

## **Development, characterization, and evaluation by cutaneous bioengineering of a natural emulsion, to provide a standardized vehicle base for topical compounded preparations**

**Desenvolvimento, caracterização e avaliação por bioengenharia cutânea de uma emulsão natural, a fim de proporcionar uma base veicular padronizada para preparações magistrais tópicas**

**Desarrollo, caracterización y evaluación por bioingeniería cutánea de una emulsión natural para proporcionar una base portadora estandarizada para preparaciones magistrales tópicas**

Received: 11/21/2022 | Revised: 12/06/2022 | Accepted: 12/08/2022 | Published: 12/16/2022

### **Guilherme Carneiro Leal**

ORCID: <https://orcid.org/0000-0001-9063-2009>  
State University of Londrina, Brazil  
E-mail: [guilhermeleal@live.com](mailto:guilhermeleal@live.com)

### **Isabela Mazarim da Costa**

ORCID: <https://orcid.org/0000-0002-8471-1218>  
State University of Londrina, Brazil  
E-mail: [isabela.mcosta@uel.br](mailto:isabela.mcosta@uel.br)

### **Jéssica Bassi da Silva**

ORCID: <https://orcid.org/0000-0003-0586-7802>  
State University of Maringá, Brazil  
E-mail: [jessicabassidasilve@gmail.com](mailto:jessicabassidasilve@gmail.com)

### **Rafaela Said dos Santos**

ORCID: <https://orcid.org/0000-0002-6473-4869>  
State University of Maringá, Brazil  
E-mail: [rafaelasaid@gmail.com](mailto:rafaelasaid@gmail.com)

### **Marcos Luciano Bruschi**

ORCID: <https://orcid.org/0000-0002-4838-5742>  
State University of Maringá, Brazil  
E-mail: [mlbruschi@uem.br](mailto:mlbruschi@uem.br)

### **João Carlos Palazzo de Mello**

ORCID: <https://orcid.org/0000-0002-7532-3395>  
State University of Maringá, Brazil  
E-mail: [mello@uem.br](mailto:mello@uem.br)

### **Celso Vataru Nakamura**

ORCID: <https://orcid.org/0000-0002-9911-7369>  
State University of Maringá, Brazil  
E-mail: [cvnakamura@gmail.com](mailto:cvnakamura@gmail.com)

### **Audrey Alesandra Stingen Garcia Lonni**

ORCID: <https://orcid.org/0000-0001-6498-2806>  
State University of Londrina, Brazil  
E-mail: [audrey@uel.br](mailto:audrey@uel.br)

### **Abstract**

The use of products containing natural and sustainable substances has shown a remarkable growth in the pharmaceutical and cosmetic market, such as in the compounding pharmacy. This research aimed to develop, characterize, and evaluate the cutaneous bioengineering of natural and sustainable emulsions, providing a vehicle base for topical preparations. Nine O/W emulsions were developed changing the nonionic self-emulsifying wax (Cetearyl Oliviate (and) Sorbitan Oliviate, Cetearyl Glucoside (and) Cetearyl Alcohol, Candelilla/Jojoba/Rice Bran Polyglyceryl-3 Esters (and) Glyceryl Stearate (and) Cetearyl Alcohol (and) Sodium Stearoyl Lactylate), with or without the anionic co-emulsifier (Sodium Stearoyl Glutamate). They were characterized through preliminary stability tests, rheology and accelerated physicochemical stability study. Four formulations were approved (FB1, FB2, FB3 and FB5), but only FB1 (Cetearyl Oliviate (and) Sorbitan Oliviate with Sodium Stearoyl Glutamate) was considered stable, being selected for preservative efficacy evaluation and the cutaneous bioengineering. The hydration and transepidermal water loss (TEWL) of stratum corneum were analyzed comparing with a conventional topical vehicle (Emulsifying Wax NF). The clinical study showed that FB1 improved the skin hydration with no significant changes for TEWL, but

demonstrated considered values. The FB1 could be classified as “skin friendly” and represents a promising natural and sustainable vehicle in compounded pharmacy preparations.

**Keywords:** Natural products; Compounding pharmacy; Cutaneous bioengineering; Case study.

### Resumo

O uso de produtos contendo substâncias naturais e sustentáveis tem mostrado um crescimento notável no mercado farmacêutico e cosmético, bem como na farmácia de manipulação. Este trabalho objetivou desenvolver, caracterizar e avaliar a bioengenharia cutânea de emulsões naturais e sustentáveis, proporcionando uma base veicular para preparações tópicas. Nove emulsões O/A foram desenvolvidas mudando a cera autoemulsionante não iônica (Cetearyl Oliviate (and) Sorbitan Oliviate, Cetearyl Glucoside (and) Cetearyl Alcohol, Candelilla/Jojoba/Rice Bran Polyglyceryl-3 Esters (and) Glyceryl Stearate (and) Cetearyl Alcohol (and) Sodium Stearoyl Lactylate), with or without the anionic co-emulsifier (Sodium Stearoyl Glutamate), com ou sem o coemulsificante aniônico (Sodium Stearoyl Glutamate). Elas foram caracterizadas através de testes de estabilidade preliminar, reologia e estudo da estabilidade físico-química acelerada. Quatro formulações foram aprovadas (FB1, FB2, FB3 e FB5), mas apenas FB1 (Cetearyl Oliviate (and) Sorbitan Oliviate with Sodium Stearoyl Glutamate) foi considerada estável, sendo selecionada para a avaliação de eficácia de preservação, e para a bioengenharia cutânea. A hidratação e a perda de água transepidermica (TEWL) do estrato córneo foram analisadas comparando com uma base veicular tópica convencional (Emulsifying Wax NF). O estudo clínico mostrou que FB1 melhorou a hidratação cutânea sem mudanças significativas para o TEWL, mas demonstrou valores consideráveis. A FB1 pode ser classificada como “skin friendly” e representa um promissor veículo natural e sustentável para preparações farmacêuticas manipuladas.

**Palavras-chave:** Produtos naturais; Farmácia de manipulação; Bioengenharia cutânea; Ensaio clínico.

### Resumen

El uso de productos que contienen sustancias naturales y sostenibles ha mostrado un crecimiento notable en el mercado farmacéutico y cosmético, así como en la farmacia de compuestos. Este trabajo tuvo como objetivo desarrollar, caracterizar y evaluar la bioingeniería cutánea de emulsiones naturales y sostenibles, proporcionando una base vehículo para las preparaciones tópicas. Se desarrollaron nueve emulsiones O/W cambiando la cera autoemulsionante no iónica (Cetearyl Oliviate (and) Sorbitan Oliviate, Cetearyl Glucoside (and) Cetearyl Alcohol, Candelilla/Jojoba/Rice Bran Polyglyceryl-3 Esters (and) Glyceryl Stearate (and) Cetearyl Alcohol (and) Sodium Stearoyl Lactylate), con o sin el coemulsionante aniônico (Sodium Stearoyl Glutamate), con o sin el coemulsionante aniônico (estearoil glutamato de sodio). Se caracterizaron mediante pruebas preliminares de estabilidad, reología y estudio de estabilidad fisicoquímica acelerada. Se aprobaron cuatro formulaciones (FB1, FB2, FB3 y FB5), pero solo FB1 (Cetearyl Oliviate (and) Sorbitan Oliviate with Sodium Stearoyl Glutamate) se consideró estable, siendo seleccionada para la evaluación de la eficacia de conservación y para la bioingeniería cutánea. Se analizó la hidratación y pérdida de agua transepidermica (TEWL) del estrato córneo comparándolo con una base de vehículo tópico convencional (Emulsifying Wax NF). El estudio clínico mostró que FB1 mejoró la hidratación de la piel sin cambios significativos para TEWL, pero mostró valores considerables. FB1 se puede clasificar como "amigable con la piel" y representa un vehículo natural y sostenible prometedor para las preparaciones farmacéuticas manipuladas.

**Palabras clave:** Productos naturales; Farmacia de manipulación; Bioingeniería de la piel; Ensayo clínico.

## 1. Introduction

Throughout history, the use of medicines has been an integral part of pharmacy and medical practice in the prevention and management of health. In the last century, the pharmaceutical industry has helped to make medical practice more consistent, facilitating access to medicines and considerably promoting the development of pharmacological therapy. But, in recent years it has become clear that, although the products of the pharmaceutical industry have reached almost all therapeutic areas, they have not been sufficient to resolve treatments at an individual level in relation to dosage, pharmaceutical form, and vehicle, or are not often suitable for the clinical condition. Therefore, compounded preparation is a personalized preparation, which shows its value in modern medicine, as a way of complementing or optimizing pharmacological treatment (Sánchez-Regañá *et al.*, 2013).

There is a growing demand for topical preparations in dermatology for pharmaceutical and cosmetic use. Therapy customization offers the means to better adapt treatment to individual needs, with the possibility of dosage adjustment, active ingredient associations, choice of excipients and ideal vehicle.

Topical preparations mainly consist of one or more active ingredients and a carrier or galenic base in which it is incorporated. These products are easy to apply and convenient in terms of patient or consumer acceptability and are used in dermatology both to treat diseases and to maintain healthy skin (Filipe *et al.*, 2022). These preparations exist in many forms, such as ointments, gels, creams, lotions, solutions, among others, and are generally classified by their physicochemical nature as powders, liquids, or semi-solids (Surber & Smith, 2005). Semi-solid emulsion is the most widely used vehicle to deliver cosmetic and pharmaceutical ingredients for topical application (Epstein, 2009).

When developing a topical galenic base, each of its components must be correctly selected to meet several requirements. In addition to being pharmacologically inert, it needs to be chemically stable, non-toxic, non-irritating, non-allergenic, cosmetically acceptable, easy to apply, and must easily release the active ingredients to ensure their correct absorption (Surber & Smith, 2005).

Meeting the above criteria, it is essential to carry out tests to evaluate the quality of the emulsion produced, such as the micelle size distribution, long-term physical and mechanical stability (centrifuge test, stress induced by temperature and rheology), microbiological (challenge test) and its interaction with the skin (cytotoxicity test and cutaneous bioengineering) (Lonni *et al.*, 2015).

Over the past decade there has been a growing awareness among consumers of the ingredients in skin care products they use. Cosmetic products containing ingredients from natural sources described as “natural”, “organic” or “vegetable” have been gaining attention from consumers, cosmetic industries, and organizations, which are constantly expanding (Amberg & Fogarassy, 2019; Barros & Barros, 2020; Furman *et al.*, 2022). They convey the impression of having better safety, skin compatibility and causing less impact on the environment (Ribeiro *et al.*, 2015), being trends for the future (Barros & Barros, 2020). The challenge in the market is to find a balance between “natural” and the “cosmetic product’s chemistry” (Dini & Laneri, 2021).

In Brazil, there are no rules or definitions for the classification of natural or organic cosmetics by ANVISA, and in several countries around the world there are also no such definitions by their regulatory agencies (Fonseca-Santos, Corrêa & Chorilli, 2015). One of the most used classifications in the world to define “natural” and “organic” cosmetics is according to the standards of ECOCERT, a French certification agency that has subsidiaries in several countries around the world, including Brazil. Being considered an organic or natural cosmetic product, the fraction of plant ingredients needs to be 95%, and in organic cosmetics all these ingredients need to come from organic agriculture and 50% for natural cosmetics (ECOCERT, 2012). For the remaining 5% there is a restrictive list of ingredients (including preservatives) authorized for use in small amounts.

