Colloid Polym Sci (2007) 285:657–663
DOI 10.1007/s00396-006-1608-2

ORIGINAL CONTRIBUTION

X-ray investigation of the role of the mixed emulsifier in the structure formation in o/w creams

Magdolna Kónya · Imre Dékány · István Erõs

Received: 7 March 2006 / Accepted: 12 October 2006 /

Abstract The aim of this research work was to clarify the role of the mixed emulsifier in the structure formation and water binding mode in the case of o/w creams prepared with different surfactants. The swelling behavior of mixed emulsifiers was examined by means of direct investigation methods such as transmission electron microscopy (TEM) and X-ray diffraction. The detailed structure image of the creams was created with the help of the latter. The influence of the structure of the hydrophilic gel phase, and the structural changes during storage were studied with rheological methods. On the basis of the results, it can be stated that the investigated creams had different structures from those mentioned in the literature: surfactant did not create a mixed bilayer with the structure to furnish fatty amphiphile; instead, micelles were formed. These results correlated well with the results of the rheological tests.

Keywords O/w creams · Microstructure · Mixed emulsifier · X-ray diffraction · Rheology

Introduction

O/w creams are complex, multicomponent preparations. They are combinations of a number of surfactants, polymers, and other additives. Mixed emulsifiers are frequently used in o/w systems; these are the combinations of an ionic or nonionic surfactant with fatty amphiphiles, such as fatty alcohols, fatty acids, or monoglycerides [1]. Phase-separation processes can be eliminated through their use, which is essential for the preparation of a product with consistently equal long lasting therapeutic properties, real storage ability, and good stability. The gel-network theory, developed by Barry and Eccleston [2–5], gave a coherent explanation for the manner in which mixed emulsifier not only stabilize o/w creams but also control their consistencies. The lamellar theory of the microstructure of creams was set up by summarizing the results of X-ray diffraction, electron microscopic, thermoanalytical, and rheological studies. O/w creams stabilized with a mixed emulsifier are at least four-phase systems, as follows: (1) *crystalline/hydrophilic gel phase*: composed of bilayers of surfactant and fatty amphiphile [6–8]. Water molecules are inserted between the bilayers, thus forming an interlamellar water layer. (2) *Bulk water layer*: mechanically bound water [9–11]. (3) *Lipophilic gel phase*: the excess of the fatty amphiphile, which is not part of the hydrophilic gel phase, builds up a matrix with lipophilic character; (4) *dispersed oil phase*: this inner phase is mainly immobilized mechanically from the lipophilic gel phase [12–14]. The literature findings indicate that this four-phase structure theory is generally valid for o/w creams prepared with ionic or nonionic surfactants. The proportions of the various water phases (interlamellarly bound or bulk water) in o/w creams depend on the composition and proportions of the constituents of the mixed emulsifier. It is assumed that

---

M. Kónya · I. Erõs
Department of Pharmaceutical Technology, University of Szeged,
Szeged, Eötvös u. 6.,
6720, Hungary

I. Dékány
Department of Colloid Chemistry, University of Szeged,
Szeged, Aradi vértanúk tere 1.,
6720, Hungary

M. Kónya (✉)
Rátz László u. 13. II/8,
Budapest 1116, Hungary
e-mail: magdolna.konya@sanofi-aventis.com

Table 1 Surfactants and their properties

Abbreviation	Surface active agent	Chemical name	Properties
EC	Eucarol® AGE/EC (Cesalpinia Chem., Lamberti Spa.)	Aqueous solution of alkylpolyglucoside citrate	Anionic, liquid, HLB~20
ET	Eucarol® AGE/ET (Cesalpinia Chem., Lamberti Spa.)	Aqueous solution of alkylpolyglucoside tartrate	Anionic, liquid, HLB~20
Tegin	Tegin® (Goldshmidt AG)	Glyceryl stearate SE	Nonionic POE-free, solid, HLB=12, Mp=77–80 °C
TCPS	Tego Care® PS (Goldshmidt AG)	Methyl glucose sesquistearate	Nonionic POE-free, solid, HLB=12, Mp=77–78 °C
T60	Tween® 60 (Sigma)	Polyoxyethylene (20) sorbitan monostearate	nonionic with POE chain, liquid, HLB=14.9

HLB Hydrophilic–lipophilic balance value from the manufacturer, *Mp* melting point value from the manufacturer

interlamellarly bound water molecules exhibit different physicochemical and biopharmaceutical properties from those of the bulk water phase because the interlamellarly fixed water may serve as a formulation reservoir (“depot”) for controlled release skin hydration [12, 13].

