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Residual film formation after emulsion application: understanding the role and fate of excipients on skin surface

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Abstract

This study focuses on the fate of excipients contained in topical emulsions once applied on the skin. The aim was thus to develop a methodology to characterize the residue left on the skin **shortly** after emulsion application. To this end, both the role and the impact of the different excipients on the formation and properties of the residue left on the skin surface once a product is applied were investigated. To that purpose, an O/W emulsion composed of an ester as oily phase, an emulsifier (alkylpolyglucoside-based vehicles), a polymer and a humectant (hydrophilic excipient) was first developed. Then, systems with fewer ingredients were prepared to understand their respective role in the residual film. This residual film was studied *in vivo* by means of biophysical instrumental methods, all being performed on the **participants'** forearm. Results highlighted the major role of the ester giving a bright and hydrophobic residue. While the surfactant structuration as the presence of glycerin and polymer provided a specific water distribution inside the residue on the skin surface. Finally, this work evidenced the ingredients organization in the residue depending on the systems

composition, with a particular stratification on skin surface which could be considered in the formulation strategy for efficient **active** delivery and skin protection.

Highlights

- In vivo biophysical methods to describe the residue left on skin
- Influence of the emulsion composition on the residue properties
- The aqueous phase is still present on skin 15 minutes after emulsion application
- The oily phase forms a durable hydrophobic and bright film on skin surface
- Ingredients stratification on skin surface after application the emulsion

Keywords

Excipients-skin interaction

Residual properties

Alkylpolyglucoside-based topical vehicles

Lamellar gel phase/ liquid crystals

In vivo characterization

Biophysical techniques

Water distribution

1. Introduction

As the extreme surface of the body, the skin is in interaction with the environment and thus with possible skin-damaging factors such as pollution, UV light, etc. Among other effects, applying a topical product on **the skin allows to moisturize, smoothen and also to bring radiance and a pleasant feel to the skin¹**. In addition to that, it can also prevent **skin damages** and protect the skin against the environment or diseases by providing overtime protection of skin surface or by efficient active delivery by using adapted excipients and vehicles^{2,3}. During and after application different phenomena occur, such as the evaporation and the absorption/penetration **of ingredients contained in the product. However, some ingredients can remain on the surface and form a residual film onto the skin**. Characterizing the corresponding film is clearly essential to allow understanding the interactions of the different excipients of the dermocosmetical or pharmaceutical products with the

skin and also their individual role in the film characteristics³. Indeed, the penetration and efficacy of the actives are widely studied in the literature but the role and fate of excipients combination on skin are poorly investigated⁴. Studying this combination of excipients on skin is however of great challenge for the future formulation strategy in order to formulate more efficient and protective products for the skin by providing a protective film or by promoting efficient active delivery.

In the literature, the pick-up and application phases of a topical product are more and more studied⁵⁻⁷ but until now only a few studies deal with the after application phase and the fate of the product on the skin^{8,9}. In this latter case different instrumental methods can be envisaged to characterize the residual film left onto the skin. For example, Lukic et al. used a combination of two biophysical methods, namely Corneometer® and Sebumeter®, to assess the influence on the skin of four distinct emulsions, each containing a different emollient. They showed that one hour after emulsion application on the skin, the skin hydration increased from 44a.u to 55a.u (arbitrary unit). Besides, the Sebumeter® measurements allowed evidencing the presence of oily material at the skin surface; nevertheless, this latter result was linked to oily compounds present in the residue left on the skin surface after emulsion application rather than an increase in skin sebum. Through this work, the authors evidenced that **shortly** after sample application on the skin it is possible to measure features of a residual film using biophysical methods like Corneometer® and Sebumeter®.¹⁰

Another way to characterize the effect of a product on the skin is the infrared spectroscopy that **provides information** on chemical **changes of** the skin surface and, potentially, on other skin parameters such as skin hydration^{11,12}. As an example, Prash et al. showed an evolution of skin hydration after application of different emulsions on the skin using FTIR spectroscopy. The authors consider that the calculation of the height ratio of amide 1 and amide 2 bands, both being related to skin proteins structures, gives an insight on skin hydration. They could show an increase in skin hydration 6h and 12h after emulsion application on skin surface; IR spectroscopy is indeed a relevant method to study the skin changes after topical product application.¹¹

The application of a product on the skin surface modifies its surface physical chemistry properties. In order to study the influence of the product on those properties, the contact angle method can be used. Indeed as the skin presents a monopolar basic behavior, the deposition of a water drop on the surface gives a contact angle from 90 to 110°¹³. Hagens et al. used this method to characterize the water-resistance of sunscreen products, 20 minutes and 60 minutes after application on the skin. A sharp modification of the water contact angle depending on the type of product applied **was observed**, with a contact angle of 20° for phase inversion temperature formulation, 40° for water in oil and 60° for oil in water emulsions¹⁴. This work clearly shows that this method is discriminant as it brings information on how a product can modulate skin surface characteristics and, finally, its efficacy.

To summarize, these studies showed that different methods such as biophysical methods, IR spectroscopy and contact angle measurements can be used to characterize skincare product effects onto the skin surface. However, no study yet has developed a methodology combining all these approaches with a common protocol to characterize the residual film on the skin surface. Besides, as illustrated by the literature, complex products are generally used within such studies **making it impossible to assess the role of each ingredient; thus, using more simple mixtures would enable to understand the role of the ingredient after product application. Indeed, characterizing the residual film would help to understand the interaction and durability of the ingredients on the skin surface in order to efficiently protect it.**

The aim of this work was twofold. First, an innovative methodology was developed to characterize the residual film left after application of different emulsions on the skin surface. This enabled thereafter to discuss the selectivity of the analytical methods regarding either a property or a given ingredient. Then, the results obtained highlighted the influence of the different ingredients contained in the systems applied, on the properties of the residues remaining on the skin. For this purpose, different systems were prepared, containing from two to six ingredients, representing

101 classical topical excipients. The study focused on the main ingredients composing an O/W emulsion:
102 surfactant, oil, humectant, polymer, water and their combination. The surfactant chosen is an
103 alkylpolyglucoside (approved by the Food and Drug Administration as a pharmaceutical excipient)
104 that showed to have interesting potential as a vehicle for drug delivery^{15,16} and to enhance skin
105 hydration¹⁷. Then, lipophilic and hydrophilic excipients, a penta ester and the glycerin respectively,
106 were added to form binary, ternary or more complex systems and their effects on the residue
107 properties were investigated. The selectivity and quantification of ingredients was measured *in vivo*
108 using different methods described below in order to understand their interaction and durability on
109 skin surface.

