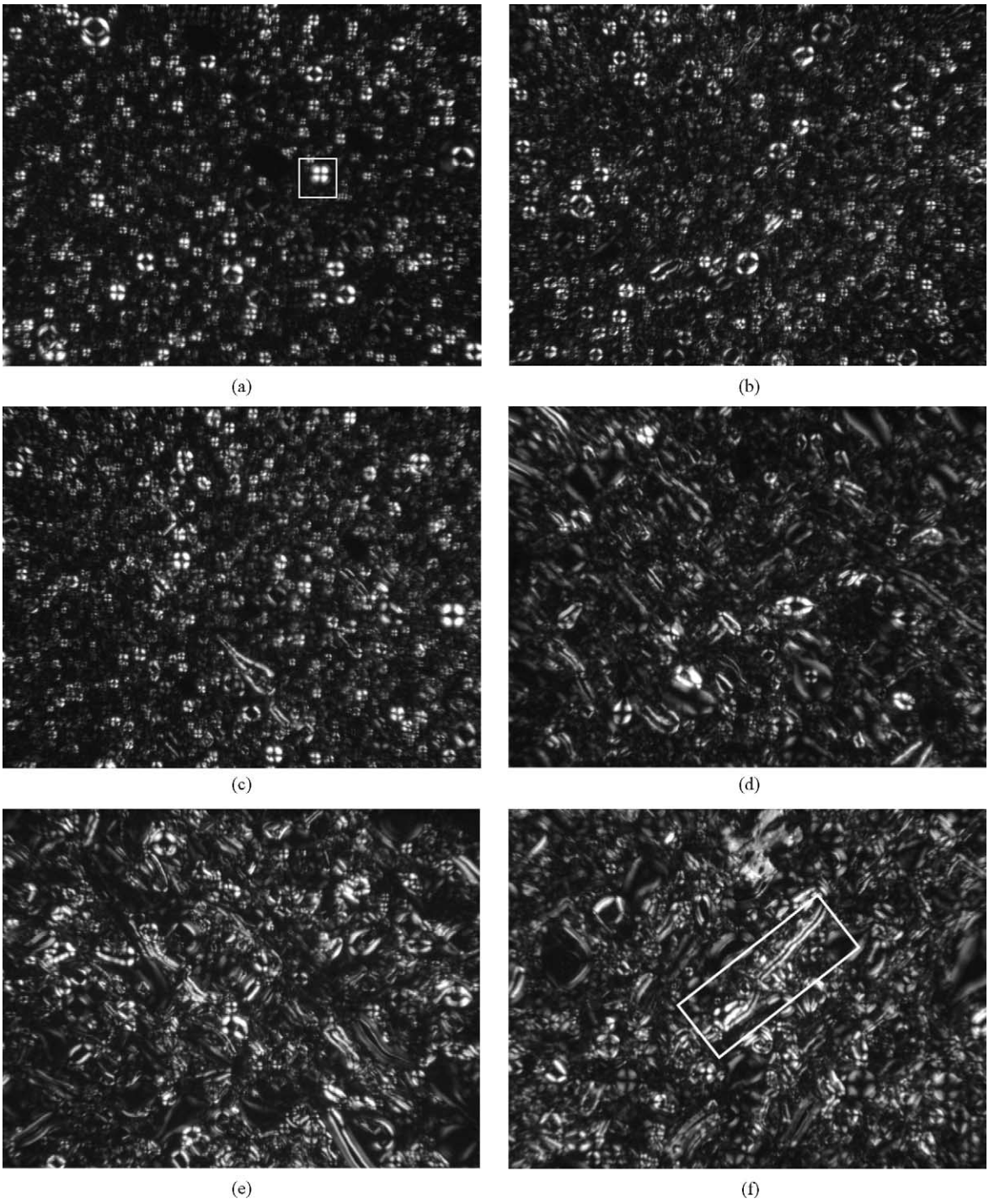

Fig. 1. Polarizing microscopic photos of MLV samples of different β-sitosterol concentrations. Sitosterol concentrations are the following: (a) 10 mol%, (b) 15 mol%, (c) 20 mol%, (d) 25 mol%, (e) 30 mol%, (f) 35 mol%.

