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Water–Tween 40®/Imwitor 308®–isopropyl myristate microemulsions as delivery systems for ketoprofen: Small-angle X-ray scattering study

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Abstract

Small-angle X-ray scattering technique has been used to study the structural properties of the quaternary microemulsion Tween 40®/Imwitor 308®/isopropyl myristate/water and of five-component system obtained by the addition of the drug ketoprofen to the original quaternary system. The results enlighten the structuration of the studied systems and represent new complementary findings to the previous study [Podlogar, F., Bešter-Rogač, M., Gašperlin, M., 2005. The effect of internal structure of selected water–Tween 40 (R)–Imwitor 308 (R)–IPM microemulsions on ketoprofene release. Int. J. Pharm. 302, 68–77] on the correlation between the structuration of these systems and the release rates of the ketoprofen. The present results indicate that in the samples with the moderate to high concentration of water where the latter is a continuous phase the addition of smaller amounts of the ketoprofen does not change their inner structuration significantly. The quaternary sample containing 46.2 wt.% of water seems to be very near the composition where the transition from the bicontinuous to the lamellar structure of the microemulsion occurs. In the samples containing from 46.2 to 62.7 wt.% of water the swelling of lamellar phases with constant thickness of double-layer can be characterized. At approximately the latter composition another noticeable transition in the inner structuration of the microemulsion has been observed. Interestingly, all these changes in the inner structuration of the studied systems did not affect the trend of the drug release rates in this regime of water concentrations.

Keywords: Microemulsions; Structural investigations; Small-angle X-ray scattering; Lamellar phases; Swelling law

1. Introduction

Microemulsions are novel colloidal drug delivery systems. Comparing to coarse emulsions, microemulsions are transparent and thermodynamically stable. They are known to increase solubility and improve drug bioavailability, especially for poorly soluble drugs (Tenjarla, 1999; Bagwe et al., 2001). The inner structuration of the microemulsions is expected to comprise the structures of various geometries (e.g. spherical or elongated shapes, rod-like micelles, bicontinuous structures, cubic phases, etc.) and at some compositions often transforms also to anisotropic phases like hexagonal phases or lamellar phases that are strictly speaking not microemulsions any more. This is one of the main reasons why the characterizations of the microemulsions are not trivial at all. Nevertheless, the knowledge on the inner structuration of the microemulsion is quite important in order to better understand a physical process like the drug delivery from such a system.

Microemulsions consisting of nonionic surfactant/cosurfactant Tween 40®/Imwitor 308® mixture with mass ratio 1:1, hydrophobic isopropyl myristate and water (all components are appropriate for peroral or dermal application) have already been the subject of our previous structural study (Podlogar et al., 2004). Latter also the correlation between the internal structuration of the microemulsion and the ketoprofen release across the hydrophilic membrane was examined following the water dilution line originating from the initial non-aqueous sample with 80% of surfactant/cosurfactant mixture and 20% of isopropyl myristate (Podlogar et al., 2005). This specific dilution line was chosen because it mimics the actual processes taking place *in vivo* after the application. The results of the methods used in this previous study, i.e. the differential scanning calorimetry (DSC) and the measurements of the density, surface tension and electric conductivity, were sensitive to the structural changes in the

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studied systems but they could not provide quantitative information about the structuration of these media. For this reason we supplement the previous investigation (Podlogar et al., 2005) by using the small-angle X-ray scattering (SAXS) method, which is widely used technique for the direct structural investigations of the systems with the inner structuration falling in the colloidal domain. The results presented in this paper therefore offer some new insights in the actual internal structuration of these colloidal drug delivery systems and should be taken as the complementary results to the previous study.

2. Materials and methods

2.1. Materials

The oil isopropyl myristate (IPM) and the nonionic surfactant Tween 40® (TW40; polyoxyethylene (20) sorbitan monopalmitate) were purchased from Fluka Chemie GmbH, Switzerland. The nonionic cosurfactant Imwitor 308® (IMW; glyceryl caprylate) from Condea Chemie GmbH, Germany, and the drug ketoprofen from Lek d.d., Slovenia, were also used. The stock solution was prepared by mixing 80 wt.% of the surfactant/cosurfactant mixture (1:1 mass ratio) and 20 wt.% of IPM. The appropriate amount of twice distilled water was then added to obtain the desired quaternary microemulsion composition. The samples were stirred for 5 min at room temperature (22 ± 2 °C). The drug ketoprofen was added to these microemulsion systems afterwards. All samples were stable over 6 months, remaining clear and transparent. The compositions of the microemulsions are given in Table 1 and marked on the dilution line in the phase diagram shown in Fig. 1. Small-angle X-ray scattering measurements were performed on these quaternary microemulsion samples as also on some five-component samples containing ketoprofene. The latter contained from 0.6 to 1.5 wt.% of ketoprofene as explained into details in Section 3.

