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An investigation into the characteristics and drug release properties of multiple W/O/W emulsion systems containing low concentration of lipophilic polymeric emulsifier

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Abstract

Multiple W/O/W emulsions with high content of inner phase ($\Phi 1 = \Phi 2 = 0.8$) were prepared using relatively low concentrations of lipophilic polymeric primary emulsifier, PEG 30-dipolyhydroxystearate, and diclofenac diethylamine (DDA) as a model drug. The investigated formulations were characterized and their stability over the time was evaluated by dynamic and oscillatory rheological measurements, microscopic analysis and in vitro drug release study. In vitro release profiles of the selected model drug were evaluated in terms of the effective diffusion coefficients and flux of the released drug. The multiple emulsion samples exhibited good stability during the ageing time. Concentration of the lipophilic primary emulsifier markedly affected rheological behaviour as well as the droplet size and in vitro drug release kinetics of the investigated systems. The multiple emulsion systems with highest concentration (2.4%, w/w) of the primary emulsifier had the lowest droplet size and the highest apparent viscosity and highest elastic characteristics. Drug release data indicated predominately diffusional drug release mechanism with sustained and prolonged drug release accomplished with 2.4% (w/w) of lipophilic emulsifier employed.

Keywords: W/O/W emulsions; Rheology; Droplet size; In vitro release; Diclofenac diethylamine

1. Introduction

Multiple emulsions are complex dispersion systems, known also as “emulsions of emulsions”. The most common multiple emulsions are of W/O/W type, although some specific applications O/W/O emulsions can also be prepared.

These emulsion systems, at least in theory, have significant potential in pharmacy and cosmetics providing prolonged release of active substances, the possibility of combining incompatible substances in one product and/or protection of fragile substances. In practice, however, significant problems may arise because of their thermodynamical instability and strong tendency for coalescence, flocculation and creaming.

Numerous factors may affect stability of W/O/W emulsions, including the method of preparation, the effect of electrolytes, phase volumes ratio, type and concentration of the emulsifier. The polymeric surfactants proved to be superior to the conventional non-ionic surfactants in maintaining the physical stability of the multiple emulsion (Tadros et al., 1998).

Rheological characteristics of the emulsions can be an important indicator of its physical stability. It is well documented that multiple emulsions can be appropriately characterized and their stability evaluated by rheological analysis (Terrisse et al., 1993; Tadros et al., 1995; Grossiord and Seiller, 1998).

Generally, two main drug release mechanisms from W/O/W emulsions can be distinguished, being diffusion and/or emulsion breakdown/membrane rupture (Florence et al., 1985; Laugel et al., 1996). Diffusion-controlled release will be dependent upon polarity and molecular weight of the drug and surfactant type/concentration, while membrane rupture will be dependent upon the physical stability of the emulsion.

In a separate study, it was shown that lipophilic polymeric emulsifier, PEG 30-dipolyhydroxystearate applied used in low concentrations gave semi-solid W/O/W multiple emulsions with good long-term stability (Vasiljevic et al., 2005).

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The objective of this study was to formulate and characterize W/O/W emulsions with low concentrations of primary polymeric emulsifier (PEG 30-dipolyhydroxystearate, PDHS) containing diclofenac diethylamine as a model drug. Following the results of the preliminary studies, the concentrations of the primary emulsifier in the W/O/W emulsions were set to 0.8, 1.6 and 2.4% (w/w). The prepared sample formulations were characterized and their stability over time evaluated by dynamic and oscillatory rheological measurements and microscopic analysis. In vitro release profiles of the selected model drug were evaluated in terms of the effective diffusion coefficients and flux of the released drug.

2. Materials and methods

2.1. Material and composition

The oil phase consisted of medium chain triglicerides (Myritol® 318, Fina, Brussels, Belgium); the polymeric surfactants used were PHS/PEO/PHS block copolymer (PEG 30-dipolyhydroxystearate, Arlacel® P135, ICI, Kortenburg, Belgium) and ethoxylated propylene oxide copolymer (Poloxamer 407, Lutrol® PE/F127, BASF, Ludwigshafen, Germany). The other substances used were propylene glycole (Vetprom Hemikalije, Belgrad, Serbia and Montenegro) and purified water. Diclofenac dethylamine was a kind gift of Hemofarm, A.D. (Vrsac, Serbia and Montenegro).

