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Influence of chlorhexidine species on the liquid crystalline structure of vehicle

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Abstract

The aim of this study was to investigate the influence of three chlorhexidine species, chlorhexidine base and its salts (diacetate and digluconate), on the physico-chemical features of liquid crystalline systems and on drug transport through lipophilic membranes. Nonionic surfactant, Synperonic A7 (PEG_7-C_{13-15}) was selected for the preparation of the liquid crystalline systems. Mixtures of different ratios of Synperonic A7 and water were prepared. The liquid crystalline systems were characterized using polarizing microscopy, small-angle neutron scattering and transmission electron microscopy. Membrane transport was also examined. The addition of chlorhexidine species to the liquid crystalline system modified the structure of the liquid crystalline system. As a result of liquid crystal–drug interaction, the solubility of chlorhexidine base and its diffusion through lipophilic membranes increased in comparison with those of the chlorhexidine salts.

Keywords: Liquid crystals; Clorhexidine base and salts; Transmission electron microscopy; Small-angle neutron scattering; Membrane transport

1. Introduction

Lyotropic liquid crystals have many advantages in formulation of semisolid vehicles. Physical stability, broad solubilization potential and delayed drug delivery characterize these systems (Shinoda and Friberg, 1986; Osborne and Ward, 1995).

Higher concentrations of amphiphile molecules form close-packed aggregated structures in water, as liquid crystals. Different liquid crystalline phases (cubic, hexagonal and lamellar) can be observed depending on the molecular packing of the aggregates (Israelachwilli et al., 1976). Liquid crystals have distinct hydrophilic and hydrophobic domains, which give these systems the possibility of dissolving or dispersing both water-soluble and water-insoluble compounds.

Novel optical and electromicroscopic investigations support the nature of the aggregates expected and confirm their highly dynamic nature (Osborne and Amann, 1990; Osborne and Ward, 1995).

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In the authors' previous study (Farkas et al., 2000), drug release, differential scanning calorimetry and rheological measurements were carried out for analysing the kinetics of chlorhexidine diacetate release from lamellar and hexagonal liquid crystalline phases in relation to the thermodynamic state and viscoelasticity of the sample.

The main goal of this paper was to study the influence of three chlorhexidine species, chlorhexidine base and its salts (diacetate and digluconate), on the physico-chemical features of the liquid crystalline system and on the drug transport through a lipophilic membrane.

2. Materials and methods

2.1. Materials

Chlorhexidine, chlorhexidine diacetate (Aldrich Chemical Co.), chlorhexidine digluconate (RE-ANAL, Hungary) were used. The base and its salts differ highly in water solubility (Table 1). The nonionic surfactant, Synperonic A7, was a gift from ICI Surfactants. It is an alcohol ethoxylate type surfactant of a mixture of C_{13} and C_{15} alkyl chains in the ratio of 6.6:3.4 and of an average of seven ethyleneoxide units per molecule.

Bidistilled water was used in all formulations. Other chemicals and reagents were analytical or high-performance liquid chromatography grade.

2.2. Sample preparation

The samples were prepared by heating Synperonic A7, water and drug to 60°C in closed glass

Table 1
Some physico-chemical properties of the examined drugs

Drug	Water solubility (% w/v)	Octanol/water partition coefficient[a]
Chlorhexidine	0.008	0.754
Chlorhexidine diacetate	1.8	0.047
Chlorhexidine digluconate	>70	0.037

[a] Relative standard deviation, <5.0%.