To understand the microstructure of o/w creams better, simple models could be used that mimic the mode action of the mixed emulsifiers in more complex systems. It was shown that ternary surfactant/amphiphile/water systems have similar structures and rheological properties to the corresponding creams, and such ternary systems have been extensively studied as structural models for the continuous phases of dermatological creams [15–22].

The object of this study was to determine the exact microstructure of the investigated creams with the help of X-ray diffraction on ternary systems and to analyse the role of the mixed emulsifier in the structure formation and water binding mechanism through the examination of its swelling ability. We studied the modifying effect of the different cream structure by means of rheological measurements.

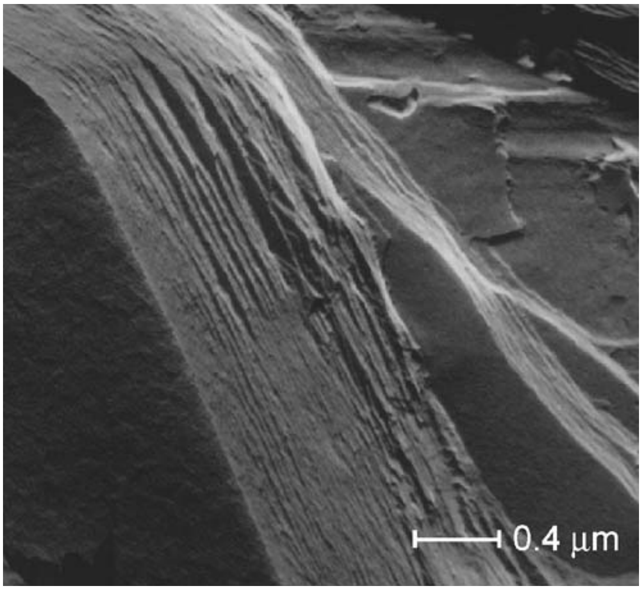

Fig. 1 Freeze-fracture electrographs of o/w creams prepared with T60, revealing the lamellar structure

Materials and methods

Materials

The following members of three different emulsifier groups were used during our research work: ionic, nonionic POE chain containing, and nonionic POE chain-free surfactants (Table 1).

Cetostearyl alcohol (CSA) as fatty amphiphile, isopropyl myristate (IM) as the lipophilic phase of the creams, distilled water, and the preservatives (methyl *p*-hydroxybenzoate and propyl *p*-hydroxybenzoate) were of Ph. Eur. 4 grade.

Sample preparation

For the swelling studies of the mixed emulsifier, a series of ternary surfactant/CSA/water systems were prepared with a constant ratio of surfactant to fatty alcohol of 1:4 and increasing water contents 25, 40, 60, 70% *w*/*w*. A

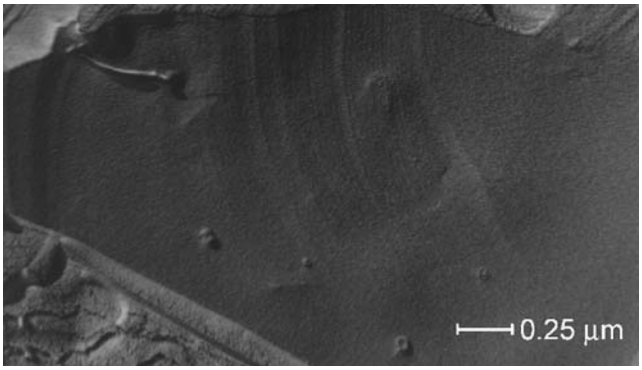

Fig. 2 Freeze-fracture electrographs of o/w creams prepared with ET, revealing the lamellar structure

