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Investigation of surfactant/cosurfactant synergism impact on ibuprofen solubilization capacity and drug release characteristics of nonionic microemulsions

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ABSTRACT

The current study investigates the performances of the multicomponent mixtures of nonionic surfactants regarding the microemulsion stabilisation, drug solubilization and *in vitro* drug release kinetic. The primary surfactant was PEG-8 caprylic/capric glycerides (Labrasol®). The cosurfactants were commercially available mixtures of octoxynol-12 and polysorbate 20 without or with the addition of PEG-40 hydrogenated castor oil (Solubilisant gamma® 2421 and Solubilisant gamma® 2429, respectively). The oil phase of microemulsions was isopropyl myristate. Phase behaviour study of the pseudo-ternary systems Labrasol®/cosurfactant/oil/water at surfactant-to-cosurfactant weight ratios (K_m) 40:60, 50:50 and 60:40, revealed a strong synergism in the investigated tensides mixtures for stabilisation of microemulsions containing up to 80% (w/w) of water phase at surfactant +cosurfactant-to-oil weight ratio (SCoS/O) 90:10. Solubilization of a model drug ibuprofen in concentration common for topical application (5%, w/w) was achieved at the water contents below 50% (w/w). Drug free and ibuprofen-loaded microemulsions M1–M6, containing 45% (w/w) of water phase, were prepared and characterized by polarized light microscopy, conductivity, pH, rheological and droplet size measurements. *In vitro* ibuprofen release kinetics from the microemulsions was investigated using paddle-over-enhancer cell method and compared with the commercial 5% (w/w) ibuprofen hydrogel product (Deep Relief®, Mentholatum Company Ltd., USA). The investigated microemulsions were isotropic, low viscous *Bingham*-type liquids with the pH value (4.70–6.61) suitable for topical application. The different efficiency of the tensides mixtures for microemulsion stabilisation was observed, depending on the cosurfactant type and K_m value. Solubilisant gamma® 2429 as well as higher K_m (i.e., lower relative content of the cosurfactant) provided higher surfactant/cosurfactant synergism. The drug molecules were predominantly solubilized within the interface film. The amount of drug released from the formulations M3 (10.75%, w/w) and M6 (13.45%, w/w) (K_m 60:40) was limited in comparison with the reference (22.22%, w/w) and follows the *Higuchi* model. Microemulsions M2 and M5 (K_m 50:50) gave zero order drug release pattern and ~15% (w/w) ibuprofen released. The release profiles from microemulsions M1 and M4 (K_m 40:60) did not fit well with the models used for analysis, although the amounts of ibuprofen released (24.47%, w/w) and 17.99% (w/w), respectively) were comparable to that of the reference hydrogel. The drug release mechanism was related with the surfactant/cosurfactant synergism, thus the lower efficiency of the tensides corresponded to the faster drug release.

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1. Introduction

Transdermal delivery of nonsteroidal anti-inflammatory drugs (NSAIDs) for the treatment of acute and chronic osteoarthritis pain has attracted great interest (Barthel and Axford-Gatley, 2010). Ibuprofen ((*RS*)-2-(4-(2-methylpropyl)phenyl)propanoic acid) is a NSAID which is applied topically usually in a form of 5% cream, foam, gel or spray solution. The extremely low water solubility of ibuprofen and low intrinsic skin permeability compromise a successful transdermal drug delivery. In order to provide consistent drug levels at the application site for prolonged periods there is a persistent interest to develop carriers with sufficient drug solubilization capacity and preferred drug release kinetics. Literature describes few successful attempts to improve solubility of ibuprofen by using microemulsion carriers (Araya et al., 2005; Chen et al., 2006). Microemulsions are isotropic, transparent,

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thermodynamically stable colloidal systems consisting of water phase, oil phase and sufficient concentrations of an appropriate surfactant in combination with a suitable cosurfactant. The microstructure of such systems is organised on the level below 100 nm providing a large interface which is available for solubilization of drug molecules (Fanun, 2009; Heuschkel et al., 2008; Neubert, 2011). Microemulsions usually contain high concentration of a surfactant and short or medium chain alcohols as cosurfactants that put a risk for skin irritation. Current investigations are focused on development of biocompatible microemulsions stabilised by nonionic tensides mixtures (Fanun, 2011). According to Hiuberts and Shah (1997) mixtures of nonionic surfactants, in spite of their nonionic character, may provide an enhanced solubilization efficiency, compared to performances of individual components. The achievement of a strong synergism in tensides mixtures is the prospective approach to reduce their total content required for microemulsion stabilisation. On the other hand, successful incorporation of drugs with poor water solubility requires a sufficient concentration of tensides (Rane and Anderson, 2008). Therefore, development of such carriers relies on achieving a fine balance among mutually contradictory requests.

The growing relevance of microemulsions stabilised by a low irritant nonionic surfactant PEG-8 caprylic/capric glycerides (Labrasol®) as carriers for transdermal drug delivery is well documented (Alvarez-Figueroa and Blanco-Méndez, 2001; Djordjevic et al., 2004, 2005; Escribano et al., 2003; Kreilgaard et al., 2000; Rhee et al., 2001; Zhao et al., 2006). This pharmaceutical excipient with potential to enhance drug absorption (Eaimtrakarn et al., 2002; Koga et al., 2006; Rama Prasad et al., 2004; Schulze et al., 2005; Yüksel et al., 2003), has been employed in solubilization of poorly soluble drugs (http://www.accessdata.fda.gov/scripts/cder/iig/getiigWEB.cfm). Literature documents that Labrasol® may be exploited for stabilisation of microemulsions in the presence of non-alcohol cosurfactants such as polyglycerol-6 fatty acid esters or diethyleneglycol monoethylether. Although scarcely investigated, the cosurfactants also recommended for stabilisation of Labrasol®-based microemulsions for topical application are commercially available mixtures of polyoxyethylene nonionic tensides Solubilisant gamma® 2421 (octoxynol-12 and polysorbate 20) and Solubilisant gamma® 2429 (octoxynol-12 (and) polysorbate 20 (and) PEG-40 hydrogenated castor oil).

The purpose of this study was to evaluate the influence of the cosurfactant composition and K_m value on the stability, drug solubilization capacity and drug release performances of Labrasol®-based microemulsions, using the commercially available mixtures of nonionic tensides Solubilisant gamma® 2421 and Solubilisant gamma® 2429 as cosurfactants. Ibuprofen was employed as a model drug.