Although pharmaceutical and cosmetic companies are adjusting to this new trend of sustainable practice and continually launching several products with natural claims on the market, a reasonable share of these products are prescribed by dermatologists in an individualized way, in the form of compounded preparation. The vehicles commonly used in these dermatological or cosmetic preparations are produced by the compounding pharmacies themselves or sold ready-made by manufacturers and suppliers in the sector. As far as we know, there is no vehicle in the national market (cream) that attests to the conditions established by the ECOCERT certification agency, so that it can be considered a “natural” vehicle. Therefore, enabling a dermatological and cosmetic vehicle that can be certified as “natural” can contribute to the consolidation and expansion of the compounding market.

In the context of dermatology and cosmetology, biophysical methods (bioengineering) of skin analysis comprise the use of different instruments that use different measurement methods. In a non-invasive way, the physicochemical characteristics of the skin and the behavior of a cosmetic product in its actual conditions of use are evaluated. The most cited

biophysical methods in scientific journals are to assess skin hydration, transepidermal water loss, skin pH, skin lipid content, skin mechanical properties and skin color (Gonçalves & Campos, 2009).

Turning the measurement practices more objective and reliable, guidelines have been published that define the technical and methodological conditions that need to be considered when performing tests using biophysical methods for skin analysis (Du Plessis *et al.*, 2013). Thus, the development of technological products of sustainable origin presents itself as an advantageous strategy for cosmetic companies and a safe option for prescribers and consumers who seek this type of consumption. Given its importance and given the lack of this type of product for the masterful market, the present work aimed to develop and evaluate topical formulations, for cosmetic and pharmaceutical use, containing natural ingredients for compounded prescription. Therefore, we sought to contribute to individualized pharmaceutical and cosmetic treatment by offering an alternative to conventional vehicles.

## 2. Methodology

### 2.1 Materials

The following ingredients were used in the formulations: Cetearyl Oliviate (and) Sorbitan Oliviate (Biovital, São Paulo, Brazil), Candelilla/Jojoba/Rice Bran Polyglyceryl-3 Esters (and) Glyceryl Stearate (and) Cetearyl Alcohol (and) Sodium Stearoyl Lactylate (I9-Magistral, São Paulo, Brazil), Cetearyl Glucoside (and) Cetearyl Alcohol (Embacaps, Rio Grande do Sul, Brazil), Sodium Stearoyl Glutamate (Embacaps, Rio Grande do Sul, Brazil), Emulsifying Wax NF (Pharmaspecial), Xanthan Gum (Biovital, São Paulo, Brazil), Tocopherol (Embacaps, Rio Grande do Sul, Brazil), Ultimate Undecane (and) Tridecane (Embacaps, Rio Grande do Sul, Brazil), Cetearyl Alcohol (Infinity Pharma, São Paulo, Brazil), Glyceryl Stearate (Embacaps, Rio Grande do Sul, Brazil), Caprylic/Capric Triglyceride (Embacaps, Rio Grande do Sul, Brazil), Dicaprylyl Carbonate (Embacaps, Rio Grande do Sul, Brazil), Glycerin vegetable (Embacaps, Rio Grande do Sul, Brazil), Propanediol (Sarfam, São Paulo, Brazil), Dehydroacetic Acid (and) Benzoic Acid (and) Benzyl Alcohol (Sarfam, São Paulo, Brazil), and Gluconolactone (Valdequímica, São Paulo, Brazil).

### 2.2 Development of formulations

Nine formulations O/W (Table 1) were prepared using ingredients validated by ECOCERT, a French certification agency, certified as a “natural cosmetic”. The type of non-ionic self-emulsifying base, with or without the addition of an anionic co-emulsifier, was used as variables. The self-emulsifying bases were Cetearyl Oliviate (and) Sorbitan Oliviate, Cetearyl Glucoside (and) Cetearyl Alcohol, and Candelilla/Jojoba/Rice Bran Polyglyceryl-3 Esters (and) Glyceryl Stearate (and) Cetearyl Alcohol (and) Sodium Stearoyl Lactylate. The co-emulsifier was Sodium Stearoyl Glutamate.

The aqueous phase (A) and the oil phase (B) were separately heated until 80 °C, using a heating platform (IKA® RH Basic 2). The oil phase was gradually poured over the aqueous phase, maintaining 80 °C, under agitation at 2,500 rpm for 3 minutes using a mechanical stirrer (Fisatom® 713D), with a dispersing element (rod) of the type centrifuge. After stirring, it was reduced to 800 rpm until reaching 40 °C. Afterwards, the preservatives of phase (C) and (D) were added, keeping 800 rpm until reaching 30 °C. The formulations were sealed and stored for 48 hours.

**Table 1** - Composition of the nine formulations, and PW as the control (FB1 to FB9), and PW as the control.

Phase	Compounds	Percentage (% w/w)									
		FB1	FB2	FB3	FB4	FB5	FB6	FB7	FB8	FB9	PW
A	Purified water qsp	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
	Vegetable Glycerin	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Propanediol	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
	Sodium Stearoyl Glutamate	.	.	.	.	.	.	0.6	0.6	0.6	.
	Xanthan Gum	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	.
B	Tocopherol	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	Cetearyl Alcohol	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	.
	Sodium Stearoyl Glutamate	0.6	.	0.6	.	0.6	.	.	.	.	.
	Cetearyl Olivatate (and) Sorbitan Olivatate	6.0	6.0	6.0	.	.	.	6.0	.	.	.
	Cetearyl Glucoside (and) Cetearyl Alcohol	.	.	6.0	6.0	.	.	.	6.0	.	.
	Candelilla/Jojoba/Rice Bran Polyglyceryl-3 Esters	.	.	.	.	6.0	6.0	.	.	6.0	.
	Emulsifying Wax NF	.	.	.	.	.	.	.	.	.	16.5
	Undecane (and) Tridecane	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	.
	Glyceryl Stearate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	.
	Caprylic/Capric Triglyceride	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	11.0
	Dicaprylyl Carbonate	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
C	Dehydroacetic Acid (and) Benzoic Acid	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
D	Gluconolactone	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Source: Authors.

### 2.3 Preliminary stability tests

The preliminary stability through organoleptic characteristics was performed with the 9 formulations developed. Subsequently, all preliminary stability tests were carried out with the formulations that presented the best physical characteristics.

#### 2.3.1 Organoleptic characteristics evaluation

The evaluation of organoleptic characteristics was observed through visual inspection, and performed in triplicate, placing 20 g of the emulsions in beakers (50 mL), and closing with plastic film. After 48 hours at controlled room temperature ( $22 \pm 5$  °C), the changes observed were: color, odor, homogeneity, and signs of instability such as creaming and phase separation. The nomenclature of the National Health Surveillance Agency (ANVISA, 2004) was followed, such as: Normal (N), no change; slightly modified (SM), presence of cream; modified (M), intense cream or discrete phase separation; intensely modified (IM), intense phase separation.

#### 2.3.2 Spin test

The spin test was made weighing 5 g of each selected formulations in triplicate and subjected to centrifugation (Baby I Fanem 206-BL Centrifuge, São Paulo, Brazil) at 3200 rpm for 30 minutes at room temperature, to detect visible changes or instabilities such as separation of phases, formation of compact sediment (caking) and coalescence (ANVISA, 2004).

### 2.3.3 Thermal stress test by freeze-thaw cycle

The freeze-thaw cycle thermal stress test was made with 20 g of formulations' samples and placed in large-mouth polypropylene pots, occupying about 2/3 of the total volume of the pot (30 mL), to monitor possible gas exchange. The samples, in triplicate, were submitted to 4 °C ± 2 °C for 24 hours and to 45 °C ± 2 °C for 24 hours, thus completing 1 cycle. Possible signs of instability were recorded before the beginning of the first cycle and at the end of the sixth cycle (12 days) (ANVISA, 2004).

## 2.4 Characterization of formulations

### 2.4.1 Determination of emulsion type

The preliminary formulations were verified whether the emulsion type was O/W or W/O, and a two-phase dispersion test was carried out in triplicate. In the first phase, about 1 g of the formulations were weighed in falcon tubes (15 mL) containing 9.0 mL of purified water (25 ± 2 °C). In the second phase, the same procedure was repeated, exchanging purified water for mineral oil. Then the samples were homogenized by means of a Vortex homogenizer (IKA®) and the final aspect of the dispersion was observed macroscopically (Lonni *et al.*, 2015).

### 2.4.2 Continuous flow rheological analysis

The continuous flow measurements of the preliminary formulations were determined using a HAAKE™ MARSTM II controlled shear gradient and shear modular rheometer (Thermo Scientific™) in continuous flow mode at temperatures of 25 °C and 37 °C ± 0.1 °C using 35 mm diameter parallel cone-plate geometry. It was performed in triplicate by inserting about 2 g of sample into the lower plate, generating minimal shear of the formulation and allowing a resting time of 1 min to ensure a relaxation of the tension generated before analysis. Readings were taken at times 0 and 15 days after the freeze-thaw cycle heat stress test. Samples stored at 45 °C and 5 °C were left at room temperature (± 25 °C) for 48 hours before reading. The choice of the shear gradient range, 0 to 2000 s<sup>-1</sup>, was based on previous tests carried out on the equipment itself and determined based on the formulation's resistance to breakage. From the values of the ascending and descending flow curves it was possible to determine the apparent viscosity, consistency index (*k*) and flow index (*n*) according to the power law (Ostwald de Waele equation). From these measurements, the continuous flow was determined according to the Equation 1:

$$\sigma = \kappa \cdot \dot{\gamma}^n \quad (1)$$

Where  $\sigma$  is the shear stress (D/cm<sup>2</sup>),  $\dot{\gamma}$  is the shear gradient (1/Sec), *k* is the consistency index, and *n* is the flow index. The obtained rheograms were also mathematically analyzed by the equations of Herschel-Bulkley (Equation 2) and Casson (Equation 3) through the *software* RheoWin 4.10.0000 (HAAKE™ MARSTM II) according to the Equations 2 and 3:

$$\sigma = \sigma_0 + \kappa \cdot \dot{\gamma}^n \quad (2)$$

$$\sqrt{\sigma} = \sqrt{\sigma_0} + \sqrt{\kappa \dot{\gamma}} \quad (3)$$

### 2.4.3 Granulometric analysis

The stable formulations were subjected to particle size distribution analysis performed by capturing 10 images using a Carl Zeiss DCM510 digital camera coupled to an optical microscope. The quantification was performed using Image Pro Plus 4 analysis software.

The samples were placed on a glass slide and submitted to image capture to perform the particle size distribution, using the diameter as a measurement parameter. Determining the size and distribution, 1200 particles were measured. All measurements were made in the same direction, regardless of the orientation of the particles (Bruschi *et al.*, 2003).

## 2.5 Accelerated stability test

The stable formulations were submitted to the Accelerated Stability Test (AST). Approximately 40.0 g were weighed and placed in wide-mouth polypropylene pots of 60 mL, occupying 2/3 of the total volume to evaluate possible gas exchanges. The analysis was performed in triplicate, under different storage conditions at room temperature ( $22.0 \pm 3.0$  °C). The samples stored under refrigeration ( $5.0 \pm 2$ °C) were taken as standard (ANVISA, 2004). The samples were submitted to three temperatures,  $22.0 \pm 3.0$  °C,  $5.0 \pm 2.0$  °C and  $40.0 \pm 2.0$  °C for 90 days, being analyzed at 1st, 30th, 60th and 90th day, evaluating the following parameters: Organoleptic characteristics (appearance, color, and odor); physicochemical characteristics (pH value); physical characteristics (average particle size variation).