Table 2 X-ray diffraction data on the ternary T60/CSA/water system

Water (%)	2Θ (°)	d_L (Å)	D (Å)	$n_b(=D/d_L)$
25	1.76	50.11	625.22	12.5
40	1.76	50.11	340.78	6.8
60	1.67	52.97	248.91	4.7
70	–	–	–	–

corresponding series of CSA/water systems without any surfactant was also prepared to identify the peaks corresponding to crystalline CSA. Creams were prepared with 4% *w*/*w* surfactant content (20% *w*/*w* mixed emulsifier, 20% IM and 60% *w*/*w* distilled water). To prepare the systems (30.0 g in each case), the mixed emulsifier or mixed emulsifier/lipophilic phase (in the case of the creams) were melted in a pan at 70 °C. The preservatives were dissolved into hot distilled water. When the temperature of both phases was 70 °C, the water was added to the melted mixed emulsifier or mixed emulsifier/lipophilic phase and the ternary system/cream was allowed to cool under continuous manual stirring. The water that evaporated off was replaced up to the desired weight.

Microscopic analysis

The microstructure of o/w creams was studied with freeze fracture techniques and TEM. Replicas were produced with BAF 400D freeze-etching device (Balzers, Liechtenstein). Specimens were mounted on 300-mesh copper grids and examined in a CEM902 (Zeiss, Germany) transmission electron microscope. Micrographs were made at a primary magnification of ×7,000–20,000.

X-ray diffraction

Qualitative investigation of the lamellar layer, and quantitative characterization of the lamellar distance: the swelling behavior of the applied surfactant and the water layer thickness, the detailed microstructure of o/w creams and were determined with the help of X-ray diffraction. The repeat distances were therefore calculated according to the Bragg equation:

$$n\lambda = 2dL \cdot \sin\Theta$$

where n is the integer (the reflection order), λ is the wavelength of the incident X-rays, d_L is the lamellar spacing, and Θ is the angle of reflection [23]. The lamellar spacing (d_L) values were determined with a Philips PW-1830 diffractometer (Cu Ka radiation, λ=1.54 Å) in the 2Θ range 1–10°.

Rheological measurements

A Haake Rheostress 1 rheometer (ThermoElectron, Germany) was used to measure the rheological properties of the creams. The yield stress test and constant stress test were carried out as viscosity measurements. Cone–plate (CP4/40 and 1/35° TI) combinations were used as measuring system. The temperature of the sample was 25±0.1 °C. The tests were performed at least in triplicate.

Results

Structural elements, primarily of the lamellar phase-mentioned in the literature, were verified by means of TEM. Figures 1 and 2 reflect the lamellar structure of the investigated o/w creams.

Ternary systems

X-ray diffractograms of CSA, cetyl alcohol (CA), and stearyl alcohol (SA) were collected and the d_L values of the typical peaks were determined from them. Values of the calculated lamellar spacing (d_{Lcalc}) were determined via the following equations in the case of CA and SA:

$$L_c = (1.27 \cdot n_c + 2.8) + h$$
$$d_{Lcalc} = 2 \cdot L_c$$

where L_c is the length of the alkyl chain containing n_c carbon atoms, 1.27 is the C–C distance in A.U., n_c is the number of carbon atoms, 2.8 is the volume of the end (methyl) group (in A.U.), and h is the volume of the head group. A comparison of the calculated values with the measured data demonstrated that CSA formed a bilayer in the investigated samples, the most likely angle of inclination of the molecules in this bilayer being 55° to the horizontal. In accordance with the results presented in the

Table 3 X-ray diffraction data on the ternary Tegin/CSA/water and TCPS/CSA/water systems

Water (%)	2Θ (°)		d_L (Å)		D (Å)		$n_b(=D/d_L)$	
	Tegin	TCPS	Tegin	TCPS	Tegin	TCPS	Tegin	TCPS
25	1.76	1.67	50.11	52.97	536.50	475.47	10.70	8.98
40	1.79	1.67	49.06	52.97	493.18	678.66	10.05	12.81
60	1.76	1.68	50.11	52.37	588.17	610.80	11.70	11.66
70	1.74	1.67	50.66	52.97	661.69	684.51	13.06	12.92

Table 4 X-ray diffraction data on the ternary EC/CSA/water and ET/CSA/water systems