110

2. Materials and methods

2.1. Materials

2.1.1. Preparation of the systems

Pentaerithrityl tetraethylhexanoate (Dub PTO) from Stéarinerie Dubois, France was used as emollient, Cetearyl glucoside / cetearyl alcohol (Montanov 68) and Phenoxyethanol and Methylparaben and Ethylparaben (Sepicide HB) from Seppic, France as emulsifier and preservative system respectively, and xanthan gum powder (Rhodicare T) from Rhodia, France as a texturing agent. All were kindly given by suppliers. Finally, glycerin as a humectant was purchased from Aroma Zone, France. The ingredients used in this study are of cosmetic grades to enable their use on the skin.

Table 1: Name and compositions of the systems

INCI Name	Function	S	S_G	Emulsion	Emulsion_G	Emulsion_G_X
Cetearyl glucoside / cetearyl alcohol	Surfactant (S)	5	5	5	5	5
Pentaerithrityl tetraethylhexanoate	Oil	-	-	20	20	20
Water	Water	94.5	89.5	74.5	69.5	68.5
Glycerin	Glycerin (G)	-	5	-	5	5
Xanthan gum	Polymer (X)	-	-	-	-	1
Phenoxyethanol and Methylparaben and Ethylparaben and Propylparaben and Butylparaben	Preservative	0.5	0.5	0.5	0.5	0.5

Five different systems were prepared with different compositions. The first system was composed of just the surfactant and water and then more complex systems were formulated with the addition of the glycerin, oil or the xanthan gum. S refers to the surfactant alone in water, while S_G corresponds to the surfactant in an aqueous phase composed of water and glycerin. Then 3 successive systems of

o/w emulsions were prepared: starting with the ternary system with surfactant, oil and water and then with the addition of glycerin; and xanthan to form respectively the Emulsion_G and Emulsion_G_X.

Emulsions were prepared according to the following protocol: first the oil phase (emulsifier and oil) and the aqueous phase (water) were heated separately up to 75°C under mechanical stirring. Then the oily phase was added to the water phase and homogenized at 11000 rpm for one minute using a T25 digital ultra-turrax (IKA, Freiburg, Germany) equipped with the dispersing head S25N-25F. The formulation was then left cooling down to 30°C under mechanical stirring. Finally, both the xanthan gum pre-dispersed in glycerin and preservative were added to the mixture^{18,19}.

2.2. Methods

In accordance with the Declaration of Helsinki, 9 healthy female volunteers, aged from 22 to 29 years old were recruited, informed of the study and asked to sign a written consent. The study was approved by the local Ethical Committee on Human Research. All the measurements were made *in vivo* with the participation of the 9 assessors. For each assessor, two squares of 4cm x 5cm were drawn on each forearm and 50 µL of product was applied by the assessor in 50 uniform rotations. The measurements were performed before (Control) and fifteen minutes after sample application to evaluate the residual film properties. All measurements were made by the same operator. Based on protocols and guidelines of the literature^{14,20-22}, a communal protocol was established for all the methods and some adjustments were needed to characterize the residue on the skin surface. Indeed, the time after application chosen for the residual properties characterization was of 15 minutes, as an evaporation phase takes place in the first 10 to 15 minutes after application of the product^{23,24}. Furthermore, the EEMCO guidelines recommend measuring the skin hydration at least 30 min after product application on the skin to avoid the measurement of the residual product present on the skin shortly after application²². That is why it was decided in this study to perform the measurements 15min after product application with the assurance to measure the residue left by the product.

2.2.1. Biophysical measurements

The biophysical measurements were taken under controlled temperature (20-22°C) and humidity (40-45%). The assessors were left acclimating for fifteen minutes prior to measurement in order to control emotional and physical factors that could disrupt the results.²²

2.2.1.1. Skin surface brightness. The skin brightness was measured using a Glossymeter® GL 200 (Courage-Khazaka electronic GmbH, Köln, Germany). **The probe emits white light created by LEDs and then measures the reflected light on the skin surface²⁰.** From a practical point of view, the probe was applied and pressed for a few seconds onto the skin surface; then the gloss value was measured on bare skin and 15 min after product application. Analyses were performed in triplicate for each application zone. Gloss result is given in arbitrary unit.²⁰

2.2.1.2. Oily material amount on skin surface. The amount of oily material on the skin surface was measured using the Sebumeter® SM 815 (Courage-Khazaka electronic GmbH, Köln, Germany). The probe is pressured on the skin surface for 30s to allow the oily substances present on skin surface absorbing onto the tape making it more or less transparent. Then, tape transparency is measured by a photocell and the device gives the amount of oily materials on the skin expressed in $\mu\text{g}/\text{cm}^2$. A dose-response curve was established for the ester chosen as oil in this study. To this purpose, different doses of the ester, ranging from 2 to 10 μL , were applied on the skin with the finger in 50 uniform rotations by the assessor. A curve presenting the Sebumeter® response ($\mu\text{g}/\text{cm}^2$) as a function of the dose of ester applied onto the skin ($\mu\text{g}/\text{cm}^2$) was built and fitted through a linear regression model. The dose applied on the skin was calculated using the oil density (0.963) that was measured using a portable densimeter (Mettler Toledo, France). Oily residue quantity was measured for the different systems applied onto the skin. All measurements were made in triplicate for each application zone²⁵.

2.2.1.3. Water amount of skin surface. The amount of water on the skin surface was determined using a Corneometer® CM 825 (Courage-Khazaka electronic GmbH, Köln, Germany) which measures the changes in dielectric constant due to skin surface hydration^{20,22}. The probe is

pressured for a few seconds (5 to 10 seconds) on the skin and the result is given in arbitrary units. Each measurement was performed five times for each application zone.

2.2.2. Infrared spectroscopy.

The measurements were performed using a FTIR spectrometer Frontier (Perkin-Elmer, Waltham, USA) using the ATR accessory. The forearm of the participant was directly pressed on the zinc selenide crystal. The beam penetration depth is only of few microns for the human skin, so the method is measuring only the outermost skin layer, which is thus adapted for measuring the residual film composition on skin surface¹². For each acquisition, four spectrums were performed and averaged. The spectrum analysis was performed on the Spectrum program. Relevant peaks height was calculated in order to determine the amide 1/ amide 2 ratio that reflects the water present at skin surface. Indeed a contribution of water with the bending vibration of –OH groups (around 1642cm⁻¹) is hidden behind the amide 1 band, thus calculating the ratio enables determining the water content of superficial skin layer²⁶ with considering that the higher the ratio, the greater the water amount. The amide 1 band (1645cm⁻¹) and amide 2 (1545cm⁻¹) and the ester band (1740cm⁻¹) from the oil phase, were used to evaluate the differences between the products after application on the skin and also to compare the results before and after sample application. Measurements were made once by assessor for each sample tested.^{11,12}

2.2.3. Contact angle (CA) measurements.

The advancing contact angles were measured on the skin surface using the Portative Goniometer PGX+ (ScanGaule, Gravigny, France) connected to the PGPlus® software. Measurements were performed at room temperature (20±1.5°C). Advancing contact angle was determined as follows: first a small water drop was deposited on the skin surface; then the drop volume was increased by adding successively five drops until a final drop volume of 6µL was reached. An image was acquired at each drop deposition resulting in five pictures and the value of each CA was determined by the software program²⁷. The advancing contact angle corresponds to the highest CA value among the five

203 pictures. The measurements were repeated at least four times for each application zone and an
204 average of these four measurements was calculated.