also refer to the β-sitosterol induced phase transition (Fig. 3). Phospholipids show a phase transition from the L-β crystalline phase to L-α liquid–crystalline phase at a well defined temperature. The addition of 7–33 mol% cholesterol was shown to decrease the heat absorbed during the crystalline to liquid–crystalline phase transition. Cholesterol causes a progressive fluidization of the lipid chains between 7 and 50 mol%

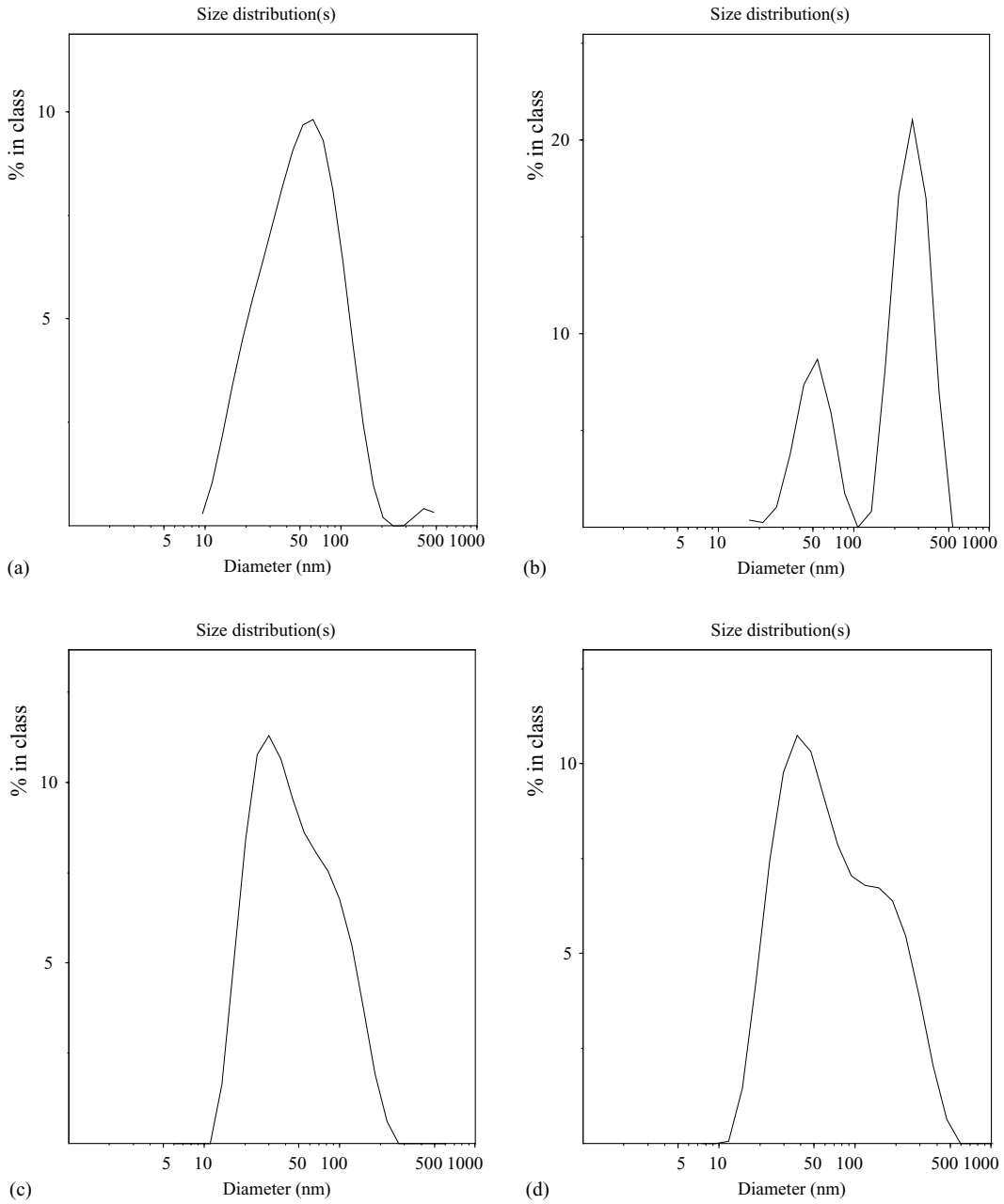

Fig. 2. Size distribution of different VPG samples. Sitosterol concentrations are the following: (a) 10 mol%, (b) 15 mol%, (c) 20 mol%, (d) 25 mol%, (e) 30 mol%, (f) 35 mol%.