As a reference preparation for in vitro drug release study commercially available Voltaren® Emulgel® (Novaris Pharma AG, Basel, Switzerland) was used. Both, primary and multiple emulsions were prepared with high content of inner phase ($\Phi 1 = \Phi 2 = 0.8$). The compositions of the primary emulsions (PE1–PE3), as well as the multiple emulsions (ME1–ME3) are given in Table 1.

2.2. Preparation of the samples

The preparation method was a two-step method first proposed by Matsumoto et al. (1976).

Table 1
Compositions of the primary (PE1–PE3) and multiple (ME1–ME3) emulsions (%, w/w of ingredients)

	Primary emulsion		
	PE1	PE2	PE3
PEG 30-dipolyhydroxystearate	1.0	2.0	3.0
Medium chain triglicerides	19.0	18.0	17.0
Propylene glycole	20.0	20.0	20.0
Purified water to	100.0	100.0	100.0

	Multiple emulsion		
	ME1	ME2	ME3
Primary emulsion	80.0 (PE1)	80.0 (PE2)	80.0 (PE3)
Poloxamer 407	0.8	0.8	0.8
Preservative[a]	2.0	2.0	2.0
Purified water to	100.0	100.0	100.0

[a] As a preservative methylhydroxibenzoate (7%) and propylhydroxybenzoate (3%) solution in propylene glycole was used.

In the first step the primary W/O emulsion (PE) was prepared by slowly adding water phase (containing the drug) preheated to $80 \pm 2\,^{\circ}C$ to the oil phase (containing the polymeric lipophilic surfactant) preheated to the same temperature. In order to solubilize the drug, propylene glycole was added to the inner water phase.

The stirring was performed by laboratory mixer (Heidolph RZR 2020, Heidolph Elektro GmbH & Co., KG, Kelheim, Germany) at 1000 and 1500 rpm until the emulsion temperature which was approximately 25 °C.

In the second step, the primary emulsion was added slowly while the system was stirred at 500 rpm at room temperature. After complete introduction of the primary emulsion the stirring was continued for 20 min.

The concentration of DDA in the final, multiple emulsions ME1–ME3 was 1.16%, being equal to the DDA content in the brand preparation Voltaren® Emulgel®.

Prepared samples were kept on the ambiental temperature over the 90 days time interval and re-examined with regards to rheological behaviour and microscopic analysis 48 h after preparation as well as after 30 and 90 days of storage.

2.3. Evaluation techniques

2.3.1. Microscopic analysis

Microscopic analysis of the investigated samples was conducted in order to gain information about the multiple character of the emulsion and the size of the particles. Optical microscope (Olympus® BX 50, Olympus Optical Co., Tokyo, Japan) with a camera (DXC-151 Single Chip Sony CCD Camera, Sony Corporation, Tokyo, Japan) was used throughout the study. Photomicrographs of the multiple emulsions were taken at various time, after carefully diluting samples with purified water. The droplet size was measured using software Microimage version 4.0 (Olympus, Japan).

2.3.2. Stability studies

Stability studies were conducted at room temperature ($22 \pm 2\,^{\circ}C$). Centrifugation test was performed at $1500 \times g$ using laboratory centrifuge (LC 320, Tehtnica, Zelezniki, Slovenia). Samples were inspected for eventual phase separation after 15 and 30 min of centrifugation.

2.3.3. Rheological measurements

Rheological measurements were performed on rotational and oscillatory rheometer Rheolab MC 120 (Paar Physica, Stuttgart, Germany), coupled with cone and plate measuring device MK 22 (diameter 50 mm, 1° angle, gap 50 μm) for rotational and MK 24 (diameter 75 mm, 1° angle, gap 50 μm) for oscillatory measurements, at $20 \pm 0.2\,^{\circ}C$.

In the steady-state measurements, the shear stress was measured as a function of the shear rate. Values of apparent viscosity (at shear rate $10.5\,s^{-1}$) were used for characterization of the samples for flow analysis.

Oscillatory measurements were performed in order to determine linear viscoelastic region of the samples (amplitude sweep). After the linear viscoelastic region was determined, the

frequency sweep procedure performed, at the constant strain within frequency range 0.1–10.0 Hz. Values of storage modulus (G'), loss modulus (G'') and loss angle (δ) were used for characterization of the samples in oscillatory measurements.

