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The Physicochemical Characterization and In Vitro/In Vivo Evaluation of Natural Surfactants-based Emulsions as Vehicles for Diclofenac Diethylamine

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ABSTRACT Two sugar-based emulsifiers, cetearyl alcohol & cetearyl glycoside and sorbitan stearate & sucrose cocoate, known as potential promoters of lamellar liquid crystals/gel phases, were investigated in order to formulate an optimal vehicle for amphiphilic drug–diclofenac diethylamine (DDA).

Physico-chemical characterization and study of vehicle's physical stability were performed. Then, the in vitro DDA liberation profile, dependent on the mode of drug incorporation to the system, and the in vivo, short-term effects of chosen samples on skin parameters were examined.

Droplets size distribution and rheological behavior indicated satisfying physical stability of both types of vehicles. Unexpectedly, the manner of DDA incorporation to the system had no significant influence on DDA release. In vivo study pointed to emulsion's favorable potential for skin hydration and barrier improvement, particularly in cetearyl glycoside-based vehicle.

KEYWORDS Liquid-crystalline gel network, Cetearyl alcohol & cetearyl glycoside, Sorbitan stearate & sucrose cocoate, Diclophenac diethylamine release, Rheology, Droplet size analysis

INTRODUCTION

To be in class of naturals, an emulsifier should be biodegradable, free of ethylene-oxide and not tested on animals (Dedern et al., 2001). The sugar-based emulsifiers become an emerging class of surfactants, which can form both, the thermotropic and the lyotropic liquid-crystalline phases (Dedern et al., 2001; Rybinski, 2001; Müller-Goymann, 2002). Beside the others, cetearyl alcohol & cetearyl glycoside (Montanov™ 68-Seppic, France) (Seppic, 2001) and sorbitan stearate & sucrose cocoate (Arlatone® 2121-ICI Surfactants, ICI Surfactants, USA, 1996), recognized as surfactants in research and applicative focus, are the oil/water (o/w) emulsifiers which could build lamellar mesophases around the oil droplets and lamellar liquid-crystalline/gel structures through the continuous phase (Loll, 1993, Savic et al., 2005).

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The mesomorphic behavior of sugar-based emulsifiers influences the properties of emulsion systems important for dermopharmaceutical use (physical stability, water distribution mode, and rheological performance), either as carriers for active substances or vehicles for enhanced stratum corneum (SC) moisturization (Savic et al., 2005). Colloid structure of these emulsion systems, according to Junginger (1997), is featured by the appearance of four main phases: (1) dispersed oil phase, (2) crystalline gel phase where the double lamellar layers are separated by the layer of so-called interlamellar or "bound"/fixed water, (3) crystalline hydrates of amphiphiles, which show limited swelling and frequently are called as "coagel" phase, and (4) pockets of bulk or "free" water. These systems contain differently bound water, designating them as systems for prolonged/controlled skin hydration (Erős et al., 1997; Savic et al., 2005). Furthermore, similarity of these emulsion structure with SC intercellular lipid organization is their advantage in use to the skin, particularly in term of potential penetration enhancer effect (Hadgraft, 2001; Norlén, 2003).

In fact, interlamellarly fixed water, present in topical emulsions stabilized by lamellar gel-crystalline and/or lamellar liquid-crystalline phases, may serve as formulation reservoir ("depot") for controlled skin hydration (Junginger, 1997). Besides the putative effects of other formulation ingredients on SC structure, prolonged skin hydration contributes to the penetration enhancer effect of a vehicle (Fluhr, Lazzerini et al., 1999; Zhai & Maibach, 2001; Savic et al., 2004; Levin & Maibach, 2005). Actually, water is commonly called as the most natural penetration enhancer (Roberts & Walker, 1993). Moreover, liquid-crystalline structures may influence the drug's solubility and chemical potential in both the vehicle and the SC, as well as its diffusion rate through the vehicle. In fact, liquid-crystalline vehicles could interact with the skin, i.e. SC (Műller-Goymann & Frank, 1986; Farkas et al., 2000, 2001; Makai et al., 2003; Savic et al., 2005).

Therefore, it was of interest to develop an optimal formulation of emulsion vehicle based on sugar type emulsifier, for an amphiphilic nonsteroidal anti-inflammatory drug–diclofenac diethylamine (DDA). Considering DDA is often indicated in therapy of pain in muscles and joints, for the reason of its therapeutic effect, it is very important to optimize release of DDA from the vehicle and to investigate the vehicle impact on the skin hydration and barrier function. The latter is of importance in term of potential enhancing the DDA skin permeation profile.

Consistently, the purpose of this study was to examine the correlation between colloidal microstructure of vehicle, presumably with structure of liquid-crystalline gel network (Savic et al., 2005), and in vitro DDA liberation profile regarding to the place of drug incorporation within the system (100% in water phase vs. 100% in oil phase vs. 50:50 in oil–water phase), as well as to evaluate the physical stability of the vehicles through the rheological and droplet size measurements. Two skin parameters (hydration level and barrier function–transepidermal water loss; TEWL) can improve DDA skin permeation. Our aim was to test the vehicle impact on these two skin parameters, by performing the in vivo short-term application study.

MATERIALS AND METHODS

Materials

The emulsifiers used for preparing of semisolids were sorbitan stearate & sucrose cocoate (Arlatone®2121-ICI Surfactants, USA) and cetearyl alcohol & cetearyl glycoside (Montanov™ 68-Seppic, France) in concentrations of 7% and 10% (w/w), respectively, according to producer's recommendation. Medium-chain triglycerides (Caprylic/Capric triglycerides, Mygliol® 812, Dinamit Nobel, Germany) were used as oil phase and purified water preserved with 0.2% (w/w) methylparaben (Nipagin, NIPA, SAD). DDA (gift from Hemofarm ad., Vrsac, Serbia) was incorporated in concentration of 1.16% (w/w). The composition of placebo emulsions and emulsions with DDA is listed in Table 1.

Preparation of Emulsions

All samples were prepared using the same procedure. According to producer's designation (which consists of water phase with emulsifier and oily phase, both heated to 75°C) (ICI Surfactants, 1996; Seppic, 2001) to design poliethylenglycol-free based emulsions with liquid-crystalline gel network successfully, two main requests should be fulfilled: preliminary emulsifier swelling in cold water phase, in duration of 30 min, followed with gradual heating of emulsifier's

TABLE 1 **Composition of Emulsions**

	Sample % (w/w)							
Composition	SA	SA/I	SA/II	SA/III	SM	SM/I	SM/II	SM/III
Arlatone®2121	7.00	7.00	7.00	7.00	–	–	–	–
Montanov™68	–	–	–	–	10.00	10.00	10.00	10.00
Mygliol®812	10.00	10.00	8.84 + 1.16 DDA	9.42 + 0.58 DDA	40.00	40.00	38.84 + 1.16 DDA	39.42 + 0.58 DDA
Methylparaben	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Purified water	82.80	81.64 + 1.16 DDA	82.80	82.22 + 0.58 DDA	49.80	48.64 + 1.16 DDA	49.80	49.22 + 0.58 DDA

water dispersion till 75°C, simultaneously with oily phase. Then oily phase was added to the water dispersion at quite low speed (50 rpm) and then stirring was maintained for another 15 min at 500 rpm. Prepared emulsions were placed into appropriate glass containers with grinded closers and stored at room temperature (20 ± 1°C) for 72 hr (structuring period according to Tadros et al., 1994) and 6 months (storage time). Drug-loaded samples were prepared using the same procedure but in different order of DDA incorporation. DDA was added into water phase after emulsifier swelling and then heated to 75°C. In oily phase, DDA was placed before the heating started. Considering the different manner of DDA incorporation into base formulations, two series of samples were created, i.e., SA and SM series in following order:

- SA/I and SM/I (complete DDA dissolved in water phase)
- SA/II and SM/II (complete DDA dissolved in oil phase)
- SA/III and SM/III (50% of DDA dissolved in water and 50% in oil phase).