Table 1
The compositions of the studied quaternary microemulsion samples

Water (wt.%)	IPM (wt.%)	TW40/IMW (wt.%)
0.0	20.0	80.0
5.0	19.0	76.0
10.0	18.0	72.0
15.0	17.0	68.0
20.0	16.0	64.0
25.0	15.0	60.0
30.0	14.0	56.0
35.0	13.0	52.0
40.0	12.0	48.0
45.0	11.0	44.0
46.2	10.8	43.1
47.5	10.5	42.0
50.6	9.9	39.6
53.0	9.4	37.6
54.9	9.0	36.1
57.3	8.6	34.2
59.7	8.1	32.3
62.7	7.5	29.8
64.8	7.0	28.1
67.4	6.5	26.1
69.8	6.0	24.2

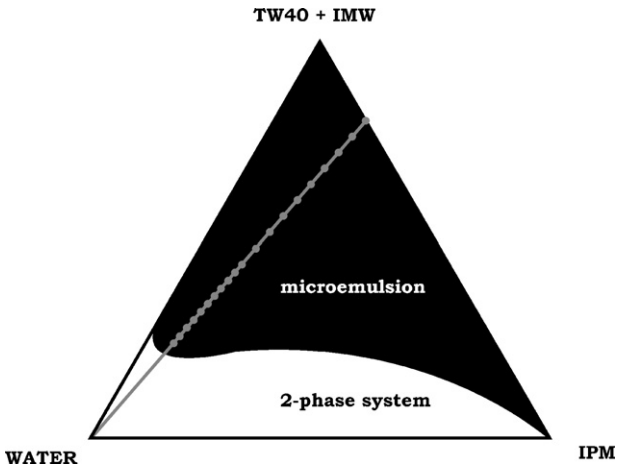

Fig. 1. The pseudo-ternary phase diagram for the quaternary mixture isopropyl myristate/Tween 40®/Imwitor 308®/water. Dots along the grey dilution line represent the investigated sample compositions given in Table 1.

2.2. Small-angle X-ray scattering measurements

Small-angle X-ray scattering spectra were measured utilizing an evacuated Kratky compact camera (Kratky and Stabinger, 1984) system (Anton Paar, Graz, Austria) with a block collimating unit, which was attached to a conventional X-ray generator (Bruker AXS, Karlsruhe, Germany). The generator was equipped with a sealed X-ray tube (Cu-anode target type) producing Ni-filtered Cu Kα X-rays with a wavelength of $\lambda = 0.154$ nm. The operating power was 35 kV × 10 mA. The samples were measured in standard quartz capillary, which was placed in a thermally controlled sample holder centered in the X-ray beam. The scattering intensities were measured with a linear position sensitive detector (PSD 50m, M. Braun, Garsching, Germany). The scattering intensity was detected within the whole range of scattering vector q ($q = 4\pi/\lambda \sin(\theta/2)$, where θ is the scattering angle between the incident beam and the scattered radiation) simultaneously. All measurements were performed at 25 °C. For each sample 15 SAXS curves with a sampling time of 5000 s were recorded in order to check the reproducibility. These curves were subsequently averaged to ensure the reliable statistics of the measurement. At this point we have to stress that the scattering intensities obtained in this way are still experimentally smeared because of the finite dimensions of the primary beam (Glatter, 1983).

2.3. Measurements of ketoprofen release

Since some data on the ketoprofene release rates from the previous study (Podlogar et al., 2005) are used in Section 3 of the present work we therefore in the following shortly describe the method used for the measurements of ketoprofen release rates. Ketoprofen release through a hydrophilic cellulose acetate membrane was determined with a Franz diffusion cell (Hanson research, Chatsworth, USA) at 25 °C. The cell held 7 ml of

phosphate buffer at pH 7.4 as an acceptor medium and 0.3 g microemulsion on the donor side. The surface area between acceptor and donor compartments was 1.18 cm^2. Microemulsions containing 1.2 wt.% ketoprofene were introduced to the donor compartment of the diffusion cell and the acceptor medium was sampled after 0.5, 1, 1.5, 2, 3, 4, 5, 6 and 8 h. These samples were assayed spectrophotometrically for ketoprofen content at 260 nm (UV spectrophotometer 8453, Hewlett Packard, Waldbronn, Germany). Experiments were carried out in triplicates and results were obtained as arithmetic means. The drug release was determined as the amount of released drug per hour averaged over an 8 h interval.