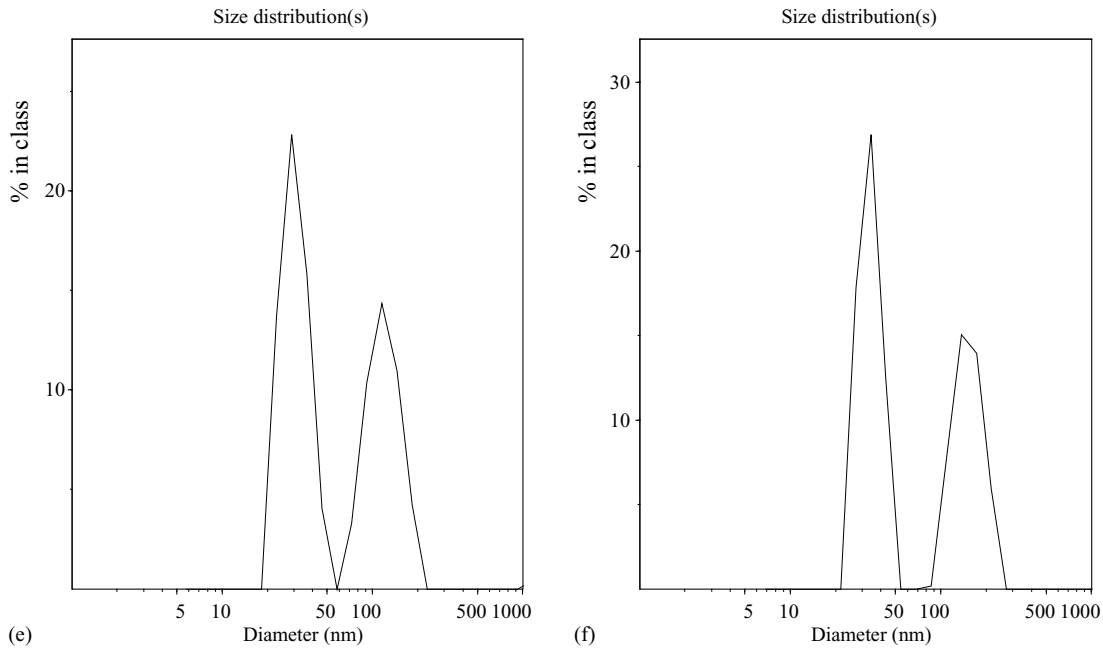

Fig. 2. (*Continued*).

(Demel and De Kruyff, 1976). Similarly, it was found that β-sitosterol, the more lipid-soluble and less planar sterol than cholesterol, could effectively prevent the rearranging and crystallization of the hydrocarbon chains observed in the case of the cholesterol-free phospholipid system below the transition temperature. The increase of β-sitosterol concentrations (10–35 mol%) is associated with the enthalpy decrease required to the thermotropic phase transition. The obtained curve is S-like indicating the transition