2. Material and methods

2.1. Material

Labrasol® (PEG-8 caprylic/capric glycerides) was used as a surfactant. Solubilisant gamma® 2421 (octoxynol-12 and polysorbate 20) and Solubilisant gamma® 2429 (octoxynol-12 (and) polysorbate 20 (and) PEG-40 hydrogenated castor oil) were used as cosurfactants. Labrasol®, Solubilisant gamma® 2421 and Solubilisant gamma® 2429 were kindly donated by Gattefosse, France. The oil phase was isopropyl myristate (Crodamol® IPM, Croda Chemicals Europe, England). All of the components were used as supplied without further purification. The aqueous phase of microemulsions was double-distilled water.

2.2. Formulation and characterization of microemulsions

2.2.1. Phase behaviour investigations

In our previous study (Djekic and Primorac, 2008) a high water solubilization efficiency of the Labrasol®/Solubilisant gamma® 2421 (or) Solubilisant gamma® 2429/isopropyl myristate mixtures was observed at K_m ranged from 40:60 to 60:40 (the values represent relative percentages of the surfactant and the cosurfactant, respectively). The current investigation characterizes phase behaviour of the pseudoternary systems Labrasol®/cosurfactant/oil/water by the construction of pseudoternary phase diagrams at three K_m values (40:60, 50:50, and 60:40), at room temperature. The corresponding surfactant/cosurfactant mixtures were prepared and mixed further with isopropyl myristate at SCoS/O: 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, and 90:10 (the values represent relative percentages of the surfactant/cosurfactant mixture and the oil, respectively). The prepared surfactant/cosurfactant/oil mixtures were titrated dropwise with water (so called water titration lines), under moderate magnetic stirring, until the microemulsion area border was reached when a transparent mixture changed to turbid or milky. The maximum percentage of water incorporated in clear, isotropic, single-phase mixtures, formed during the titrations of the investigated surfactant/cosurfactant/oil mixtures just before the breakdown of the microemulsion (W_{max}) was related with the microemulsion area border.

2.2.2. Drug solubilization capacity evaluation

Drug solubilization capacity was evaluated in numerous microemulsion samples formed along the water titration line where the maximum efficiency of the surfactant/cosurfactant mixture for microemulsion stabilisation was observed. Ibuprofen-free microemulsions were prepared by stirring the required quantities of the components until it forms a clear and transparent liquid, at room temperature. The drug powder was added into the preweight microemulsion vehicles to give a final concentration of 5% (w/w), under continuous mixing using a magnetic stirrer, at room temperature. The samples were kept in well-closed glass containers and stored at room temperature for 48 h until the time of visual examination. Microemulsions with the sufficient capacity for solubilization of the drug were identified as clear and transparent liquids free of blurriness and/or precipitate.

2.2.3. Determination of ibuprofen solubility in the excipients (surfactant, cosurfactans and oil)

The excess amount of the drug was added in 5 ml of each excipient and samples were continuously shaken (HS 260 control, IKA, Germany) for 6 h at room temperature. The samples were then kept at ambient temperature for 18 h and after that centrifuged (3000 rpm × 30 min) to remove the undissolved drug. Concentration of the drug remained in the supernatant was determined by thin layer chromatography (TLC). TLC was performed on aluminium-backed silica gel $60F_{254}$ plates (Merck, Darmstadt, Germany). Ascending chromatography was performed with toluene/acetone/formic acid, 35:14:1 (v/v/v), as mobile phase in a twin-trough TLC chamber previously saturated with mobile phase vapour for 15 min. After the development the plates were air-dried and the spots were detected under UV light at 254 nm. For quantitative analysis chromatograms were scanned at 220 nm with a Camag TLC Scanner II which is integrated with the computer system and CATS software V.3.15 (Camag, Switzerland). Peak areas were used for quantification. The peak area correlated polynomial with ibuprofen concentrations ($r^2 > 0.99$) in the range 1.52–6.84 mg/l.

Table 1
Mathematical models applied in drug release analysis.

Release models	Equation
Higuchi model	$Q = k_h t^{1/2}$
Zero order release model	$Q = Q_0 - k_0 t$
First order release model	$\ln Q = \ln Q_0 - k_1 t$

Q – cumulative amount of drug released at time t; t – time (h); Q_0 – initial amount of drug ($t = 0$); k_h – *Higuchi* constant; k_0 – zero order release constant; k_1 – first order release constant.

2.2.4. Characterization of the drug-free microemulsions and ibuprofen-loaded microemulsions

Optical isotropy of the investigated microemulsions was verified using cross-polarized light microscopy (Leitz Wetzlar 307–083.103 514652, Germany). Electrical conductivity (σ) was measured using the conductometer CDM230 (Radiometer, Denmark) at 20 °C. The pH value was measured by the pH meter HI 9321 (Hanna Instruments Inc., USA) at 20 °C. Flow behaviour of the microemulsions was determined using a rotational rheometer (Rheolab MC120, Paar Physica, Germany) equipped with a cup and bob measuring device Z3 DIN at 20 °C. The shear stress measurements were performed within the shear rate ranging from 0 to 200 s^{-1}, for both, up and down curves. The obtained flow curves were evaluated by fitting the experimental data to *Newtonian* model, *Bingham* model and *Ostwald* (or *power-law*) model using the software which is an integral part of the rheometer. The droplet size of microemulsions was characterized by photon correlation spectroscopy (PCS) using the apparatus Nano ZS90 (Malvern Instruments, U.K.) equipped with a He–Ne laser at 633 nm. The size measurements were carried out at fixed angle of 90° after external standardization with spherical polystyrene beads (63 nm). The measurements were performed at 20 °C. Samples were suitably diluted with distilled water to avoid multi-scattering phenomena. The predetermined viscosity of microemulsions was incorporated into the associated computer software which performs statistical analysis of data and calculates the average droplet size (Z-Ave) and polydispersity index (PDI) from intensity distribution. The results are the mean and standard deviation (S.D.) of three consecutive measurements for each sample.

2.2.5. In vitro drug release investigations

Evaluation of *in vitro* ibuprofen release from investigated microemulsions and the referent hydrogel product (Deep Relief®, Mentholatum Company Ltd., USA) was performed in the rotating paddle apparatus (Erweka DT70, Germany) using enhancer cell (VanKel Technology Group, USA), at 32 °C. Two grams of the tested microemulsion or the reference was loaded into the Teflon cell with 4 cm^2 diffusion area, covered with a regenerated cellulose membrane (Cuprophan®, Medicell, UK) and dipped in 750 ml of the receptor medium (phosphate buffer pH 7.4, USP). The rotating paddle speed was 100 rpm. Five millilitres samples were withdrawn from the buffer medium at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, and 6 h and replaced with the equal volume of fresh buffer solution to maintain sink conditions. The samples were analysed spectrophotometrically (Carry 50, Varian, Germany) (λ = 220 nm). The obtained release profiles were compared by applying model-dependent approach using *Higuchi* model, zero order and first order mathematical equations (Table 1).