3. Results and discussion

Small-angle X-ray scattering technique has been applied to study the structural properties of the quaternary systems consisting of nonionic surfactant/cosurfactant Tween 40®/Imwitor 308® mixture (mass ratio 1:1), hydrophobic isopropyl myristate and water as also the corresponding five-component systems obtained by the addition of the drug ketoprofen to the original quaternary systems. The pseudo-ternary phase diagram of the basic quaternary system is shown in Fig. 1. We examined the samples with the compositions along the marked water dilution line starting from the initial non-aqueous system and following the direction of increasing water concentration. In this way the effect of diluting of the initial non-aqueous system on the internal structuration of the media could be explored. Such a dilution would be expected to occur under *in vivo* conditions after peroral application, moreover the opposite concentrating effect (due to the evaporation of water) would be observed when microemulsions are applied topically. In Fig. 2, the experimental SAXS curves for the quaternary system at different water concentrations are displayed in a plot of intensity versus the scattering vector q. These curves were previously corrected for the empty capillary and background scattering; however, corrections for slit length and width smearing effects stemming from the slit collimating optics of the Kratky compact camera were not made. In the case of the diffuse scattering characteristic for colloidal dispersions, it is usually rather difficult to make the structural analysis directly from the course of the raw experimental scattering functions. This problem is especially important when the samples show broad scattering peaks. Nevertheless, even from these raw experimental data we can immediately observe some very interesting features referring to the evolutions and changes of the scattering peaks when starting from the non-aqueous sample and following the ones with increasing water content. This behavior is due to different scattering patterns of the individual solutions and can be ascribed to the considerable influence of the water on the structuration of these systems. The broad and unexpressive peak, seen at low water content, gradually straitens and shifts towards lower q values upon increasing the concentration of water up to approximately 40 wt.%. From the scattering theory we learn that the real space information, i.e. the actual distances, dimensions and shapes of the scattering objects, and the information reflected in q space, i.e. the scattering intensity, are mutually connected in a reciprocal relationship. For this reason,

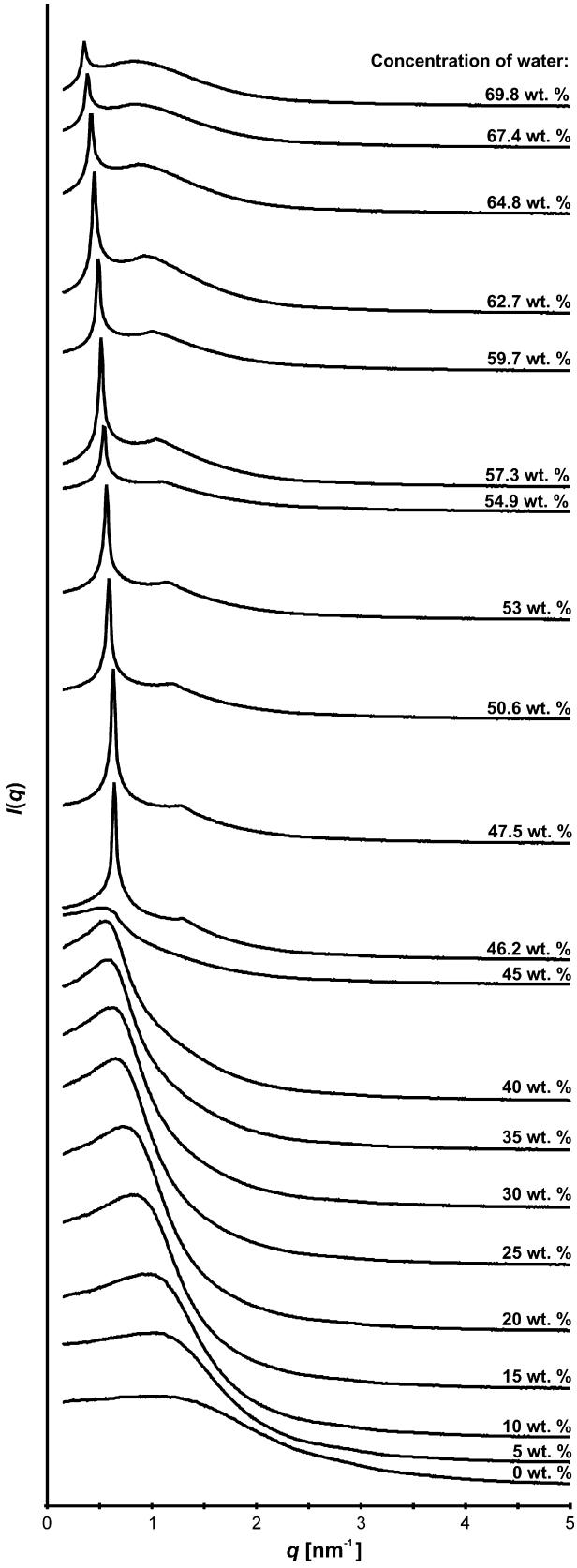

Fig. 2. The experimental small-angle X-ray scattering intensities of the studied quaternary isopropyl myristate/Tween 40®/Imwitor 308®/water microemulsions. The actual compositions are indicated in Fig. 1 and Table 1. In the legend only the fraction of water is indicated. The curves were shifted for the sake of clarity—original curves coincided at the largest measured q-values.