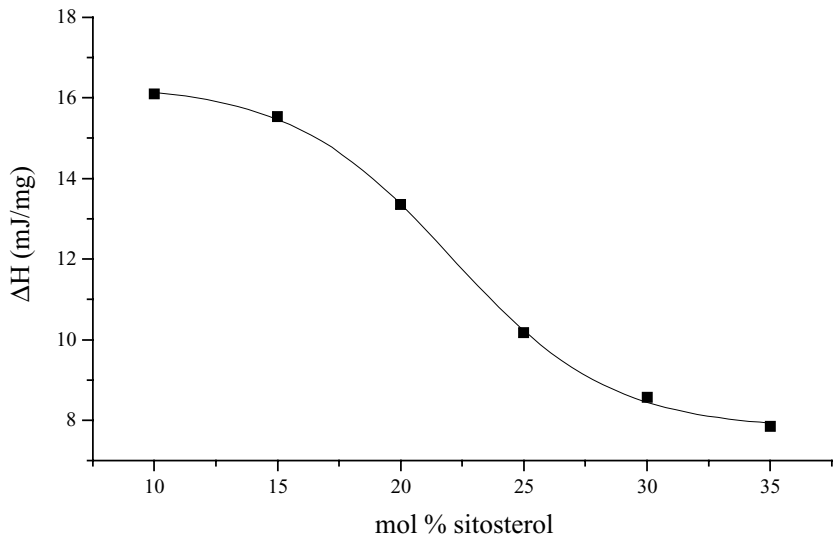

Fig. 3. Enthalpy changes of various VPG samples (average values, $n = 3$).

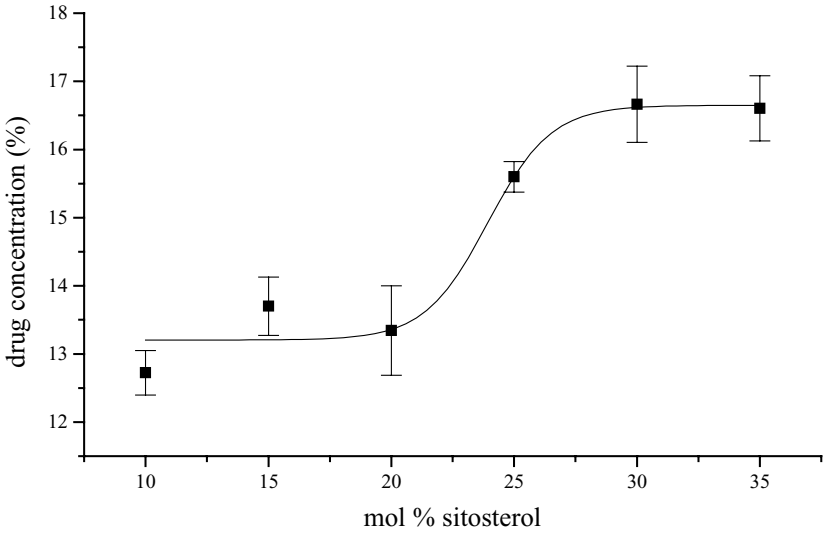

Fig. 4. Release of chlorhexidine gluconate from various VPG samples.

of more ordered rigid state to the less ordered more mobile liquid like phase.

3.3. The effect of β-sitosterol concentration of redispersed VPG-s on the chlorhexidine gluconate release

The found transition altered the extent of chlorhexidine release from the VPG systems. Fig. 4 also can be characterized by sigmoid curve. The low concentration of β-sitosterol causes the packing of the lipid chains to change from a tilted configuration to a vertical configuration. The increased packing and decreased mobility of the hydrocarbon chains result in reduced permeability. Along with the increase of β-sitosterol, the packing decreases and consequently the permeability increases. The obtained transition to the less ordered structure increased the amount of released

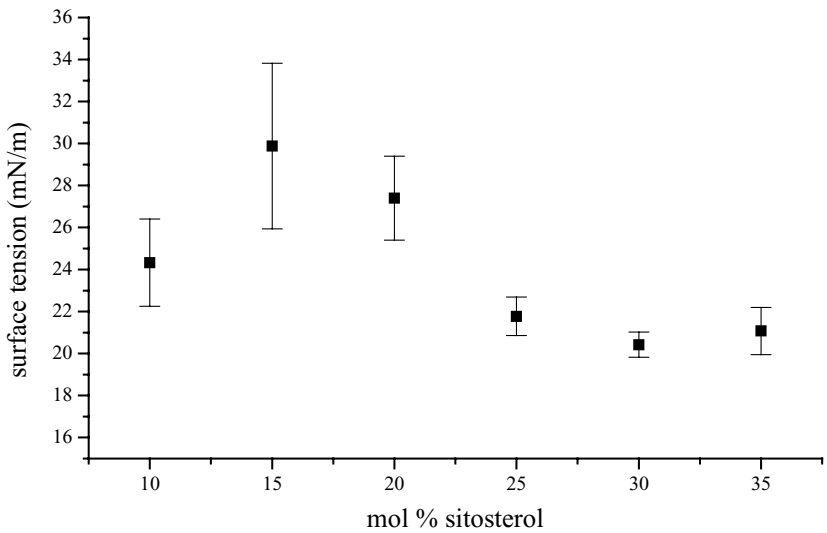

Fig. 5. Surface tension values of VPG samples of different sitosterol concentrations.

