Original article

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THE INFLUENCE OF POLAR AND NON-POLAR EMOLLIENTS ON THE STRUCTURE AND SKIN MOISTURIZING POTENTIAL OF THE EMULSIONS STABILIZED BY MIXED EMULSIFIER

Dragana Stojiljković[1], Ivana Arsić[2], Marija Tasić-Kostov[2]

The appropriate moisture content in the stratum corneum, as a superficial layer of the epidermis, provides softness and flexibility of the skin in different environmental conditions, and maintaining of skin humidity is very important in dermatology and dermocosmetology. In this paper, we investigated the skin moisturizing potential after a single application and structure of the emulsion of o/w type, stabilized by mixed emulsifier glycerylmonostearate self-emulsifying (GMSse), which contained polar emollients (PEG-7 glicerylcocoate and myristyl myristate) and non-polar emollient (liquid paraffin), in a concentration of 10% (emulsions E1-E3, respectively). The emulsion structure was investigated by polarization microscopy, and the presence of different anisotropic structure was observed. The moisturizing potential after a single application and skin pH were investigated by skin bioengineering. Emulsions with polar emollients (E1 and E2) showed a statistically significant increase in skin moisture content after 30 minutes; 300 min after applications it did not exist; emulsion with a non-polar emollient (E3) showed significant moisturizing potential after 30 min and after 300 min probably as a consequence of occlusion. Nature and polarity of emollients affected the structure and properties of emulsions stabilized by anisotropic structures, and also the moisturizing level and pH of the skin immediately after their application. *Acta Medica Medianae 2016;55(2):25-30.*

Key words: emulsion o/w type, emollients, mixed emulsifier, liquid-crystalline phase, skin bioengineering

Farmakop Public Pharmacy, Niš, Serbia[1]
University of Niš, Faculty of Medicine, Department of Pharmacy, Niš, Serbia[2]

Contact: Dragana Stojiljković
Duška Taskovića 18, Niš, Serbia
e-mail: s.dragana83@yahoo.com

Introduction

The degree of hydration in the superficial layer of the epidermis, the stratum corneum (SC), provides softness and flexibility of the skin under different environmental conditions, and maintaining of skin humidity is very important in dermatology and dermocosmetic industry (1). One of the most important functions of the skin is to prevent the excessive water loss into the environment (2). The water comes to the skin surface through the sweat ducts and passive diffusion through epidermis, where the integrity of the SC determines the level of transepidermal water loss. Another important barrier function of the skin is to prevent the entry of substances from the external environment (3). Water is necessary for the lipid enzymatic hydrolysis and other skin constituents. For this reason, the maintenance of moisture in the SC, which is composed of corneocytes, cells and intercellular lipids with complex composition as well as the maintenance of the barrier function of the skin are important cosmetic, dermocosmetic, and dermatological tasks (4, 5).

The term skin moisturizer refers to the product whose role is to increase the humidity of the healthy, and dry and rough skin, too, after the application (6, 7). Moisturizers in the form of emulsions are hydrophilic products that dermatologists commonly prescribe to patients with various pathological conditions of the skin, and they are the preparations that are commonly used by the users (8). Preparations which have skin moisturizing effect contain emollient substances, which can act through two principal mechanisms, such as occlusion and water binding from the atmosphere (9).

The correct choice of emollients is essential for the efficacy of the product in terms of the skin moisturizing, but also achieving the satisfactory physical and chemical stability of the emulsion, where the polarity of the emollients, or their chemical composition, affects the mechanism of the interaction with the skin and the structural organization and organoleptic characteristics of the emulsion. Many studies have shown that there is a

connection between the application of different emollients and structure of emulsions stabilized by phase liquid crystals as well as their skin moisturizing potential (10, 11).

The presence of the mixed emulsifiers in the emulsion leads to stabilization by the liquid crystal phase, which corresponds to the structural organization of the SC lipids of healthy skin, and they are often used in emulsions that moisturize the skin. GMSse (glycerylmonostearate self-emulsifying) is a mixed oil/water (o/w) emulsifier, which consists of lipophilic emulsifier glycerolmonostearate and hydrophilic glycerolpotas-siumstearate. The polarization microscopy is one of the methods for characterization of the system liquid crystal type, since all lyotropic liquid crystals, except cubic, show an ability to refract the polarized light (12-14).