Water (%)	2Θ (°)		d_L (Å)		D (Å)		$n_b(=D/d_L)$	
	EC	ET	EC	ET	EC	ET	EC	ET
25	1.76	1.78	50.11	49.58	601.54	742.08	12.00	14.98
40	1.78	1.74	49.58	50.66	583.84	728.47	11.78	14.38
60	1.72	1.72	51.22	51.22	547.61	305.40	10.69	5.96
70	1.78	1.78	49.58	49.58	352.90	487.13	7.12	9.83

literatures [24], CSA alone in excess water exhibited minimal swelling on hydration on increase of the water content. In the presence of 25% water, a sharp diffraction peak was present at 49.06 Å, representing hydrated crystalline bilayers of CSA. Upon addition of 40% water, the crystalline hydrate swelled to 50.11 Å. At water concentrations above 40% *w*/*w*, two separate phases formed: a water phase and a CSA phase.

Table 2 contains first-order reflection values of ternary systems prepared with T60, where D is the size of the domain and n_b is the number of bilayers within a domain.

On the basis of these data, it can be stated that the ternary T60/CSA/water systems did not exhibit a significantly higher swelling ability than that for systems containing only CSA/water. This indicates that a bilayer was not formed by the mixed emulsifier, but rather, the lamellar phase was built up merely from CSA domains containing 12–4 connected bilayers, depending on the water content. The presence of the micelles leads to a decrease in the domain size, and the formation of an ever more disordered structure. Thus, part of the water exists between the lamellas because the polar groups of the CSA are hydrated. T60 forms micelles and the residual water is chemically bound (hydrogen-bonded) to the POE chains [25], and this layer is also capable of mechanically entrapping a considerable number of water molecules.

Table 3 depicts the first-order reflection values of ternary systems prepared with Tegin and TCPS.

The results clearly showed, that CSA formed 9–13 connected bilayers alone in the lamellar phase and the value of D increased with increasing water content. The structure became more ordered as a result of the increasing water content. The explanation of this is the hydrophobic structure and the HLB value of the surfactant, which resulted in increasing ordering. It can be stated that apart from the semihydrate water of the CSA in the structure, most of the incorporated water was mechanically dispersed in the structure [25].

Table 4 presents the first-order reflection values of ternary systems prepared with EC and ET.

CSA formed domains alone containing 12–7 (EC) and 15–10 (ET) bilayers, depending on the water content and the type of ionic surfactant applied. Thus, the structure was more ordered than in the case of T60. The micelles formed by Eucarols were smaller because of the lack of POE chains. Part of the incorporated water was positioned around the head group of the CSA as semihydrate water. The incorporated water, which is situated close to the micelle, is bound by electrostatic forces. The surplus is bound mechanically as bulk water in the gel structure [25].

The chemical structure and hydrophilicity play important roles in the structure formed; this is demonstrated by the results of rheological measurements. The results on ternary systems containing 60% *w*/*w* water are listed in Table 5. Considerable differences in viscosity were observed, though the compositions of the ternary systems differed only in the nature of the surfactant.

For the samples containing T60, which has a nonionic character, larger and more asymmetric micelles were formed than in the case of ionic ones. This led to a change in the degree of order of the structure and the value of D was smaller. The change in the rheological index correlated well with the change in the domain size: the greater thixotropic area indicates that the system took longer to restore its original viscosity and structure. The reason for this is the presence of the POE chains, which increase the viscosity of the system; at the same time, this means a more breakable structure.

For the less hydrophilic Tegin and TCPS, high thixotropic areas and viscosities were observed. This was connected not so much with the domain size, but rather with the lower HLB values of the surfactants and the closely hydrophobic character because such hydrophobic units tend to connect and aggregate.