### 2.5.1 Macroscopic analysis

The organoleptic characteristics and the homogeneity of the formulations were visually observed to identify possible signs of instability such as color change, odor, homogeneity, creaming, phase separation and consistency change (ANVISA, 2004).

### 2.5.2 Determination of pH

The pH values were obtained by inserting the electrode directly into the samples. The samples stored in the refrigerator ( $5 \pm 2$  °C) and in the oven ( $40 \pm 2$  °C) were removed from the temperature to which they were submitted and remained on the bench for about 30 minutes until reaching the laboratory room temperature ( $22 \pm 2$  °C). 3 °C) to carry out the measurements. The percentage of pH change was calculated according to the Equation 4:

$$pH = \frac{(pH_f - pH_i) \times 100}{pH_i} \quad (4)$$

Where  $pH_i$  is the pH value in  $T_0$  (initial), and  $pH_f$  is the final pH value at 48 hours after preparation.

### 2.5.3 Medium particle size analysis

The quantitative microscopy technique was used to determine the average particle size according to item 2.4.3 (Groulometric analysis). The mean and standard deviation of 1200 measurements of each sample were calculated during the times 0, 30, 60 and 90 days.

## 2.6 Challenge test

This assay was performed in accordance with the recommendations of the Brazilian Pharmacopoeia, in duplicate with the selected formulation. The microorganisms used to perform the test were *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Candida albicans* (ATCC 10231), and *Aspergillus brasiliensis* (ATCC 16404).

## **2.7 Evaluation of formulations effectiveness by cutaneous bioengineering**

The cutaneous bioengineering was evaluated using the formulation FB1, because it was the only one considered stable. The study case was realized with 26 volunteers, from 20 to 44 years old, with phototype between I and VI (Fitzpatrick scale, 1975), selected and randomly divided in two groups (n=13): the PW group (control group), who used exclusively a conventional topical use vehicle based on Polawax® wax, and the FB group (experimental group), who used the formulation FB1 elaborated in the study. After approval by the Standing Committee on Ethics in Research Involving Human Beings of the State University of Maringá - UEM (ANNEX I), the volunteers were instructed on the objectives and methods of the research, agreeing to participate in it, they signed the Free and Informed Consent Term (CEP/ UEM - Protocol No. 2,968,234). This experimental procedure was approved by the Human Experimentation Ethics Committee of the State University of Maringá (Protocol No 2.968.234/2018).

The difference in Transepidermal Water Loss (TEWL) and stratum corneum (SC) hydration was evaluated. The volunteers applied 1.0 mL (total valve activation) in the volar region of forearm (left), with round moves for 15 seconds. The volar region of the right forearm was the control. Measurements were taken at times 0 (baseline) and 28 days after daily self-application (two times a day) of FB1 or PW, after 20 minutes of acclimatization to controlled conditions of temperature (22 °C to 24 °C ± 2 °C) and humidity (40 °C to 60 °C ± 5 %). The TEWL (g/m<sup>2</sup>.h) was measured by MPA probe Tewameter® based on the diffusion principle proposed by Adolf Fick, and the SC hydration, expressed in Arbitrary Units (AU), by Corneometer® probe. Six measurements were taken in each region of the forearms, and the average calculated. The volunteers didn't use any cosmetic products or underwent dermatological procedures, avoiding interference. All participants used the same liquid soap to minimize the interference of other surfactants on skin.

### **2.7.1 Volunteer screening**

After approval by the Permanent Committee on Ethics in Research Involving Human Beings of the Universidade Estadual de Maringá – UEM (ANNEX I), 26 volunteers, aged 20 to 44 years, with phototypes between I and VI (Fitzpatrick scale, 1975) were selected and randomly divided (13 women each) into two treatment groups: The PW group (control group), that exclusively used a conventional topical vehicle based on Polawax® wax, and the FB group (experimental group), that used the formulation FB1 developed for the study. The volunteers were instructed on the objectives and methods of the research and, agreeing to participate in it, they signed the Free and Informed Consent Term (CEP/ UEM - Protocol No. 2,968,234). The Study was conducted at the Research Support Center Complex (COMCAP) of the State University of Maringá, Maringá, Brazil.

### **2.7.2 Inclusion and exclusion criteria**

The participants were selected according to the inclusion criteria, being healthy adults, female, aged between 20 and 44 years, covering all skin phototypes (I to VI), according to the Fitzpatrick scale (1975), which worked or studied at the State University of Maringá (UEM). The volunteers provided their free and informed consent in writing, after being informed for voluntary participation in the study. All volunteers were aware that they could not use any dermocosmetics or topical therapy at the measurement sites (forearms) for at least 10 days, as well as not having performed any aesthetic and dermatological procedures that could interfere with the research. The volunteers received information (oral and written) about all the methodologies to be used during the research. The selected volunteers were previously examined by the coordinator of this study, who has knowledge in cosmetology to assess any damage to the skin.

The volunteers who were pregnant or suspected of being pregnant, lactating women, patients with chronic, systemic, or skin diseases, under hormonal or antibiotic treatment, with a history or signs of dermatopathologies, previously known,

hypersensitivity of any component of the formulations or that, during the evaluation period show signs or symptoms of irritation, were excluded. The volunteer could withdraw at any time during the study period, without causing any burden or harm to her person. The volunteers who reported not using the product correctly could also be excluded.

### **2.7.3 Determination of the aqueous content of the stratum corneum and the transepidermal water loss**

The Corneometer® CM 820 probe (Courage-Khazaka, Germany) was used to assess the level of skin hydration, which uses the principle of electrical capacitance. The dried stratum corneum is a dielectric medium, and its dielectric properties change with changes in moisture content. The Corneometer® CM 820 capacitor, when placed between the probe base and the skin, measures the change in the dielectric constant, due to changes in the amount of water on the skin surface. The result is expressed in Arbitrary Units (AU), and it is estimated that 1 AU corresponds to 0.2 - 0.9 mg of water per gram of stratum corneum (Du Plessis *et al.*, 2013).

In order to assess the transepidermal water loss (TEWL), the Tewameter® probe was used, which assesses the rate of water evaporating from the skin surface, based on the diffusion principle proposed by Adolf Fick. Values are expressed in g/m<sup>2</sup>.h. The measurement of TEWL is an important tool to assess skin barrier function, since an increase in the rate of transepidermal water evaporation can signify a dysfunction of the skin barrier (Logger *et al.*, 2019).

### **2.7.4 Evaluation of formulations effectiveness in a short time**

The TEWL and SC hydration were evaluated in the application of FB1 and PW formulations for 28 days. The volar region of the forearm was chosen because it is considered representative of the face to measure EC hydration and other biomechanical properties, thus being relevant for the evaluation of the effectiveness of cosmetic products intended for facial use (Bazin & Fanchon, 2006). The volunteers applied equivalent to 1.0 mL of the formulations (by fully activating the valve on the package that provides this nominal volume), in the instructed region (proximal area of the left forearm) with circular movements for 15 seconds. The proximal region of the forearm was chosen for the application because it suffers less interference from surfactants when washing hands.

Measurements were performed at times 0 (baseline) and 28 days after daily self-application (twice a day) of the formulations (FB1 or PW). Two measurement areas were defined on each forearm of the participants, being in the proximal region of the volar part of the right forearm (control), and the left forearm (treatment). Measurements were performed after 20 minutes of acclimatization under controlled conditions of temperature (22 °C to 24 °C ± 2 °C) and humidity (40 to 60 ± 5 %). For the evaluation of TEWL and SC hydration, 6 measurements were performed in each forearm, and the average was calculated.

## **2.8 Statistical analysis**

The results of the rheological measurements and the clinical study were expressed as the mean ± standard deviation and were statistically analyzed by analysis of variance (ANOVA). The means were compared using Tukey's test, with a significance level set at p<0.05.

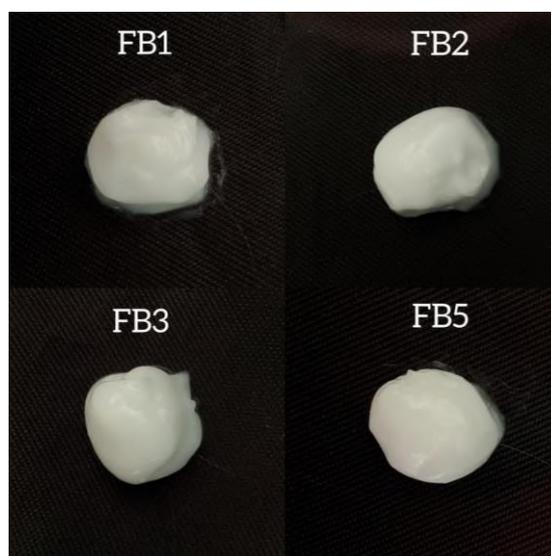
## **3. Results and Discussion**

### **3.1 Development of formulations**

The formulations FB1, FB2, FB3 and FB5 (Figure 1) showed superior performance when compared to the others because they showed no signs of instability, adequate texture, and consistency profiles and, therefore, were selected to proceed with further stability tests. FB4 and FB6 were rejected in the development stage because of their low consistency, which makes

it difficult or impossible to add large amounts of adjuvants and active ingredients without maintaining their cream consistency. FB7, FB8 and FB9 showed signs of instability after preparation such as coalescence and creaming, thus being discarded from the study.

**Figure 1** - Morphology of emulsion systems displaying better microscopic characteristics: FB1, FB2, FB3 and FB5.



Source: Authors.

In Figure 1, we can observe that all of them showed similar physical-chemical characteristics, and the expected characteristics. That is why they were chosen to continuous the subsequently tests.

### 3.2 Preliminary stability tests

The stability test aims to determine any signs of instability (ANVISA, 2004). When the samples submitted to centrifugation are not stable, there will be a tendency for phase separation, loss of other parameters, such as apparent viscosity and changes in the organoleptic characteristics of the components. According to Stoke's law, centrifugation acts on the emulsion destabilizing the dispersed phase, causing an increase in the number of collisions between droplets and, therefore, accelerating these signs of instability (Lonni *et al.*, 2015). The selected formulations did not show any change in the centrifugation test, being considered stable and therefore, submitted to the thermal stress test by freezing and thawing cycle.

#### 3.2.1 Organoleptic characteristics evaluation

The organoleptic characteristics of FB1, FB2, FB3 and FB5 were observed macroscopically after resting 48 hours at room temperature ( $25 \pm 3$  °C), so that the emulsification process was completed, and its maximum apparent viscosity was reached. None of them showed visual changes such as color, odor, and signs of instability (phase separation or heterogeneity). Thus, the selected formulations did not show any changes after centrifugation, being considered stable, therefore, submitted to the thermal stress test by freeze-thaw cycle.

#### 3.2.2 Thermal stress test by freeze-thaw cycle

The formulations FB1, FB2, FB3 and FB5 remained stable to the thermal stress test by freeze-thaw cycle, with no signs of instability, which is, withstanding the changes in temperature to which they were submitted.

