

## NEW NATURAL SUGAR-BASED SURFACTANTS INTENDED FOR STABILIZATION OF COSMETIC/DERMOPHARMACEUTICAL VEHICLES – SAFETY AND EFFICACY ASSESSMENT

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Despite a large number of different vehicles available nowadays, conventional emulsion systems remain one of the most commonly used for cosmetic and dermatological preparations. Popularly labelled as skin- and environmentally-friendly, alkyl polyglucoside (APG) sugar-based emulsifiers have attracted considerable interest with regard to their dermatological properties, since irritation potential of commonly used emulsifiers could affect the functionality and safety of dermopharmaceutics. The aim of this study was to promote the emulsion based on C16/18 APG as a prospective vehicle for topical drugs and cosmetic actives assessing the safety for use and skin hydration capacity. In accordance with the requirements of newer legislation *in vitro*, acute skin irritation test was performed using cytotoxicity assay on artificial skin. The results were compared with *in vivo* data obtained by measuring the skin biophysical parameters, such as: stratum corneum hydration (SCH), erythema index (EI), and transepidermal water loss (TEWL). Parameters were measured prior to (baseline values) and upon cessation of a 24-h occlusive treatment in 14 healthy human volunteers. *In vivo* moisturizing capacity of the emulsions was assessed in 16 healthy volunteers in a long-term trial measuring of SCH.

This study showed, investigating the most frequently used APG, that emulsions based on these emulsifiers could probably be promoted as safe cosmetic/dermopharmaceutical vehicles. Prospective safety for human use with the correlation between *in vivo* and *in vitro* findings was shown. In addition, the investigated vehicle *per se* showed an excellent skin moisturizing capacity which is essential in maintaining healthy skin, but also in improving dermatitis, which follows most pathological skin conditions. *Acta Medica Medianae* 2014;54(1): 34-39.

**Key words:** emulsifiers, alkyl polyglucosides, dermatological preparations, cosmetics, *in vivo/in vitro* skin safety and efficacy, biophysical skin parameters, skin hydration potential

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### Introduction

Creams are a common type of delivery systems in cosmetic and dermatological preparations, although a large number of different vehicles are available nowadays (1). Particularly, emulsions stabilized by lamellar liquid crystals (LLC) are intensively investigated as carriers for various dermopharmaceutics/dermocosmetics, considering their extensive similarity to the systems found in a living organism (e.g. stratum

corneum (SC) lipid matrix), significant solubilisation capacity for lipophilic and hydrophilic substances and particularly the potential for sustained skin hydration (2,3). A formulation of emulsions presents a real challenge, since the whole series of general demands must be fulfilled: physicochemical stability, chemical inertness, efficacious delivery of the active, satisfactory aesthetic characteristics (a pleasing appearance which is retained during storage), optimal sensory attributes, pleasant skin feeling during application, the optimal skin hydration potential (4,5). Alongside the listed quality standards and expectations of demandable consumers, those emulsions must, in the first place, have a satisfactory safety profile.

If emulsion vehicles are used as drug carriers, they are then considered to be medicinal products, and they are covered by special docu-

mentation and registration requirements (6). Pharmaceutical emulsion-type topical products must fulfil the demands for the satisfactory safety profile. On the other hand, cosmetic emulsions must meet the safety requirements of the Regulation (EC) 1223/2009 on cosmetics.

Among all the pharmaceutical and cosmetic excipients used for emulsions, emulsifiers are most often recognized as potential skin irritants (7,8). The first step and a key feature in the irritation process is the interaction of the surfactant with the stratum corneum (SC) lipids. The interaction leads to the damage of the skin barrier, it reflects in the increased transepidermal water loss (TEWL) and is often followed by an inflammatory response. The application of emulsions stabilized with anionic emulsifiers is accompanied by adverse skin reactions (9), so, today, they are considered obsolete. Non-ionic surfactants, particularly polyethylene glycol (PEG)-based ones (e.g. etoxylated fatty alcohols and fatty amphiphiles), represented for a long time the emulsifiers of choice, as those with the least potential for irritancy (10) and with the ability to form lyotropic liquid crystals in the presence of water as well. However, the PEG-emulsifiers' potential to induce the barrier impairment and sporadic erythema on exposed healthy skin was indicated (9), which could be a limiting application factor. Some commonly used emulsifiers also have the ability to induce phospholipid emulsification contributing to cellular damage, which can result in cytolytic process and the release of proteins, lysosomal and cytoplasmic enzymes and inflammatory mediators (11-13). PEG-free sugar-based surfactants - Alkyl Polyglucosides (APGs) gain increasing attention due to advantages with regard to their biodegradability and health of consumers (14).