q space is often called as the reciprocal space. This means that the information about the bigger dimensions of the scattering structures is predominantly expressed in the regime of lower q values. Correspondingly, according to the initial trend of the shift of the scattering peak illustrated in Fig. 2 we could assume that the dimension of the scattering structures increases with increasing the water concentration. However, observing the shape of the scattering peaks (they are somewhat flattened) and considering the fact that the concentration of the surfactant/cosurfactant mixture is rather high in these samples (see Table 1) it is hard to expect the formation of the particulate structures (i.e. various micellar structures) especially in the systems with somewhat higher water concentration. Accordingly, our hint is a bicontinuous structure of these samples (knitted continuous water and oil regimes) where the average size of the coherent scattering domains slowly increases with the water concentration. This speculation is supported also with the assumption made in the previous article (Podlogar et al., 2005). Namely, on the basis of some trends in the change of electric conductivity and DSC results some samples from this concentration regime were considered to be above the percolation threshold. Interestingly, these samples were reported to show also very similar excess volumes and were found to yield similar drug release rates. The ketoprofen release rate versus the concentration of water in the microemulsion are presented also in Fig. 3 (the data are taken from Table 2 of Podlogar et al., 2005).

The SAXS data of the studied quaternary samples with higher water concentration show that at approximately 45 wt.% of water some more pronounced changes in the inner structuration of these systems occur. At the sample containing 46.2 wt.% of water a very sharp scattering peak evolves at $q = 0.6392\,\mathrm{nm}^{-1}$ which is followed by another less pronounced but still clearly recognizable peak at larger $q = 1.2784\,\mathrm{nm}^{-1}$. These two peaks are practically perfectly equidistant within the resolution of our experiment ($\Delta q = 0.0052\,\mathrm{nm}^{-1}$), which makes possible the more reliable structural interpretation of the scattering data in this regime of the studied dilution line. Namely, the equidistant peaks indicate the presence of lamellar structures in this sample. Upon increasing the water concentration up to around 62.7 wt.% the two scattering peaks harmoniously move towards smaller q-values but remain at equidistant positions. This feature confirms the presence of the lamellar phases in all samples in this concentration regime. With further increase of the water concentration the second scattering peak broadens and becomes non-equidistant to the first peak indicating some distinct structural changes in these samples. In parallel the first peak remains very sharp, which means that these systems still retain a high level of order. As can be seen from the phase diagram (Fig. 1), the microemulsion forms only up to approximately 73% of water, afterwards the system macroscopically phase separates into a two-phase system.

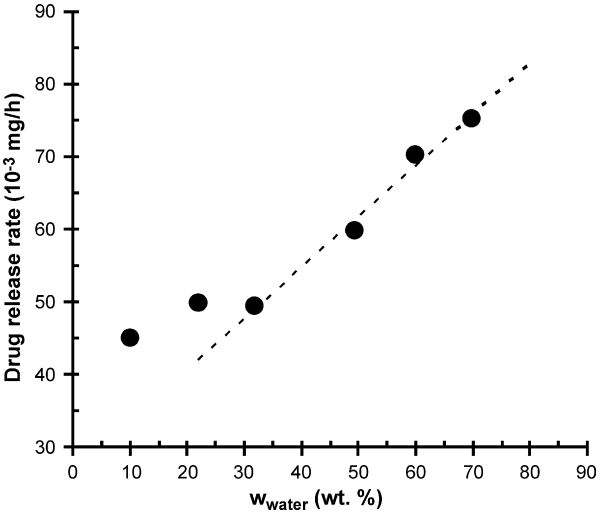

Fig. 3. The ketoprofen release rates (taken from Table 2 of Podlogar et al., 2005) vs. the concentration of water in the samples. The error of the drug release rate data was assessed to be inside the dot size. Methodology of the drug release rate measurements is briefly explained in Section 2.3.