The freeze cycle thermal stress testing is used to accelerate the rate of destabilization of emulsions. The samples are

stored under different temperature conditions and rotated at regular intervals of time. The final structure of the product subjected to this test depends heavily on the storage conditions (Lerche & Sobisch, 2011). Under extreme temperature conditions, such as 24-hour cycles in an oven at  $40 \pm 2$  °C and in a freezer at  $-10 \pm 2$  °C, changes in its structure are common and even predicted. These modifications need to be carefully evaluated and defined within specified limits, as they may not be grounds for sample failure (Dickinson et al., 2019).

The criteria for choosing the storage conditions were to reach different climatic zones in which the product can be stored and for being among the most used temperature ranges in this test (ANVISA, 2004). The samples were submitted to  $4 \pm 2$  °C in 24 hours (Refrigerator Consul Facilite CFF 300L) and  $40 \pm 2$  °C in 24 hours (Estufa Ethik series 400) until completing 6 cycles (12 days).

### 3.3 Characterization of formulations

#### 3.3.1 Determination of the emulsion type

After the final preparation, both types of emulsions (W/O or O/W) have the same appearance to the naked eye, requiring certain tests to differentiate them and get a conclusion. In this study, it was found that FB1, FB2, FB3 and FB5 appear as O/W type emulsions, which means their external phase is aqueous, since they all remained homogeneous after dilution in purified water and heterogeneous in the presence of mineral oil.

#### 3.3.2 Continuous flow rheological analysis

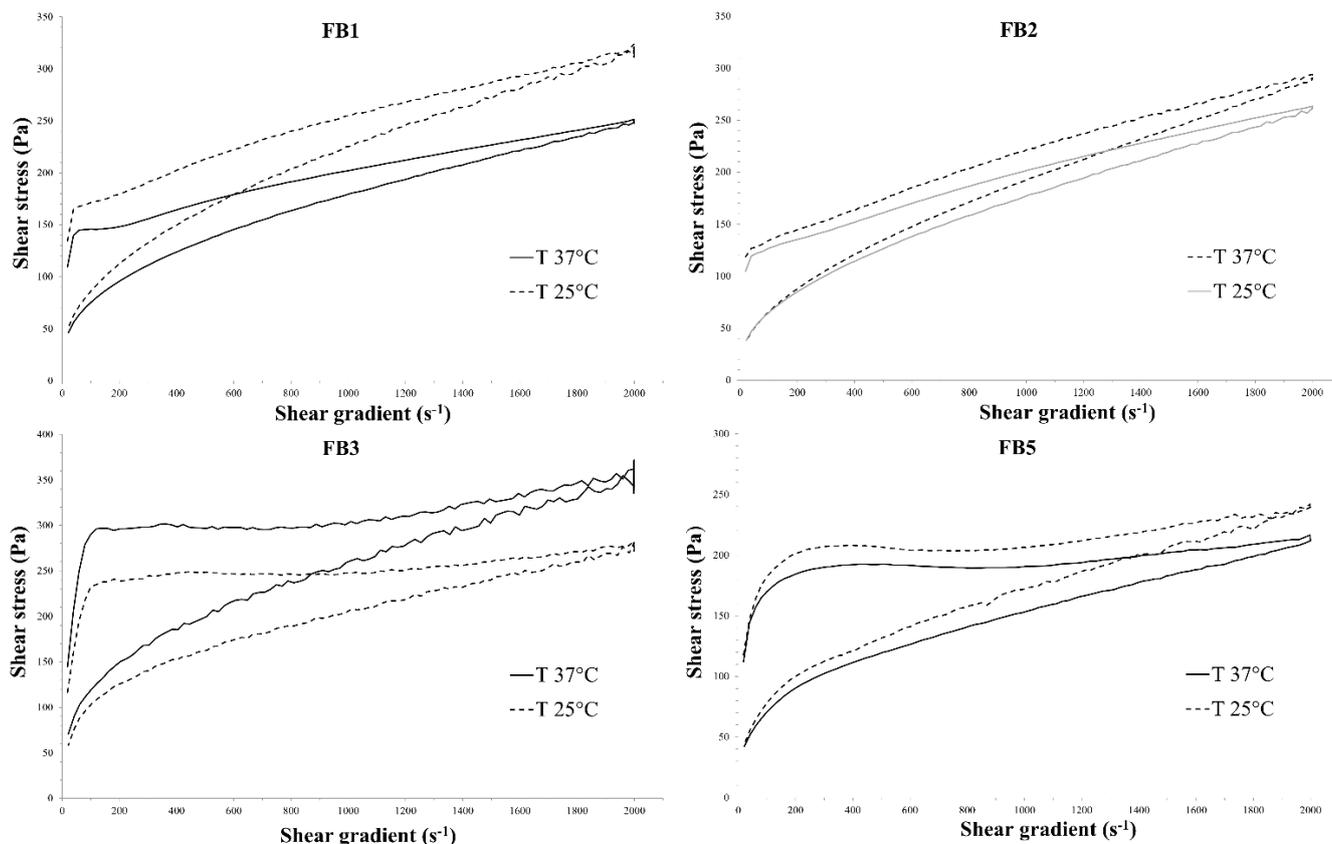
The knowledge of the rheological properties of emulsions has become a fundamental tool of the pharmaceutical and cosmetic industry. The rheological behavior of emulsions influences the various stages of the product development process, both in technical terms (manufacturing, packaging, storage) and aesthetics (Bruschi *et al.*, 2003). Consequently, the ability to measure the structure and consistency of an emulsion can be instrumental in evaluating, optimizing, and even predicting the in vivo performance of a formulation. Information obtained from rheological measurements has also been shown to be useful for estimating the long-term stability of the emulsion structure (Isaac *et al.*, 2013).

Emulsifiers significantly influence the rheological characteristics of emulsions, as they are what enable the emulsion to be formed, in addition to being the main responsible for the physical stability during the established storage time. Considering that also the association of different emulsifiers is more effective in stabilizing emulsions, than using only one type of emulsifier, since the of association each emulsifier can complement the action of other.

According to the flow and viscosity rheograms (Figures 2 and 3) all formulations showed non-Newtonian flow behavior, with shear thinning (flow index  $<1$ ), and hysteresis area (Table 2). The non-Newtonian behavior implies that the viscosity of fluids changes according to the shear rate, consequently they do not have constant viscosity (Bruschi *et al.*, 2003).

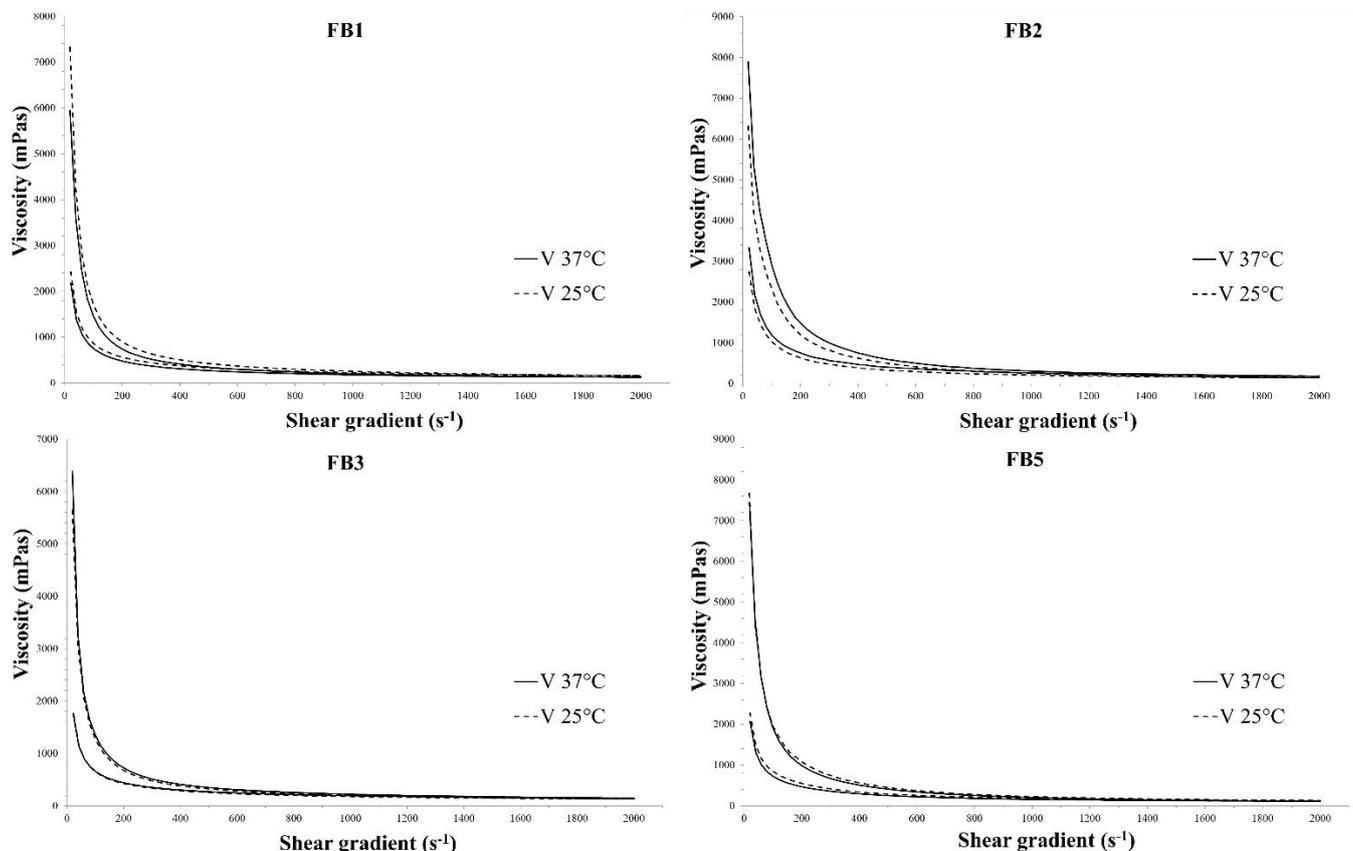
The pseudoplastic behavior of a material is characterized by the decrease in viscosity as the shear rate increases. This behavior is suitable in cosmetic emulsions because their apparent viscosity decreases with increasing stress, making the system more fluid and sensitive to flow, which helps with product application. The pseudoplasticity is generally verified by analyzing the value of the flow index ( $n$ ). This value is dimensionless and can be obtained using the mathematical equation of Oswald de Waele. According to the results presented in Table 4, the FB3 and FB5 formulations exhibited the lowest flow index values at both temperatures. Formulations with values of this parameter close to “1” indicate that the fluid approaches Newtonian behavior. As long as the flow index of a material is greater than 1, it is characterized as dilatant (Siska *et al.*, 2019).

**Figure 2** - Flow rheograms of formulations, represented as shear stress in function of shear gradient at temperatures 25°C and 37°C.



Source: Authors.

**Figure 3** - Flow rheograms of formulations, represented as the viscosity in function of shear gradient at temperatures 25°C and 37°C.



Source: Authors.

According to the Figures 2 and 3, the flow rheograms of FB1, FB2, FB3 and FB5 had shown the expected results for this research, with the adequate pseudoplastic behavior: non-Newtonian flow behavior, with shear thinning (flow index <1), and hysteresis area. Considering the rheological measurements (Figures 2 and 3) of the formulations at temperatures of 25 °C and 37 °C after 48 hours after preparation, all samples presented ascending curves superior to descending, characterizing emulsions with moderate (FB1, FB2) to pronounced (FB3 and FB5) thixotropy.

