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Original article ■

Investigation of the Effects of Different Emollients on the Structure and Skin Moisturizing Potential of the Cosmetic Creams

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SUMMARY

In this study we investigated the effects of four emollients (cetearyl octanoate, decyl oleate, C12-15 alkyl benzoate and olive oil) incorporated at a concentration of 10% on the structural properties of the cosmetic oil/water (o/w) creams (creams K1-K4, respectively) as well as the *in vivo* effects of those creams on pH and hydration level of the healthy volunteer's skin after a single application.

Creams' structure was investigated throughout polarization microscopy. Anisotropic structures were found, i.e. stabilization of creams' structure was performed by forming lamellar gel-crystalline and liquid-crystalline phase, different for different creams, which was probably caused by the presence of different emollients.

The effects of creams (K1-K4) on skin hydration level and pH (30, 90, 150 and 300min after single application) were examined using Multi Probe Adapter (MPA[®]9, Courage&Khazaka Electronic, Germany), with appropriate sonde, Corneometer[®] CM 825 for measuring capacitance as a measure of skin moisture level, while the skin pH was measured using Skin-pH-Meter, PH 900 sonde. The results indicate that the presence of the different emollients in o/w creams (10%) led to the creams' different effects on the skin hydration level. Cream with decyl oleate leads to a good initial hydration effect and probably rapid release of large amounts of water from the cream. Creams containing both C12-C15 alkyl benzoate or olive oil had a weaker initial effect on the skin moisture, showing good occlusive properties. A cream containing 10% cetearyl octanoate showed the lowest skin hydration potential.

Key words: emollients, hydrophilic creams, liquid-crystalline phase, skin hydration

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INTRODUCTION

The stratum corneum (SC) is the surface layer of the skin, composed of corneocyte cells and intercellular lipids, complex composition organized in liquid crystalline structures. SC protects the skin from drainage and gives it softness and flexibility (1, 2). SC hydration state affects the barrier function of the skin, the penetration of active substances and skin mechanical properties. The increase in skin hydration leads to the swelling of the SC and softening of its structure (3, 4). When the moisture content of the skin drops below a certain level, the liquid crystalline structures, present in the SC lipids organization, are destroyed and skin becomes dry (5, 6). Dry skin is characterized by rough appearance, dandruff, turgor loss and reduced elasticity. Subjectively, there is a feeling of burning and skin tightening (7-9). Maintaining an optimal SC hydration level and the preservation of the skin barrier function is an important cosmetic and dermatological goal (4, 6).

The term moisturizers refers to the products which are applied to the skin (mainly emulsions, lotions, creams) in order to increase the humidity in healthy and dry, rough skin (4, 8). Moisturizers, in the form of creams, are the most commonly prescribed products by dermatologists to patients with a variety of pathological skin conditions, in order to maintain optimal skin hydration which is threatened by the damage of its surface (4, 10). In fact, emollient creams are often used to alleviate the objective and subjective characteristics of dry skin (11, 12). They contain emollient ingredients that enhance skin hydration and plasticity showing twofold action: occlusive effect - covering the skin surface by reducing water loss from the SC, and tying water from the atmosphere (moisturizing effect) (4, 13).

The chemical structure and polarity of emollients, as common ingredients of the moisturizing creams, may affect the structure of the o/w cream, its sensory characteristics, aesthetic features and mechanism of interaction with skin (6, 12, 14). Therefore, the proper choice of emollients or their combination is essential for the product efficiency in skin moisturizing (14). The presence of mixed emulsifiers such as GMS-se (glycerolmonostearate self-emulsifying) stabilize creams by liquid crystal phases, corresponding to the structural organization of SC lipids of healthy skin, so those emulsifiers are often used in the stabilization of the creams intended for effective skin hydration (6, 12, 15).

In the context of determining the moisturizers efficiency, the 6th Amendment to the EU Cosmetics Directive impose an obligation to producers who sell these products on its market for a clear quantification of the exhibited effects of cosmetic and dermatological preparations (*claim substantiation*) (16). Objective *in vivo* methods, based on the measurements of biophysical properties of the skin, are a way to quantify certain effects of cosmetic products. Previous studies have already identified the connection between effects of different emol-

lients on the cream structure and their skin moisturizing potential (17, 18).

The aim of this study was to investigate the effects of four different emollients (cetearyl octanoate, decyl oleate, C12-C15 alkyl benzoate and olive oil) on the structural properties of cosmetic o/w creams stabilized with liquid-crystalline phases as well as the effects of the creams on the skin hydration level and pH of the skin of healthy volunteers after a single application.