3. Results and discussion

3.1. The influence of the cosurfactant, K_m and SCoS/O values on microemulsion area in pseudo-ternary phase diagrams

The regions of transparent systems formed along the investigated titration lines are depicted as coloured sequences in the pseudo-ternary phase diagrams in Fig. 1.

Table 2
Solubility of ibuprofen in the components of the microemulsions.

Component	Ibuprofen (%, w/w)
Isopropyl myristate	9.4
Labrasol®	14.6
Solubilisant® gamma 2421	12.3
Solubilisant® gamma 2429	12.4

The area of microemulsion systems was mainly affected by the cosurfactant and the SCoS/O value. The microemulsion area observed along the titration lines in oil-reach region of the phase diagrams was very limited in the presence of both cosurfactants. A significant increase of the microemulsion area (i.e., the water solubilization capacity) in the system containing cosurfactant Solubilisant gamma® 2421 was detected at SCoS/O ≥ 80:20 for K_m 40:60 and K_m 50:50, and at SCoS/O ≥ 70:30 for K_m 60:40. The highest capacity for water solubilization (~80%, w/w) was observed at SCoS/O 90:10 for all K_m values (Fig. 1a). The water solubilization capacity in the presence of Solubilisant gamma® 2429 was also very limited at the investigated K_m values at SCoS/O ≤ 50:50. As SCoS/O increases, the efficiency of surfactant/cosurfactant/oil mixture for solubilization of water phase increases and at SCoS/O 90:10 a maximum of ~80% (w/w) of solubilized water was determined (Fig. 1b). Therefore, both cosurfactants enhance the efficiency of the surfactant for stabilisation of microemulsions with high content of water phase when the concentration of tensides mixture was significantly higher compared to the oil. We assumed in both cases that at very high water contents (>80%, w/w) in oil-in-water microemulsions occurred a migration of the hydrophilic tensides molecules from the interface into the surrounding water phase disturbing the interface film integrity. Comparing the influence of the cosurfactants on the microemulsion area, the wider transparent sequences were observed in the systems prepared with Solubilisant gamma® 2429. Solubilisant gamma® 2429 represents a mixture of octoxynol-12, polysorbate 20, and PEG-40 hydrogenated castor oil, while Solubilisant gamma® 2421 contains octoxynol-12 and polysorbate 20. Therefore, the increased water incorporation along the investigated dilution lines was ascribed to PEG-40 hydrogenated castor oil. The latter tenside has larger both hydrophilic and lipophilic groups compared to octoxynol-12 and polysorbate 20, and thus enhanced solubilization power (Kahlweit, 1999; Sjöblom et al., 1996). The interface film containing PEG-40 hydrogenated castor oil is probably less sensitive on water dilution and the microemulsion structure exists at higher concentrations of water phase. Enhancing influence of the increase in the size of the hydrophilic chain lengths and/or hydrocarbon chain length in the molecule of alkyl polyoxyethylenes on the size of the single-phase region was also demonstrated by other investigators (Bayrak and Iscan, 2005).

3.2. Solubilization of ibuprofen in the investigated microemulsion

In accordance with the results of phase behaviour characterization, ibuprofen solubilization capacity was evaluated in microemulsion samples selected from the water titration line at SCoS/O 90:10. It was observed that the solubilization capacity for ibuprofen decreases as surfactant/cosurfactant/oil content decreases as well as the water fraction increases. Roughly equal contents of water phase and surfactant/cosurfactant/oil mixture was found as the highest acceptable for complete solubilization of ibuprofen. Further increase in water content was followed by the formation of a drug precipitate. The loading capacity for ibuprofen was very similar in the presence of both cosurfactants at the investigated K_m values.

Furthermore, solubility of ibuprofen in the excipients was determined and the obtained results are shown in Table 2.