Thixotropy is a property of some fluids that is characterized by the continuous decrease of viscosity, with the increasing in shear stress applied to a sample resting and recovers its viscosity once this stress is stopped (Larson & Wei, 2019). A commonly observed phenomenon associated with thixotropy is the hysteresis area, which is the area generated between the two rheogram curves (shear stress versus shear rate). The presence of thixotropy in formulations is a desirable feature for topical products, as they promote a longer shelf life of the formulation – during storage the viscosity is constant, making it difficult to separate the formulation components (Isaac *et al.* 2013). In addition to facilitating the use of the product due to the deformation suffered during application and flow and the recovery of the initial viscosity after shearing has ceased, which prevents product leakage.

Viscosity is related to various properties of emulsions and depends on the shear rate range. In the case of a cream used as a vehicle in magistral preparations, it needs to have a high viscosity, since it is a semi-finished product, to which adjuvants and active ingredients (dermatological or cosmetic) will be incorporated in different concentrations (Jaksic *et al.*, 2012).

The apparent viscosity of the formulations was determined by selecting the viscosity value at the maximum shear point (2000 S<sup>-1</sup>) (Table 3).

**Table 2** - Results of consistency index (K), flow index (n), yield stress ( $\tau_0$ ), and hysteresis area (Pa/s) at temperatures 25 °C and 37 °C for formulations FB1, FB2, FB3 and FB5.

Temperature (°C)	K (Pa.s)	Flow index (n)	Yield stress ( $\tau_0$ )	Hysteresis area (Pa/s)
25	52.91 ± 0.96	0.2305 ± 0.029	128.46 ± 3.00	65880 ± 1809
37	49.78 ± 2.41	0.2064 ± 0.0059	105.33 ± 2.40	52397 ± 2169
25	28.50 ± 0.78	0.3006 ± 0.0039	91.12 ± 3.29	61247 ± 642
37	30.90 ± 1.20	0.2689 ± 0.0028	85.57 ± 0.75	53170 ± 8495
25	170.43 ± 15.35	0.0967 ± 0.0076	240.13 ± 16.85	119566 ± 3758
37	145.30 ± 6.95	0.0816 ± 0.0045	118.20 ± 6.22	101433 ± 1250
25	81.37 ± 60.74	0.0914 ± 0.0064	160.90 ± 7.21	88026 ± 447
37	108.33 ± 0.77	0.0868 ± 0.0014	149.30 ± 0.46	88960 ± 490

Source: Authors.

FB1 made with Cetearyl Olivatate (and) Sorbitan Olivatate, and the anionic co-emulsifier Sodium Stearoyl Glutamate, showed a higher apparent viscosity compared to FB2 (without the anionic co-emulsifier). This effect demonstrates that the association of this anionic co-emulsifier promoted a higher apparent viscosity value, and also an increase in parameters such as consistency index (k), yield stress ( $\tau_0$ ) and hysteresis area (Pa/s) (Table 2).

The Table 2 also shows the yield stress values of the formulations at the two different temperatures. The yield value ( $\tau_0$ ) represents the minimum shear stress required to trigger the flow of a fluid, below that it behaves like a solid. It is considered an important parameter to evaluate the physical stability of semi-solid formulations (Savic *et al.*, 2008). In the group of emulsions based on the primary emulsifier Cetearyl Olivatate (and) Sorbitan Olivatate, samples FB1 and FB2, presented the highest values of flow index (n). According to Silva *et al.* (2012), high values of n mean higher yield stress values and it was reported to be advantageous for the stability of emulsions during storage time.

**Table 3** - Minimum apparent viscosity values of the formulations at 25 °C.

Formulation	Minimum apparent viscosity (mPa.s)
FB1	2425.67 ± 7.41 b
FB2	1770.33 ± 8.38 d
FB3	3334.67 ± 49.77 a
FB5	2104.67 ± 11.14 c

Legend: The means followed by the same letters in the column do not differ by Tukey's test at 5 % ( $p < 0.05$ ). Source: Authors.

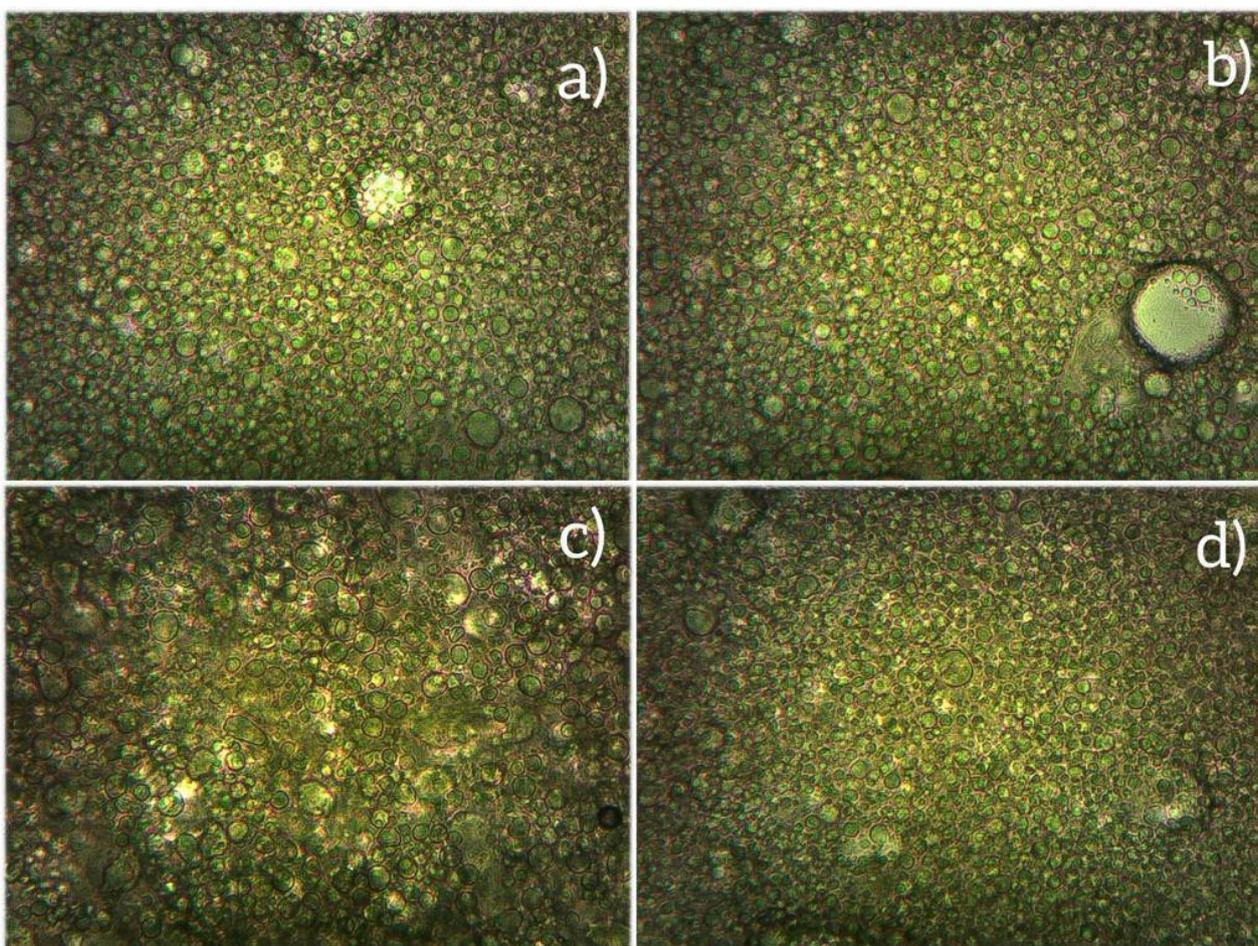
According to Table 3, at 25 °C, FB3 showed the highest apparent viscosity value (3334.67 ± 49.77 mPa.s), FB1 and FB5 showed an intermediate value (2425.67 ± 7.41 and 2104.67 ± 11.14 mPa.s respectively), and FB2 had the lowest value (1770.33 ± 8.38 mPa.s). These results were statistically different ( $p < 0.05$ ), demonstrating that the type of self-emulsifying base used can influence this parameter. In addition, it was found that FB3 presented very irregular shear bands and this behavior may be related to unsatisfactory preliminary physicochemical stability, as well as unpleasant aesthetic characteristics (Willenbacher & Georgieva, 2013).

### 3.3.3 Granulometric analysis

The particle size and its distribution are of great importance to measure the quality of a cosmetic or dermatological emulsion. There are several instrumental techniques that measure the size and distribution of particles (micelles) such as laser diffraction, light obscuration, and ozone detection. Analysis by these instrumental methods are considerably fast and accurate, as many particles are sized in a short period. However, these methods do not allow to examine the droplet diameter as in microscopic analysis. For this reason, microscopic analysis cannot be compared with these other instrumental methods (Prestes *et al.*, 2016).

The particles size and their distribution in the formulations, was conducted according to the procedure described by Lonni *et al.* (2015). The diameter measurement was used as a measurement parameter and quantified using the Image Pro Plus 4 analysis software (Figure 4).

**Figure 4** - Emulsion micrographs: a) FB1, b) FB2, c) FB3 and d) FB5. 400X magnification.

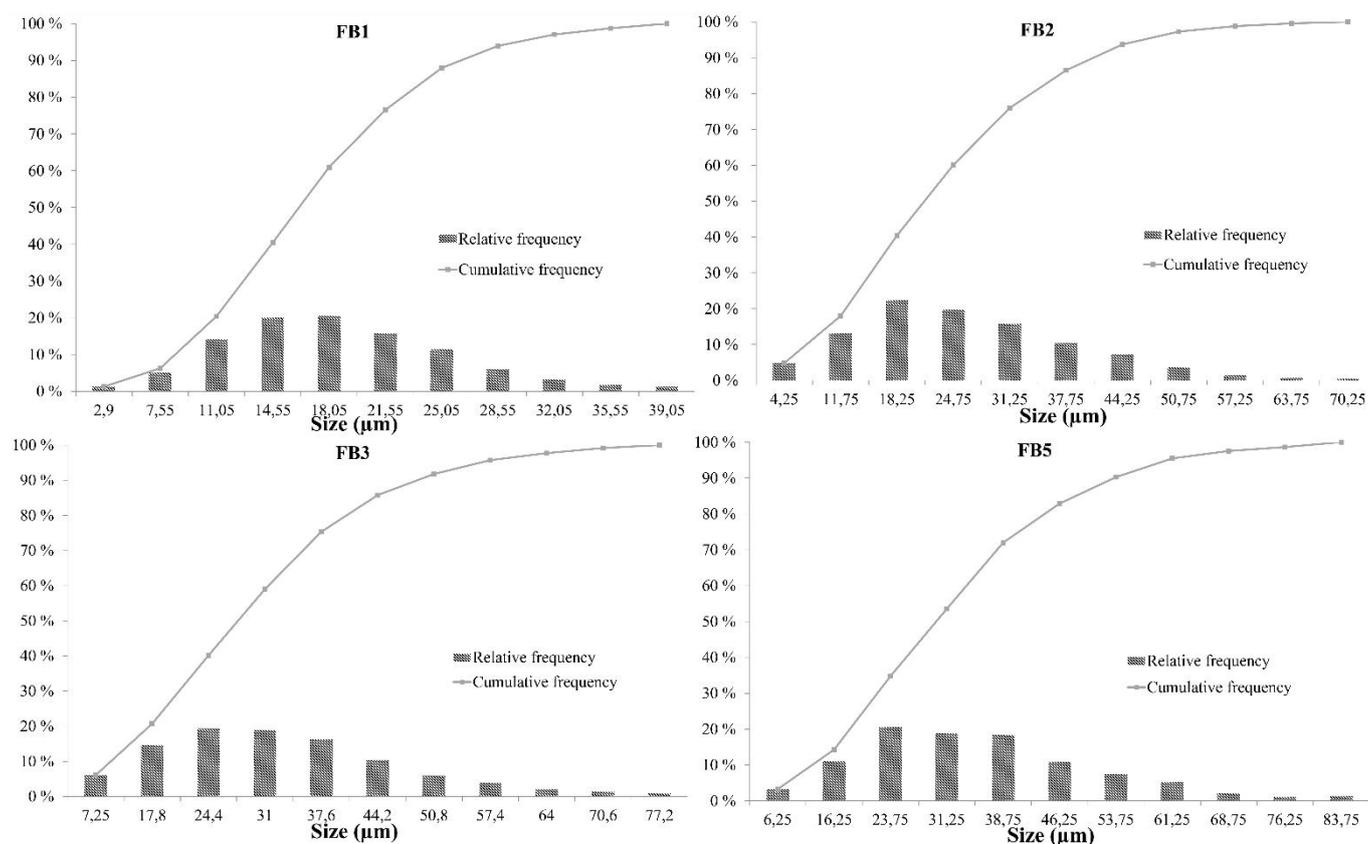


Source: Authors.