This study investigated one of the most frequently used APG mixed emulsifier - Sepineo SETM68 INCI/ Cetearyl Glucoside & Cetearyl alcohol (Seppic, France). There is insufficient data about its performances and skin safety aspects assessed by manufacturer. The aim of this study was to formulate multicomponent emulsion based on this emulsifier, and to assess its skin irritation profile and skin moisturizing capacity. Safety profile was evaluated *in vitro* using an alternative method for acute skin irritation test (a cytotoxicity assay) (15), and *in vivo* on healthy human volunteers in a 24h-study under occlusion using the measurements of the following parameters: SC hydration (SCH), transepidermal water loss (TEWL), and skin erythema index (EI) (9). In addition, we checked the *in vivo* moisturizing efficacy of this emulsion sample in short-term (3 hours) study measuring SCH.

### Material and methods

A multiphase oil/water (o/w) cream based on Sepineo SETM68 (kindly provided by Seppic, France) sugar emulsifier in the concentration of

7% (w/w) was formulated and marked as M68. The sample was adequately preserved using Euxyl K<sup>®</sup>300 (Schülke&Mayr, Germany) preservative blend. The humectant used was glycerol (BASF, Germany) at 2% (w/w). For the purpose of the preparation of the sample, emulsifier and 16% (w/w) oil phase which consisted of: isopropylmiristate (Unichemcom, Austria), caprylic-capric triglycerides (MyrtilTM 318, Henkel, Germany), decyl oleate (SabodermTM DO, SABO, Italy), mineral oil (BASF, Germany), cetearyl alcohol (Lanette 0, Cognis, Germany) and dimethicone (Abil<sup>®</sup>100, Cognis, Germany) were heated at 70°C, and then added to the preserved water phase at the same temperature by stirring (stirrer RW16 basic, IKA<sup>®</sup>WERKE, Germany) at constant temperature for 5min (800rpm). Cooling was started while mixing at 500rpm 3min, then at 300rpm to the room temperature.

For the cell culture experiments, human dermal fibroblasts from the foreskin of newborns were used. These cells were obtained from Cascade Biologics (Mansfield, UK), cultured according to standard conditions and used from the third to the twelfth passage. Immortalised keratinocytes from the HaCaT-cell line (Human adult, low Calcium, elevated Temperature) were used according to a standard cell culture method during passages 68-84 (16). For cytotoxicity (MTT) experiments, 3-(4,5)-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma, Steinheim, Germany), sodium dodecylsulphate (Acros, B-Geel), isopropanol (RiedeldeHaën, Seelze, Germany), hydrochloric acid (Merck, Darmstadt, Germany) and freshly double distilled water were used. Artificial skin construct (ASC) was manufactured and a modified version of Mosman's method (17) was used for the *in vitro* acute skin irritation test-a cytotoxicity assay as previously described (3).

Additionally, an *in vivo* bioengineering study was also performed. Skin parameters TEWL, EI, SCH were measured prior to (baseline values) and 60min upon cessation of 24h occlusive treatment. Fourteen healthy female volunteers (27.4±1.1 years) were recruited. The flexor side of their left forearms was treated with investigated emulsion using precisely delineated and marked cardboard ruler (with empty spaces in the form of rectangles, 16cm<sup>2</sup> each). Two additional sites were left as a non-treated control under occlusion (UCO) on the right forearm and without occlusion (UC) on the left. Sample was applied in quantity of 0.016g/cm<sup>2</sup>, spread vigorously with rubber glove, and immediately covered with Parafilm<sup>®</sup> and then with cotton adhesive tapes. All parameters were measured according to the published guidelines and documents (18).

To estimate the moisturizing efficacy of the same sample *in vivo* by measuring EC, additional group of 16 healthy volunteers (24.2±1.6 years) was recruited. They all took part in short- (3h) term trial. The flexor side of their left forearm was treated with the emulsion sample using cardboard ruler as described. One rectangle was left as an

untreated control (UC) on the right forearm. The amount of applied sample was 0.016g/cm<sup>2</sup>, and the measurements conducted were: baseline, 1,2, and 3 hours after application.