Table 5 X-ray diffraction and rheological data on ternary systems

Ternary system (60% water)	d_{001}		Viscosity	Thixotropic area
	d_L (Å)	D (Å)	At D=10 1/s (Pa s)	(Pa/s)
T60	52.97	248.91	29.63	2,094.61
EC	51.22	547.61	4.18	773.27
ET	51.22	305.40	2.33	346.49
Tegin	50.11	588.17	64.99	6,548.99
TCPS	52.37	610.80	38.05	3,942.90

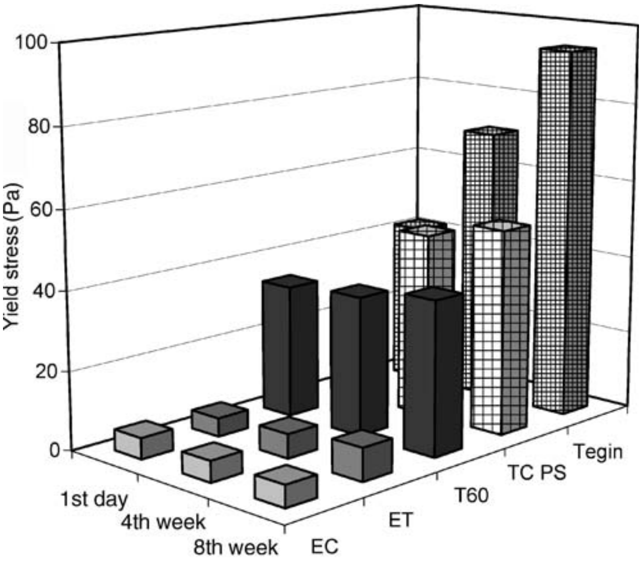

Fig. 3 Yield stress values of creams prepared with different surfactants

Creams

X-ray measurements were made on creams; their structure images were close to those of the ternary systems. CSA alone formed a bilayer, the structure of which was not influenced by the presence of the lipophilic phase. IM was entrapped inside the micelles and incorporated between the apolar chains of CSA.

The above-mentioned X-ray diffraction results and the previously published thermoanalytical results [26] provide a good explanation for the changes that occurred in the measured rheological parameters (yield stress, thixotropic area, and viscoelastic behavior) during storage. Each investigated system was characterized by plastic-thixotropic behavior.

The magnitude of the yield stress is related to the strength of the interparticle interaction in the 3D network of the microstructure of the creams. The following tendency was observed for the yield stress: samples prepared with nonionic POE chain-free surfactants had the highest yield stress (Tegin and TCPS). These values were significantly higher than the others ($p<0.01$). They were followed in order of magnitude by the results for the samples prepared with a nonionic POE chain-containing surfactant (T60). The yield stress was smallest for ionic surfactants (EC and ET). Significant changes in yield stress during storage were observed only for samples containing nonionic POE chain-free surfactants (Fig. 3), where the yield stress was significantly higher by the fourth week ($p<0.05$). After this, there was no further change up to the eighth week.

The higher yield stress values for the creams prepared with Tegin and TCPS indicated larger contact surfaces and stronger packing between the structural elements. On the other hand, the lower yield stress values for the creams prepared with the Eucarols, independently of the concentration and the storage time, suggested that a small stress is needed to initiate flow, which may be better in terms of the applicability of the formulation to the skin. A further advantage of these systems is that the yield stress did not change significantly with change of the surfactant concentration or during storage.