The relationship between G', G'' and $\tan\delta$ is shown in Eq. (1):

$$\tan\delta = \frac{G''}{G'} \quad (1)$$

2.3.4. In vitro drug release study

Dissolution profiles of the investigated multiple emulsions and reference preparation were determined using the rotating paddle apparatus (Erweka DT70, Hausenstamm, Germany), equipped with dissolution cell (enhancer cell, VanKel Industries Inc., Edison, USA) (Rege et al., 1998; Rapedius and Blanchard, 2001; Djordjevic et al., 2005). Dissolution cell was filled with the 2 g sample and covered with a regenerated cellulose membrane (surface area 4.906 cm^2) (Cuprophan®, Akzo, Wuppertal, Germany). The cell was capped and placed in the dissolution vessel containing 500 ml of the receptor medium (pH 7.4 phosphate buffer). The receptor medium was maintained at a constant temperature of 32 °C. The paddle rotation speed was 100 rpm. At fixed time intervals (30, 60, 120, 180, 240, 300, and 360 min), 4 ml samples were withdrawn and were immediately replaced by fresh buffer solution. Sink conditions were maintained at all times. All samples were filtered using 0.2 μm membrane filter (CST, Belgrade, Serbia and Montenegro) and assayed for DDA. DDA concentration was determined spectrophotometrically at 275 nm (Spectrophotometer Cary 50, Varian, Darmstadt, Germany). The vehicle without drug substance, treated the same way as the investigated samples was used as the blank. The dissolution experiments were carried out in triplicate, and data results were expressed as mean value ± S.D. The obtained drug release data were fitted to Eq. (2), described by Higuchi (1962) in order to determine the diffusion coefficient (D) of the investigated samples:

$$Q = 2C_0\sqrt{\frac{Dt}{\pi}} \quad (2)$$

where Q is the amount of drug released per unit area, C_0 the initial drug concentration, D the effective (apparent) diffusion coefficient and t is the time.

The effective diffusion coefficients, for each formulation, were calculated from the slope of the cumulative amount of drug released versus square root of time plot. Flux was calculated as the slope of the cumulative amount of drug released per unit area versus time. Both parameters were calculated from the drug release data obtained after 60 and 360 min of investigation.

3. Results and discussion

3.1. Characteristics of the multiple emulsions

Immediately after processing, multiple emulsions ME2 and ME3 were apparently white, soft and homogenous creams. The sample ME1 was particularly soft and homogenous. The samples did not show any changes in appearance, homogeneity and consistency over the investigation time period at room temperature.

The consistency and homogeneity were also preserved after centrifugation of the samples after 48 h, 30 and 90 days storage, as no phase separation could be detected.

3.2. Microscopic analysis

Photomicrographs for multiple emulsions 48 h after the preparation are given in Fig. 1. Multiple emulsion drops with a large number of small internal droplets can be observed. With the increased concentration of PDHS in the oil phase, considerable reduction of both the inner dispersed water droplets and the multiple droplets size appeared.

The average particle diameters of the 48 h-old emulsions were 11.2, 8.9 and 5.1 μm for samples ME1–ME3, respectively (Table 2). When the concentration of lipophilic emulsifier was increased to 2.4% (w/w), very fine inner aqueous droplets formed. The size of inner droplets was about 1.7, 1.0 and 0.7 μm for the samples ME1–ME3, respectively, but the size of the droplets that were in contact could not be determined accurately.

The microscopic analysis was repeated at days 30 and 90. Droplet size remained almost constant with time for the sample ME3. In the case of samples ME1 and ME2, the minor change in droplets diameter noticed 30 days after the preparation probably may be explained with of drug across oily membrane during this interval.

3.3. Rheological measurements

Steady-state rheological measurements revealed that all the investigated samples exhibited non-Newtonian plastic flow behaviour as it is demonstrated by the stress–shear curves given in Fig. 2. It can be observed that the download curve is below the upward curve, indicating some thixotropy in the systems. All prepared samples showed shear-thinning behaviour: the apparent viscosity decreases with the increase in shear rate.