205 2.2.4. Data analysis

206 All the values were reported in tables and graphs as mean \pm standard deviation (SD). Statistical
207 analysis of differences was performed using XLSTAT software from Addinsoft (version 2012.1.01). As
208 sufficient data were obtained to confirm the normality²⁸, one or two-factor analyses of variance
209 (ANOVA) were performed on instrumental data to evaluate the effect of the residual film on the skin
210 properties depending on the formulation applied on the skin. When discrimination between products
211 was significant ($p < 0.05$), the mean scores obtained were compared using the Fisher multiple
212 comparison post-test to form groups of products.

213 The box-plot representation was chosen for the Corneometer® and Glossometer® results expressed
214 in percentage change compared to the bare skin. The line inside the box represents the median, the
215 lower part of the box represents the first quartile *i.e.* 25% of the values are below this line, and the
216 upper part of the box represents the third quartile *i.e.* 75% of the values are below this line. The Stars
217 represent the min and max values and the square inside the box represents the mean. Besides, the
218 raw mean values in arbitrary units and the standard deviation are also presented in Table 2.

3. Results and Discussion

3.1. Composition and structure of the systems

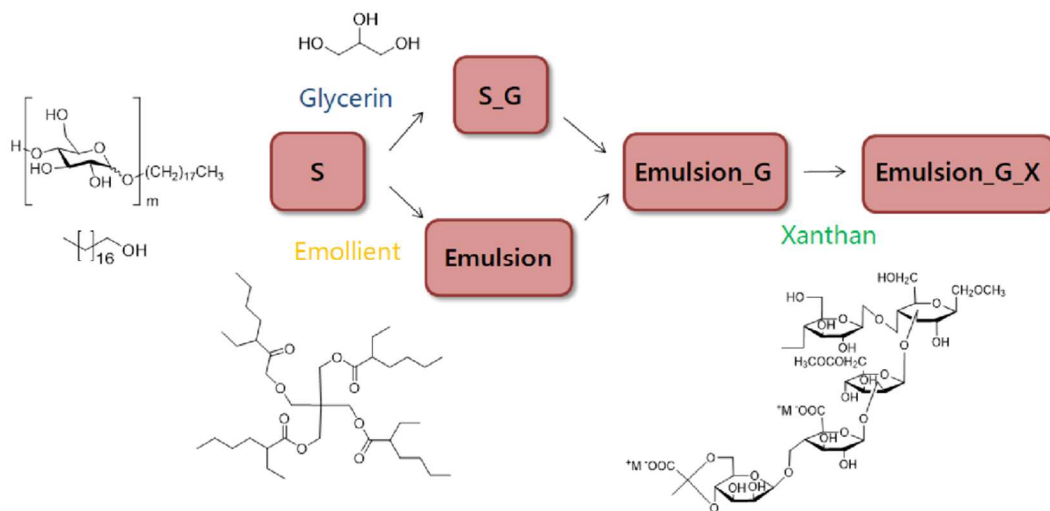


Figure 1: Presentation of the Ingredients and systems compositions with the surfactant “S” corresponding to a cetearyl glucoside / cetearyl alcohol, the emollient to a pentaerithrityl tetraethylhexanoate, the glycerin referred as “G” and the xanthan gum as “X” on the figure

The first system formulated was a solution of surfactant in water, a binary system called “S”. Then other ingredients were added gradually to this first system like an oil, a humectant and a polymer to form more complex systems as shown in Figure 1. The surfactant used in the present study is a mix of alkyl polyglucoside and fatty alcohol that forms an auto emulsion with α -gel structures²⁹ in water. The surfactant is at the interface between the water and the residual fatty alcohol; the corresponding mixture is the so-called “S”. The glycerin is a polyol that promotes hydrogen bonding in the presence of water. This is the main reason why it is currently added to topical products as a moisturizer, skin protectant and humectant as such H-bonding formation enables retaining water, so preventing evaporation in the system³⁰. Then, glycerin was added to form the S_G system: the microstructure remained unchanged as the glycerin is present in the aqueous phase. In parallel, an ester is added to the surfactant dispersion thus leading to an O/W emulsion, so-called “Emulsion”. The ester as emollient in emulsion “helps to maintain the soft, smooth, and pliable appearance of the

skin. Emollients function by their ability to remain on the skin surface or in the stratum corneum to act as a lubricant, to reduce flaking, and to improve the appearance of the skin” as described by CTFA dictionary³¹. The ester chosen in this study presents a log P of 11.4, which will be dispersed in droplets in the aqueous phase thanks to the interface stabilization with the surfactant. The addition of the ester is changing the microstructure compared to the surfactant alone in water, with the formation of lamellar liquid crystals around the droplets, together with α -gel in the continuous phase⁷. The influence of the microstructure will be further discussed below. Then a combination of these two latter systems to form the Emulsion_G system was performed, to study the influence of the combination of those two ingredients on the balance of the system properties. Finally a polymer, the xanthan gum, which is a hydrophilic polymer widely used in O/W emulsions for its thickening and stabilizing properties³² was added, thus leading to the complete mixture referenced as Emulsion_G_X. The aim was to study the influence of a well-known texturizer on the residual properties on the skin.

Then, each of these different systems was applied *in vivo* onto the skin in order to characterize the residue left after application on following the methodology described in the material and methods section; results are described and discussed in the following parts.

3.2. Characterization of the residue left on the skin

In this work, an approach using several analytical methods was developed in order to identify and get an accurate characterization of the residual film left on the skin after product application. The results of the different measurements using biophysical probes, IR spectroscopy and advancing contact angle, are described below in this section. Results from the biophysical analysis are reported in table 2.

3.2.1. Skin surface brightness

The Glossymeter® was used to characterize the skin surface brightness 15 min after application of products of different compositions. The mean results given by the Glossymeter® are gathered in table 2 with values ranging from 4.4 to 5.9 u.a. Results were computed and expressed as percentage of brightness changing when compared to bare skin after application of the different systems as presented in Figure 2.

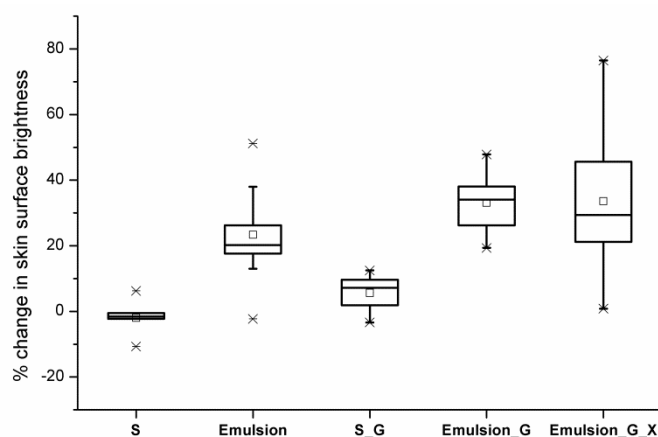


Figure 2: % change in skin surface brightness 15 min after application of the different systems on the skin compared to the bare skin.