Fig. 4 Flow curves of creams prepared with different surfactants, after 8 weeks

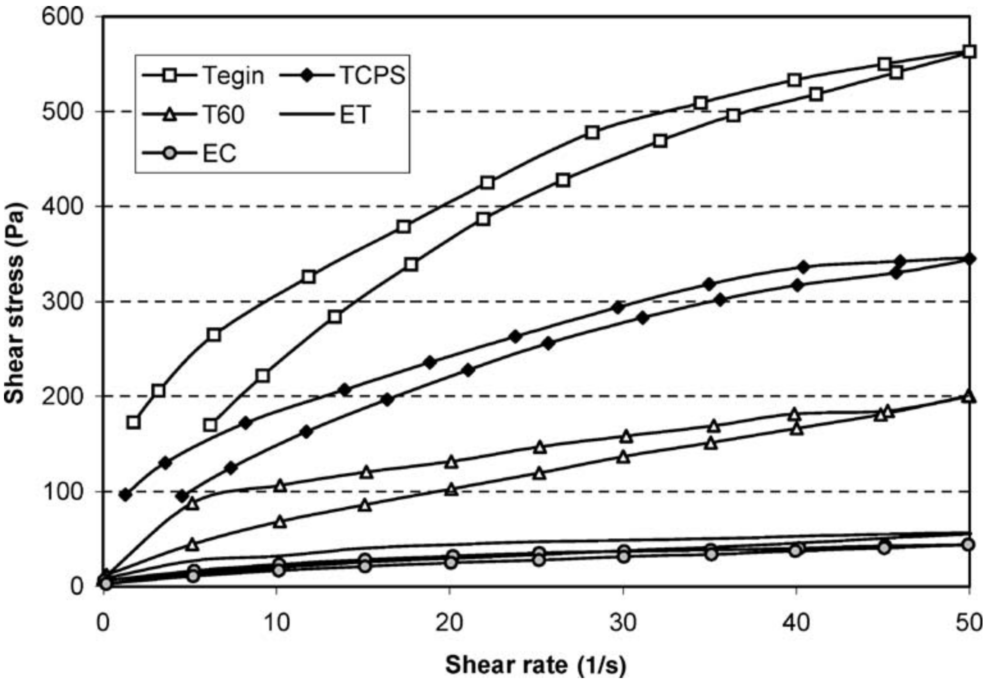

Table 6 Thixotropic values of creams prepared with different surfactants (mean values±SD, $n=3$)

Thixotropic area (Pa/s)	Tegin	TCPS	T60	EC	ET
1st day	2,138.44 SD±10.30	1,952.60 SD±11.91	1,004.50 SD±5.73	374.00 SD±8.11	273.50 SD±2.56
4th week	2,230.82 SD±5.59	2,566.70 SD±4.03	1,298.00 SD±5.56	459.00 SD±6.98	259.67 SD±5.45
8th week	2,629.24 SD±2.84	3,496.73 SD±4.45	1,201 SD±2.45	505.50 SD±2.21	261.67 SD±4.98

The most desirable type of flow behavior encountered in pharmaceutical or cosmetic products is thixotropic flow. All formulations behaved thixotropically (Fig. 4).

A thixotropic loop illustrates the deformation history of a material, and the thixotropic area provides qualitative information about its time dependence; it is proportional to the invested work necessary to change the structure. A significantly greater thixotropic area was observed for the samples prepared with nonionic POE chain-free surfactants ($p<0.01$), independently of the storage time. These were followed by the samples prepared with nonionic POE chain-containing surfactants, and the smallest thixotropic area was found for creams containing ionic surfactants (Table 6). This indicates that these latter creams had better structure-recovery properties. The higher thixotropic values for the creams containing POE chain-free surfactants imply that these formulations took longer to restore their viscosity at rest after being sheared, which is potentially an undesirable property from the aspect of application.

No significant change was observed in the values during storage for 4 weeks in any case, in accordance with the structure-forming mechanism (hydration of the POE chains, change in form of the micelles, and binding of further aggregates of the surfactant).

Discussion

Detailed microstructural images of o/w creams prepared with different mixed emulsifiers were created with the help X-ray diffraction. The results revealed that the investigated creams had different structures from those mentioned in the literature. The surfactant of the mixed emulsifier formed micelles, instead of mixed bilayers with the fatty amphiphile (Fig. 5). Bilayers were built up merely from CSA. Thus, chemically bound water was present in the creams prepared with nonionic POE chain-containing or ionic surfactants (besides the bulk water). In the case of POE chain-free surfactants, only the latter was present. The chemical structure and hydrophilicity of the surfactant played important roles in the structure formed; this was demonstrated by the results of rheological measurements.