The values of apparent viscosity of the samples ME1–ME3, 48 h, 30 and 90 days after preparation are given in Table 3.

The rheological characteristics of the investigated W/O/W emulsions were markedly influenced by the concentration of lipophilic emulsifier. The highest apparent viscosity was observed for W/O/W emulsion prepared with highest concentration of lipophilic emulsifier.

The observed trend towards the gradual decrease of apparent viscosities of the samples with aging, could be attributed to the coalescence of inner water droplets with the external

Table 2
Droplet size of the samples ME1–ME3 during aging time

Time	Droplet size (μm) ±S.D.		
	Sample ME1	Sample ME2	Sample ME3
48 h	11.2 (2.85)[a]	9.0 (2.27)	5.1 (1.24)
30 days	10.0 (2.63)	7.8 (1.85)	5.2 (1.36)
90 days	9.9 (2.74)	7.8 (2.06)	5.4 (1.42)

[a] $n = 500$.

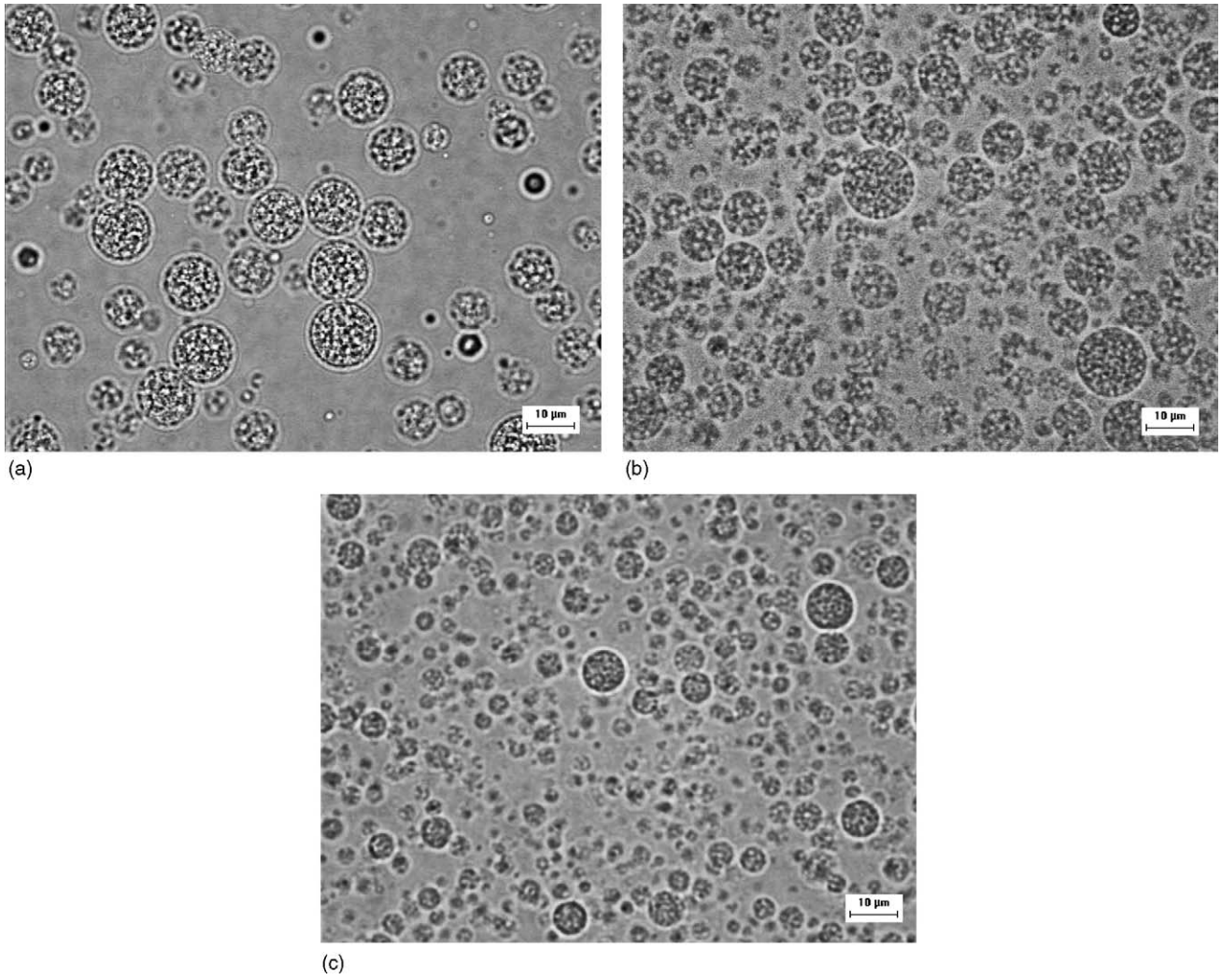

Fig. 1. Photomicrographs of multiple emulsions, 48 h after preparation (magnification 1000×): (a) ME1, (b) ME2, (c) ME3.

water phase as discussed by Jiao and Burgess (2003). The subsequent increase in the volume of the external water phase of W/O/W emulsions may lead to the decrease of apparent viscosity.