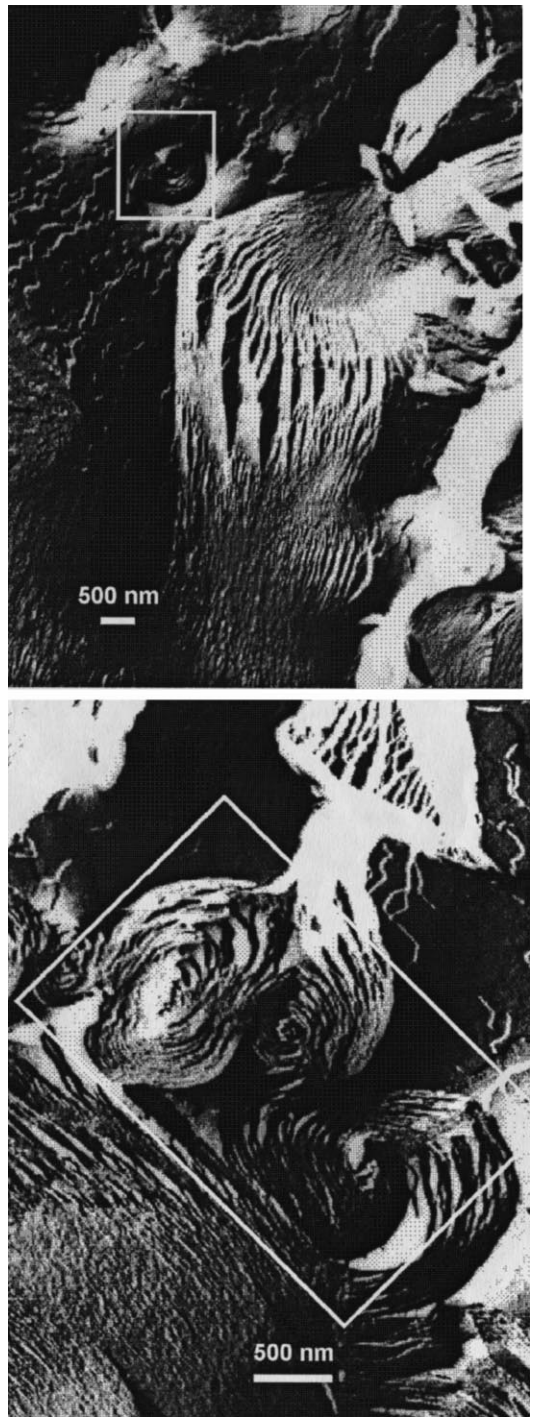

Fig. 1. Electron micrographs of liquid crystalline systems containing chlorhexidine base: (a) 50% w/w Synperonic A7; (b) 70%w/w Synperonic A7.

vials, and they were stirred until the drug dissolved and clear solutions were obtained. The solutions were then cooled down to room temperature. The concentration of the incorporated drug was 4%w/w.

2.3. Microscopic analysis

The texture of the samples was observed by polarizing microscope (HUND, Germany). The measurements were carried out at room temperature, under magnification of 200–400 ×.

2.4. Small-angle neutron scattering

For structural investigation of the liquid crystalline samples, the small-angle neutron scattering (SANS) method was used. Small-angle scattering of the neutrons has the advantages of being non-destructive and of providing structural information with high statistical accuracy.

The liquid crystalline samples were prepared using deuterium in order to achieve good contrast between the hydrogenated surfactant molecule and the solvent (Jacrot, 1976).

The measurements were carried out on the SANS instrument at the Budapest Research Reactor. The intensities were observed as a function of the Bragg wavenumber (Q):

$$Q = (4\pi/\lambda)\sin(\theta/2) \quad (1)$$

where λ is the neutron radiation wavelength and θ is the scattering angle.

Structural information can be obtained from the peak position Q_0, i.e. the repeat distance d, with the relation $Q_0 = 2\pi/d$.

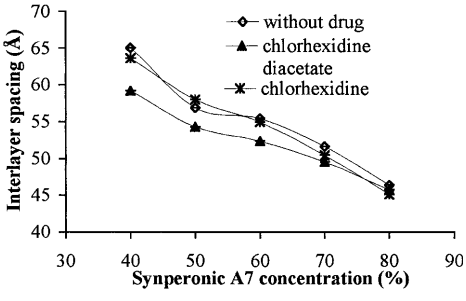

Fig. 2. Interlayer distance of a liquid crystalline sample containing different drugs (relative standard deviation, $< 0.06\%$).

