THERMOANALYTICAL INVESTIGATION OF LYOTROPIC LIQUID CRYSTALS AND MICROEMULSIONS FOR PHARMACEUTICAL USE

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The interactions between surfactant and water were studied thermoanalytically focusing on the lyotropic liquid crystalline and microemulsion region in four ternary systems containing Cremophor EL and Cremophor RH40 as surfactants, neutral oil and isopropyl myristate as oily components. Subzero temperature DSC (SZT-DSC) measurements were carried out to determine the quantity of the bound water forming a hydration layer in surfactant microstructures, and the amount of free water, which has physico-chemical properties not much different from those of pure water. The variation of the surfactant:bound water ratio in the function of water concentration was also investigated. Phase changes detected by the SZT-DSC measurements were confirmed by polarization-microscopic and rheological investigations.

Keywords: free and bound water, lyotropic liquid crystals, SZT-DSC measurements

Introduction

Surfactant-based lyotropic liquid crystalline systems and microemulsions are becoming more widely used in the cosmetic and chemical industries and also in the field of pharmaceutical technology and this kind of systems can be characterized by using of calorimetrical methods [1–4]. Lyotropic liquid crystals are usually formed from water and one or two surfactants and possibly cosurfactants and oils within a definite concentration and temperature range. Depending on the temperature and the concentration of the solvent (generally water or an aqueous solution), there are many types of liquid crystal states called mesophases, which are characterized by different degree and way of order in the material. Cubic, hexagonal, lamellar and micellar phases can be observed in most cases [5, 6]. Different mesophases show different rheological properties, therefore by varying the solvent concentration their consistency can be changed, as required [7, 8]. The term microemulsion is used to describe a system of water, oil and amphiphile, which is a single optically isotropic and thermodynamically stable liquid solution [9, 10]. From the pharmaceutical point of view lyotropic liquid crystals and microemulsions possess several advantageous properties. They are able to incorporate and control the release of drugs of varying size and polar characteristics as they contain lipophilic, hydrophilic and amphiphilic domains as well. Through the use of non-ionic surfactants, a reversible increase in the permeability of the stratum corneum can be achieved. Due to the very low interfacial tension arising at the oil/water interface, they

may facilitate the progressive diffusion of biologically active substances into the skin or systemic circulation [11–13]. They are formed usually with low energy input or by means of spontaneous structural organization; their preparation is therefore simple and cost-effective. Due to their thermodynamical stability, these systems can be stored for long periods of time without phase separation [5, 6]. These advantageous features make them interesting to be used as drug delivery systems for various routes of administration.

In view of the above-mentioned facts it can be stated that one of the principal components of these surfactant-based systems is water, the behavior of which is sensitive to the presence of adjacent interfaces of varying types. Different forms of water have been detected in surfactant-based microstructures such as microemulsions and liquid crystalline phases [14]. Castoro *et al*. and Atkas *et al*. classified water into two categories, free and bound water, based on a temperature of –40°C [15]. The part of water which achieves complete freezing at this reference temperature accepted by numerous researchers is termed free water, and the remaining unfrozen water is termed bound water. When the system is sufficiently diluted, free water is present in it, which is assumed to have similar physico-chemical properties like pure water. Its presence can be detected by so-called subzero temperature differential scanning calorimetry (SZT-DSC) by the appearance of the melting peak on the DSC curve at about 0°C. The disappearance of the melting enthalpy of water (at the disappearance of free water) coincides with phase changes occurring in the liquid crystalline structure [15, 16].

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Phase changes caused by the change of water content can be detected as well by means of polarization microscopic and rheological investigations [17–23]. Németh *et al*. reported a dynamic rheological method for the identification of pharmaceutically important lamellar phases [21].

The aim of our research was to study the effect of different water concentration on the liquid crystalline structure and to determine the amount of free and bound water in compositions with different water content. The knowledge of the proportion of varying types of water is important to get information of the structure, which has a strong effect on drug release from pharmaceutical formulations. The quantity of free and bound water was determined by means of SZT-DSC measurements, phase changes detected by the SZT-DSC experiments were confirmed by polarisation light microscopy and oscillation rheological investigations.