In the context of determining the efficiency of the moisturizers, investigation of skin humidity is an important task. The objective methods of bioengineering, based on the biophysical properties of the skin, are a way to quantify the effects of certain cosmetic products. Measurement of the electrical properties of the skin with appropriate devices, primarily its capacitance, is used as a bioengineering method for the assessment of skin humidity. The importance of moisturizing products (primarily hydrophilic emulsions, creams and lotions) in cosmetology, dermocosmetology, and der-matology is high (10, 14, 15).

Experimental section

Investigated emulsions

Three emulsions of o/w type stabilized by mixed emulsifier GMSse (Cognis, Germany), with the same basic formulation by varying one of the present emollients, in the same percentage (10%), were made. Emulsions E1 and E2 contained polar emollients (E1 contained PEG-7 glicerylcocoate and E2 myristyl myristate), whereas the emulsion E3 contained non-polar emollient liquid paraffin. All samples were preserved using liquid preservative Euxyl K®300 (Schülke Mayr, Germany) (INCI-phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, isobytilparaben). Qualitative and quantitative compositions of the emulsions are shown in Table 1.

Samples were made using standard procedure for making a hydrophilic emulsion, with the same composition of aqueous and oil phase and by varying the emollient (16). Lipophilic components and emollient as one of the lipophilic components (Table 1) were first mixed and then heated to 70^{o}C under stirring. Components of the aqueous phase (Table 1) were separately heated to 72^{o}C under stirring. The water loss due to evaporation was compensated by adding water to the aqueous phase, before emulsification. Then, aqueous phase was gently added to the warm oily phase under stirring. Preservative was added at 40^{o}C and the emulsions were kept under stirring for an additional period of 5 min until their temperature dropped to ambient temperature (11).

In vitro investigation of the emulsions

PH values of the samples were measured potentiometrically, using pH 211 Microprocessor pH Meter Hanna Instruments, USA. The values of electrical conductivity were measured using apparatus Hanna HI 98311, Hanna Instruments, USA. The organoleptic examination was carried out by observing the characteristics: color, consistency, spreadability, and homogeneity of the samples. Measurements were taken immediately after preparation, and after four weeks of storage at 22±2^{o}C. The results represent the average of three measurements.

Table 1. Qualitative and quantitative composition of the investigated emulsions

Ingredients/INCI	Samples % (w/w)		
	E1	E2	E3
Oil phase			
Glyceryl Stearate SE	5.00	5.00	5.00
Cetearyl alcohol	3.00	3.00	3.00
PEG-7 glicerylcocoate	10.00		
Myristyl myristate		10.00	
Liquid paraffin			10.00
Water phase			
Glycerol	2.00	2.00	2.00
Phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, isobytilparaben (Euxyl K® 300)	0.50	0.50	0.50
Aqua purificata ad	100.00	100.00	100.00

Physical stability of the investigated emulsions with the emollient was examined by performing the test of centrifugation (two times per 15 minutes, at 3000 rev/min, at room temperature) using a laboratory centrifuge LC 320 (Libra, Slovenia). The stability of the emulsion was examined immediately after preparation, four weeks after preparation, and storage at room temperature (22±2°C). After 30 minutes, the final phase separation was observed.

Microscopy of the samples was performed using polarization microscope LEICA DMR, Germany using a light polarizer and λ- plates, four weeks after preparation and storage at room temperature (ampilification – 500x).

In vivo investigation of the emulsions

Investigation of the effects of the samples E1, E2 and E3 (after four weeks of their preparation) and humidity and pH of the skin after a single application by corneometer method was performed in 12 healthy female volunteers (average age 45.25±3.45) after obtaining their consent, in accordance with the Helsinki Declaration. Samples were applied to the inner side of the forearms, and the volunteers were told not to use other products on these places three days before the start and during the measurement. Before the measurement, volunteers stayed 20 min in a climate-controlled room (temperature 22±2°C, humidity 50±5%) where the measurements took place. Electrical capacitance (EC), as a reflection of hydration state in SC, was measured by Corneometer®CM 825 (Courage&Khazaka Electronic GmbH, Germany), and the pH of the skin was measured using Skin-pH-meter PH 900 (Courage&Khazaka Electronic GmbH, Germany). The first measurement was the baseline measurement (initial values, before application of emulsions). Then, 2 mg/cm^2 of emulsion were applied on the specific places (area of 9 cm^2), using a single-step randomization, and measurements were performed 30, 90, 150 and 300 minutes after the application of emulsions. The results represent the average of the three measurements.