It is striking that the residues of S and S_G systems show the lowest brightness. Indeed, the surfactant alone (S) in water does not bring any brightness, **with no significant difference compared to the bare skin**. While the addition of glycerin to the system (S_G) induces a slight increase in the brightness compared to the bare skin **with a significant difference from the bare skin**. The residues of Emulsion, Emulsion_G and Emulsion_G_X systems present the highest brightness with an increase between 20 to 50% of the glossiness **that is statistically different from the bare skin**. One can notice that each of these latter systems contain the ester and leave a bright residue on the skin. These results evidence the major role of the oil phase and the glycerin in the brightness of the residue left after application. Indeed, only emulsions containing these two components showed to be significantly different from the bare skin (Table 2). Besides, the presence of glycerin and/or polymer with the ester in the system (Emulsion_G and Emulsion_G_X) enhances the glossiness of the residue.

Therefore, the Glossymeter® is unambiguously a quantitative method allowing measuring the skin brightness and to show the presence of a bright, greasy or humid, residue at skin surface.

Table 2: Average values and statistical groups from the Fisher test for the Glossymeter® and Corneometer® measurements. Different letters in the same column means significant difference between the systems ($p < 0.05$).

Product	Glossymeter® average value (u.a)	Groups	Corneometer® average value (u.a)	Groups
Control	4.4±0.3	D	35.6±5.0	B
S	4.4±0.3	D	34.5±4.3	B
Emulsion	5.3±0.8	B	45.8±4.5	A
S_G	4.6±0.3	C	49.2±9.3	A
Emulsion_G	5.9±0.7	A	47.4±5.2	A
Emulsion_G_X	5.6±0.7	A	49.2±8.2	A

3.2.2. Water amount on skin surface

Basically, the Corneometer® measures the electrical capacitance in the upper layers of the skin, thus giving access to the *stratum corneum* hydration. The aim in this study was to measure the water amount or the electrical capacitance of the residual film remaining 15 min after application of different systems: values given by the Corneometer® as the statistical results are given in Table 2 and are presented in a graph (Figure 3), as the change (in percentage) in skin surface water amount after application of different systems compared to the bare skin.

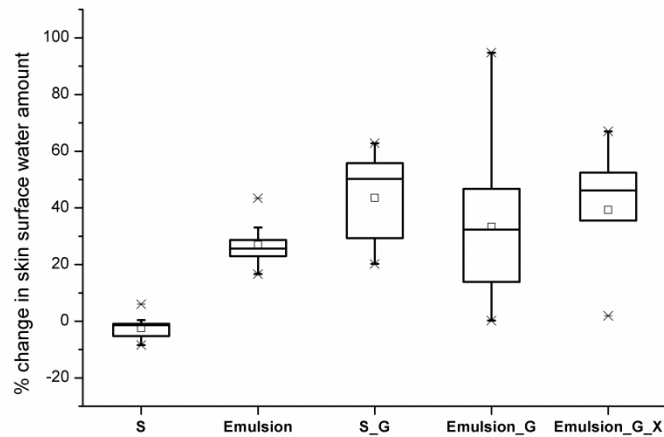


Figure 3: % change in skin surface water amount 15 min after application of the different systems on the skin compared to the bare skin.

One can observe an increase of 25% of the water amount measured between the surfactant system (S) and the surfactant-ester system (Emulsion). Indeed, the structuration of the emulsion with the oil droplets dispersion clearly impacts the water distribution in the residual film left after application as illustrated with the increase of skin water amount for the Emulsion system. Concerning the surfactant-water system (S), the water is probably weakly bonded and evaporates rapidly after application, thus leading to no significant change in the hydration state of treated skin compared to the bare skin. S_G, Emulsion_G and Emulsion_G_X systems present about a 50% increase in skin water amount compared to bare skin. Thus, adding glycerin to the systems (S_G, Emulsion_G) sharply increases the Corneometer® values; since the glycerin is a humectant, it undoubtedly explains the high amount of water measured by the Corneometer® in the residual film^{33,34}. The presence of the polymer in the emulsion (Emulsion_G_X) also gives a high Corneometer® value (around 50u.a), this latter value being slightly higher than the one corresponding to the emulsion without polymer (Emulsion_G), therefore showing its influence on the water distribution in the residual film left on the skin. Indeed, the water amount is different in every product, since additional ingredients are incorporated in the different systems, but still, the Corneometer® measurements were not significantly different except concerning the surfactant-water system (see the statistical groups in Table 2). The structure as well as the composition of the systems has undoubtedly an

impact on the water retention in the product and in the residue left on the skin, but corneometry measurements remain lowly discriminating for identifying and rank the role of each ingredient.

3.2.3. IR spectroscopy

The infrared spectroscopy acquisition was performed *in vivo*, putting directly the forearm skin of volunteers in contact with a zinc selenide crystal. The aim of this method was first to evidence the presence of the residual film and then to determine its chemical composition, 15min after application. The oil used in this study is an ester, its functional group is well differentiated from the skin components peaks at 1738cm^{-1} thus enabling easy identification of its presence on the skin as illustrated on the spectra in Figure 4.

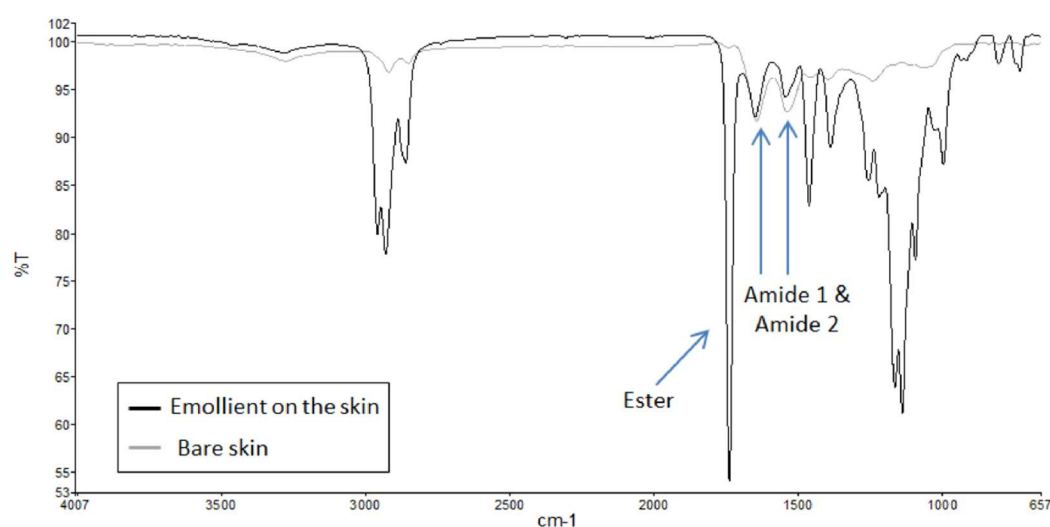


Figure 4: Infrared spectra of the bare skin (grey) and 15 min after application of the oil on the skin (black).