Experimental

Materials and methods

Materials

The non-ionic surfactants were polyethoxylated 35 castor oil (BASF, Cremophor EL official in USP/NF) and polyethoxylated 40 hydrogenated castor oil (BASF, Cremophor RH40 official in USP/NF). The applied oils were neutral oil (Ph. Eur. 4) and isopropyl myristate (Ph. Eur. 4). Distilled water was produced by double distillation in glass equipment.

Sample preparation

The oil-surfactant mixture was homogenised with a magnetic stirrer (Ikamag RET-G, 500 r/s) at room temperature. The oil:surfactant ratio was 1:4. Compositions with various water content (from 10 to 90%) were prepared in order to study the effect of water on the properties of the samples. In the course of this the given quantity of water was added to the oil-surfactant mixture in small amounts while stirring. The samples were stored at room temperature for 1 week before investigation, until the subsequent spontaneous structural organizations had finished.

Polarization microscopic examinations

The structure of the samples was examined with a polarization microscope (LEICA Q500 MC Image Analyser system) at room temperature. The magnification was 40 fold.

Differential scanning calorimetry

The measurements were carried out with a Mettler-Toledo DSC 821^e instrument. Samples (5–10 mg) were weighed in aluminium pans and immediately sealed by press. The reference was an empty pan. The samples were cooled at a heating rate of $-5°C$ min^{-1} to $-40°C$. They were kept 2 min at this temperature and then samples were heated to 25°C. The heating rate was 5°C min^{-1}. The heat flow was measured as a function of the temperature.

Rheological investigations

Rheological measurements were carried out with a RheoStress 1 HAAKE rheometer. A cone-plate measuring device was used in which the cone angle was 1 degree, and the thickness of the sample was 0.048 mm in the middle of the cone. The measurements were performed at room temperature. The samples were kept in a space saturated with water vapour during measurement in order to prevent evaporation. The linear viscoelastic range was determined in the first step by examining the complex modulus as the function of shear stress at a given frequency (1 Hz). Based on these experiments, the value of shear stress was set at 2.5 Pa during the dynamic test as this value was always within the linear viscoelastic range, then the values of the storage and loss moduli were examined as the function of frequency. Besides the oscillation tests, flow curves and viscosity curves of the different samples were also determined.

Results and discussion

The ternary phase diagrams of the investigated systems can be seen in Fig. 1. The dilution line along which the composition changes with increasing water concentration is marked with a black line. On the basis of the phase diagrams the occurring phase changes can be followed. The exact composition of the studied systems is summarized in Table 1.

In the course of SZT-DSC experiments we observed the sharp exothermic and the wider endothermic peak caused by freezing and melting of water just above a definite water concentration. This phenomenon is caused by the fact that under a certain water content there is a strong interaction between the surfactant and water molecules. This type of water is termed non-freezable, or bound water and it has been interpreted as water forming a hydration layer in surfactant microstructures. When the saturation of surfactant molecules with water occurs, a new state of water appears which possesses similar features like the pure solvent. The water concentration at which this free or bulk water appears coincides
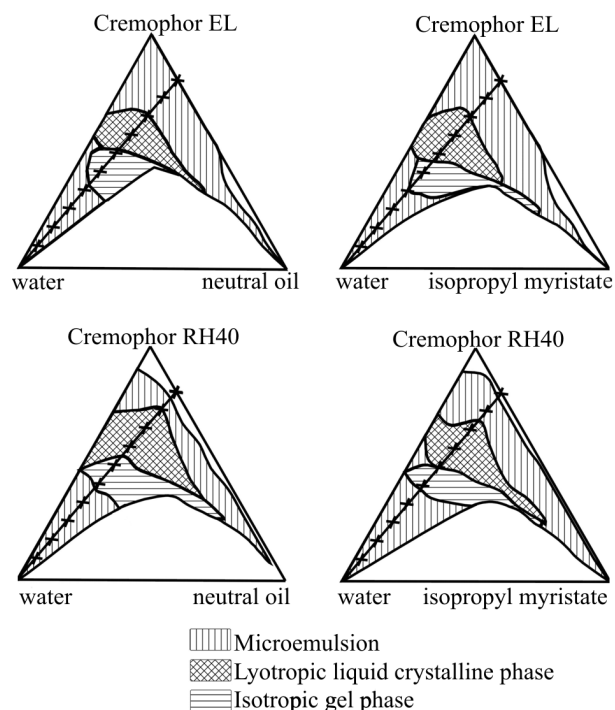
Microemulsion
Lyotropic liquid crystalline phase
Isotropic gel phase