The initial rheological and microstructure characterization of the vehicles was performed for 72 hr upon the sample preparation and then after 6 months storage under the same conditions, whereas other investigations (DDA release and in vivo study) were performed with samples stored for 72 hr.

Conductivity Measurement

In order to obtain data about structural changes/physical stability of the vehicles, electrical conductivity measurements were performed directly in prepared samples at aforementioned time intervals using conductometer CDM 230 (Radiometer, Copenhagen, Denmark).

Droplet Size Analysis

Samples were examined microscopically in bright field (ordinary light microscope/OLM) and between crossed polarizers (polarized light microscope/PLM) using the photomicroscope (ORTHOMATE E, Laitz, Germany), integrated with software package Image Analysis 4.0. The droplet size was determined by microscopic analysis of undiluted emulsions counting the 500 randomly chosen droplets per sample. Each measurement was done in triplicate and results are presented as mean ± standard deviation (SD).

Rheological Measurements

Rheological behavior was investigated using controlled stress/rate rheometer (Rheolab MC 120, Paar Physica, Stuttgart, Germany) coupled with the cone and plate measuring device MK 22 (radius 40 mm, 1° angle) for continual and MK 24 (radius 40 mm, 1° angle) for oscillatory measurements. All measurements were performed with sample thickness of 0.050 mm at 20 ± 0.1°C and repeated three times.

During continual testing, controlled shear rate procedure was applied (shear rate from 0 to 200 s^{-1} and back to the starting point, each stage lasting 120 s). Flow curves, minimal and maximal apparent viscosities and yield stress values were used as comprehensive denominators for the samples characterization, particularly in term of their physical stability.

Oscillatory (dynamic) measurements were conducted in order to determine linear viscoelastic region of the samples (amplitude sweep) at constant frequency of 1 Hz and amplitude sweep ramp from 0.6 to 100%. A frequency sweep ramp from 0.1–10 Hz was performed at the constant strain of 10%, which was within the previously marked linear viscoelastic region for all samples. Storage-elastic (G′) and loss-viscous (G″) modulus as well as the damping factor (tanσ) were used for analysis of the structural properties of emulsion systems.

In Vitro DDA Release Study

DDA liberation profiles from emulsion systems based on two different sugar emulsifiers distinguished also by place of drug incorporation, were evaluated using the rotation paddle apparatus (Erweka DT70, Hausenstamm, Germany), modified by addition of diffusion cells (Enhancer cell, VanKel Industries Inc., Edison, USA). The cell was filled with the sample (2 g) and covered with regenerated cellulose membrane (Cuprophan®, Akzo, Wuppertal, Germany), capped and then placed to the dissolution vessel containing the receptor medium (pH 7.4 phosphate buffer). Constant paddle rotation speed of 100 rpm and receiver phase temperature of 32°C were maintained through entire experiment. At fixed time intervals (30, 60, 120,

180, 240, 300, and 360 min), 4 ml aliquots were withdrawn from the acceptor compartment and replaced with fresh buffer, thus the sink conditions were retained at all times. Samples were filtered using 0.45 μm MF-Millipore® membrane filter (Milipore Corporation, Bedford, Massachusetts, USA) and assayed for DDA (spectrophotometrically at 275.1 nm, Spectrophotometer Cary 50, Varian, Darmstadt, Germany). The in vitro release study was performed for each sample in triplicate and data were expressed as mean ± SD.

In Vivo Performance Study

The skin hydration and barrier-function dependence on short-term treatment with both placebo (SA and SM) and active samples SA/II and SM/II (chosen from previous experiment) were tested within this study.

Ten healthy volunteers (mean age: 25.6 ± 3.6) without any signs of skin diseases were involved in the experiment. The flexor sides of both forearms were treated with four samples using precisely delineated and marked cardboard ruler (with three empty spaces in the form of rectangles, each being 9 cm^2). Samples SA and SA/II (in quantity of 2 mg/cm^2) were applied at the left, and SM and SM/II at the right forearm. At each forearm one rectangle was left as an untreated control.

Two parameters were measured: electrical capacitance implying the hydration level of the outer epidermis (expressed in relative corneometer units, rcu) (Corneometer® CM 825, Courage-Khazaka, Germany) and TEWL as indicator of skin barrier integrity (Tewameter® TM 210, Courage-Khazaka, Germany). Skin evaluation was performed after adaptation of participants to room conditions (30 min at 21 ± 1°C and 45 ± 5% RH), and then baseline values of both parameters were taken for each treated site. Measurements were repeated 30, 60, 120, and 180 min upon the application of the samples. The following order of measurements was performed: TEWL and then electrical capacitance at each site. All measurements were carried out according to the relevant guidelines (Pinagoda et al., 1990; Berardesca, 1997; Rogiers, 2001) and performed in triplicates.

Statistical Analysis

Whenever applicable, data are given as mean ± standard deviation (SD).

In vitro drug release data were compared by Student t-test for independent samples. In vivo effects (TEWL, skin hydration) of placebo (SA and SM) and chosen active samples (SA/II and SM/II) at distinct time points were analyzed by the one-way subjects (repeated measures) ANOVA, followed by Tukey's *t*-test, where appropriate, comparing them mutually and related to the untreated controls. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Presented PLM micrographs (Fig. 1) taken 72 hr after sample preparation, revealed an anisotropic texture within both type of emulsions independent on emulsifier used. Namely, liquid lamellar phase is characterised by mosaic texture, oily streaks and Maltese crosses (Tadros et al., 1994; Fairhust et al., 1998; Eccleston et al., 2000; Müller-Goymann, 2004). In this case, structures so-called distorted Maltese crosses were recorded, implicating lamellar phase, too (Eccleston et al., 2000). According to Savic et al. (2005) in model cream based on 7% cetearyl alcohol & cetearyl glycoside with 20% of medium chain triglycerides as oil phase, the equilibrium between liquid lamellar and lamellar gel-crystalline phases was approved using, beside a number of the other research techniques, PLM, OLM and rheological measurements as well. However, a clear difference is noticed regarding to anisotropic structures attributes in both polarized and ordinary light micrographs (Figs. 1 and 2). In sample with disaccharide mixed emulsifier (SA), thinner anisotropic layers are present at the edges of oil droplets and, at the same time, it is visible that gel structure formed within continual phase of the system is somehow less developed in sample SA, compared to that of sample SM. This finding may be the result of fact that emulsifier concentration in sample SA is lower than in sample SM (7% vs. 10%), as well as the oil content (10% vs. 40%). It should be emphasized that such formulation approach arose from the results of physical stability preliminary investigations. It could be that hydration and swelling capacity of sucrose cocoate mixed emulsifier is more expressed than in monosaccharide emulsifier, at least because of higher percentage of water phase in the sample SA and also as result of numerous hydroxyl groups within disaccharide.