A characteristic feature of the samples containing the lamellar phases with the constant thickness of the hydrophobic double-layer is that upon dilution they obey the following linear swelling law (Hyde, 1995; Fairhurst et al., 1997; Grillo et al., 2000; Ficheux et al., 2001; Castelletto et al., 2002; Yamashita et al., 2004):

$$D = \frac{\delta}{\phi_{\mathrm{hc}}}, \tag{1}$$

where δ and ϕ_{hc} represent the constant thickness and the volume fraction of the hydrophobic layer, respectively. In order to check whether this swelling law holds for the lamellar samples studied here we first had to calculate the inter-layer repeating distance D utilizing the Bragg law:

$$D = \frac{2\pi}{q_{\mathrm{peak}}}, \tag{2}$$

where the q_{peak} is the q-value at the position of the first scattering peak. At this point we have to mention that the presented scattering data are experimentally smeared because of the slit collimation used in our experimental setup (Glatter, 1983). Calculating the inter-layer repeating distances D according to Eq. (2) one should in principle need the desmeared scattering functions free from experimental effects. Of course, such experimental data could be obtained utilizing the so-called pinhole measuring system with a point-like primary beam. However, such system would be hampered by the low scattering intensities being typical for the liquid systems. Nevertheless, in the case of very sharp scattering peaks, typical for the lamellar samples, the usual smearing effect due to the slit collimation practically does not affect the position of these sharp equidistant peaks. Therefore, the inter-layer repeating distances D presented in Fig. 4 were calculated from Eq. (2), where q_{peak} values were obtained simply from the positions of the first sharp peaks in the scattering curves for different concentrations shown in Fig. 2. Before testing the swelling law given by Eq. (1) on these data for the studied lamellar samples the volume fraction of hydrophobic layers ϕ_{hc} had to be assessed. For this purpose we used the usual approach assuming the conservation and additivity of the volumes of the components forming the microemulsion (Grillo et al., 2000; Castelletto et al., 2002; Yamashita et al., 2004).

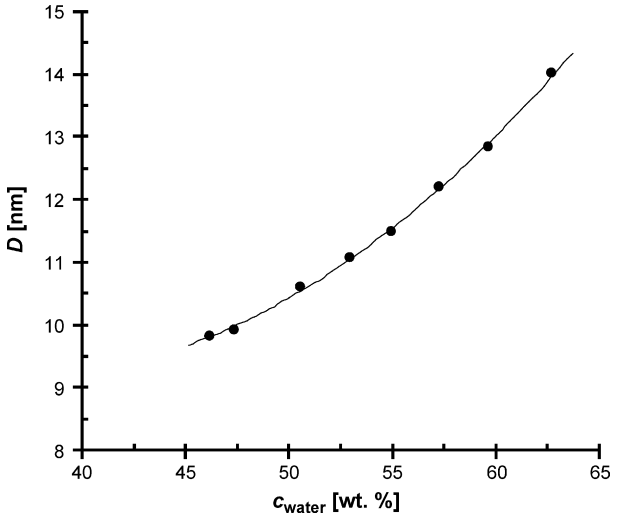

Fig. 4. The dependence of distances D obtained according to the position of the first sharp scattering peak (see Eq. (2)) on the concentration of water in the sample.

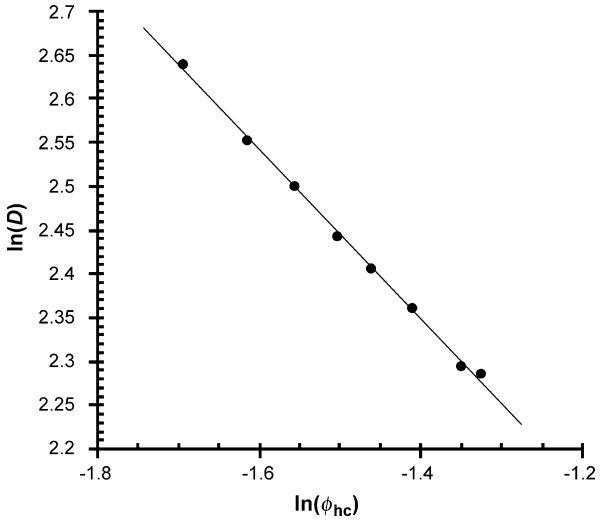

Fig. 6. Linear swelling law for the samples with different concentrations of water in the lamellar regime.