The Figure 4 shows great importance for this research, in a way that the particle size of formulations is visible and measurable, improving the specification of each one.

The formulations were classified as macroemulsions, with particle size in the range of 2.4 to 84  $\mu\text{m}$ . The droplets showed regular morphology with a mean diameter of  $18.63 \pm 6.89 \mu\text{m}$  for FB1,  $26.38 \pm 12.48 \mu\text{m}$  for FB2,  $33.02 \pm 13.86 \mu\text{m}$  for FB3, and  $35.56 \pm 15.40 \mu\text{m}$  for FB5. The droplet size distribution graphs are shown in Figure 5.

**Figure 5 - Particle size distribution of formulations FB1, FB2, FB3 and FB5.**



Legend: (■) histogram of class intervals and (—●—) representation of the cumulative distribution of particle sizes. Source: Authors.

In Figure 5, FB1 presented smaller particle size distribution, in the range of 2.4 to 40.02 µm and greater homogeneity (smallest standard deviation). FB5 showed the most heterogeneous distribution and the largest droplets. The analysis of variance showed significant differences in droplet size ( $p = 0.05$ ) in relation to the type of emulsifying system used.

The droplet sizes of all emulsions were larger than indicated for a good quality O/W cosmetic emulsion (0.1 to 10 µm). This result can be explained by the high concentration of emulsifiers, and by the preparation technique, which carried out the emulsification process in an open system and with low mechanical energy (stirring with low rotation speed). The choice to reduce the stirring speed was to avoid the incorporation of air in the emulsion during the cooling process. The impossibility of using foam-reducing ingredients or air in the emulsion, such as silicones, also supported the choice of preparation technique.

One of the fundamental parameters in obtaining O/W emulsions with good stability is to reduce the droplet sizes of the internal phase to a very narrow size range, as mentioned above. A well-homogenized emulsion is also a relevant factor for its physical stability, with reduced size droplets showing a low tendency to increase in size. Given the components of an emulsion and the equipment used in the emulsification, the most influential parameter to obtain emulsions with smaller particles is the agitation intensity. Temperature also has a significant influence on emulsification, contributing to the change in interfacial tension, emulsifier adsorption and viscosity (Chen & Tao, 2005). Emulsifying at a temperature below the recommended temperature can also cause coagulation of the emulsifiers, preventing their formation.

In general, an increase in the concentration of emulsifiers promotes a decrease in surface tension and, consequently, a reduction in the droplet size (Hecht *et al.*, 2011). However, according to Chen and Tao (2005), there is a window in the concentration of emulsifiers in obtaining stable emulsions with low droplet size, outside of which there is an increase in droplet size and emulsion destabilization.

Some studies also show that the emulsion droplet size influences skin permeation, with emulsions with smaller droplets having a greater penetration into the skin. However, the vehicle used in most of these comparative studies differs in its composition, making it difficult to confirm the influence of droplet sizes on skin permeation (Otto *et al.*, 2010). A study by Izquierdo *et al.*, (2007) evaluated the effect of droplet size on transdermal tetracaine permeation in identical emulsifier vehicles, but with different droplet sizes. Interestingly, they found no correlation between vehicle droplet size and transdermal permeation.

### 3.4 Accelerated stability test (AST)

The AST also called Normal Stability Test (NST), aims to evaluate the physical and physicochemical stability of the product, estimating the shelf life and the formulation compatibility with the conditioning material. This test is performed during the product development phase, usually using small-batch prototypes produced on a laboratory scale. The duration of the AST is usually 90 to 120 days and can extend to six months or more. The storage conditions of the test formulations in the AST are less extreme than in the Preliminary Stability Test (ANVISA, 2004). The results of the AST are described in Table 4.

**Table 4** - Accelerated stability test of the formulations FB1, FB2, FB3 and FB5.

Accelerated stability test - storage conditions										
FB1										
Variables	Room temperature (22 ± 3 °C)				Low temperature (5 ± 2 °C)			High temperature (40 ± 2 °C)		
	T0	T30	T60	T90	T30	T60	T90	T30	T60	T90
Time (days)										
Aspect	Homogeneous	N	N	N	N	N	N	N	N	N
Color	White	N	N	N	N	N	N	N	N	N
Odor	Characteristic	N	N	N	N	N	N	N	N	N
pH	5,37 ± 0,03	5,25	5,22	5,15	5,31	5,26	5,18	5,26	5,14	5,03
Size (µm)	18,63 ± 6,89	18,86 ± 7,18	20,08 ± 5,18	21,25 ± 7,71	19,02 ± 6,98	21,30 ± 7,78	21,89 ± 6,58	20,38 ± 5,02	22,41 ± 6,52	25,09 ± 7,31
FB2										
Variables	Room temperature (22 ± 3 °C)				Low temperature (5 ± 2 °C)			High temperature (40 ± 2 °C)		
	T0	T30	T60	T90	T30	T60	T90	T30	T60	T90
Time (days)										
Aspect	Homogeneous	N	N	N	N	N	N	N	M	M
Color	White	N	N	N	N	N	N	N	SM	M
Odor	Characteristic	N	N	N	N	N	N	N	N	SM
pH	4.74 ± 0.02	4.68	4.75	4.95	4.67	4.7	4.56	4.6	4.61	4.44
Size (µm)	26.38 ± 12.48	26.67 ± 7.18	27.47 ± 5.18	29.52 ± 6.46	28.85 ± 6.98	29.44 ± 7.30	33.2 ± 5.66	37.38 ± 15.02	N/A	N/A
FB3										
Variables	Room temperature (22,0 ± 3°C)				Low temperature (5 ± 2°C)			High temperature (40 ± 2°C)		
	T0	T30	T60	T90	T30	T60	T90	T30	T60	T90
Time (days)										
Aspect	Homogeneous	N	N	N	N	N	N	SM	M	M
Color	White	N	N	SM	N	N	N	N	M	M
Odor	Characteristic	N	N	N	N	N	N	N	SM	SM
pH	5.03 ± 0.03	5.07	5.08	5.04	5.09	5.04	5.04	5.04	4.98	5.07
Size (µm)	33.02 ± 13.86	36.41 ± 13.21	34.3 ± 13.78	34.44 ± 14.35	33.02 ± 11.71	35.76 ± 14.20	33.72 ± 15.68	39.05 ± 22.71	N/A	N/A

		FB5								
Variables		Room temperature (22 ± 3 °C)			Low temperature (5 ± 2 °C)			High temperature (40 ± 2 °C)		
Time (days)	T0	T30	T60	T90	T30	T60	T90	T30	T60	T90
Aspect	Homogeneous	N	N	N	N	N	N	SM	M	M
Color	White	N	N	SM	N	N	N	M	M	M
Odor	Characteristic	N	N	N	N	N	N	N	SM	SM
pH	5.03 ± 0.03	5.14	5.05	4.98	5.08	5.12	5.04	5.13	5.14	5.07
Size (µm)	35.56 ± 15.40	28.04 ± 8.96	31.13 ± 9.85	30.44 ± 10.46	28.11 ± 7.39	26.04 ± 8.96	28.98 ± 9.40	36.43 ± 16.80	N/A	N/A

Legend: N = Normal (no modification in relation to the initial appearance); SM = Slightly modified and M = Modified from the initial appearance. Source: Authors.

According to Table 4, we can see that the variables aspect, color, and odor of all formulations remained normal almost in all different temperatures and times. FB2 showed the color and odor slightly modified, after 60 days and 90 days respectively, at high temperature. FB3 and FB5 showed the color slightly modified after 90 days at room temperature, the aspect after 30 days at high temperature, and the odor after 60 and 90 days at high temperature. The pH and the size of the formulations remained normal in all analysis.

### 3.4.1 Macroscopic analysis

FB1 proved to be stable and without changes in organoleptic characteristics (appearance, color and odor) during the entire test period and under all temperature conditions. The emulsion continued with a bright white appearance, homogeneous and with a pleasant sensation to the touch. FB2 (without the secondary emulsifier *Sodium Stearoyl Glutamate*) was stable throughout at room temperature. However, at high temperature (40° C), it showed a change in appearance, from the 60th day, with a slight dryness on the surface and color change. In the last period of analysis (90th day), the presence of oil droplets on the surface (creamed) was observed.

FB3 prepared with the combination of the self-emulsifying base Cetearyl Glucoside (and) Cetearyl Alcohol and the anionic emulsifier *Sodium Stearoyl Glutamate*, showed signs of instability only when subjected to a thermal stress temperature of 40° C. In the visual inspection on the 60th day, the formulation showed apparent dryness on the surface, but without the presence of creamation. At the end of the test, on the 90th day, it was noticed the presence of cream, color change and intensity of the opaque aspect of its surface due to a visible sign of dryness.

FB5 samples prepared with the combination of the self-emulsifying base (Candelilla/Jojoba/Rice Bran Polyglyceryl-3 Esters (and) Glyceryl Stearate (and) Cetearyl Alcohol (and) Sodium Stearoyl Lactylate) with the anionic secondary emulsifier (*Sodium Stearoyl Glutamate*) also showed adequate stability at room temperature, maintaining their homogeneous appearance. At 40° C, it was found that the base emulsion showed a slight color change on its surface on the 60th day, but without the presence of cream. In the evaluation of the 90th day, there was the presence of droplets on the surface – a sign of physical instability called creamation.

Among the possible factors that may have caused the signs of instability in the formulations, the inadequate balance of the emulsifying system, emollient, and consistency agents (water and oil ratio) can be highlighted, as well as the equipment used to emulsify the system (mechanical stirrer), the position of the stirrer rod, the rotation speed used to emulsify the system and also the presence of air in the emulsion. The presence of air bubbles in cosmetic emulsions not only alters its appearance, leaving it unsightly, but can also affect the stability of the emulsion by adsorbing the emulsifying molecules at the air-liquid

interfaces.

Thus, FB1, based on the emulsifier Cetearyl Olivatate (and) Sorbitan Olivatate and Sodium Stearoyl Glutamate, which performed better in the stability tests and presented the best rheological characteristics, was selected for the challenge test of the preservative system (*Challenge Test*) and the evaluation of effects on human skin by cutaneous bioengineering.