According to Prasch et al., the skin hydration level is accessible by IR spectroscopy experiments using the amide 1/ amide 2 ratio (Figure 4) as explained in the introduction of this work ^{11,12}. The calculation of the ratio of the amide peaks intensities for the five emulsions once applied on the skin surface was studied and reported in Figure 5.

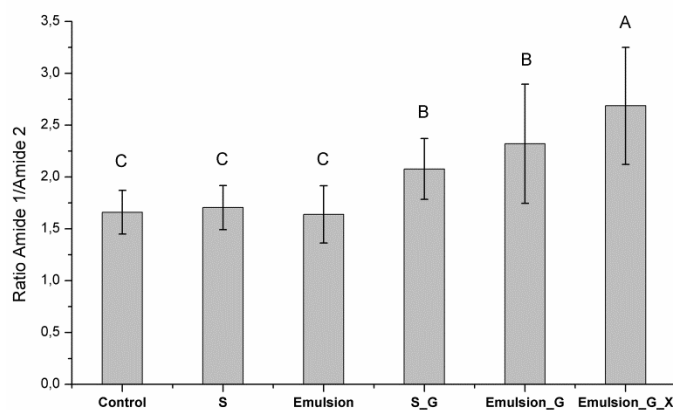


Figure 5: Skin water amount measured by the amide 1 / amide 2 band ratio for the bare skin (control) and after application of different systems on the skin. Different letters means significant difference between systems ($p < 0.05$).

The presence of glycerin in the systems studied increases this ratio, with a significant difference for the systems containing glycerine compared to the bare skin or the systems without it. Indeed, the pure glycerin presents a little contribution around 1630cm^{-1} . Thus, not only the water from the skin and the product residue on the skin contributes behind the amide 1 peak intensity, but also the presence of glycerin by itself. Besides, the emulsion structure for the complex emulsion system (Emulsion_G_X) has an impact on the ratio, in addition to the presence of glycerin. It means that at an equal amount of glycerin, the water distribution in the residual film is changing depending on the system composition, with the addition of the ester and then, in a higher extends, of the polymer. Indeed, the ratios for these two systems are statistically different showing the impact of the polymer on the residue properties.

Then the ester peak contribution of the oil phase was calculated for each system after application on the skin (Figure 6). As expected the intensity was positive only for the products containing the ester, but it interestingly varied depending on the composition of the product applied, even at constant ester content. Giving the statistical analysis, the complex emulsion, Emulsion_G_X, showed the lowest intensity for the ester function followed by the Emulsion_G and Emulsion systems presenting the highest intensity around 35%. Such a difference in the ester intensity peaks can suggest a

difference in the ester distribution in the residual film left on the skin surface, this latter depending on the system composition.

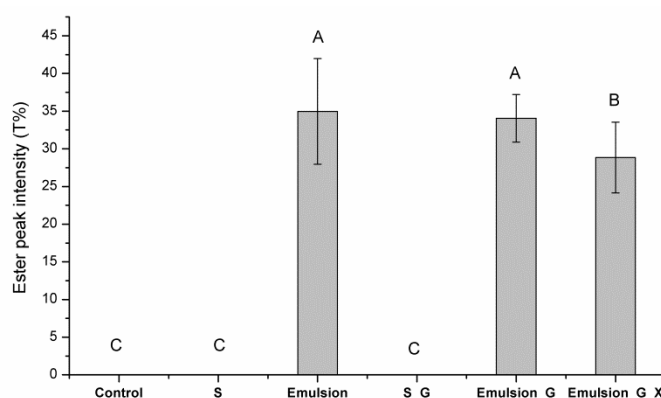


Figure 6: Intensity of the ester peak (%T) for the bare skin (control) and after application of different systems on the skin.

Different letters mean significant difference between formulations ($p < 0.05$).

As a conclusion, the IR spectroscopy is a semi-quantitative method that gives access to information for both the skin and the residue hydration, and also make it possible to identify the presence and the distribution of the ester depending on the system applied on the skin.

3.2.4. Oily material amount on skin surface

The Sebumeter® is a probe normally used to assess the sebum amount on the skin surface; nevertheless, in the present study, it was used as a method to measure the amount of oily compounds (expressed in $\mu\text{g}/\text{cm}^2$) present on the skin surface after emulsion application^{21,24}. A dose-response curve for the oil considered in this study was first built by measuring the response 15 min after application of different doses of oil on the skin using the Sebumeter® (Figure 7). A linear regression model was obtained between the dose of oil applied on the skin and the Sebumeter® values measured. This result highlights that the Sebumeter® is a quantitative method for the characterization of the oily component applied on the skin as previously demonstrated by Crowther²¹. Then the different systems formulated were tested and the results were gathered in Figure 8.

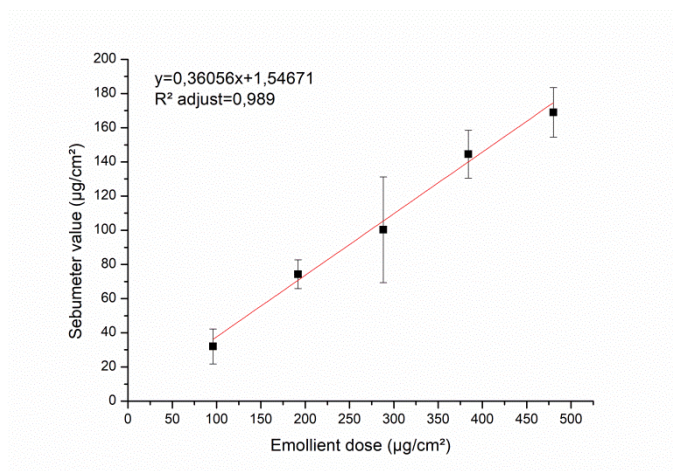


Figure 7: Dose-response curve showing the Sebumeter® value against the oil dose applied on skin in µg/cm²

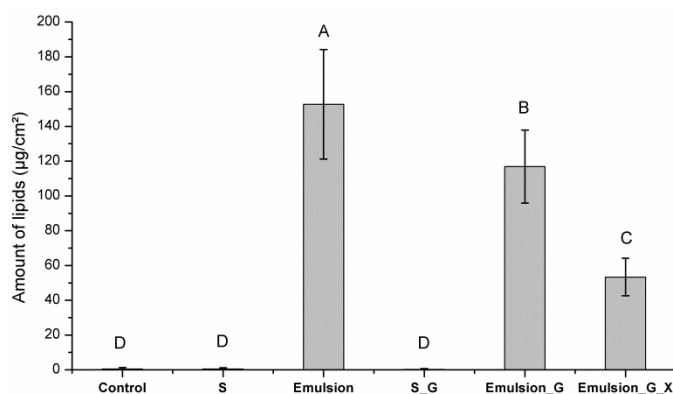


Figure 8: Amount of oily materials on skin surface measured using the Sebumeter® measured for the bare skin and 15 min after application of different systems on the skin. Different letters mean significant difference between systems ($p < 0.05$).