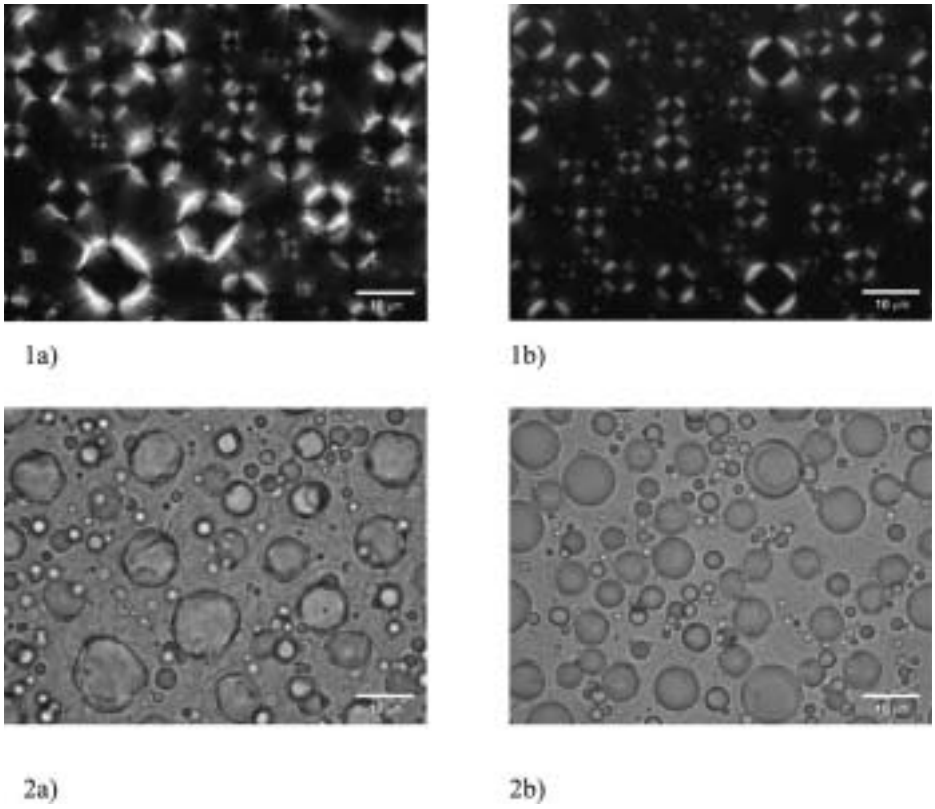

FIGURE 1 **Micrographs of o/w Emulsion Systems, 72 hr after Preparation: (1a) PLM and (1b) OLM—Sorbitan Stearate & Sucrose Cocoate (Arlatone® 2121); (2a) PLM and (2b) OLM-Cetearyl Alcohol & Cetearyl Glucoside (Montanov™ 68); bar 10 μm.**

Otherwise, this type of colloid structure featured by anisotropic droplets ("onion rings") uniformly dispersed into the continuous phase, together with the remnants of the gel network, surrounding larger and floccules of smaller oil drops is typical for the semisolids stabilized with traditional ionic or nonionic mixed emulsifiers, so called emulsifying waxes (Eccleston et al., 2000). It seems that in case of both sugar emulsifiers similar type of interaction occurs, like in the case of cetrimide/cetostearyl alcohol or polyoxyethylene alkyl ether surfactants/cetostearyl alcohol mixed emulsifiers (Eccleston et al., 2000). Accordingly, it sounds reasonable that in investigated emulsion systems, oil droplets act as a focus for multilayers of gel phase, which become more randomly oriented as they progress into the continuous phase (Eccleston et al., 2000; Savic et al., 2005). Thus, these systems are stabilized by synergistic effect of lamellar liquid crystals (L_α), present at the oil droplets border and lamellar α-gel-crystalline phase (L_β), predominantly existed within continuous compartment. Obtained creams were shiny, white semisolids, with consistency corresponding to different contents of emulsifiers and oil within formulations. In fact, consistency in this kind of emulsion systems is controlled more by strength of the lamellar gel-crystalline structure, than by density of droplets packaging (Eccleston et al., 2000; Savic et al., 2005). Consistently, from OLM micrographs (Figs. 1 and 2) it is visible that gel structure dominates within continual phase of sample, based on alkylpolyglucoside mixed emulsifier (SM), which is in accordance to the increased consistency of this sample comparing to sample SA.

This observation was substantiated by electrical conductivity and rheological measurements as well as by determination of average droplet size.

Namely, it is well established that complex gel-crystalline matrix prevails in stabilization of multiphase o/w emulsions, based on either ionic or nonionic mixed emulsifiers regarding to the significance of droplets size and density of their packaging (Junginger, 1997; Eccleston, 1997, 2001; Eccleston et al., 2000). Furthermore, Eccleston (1997, 2001, 2000) underlines that α-crystalline lamellar gel phase is dominant for stabilization compared to the multilayer of lamellar liquid-crystals, placed around the oil droplets. Nevertheless, there are

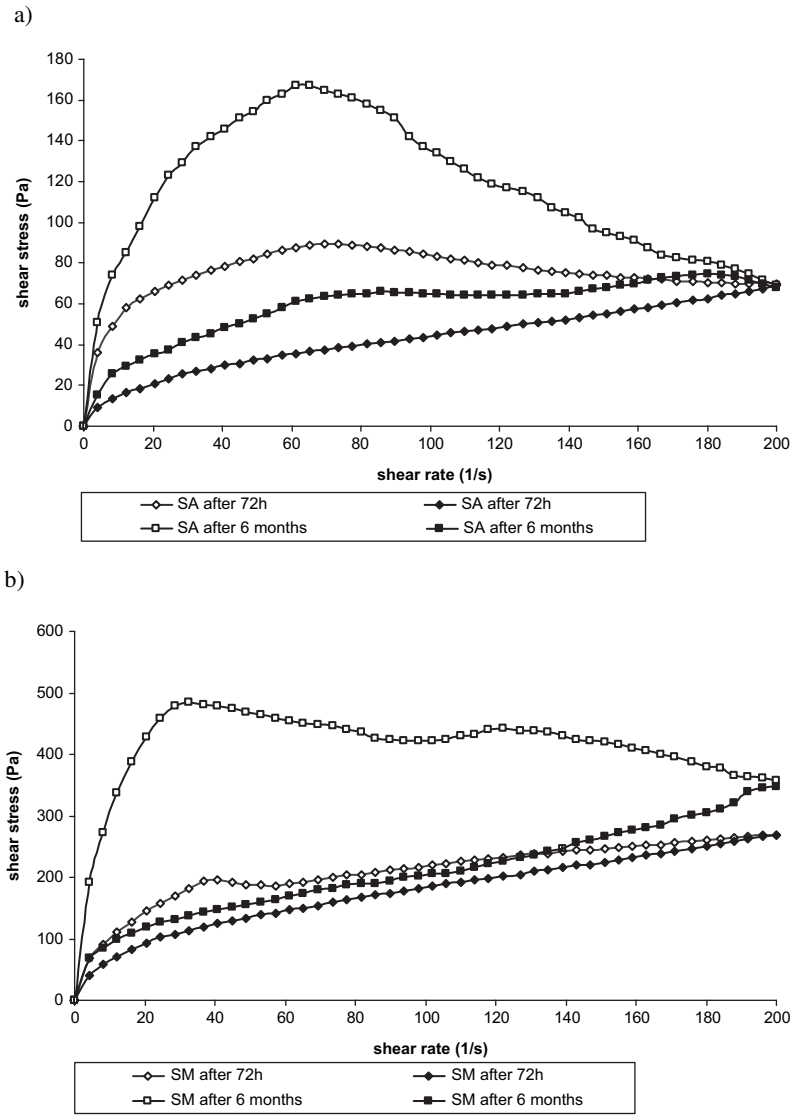

FIGURE 2 **Rheograms of Cream (a) SA and (b) SM, 72 hr and 6 Months after Preparation; (Samples Stored at Room Temperature).**

different literature data showing the necessity of measuring the droplets size and droplets size distribution, particularly in term of physical stability examination (Tadros et al., 1994; Korhonen et al., 2000, 2002).