EXPERIMENTAL SECTION

Material and methods

Tested creams

Four o/w creams were made, each containing 10% of one of the emollients: cetearyl octanoate (K1), decyl oleate (K2), C12-C15 alkyl benzoate (K3) or olive oil (K4). 5% of mixed emulsifier GMS-se-Cognis, Germany (consisting of lipophilic emulsifier glycerolmonostearate and hydrophilic potassium stearate) was used in order to stabilize each of the creams. The optimum cream viscosity was achieved using 3% cetostearyl alcohol (Lanette O - Cognis, Germany). Samples were made using standard procedure for making a hydrophilic cream (emulsion o/w type), with the same composition of aqueous and oil phase and by varying the emollient (19). Composition of tested creams K1, K2, K3 and K4 is shown in Table 1. Lipophilic components, and emollient as one of the lipophilic component (Table 1) were first mixed and then heated to 70°C under stirring. Components of the aqueous phase (purified water and glycerine) (Table 1) were separately heated to 72°C under stirring. Before emulsification, the water loss due to evaporation was compensated by adding water to the aqueous phase. Then, aqueous phase was gently added to the warm oily phase under stirring. All samples were preserved using liquid preservative Euxyl K[®]300-Schülke Mayr, Germany (INCI-Phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, isobutylparaben), which was added at 40°C and the emulsions were kept under stirring for an additional period of 5 min until their temperature dropped to ambient temperature (14).

Investigation of the creams

Organoleptic, physicochemical properties and structure of the creams

Organoleptic appearance of the creams was determined observing the characteristics: color, consistency, ability to spread and homogeneity of the samples. pH values of the samples were measured potentiometrically by pH 211 Microprocessor pH Meter, Hanna Instruments, USA. The values of electrical conductivity

were measured by the device Hanna Hi 98311, Hanna Instruments, USA. Measurements of pH and conductivity were performed immediately after preparation of the creams (CDM 230, Radiometer, Copenhagen, Denmark), and after four weeks of storage at room temperature. The results represent the average of three measu-

rements. The cream structure was investigated by polarization microscope Leica DMR, Germany, using light polarizer and λ -plates, four weeks after preparation and storage at room temperature (amplification-500x).

Table 1. Composition of tested creams K1, K2, K3 and K4

Ingredients/INCI	Samples % (w/w)			
	K1	K2	K3	K4
Glyceryl Stearate SE	5,00	5,00	5,00	5,00
Cetearyl alcohol	3,00	3,00	3,00	3,00
Cetearyl octanoate	10,00			
Decyl oleate		10,00		
C12-C15 alkyl benzoate			10,00	
Oleauropea oil				10,00
Glycerol	2,00	2,00	2,00	2,00
Phenoxyethanol, methylparaben, ethylparaben, propylparaben, butylparaben, isobutylparaben (Euxyl K® 300)	0,50	0,50	0,50	0,50
Aqua purificata ad	100,00	100,00	100,00	100,00

In vivo investigation of creams' effects on skin

Since the skin surface is covered with a hydrophilic-lipophilic cover (hydrosoluble substances in the horny layer, sweat, sebum) with a relatively high moisture content, direct electrochemical measurement of the pH value of the skin is possible. The effect of each of the samples K1, K2, K3 and K4 on the pH value of the skin of healthy volunteers after a single application was measured using Skin-pH-meter, PH 900, Courage& Khazaka Electronic GmbH, Germany. Investigation of effects of the samples K1, K2, K3 and K4 on skin moisture after a single application was determined by measuring skin electrical capacitance (EC), by applying Corneometer®CM 825, Courage&Khazaka Electronic GmbH, Germany.

Examination of the effects of samples on pH and moisture of the skin were conducted on 12 healthy female volunteers (average age 45.25 ± 3.45) after obtaining their consent, in accordance with the Declaration of Helsinki and approved by the local Ethics committee. Samples were applied to the different skin sites of the inner side of the forearms, and the volunteers were told

not to use other cosmetic or dermatological preparations on this place three days before, as well as during the measurements. Participants had healthy skin without any dermatological diseases. Before the measurements, the volunteers stayed 20 minutes in the room where the measurements took place, in order to acclimatize (temperature $21 \pm 1^\circ\text{C}$, humidity $50 \pm 5\%$). The first measurement was performed after acclimatization of volunteers, before samples application (initial values of moisture and pH of the skin). Then 2 mg/cm^2 of the samples were inflicted on the exact position of the forearm inside (area of 9 cm^2) using a single-step randomization, and measurements were taken 30, 90, 150, and 300 minutes after application of the preparation. The results represent the average of three measurements.