The storage modulus G' and loss angle δ provide a quantitative characterization of the balance between the viscous and elastic properties of the multiple emulsion. The loss angle δ is a very precise indicator of this balance. The lower the δ value, the more pronounced the elastic character, and vice versa (Grossiord and Seiller, 1998).

The values of the basic viscoelastic parameters of the investigated samples are given in Table 3.

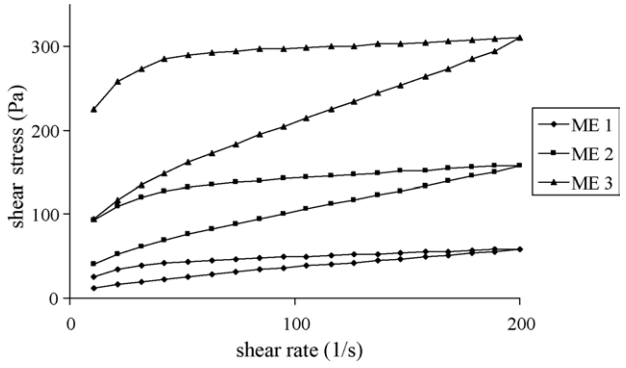

Fig. 2. Flow curves of the multiple emulsion samples, 48 h after preparation.

Table 3
Apparent viscosity (η_{app}), storage modulus (G'), loss modulus (G'') and loss angle (°) of the samples ME1–ME3 during ageing time (at 1 Hz)

Time	η_{app} (Pas) (at 10.5 s^{-1})	G' (Pa)	G'' (Pa)	δ (°)
Sample ME1				
48 h	2.5 (0.17)[a]	204 (11.1)	172 (22.3)	40.1 (2.27)
30 days	2.4 (0.005)	199 (36.4)	178 (53.3)	41.8 (1.76)
90 days	2.3 (0.05)	198 (34.0)	182 (27.8)	42.6 (2.52)
Sample ME2				
48 h	8.8 (0.77)	447 (74.1)	219 (22.3)	26.1 (1.76)
30 days	8.5 (0.55)	412 (45.1)	212 (19.4)	27.2 (1.70)
90 days	7.6 (0.57)	447 (46.7)	247 (43.4)	28.9 (0.71)
Sample ME3				
48 h	21.5 (0.23)	1060 (40.4)	384 (26.1)	19.9 (1.19)
30 days	19.7 (0.62)	1220 (21.2)	435 (24.0)	19.6 (1.34)
90 days	18.2 (0.43)	1120 (101.7)	381 (46.7)	18.8 (0.49)

[a] ±S.D. ($n = 3$).

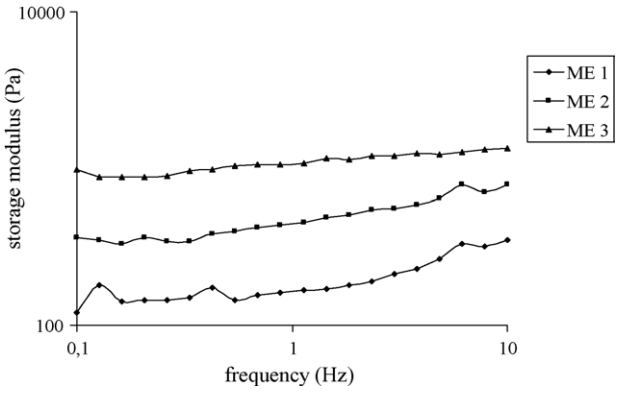

Fig. 3. Storage moduli (G') of the multiple emulsion samples, 48 h after preparation.