Firstly, products without the oil phase (S and S_G), presented the same value as the bare skin, near 0µg/cm² of oily materials. Then, results show that only the products containing the ester (Emulsion, Emulsion_G, Emulsion_G_X) induced an increase of the Sebumeter® response. Furthermore, significantly different values were obtained depending on the composition of the products from 150µg/cm² for Emulsion to 120µg/cm² for Emulsion_G and 60µg/cm² for Emulsion_G_X, even if the amount of ester was kept constant for each system (20% w/w). Thus, different results for the same amount of ester means that its distribution and more generally the organization of the different ingredients in the residual film on skin surface is significantly dependent on the composition of the

system i.e. dependent on the presence of both the glycerin and the polymer in the system in our case.

As a conclusion, the Sebumeter® is a quantitative method that can efficiently evidence the presence and the distribution of oily material in a system once applied onto the skin surface.

3.2.5. Skin surface physical chemistry properties

Advancing contact angle is currently used to study the physical chemical properties of the skin surface and therefore can be useful to characterize the changes induced by product application on the skin surface. This method consists of measuring the contact angle between a drop of water deposited on the skin surface and the surface. The skin presents a monopolar basic behavior, giving a high water contact angle ranging from 90° to 110°. Indeed, lower contact angles evidence a change in skin surface properties^{13,35}. In this work different mixtures or emulsions of various compositions were applied on the skin surface, and water contact angles were measured 15 min after application. Results gathered in Figure 9 present the advancing contact angles obtained for the five systems and the bare skin. All the systems present an average contact angle significantly different from the bare skin and the other systems.

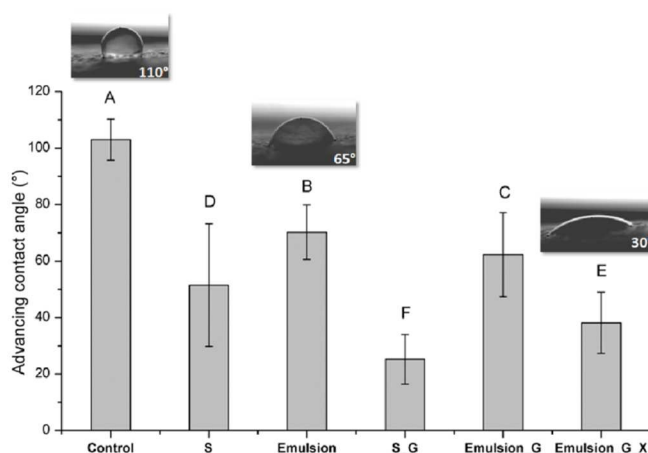


Figure 9: Graph of the water advancing contact angles (°) for the bare skin and 15 min after application of different mixtures or emulsions on the skin surface. Different letters mean significant differences between systems ($p < 0.05$).

Firstly, the surfactant alone in water (S) induced a sharp decrease in skin hydrophobia as a result of the surfactant amphiphilic properties^{36,37}. Then, adding the glycerin to this water-surfactant system (S_G) led to a highly hydrophilic character for the skin, with an angle measurement of less than 30°; these may be explained by the humectant properties of glycerin. On the contrary, the Emulsion system induced an intermediate contact angle, around 70°, which means that the ester plays a predominant role in the residue left on the skin. Indeed, the ester (oily component) is a hydrophobic compound that gives a high contact angle around 80-90° when applied alone on human skin³⁵. Then, the addition of glycerin to this system (Emulsion_G) induced a decrease of the angle around 60° due to the presence of glycerin and its properties in the formulation. Thus, the behavior of these two systems (Emulsion and Emulsion_G) again highlights the key role of the ester in the residue properties with higher angles when compared to the other systems. Finally, the complete emulsion (Emulsion_G_X), gave a hydrophilic residue, with an angle around 40°. This can be explained by the presence of the xanthan, a hydrophilic polymer that, among the other ingredients, remains on the skin surface and forms a highly hydrophilic film.

To sum up, the contact angle measurement can unambiguously evidence the role of each ingredient and their combination in the hydrophilic/hydrophobic balance at the skin surface. This technic allows understanding the changes in skin surface properties after product application depending on the system composition, with elucidating the role of each ingredient on the organization of the residue remaining on the skin surface.

3.3. General discussion

In this work, a methodology with complementary tools was developed to characterize the residual film left on the skin surface after emulsion application. Indeed, understanding the fate of these excipients once applied on the skin would provide interesting insight concerning: active

incorporation, delivery and efficiency in pharmaceutical emulsions. To this end, emulsions of different compositions were prepared and applied on the skin surface to understand the role of each excipient on the formation and the properties of the residual film. The role of the ester, glycerin, polymer and their combination will be discussed in this section.

First, results indicate that the surfactant alone in water (S) does not significantly change the skin properties as measured by both biophysical methods and IR spectroscopy. However, the contact angle measurements showed a much lower contact angle if compared to the bare skin, thus meaning a change for the skin surface properties after product application. Since the surfactant is an amphiphilic compound, it undoubtedly remains on the skin surface and influences the physical chemistry properties¹³, this without changing the skin brightness or the amount of oily materials left on the skin surface. Besides, even if the surfactant is forming an auto emulsion with α -gel structures in solution²⁹, the surfactant alone does not retain water in the residue present on the skin surface as illustrated with unaffected Corneometry and Infrared Spectroscopy results. In this case, the water contained in the mixture does rapidly evaporates after application on the skin as far as it is not retained through any specific interactions with the other ingredients. Generally through evaporation of some ingredients after application, the drug is concentrated in the film remaining on the skin and so the drying out of the formula such as this binary system can prevent drug delivery^{3,4}. This is why it is important to consider the fate of the residue. Thereby, it is important to highlight that the combined methodology developed in this work is crucial to whom ambitioning to understand the phenomena occurring on the skin surface as some methods taken alone cannot detect the presence of an ingredient on the skin while some others such as the contact angle assessment will.