Statistics

Values obtained by measuring the electrical capacitance were statistically analyzed using computer software SPSS 14.0 for Windows 2003. Values are presented as arithmetic mean (\bar{X}) \pm standard deviation (SD); comparison of values obtained after a certain time interval to baseline values and their mutual

comparison were conducted using non-parametric Friedman's test, and for comparison of two groups, Wilcoxon's test was used where it was necessary. $p < 0,05$ was considered statistically significant for all applied statistical tests.

RESULTS AND DISCUSSION

Organoleptic, physicochemical properties and structure of the creams

All prepared creams were white, odorless, without shine, of semi-solid consistency, homogeneous, with good spreadability. There were no differences in these characteristics regardless of the type of emollients that creams contain. After four weeks of storage at room temperature, color, consistency and odor of the samples remained unchanged, while the improvement of spreadability was observed in all samples, probably as a result of cream structure. Homogeneity (immediately after preparation as well as after four weeks of storage) was satisfactory for all samples, without any signs of phase separation.

pH values of the creams were within the recommended values for preparations intended for skin appli-

cation. Depending on the used emollient type, cream pH values, measured after preparation, were in the range of 7.41 for sample K2 to 7.67 pH units to sample K1. After weeks of storage of the samples at room temperature, a slight decrease in pH was recorded, but the values were still within the recommended values for the skin preparations. The values of electrical conductivity and pH values of the creams after preparation and after four weeks of storage at room temperature are given in Table 2.

Measured values of electrical conductivity of K1-K4 creams, immediately after preparation, were above 50 $\mu\text{S}/\text{cm}$, confirming that prepared creams were o/w type. Values ranged from 79 $\mu\text{S}/\text{cm}$ for sample K1 to 98 $\mu\text{S}/\text{cm}$ for K2 sample. Changes in the value of electrical conductivity (its increase) may indicate a separation of the dispersed phase droplets on the emulsion surface, sedimentation of the droplets to the bottom, or phase inversion (20). During cream storage for four weeks at room temperature, a slight decrease in conductivity of all the samples was registered, but their values were still within the range specified for the o/w systems. The most dominant conductivity value decrease was found in sample K2 88 $\mu\text{S}/\text{cm}$, probably as a result of subsequent structuring of the system (Table 2).

Table 2. The pH value and the electrical conductivity of tested creams

Samples	pH value		Conductivity ($\mu\text{S}/\text{cm}$)	
	after preparation	after 4 weeks	after preparation	after 4 weeks
Cream K1	7,67	7,27	79	75
Cream K2	7,41	7,25	98	88
Cream K3	7,50	7,12	82	86
Cream K4	7,63	7,24	87	84

Polarization microscopy is one of the methods used for the characterization of liquid crystal type system, since all lyotropic liquid crystals, except cubic, show polarized light refraction property (3, 6). Polarization micrographs of the samples at 500x magnification are shown in Figure 1.

Polarization microscopy of samples indicated the presence of anisotropic structures in all tested creams, i.e. the stabilization of the creams structure with lamellar gel-crystalline and liquid-crystalline phases, which were different for different creams, probably because of the presence of different emollients. Observed structures, the so-called Maltese crosses and inserted oil lines in the external phase, indicated the existence of the lamellar type of a liquid-crystalline and/or gel-crystalline

phase (polarization light refracts these structures). The effects of different emollients are reflected on a different kind/shape of deformation of observed structures, probably due to different mechanisms of their formation in samples with different emollients (3).

In vivo investigation of cream effects on skin

The average pH value of skin of healthy volunteers before the sample application was 5.64 ± 0.56 pH units. Since the pH values of investigated creams were more than average skin pH values of volunteers, their influence on the pH of the skin of volunteers was expected

ted. Maximum change in pH of the skin of the volunteers was registered 30 minutes after cream application. Between 150 and 300 minutes after cream application, the skin gradually established its initial pH values, probably by activation of its own buffering capacity (difference between pH values of the skin 300 minutes after the application of the creams K1 and K2 and the value before application was 0.1 pH units, 0.12 pH units for cream K4 and 0.17 pH units for K3 cream). The changes of the skin pH measured on healthy volunteers, as a

function of time, recorded 30, 90, 150 before, and 300 minutes after a single application of tested creams are shown in Figure 2.