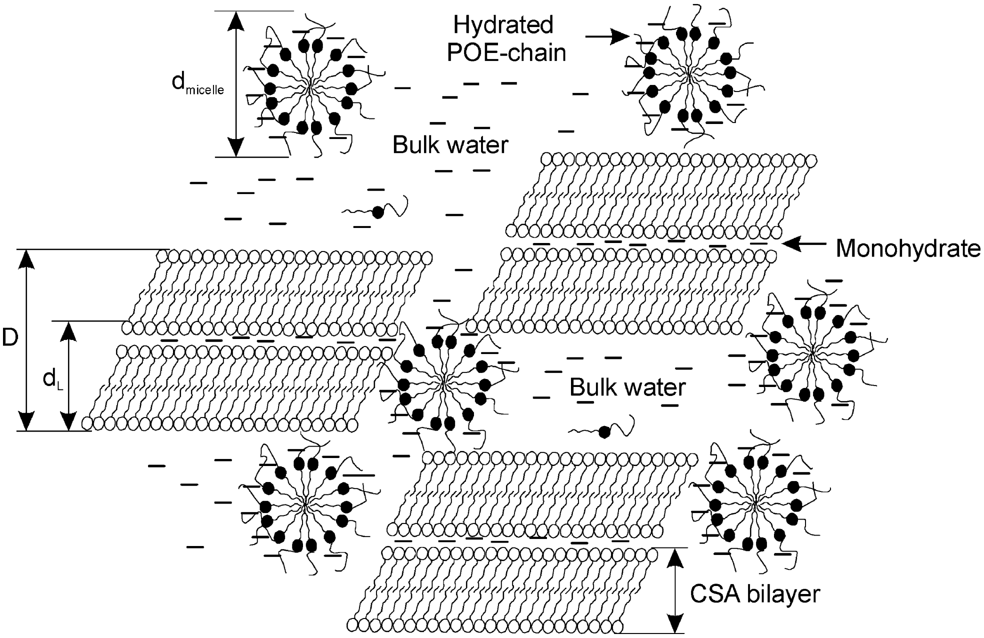

Fig. 5 Structure of the POE chain-containing nonionic ternary system

Acknowledgement The work was supported by the Hungarian Science Fund (OTKA) under grant number T046908.

References

1. Eccleston GM (1997) Colloid Surf A Physicochem Eng Asp 123–124:169
2. Barry BW (1983) Dermatological formulations. Percutaneous absorption. Marcel Dekker, New York
3. Eccleston GM (1986) Pharm Int 7:63
4. Eccleston GM (1985) Cosmet Toilet 101:73
5. Barry BW, Eccleston GMJ (1973) J Texture Stud 4:53
6. Kallionen S, Helenius K, Yliruusi J (1995) Pharmazie 50:478
7. Junginger HE, Akkermans AAMD, Heering W (1984) J Soc Cosmet Chem 35:45
8. Niemi L, Laine E (1991) Int J Pharm 68:205
9. Junginger HE (1984) Ber Bunsenges Phys Chem 88:1070
10. Junginger HE (1985) Pharm Weekbl Sci Ed 7:59
11. Eccleston GM.J (1977) J Pharm Pharmacol 29:157
12. Swarbrick J, Boylan JC (1988) Encyclopedia of pharmaceutical technology 5. Band. Marcel Dekker, New York
13. Junginger HE (1984) Pharm Weekbl Sci Ed 6:141
14. Peramal VL, Tamburic S, Craig DQM (1997) Int J Pharm 155:91
15. Eccleston GM (1985) Int J Pharm 27:311
16. Junginger HE (1984) J Phys Chem 88:1070
17. Vringer T, Joosten JGH, Junginger HE (1986) Colloid Polym Sci 264:691
18. Vringer T, Joosten JGH, Junginger HE (1987) Colloid Polym Sci 265:448
19. Rowe RCJ (1987) J Pharm Pharmacol 38:50
20. Vringer T, Joosten JGH, Junginger HE (1984) Colloid Polym Sci 262:56
21. Vringer T, Joosten JGH, Junginger HE (1987) Colloid Polym Sci 265:167
22. Rowe R, Barry MD (1989) Int J Pharm 53:139
23. Evans DF, Wennerström H (1999) The colloidal domain: where physics, chemistry, biology, and technology meet. Wiley, Canada
24. Eccleston GM, Behan MK, Jones GR, Towns-Andrews E (2000) Int J Pharm 203:127
25. Florence AT, Attwood D (1985) Physicochemical principles of pharmacy. Macmillen Publishers, London
26. Kónya M, Sorrenti M, Ferrari F, Rossi S, Csóka I, Caramella C, Bettinetti G, Erõs I (2003) J Therm Anal Calorim 73:623