Statistics

The values obtained by measuring the electrical capacitance were statistically analyzed using SPSS for Windows 13.0. Testing the statistical differences in the moisture content of the skin treated with a variety of samples after the same time intervals was carried out by the analysis of variance (ANOVA) ($p<0.05$).

Results and Discussion

In vitro investigation of the emulsions

The measured values of pH and conductivity of the emulsions remained at approximately the same level after four weeks of storage at room temperature, and the values were within the range recommended for preparations intended for use on the skin. A slight decline in the value of conductivity was most visible in the sample E3, probably caused by subsequent structuration of the system, forming lamellar gel phases and binding of free water, which is in line with the organoleptic findings. The values of electrical conductivity and pH values of the emulsions after preparation and after four weeks of storage at room temperature are given in Table 2.

Table 2. pH values and electrical conductivity of the investigated emulsions

Samples	pH value		Conductivity (μS/cm)	
	after preparation	*after 4 weeks*	*after preparation*	*after 4 weeks*
E1	7,69	7,15	78	73
E2	7,55	6,95	98	76
E3	7,45	6,80	76	71

Color of samples remained unchanged after four weeks. Change of emollients also led to a change in the viscosity of the emulsion, that we observed in the emulsion E1, which has lower viscosity than the other emulsions, and in the emulsion E2 (containing myristyl myristate), which has a higher viscosity compared to other emulsions. Consistency of the samples E2 and E3 was similar, while the consistency of the sample E1 was considerably lower, and spreadability on the skin much better. A change in spreadability, probably due to changes in density, occurred in all samples. Homogeneity was satisfactory in all the samples, both immediately after preparation and after four weeks, without the sign of phase separation.

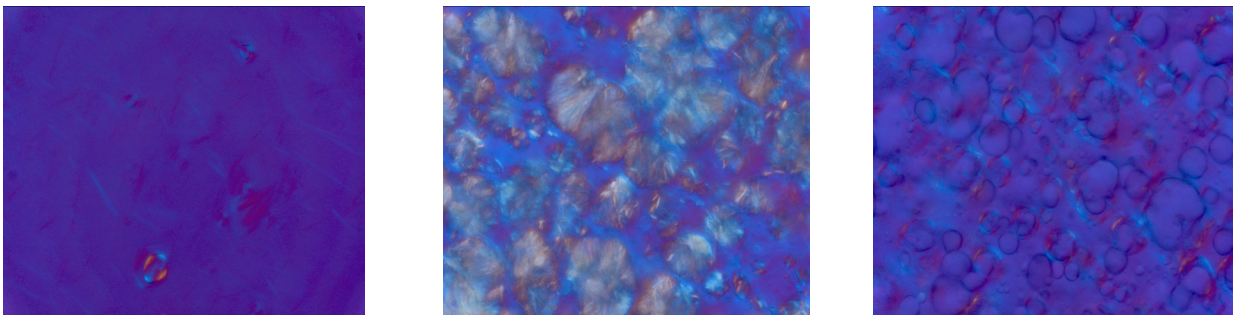

Figure 1. Polarization micrographs of emulsion samples a) E1, b) E2, c) E3

All investigated emulsions retained their structure after centrifugation, and there were no changes in their organoleptic properties and pH values. A phase separation of emulsions did not occur in any of the samples. Type and polarity of used emollients had no impact on the physical stability of investigated emulsions.Polarization micrographs of the investigated emulsions (ampilification – 500x) were given in Figure 1

Anisotropic layers on the edge of droplets of the inner (oil) phase, i.e. deformed Maltese crosses were observed on polarization micrographs of the sample E1 (Figure 1, a), which indicates the presence of lamellar mesophases, by the presence of structures which reflect polarized light, presented in a continuous phase within the dispersed droplets, which corresponds to the lamellar gel phase, and this is correlated with the findings of organoleptic tests. The polarization micrograph of the sample E2 (Figure 1, b) indicated the anisotropy type of mosaic structure, probably due to the partial presence of partially rehydrated crystals of cetostearyl alcohol, i.e. koagel phase, due to the presence of a specific type of an emollient, ester of a long-chain fatty acid and the appropriate alcohol. In fact, a specifically organized gel phase might be seen, which strongly resembled a stable gel phase, seen in some distilled monoglycerides (14). The polarization micrograph of the sample E3 (Figure 1, c) showed the presence of anisotropic layers around oil droplets ("onion rings"), indicating a low presence of lamellar phase liquid crystals, as well as the remains of the gel network in the continuous phase which randomly surrounded the larger droplets.