The average value of the skin moisture level was 67.03 ± 10.12 (expressed in relative corneometer units) before the application of the samples. A graphical representation of the results of measurements of electrical capacitance (EC) of the skin before application and after a single application at specified time intervals is shown in Figure 3.

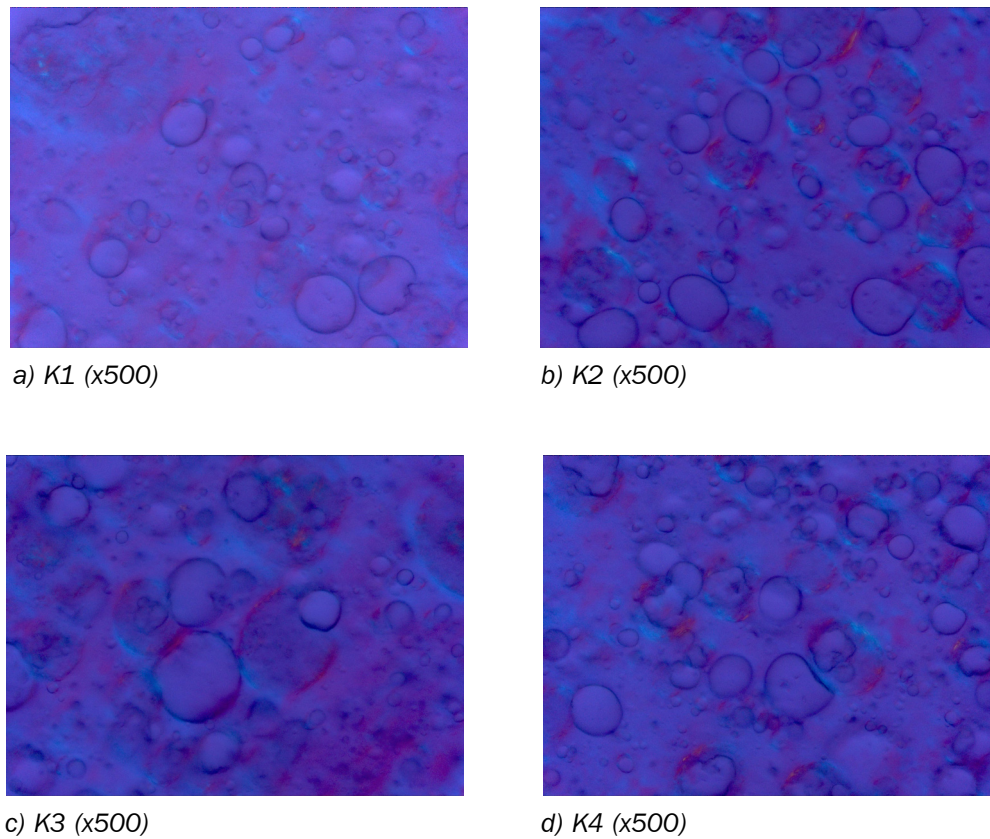


Figure 1. Polarization micrographs of cream samples a) K1, b) K2, c) K3, d) K4

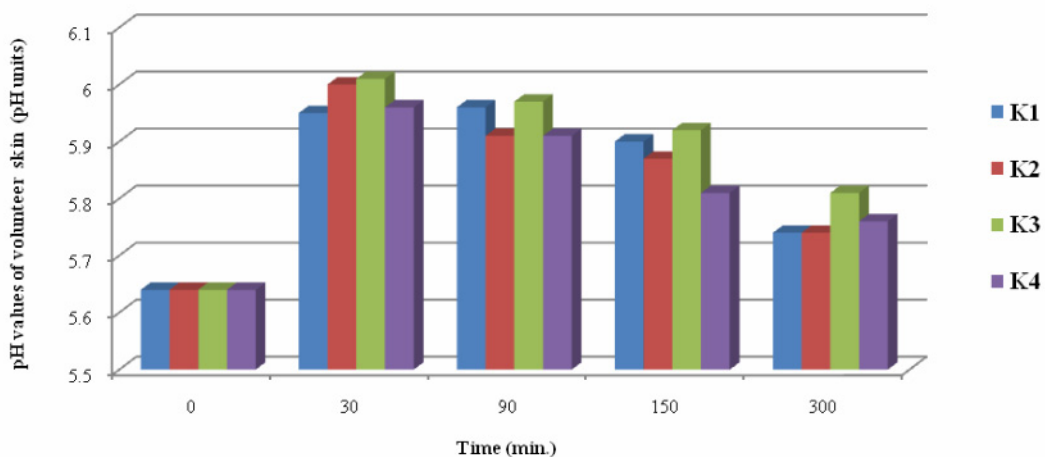


Figure 2. Changing the skin pH after a single application of the creams K1-K4

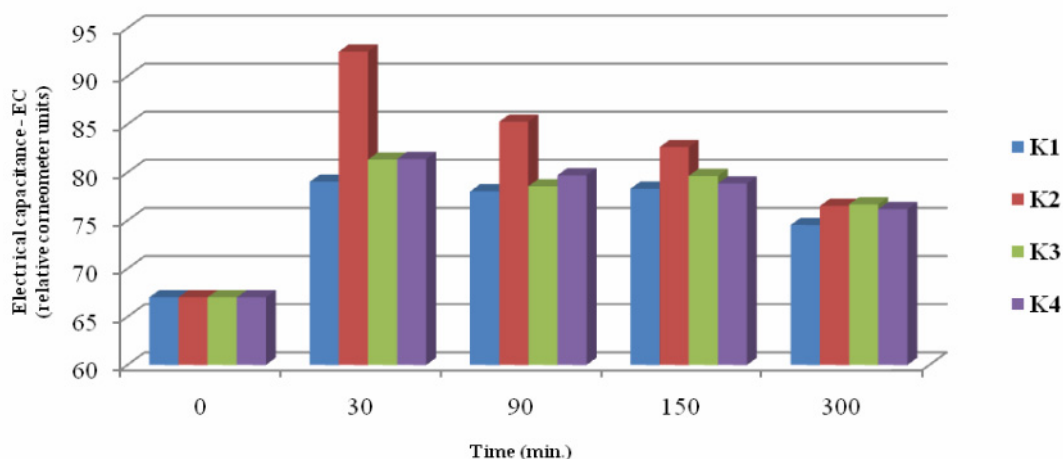


Figure 3. Electrical capacitance of the skin after a single application of creams K1-K4

The increase in skin moisture, which is achieved 30 minutes after administration of hydrophilic creams, is a consequence of the absorption of the water released to the skin surface after creams application (10, 13). That increase obviously depended on the presence of moisturizers in different creams. As our creams showed a significant increase in skin moisture level 30 minutes after application, it can be presumed that a considerable amount of water was released, which then contributed to the hydration state of the skin surface. Since all the creams do not cause the same skin hydration at the moment, it can be assumed that the emollients used affected the creams' structures and speed and amount of water which have been released immediately after application. The largest increase in volunteer skin moisture was registered 30 minutes after application of cream K2.

The gradual decline in the skin hydration level after this period occurs due to evaporation of water, and formation of a plateau in skin hydration level could be explained by the occlusive effect that product or component of oil phase exercise on the skin surface. Forming an occlusive film on the skin surface, creams prevent water evaporation from the skin surface (13).