In vivo measurements were performed in accordance with the Declaration of Helsinki, and the volunteers signed a written consent. They were informed of the study and instructed not to use any skin cleansing or skin care product on the test sites for the whole week before the study, and during the experiment. The study was approved by the local Ethical Committee on Human Research. All subjects had healthy skin and no known allergy to any ingredient of the sample. Before any measurements were taken, the subjects were asked to acclimatize for 30min under controlled conditions (21±1°C and 50±5% RH). TEWL was measured using Tewameter®TM 210, EI using Mexameter®MX 18, and SCH by means of Corneometer®CM 825 (all Courage + Khazaka, Germany).

All data were presented as means +/- standard error of the means (SEM). In vivo measured parameters (SCH, TEWL, EI) were expressed as absolute changes to baseline ( $\Delta$  values) values, second versus first day for irritation testing. The values obtained for M68 to UCO and UC were compared using one-way ANOVA, followed by Tukey's t-test, where appropriate. For moisturizing efficacy testing SCH was presented as percent change compared to baseline for M68 and UC. SCH for the sample in distinct time points was compared using a paired sample t-test.

The differences were accepted as statistically significant at  $p < 0.05$ . Statistical analysis was performed with commercial statistical software SPSS for Windows 13.0.

**Results**

The sample was applied to the ASCs in three different concentrations: 0.25%, 2.5% and 25%. The results for the in vitro skin irritation test are presented in Figure 1.

The results for in vivo investigation of skin irritation potential are shown in Figures 2a, 2b. Parameters were shown as  $\Delta$ TEWL,  $\Delta$ SCH and  $\Delta$ EI - absolute changes of mean values for treated

sites and untreated controls, after 24h occlusion related to the baseline values. All participants reported strict compliance with the initially given instructions.

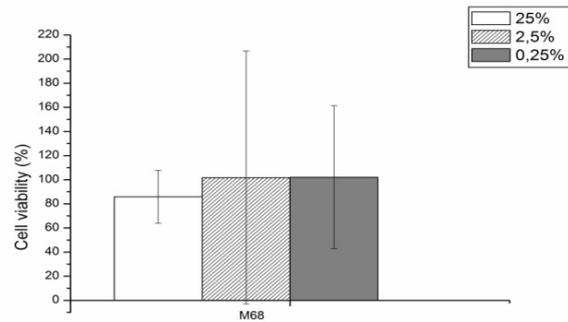


Figure 1. In vitro skin irritation test, concentration-viability histograms for the sample M68

The results of the moisturizing efficacy of the sample M68 are presented in Figure 3. The application resulted in a significant improvement of skin moisture (increase of SCH), compared to both UC and baseline in a short-term study.

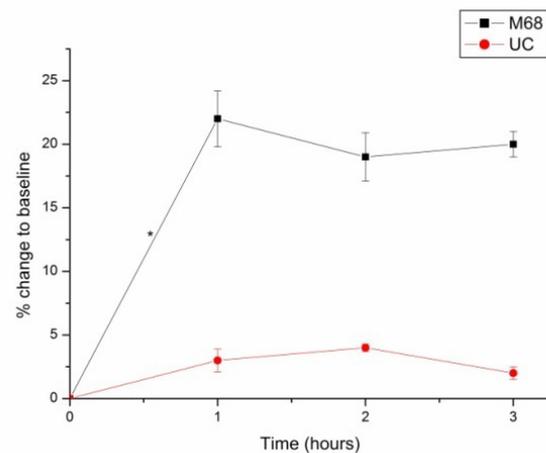


Figure 3. The effect of topical application of the sample M68 related to baseline (percent change) in a short-term study (SCH measurements). Significant differences ( $P < 0.05$ ) are marked with (\*)