Fig. 1 Ternary phase diagrams of the investigated systems

Fig. 2 Variation of melting enthalpy in the function of water concentration

with phase changes in the liquid crystalline texture [16]. This change of the structure can be detected by means of rheological measurements and polarisation microscopic examinations as well [21, 22].

On the basis of curves the melting enthalpy of water ($\Delta H_{f(exp)}$) was calculated and plotted in the function of water concentration. In Fig. 2 it can be seen that there is a linear relationship between the measured enthalpy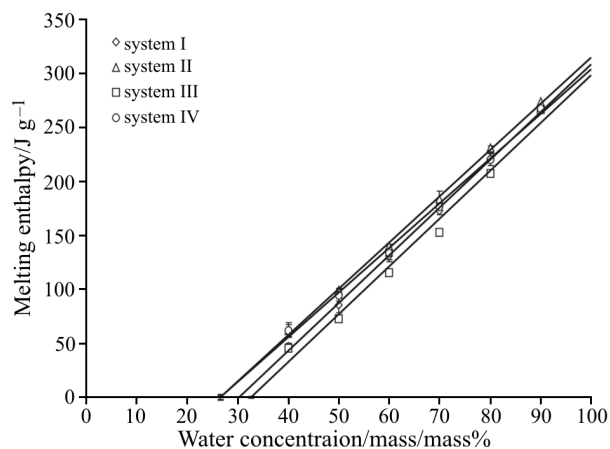 change and the water content of samples. By means of equations describing this relationship the water concentration at which the melting enthalpy disappears and the melting enthalpy of systems containing 100% water were calculated. This calculated melting enthalpy was similar to the value which can be measured in the case of pure water (320 J g^{-1}). The above mentioned data are summarized in Table 2.

On the bases of the results we were able to calculate the amount of free and bound water using the equations

$$W_f = \frac{\Delta H_{f(exp)}}{\Delta H_f^0} \cdot 100 \tag{1}$$

$$W_t = W_f + W_b \tag{2}$$

where W_f is the mass percent of free water, W_t is the mass percent of total water content, W_b is the mass

Table 1 Compositions of the studied systems

System	Surfactant/%		Oil/%	
I	Cremophor EL	80	neutral oil	20
II	(polyethoxylated 35 castor oil)	80	isopropyl myristate	20
III	Cremophor RH40	80	neutral oil	20
IV	(polyethoxylated 40 hydrogenated castor oil)	80	isopropyl myristate	20

Sample number	Surfactant/%	Oil/%	Water/%
1	80	20	0
2	72	18	10
3	64	16	20
4	56	14	30
5	48	12	40
6	40	10	50
7	32	8	60
8	24	6	70
9	16	4	80
10	8	2	90

**Table 2** Results calculated from the relationship between the water concentration and the measured melting enthalpy

System	Equation	R^2	Water concentration at zero enthalpy/mass/mass%	Calculated melting enthalpy of samples with 100% water content/J g^{-1}
I	$y=4.4181x-133.31$	0.9989	30.17	308.5
II	$y=4.2827x-113.63$	0.9985	26.53	314.64
III	$y=4.4195x-143.97$	0.9844	32.58	297.98
IV	$y=4.1362x-109.85$	0.9964	26.56	303.77

percent of bound water, $\Delta H_{f(exp)}$ is the measured enthalpy change for the melting peak, and ΔH_f^0 is the melting enthalpy calculated by extrapolation to the 100% water content [16]. Knowing the amount of bound water and the composition of samples, we examined how the surfactant:bound water ratio varies with the increasing water content. On the basis of Fig. 3 it can be stated that after the saturation of the surfactant and below 80% water concentration the amount of bound water forming the hydration layer around the surfactant molecules is independent of the total water content of samples. Above 80% water content the decrease of the amount of the bound water related to the surfactant concentration can be explained by the fact that these systems are situated at the border region of colloid systems and can be characterized by lower stability.