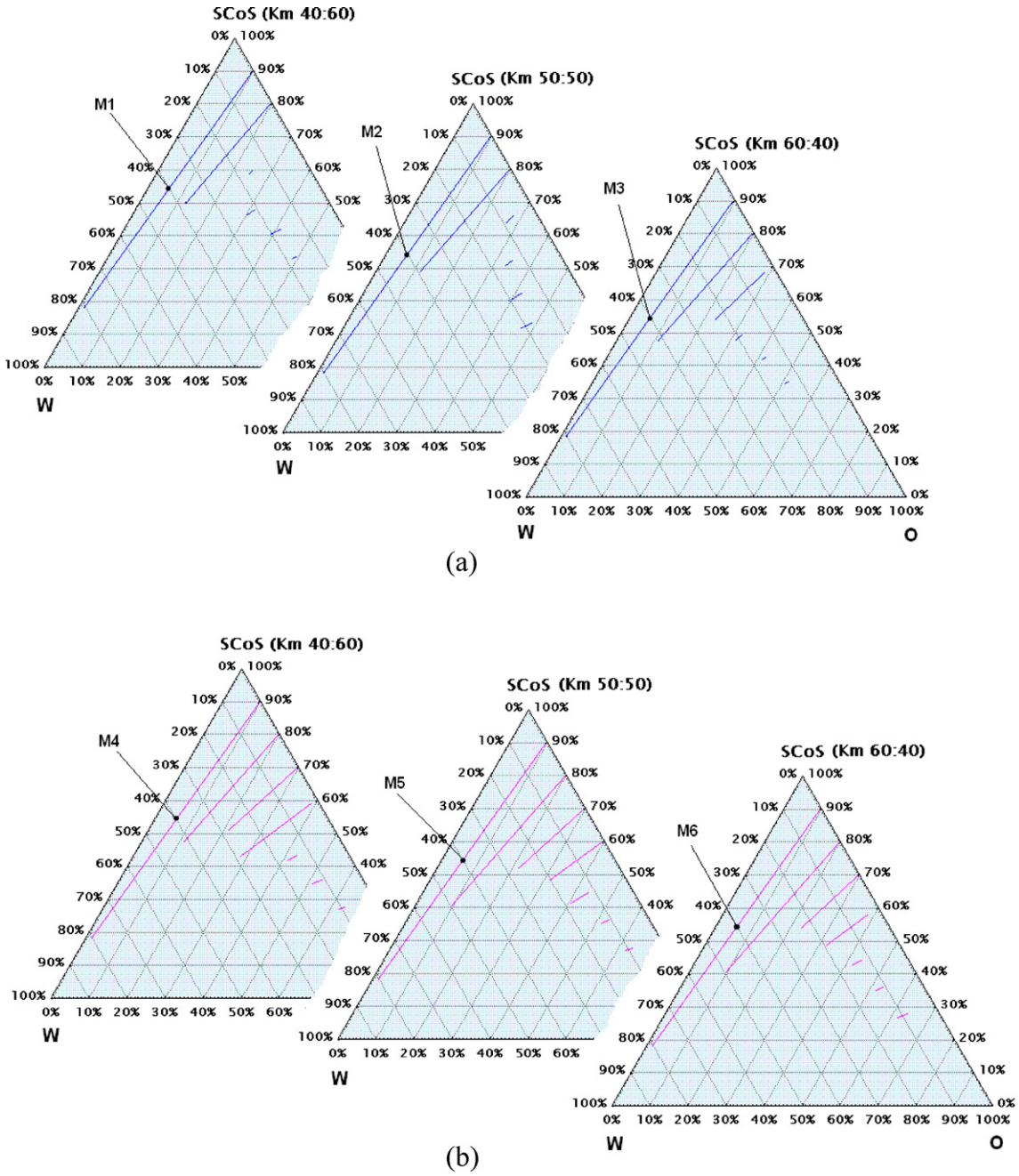

Fig. 1. Pseudo-ternary phase diagrams representing the extent of the transparent sequences on the investigated dilution lines for the system Labrasol®/cosurfactant/isopropyl myristate/water using as a cosurfactant: (a) Solubilisant gamma® 2421; (b) Solubilisant gamma® 2429 (W – water; O – oil; SCoS – the surfactant + cosurfactant mixture) (the black spots in the phase diagrams correspond with compositions of the selected microemulsions M1–M6).

The solubility of ibuprofen was the highest in Labrasol®, followed by the cosurfactants and the oil, and it was approximately 2–3 times higher than the targeted therapeutic concentration. Although the solubility of the drug in microemulsion components was not directly correlated with the carriers capacity for drug solubilization, we consider the obtained results in order to elucidate the drug distribution between the water phase, oil phase and the surfactant/cosurfactant film at the oil/water interface. Considering the solubility of ibuprofen in the microemulsion constituents (Table 2), the value of $\log P_{n\text{-octanol/water}}$ (3.51) (Hadgraft et al., 2000) and the high SCoS/O (90:10), it was assumed that the surfactant/cosurfactant film should be the most significant for the drug solubilization. The latter observation was supported by the observation of Rane and Anderson (2008) that only extremely lipophilic substances are predominantly dissolved in oil phase, while majority of poorly soluble drug molecules are located at the interface. On the other hand, ibuprofen has very limited solubility in water (European Pharmacopoeia, 2011) and it was reasonable that the solubilization capacity for ibuprofen was decreased at higher water concentrations since the content of the tensides at interface available for the drug solubilization decreases simultaneously. Similar solubility of ibuprofen in the surfactant and the cosurfactants (Table 2) was related with the observed similarity in drug solubilization capacity at different K_m values.

In order to investigate further the influence of the cosurfactant composition and K_m value on microemulsion vehicle performances, six microemulsion formulations M1–M6, the one from each of the phase diagrams (Fig. 1), were selected. The microemulsions

Table 3
Composition of the microemulsion formulations M1–M6.

Microemulsion	Surfactant[a] (%, w/w)	Cosurfactant (%, w/w)	Oil[d] (%, w/w)	Water (%, w/w)
M1	19.80	29.70[b]	5.50	45.00
M2	24.75	24.75[b]	5.50	45.00
M3	29.70	19.80[b]	5.50	45.00
M4	19.80	29.70[c]	5.50	45.00
M5	24.75	24.75[c]	5.50	45.00
M6	29.70	19.80[c]	5.50	45.00

[a] Labrasol®.
[b] Solubilisant gamma® 2421.
[c] Solubilisant gamma® 2429.
[d] Crodamol® IPM.

contain the same concentration of water phase (45%, w/w) and surfactant/cosurfactant/oil mixture (55%, w/w) at SCoS/O 90:10 and mutually differ by the cosurfactant composition and/or K_m value. Composition of microemulsions M1–M6 is given in Table 3.

The investigated microemulsion vehicles M1–M6 and the corresponding ibuprofen-loaded microemulsions were homogeneous and transparent liquids at room temperature. The absence of interference of the microemulsions with the polarized light confirmed their homogeneity and optical isotropy. Visual inspection of the samples after 48 h, once weekly during the first month and after that once monthly up to one year, did not reveal any changes in their appearance (e.g., blurriness, phase separation, formation of a precipitate).

The data obtained within the physico-chemical characterization of the microemulsions are listed in Table 4.

Electrical conductivity of the microemulsion vehicles M1–M6 ranged from 26.15 to 57.0 μS/cm. The incorporation of ibuprofen into vehicles led to a small decrease in conductivity (from 20.49 to 48.12 μS/cm) (Table 4) which may indicate that the droplet size increases in the presence of the drug. The pH values of microemulsion vehicles M1–M6 ranged from 5.85 to 6.61, while they were decreased in corresponding ibuprofen-loaded microemulsions (4.7–5.23) (Table 4). This was ascribed to weak acidic character of the drug molecule (pKa ~ 4.50) (Brittain, 2001). Nevertheless, pH value of the investigated microemulsions was acceptable for dermal application.

Figs. 2 and 3 show the flow curves of ibuprofen-free and ibuprofen-loaded microemulsions.

The obtained rheological profiles revealed that investigated microemulsions were non-*Newtonian* low viscosity fluids that do not exhibit thixotropy. The best fit ($R_{xy} > 0.995$) for all microemulsions gives *Bingham* model, which describes a linear shear-stress (τ)/shear-rate (γ) behaviour beyond an initial shear-stress threshold (τ_0) Eq. (2):

$$\tau = \tau_0 + \eta\gamma \quad (2)$$

where η represents *plastic viscosity*. The observed flow behaviour was ascribed to the deviation of the oil droplet shape from spherical symmetry as well as to interactions between droplets. The low values of τ_0 (from 1.5848 to 3.1433 Pa) indicate weak attractive interdroplet interactions probably via hydrogen bonding between hydrated polyoxyethylene chains of the tensides molecules at the interface, as proposed recently by Boonme et al. (2006). The values of η are given in Table 5.