The average droplet size in test emulsions (Table 2), confirms impression obtained after insight to the micrographs (not shown), bringing the conclusion on certain diminution of drops after 6 months of storage at room temperature. However, this change was not statistically significant ($p > 0.05$, Table 2). A different finding is imposed upon the droplets size distribution have been analyzed, dependent on storage time. A distinct increase ($p < 0.05$) of smaller droplets ratio was noticed in cream SA, where, after 6 months storage, percentage of the smallest drops changed from 49.32% (72 hr after preparation) to 92.9%. On the other side, a moderate, but insignificant increase of the smallest droplets percentage (from 84.16% to 92.12%) was recorded in cream SM. This is a different comparing

TABLE 2 **Average Droplet Size in Emulsion Vehicles SA and SM, 72 hr and 6 Months after Preparation**

	Average droplet size (μm)	
Sample	72 hr	6 months
SA	3.17 ± 0.42	2.15 ± 0.43
SM	2.90 ± 0.29	2.34 ± 0.54

to classic emulsions where oil droplets could only become larger during storage because of flocculation and coalescence. Mandani & Friberg (1971) and then Cook (1997), explained that probably extremely low interfacial tension between the oil and water phase, existing in emulsions with liquid-crystalline gel network, may provoke a decrease in droplet sizes followed by more homogeneous distribution. According to some authors (Krog, 1997; Georgalas, 2001), until the droplets have been incorporated into liquid-crystalline gel network, they are protected from coalescence mostly thank to viscoelastic properties of gel matrix that minimize Van der Waals attractive forces. Studies conducted by Tadros (1991) and Tadros et al. (1994) also showed that because of aforementioned, almost negligible interfacial tension in multiphase emulsions, oil droplets continue to be smaller after process of emulsification. This process is the most intensive during the period of emulsion structuring and then goes on over the period of storage until the droplet size is not highly uniformed (Tadros, 1991; Tadros et al., 1994).

The process of multiphase emulsion additional structuring also includes migration of bulk/free water to the interlamellar spaces (Junginger, 1997; Eccleston, 1997, 2001; Eccleston et al., 2000; Savic et al., 2005). This was confirmed by the results of electrical conductivity measurement (Table 3).

TABLE 3 **Electrical Conductivity of Vehicles SA and SM, 72 hr and 6 Months after Preparation**

	Electrical conductivity (μs/cm)	
Sample	72 hr	6 months
SA	66.39	48.59
SM	8.29	4.32

The clear decrease of electrical conductivities, which occurred in both samples (SA and SM) during the storage, is distinct indicator of two simultaneous processes happening during the emulsions structuring: an increase of the water content between lamellae either in gel-crystalline or in liquid-crystalline state, and an increase of apparent viscosities. It is known that conductivity is a measure of amount of free water and free ions within the system (Eccleston et al., 2000; Korhonen et al., 2000, 2002). Moreover, high conductivity values reveal less lamellar/fixed water and more free water within the vehicle, reflecting its consistency decrease (Korhonen et al., 2000, 2002). Hence, it could be expected that formulation SM may be with higher content of "depot"/reservoir water and more pronounced potential for controlled/prolonged skin hydration. A marked difference in initial conductivities in creams SA and SM (Table 3) points at higher ratio of free/bulk water within system based on disaccharide mixed emulsifier and presumably the different colloid structure of two different formulations.

Both samples (SA and SM) exhibited the "shear-thinning" pseudoplastic flow behavior (Table 4, Fig. 3a,b), with pronounced thixotropy, but as it was expected with marked differences in yield values and apparent viscosities between these two formulations. Two-fold higher yield value in sample SM, compared with that of sample SA, and similar trend with viscosities (Table 4) is a sign of α-crystalline gel phase predominance within the colloid structure of the system and also correlates well with conductivity values decrease and previously described change of droplets size distribution. On the other side, a relatively uniform increase of rheological parameters dependent on storage, generally, point at acceptable physical stability of the samples (Savic et al., 2005), although significant enlargement of critical point and apparent viscosities

TABLE 4 **Flow Parameters of Emulsion System without DDA (SA and SM), 72 hr and 6 Months after Preparation, and with DDA (SA/II and SM/II), 72 hr after Preparation**

			Apparent viscosity (Pas)	
Sample	Yield stress value (Pa)	Time	Min	Max
SA	72 hr	89.10 ± 1.54	0.35 ± 0.01	8.87 ± 0.08
	6 months	167.00 ± 1.73	0.34 ± 0.01	7.05 ± 0.10
SA/II	72 hr	79.13 ± 1.16	0.28 ± 0.01	8.39 ± 0.30
SM	72 hr	195.00 ± 3.61	1.65 ± 0.10	17.30 ± 0.42
	6 months	485.00 ± 10.58	1.78 ± 0.13	46.70 ± 0.80
SM/II	72 hr	169.00 ± 12.80	1.34 ± 0.07	11.30 ± 1.72

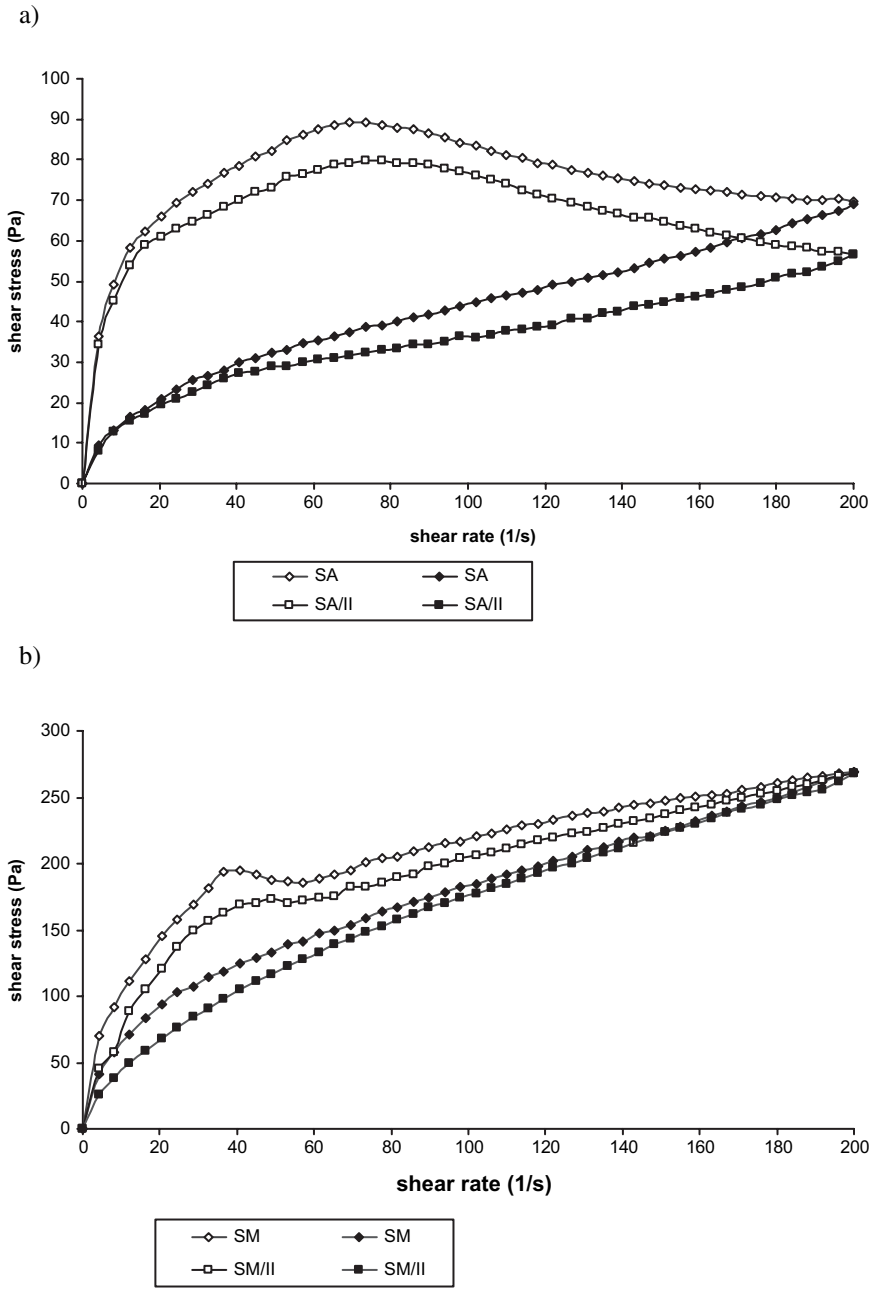

FIGURE 3 **Rheograms of Cream with and without DDA. (a) SA (without DDA) and SA/II (with DDA), (b) SM (without DDA) and SM/II (with DDA).**