In vivo investigation of the emulsions

Change in the pH values of the volunteers' skin occured within 30 minutes after the application of the tested emulsions (the biggest change to 0.4 pH units in the emulsion E3, and the lowest in the emulsion E2 0.24 pH units). In the period from 150 to 300 minutes after the application of emulsions and the activation of its buffering capacity, the skin gradually established the original pH values, and in this period a lower change of the skin pH was recorded. The pH values of the skin of the volunteers are shown in Figure 2.

The application of the sample E1 led to a statistically significant increase in EC after 30 minutes of application compared to the baseline. Further, humidity decreased over time, which is probably

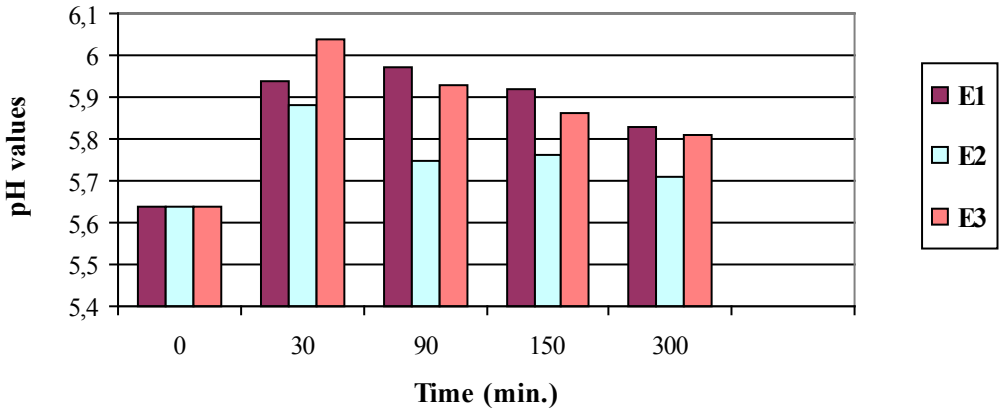

Figure 2. Changing the skin pH after a single application of the emulsions E1-E3 in the function of time

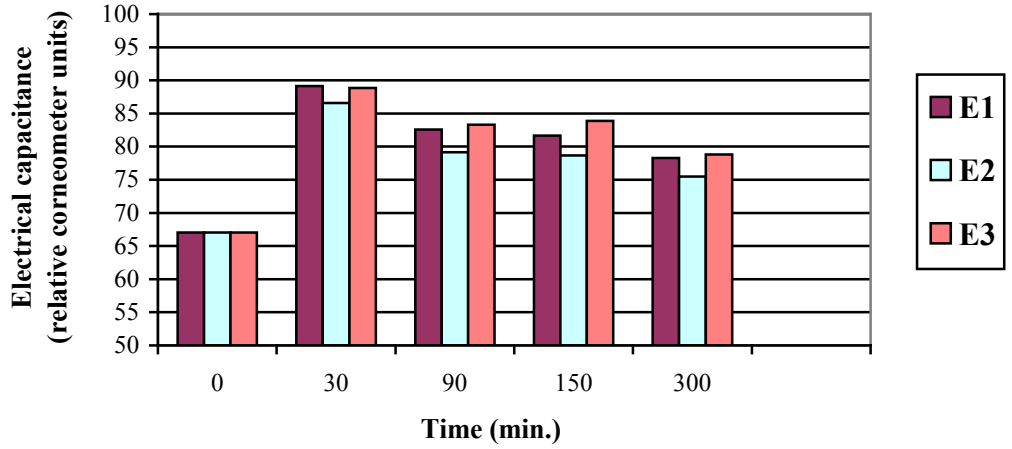

Figure 3. Electrical capacitance of the skin after a single application of the emulsions E1-E3 in the function of time

due to the partial collapse of the emulsion and release of water immediately after the application. There was a statistically significant reduction in the value of this parameter measured after 30 min in relation to the value measured after 90 minutes, 30-150 minutes, 30-300 minutes (Figure 3). The sample E2 showed almost identical results. The application of sample E3 has led to a statistically significant increase in the EC compared to the baseline; the most significant increase was achieved after 150 minutes of sample application, significantly more than the changes in skin moisture content after 30 and 90 minutes of application, indicating the nature of the used occlusive emollients (liquid paraffin) and the emulsion, but also the possibility of a slower collapse of emulsions stabilized by the present phase liquid crystals. After 300 minutes of application, skin moisture remained significantly higher than the basal measured value, but showed a decrease compared to that measured 150 min after the application. Analysis of differences in the humidity of the treated skin 30 min after the application showed that in the sample E2 a statistically significant increase in skin moisture was found ($p<0.05$) compared to the increase in sample E1. Results of electrical capacitance are shown in Figure 3.