Considering the results it can be seen that in the case of the four investigated systems the free water will appear above 30% water content. We tried to confirm the occurring phase change at this point by means of independent methods as well. In Fig. 4 we can see the polarisations micrographs of samples (system II) with 20 and 30% water concentration. In the case of 20% water content a typical pattern of the lamellar liquid crystalline structure can be observed with Maltese crosses. At 30% water content we can see a hexagonal texture.
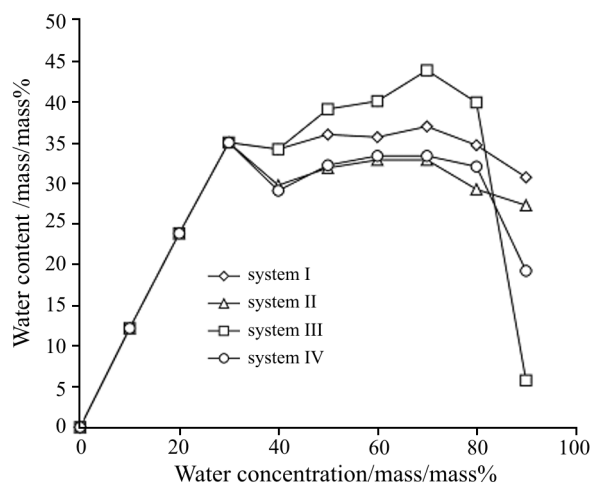
The phase change can be demonstrated also with rheological examinations. Oscillation tests were carried out with samples of different water content. The change of storage modulus (G') and loss modulus (G'') was investigated. These parameters describe the elastic and plastic behavior of materials. In Fig. 5 the oscillation test of samples with 10, 20 and 30% water concentration can be seen. In the case of 10% water content the G'' value is higher than the G' value, that is the system shows plastic behavior. At 20% water content the value of G' will exceed with about one order of magnitude that of G'', and the G'' curve shows a minimum. This rheological behavior characterizes each of our samples possessing