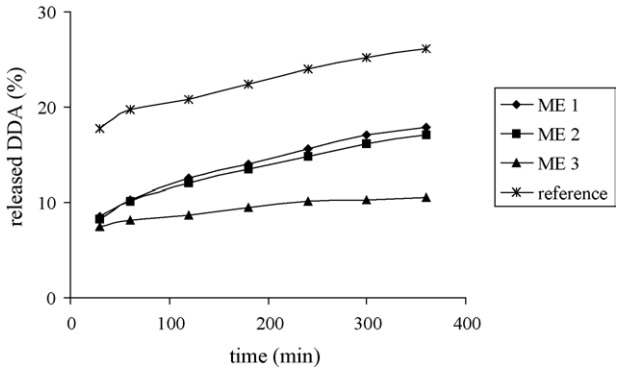

Fig. 4. DDA release profiles for ME1–ME3 and reference.

In Fig. 3, storage moduli values (G') of multiple emulsion systems are presented as a function the frequency, 48 h after the preparation.

The concentration of the primary emulsifier exhibited significant influence on the oscillatory parameters of the investigated samples.

At 0.8% (w/w) PDHS, the formulation exhibited low viscoelasticity. However, at 2.4% (w/w) the formulation was highly viscoelastic or "solidlike", as indicated by considerably higher G' values and lower δ values.

The observed increase in the storage modulus values with an increase in the concentration of the primary emulsifier was accompanied by decrease in the mean droplet diameter. It was reported that, as the diameter of the multiple emulsion droplets becomes smaller, the number of contact points between them increases leading to the increase in storage modulus values (Tadros et al., 1995).

Certain decrease in the storage modulus values with ageing were observed in the case of samples ME1 and ME2. This reduction could be explained by the expulsion of some of the primary emulsion water droplets from the multiple emulsion drops into the continuous medium, which results in a decrease in the volume fraction of the multiple emulsion and a reduction in the modulus value. It was recognized that any small change in the volume fraction may cause a large change in the modulus value (Tadros et al., 1995).

In contrast, sample ME3 exhibited certain increase in elasticity during ageing time. In this case it appears that during time, water may penetrate from the outer water phase to the inner one by virtue osmotic pressure difference, following the droplet swelling. This slight swelling leads to an increase in elasticity of the system.

3.4. In vitro drug release study

DDA release profiles from investigated multiple emulsions and reference preparation are shown in Fig. 4. Drug release kinetics were almost superimposable in the case of samples ME1 and ME2, while more sustained drug release occurred from the sample ME3. The cumulative percent of drug released after 6 h was 17.89 ± 0.41, 17.09 ± 0.49, 10.58 ± 0.13 and 26.12 ± 0.63 for samples ME1–ME3 and reference preparation, respectively.

Parameters describing drug release kinetics from investigated samples are presented in Table 4.

Linear relationship between amount of drug released and square root of time, characterized with the regression coefficients higher than 0.99, was obtained for each formulation. The obtained results indicate that DDA release from investigated formulations could be described by the diffusional model, and, that rate-controlling step in the release process is diffusion of the dissolved drug through the vehicle (Higuchi, 1962). Analogous results, describing diffusional release as the dominating drug release mechanism from W/O/W emulsion systems, were also reported from the studies of Magdassi and Garti (1986) and Laugel et al. (1996).

The values of diffusion coefficients calculated from the entire dissolution profiles (D, F) did not correlate well with percentage of drug released. The obtained diffusion coefficients for ME1 and ME2 were close to that obtained for reference preparation. However, data calculated from the initial segment of the dissolution curves where distinct differences among the obtained profiles can be observed ($D_{60\,\mathrm{min}}$, $F_{60\,\mathrm{min}}$) were better indicator of the release behaviour of the investigated preparations. The obtained values of diffusion coefficients indicate that highest drug release rate was achieved in the case of Voltaren®

Table 4
Drug release characteristics of the samples ME1–ME3 and reference preparation