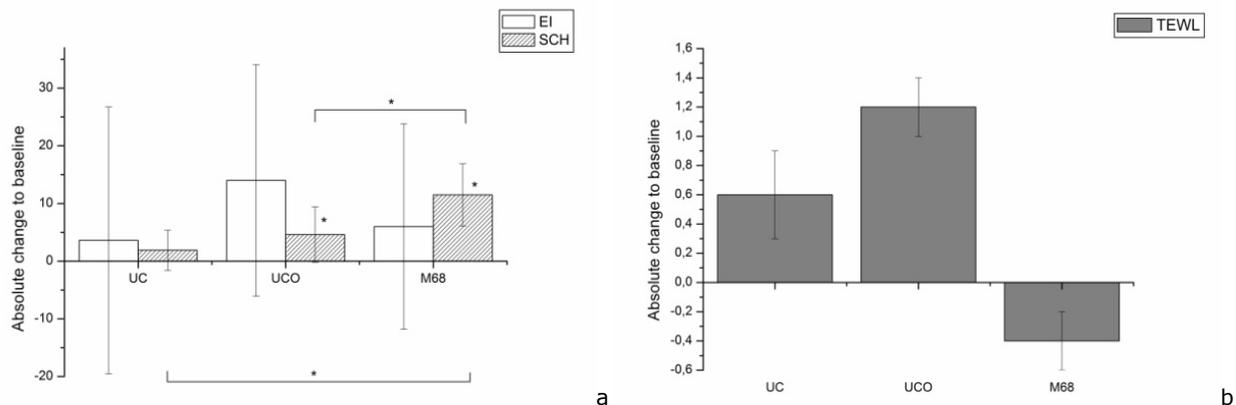


Figure 2. In vivo skin irritation test - the influence of the sample M68 on parameters a) SCH, EI and b) TEWL and both controls (under occlusion UCO and without occlusion UC); the results are shown as absolute changes of mean values on the second vs. first day and standard error of means. Significant differences ( $P < 0.05$ ) are marked with (\*)

(\*) for denoting the differences between the baseline and obtained values, and also to mark the mutual differences.

## Discussion

Dermal irritation is defined as the production of "reversible damage of the skin following the application of a test substance for up to 4 hours". It was generally assessed by the potential of a certain substance/product to cause erythema/eschar and/or oedema after a single topical application on rabbit skin and based on the Draize score (19). In fact, skin irritation profile of topicals and raw materials was, alongside other aspects of toxicity, assessed routinely and reliably using *in vivo* animal testing until recently. Ethical concerns involving the use of laboratory animals, the validity of animal skin as human skin models, and the need for more efficient validation methods have promoted the development of alternative methods to assess irritation (15). Moreover, the sales ban on animal-tested cosmetics prohibits the sale on the EU market of any cosmetic product that has been tested on animals or using alternative methods other than those validated by the European Centre for Validation of Alternative methods (ECVAM) or the Organisation for European Economic Co-operation and Development (OECD) (20). Nowadays, there are alternative ways to perform irritancy tests. The artificial skin constructs are being used, employing alternative method for acute skin irritation test (cytotoxicity assay) validated by ECVAM (21). In order to compare the value of this *in vitro* test with the actual outcome, parallel *in vivo* tests on humans should normally be employed (22).

In accordance with the requirements of current legislation, the determination of skin irritation potential of APG based emulsion was performed in our study using both described *in vivo* and *in vitro* methods, which is compulsory in order to draw a definitive conclusion on irritation potential of the product. In this study, an *in-house* three dimensional human skin model, involving the reconstructed epidermis with functional stratum corneum, was used for *in vitro* study. More precisely, quantitative cell viability was determined as a percentage of negative control and is used to predict the irritation potential – if the mean cell viability is less than or equal to 50%, a product can be defined as a local irritant; otherwise, its skin irritation profile could be characterized as favourable (19) which was shown for our sample (Fig. 1).

Regarding *in vivo* measurements of the skin, there is not a single biophysical parameter efficient to embrace fully all pathophysiologic aspects of skin irritancy. A multi-parametric approach in the assessment of the skin irritation potential of topical emulsions is proposed when employing biophysical measurements (14). The effects of irritants have previously been studied by measurements of transepidermal water loss (TEWL) and skin color (23). Since the increase in TEWL is observed after the application of skin irritants,

TEWL measurements are often used in support of cosmetic claims of product mildness (18). Erythema index (EI) measured by Mexameter<sup>®</sup> was shown to be an adequate parameter for objective evaluation of skin irritation (24).

Investigated APG- based emulsion have shown increased EI value after application, but no statistically significant change was found (Fig. 2a). This could support the claim that the investigated emulsion is not expected to cause immediate irritation of the skin. At the same time, the emulsion efficiently hydrated the skin which is shown as the SCH increase after 24h (Fig 2a).

Barrier function of the skin is dependent on the intercellular lipid phase of SC; it has been shown that changes in the lipid content and organization of the intercellular lipids influence the performance of the barrier (23). After the application of the APG-based emulsion, the decrease of TEWL was noticed (Fig. 2b), and it could be interpreted as a repair of the barrier function. This could probably be attributed to the specific, skin-friendly colloidal structure of those emulsion systems. It was shown that APG-based vehicles mimic SC organization well, as the result of lyotropic liquid crystals formation (3,15). Similarity between the emulsions' liquid crystalline structure and the structure of physiological skin lipids, rather than a simple deposition of lipid material to the surface, is probably responsible for the decrease in TEWL, that is, for a "strengthening" of the SC barrier. It is possible that this beneficial effect is also the result of the increased skin hydration, which consequently improves SC elasticity.