The volume fraction of hydrophobic layers ϕ_{hc} was estimated according to the following expression:

$$\phi_{hc} = \frac{(v_{IPM} w_{IPM}/M_{IPM}) + (v_{hc,TW40} w_{TW40}/M_{TW40}) + (v_{hc,IMW} w_{IMW}/M_{IMW})}{(v_{IPM} w_{IPM}/M_{IPM}) + (v_{TW40} w_{TW40}/M_{TW40}) + (v_{IMW} w_{IMW}/M_{IMW}) + (v_{water} w_{water}/M_{water})}, \quad (3)$$

where $v_{hc,i}$ represents the molar volume of the hydrophobic part of the component i, w_i the weight fraction, M_i the molar mass, and v_i is the molar volume of the component i. The molar volumes for the individual components in water at 25 °C were assessed according to the scheme drawn in Fig. 5 and to the method reported in the literature (Durchschlag and Zipper, 1994, 1995). The swelling law (Eq. (1)) was tested in a logarithmic plot of ln(D) versus ln(ϕ_{hc}) depicted in Fig. 6. As clearly seen, this linear law almost perfectly holds for the samples with water concentration in the range from approximately 46.2 to 62.7 wt.%, which can be taken as additional evidence for the presence of lamellar phases in the samples from this range of the studied dilution line.

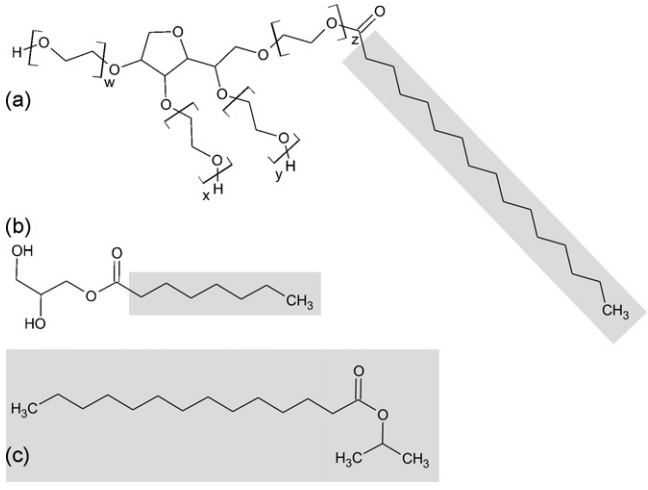

Fig. 5. Schematic representation of: (a) nonionic surfactant Tween 40® (the total number of oxyethylene units is 20: $x + y + z + w = 20$), (b) nonionic cosurfactant Imwitor® 308 and (c) oil isopropyl myristate. The parts of the molecules that were taken as hydrophobic in the test for the swelling law (Eq. (1)) are marked with grey areas.

To summarize, our findings based on the SAXS data for the studied quaternary samples indicate that in the systems with moderate to high concentrations of water the water phase seems to be a continuously connected phase and further point out the two distinct changes in the inner structuration of these systems in this regime of water concentration. In parallel, as can be observed in Fig. 3, the ketoprofen release rates for the samples with higher water concentrations increase in practically constant steps when the water concentration is gradually increased. This is a very interesting fact, which indicates that the trend in the ketoprofen release rates does not change even upon some distinct geometrical changes in the inner structuration of the samples in this concentration regime. It follows that the release rates obtained for ketoprofen are not directly dependent on the inner structuration of the media in this regime and seem to depend mainly on the overall concentration of water in the microemulsion. Namely, ketoprofen is a drug that is soluble in the hydrophobic surrounding (solubility in IPM is 10.7 mg/ml at 20 °C) (Rhee et al., 2001), but has a very low solubility in water (294 μg/ml at 20 °C) (Cordero et al., 1997). One would therefore expect the ketoprofen to be dissolved mainly in the hydrophobic surroundings of the microemulsion (oil phase), whereas its concentration in the hydrophilic part of this system (water phase) would be substantially lower. In the previous investigation the rate determining step of the drug release was ascribed to the transport of the ketoprofen from oil to water phase of the microemulsion and not to its transmission over the hydrophilic membrane (Podlogar et al., 2005). Since the water is a continuous phase in the samples with its moderate concentration and consequently not captured inside the nanostructures of microemulsion this assumption seems very likely. Since all the samples contain the

same amount of the drug, the concentration of the latter in the oil phase of the microemulsion increases with increasing the concentration of water in the system. Consequently, the difference between the concentration of ketoprofen in the oil phase and that in the water phase of microemulsion also increases thus giving rise to a faster diffusion of the drug from the oil nanostructures into the water continuous phase. In parallel, the measured overall rate of the drug release at the experiment also increases when this process is supposed to be the rate determining step. Such trend can be actually observed in Fig. 3.