Sample	Percent released per 6 h, $Q_{6\,\mathrm{h}} \pm$ SD (%)	r_H	Diffusion coefficient, D (cm^2/s)	Diffusion coefficient for 60 min, $D_{60\,\mathrm{min}}$ (cm^2/s)	Flux, F (mg/cm^2 s)	Flux for 60 min, $F_{60\,\mathrm{min}}$ ($\times 10^{-4}$ mg/cm^2 s)
ME1	17.89 ± 0.41	0.9978	1.59×10^{-7}	4.48×10^{-7}	2.17×10^{-5}	1.41
ME2	17.09 ± 0.49	0.9989	1.39×10^{-7}	3.97×10^{-7}	2.02×10^{-5}	1.33
ME3	10.58 ± 0.13	0.9951	1.95×10^{-8}	2.63×10^{-7}	7.51×10^{-6}	1.06
Voltaren® Emulgel®	26.12 ± 0.63	0.9953	1.26×10^{-7}	1.56×10^{-6}	1.93×10^{-5}	2.59

Emulgel®, while in the case of sample ME3 sustained drug release was accomplished.

Literature data suggests that in multiple emulsion systems, the drug is available for absorption after a two step partitioning phenomena (Nakhare and Vyas, 1994). The first partitioning is of the drug between the internal water phase and the middle oily phase and the second occurs between the middle oily phase and the outer water phase. In the case of investigated multiple emulsion systems, certain lag time corresponding to the time required for partitioning through the complex barriers can be observed when compared to the reference preparation. DDA release rate from the investigated samples was affected by the concentration of the lipophilic emulsifier applied. Higher PDHS concentration led to a more sustained drug release. The above observations may be additionally affected by the viscosity of the sample. Sample ME3 exhibited the highest viscosity, and in this case the diffusion of the drug may be suppressed.

The concentration gradient caused by all the dissolved species is responsible for water flow from the external to the internal phase. This aqueous transport produces an increase of the internal microglobule size, and therefore the oil globules swell until a critical size is reached (Geiger et al., 1998). According to authors (Jager-Lezer et al., 1997; Geiger et al., 1998) that the more lipophilic surfactant is increases, the more the oil globule swelling capacity is increases, and the more the release is delayed. In order to explain the influence of the lipophilic surfactant concentration on the swelling of the oil globule, two different mechanisms have been proposed. The first one consists in an increase of the rigidity of the second interface by the progressive migration of the lipophilic surfactant. During the second step of multiple emulsion preparation, lipophlic surfactant molecules can diffuse from the first to second interface, were they produce a synergistic effect resulting in membrane strengthening. The second one involves a delay in the in the aqueous droplet coalescence. In course of swelling of the oil globule, the lipophilic surfactant molecules, which are in excess in oily phase, can diffuse to the first interface to fill up free spaces caused by swelling, when required.

The results of this study indicate that sample ME3 exhibited the highest swelling capacity of the oil globule which lead to a more sustained drug release.

However, it should be considered that DDA, as an amphiphlic substance, may play the same role as a cosurfactant; in this case, it could also rigidify the interface, thus further suppressing the diffusion process. Such interaction is probably more pronounced at the higher content of lipophilic emulsifier, as drug release profiles obtained in the case of samples ME1 and ME2 were almost superimposable.

These results show that by changing the PDHS concentration, it is possible to modulate and to prolong the release of diclofenac diethylamine.

4. Conclusion

In this study, W/O/W multiple emulsion systems containing low concentration levels of lipophilic polymeric primary emulsifier PEG 30-dipolyhydroxystearate and diclofenac diethyamine as a model drug were evaluated. The prepared sample formulations were characterized and their stability over time evaluated by dynamic and oscillatory rheological measurements and microscopic analysis. In vitro release profiles of the selected model drug were evaluated in terms of the effective diffusion coefficients and flux of the released drug. The multiple emulsion samples exhibited good stability during the ageing time. Concentration of the lipophilic primary emulsifier markedly affected rheological behaviour as well as the droplet size and in vitro drug release kinetics of the investigated systems. The multiple emulsion systems with highest concentration of the primary emulsifier had the lowest droplet size and the highest apparent viscosity and highest elastic characteristics. Drug release data indicated predominately diffusional drug release mechanism with sustained and prolonged drug release accomplished with 2.4% (w/w) of lipophilic emulsifier employed.

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