The increase in EC compared to the value before the application was noticed after the application of the sample K1. That effect was the weakest for the cream K1, compared to the effects of the other investigated samples. We can assume that cetearyl octanoate affected the cream structure in a manner that relatively small amounts of water could be released from the cream immediately after its application to the skin. This yielded a negligible effect of the K1 cream to the skin moisture level, and thus increased slightly the skin hydration degree. Probably, this hydration level was achieved by a subsequent occlusive effect of the K1 cream (Figure 3).

Cream K2 led to a significant increase in EC (compared to baseline) 30 minutes after its application. Skin moisture, measured 30 minutes after application, was

increased to 38.11% from the baseline values, indicating that the decyl oleate has a positive effect on the emulsions structuring and significantly increases its moisturizing potential, allowing the initial release of large amounts of water and quick hydration of the skin. After this period the skin moisture decreased. There is a statistically significant decrease in skin moisture 90 min, 150 min, and 300 min after applying the cream, but the EC value after applying the cream K2 was higher compared to the other samples during the entire observation period, which indicates that the occlusive effect of decyl oleate is relatively weak, but its overall moisturizing effect is very good, probably due to strong initial hydration effect (Figure 3).

Application of the samples K3 and K4 leads to an almost similar effect on skin hydration. The highest skin moisture level was achieved 30 minutes after administration of the creams and those values were greater than after the application of the cream K1, and significantly lower than after application of the cream K2. It was reported that olive oil has a positive influence on the moisture of the skin, which was shown on healthy as well as sodium lauryl sulfas irritated skin (21). Statistically significant differences in the values of EC 300 minutes after the application of cream K3 were registered compared to the baseline values ($p < 0,05$) (Figure 3), indicating its excellent occlusive effect.