### 3.4.2 Determination of pH

According to the pH values of formulations, it was found that regardless of the type of emulsifier used, all were considered compatible with the pH of the skin, since stratum corneum is slightly acidic, with pH values ranging from 4.0 to 6.0 (Ali & Yosipovitch, 2013). According to the AST (Table 4) the formulations did not show a pH percentage variation greater than  $\pm 10\%$  of the initial pH value (ANVISA, 2004). The absence of large fluctuations in pH values during the test period demonstrated that the formulations had a stable behavior in this parameter, which may indicate stability of the components.

### 3.4.3 Analysis of the average particle size variation

The experimental result shows that there was a significant increase in droplet size in all samples when subjected to thermal stress temperature ( $40\text{ }^{\circ}\text{C} \pm 2^{\circ}\text{C}$ ). As shown in Table 4, the FB1 formulation showed the smallest particle size and the smallest variation during all conditions to which it was subjected. Although optical microscopy is still used in several studies to evaluate droplet coalescence, it is a method that has statistical problems (Lonni *et al.*, 2015).

## 3.5 Challenge test

The microbiological specifications for final cosmetic products, and the results of microbiological challenge tests are mandatory and defined by organ's health bodies of each country. In Brazil, the microbial limits for cosmetics, perfumes and personal hygiene products are established by the National Health Surveillance Agency (ANVISA), through Resolution No. 481/99 (ANVISA, 2022). The preservatives used must comply with the provisions of Resolution No. 29/2012 (ANVISA, 2012) and its updates. As for the methods of analysis, those described in official compendia and guidance guides for microbiological analysis of cosmetics and for testing the effectiveness of the preservative system (Pharmacopoeias, ABC Guide (Brazilian Association of Cosmetology) of Microbiology, CTFA Guide (Cosmetology Products for Personal Care), among others) (ANVISA, 2004).

Into the detriment of the cost of carrying out the challenge test by outsourced quality control companies and by the evaluated parameters of the studied, FB1 was selected to perform the microbiological test and the effects on human skin. The number of viable microorganisms was counted in function of the time (days) the experiment was carried out. At time 0 the UFC/mL to *Escherichia coli* was  $8,0 \times 10^4$ , to *Pseudomonas aeruginosa* was  $1,5 \times 10^5$ , to *Staphylococcus aureus* was  $1,8 \times 10^5$ , to *Candida albicans* was  $1,8 \times 10^5$  and to *Aspergillus niger* was  $9,5 \times 10^3$ . At times 7, 14 and 28 days the number of UFC/mL of all microorganisms was absent. For topical formulations (category 2), there must be a reduction of 2 logs of the number of CFUs initially inoculated after 14 days, followed by no increase in the count over the period from 15 to 28 days in the case of bacteria. For yeasts and fungi, the results should not indicate any increase in the initial count after the period between 14 and 28 days (ANVISA, 2019).

For bacteria, on the 14th day there was a reduction in the number of microorganisms greater than  $2 \log^{10}$  in relation to the initially inoculated and there was no increase in the count on the 28th day in relation to the 14th day. For fungi and yeasts, there was no increase in the microbial count on the 14th and 28th days in relation to the initially inoculated. Evaluating the results obtained in the study, it is concluded that the preservative system used (Sharomix® 706 associated with gluconolactone) showed excellent antimicrobial efficacy at the studied pH  $5.14 \pm 0.04$ , because in addition to meeting the test

specifications, no growth was observed microbiological at any of the post inoculation analysis times.

Thiemann & Petersen (2016) evaluated the influence of the emulsifying system on the conservation of emulsions in the face of the microbial challenge test. Twelve formulations with different amounts and types of emulsifier systems were tested to verify if there was a positive or negative interaction in the preservative performance. The emulsifying system showed a significant influence on the antimicrobial activity of a preservative system based on emulsifiers and organic acids. The mixtures of non-ionic emulsifiers based on hydrocarbons showed unfavorable performance on the antimicrobial performance of the preservative system tested. Anionic emulsifiers had better preservative activity than non-ionic ones. Thus, the association of the anionic co-emulsifier Sodium Stearoyl Glutamate in the FB1 formulation, in addition to the benefits of physical stability, may also have benefits in microbial stability.

### **3.6 Evaluation of formulations on human skin by cutaneous bioengineering**

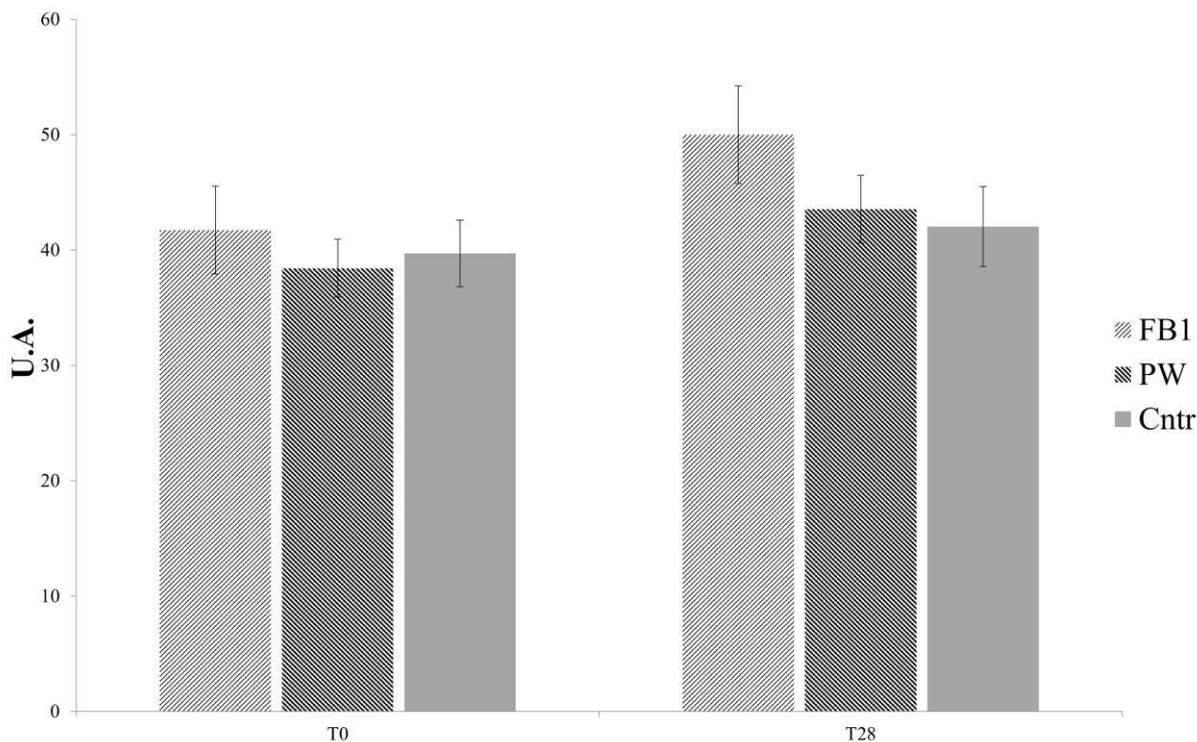
#### **3.6.1 Assessment of short-term effects of formulations**

As it is not only the active ingredients but also the vehicle that influences skin conditions, it is recognized that the vehicle can have a significant impact on the moisturizing effect and water-holding ability of EC. Measurements of TEWL and stratum corneum (SC) hydration are relevant approaches to assess vehicle effects on SC barrier function. The performance of FB1 in terms of improving skin condition was studied by comparing it to a conventional formulation (PW) used in dermatological vehicles. The hydration values, resulted by corneometry (Corneometer® CM 825), are expressed in arbitrary unit (AU), where AU <30 means very dry skin, AU between 30 – 40 means dry skin, and AU >40 means normal skin (Heinrich *et al.*, 2003).

#### **3.6.2 Determination of the aqueous content of the stratum corneum**

By analyzing the results (Figure 6), after 28 days of treatment, FB1 showed a higher aqueous content in the SC than the PW treated group and the control group. However, a statistically significant difference was found between FB1 and the control group. In addition, it was demonstrated that the PW group did not present a significant difference in relation to the control area, indicating that the application of the conventional base emulsion (PW) did not contribute to hydrate the SC, while FB1 emulsion has a positive effect on the aqueous content of SC.

**Figure 6** - Aqueous content of volunteers' forearms SC using the Corneometer® at time 0 and after 28 days of using the formulations.



Legend: FB1 (study formulation), PW (conventional vehicle) and Cntrl (control – no treatment). Source: Authors.

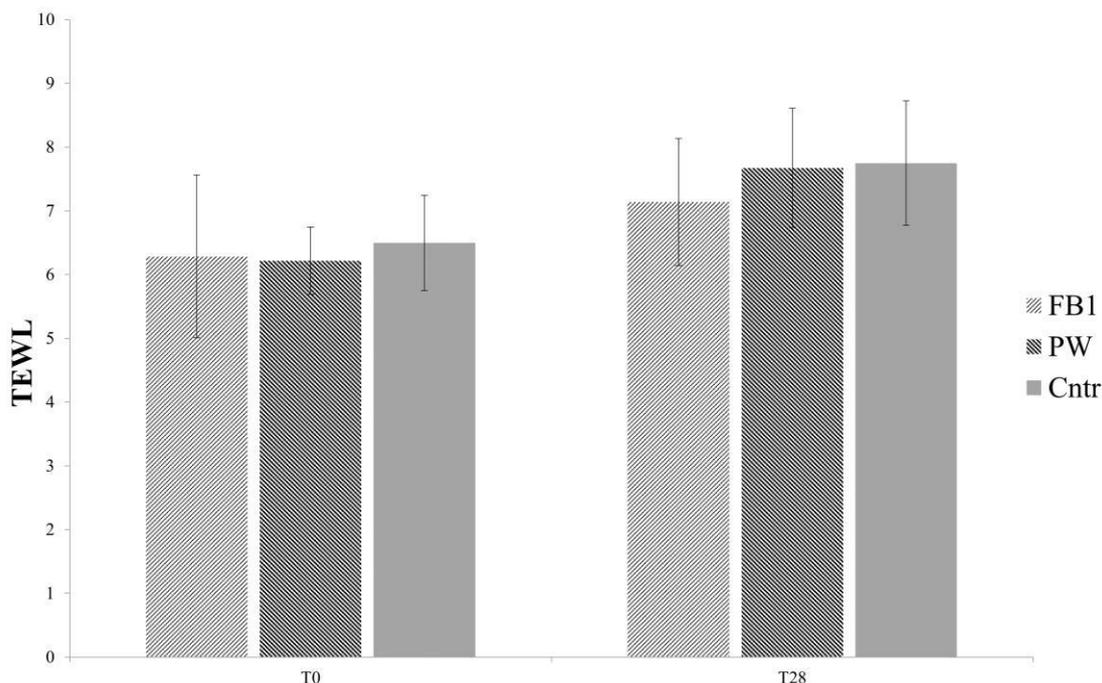
Although there are several factors to consider, we can attribute this difference showed in Figure 6 in hydration of the SC of FB1 in relation to the untreated area to the emollients and emulsifiers. While PW contains only one emollient (capric and caprylic acid triglycerides), FB1 was composed of an association of emollient esters with different chain lengths (long, medium, and short), called the emollient cascade method. This association method, in addition to providing better sensory aesthetics, can positively contribute to the long-lasting moisture effect. In addition, FB1 was also added with an aqueous phase rheology modifier (xanthan gum), which may be contributing to the increase in skin contact time.