chlorhexidine. The linear portions of the two S-curves coincide because the configuration changes of the lipid chains are associated with the permeability changes, thus, the alteration of the extent of drug release.

Along with the changes of the β-sitosterol ratio in the vesicles, the surface tension of the whole system was also altered. The initial increase of the surface tension between 10 and 15 mol% β-sitosterol concentration refer to that there might exist a concentration limit where the β-sitosterol does not modify the original tilted configuration, but increases the hydrophilic behavior of the lipid bilayer. Above 20% β-sitosterol ratio the surface tension gradually decreased (Fig. 5). Decreasing surface tension of the redispersed VPG-s resulted in the increase of the membrane surface, and consequently the particle size of the vesicular dispersions was shifted to smaller size distributions (Fig. 2a–f). The smaller, less ordered, more fluid structure allowed the release of chlorhexidine in greater extent (Fig. 4).

4. Conclusions

The interfacial surface tension of the local vesicular gels determines the structure of the supramolecular elements of multilamellar vesicles. The phase transition of the vesicular gels 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. Due to the surface tension-decreasing effect of β-sitosterol, the membrane deformability and the dispersity of the VPG-s were increased. The increased dispersity and fluidity significantly increased the extent of chlorhexidine release from the vesicles.

Acknowledgements

We like to thank to Wolf Michaelis for his valuable help in the DSC experiments.

References

Bouwstra, J.A., Honeywell-Nguyen, P.L., 2002. Skin structure and mode of action of vesicles. Adv. Drug Deliv. Rev. 54, S41–S55.

Brandl, M., Bachmann, D., Drechsler, M., Bauer, K.H., 1990. Liposome preparation by a new high pressure homogenizer Gaulin Micronlab 40. Drug Dev. Ind. Pharm. 16, 2167–2191.

Catuogno, C., Jones, M.N., 2003. The antibacterial properties of solid supported liposomes on Streptococcus oralis biofilms. Int. J. Pharm. 257, 125–140.

Demel, R.A., De Kruyff, B., 1976. The function of sterols in membranes. Biochim. Biophys. Acta 457, 109–132.

Kaiser, N., Kimpfler, A., Massing, U., Burger, A.M., Fiebig, H.H., Brandl, M., Schubert, R., 2003. 5-Fluorouracil in vesicular phospholipid gels for anticancer treatment: entrapment and release properties. Int. J. Pharm. 256, 123–131.

Maitani, Y., Nakamura, K., Suenaga, H., Kamata, K., Takayama, K., Nagai, T., 2000. The enhancing effect of soybean-derived sterylglucoside and β-sitosterol β-D-glucoside on nasal absorption in rabbits. Int. J. Pharm. 200, 17–26.

Meisner, D., Mezei, M., 1995. Liposome ocular delivery systems. Adv. Drug Deliv. Rev. 16, 75–93.

Mora, M.P., Tourne-Peteilh, C., Charveron, M., Fabre, B., Milon, A., Muller, I., 1999. Optimisation of plant sterols incorporation in human keratinocyte plasma membrane and modulation of membrane fluidity. Chem. Phys. Lipids 101, 255–265.

Schuler, I., Duportail, G., Glasser, N., Benveniste, P., Hartmann, M.A., 1990. Soybean Phosphatidylcholine vesicles containing plant sterols: fluorescence anisotropy study. Biochim. Biophys. Acta (BBA)-Biomembr. 1028, 82–88.

Touitou, E., Junginger, H.E., Weiner, N.D., Nagai, T., Mezei, M., 1994. Liposomes as carriers for topical and transdermal delivery. J. Pharm. Sci. 83, 1189–1203.