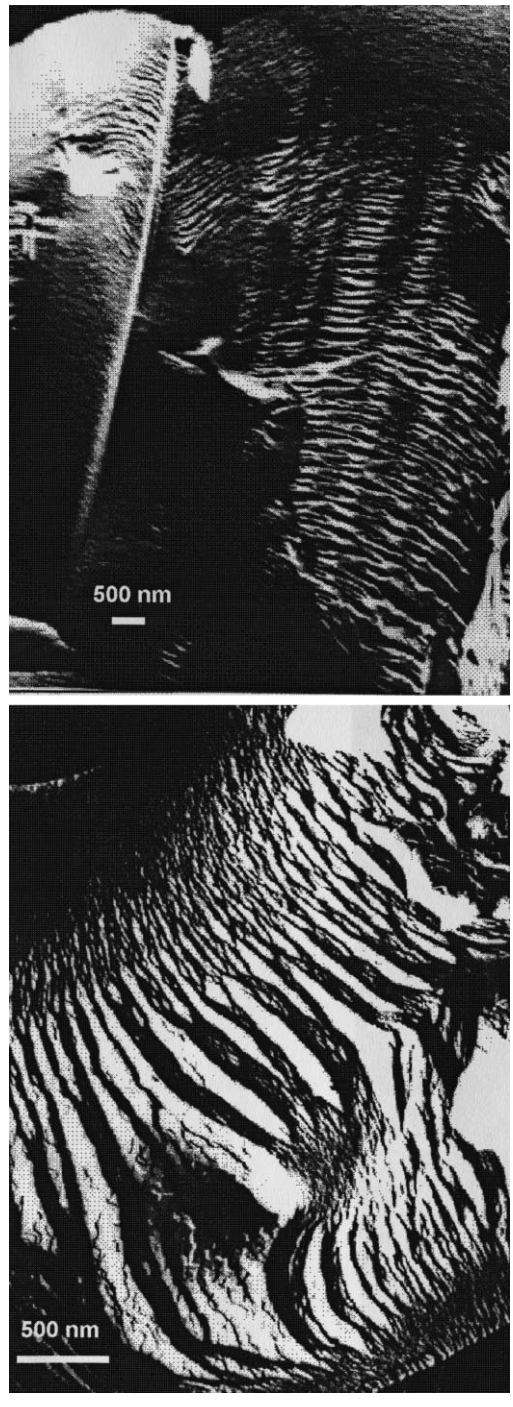

Fig. 3. Electron micrographs of liquid crystalline systems containing chlorhexidine diacetate: (a) 50%w/w Synperonic A7; (b) 70%w/w Synperonic A7.

2.5. 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 they were shadowed at −110°C with Pt/C (2 nm) at 45° elevation and with C (25 nm) at 90° elevation. The obtained Pt/C layer was then cleaned with distilled water and mounted on 200 mesh copper grids, and it was viewed in a transmission electron microscope (CM10; Philips).

2.6. Partition studies

The octanol/water partition coefficients ($P_{o/w}$) of chlorhexidine and its salts were determined by the shake-flask method. The phases were analysed spectrophotometrically. $P_{o/w}$ was calculated from the equation $P_{o/w} = C_{octanol}/C_{water}$, where $C_{octanol}$ and C_{water} are the drug concentrations measured in octanol and in water, respectively.

2.7. Membrane transport study

The drug diffusion experiments were carried out in SartoriusModell Apparatus (Göttingen, Germany) as described earlier (Loth and Holla-Benninger, 1978). The donor chamber of the flow-through cell contained 8.0 g liquid crystalline sample. The acceptor medium was Britton–Robinson buffer (pH 6.0 ± 0.05) and it was stirred by a teflon-coated magnetic bar. Cellulose nitrate membrane (Sartorius SM 16754; Göttingen, Germany) impregnated with dodecanol was placed between the two phases. The effective surface area of the membrane was 15.9 cm^2. The measurement was carried out at 32°C for 6 h.

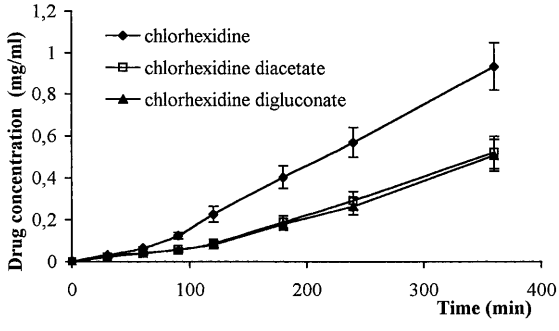

Fig. 4. Drug transport from liquid crystalline systems containing 70%w/w Synperonic A7.

2.8. Quantitative determination of chlorhexidine diacetate

Chlorhexidine species were analysed spectrophotometrically at 255 nm using a Shimadzu UV-160A (Japan) spectrophotometer. The method gave a linear response over a concentration range of 1–20 μm/ml.

3. Results and discussion

The octanol/water partition coefficient of chlorhexidine base is higher than that of the chlorhexidine salts (Table 1). As a result of the higher lipid solubility, the base could locate between the Synperonic molecules in the liquid crystalline structure. This phenomenon could be confirmed by the transmission electron microscopy (TEM) photographs of the freeze-fractured sample and by the results of the small-angle neutron scattering. The domains consist of regions of vortex-type fluid motion (Gray and Winsor, 1974) that are visible on the TEM photos (Fig. 1). This orientation could not significantly change the interlayer distance (Fig. 2). Chlorhexidine salts could partially locate between the liquid crystal layers because of their ionic molecular part, and consequently they induce less vortex-type fluid motions (Fig. 3). As a result of their ionic structure, H-bonds between the chlorhexidine salts and the liquid crystal molecules can be formed, thus decreasing the interlayer distance (Fig. 2).

The addition of chlorhexidine base to the liquid crystal may lower the structure-dependent part of the free energy and give a higher solubility than can be achieved in an isotropic liquid with a

similar chemical composition. The results of the membrane transport studies indicate that the extent of diffusion of chlorhexidine base increased from the prepared liquid crystalline system. Fig. 4 illustrates the transport of chlorhexidine base and its salts from liquid crystalline systems containing 70% nonionic surfactants through a hydrophobic membrane. The results indicate that, within the examined Synperonic A7 concentration range (40–80%), the drug diffusion profile was similar in each case.

4. Conclusion

The results indicate that the location of the chlorhexidine base is between the Synperonic molecules, and that diffusion mainly takes place within the constrains of the hydrophobic parts of the lamellar structure. The drug–vehicle interaction modified the liquid crystalline structure, thus solubilizing the active substance and consequently increasing the extent of diffusion through a lipophilic membrane.

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