Of course, we could state the above discussion and comparison of the SAXS and drug release rate results much more firmly if we would dispose of the reliable scattering data also for the five-component systems containing ketoprofen. However, ketoprofen is a well known photosensitive drug that in some media undergoes a photo-degradation when exposed to the light (Bosca et al., 1994; Baudot et al., 1998; Cosa et al., 1999; Bagheri et al., 2000; Loden et al., 2004, 2005). Therefore, it is difficult to study the systems containing ketoprofen with the light scattering experiments. Nevertheless, in order to get some deeper insight into the structuration of these media it seems worthwhile to carry out SAXS experiments on these five-component systems. As proven in the previous text SAXS results yielded some very useful information about the inner structure of the studied quaternary systems. Microemulsions used for the drug release tests (Podlogar et al., 2005) contained also the photo-degradable drug ketoprofen as the fifth component, which should surely have a certain effect on their internal structuration. We therefore attempted to benefit with the SAXS results also for the systems containing ketoprofen. The experimental scattering intensities versus scattering vector q of the five-component sample with 0.6 wt.% of ketoprofen obtained with the addition of ketoprofen to the isopropyl myristate/Tween 40®/Imwitor 308®/water quaternary sample containing 53 wt.% of water (see Table 1) are presented in Fig. 7a. It is certainly not surprising that the photosensitive drug ketoprofen suffered from a very high sensitivity also to the X-rays. We should mention here that these scattering curves refer to the successive measurements, each taking time of 5000 s, during the period of more than 55 h of constantly irradiating the sample with the Cu Kα radiation of wavelength $\lambda = 0.154$ nm. A comparison of the first few scattering curves from Fig. 7a to those referring to the basic quaternary system (see Fig. 2) indicates that the addition of this small amount of ketoprofen to the quaternary system does not significantly alter the inner structuration of the sample. However, as the time passes the photosensitive ketoprofen degrades. The products of this degradation obviously cause some significant structural changes pointing in the direction of the more disordered structure of the system. We performed also the SAXS measurements on the samples with higher concentrations of ketoprofen (0.8, 1.0, 1.2 and 1.5 wt.%). These data showed and confirmed the same trend of structural changes due to the photo-degradation of the ketoprofen as depicted in Fig. 7a, with only a slight difference, namely, it was getting much more pronounced with increasing concentration of the drug (see Fig. 7b). The scattering curves for the samples with the highest concentration of ketoprofen (1.2 and 1.5 wt.%) recorded during the first 5000 s of their

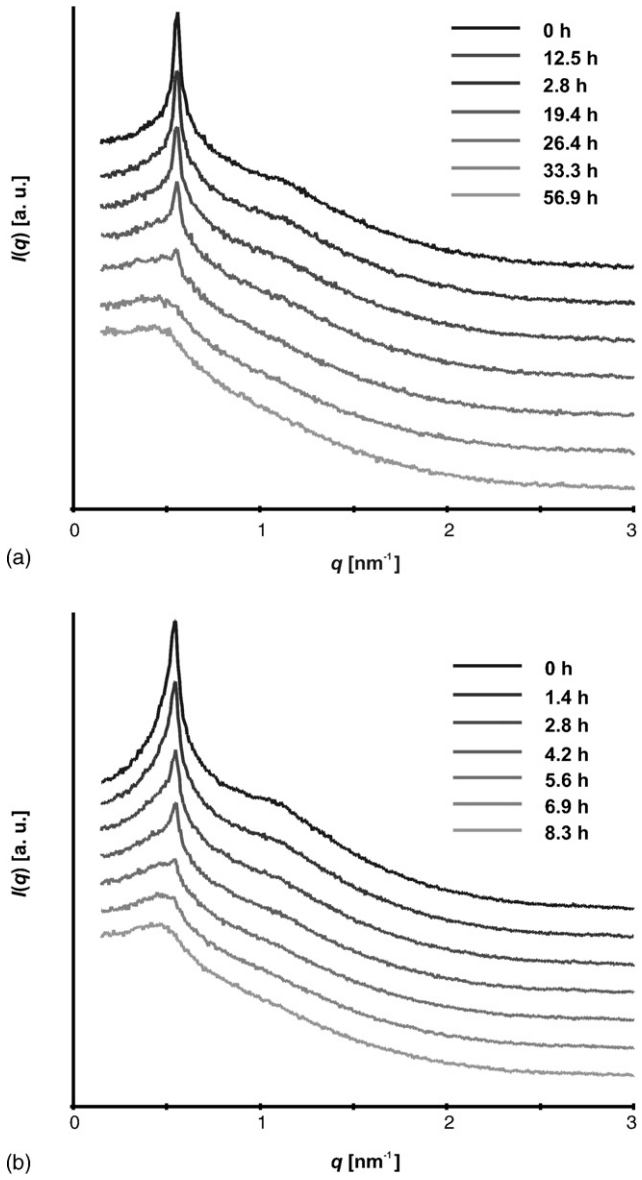

Fig. 7. The time-dependent experimental small-angle X-ray scattering intensities of the five-component system with (a) 0.6 wt.% and (b) 1.0 wt.% of ketoprofen obtained with the addition of ketoprofen to the quaternary isopropyl myristate/Tween 40®/Imwitor 308®/water sample with 53 wt.% of water (see Table 1).