It was noted that viscosity of the microemulsions M1–M3, which were prepared with Solubilisant® gamma 2421, was lower compared to the corresponding microemulsions M4–M6 prepared with Solubilisant® gamma 2429 at the same K_m value. Furthermore, as the K_m value increases (i.e., as the relative concentration of cosurfactant in the surfactant/cosurfactant mixture decreases) the

Table 4
Electrical conductivity (σ) and pH values of the drug-free and ibuprofen-loaded microemulsions (IB).

Microemulsion	σ (μS/cm)	σ^{IB} (μS/cm)	pH	pH^{IB}
M1	26.99	20.49	6.05	4.75
M2	33.60	27.24	5.85	4.70
M3	26.15	21.14	5.87	4.77
M4	54.45	40.56	6.61	5.23
M5	57.00	48.12	6.33	5.16
M6	43.32	31.53	6.26	4.99

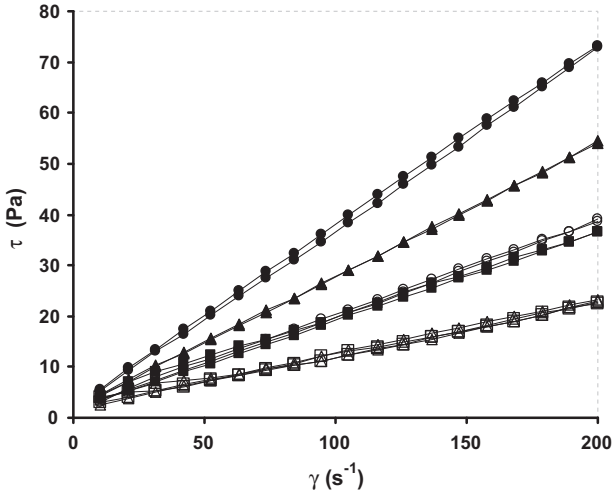

Fig. 2. Flow curves of microemulsion vehicles M1 (●), M2 (▲), and M3 (■) and corresponding ibuprofen-loaded microemulsions (5%, w/w) (open symbols).

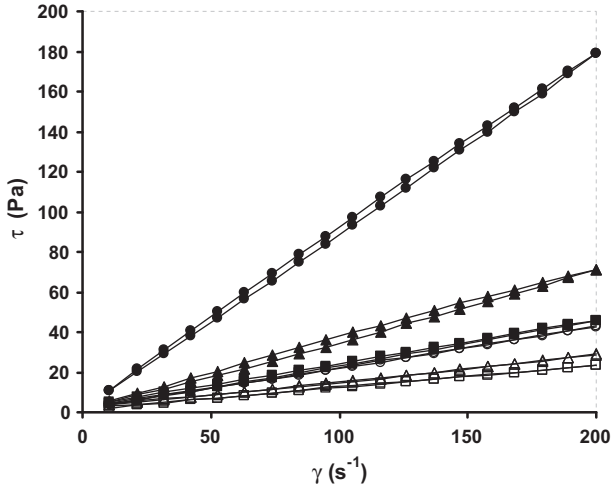

Fig. 3. Flow curves of microemulsion vehicles M4 (●), M5 (▲), and M6 (■) and corresponding ibuprofen-loaded microemulsions (5%, w/w) (open symbols).

Table 5
Bingham plastic viscosity (η) for ibuprofen-free and ibuprofen-loaded microemulsions.

Microemulsions	Ibuprofen-free microemulsions η (Pas)	Ibuprofen-loaded microemulsions η (Pas)
M1	0.4135 ± 0.005	0.1915 ± 0.003
M2	0.2589 ± 0.003	0.0934 ± 0.002
M3	0.1690 ± 0.004	0.0957 ± 0.004
M4	0.9028 ± 0.002	0.2025 ± 0.003
M5	0.3330 ± 0.003	0.1239 ± 0.001
M6	0.2142 ± 0.003	0.1166 ± 0.005

viscosity value decreases, for both cosurfactants. The increased viscosity in the presence of Solubilisant® gamma 2429 was ascribed to the influence of PEG-40 hydrogenated castor oil. The introduction of the latter surfactant into the interface film increases progressively a number of ethyleneoxide units available for bonding with the surrounding water molecules, providing a much higher extent of hydration as well as droplet interactions compared to octoxynol-12 and polysorbate 20.

Rheological properties of ibuprofen-loaded microemulsions were compared with the drug-unloaded vehicles (Figs. 2 and 3, Table 5). Although the flow behaviour of the microemulsions remains unchanged after solubilization of ibuprofen, the incorporation of the drug reduced their viscosity. The obtained results indicate that drug solubilization does not cause much change in the microstructure of the microemulsions but most likely reduce interdroplet interactions. Similar influence of ketoprofen on rheological properties of mixed nonionic microemulsions was reported by Tomšič et al. (2006), and it was interpreted by the insufficient interactions within the vehicle for the formation of gel-like structure after the drug incorporation. As previously assumed the relatively small molecules of ibuprofen (M_r 206.29 g/mol) were predominantly solubilized at the interface. The drug molecules were likely located deeper inside polyoxyethylene chains region and thus their contact with water phase was minimized. Therefore, the solubilized drug molecules might affect the geometry of the interface and the polyoxyethylene chains of tensides became more closely packed and less exposed to water molecules reducing H-bonding and hence decreasing the viscosity.

PCS analysis was performed on the drug-unloaded and ibuprofen-loaded microemulsions M1–M6 which were diluted with water up to 80% (w/w). Droplet sizing data are summarized in Figs. 4 and 5 and in Tables 6 and 7.

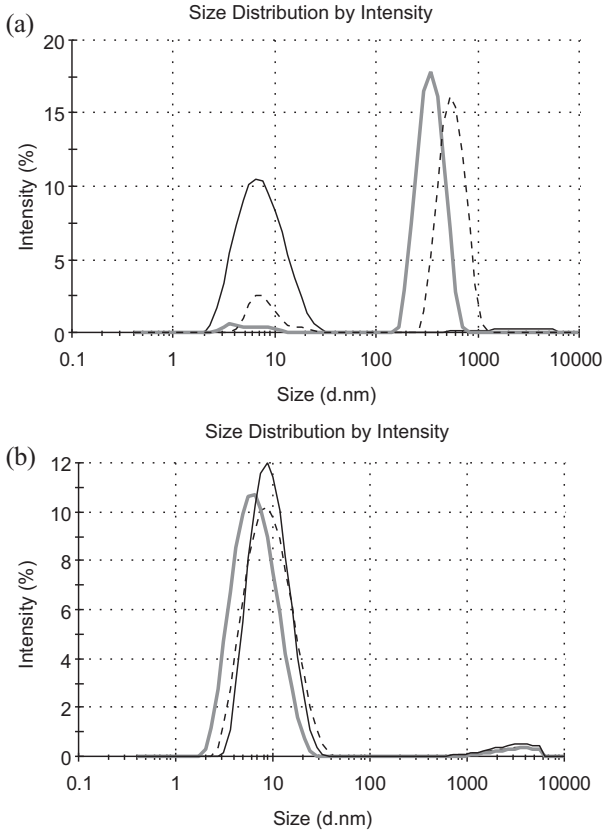

Fig. 4. The droplet size distribution by intensity for microemulsions M1 (——), M2 (– – –), and M3 (——): (a) without ibuprofen and (b) in the presence of the drug.