*In vivo/in vitro* investigations in this study showed the absence of skin irritation, pointing to the satisfying skin safety profile of this emulsifier and additional APG-based emulsions benefits to the skin.

Topical moisturizers are essential in enabling the skin to stay intact as well as in the topical treatment of some diseases such as psoriasis, ichthyosis, atopic dermatitis (18). Thus, it could be of great benefit for emulsion vehicle to show good skin moisturizing potential and to hydrate the skin even a few hours after its topical application. Regarding our investigation, three hours after the application of the sample M68, significantly higher SCH compared to baseline was measured, indicating significant potential of the sample M68 for prolonged skin hydration (Fig. 3).

## Conclusion

Overall, this study showed that C 16/18 APG-based vehicles could be promoted as safe for cosmetic/dermopharmaceutical use. So far, there has been a considerable body of evidence to support the claim that APG emulsifiers, particularly in the form of emulsifying waxes, could be used in the formulation of advanced, sophisticated phar-

maceutical and cosmetic emulsion-type topical products. Comparison of the results from in vivo skin irritation potential assessment for APG-based emulsions with complementary in vitro cytotoxicity assay from this study reveals a correlation between the two confirming that topical emulsions based on the most commonly used APG mixed emulsifier are not expected to cause irritation of the skin, when used in concentrations correspon-

ding to the therapeutic need, i.e. to the need of the cosmetic treatment. Besides being mild to the skin, the investigated agent plays an ameliorative role in improving the overall health of the skin barrier. Its use could be even taken into consideration when formulating emulsion vehicles for cosmetic actives and drugs with unfavourable safety profile.

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## NOVIJI PRIRODNI ŠEĆERNI EMULGATORI NAMENJENI IZRADI NOSAČA ZA DERMATOLOŠKE LEKOVE I KOZMETIČKI AKTIVNE SUPSTANCE – ISPITIVANJE BEZBEDNOSTI I EFIKASNOSTI

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Emulzije su najčešće korišćeni nosači u izradi kozmetičkih proizvoda i dermatoloških lekova. Emulgatori, obavezni sastojci emulzija, mogu da poseduju visok potencijal da iritiraju kožu i oštećuju njenu barijeru. Nasuprot konvencionalnim, alkil poliglukozidi (APG) kao noviji, blagi, biodegradabilni emulgatori, danas privlače veliku pažnju. Smatra se da, osim što ne iritiraju, pokazuju dodatne pozitivne efekte pri lokalnoj aplikaciji kao što je vlaženje kože. Cilj studije bio je ispitivanje emulzija stabilisanih jednim od najčešće korišćenih C16/18 APG, i to u smislu bezbednosti za upotrebu i potencijala za hidrataciju kože. U skladu sa najnovijom zakonskom regulativom u ovoj oblasti, potencijal za lokalnu iritaciju određen je in vitro testom citotoksičnosti na veštačkoj koži, kao i in vivo merenjem biofizičkih parametara kože na humanim dobrovoljcima: električna kapacitivnost (SCH), eritema indeks (EI) i transepidermalni gubitak vode (TEWL). Merene su bazalne i vrednosti parametara 24h nakon aplikacije uzorka okluzijom. Kapacitet uzorka da efikasno i produženo hidratiše kožu određivan je in vivo merenjem parametra SCH nakon jednokratne aplikacije.

Ispitujući emulzije stabilisane jednim od najčešće korišćenih APG, studija je pokazala da se ovi emulgatori verovatno mogu smatrati bezbednim u izradi kozmetičkih/dermofarmaceutskih nosača, pri čemu su rezultati in vivo i in vitro ispitivanja bili su u međusobnom skladu. Nije registrovan iritacioni potencijal korišćenog APG, inače čest problem pri upotrebi konvencionalnih emulgatora starijih generacija, a pokazan je odličan potencijal emulzija sa C16/18 APG za efikasnu i prolongiranu hidrataciju. Poslednje može biti od velikog značaja u tretmanu zdrave, ali naročito kože zahvaćene dermatitisom koji je pratilac većine patoloških stanja kože. *Acta Medica Medianae 2015;54(1):34-39.*

**Ključne reči:** emulgatori, alkil poliglukozidi, dermatološki lekovi, kozmetički proizvodi, in vivo/in vitro bezbednost i efikasnost na koži; biofizički parametri kože; hidratacija kože