could be an indicator for potentially unstable structure (Korhonen et al., 2000, 2002).

According to literature, the main reason for such increase of rheological parameters during the storage might be in additional hydration and swelling not only of hydrophilic amphiphiles (sucrose cocoate, i.e., cetearyl glucoside), but also, of lipophilic parts of the mixed emulsifiers (sorbitan stearate, i.e., cetearyl alcohol) (Tadros, 1994; Eccleston, 1997, 2001; Eccleston et al., 2000). Generally, it was suggested for Montanov^{TM} 68 that stabilization of the glucolipid nonionic gel phase is, essentially, attributed to the hydrogen bonding of water to the monosaccharide hydroxyl moieties, causing the specific conformation, and could be assigned like steric hindrance. This gel phase gives the structured continuous phase with increased viscosity, thus contributing to the oil droplets immobilization and both, the flocculation and coalescence are inhibited

(Eccleston et al., 2000; Savic et al., 2005). The similar interactions and mechanism of stabilization may be expected in sample SA, based on disaccharide derivative–sucrose cocoate. However, it is known that mixture of fatty alcohols, particularly in presence of hydrophilic surfactants, has a high capacity of hydration and swelling (Eccleston et al., 2000). In such way, new portions of free water appeared to be incorporated between amphiphile bilayers, producing the increase of lamellae thickness and consequently, the apparent viscosities, too (Savic et al., 2005). As Montanov^{TM} 68 contains at least 42% of cetearyl alcohol (Anconturier & Amerlic Roso, 2002), marked yield value and minimal and maximal apparent viscosities reached in sample SM, could result not only from formulation factors variation (10% of emulsifier and 40% of oil phase) compared to sample SA, but also from pronounced mesomorphic behaviour of this emulsifier (Savic et al., 2005). This kind of behavior presumably contributes to the strength of both part of the complex gel matrix, the hydrophilic and the lipophilic one, reflected in clear differences between viscoelastic parameters of two formulations, particularly after 6 months storage.

Continuous rheological measurements of emulsions with DDA (samples SA/II and SM/II) were done after the structuration period, in order to estimate the influence of DDA on physical stability of the vehicles (Fig. 3a,b). The results show that the samples maintained "shear-thining" pseudoplastic flow behavior with pronounced thixotropy (Figs. 3a,b). There is no significant distinction of yield stress values, minimal and maximal apparent viscosity in samples with DDA comparing with vehicles alone (Table 4). This can be the consequence of preserved predominant α-crystalline gel phase within the colloid structure of the system with DDA.

The results of oscillatory rheological measurement are depicted in Fig. 4 and Table 5.

Both samples (SA and SM) were with clear viscoelastic profiles, and according to values of damping factor (tanδ, Table 5), with predominant elastic over viscous component. Thus, in sample SA there is a trend of increase of both the elastic and viscous modulus in similar direction, leading to the insignificant changes of damping factor (Table 5). Namely, the elastic/viscous modulus ration altered a little from 2.32-fold at the beginning to the 2.54-fold after 6 months storage. At the same time, in sample SM this ratio moved

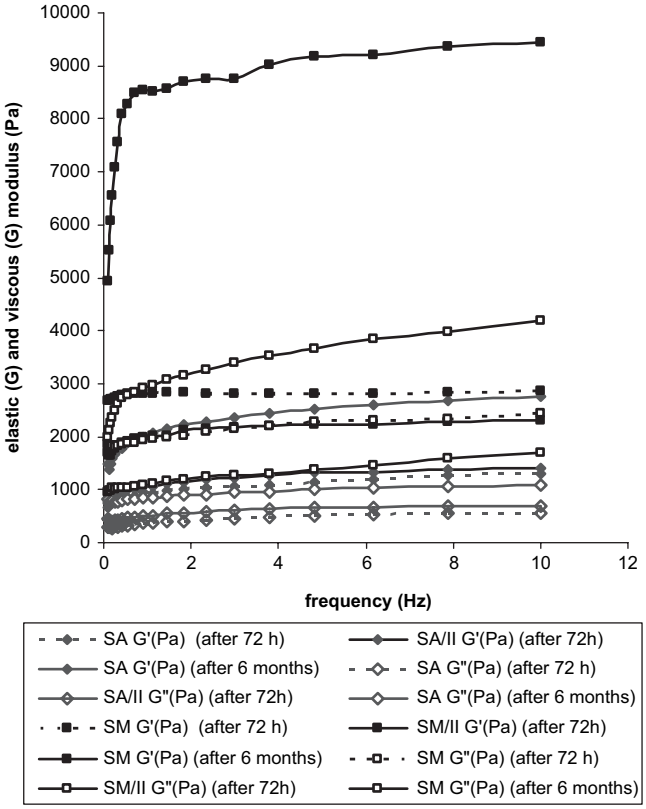

FIGURE 4 **The Viscoelastic Behavior of Samples with DDA (SA/II and SM/II) and without (SA and SM), 72 hr and 6 Months after Preparing (Stored at Room Temperature); the Elastic-Storage (G′) and Loss-Viscous Modulus (G″) Dependent on Frequency (Range 1–10 Hz).**

from 1.43 to 2.85-fold in favor of elastic component. These results are consistent with findings of continual rheological measurements (yield stress) and droplets size distribution, particularly for those obtained after the period of systems structuring. Furthermore, these results proved the previous supposition that α-crystalline gel phase is better developed in cream based on glucolipid emulsifier (sample SM), presumably because of higher swelling capacity of fatty alcohols (Eccleston et al., 2000; Savic et al., 2005). As it was described, such behavior provides the additional structuring of the system during storage, by entrapping the new amounts of water through the hydrogen bonding, which was more peculiar in sample SM. This assumption is supported by significant increase of elastic modulus and less pronounced alteration of viscous component (Fig. 4, Table 4). Consequently, it is expected from all these experiments (electrical conductivity, continual and oscillatory rheological measurements) and according to literature (Juginger, 1997;

TABLE 5 Oscillatory Parameters of Samples with DDA (SA/II and SM/II) and without DDA (SA and SM), at the Frequency of 1 Hz

Sample	Time	tanδ	G′ (Pa)	G″ (Pa)
SA	72 hr	0.39 ± 0.01	938.00 ± 13.08	369.00 ± 8.89
	6 months	0.41 ± 0.01	2060.00 ± 25.06	844.00 ± 23.81
SA/II	72 hr	0.48 ± 0.01	1060.00 ± 17.90	510.00 ± 18.40
SM	72 hr	0.69 ± 0.01	2820.00 ± 17.32	1960.00 ± 36.06
	6 months	0.35 ± 0.01	8520.00 ± 85.44	2980.00 ± 41.63
SM/II	72 hr	0.55 ± 0.01	2213.00 ± 66.40	1183.00 ± 20.80