Fig. 4a and Table 6 indicate that among microemulsions prepared with Solubilisant gamma® 2421 the diluted samples M1 (K_m 40:60) and M2 (K_m 50:50) have large droplets and high polydispersity, while the microemulsion M3 (K_m 60:40) has a monomodal distribution (PDI < 0.250) with average droplet size 6.57 nm. In the case of Solubilisant gamma® 2429 containing microemulsions, bimodal distribution was detected for M4 (K_m 40:60) and M5

Table 6
The average droplet size (Z-ave ± S.D., $n = 3$), and polydispersity index (PDI ± S.D., $n = 3$) of the drug-free microemulsions (ME) M1–M6.

ME	Cosurfactant (K_m)	Z-ave ± S.D. (nm)	PDI ± S.D.	Peak mean int (nm)			Area int (%)		
				I	II	III	I	II	III
M1	Solubilisant gamma® 2421 (40:60)	261.00 ± 12.18	0.337 ± 0.021	355.00	4.09	7.84	95.90	2.30	1.80
M2	Solubilisant gamma® 2421 (50:50)	125.80 ± 19.68	1.000 ± 0.000	575.1	8.59	0.00	86.00	14.00	0.00
M3	Solubilisant gamma® 2421 (60:40)	6.57 ± 0.01	0.237 ± 0.001	8.11	2547	0.00	96.60	3.40	0.00
M4	Solubilisant gamma® 2429 (40:60)	4.52 ± 0.02	0.322 ± 0.003	5.16	78.24	3268	88.70	10.70	0.60
M5	Solubilisant gamma® 2429 (50:50)	8.67 ± 0.07	0.354 ± 0.010	9.93	111.50	1.68	84.60	14.60	0.70
M6	Solubilisant gamma® 2429 (60:40)	7.00 ± 0.01	0.247 ± 0.007	8.39	2003	0.00	95.10	4.90	0.00

Table 7
The average droplet size (Z-ave ± S.D., $n = 3$), and polydispersity index (PDI ± S.D., $n = 3$) of the ibuprofen-loaded microemulsions (ME) M1–M6.

ME	Cosurfactant (K_m)	Z-ave ± S.D. (nm)	PDI ± S.D.	Peak mean int (nm)			Area int (%)		
				I	II	III	I	II	III
M1	Solubilisant gamma® 2421 (40:60)	6.00 ± 0.03	0.222 ± 0.007	7.31	3289	–	97.3	2.7	–
M2	Solubilisant gamma® 2421 (50:50)	8.29 ± 0.01	0.236 ± 0.004	10.24	2887	–	96.6	3.4	–
M3	Solubilisant gamma® 2421 (60:40)	8.59 ± 1.29	0.230 ± 0.059	6.70	3335	46.75	76.7	10.7	7.1
M4	Solubilisant gamma® 2429 (40:60)	5.17 ± 0.01	0.216 ± 0.01	6.11	2870	–	96.7	3.3	–
M5	Solubilisant gamma® 2429 (50:50)	8.40 ± 0.03	0.224 ± 0.005	10.03	3398	–	96.8	3.2	–
M6	Solubilisant gamma® 2429 (60:40)	8.75 ± 0.00	0.240 ± 0.001	10.42	2961	237.10	95.0	2.8	2.2

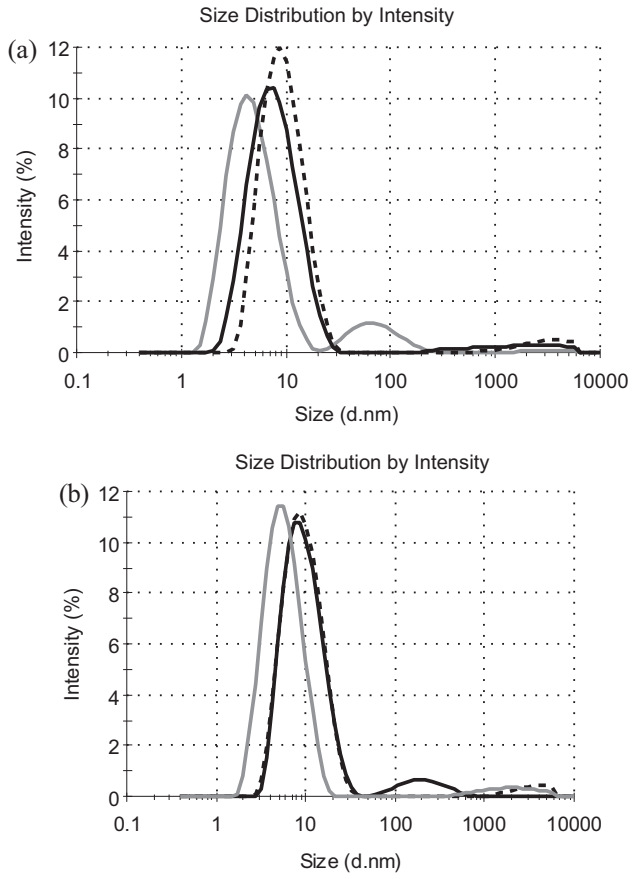

Fig. 5. The droplet size distribution by intensity for microemulsions M4 (▬), M5 (▬ ▬), and M6 (▬): (a) without ibuprofen and (b) in the presence of the drug.

(K_m 50:50) and a monodispersed droplet size distribution with small average droplet diameters (7 nm) was seen only for M6 (K_m 60:40) (Fig. 5a and Table 6). PCS analysis confirmed that at K_m 40:60 and 50:50 the dilution leads to disturbance of the interfacial film, destabilisation of microemulsion and formation of kinetically stable nanoemulsion. The latter results were quite consistent with the results of phase behaviour characterization. The investigated diluted samples belong to the microemulsion area border. However, in the presence of Solubilisant gamma® 2429 the polydispersity of the samples was lower (Table 6) confirming that interfacial film containing PEG-40 hydrogenated castor oil was less sensitive during the water dilution. Also, at K_m value 60:40 the diluted samples were monodispersed microemulsions in the presence of both cosurfactants (Table 6). In this case, the influence of the cosurfactant was decreased and the higher relative content of the less hydrophilic constituents of the primary surfactant Labrasol® (i.e., mono-, di- and triglycerides of saturated C6–C14 fatty acids (predominantly C8 and C10 fatty acids), mono- and di-fatty acid esters of polyethylene glycol) at the interface likely improve the integrity of the interface film at high water contents. Therefore, the obtained results indicate that the synergism, observed for the investigated nonionic tensides mixtures, probably was based on their enhanced partitioning at the oil/water interface.