Conclusion

In this study, we examined the effects of three different emollients on the structure and organoleptic properties of emulsions potentially stabilized by lamellar phase of liquid crystals or by gel crystalline phase, and also the impact of different emollients on skin moisturizing potenitial of emulsions. It has been shown that the nature and polarity of emollients affect the structure and organoleptic properties of the emulsion stabilized by mesophases, and significantly influences the skin pH values of the volunteers and the ability of the emulsion to moisturize the skin. Emulsions which contain occlusive emollients have the occlusive effect after applications. They reduce the currently moisturizing potential, however, a significant skin moisturizing comes after a long time, as was shown by the analysis of the sample E3 with a non-polar emollient. However, based on differences in humidity of the skin treated with samples E1, E2 and E3, it might be postulated that, in addition to the emollients, the type of liquid crystal, which is present in the structural organization of emulsion, affects its ability to currently hydrate the skin, i.e. after the application of the sample E3, water was being gradually released from the structure of the lamellar liquid crystal. This may indicate that emulsion with such structural organization has the ability of prolonged skin moisturizing compared to the emulsion stabilized by other types of liquid crystal phase. One should also bear in mind that due to the similar index of polarity, inherent emollient ability of used emollients in the samples E1 and E2 (containing polar emollients) was not significantly different, so it can also be concluded that after the application of emulsion with the structural organization as in sample E2, the current skin moisturizing will occur faster as well as the withdrawal of unpleasant symptoms associated with dryness of the skin of the patient i.e. user.

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UTICAJ POLARNIH I NEPOLARNIH EMOLIJENASA NA STRUKTURU I VLAŽEĆI POTENCIJAL EMULZIJA STABILISANIH MEŠANIM EMULGATOROM

Dragana Stojiljković[1], Ivana Arsić[2], Marija Tasić-Kostov[2]

Apoteka Farmakop, Niš, Srbija[1]
Univerzitet u Nišu, Medicinski fakultet u Nišu, Odsek za farmaciju, Niš, Srbija [2]

Kontakt: Dragana Stojiljković*
Duška Taskovića 18, Niš, Srbija
e-mail: s.dragana83@yahoo.com

Odgovarajući sadržaj vlage u stratumu corneumu (SC), kao najpovršnijem sloju epidermisa, obezbeđuje mekoću i fleksibilnost kože pri različitim uslovima spoljašnje sredine i održavanje vlažnosti kože. U ovom radu ispitivan je potencijal vlaženja kože nakon jednokratne aplikacije i struktura emulzija u/v tipa, stabilizovanih mešanim emulgatorom glicerolmonostearat samoemulgujući (GMSse), koji su sadržali polarne emolijense (PEG-7 glicerilkokoat i miristilmiristat) i nepolarni emoli-jens (tečni parafin), u koncentraciji od 10% (emulzije E1-E3, redom). Struktura emulzija je ispitivana polarizacionom mikroskopijom i uočeno je prisustvo različitih anizotropnih struktura. Vlažeći potencijal nakon jednokratne aplikacije i pH kože ispitivan je bio-inženjeringom kože. Emulzije sa polarnim emolijensima E1 i E2 pokazuju statistički značajno povećanje vlažnosti kože nakon 30 min; 300 min od aplikacije ono izostaje; emulzija sa nepolarnim emolijensom (E3) pokazuje značajni potencijal vlaženja nakon 30 min i nakon 300 min verovatno kao posledicu okluzije. Priroda i polarnost emolijenasa utiče na strukturu i osobine emulzija stabilisanih anizotropnim strukturama koje ih sadrže, ali i na nivo vlažnosti i pH kože neposredno nakon njihove aplikacije. *Acta Medica Medianae 2016;55(2):25-30.*

Ključne reči: *emulzije u/v tipa, emolijensi, mešani emulgator, tečno-kristalna faza, bioinženjering kože*