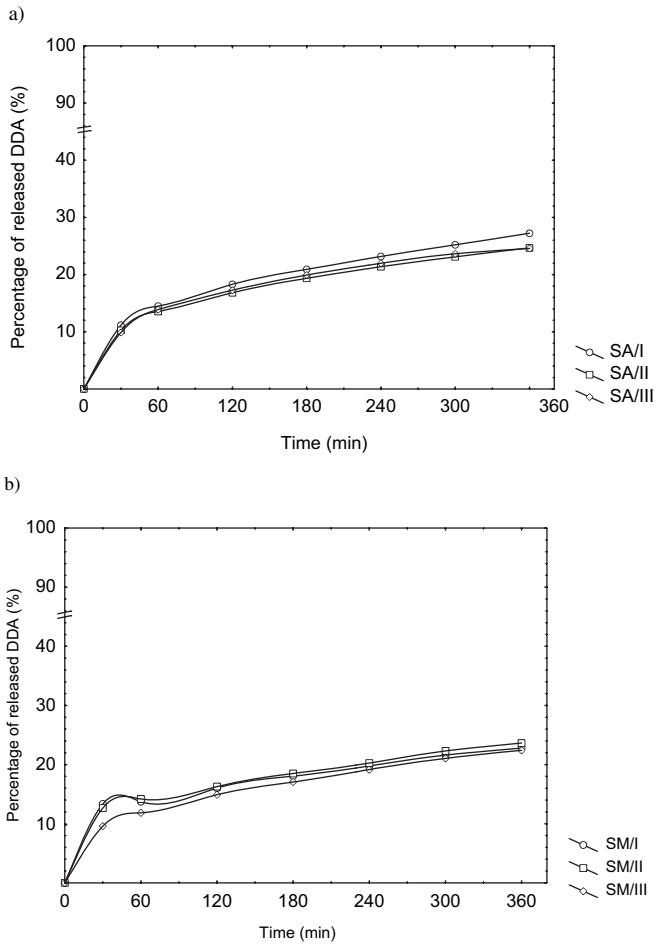

FIGURE 5 (a) Stratum Corneum (SC) Hydration after Single Application—Short-term Application Course (180 min), (b) TEWL Changes During 180 min after Application of Placebo (SA and SM) and Corresponding Active Samples (SA/II and SM/II); NCL—Nontreated Control Left and NCR—Nontreated Control Right.

Savic et al., 2005) that sample SM could dispose with relatively higher amount of "depot" water.

In order to estimate if the DDA influences the structural characteristics of examined vehicles, oscillatory parameters of emulsions with DDA (samples SA/II and SM/II) were determined. Samples of the vehicles with both emulsifiers and DDA maintain pseudoplastic behavior with thixothropy (Fig. 4, Table 5). When DDA was incorporated in vehicles with both types of emulsifiers, elastic characteristics were still predominant over viscosity. The systems remain within predominantly elastic area, confirmed by phase angle which is 28.8° for SM/II and 25.8° for SA/II.

TABLE 6 Parameters of DDA Releasing from Samples SA and SM Dependent on the Place of Drug Incorporation

Sample	Diffusion coefficient ($mg/cm^2/h$)	Releasing rate ($mg/cm^2/h^{1/2}$)	Cumulative amount (mg/cm^2)
SA/I	0.40 ± 0.33	0.53 ± 0.23	0.83 ± 0.42
SA/II	0.37 ± 0.30	0.49 ± 0.21	0.76 ± 0.38
SA/III	0.38 ± 0.30	0.50 ± 0.21	0.78 ± 0.40
SM/I	0.40 ± 0.40	0.49 ± 0.25	0.74 ± 0.34
SM/II	0.40 ± 0.37	0.50 ± 0.24	0.76 ± 0.35
SM/III	0.34 ± 0.28	0.44 ± 0.19	0.69 ± 0.35

The DDA release profiles from both samples are given in Fig. 5a,b. In addition, values of calculated diffusion coefficients and releasing rates are listed in Table 6, and correlation coefficients, obtained by mathematical fitting of DDA liberation profiles using different kinetic models, are presented in Table 7.

Although the differences in rheological profiles of vehicles stabilized with different emulsifiers were distinctive, there were no significant differences in DDA liberation profiles and corresponding parameters regarding to both the type of used emulsifier (sorbitan stearate & sucrose cocoate vs. cetearyl alcohol & cetearyl glycoside) and place of drug incorporation (Table 6, Fig. 6, $p < 0.05$). Namely, after 6 h, the highest DDA percentage was released from sample SA/I (27.23%), where drug was completely dissolved within water phase, whereas the lowest quantity of drug (22.42%) diffused from sample SM/III (drug equally incorporated into both phases of the system). Indeed, the sample SA/I stabilized with disaccharide mixed

TABLE 7 **Parameters of DAA Releasing Fitted by Different Kinetic Models**

Sample	Zero-order kinetic	First-order kinetic	Higuchi's model	Hixon-Crowell's model
SA/I	0.9105	0.9300	0.9885	0.9237
SA/II	0.9045	0.9226	0.9867	0.9167
SA/III	0.9081	0.9259	0.9883	0.9202
SM/I	0.8528	0.8698	0.9524	0.8626
SM/II	0.8669	0.8881	0.9642	0.8812
SM/III	0.9117	0.9278	0.9883	0.9226

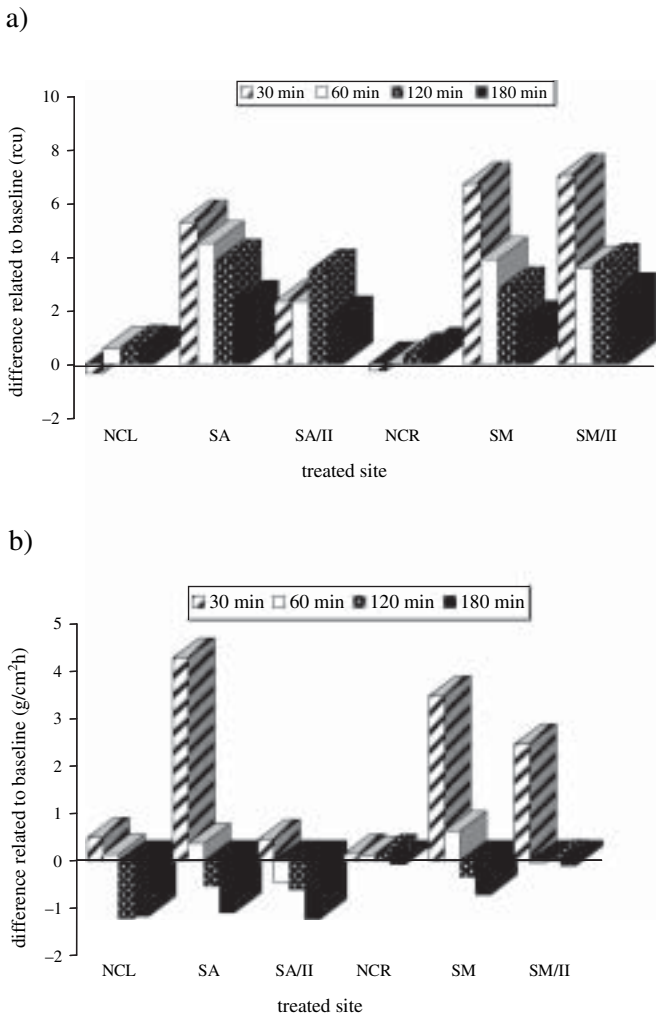

FIGURE 6 **Percentage of Released Amount of DDA (%) from (a) Cream SA and (b) Cream SM, Considering the Different Place of Incorporation.**