Following these first observations, a ternary system combining water, surfactant and the ester oily phase (Emulsion) was studied. The results obtained showed that the Sebumeter®, the IR spectroscopy and the Glossymeter® were sensitive to the presence of the ester. Results evidenced that 15 min after spreading on the skin, it remained present on the surface. Besides, adding the ester

448 to a surfactant-water system has a strong impact on the microstructure of the emulsion^{7,38}. In fact,
449 the Corneometer® measurements, related to the water amount in the residue, presented higher
450 values in the presence of emollient in the system if compared to the system without oil phase.
451 However, the ester by itself doesn't bring any hydration or water but it is indeed changing the
452 structure of the system and thus the water distribution within the emulsion is different. As liquid
453 crystalline structures of lamellar types are formed by the surfactant (alkylpolyglucoside) around the
454 oil droplets, together with the gel phase, a portion of the water is entrapped between the lamellar
455 sheets^{17,39}. This particular structuration enables the water to remain in the system even once the
456 product is spread on the skin surface which can be important for active solubilization and efficient
457 delivery³. Indeed, Savic et al. showed that the special colloidal organization of alkylpolyglucoside-
458 based systems as the water distribution in different fractions alongside the different surfactant
459 structures have an impact on drug mobility and hence on its skin permeation¹⁶. Besides, the ester is a
460 mainly hydrophobic compound giving a high contact angle when applied alone on the skin³⁵, this
461 result clearly evidenced the role of the emollient present in the residue. Indeed, the Emulsion system
462 gave the highest contact angle values among the systems, meaning that the emollient forms a
463 durable residual film on the skin surface for the ternary system.

464 In parallel, the glycerin was added to the surfactant solution forming the S_G system and its
465 influence on the residue was studied. Adding the glycerin brings brightness, while the Corneometer®
466 as the IR spectrum showed a significant increase of hydration for this system if compared to the
467 surfactant alone in water. Since the glycerin is bonding water in the system³⁰, the residue presents a
468 high water amount. This also explains the low contact angle obtained since depositing a water drop
469 on the residue that contains more water, e.g. more hydrophilic surface, induces a lower contact
470 angle³⁵. To sum up, the S_G system is a simple system that leaves a slightly bright and highly
471 hydrophilic residue on the skin surface.

Then, the two latter systems were combined to make the Emulsion_G system in order to study the interaction of the two ingredients and the impact on the residue characteristics. A statement may be that the oil (20% w/w) would take over the glycerin (5% w/w) contribution to residue properties. Nevertheless, the results showed that the residue properties are not only linked to the ingredients respective proportions. Indeed, the Glossymeter®, IR spectroscopy and Sebumeter® analysis clearly evidenced the presence of the ester in the residue present at the skin surface, as observed for the Emulsion system. However, the residue presented some differences compared to the Emulsion system, showing the impact of the glycerin. First, the residue left in the presence of glycerin was brighter exhibiting a synergistic effect of both ingredients on the glossiness. Besides, the Sebumeter® values showed a lesser amount of oily material measured on skin surface for the Emulsion_G system, meaning that the presence of glycerin actually diminishes the detection of the ester in the residue. At this stage it was assumed that the ester was hidden by the glycerin that formed a layer on the upper part of the residue. This statement could be confirmed by the contact angle measurements as the presence of glycerin induced a decrease of the water contact angle as a proof of a more hydrophilic surface. The lower contact angle values obtained can also be linked to the Corneometer® and the IR spectroscopy (amides band ratio) results. Indeed both methods showed high residue water content and a ratio significantly different from the Emulsion system too, highlighting the role of the glycerin which may form a reservoir together with the entrapped water that increases and maintains hydration in the residual film on the skin¹⁷. These results indicate that both ingredients play a key role in the residue properties and that even a low content of glycerin plays a crucial role in the residue properties, comparable to the one of the ester.

Finally, the last system studied was the complete emulsion (Emulsion_G_X), the only difference with the Emulsion_G system being the presence of the xanthan gum; this allowed investigating the influence of this hydrophilic macromolecule on the residue. It is striking that the presence of the polymer combined with the glycerin and the ester forms a strong hydrophilic film at the skin surface as evidenced through the contact angle results. Besides, xanthan polysaccharide forms a microgel

structure in emulsions⁴⁰, entrapping more strenuously the continuous phase within its network, bringing to the film a high water amount as evidenced by Corneometer® and spectroscopy measurements. The polymer has therefore a strong impact on both the water distribution within the system and the surface residue organization. Indeed, the Sebumeter® value showed a decrease for the Emulsion_G system compared to the Emulsion, but an even lower value was obtained for the Emulsion_G_X system, even if the same amount of ester is present in these three systems. Xanthan being a macromolecule remains on the skin surface after product application thus forming a film that probably covers the ester, this latter being therefore entrapped in deeper layers of the residue. This scenario was also confirmed by the ester IR peak intensities with lower intensity for the Emulsion_G_X residue if compared to the Emulsion and Emulsion_G systems. These complementary results led to the hypothesis of a stratification of the different ingredients in the residual film on the skin surface depending on the composition of the system. The glycerin and polymer network remain at the extreme surface of the residue whereas the ester dispersion is in the deeper layers of the residue, making it less present and therefore less detectable on the surface by the different analytical methods. A schematic representation of the residual film stratification onto the skin surface is proposed in Figure 10 together with an illustration of the microstructure of the emulsion Emulsion_G_X.

Based on the entire results exposed in this paper, it is obvious that each of the different ingredients plays a role in the formation and the properties of the residue left on the skin surface. The global film properties undoubtedly depend on the composition and the characteristics of each ingredient and their combination but are not necessarily linked to their respective proportions. Table 3 reports an attempt to summarize the capacity of the different analytical methods used; it also presents the influence of the different excipients on the residual film properties characterized using each method.

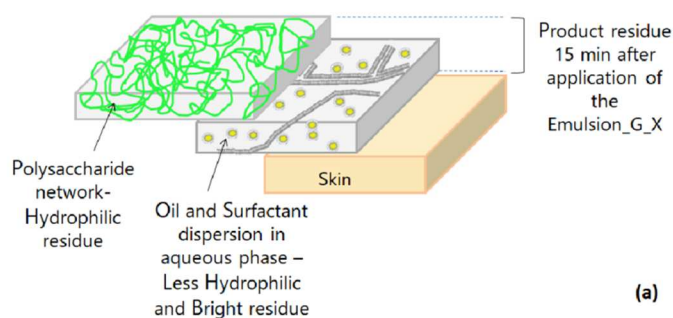
521 Table 3: Summary table of the aim and conclusions are drawn for each analytical method to study the residual film on the
 522 skin surface