CONCLUSION

This study investigated the effects of four different emollients to the structure of the hydrophilic o/w creams stabilized with lamellar liquid-crystal phase as well as their influence on the moisture content and pH of the skin of healthy volunteers after a single application of the creams. It was shown that the nature of emollients used in the preparation of o/w creams at a concentration of 10% influenced the structure of the creams stabilized with mesophases, and significantly affe-

cted the cream ability to moisturize the skin of healthy volunteers after the single application.

Polarization microscopy of the samples indicated the presence of anisotropic structures in all tested creams, i.e. stabilization of the creams with lamellar gel-crystalline and liquid-crystalline phases, which were different for different creams, probably because of the presence of different emollients. The so-called Maltese crosses and inserted oil lines were observed in the external phase, confirming the stabilization with lamellar type of a liquid-crystalline and/or gel-crystalline phases (polarization light refracts this structures).

O/w cream containing decyl oleate, incorporated in a concentration of 10% led to the most significant skin hydration increase immediately after the application. The application of the cream with 10% of cetearyl octanoate caused the weakest skin moisture increase

during the entire treatment. The samples with C12-C15 alkyl benzoate and olive oil had a weaker initial effect to the skin moisture increase compared to cream K1, but the moisturizing effect was maintained almost constant during the entire test period (especially in sample K3). The above findings could indicate that decyl oleate led to a rapid release of large amounts of water from the cream and initially good skin hydration effect. Creams with C12-C15 alkyl benzoate or olive oil had modest initial effect on the increase of the skin moisture level (probably because they affected the structure of the creams in a way that creams did not release a significant amount of water initially), which could point to the good occlusive properties of those emollients. During the study period (300 min) they disallowed skin moisture reduction which was achieved after their application.

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ISPITIVANJE EFEKATA RAZLIČITIH EMOLIJENASA NA STRUKTURNA SVOJSTVA KREMOVA I NJIHOV EFEKAT NA VLAŽNOST I PH KOŽE

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Sažetak

Ispitivan je uticaj četiri emolijensa (cetearil oktanoat, decil oleat, C₁₂-C₁₅ alkil benzoat i maslinovo ulje) inkorporirana u kozmetički u/v krem, u koncentraciji od 10% (kremovi K1-K4, redom), na strukturalna svojstva kremova i njihov efekat na vlažnost i pH kože zdravih ispitanika nakon jednokratne aplikacije.

Struktura kremova ispitivana je polarizacionom mikroskopijom. Utvrđeno je prisustvo anizotropnih struktura, tj. potvrđeno je da se stabilizacija strukture ovih kremova vrši formiranjem lamelarne gel-kristalne i tečno-kristalne faze, koje su bile različite kod različitih kremova, verovatno kao posledica prisustva različitih emolijenasa. Ispitivanje efekata kremova K1-K4 na vlažnost i pH kože nakon jednokratne aplikacije (30, 90, 150 i 300 min. po primeni) vršeno je korišćenjem aparata MultiProbeAdapter(MPA[®]9, Courage&Khazaka Electronic, Nemačka), upotrebom odgovarajuće sonde, Corneometer[®] CM 825 za merenje kapacitativnosti, kao mere vlažnosti kože, dok je pH kože meren sondom Skin-pH-meter, PH 900. Rezultati ispitivanja ukazuju da prisustvo različitih emolijenasa u u/v kremovima, u koncentraciji od 10%, dovodi do različitog nivoa hidratacije kože nakon aplikacije, kao posledice različite strukturacije kremova, okluzivnog efekta kremova različite efikasnosti i različite brzine oslobađanja vode iz kremova. Krem sa decil oleatom dovodi do inicijalno dobrog hidrirajućeg efekta samog krema, pretpostavljamo zbog brzog oslobađanja velike količine vode iz krema. Kremovi sa C₁₂-C₁₅ alkil benzoatom i maslinovim uljem imaju slabiji početni efekat na vlažnost kože, ali pokazuju dobra okluzivna svojstva u toku ispitivanog perioda, ne dozvoljavaju pad vlažnosti kože dostignute neposredno po aplikaciji kremova. Do najslabije kratkotrajne hidratacije kože dovodi krem sa 10% cetearil oktanoata.

Ključne reči: emolijensi, hidrofilni kremovi, tečno-kristalna faza, vlažnost kože