emulsifier, attributed with markedly lower viscosity than other samples of both series, exhibited the highest DDA releasing rate, but insignificantly compared to the other formulations. Opposite to series SA, in series SM, sample SM/II with drug dissolved in oil phase showed the highest dynamic of drug release (Table 6), but also without significant difference related to the others. Relying to the findings of other authors (Kriwet & Müller-Goymann., 1993; Papantoniou & Müller-Goymann, 1995; Parsaee et al., 2002; Makai et al., 2003) and results obtained in physicochemical characterization of investigated vehicles, such trend of DDA release was somehow expected. It is well established that, amphiphile drugs like DDA, are attributed with mesomorphic behavior and potential for interactions with lyotropic liquid-crystalline phases, influencing the sustained drug release (Kriwet & Müller-Goymann, 1993; Papantoniou & Müller-Goymann, 1995; Makai et al., 2003). Namely, the part of DDA could be solubilized within mostly hydrophobic lamellar bilayers existed in liquid-crystalline or gel-crystalline state, as lipophilic compartment of investigated vehicle. The quantity of drug solubilized in this way varies in different formulations, at least in few percentages, probably dependent on starting differences in type of vehicles and place of drug incorporation. Consequently, insignificant differences in DAA dissolution profiles from investigated samples were recorded. On the other side, it should be reminded that water phase dominates in these formulations. According to the previous findings (Savic et al., 2005), water is specifically distributed within these systems interacting with other components of colloid structure and producing at least two thermodynamically different sorts of so-called "depot" water. It is possible that part of DDA is simply entrapped within these compartments, serving as reservoir for prolonged drug release. In that sense, it seems reasonable to suggest that part of DDA, present in bulk or free water, mostly diffuses into receiver in first hours of drug release. According to its thermodynamic potential, this part of water evaporates in first 15–20 min after application (Savic et al., 2004), thus part of DDA from that phase forms the reservoir for releasing in following hours. The rest of water and drug remain entrapped within colloid structure of the systems, which hampers the amphiphile

drug release. This is consistent with finding of Parsaee et al. (2002), showing that DDA exhibited the superior diffusion coefficients from simple o/w emulsion lotion and lipogel lecithin formulation, in contrast to the lowest release rate found from mixed micellar aqueous gel. Such result was addressed to the possible drug-vehicle interaction (Parsaee et al., 2002).

Analyzing the DDA release with corresponding mathematical models (Table 7), the best fit was obtained with Higuchi's diffusion model in both groups of investigated samples. According to this model, the limitation factor influencing the drug kinetic is DDA diffusion through the carrier (Woolfson et al., 2000). Additionally, this result supports the previous finding on complexity of vehicle's colloid structure and possible drug-vehicle interactions.

In order to evaluate the influence of vehicles and chosen active samples (SA/II and SM/II) on skin hydration and TEWL, the short-term application study was carried out. Results of testing were expressed as absolute differences in relation to corresponding basal values for each time point and analyzed statistically (*t*-test, $p < 0.05$, Fig. 5a,b). Generally in first 30 min, in all treated sites a significant increase of skin hydration level as well as of TEWL (except for sample SA/II) was recorded, indicating the evaporation of the free water from the systems. This is the most important phenomenon taking place after application of dermatological emulsion vehicles, leading to pronounced structural changes of emulsion placed on the skin surface (Aikens & Friberg, 2000; Held et al., 2001). Afterwards, a decrease of both parameters occurred, thus at all measured sites hydration level remained significantly higher compared to hydration changes in untreated controls during entire test period. On the other side, starting from 60 min, TEWL decreased significantly comparing to its alteration in first 30 min upon the samples application, but insignificantly related to corresponding control. Thus, in all treated sites, TEWL, as measure of skin barrier integrity, kept favorable values to the end of experiment. Interestingly, samples based on monosaccharide mixed emulsifier (cetearyl glucoside & cetearyl alcohol) produced less fluctuation in TEWL values and more pronounced skin moisturization than their counterparts stabilized with disaccharide emulsifier (sorbitan stearate & sucrose cocoate). Although the latter effect was not significant, the trend demonstrated in this study could be explained through the impact that lipidization phase may have on measured skin parameters. Namely, it is known that the evaporation phase is followed by the lipidization phase, in which emulsion lipids (originated from emulsifier and/or oil) penetrate into the epidermis and cause the increase of skin hydration level (Aikens & Friberg, 2000; Held et al., 2001). Also, it could be speculated that a pronounced lipidization phase in these samples (SM and SM/II) has led to desirable TEWL levels (higher contents of emulsifier and oil). Generally, results of TEWL measurements approved declared mildness of both sugar-type emulsifiers. Furthermore, preliminary short-term study results confirm valuable contribution of this novel class of surfactants to the moisturization potential of final formulations and possibly a feasible impact on their potential for prolonged skin hydration and drug penetration enhancement.

CONCLUSION

The present physico-chemical characterization of two vehicles stabilized with sorbitan stearate & sucrose cocoate or cetearyl glucoside & cetearyl alcohol showed that these new, natural surfactants of sugar type are promising tools in tailoring of vehicles with complex colloid structure, based on synergism of lamellar liquid-crystalline and lamellar gel-crystalline phases. Having in mind the results of droplet size distribution analysis and rheological measurements, the both vehicles exhibited satisfying physical stability. Although rheological i.e., viscoelastic profiles of two vehicles differed significantly, it appeared that it had no significant influence on DDA release. Moreover, considering the amphiphile nature of DDA and complex microstructure of the samples, it was concluded that place of drug incorporation within the vehicle did not play an important role in managing of its liberation profile. Therefore, it was assumed that, in the first 6 hr of experiment, only the part of DDA, predominantly contained within the free fraction of water, diffused into the acceptor phase.

A favorable potential for skin hydration and skin barrier improvement was proved for both vehicles, but more pronounced in case of sample stabilized with alkylpoliglucoside mixed emulsifier. This feature, accompanied with desirable rheological profile may predict eventual regulatory appreciation of alkylpolyglucoside-type vehicles.

Based on these entire findings sample stabilized with alkylpolyglucoside emulsifier cetearyl glucoside & cetearyl alcohol and with DDA incorporated in oil

phase (SM/II) was chosen as an optimal formulation of emulsion carrier, at least within this study.

However, the complete information on impact that this new generation of vehicles could have on drug delivery, still remains a subject of further, more detailed investigations.

REFERENCES

Aikens, P. A. & Friberg, S. E. (2000). Emulsifiers. In *Dry skin and Moisturizers* Lodén, M., Maibach, H. I., (Eds.), Boca Raton: CRC Press LLC, pp. 183–201.

Anconturier, J. & Amerlic, R. (2002). A. Montanov 68 PHA a glucolipid emulsifier for O/W emulsions. Proc.4th world meeting ADRITELF/ APHI/APV. Florence, pp. 1473–1474.

ICI Surfactants, (1996). Arlatone®2121 – natural emulsifier for oil-in-water milks and creams, Everberg, publication 41-5 E,

Berardesca, E. (1997). EEMCO guidance for the assessment of stratum corneum hydration: electrical methods. *Skin. Res. Tech., 3*, 126–132.

Cook, W. J. (1997). Acyl lactate index; Liquid crystal gel networks. *Cosmet. Toiletries, 112*, 69–72.

Dedern, J. C., Wetzel, T., Peeters, H., Smits, R., Davies, S., Serrien, G., Taelman, M. C., & Tadros, T. (2001). Changing emulsifiers from mouth to skin: a new technology – generation for cosmetic emulsion stabilisation. Proceedings 2001 IFSCC Conference, Stockholm, 13–24.