Method	Characterization	Conclusion of this work
Glossometer®	Skin surface brightness	Identification of a humid or greasy bright residue- selective method of the presence of <u>ester</u> and <u>glycerin</u>
Corneometer®	Water amount on skin surface	Impact of <u>glycerin</u> on water retention - Characterization of the impact of the <u>ester</u> with emulsion structuration and changes in water distribution– but no-discriminating method (no significant differences between the systems)
IR spectroscopy	Chemical composition of residue	Quantitative and selective method of the role of <u>glycerin</u> and <u>polymer</u> in the water distribution - Identification and quantification of the <u>ester</u> with the ester band
Sebumeter®	Amount of oily materials on skin surface	Selective method of the presence of the <u>ester</u> - Give an insight on the emollient distribution in the residue depending on the composition of the system
Contact angle	Skin surface physical chemistry properties – skin hydrophilic/hydrophobic balance	Hydrophilic residue with <u>glycerin</u> and <u>polymer</u> and more hydrophobic residue in the presence of <u>ester</u> in the systems – selective method of the role of each ingredient in the organization of the residue

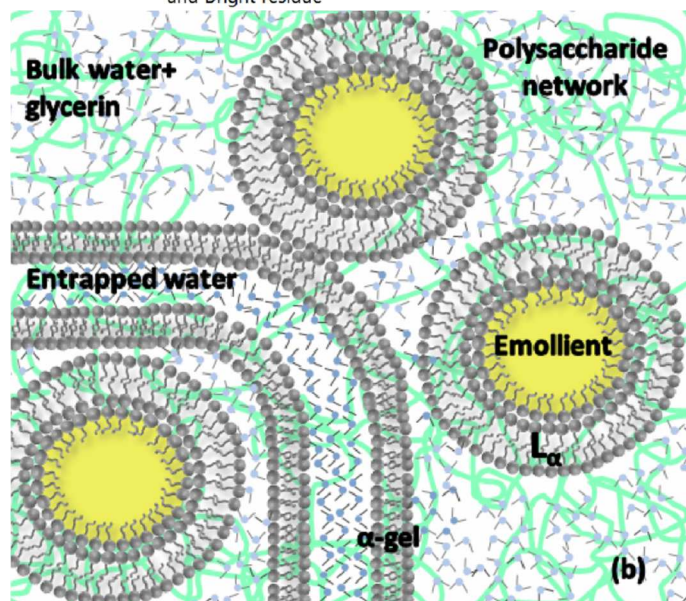
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528 Figure 10: Schematic representation of the stratification of the residual film on the skin (a) and the product microstructure
529 illustration (b)

530 4. Conclusion

531 The aim of this work was to develop an original combination of analytical approaches to characterize
532 the residue left after emulsion application on the skin, and then to understand the effect of the
533 different excipients on the residue formation and final properties. For this purpose, five mixtures of
534 different compositions and structures were prepared, and applied *in vivo* to characterize the residue
535 using the different methods.

536 Biophysical methods, infrared spectroscopy and contact angle measurements combined together
537 permitted an overall characterization of the residual film properties. Indeed, the Glossymeter®

showed the presence of a residue by characterizing the humid or oily brightness of the film left on skin surface. The Corneometer® and IR spectroscopy gave information related to the water amount present at the skin surface. Savic et al. showed that a similar surfactant to the one used in this study was capable of entrapping water within the emulsion in a specific supramolecular organization¹⁷. And interestingly, both methods, the Corneometer® and IR spectroscopy allowed evidencing the water entrapment inside the residue depending on the system composition, and therefore also pointed out the water binding in the presence of glycerin and polymer species. It means that even after spreading the product on the skin the special structure of the system, with the presence of certain components, is retaining water inside the residue on the skin surface which is of high interest for pharmaceutical excipients bases for active solubilization and delivery. Then, the Sebumeter® and a specific band on IR spectra were established to be sensitive to the oil present in the residue, as hypothesized by Crowther²¹ in his study on the quantification of different oils on the skin using the Sebumeter®.

All the results combined together clearly suggest a stratification of the residue depending on the composition of the mixture applied onto the skin surface. Among other striking observations, a lesser impact of the oil in the residual film properties in the presence of glycerin and/or polymer was evidenced. This latter observation was confirmed by the contact angle measurements that evidenced different hydrophilic/hydrophobic balance depending on the system composition, with a hydrophilic residue in the presence of glycerin and polymer on the one hand, and more hydrophobic residue in the presence of ester in a ternary system on the other hand. One can assume that depending on the system composition, specific ingredients play a predominant role in the residual film characteristics, conferring specific properties to the film left on the skin surface. The whole results of this study were obtained using our original combined methodology. This latter is indeed interesting in the formulation strategy to understand the fate of excipients once applied on the skin. This gives insight for efficient active incorporation in emulsion and solubilization once applied on the skin.

562 For future work, it would be interesting to study further the stratification by adding active and
563 testing the efficacy of these specific systems, their residence time on skin and penetration through
564 the *stratum corneum*, knowing the organization and fate of the excipients on the skin surface.

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Figures captions

- Figure 1: Presentation of the Ingredients and systems compositions with the surfactant “S” corresponding to a cetearyl glucoside / cetearyl alcohol, the emollient to a pentaerithrityl tetraethylhexanoate, the glycerin referred as “G” and the xanthan gum as “X” on the figure
- Figure 2: % change in skin surface brightness 15 min after application of the different systems on the skin compared to the bare skin.
- Figure 3: % change in skin surface water amount 15 min after application of the different systems on the skin compared to the bare skin.
- Figure 4: Infrared spectra of the bare skin (grey) and 15 min after application of the oil on the skin (black).
- Figure 5: Skin water amount measured by the amide 1 / amide 2 band ratio for the bare skin (control) and after application of different systems on the skin. Different letters means significant difference between systems ($p < 0.05$).
- Figure 6: Intensity of the ester peak (%T) for the bare skin (control) and after application of different systems on the skin. Different letters mean significant difference between formulations ($p < 0.05$).

Figure 7: Dose-response curve showing the Sebumeter® value against the oil dose applied on skin in $\mu\text{g}/\text{cm}^2$

Figure 8: Amount of oily materials on skin surface measured using the Sebumeter® measured for the bare skin and 15 min after application of different systems on the skin. Different letters mean significant difference between systems ($p < 0.05$).

Figure 9: Graph of the water advancing contact angles ($^\circ$) for the bare skin and 15 min after application of different mixtures or emulsions on the skin surface. Different letters mean significant differences between systems ($p < 0.05$).

Figure 10: Schematic representation of the stratification of the residual film on the skin (a) and the product microstructure illustration (b)

Tables captions

Table 1: Name and compositions of the systems

Table 2: Average values and statistical groups from the Fisher test for the Glossymeter® and Corneometer® measurements. Different letters in the same column means significant difference between the systems ($p < 0.05$).

Table 3: Summary table of the aim and conclusions are drawn for each analytical method to study the residual film on the skin surface



Biophysical probes



IR Spectroscopy



Contact angle

In vivo product
residue
characterization
on skin



Emulsion_G_X
residue:
Polysaccharide
network-
Hydrophilic residue

Emulsion residue :
Oil and Surfactant
dispersion – Less
hydrophilic and
bright residue