The droplet size distribution by intensity for the samples containing ibuprofen was monodisperse (Fig. 4b and Fig. 5b) with average droplet size up to 8.75 nm and low polydispersity (PDI < 0.250) (Table 7). Incorporation of ibuprofen decreases polydispersity in comparison with the drug-free samples. The comparison of the PCS analysis parameters of the samples M1, M2, M4 and M5 without the drug and in the presence of ibuprofen, pointed that the solubilized drug increases the water solubilization capacity i.e., enlarges the microemulsion area. The dilution of the drug-free and ibuprofen-loaded microemulsions was followed with the partition of the hydrophilic tensides molecules from the interface into the surrounding water phase. However, the drug molecules likely remains entrapped at the interface ensuring the monodisperse spherical droplet structure. It was rationale, since ibuprofen molecules are amphiphilic (Rao et al., 1992) and it may act as an additional cosurfactant. The similar influence of the solubilized drug was observed at all investigated K_m values and in the presence of both cosurfactants. Comparison of the monodisperse samples (M3 and M6) without the drug (Table 6) and with the drug (Table 7) pointed the small increase of droplet size in ibuprofen-containing microemulsions. This supports well the previous assumptions regarding the predominant drug solubilization within the interfacial film and its influence on the interface geometry and surface.

3.3. In vitro ibuprofen release

It is generally agreed that IVRT is useful as a more economic alternative for *in vitro* and *in vivo* studies of percutaneous penetration in development of dermopharmaceutics, particularly in early phase when is necessary to evaluate different formulation variables (Flynn et al., 1999). Although there are numerous examples in the literature for application of non-pharmacopoeial apparatus containing Franz diffusion cell, a Teflon cell with adjustable volume designed by VanKel Technology Group (enhancer cell) has also been used for IVRT, providing release profiles generally comparable to Franz diffusion cell. VanKel enhancer cell is less fragile and suitable for routine testing. Moreover, it is compatible with the standard USP dissolution apparatus with relatively large volumes of acceptor medium (200–900 ml) which are required to maintain "sink" conditions (i.e., the concentration of drug in the medium represents ≤10% of the saturation solubility) as well as to fulfil a "30% rule" (i.e., a concentration of the released active substance in the medium at the end of the experiment should not be more than 30% of the total amount of the dose applied in order to avoid receptor back diffusion into donor compartment) (Azarmi et al., 2007; Rapedius and Blanchard, 2001; Siewert et al., 2003). The enhancer cell in IVRT of the microemulsion formulations is exploited scarcely. In the current study the paddle-over-enhancer cell-method was applied. The obtained ibuprofen release profiles over time from the investigated microemulsions M1–M6 and the referent hydrogel formulation are shown in Fig. 6.

The investigated microemulsions M1–M6 were low viscous formulations stabilised by the same total concentration of the tensides. Ibuprofen release profiles observed for the microemulsions M1–M3, prepared with Solubilisant gamma® 2421 as a cosurfactant, were similar during the first 3 h and the release rate was much slower in comparison with the reference hydrogel (Fig. 6a). Surprisingly, during the last 3 h the drug release rate from the microemulsion M1 (K_m 40:60) was significantly increased compared with the two other microemulsions, M2 (K_m 50:50) and M3 (K_m 60:40), and at the end of the experiment became comparable to the reference (Fig. 6a). The drug release profiles of the microemulsions M4–M6, containing as a cosurfactant Solubilisant gamma® 2429, revealed a generally slow release kinetic compared to the hydrogel, although some increase in release rate was observed during the last 3 h of IVRT (Fig. 6b). In this case it was noted that as the relative content of the cosurfactant increases, the drug release becomes faster.

Table 8
The values of correlation coefficients (r^2), drug release rate constant (k) and transmembrane flux (F) obtained by fitting the ibuprofen release data from the investigated microemulsions (ME) M1–M6 and Deep Relief® hydrogel (the referent product) with *Higuchi*, zero order and first order mathematical models.

ME	*Higuchi*			Zero order			First order		
	r^2	k (mg h$^{-1/2}$)	F (mg cm^{-2} h$^{-1/2}$)	r^2	k (mg h^{-1})	F (mg cm^{-2} h^{-1})	r^2	k (h^{-1})	F (cm^{-2} h^{-1})
M1	0.9401	10.083	2.521	0.9791	4.153	1.038	0.9737	0.048	0.012
M2	0.9953	7.005	1.751	0.9981	2.149	0.537	0.9971	0.024	0.006
M3	0.9956	5.110	1.303	0.9806	1.486	0.372	0.9825	0.017	0.004
M4	0.9664	8.954	0.708	0.9928	2.830	0.708	0.9901	0.032	0.008
M5	0.9850	7.198	1.800	0.9972	2.232	0.558	0.9966	0.025	0.006
M6	0.9972	5.948	1.487	0.9961	1.821	0.455	0.9962	0.020	0.005
Deep Relief® hydrogel	0.9988	9.624	2.406	0.9861	2.910	0.728	0.9899	0.034	0.009

Generally, mathematical modelling of drug release profiles from microemulsions is poorly documented (Grassi et al., 2000). In the current study was applied common analysis of drug release data from topical semisolid formulations using *Higuchi*, zero order and first order mathematical models (Table 1). The values of correlation coefficients, drug release rate and transmembrane flux calculated for the investigated microemulsions M1–M6 and the referent hydrogel are collected in Table 8.