Fig. 3 Water content of the hydrated surfactant in the function of water concentration
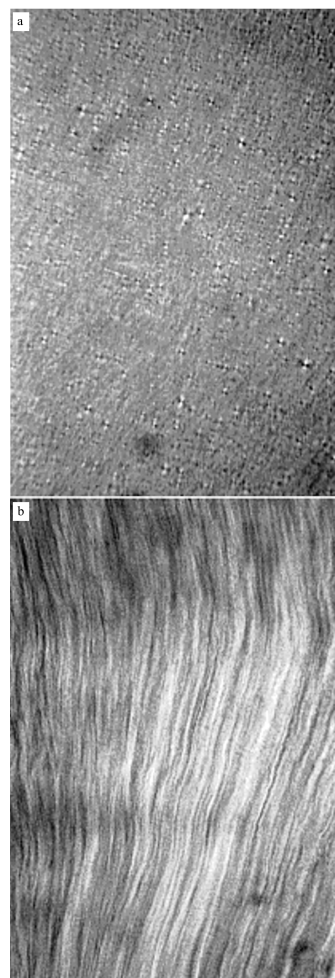
Fig. 4 Polarization micrographs of sample II/3 a – with 20% water content, and II/4 b – with 30% water content at magnification of 40×
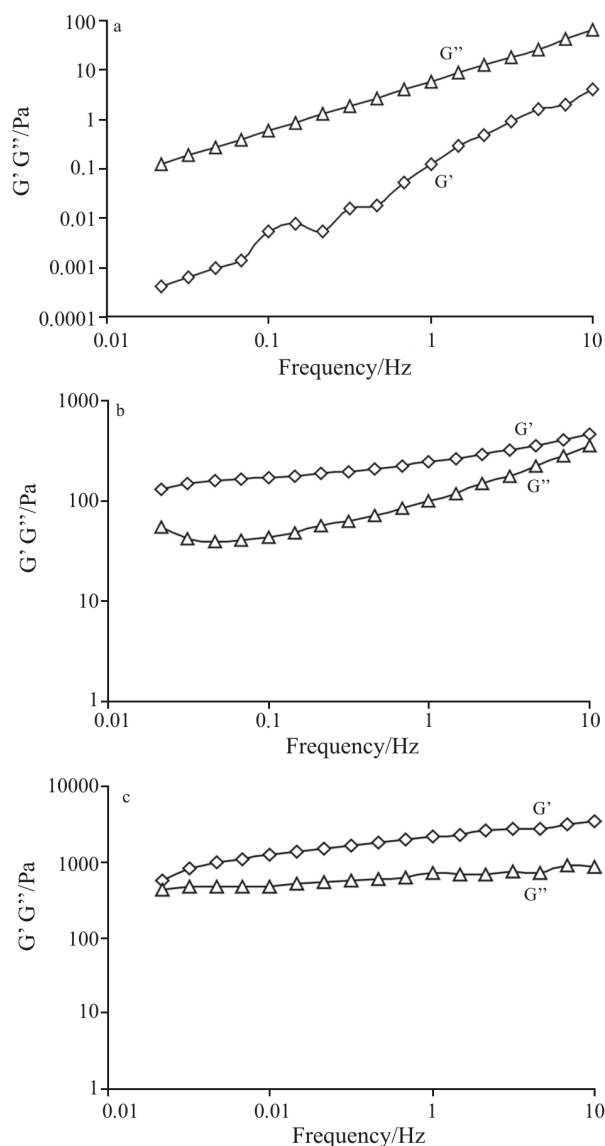
Fig. 5 Oscillation test of sample II/2 a – with 10% water content, II/3 b – with 20% water content and II/4 c – with 30% water content
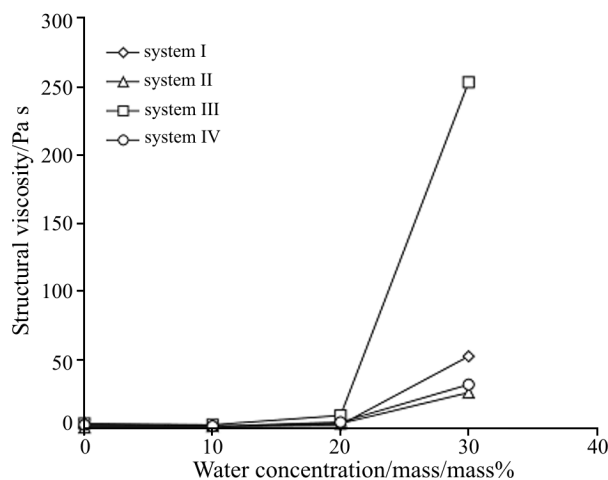

Fig. 6 Variation of structural viscosity *vs.* water content

lamellar structure. When the water concentration is 30%, higher G' values can be observed, which indicates the presence of the more elastic hexagonal structure [21]. The phase change can be detected by considering the viscosity values in the function of water content as well. In Fig. 6 we can see the rapid increase in the viscosity values at 30% water concentration. Indeed, the lamellar phase shows low viscosity, and the hexagonal structure forms a more rigid phase characterized by high viscosity values. According to our results it can be stated that rheological measurements applied for the structure examination of lyotropic liquid crystals are well completed by thermoanalytical examinations. SZT-DSC measurements are suitable to determine the pharmaceutically important free and bound water ratio, and to follow the occurring phase changes in surfactant-water based systems.

Conclusions

On the bases of the results it has been established that SZT-DSC measurements are suitable to detect the water concentration at which the saturation of surfactants by water occurs and the free water appears in the system. In the case of the investigated compositions these phenomena coincide with the transformation of the liquid crystalline texture from lamellar arrangement into hexagonal structure. Upon the examination of the water content of the hydrated surfactant in the function of water concentration it can be stated that after the saturation of the surfactant and below 80% water content its value is practically constant. Above 80% water concentration a reduction of the hydration layer can be observed, nevertheless bound water has also been found in the microemulsion area.

Acknowledgements

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