exposure to X-rays looked very similar to the very last measurements from the two sets with lower concentration of ketoprofen shown in Fig. 7. Further measurements corresponding to longer exposure times did not show the time dependence any more. This obviously means that the concentration of the ketoprofene photo-degradation products has rapidly risen to a level to significantly influence the structuration of the media in these samples with the highest concentrations of ketoprofene. For this reason, it was not possible to follow the dynamics of this process by our experimental setup any more. It is well known that ketoprofen degrades to 1-(3-benzoylphenyl)ethanone and 3-benzoyl-α-methylbenzeneacetamide (Pietta et al., 1987; Matak et al., 1994; Dvorjak et al., 2004) that are both highly aromatic molecules and most probably preferably distribute to the hydrophobic regions

inside the lamellarly nano-structured systems. Due to such organized distribution they change the curvature of the interfacial surfactant film, consequently cause the lamellar structures to become unstable and finally bring them to a complete break down. The latter complete break down of the lamellar structures is clearly indicated by the disappearance of the sharp equidistant scattering peaks with time that is clearly depicted in Fig. 7. Nevertheless, if we analyze the first measurements from Fig. 7 we find out that the inter-lamellar repeating distances D (11.3 nm for 0.6 and 11.5 nm for 1.0 wt.% of ketoprofene in the sample) are slightly larger than in the corresponding quaternary samples without ketoprofen (11.1 nm). These data therefore indicate that the small additions of ketoprofen to the quaternary samples do not change the structuration of these media considerably even at higher water concentration. At this point we have to mention that in the previous study (Podlogar et al., 2005) it was reported that for the ketoprofen samples with higher concentrations of water the methods used (i.e. DSC and the measurements of electric conductivity) showed some changes in the measured quantities in comparison to the samples without ketoprofen. The results of these methods, of course, could only be interpreted as an indirect indication about the possible changes in the microemulsion structuration where the diverse measured properties differently 'feel' and consequently differently respond to such changes. Moreover, the changes in DSC and electric conductivity values do not necessarily arise from structural changes in the media but can also originate from the change of some other quantities (e.g. viscosity, pH). On the contrary the SAXS method allows a direct structural determination of the studied systems and in our case suggests no noticeable structural changes in the samples with addition of the drug ketoprofene. The latter is true only at the condition that the samples are kept in the dark in order to prevent the photo-degradation of ketoprofene leading to the formation of such substances that can significantly affect the structuration of the media. These SAXS results therefore confirm a feasibility of a direct comparison of the ketoprofen release rate trends to the SAXS results on the structure of the quaternary systems without ketoprofen shown and discussed previously in the text.

4. Conclusions

Small-angle X-ray scattering (SAXS) method was used to study the structural properties of the quaternary microemulsion system Tween 40®/Imwitor 308®/isopropyl myristate/water and five-component systems obtained by adding the drug ketoprofen to the original microemulsions. The SAXS results indicate that the addition of smaller amounts of the ketoprofen, which is highly sensitive to the exposure to radiation, does not change the inner structuration of these microemulsions significantly. Because of the photosensitivity of the drug, this is true only at the condition that the samples are kept in the dark. Otherwise the products forming at the photo-degradation of ketoprofene can significantly affect the structuration of the media. The findings about the structure of these systems complement the results presented in the previous study (Podlogar et al., 2005). The comparison of the results of both studies indicates that the trends of the ketoprofen release rates are not directly dependent on the inner structuration of these media with moderate concentrations of water. Namely, at the samples with moderate concentrations of water the ketoprofen release rate increased with practically constant slope with increasing the overall concentration of water in the microemulsion, despite of the fact that two distinct transitions in the inner structuration of these systems were detected in this concentration regime. Appearance of the two equidistant peaks in the SAXS spectra indicated a transformation of bicontinuous structures to the lamellar phases with constant thickness of the double-layer that were characterized for the samples containing from 46.2 to 62.7 wt.% of water. The second transition was observed at the concentration around that of the latter sample, where the scattering peaks became non-equidistant indicating the transition to some different still highly ordered structures. We can conclude that all these findings nicely conform to the results of the complementary methods that were presented in the previous study (Podlogar et al., 2005).

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