The release of ibuprofen from the referent semisolid preparation and microemulsions M3 and M6 followed the *Higuchi* model, which describes a linear relationship between the square root of time (on *x*-axis) and the cumulative percentage of drug release (on *y*-axis), suggesting that the drug releases by diffusion and the process was controlled by the vehicle. The corresponding release rate and the transmembrane flux were lower when compared with the reference. The latter observation was ascribed to a significantly different mobility of the drug within the vehicles. Deep Relief® contains 5% of ibuprofen and 3% of levomenthol in the hydrogel consisting of propylene glycol, carbomer, diisopropanolamine, ethanol and water. It has previously been shown that ibuprofen solubility is enhanced by the addition of menthol, probably due to the formation of the eutectic mixture in aqueous solution (Abdul Rasool et al., 2010). Also, propylene glycol enhanced the drug solubility and mobility by co-solvency effect. Therefore, a relatively fast release was ascribed to a high diffusion rate of the ibuprofen molecules dissolved in the hydrogel vehicle. In contrary, the mobility and release of the ibuprofen from the microemulsion vehicles M3 and M6 (K_m 60:40) was hindered probably by its entrapment at the interface film. As Hu et al. (2006) proposed, the *in vitro* release of lipophilic drugs disposed on the interfacial film (and in the internal phase) into the aqueous medium is prolonged by diffusion across interfacial structure and correlates straight with the contents of oil and tensides in microemulsions. Interestingly, in this study, at K_m 50:50 (microemulsions M2 and M5) the plot of the cumulative ibuprofen permeation through the synthetic membrane versus time was linear. In this case, the data fitted better to the zero order equation (Table 8) describing the systems where the drug release is controlled by the diffusion across the membrane, while it is independent on the vehicle. The same ibuprofen release model was observed by few other authors for the microemulsion-based hydrogels (Chen et al., 2006) and polysorbate 40/glyceryl caprylate/isopropyl myristate/water microemulsions (Gašperlin and Bešter-Rogac, 2009), although IVRT was performed by different techniques. The release profile of ibuprofen from the microemulsions M1 and M4 (at K_m 40:60) was significantly different from the other microemulsions and the correlation of the drug release data with the mathematical models was poor ($r^2 < 0.95$) (Table 8). This was related with a more complex dynamic of structural transformations within the vehicles during the IVRT. The most important process is penetration of water phase from receptor compartment into the enhancer cell which may lead to the break down of the carrier structure and subsequently affects the drug solubilization capacity and *in vitro* drug release rate (Trotta, 1999). Since the quantitative composition of the investigated microemulsions was the same, the observed release profile heterogeneity might be attributed to the different solubilization power of the employed surfactant/cosurfactant mixtures. Stronger surfactant/cosurfactant synergism lead to slower ibuprofen release. As the relative content of the cosurfactant increases (i.e., lower K_m), the efficiency of the surfactant/cosurfactant mixtures to preserve the interface integrity during water dilution decreases while the potential risk for destabilisation as well as the drug release rate increases. Although Solubilisant gamma® 2429 provides a stronger synergism, the latter was observed for both cosurfactants. The amount of the drug released at the end of the experiment from the Solubilisant gamma® 2421 microemulsions (M1–M3) was 24.47%, 14,47%, and 10.75%, respectively, and for the Solubilisant gamma® 2429 microemulsions (M4–M6) 17.99%, 15,33%, and 13.45%, respectively. Considering the amount of ibuprofen released, microemulsions M1 and M4, at K_m 40:60, were very close with the reference (22.22%), thus could be further developed as promising carriers for transdermal delivery of ibuprofen.

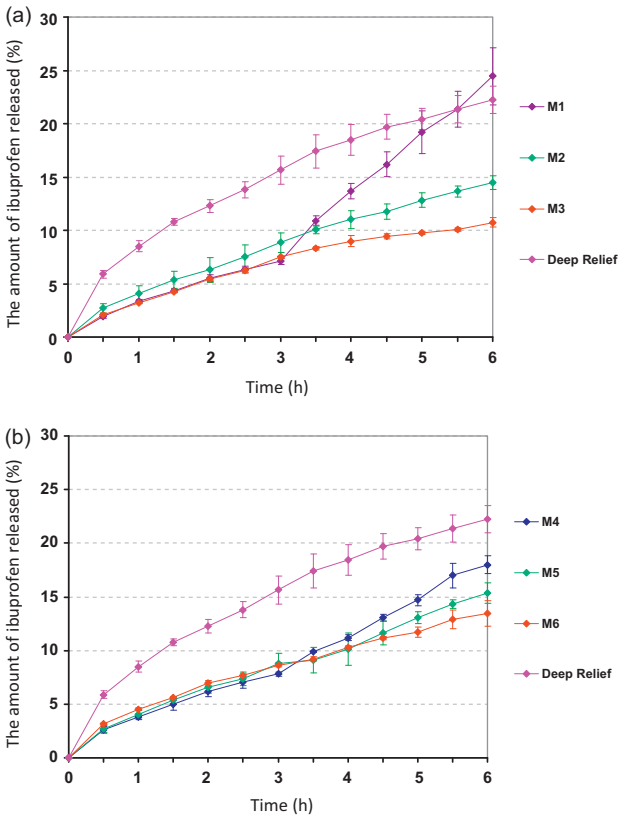

Fig. 6. *In vitro* ibuprofen release profiles from microemulsions M1–M3 (a) and M4–M6 (b) compared with the reference (Deep Relief® hydrogel).

4. Conclusion

The results of the experiments carried out in this study reveal the synergism of the investigated Labrasol®/Solubilisant gamma® 2421 or Solubilisant gamma® 2429 (K_m 40:60, 50:50 or 60:40)/isopropylmyristate mixtures for water solubilization at SCoS/O 90:10. The maximum concentration of water incorporated was roughly 80% (w/w). However, the capacity of the investigated microemulsion systems for solubilization of 5% (w/w) ibuprofen was limited at the concentration of the water phase >50% (w/w). Therefore, six microemulsions (M1–M6) were prepared with 45% (w/w) of water phase, two different cosurfactants and at three K_m values, and characterized as potential vehicles for ibuprofen. The investigated drug-free and ibuprofen-loaded microemulsions were oil-in-water, isotropic, low viscous *Bingham*-type liquids with the pH value acceptable for topical application. The analysis of the results of phase behaviour characterization, conductometric, rheological and droplet size measurements, indicate that the observed synergism was based on increased partitioning of the tensides molecules on the oil/water interface and correlated with the cosurfactant composition and K_m value. The stronger nonionic tensides synergism was observed in the presence of Solubilisant gamma® 2429 and at K_m 60:40. Also, the interface film represents the main locus for the drug solubilization, which additionally improves its flexibility. Model-dependent analysis of the *in vitro* release profiles of ibuprofen revealed *Higuchi* kinetics at K_m 60:40, zero-order kinetics at K_m 50:50 and more complex kinetic at K_m 40:60, for both cosurfactants. The observed differences regarding the release kinetics were directly related with the different efficiency of the nonionic tensides mixtures for formation of thermodynamically stable carriers i.e., different risk for the disturbance of the microemulsion state on water dilution. The lower surfactant/cosurfactant synergism corresponded to the faster drug release.

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