Eccleston, G. M. (1997). Functions of mixed emulsifiers and emulsifying waxes in dermatological lotions and creams. *Colloid Surface A, 123–124*, 169–182.

Eccleston, G. M. (2001), The importance of mesomorphic (lamellar) phases in emulsion stability. *J. Cosmet. Sci., 52*, 142–143.

Eccleston, G. M., Behan-Martin, M. K., Jones, G. R., & Towns-Andrews, E. (2000). Synchrotron X-ray investigations into the lamellar gel phase formed in pharmaceutical creams prepared with cetrimide and fatty alcohols. *Int. J. Pharm., 203*, 127–139.

Erős, I., Cserne, A., Kövér, T., Csoka, I. & Abu-Eida, E. Y. (1997). Cremofor surfactants – as wetting, solubilising and stabylising agents in pharmaceutical technology. *Farm. Vestn., 48*, 382–384.

Fairhurst, C. E.; Fuller, S., Gray, J., Holmes, M. C., & Tiddy, G.J. (1998). Lyotropic surfactant liquid crystals. In Demus, D., Goodby, J., Gray, G. W., Spiess, H. W., Vili, V. (Eds.); *Handbook of liquid crystals*, Wiley-VCH: Weinheim, Vol. 3, pp. 341–392.

Farkas, E., Zelko, R., Torok, G., Racz, I., & Marton, S. (2001). Influence of chlorhexidine species on the liquid crystalline structure of vehicle. *Int. J. Pharm., 213*, 1–5.

Farkas, E., Zelko, R., Nemeth, Z., Palinkas, J., Marton, S., & Racz, I. (2000). The effect of liquid crystalline structure on chlorhexidine diacetate release. *Int. J. Pharm., 193*, 239–245.

Fluhr, J. W., Lazzerini, S., Distante, F., Gloor, M., & Berardesca, E. (1999). Effects of prolonged occlusion on stratum corneum barrier function and water holding capacity. *Skin Pharmacol. Appl. Skin Physiol., 12,* 193–198.

Georgalas, C. W. A. (2001). Experience with amphiphilic gel network emulsifier chemistry. 2001 IFSCC Conference. Stockholm, pp. 27–33.

Hadgraft, J. (2001) Modulation of the barrier function of the skin. *Skin Pharmacol. Appl. Skin Physiol., 14*(Suppl 1), 72–81.

Held, E., Lund, H., & Agner, T. (2001). Effect of different moisturizers on SLS-irritated human skin. *Contact Dermat., 44*, 229–234.

Junginger, H. E. (1997). Multiphase emulsions. In Rieger, M. M., Rhein L. D., (Eds.), *Surfactants in Cosmetics,* New York, Marcel Dekker.

Korhonen, M., Niskanen, H., Kiesvaara, J., & Yliruusi, J. (2000). Determination of optimal combination of surfactants in creams using rheology measurements. *Int. J. Pharm., 197*, 143–151.

Korhonen, M., Lehtonen, J., Hellen, L., Hirvonen, J., & Yliruusi, J. (2002). Rheological properties of three component creams containing sorbitan monoesters as surfactants. *Int. J. Pharm., 247,* 143–151.

Kriwet, K. & Müller-Goymann, C. C. (1993).Binary diclofenac diethylamine water systems: micelles, vesicles and lyotropic crystals. *Eur. J. Pharm. Biopharm., 39*, 234–238.

Krog, N. (1997). In Friberg, S. E., Larson, K. (Eds.), *Food Emulsions*, (3 Ed.), New York, Marcel Dekker, pp.141–188.

Levin, J. & Maibach, H. (2005). The correlation between transepidermal water loss and percutaneous absorption: an overview. *J. Control. Release, 103*, 291–299.

Loll P. (1993). Liquid crystals in cosmetics emulsions. ICI Surfactants, publication RP 94/93 E, Everberg.

Makai, M., Csanyi, E., Nemeth, Z., Palinkas, J., & Erős, I. (2003). Structure and drug release of lamellar liquid crystals containing glycerol. *Int. J. Pharm., 256*, 95–107.

Mandani, K. & Friberg, S. E. (1971). *Progr. Colloid Polym., 244*, 233–239.

Müller-Goymann, C. C. (2002). Drug delivery-liquid crystals. In *The Encyclopedia of pharmaceutical technology*. New York, Marcel Dekker, Vol. 1, pp. 834–853.

Müller-Goymann, C. C. (2004). Physicochemical characterization of colloidal drug delivery systems such as reverse micelles, vesicles, liquid crystals and nanoparticles for topical administration. *Eur. J. Pharm. Biopharm., 58*, 343–356.

Müller-Goymann, C. C. & Frank, S. G. (1986). Interaction of lidocaine and lidocaine-HCl with the liquid crystal structure of topical preparations. *Int. J. Pharm., 29*, 147–159.

Norlén L. (2003). Molecular skin barrier models and some central problems for the understanding of skin barrier structure and function. *Skin Pharmacol. Appl. Skin Physiol., 16*, 203–211.

Papantoniou, I. & Müller-Goymann, C. C. (1995). Influence of the phase transformation from reverse micellar solution into lamellar liquid crystal on sustained release. *Pharm. Pharmacol. Lett., 1*, 28–31.

Parsaee, S., Sarbolouki, N. M., & Parnianpour, M. (2002) In-vitro release of diclofenac diethylammonium from lipid-based formulations. *Int. J. Pharm., 241*, 185–190.

Pinagoda, J., Tupker, R. A., Agner, T., & Serup, J. (1990). Guidelines for transepidermal water loss (TEWL) measurement. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact. Dermat., 22*, 164–178.

Roberts, M. S. & Walker, M. (1993). Water: the most natural penetration enhancer. In *Pharmaceutical Skin Enhancement*. Walters, K. A., Hadgraft, J., (Eds.), New York, Marcel Dekker, pp. 1–30.

Rogiers, V. (2001). EEMCO Group, EEMCO guidance for the assessment of transepidermal water loss in cosmetic sciences. *Skin Pharmacol. Appl. Skin Physiol., 14*, 117–128.

Savic, S., Tamburic, S., Savic, M., Cekic, N., Milic, J., & Vuleta, G. (2004). Vehicle-controlled effects of urea on normal and SLS-irritated skin. *Int. J. Pharm., 271*, 269–280.

Rybinski, W. (2001). Natural surfactants. Curr. Opin. *Colloid Interface Sci., 6*, 146–147.

Savic S., Vuleta G., Daniels R., & Müller-Goymann, C. C. (2005). Colloidal microstructure of binary systems and model creams stabilized with an alkylpolyglucoside nonionic emulsifier. *Colloid Polym. Sci., 283*, 439–451.

Seppic, 1774/GB/01/August/2001.

Tadros, T. (1991). Future developments in cosmetic formulations, ICI Surfactants, publication RP 68/91 E, Everberg.

Tadros, T. F., Dimitrova, G., Luckham, M. C., & Loil, P. (1994). Investigations of liquid crystalline phases using microscopy, differential scanning colorimetry and rheological techniques. 18th International IFSCC Congres. Venice. 1–14.

Woolfson, A. D., Malcolm, R. K., Campbell, K., Jones, D. S., & Russel, J. A. (2000). Rheological, mechanical and membrane penetration properties of novel dual systems for percutaneous delivery. *J. Control. Release, 67*, 395–408.

Zhai, H. & Maibach, H. I. (2001). Effects of skin occlusion on percutaneous absorption: an overview. *Skin Pharmacol. Appl. Skin Physiol., 4*, 1–10.