### 3.6.3 Determination of transepidermal water loss (TEWL)

The TEWL assessment provides information on the skin barrier status, its measurements can be used under normal, pathological conditions or also experimentally in an indirect way to analyze the influence of substances applied topically on the skin (Fluhr, Feingold & Elias, 2006). The ability of TEWL reflects the skin barrier properties, justifying its use in the efficacy testing of pharmaceutical and cosmetic products for topical use (Darlenski *et al.*, 2009).

The TEWL as a parameter indicative of the state of the skin barrier, was analyzed by measuring the Tewameter® 300 probe in the volar region of the volunteers' forearms. According to the results presented (Figure 7) a small increase in transepidermal water loss was observed after 28 days for all treated and control areas. Also, it was observed that this loss was greater for treatment with PW and control compared to FB1. However, there were no significant differences in TEWL values between treatments and control area (one-way ANOVA,  $p > 0.05$ ).

**Figure 7** - Transepidermal water loss (TEWL) from the volunteers' forearms using the Corneometer® at time 0 and after 28 days of using the formulations.



Legend: FB1 (study formulation), PW (conventional vehicle) and Cntr (control – no treatment). Source: Authors.

The results of the present study in Figure 7, showed a small variation of TEWL, not being possible to affirm that with the use of the formulations, there is an influence on the skin barrier function, in terms of moisture loss. The means of all analysis remained in the range of 0 to 10 (very healthy condition). A variable that may have had a greater influence in this study was the airflow in the test field, which was not fully controlled. In addition to the variables related to the environment and the instrument, individual factors are also recognized for intervening in the results (Rogiers, 2001).

These results are in agreement with studies carried out by Lóden and Wessman (2001). In the first study, the short-term effect (20 days) on the skin barrier properties, of the use of moisturizing cream with different urea concentrations, was evaluated. In the second study, the same parameter was evaluated, but by the application for 10 days of a cream containing 20% urea, compared to its vehicle. In both studies, no influence of repeated application of these moisturizing formulations to human skin on skin barrier function, in terms of TEWL, was demonstrated.

#### 4. Conclusion

The development and characterization of formulations containing different types of emulsifiers demonstrated that the choice of primary emulsifier considerably alters the particle size and consequently its physical stability in long-term storage. The association of an anionic co-emulsifier in the formulations revealed a potential benefit in the formation of emulsions, reduced particle size, increased viscosity, and physical stability. Among the nine formulations tested, four were approved (FB1, FB2, FB3 and FB5). All of them showed rheological behavior of pseudoplastic non-Newtonian flow, with the presence of thixotropy and yield strength, but only FB1 was considered stable by the accelerated stability study.

The preservative system used obtained excellent performance in the test of the preservative system challenge, with no microbiological growth being observed under the conditions and period evaluated. In addition, it had a pH compatible with the skin ( $5.14 \pm 0.04$ ).

The results of the clinical efficacy study showed that the FB1 formulation, developed with natural ingredients, improved the skin hydration content within 28 days of application in the volar region of the volunteers' forearm, compared to the untreated control region. By evaluating the TEWL results, it was not possible to observe significant changes between the formulations in this parameter, however they demonstrated to remain at values considered ideal.

According to the results of the physical-chemical analyses, accelerated stability and clinical efficacy, FB1 was adequate to suggest its use as a topical dermatological and cosmetic vehicle. Thus, this study showed that FB1 had essential characteristics to be applied in the master sector, such as high viscosity, high stability, skin compatibility ("Skin friendly") and pleasant sensory, using only ingredients considered natural and sustainable.

This paper may contribute with future researches of new natural and sustainable products in cosmetics and pharmaceutical industries, besides the strengthening of the compounded topical preparations in pharmacy.

## Acknowledgments

The authors are grateful to the Brazilian funding agencies CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/Coordination for the Improvement of Higher Education Personnel), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico/National Research Council), for their support.

## References

- Ali, S. M., & Yosipovitch, G. (2013). Skin pH: From basic science to basic skin care. *Acta Dermato-Venereologica*. 93(3), 261-267. [10.2340/00015555-1531](https://doi.org/10.2340/00015555-1531)
- Amberg, N., & Fogarassy, C. (2019) Green Consumer Behavior in the Cosmetics Market. *Resources*. 8(3), 137. <https://doi.org/10.3390/resources8030137>
- ANVISA (2004). Cosmetic product stability guide. National Health Surveillance Agency: Brasília, Brazil.
- ANVISA (2012). Cosmetic product quality control guide. National Health Surveillance Agency: Brasília, Brazil.
- ANVISA (2019). Cosmetic product quality control guide. National Health Surveillance Agency: Brasília, Brazil.
- ANVISA (2022). Resolution No. 630, of March 10, 2022. National Health Surveillance Agency: Brasília, Brazil.
- Barros, C., & Barros, R. B. G. (2020). Natural and Organic Cosmetics: Definition and Concepts. *Preprints*. 2020050374 [10.20944/preprints202005.0374.v2](https://doi.org/10.20944/preprints202005.0374.v2)
- Bazin, R., & Fanchon, C. (2006). Equivalence of face and volar forearm for the testing of moisturizing and firming effect of cosmetics in hydration and biomechanical studies. *International Journal of Cosmetic Science*. 28(6), 453-460. <https://doi.org/10.1111/j.1467-2494.2006.00352.x>
- Bruschi, M. L., Cardoso, M. L. C., Lucchesi, M. B., & Gremião, M. P. D. (2003). Gelatin microparticles containing propolis obtained by spray-drying technique: Preparation and characterization. *International Journal of Pharmaceutics*. 264(1-2), 45-55. [https://doi.org/10.1016/S0378-5173\(03\)00386-7](https://doi.org/10.1016/S0378-5173(03)00386-7)
- Chen, G., & Tao, D. (2005). An experimental study of stability of oil-water emulsion. *Fuel Processing Technology*. 86(5), 499-508. <https://doi.org/10.1016/j.fuproc.2004.03.010>
- Darlenski, R., Sassning, S., Tsankov, N., & Fluhr, J. W. (2009). Non-invasive in vivo methods for investigation of the skin barrier physical properties. *European Journal of Pharmaceutics and Biopharmaceutics*. 72(2), 295-303. <https://doi.org/10.1016/j.ejpb.2008.11.013>
- Dickinson, K. L., Wiegand, U. K., & Thijssen, J. H. J. (2019). Soft meets hard -- how does freeze-thaw cycling affect the microstructure of particle-stabilised emulsions? 1-31. <https://doi.org/10.48550/arXiv.1902.08531>
- Dini, I., & Laneri, S. (2021). The New Challenge of Green Cosmetics: Natural Food Ingredients for Cosmetic Formulations. *Molecules*. 26(13), 3921. <https://doi.org/10.3390/molecules26133921>
- Du Plessis, J., Stefaniak, A., Eloff, F., John, S., Agner, T., Chieh Chou, T., Nixon, R., Steiner, M., Franken, A., Kudla, I., & Holness, L. (2013). International guidelines for the in vivo assessment of skin properties in non-clinical settings: Part 2. transepidermal water loss and skin hydration. *Skin Research and Technology*. 19(3), 265-278. <https://doi.org/10.1111/srt.12037>
- ECOCERT. [S. l.] (2012). Ecocert benchmark for natural and organic cosmetics. Available at: <http://brazil.ecocert.com/system/files/Referencial-Cosmeticos-Naturais-e-Organicos-Ecocert.pdf>. Access on: 12 Jul. 2020.
- Epstein H. (2009). Cosmeceutical vehicles. *Clin Dermatol*. 27(5), 453-460.
- Filipe, G. A., Bigotto, B. G., Rocha, C. B., Chue-Goncalves, M., Kobayashi, R. K. T., Lonni, A. A. S. G., & Celligoi, M. A. P. C. (2022). Development of a multifunctional and self-preserving cosmetic formulation using sophorolipids and palmarosa essential oil against acne-causing bacteria. *Journal of Applied Microbiology*. 132, 10 - 25. <https://doi.org/10.1111/jam.15659>

- Fluhr, J. W., Feingold, K. R., & Elias, P. M. (2006). Transepidermal water loss reflects permeability barrier status: Validation in human and rodent in vivo and ex vivo models. *Experimental Dermatology*. 15(7), 483–492. <https://doi.org/10.1111/j.1600-0625.2006.00437.x>
- Fonseca-Santos, B., Corrêa, M. A., Chorilli, M. (2015). Sustainability, natural and organic cosmetics: Consumer, products, efficacy, toxicological and regulatory considerations. *Brazilian Journal of Pharmaceutical Science*. 51(1), 17–26. <https://doi.org/10.1590/S1984-82502015000100002>
- Furman, A. C., Veit, M. T., Palácio, S. M., Gonçalves, & G. C., Barbieri, J. C. Z. (2022). Sustainability in the production process of the cosmetic industry: a literature review. *Research, Society and Development*. 11(13), pág. e586111335852. <https://doi.org/10.33448/rsd-v11i13.35852>
- Gonçalves, G., & Campos, P. (2009). Application of biophysics methods in the study of the effectiveness of dermocosmetic products. *Brazilian Journal of Pharmaceutical Sciences*. 45(1), 1-10. <https://doi.org/10.1590/S1984-82502009000100002>
- Hecht, L. L., Wagner, C., Landfester, K., Landfester, K., & Schuchmann, H. P. (2011). Surfactant concentration regime in miniemulsion polymerization for the formation of MMA nanodroplets by high-pressure homogenization. *Langmuir*. 27(6), 2279–2285. <https://doi.org/10.1021/la104480s>
- Heinrich, U., Koop, U., Leneveu-Duchemin, M. C., Osterrieder, K., Bielfeldt, S., Chkarnat, C., Degwert, J., Häntschel, D., Jaspers, S., Nissen, H. P., Rohr, M., Schneider, G., & Tronnier, H. (2003). Multicentre comparison of skin hydration in terms of physical-, physiological- and product-dependent parameters by the capacitive method (Corneometer CM 825). *International Journal of Cosmetic Science*. 25(1-2), 45-53. <https://doi.org/10.1046/j.1467-2494.2003.00172.x>
- Isaac, V. L. B., Cefali, L. C., Chiari, B. G., Almeida, M. G. J., Ribeiro, H. M., & Corrêa, M. A. (2013). Effect of various thickening agents on the rheological Properties of oil-in-water emulsions containing nonionic emulsifier. *Journal of Dispersion Science and Technology*. 34(6), 880–885. <https://doi.org/10.1080/01932691.2012.695952>
- Izquierdo, P., Wiechers, J. W., Escribano, E., Garcia-Celma, M. J., Tadros, T. F., Esquena, J., Dederen, J. C., & Solan, C. (2007). A Study on the Influence of Emulsion Droplet Size on the Skin Penetration of Tetracaine. *Skin pharmacology and physiology*. 20, 263–270. <https://doi.org/10.1159/000106076>
- Jaksic, I., Lukic, M., Malenovic, A., Reichl, S., Hoffmann, C., Muller-Goymann, C., Daniels, R., & Savic, S. (2012). Compounding of a topical drug with prospective natural surfactant- stabilized pharmaceutical bases: Physicochemical and in vitro/in vivo characterization - A ketoprofen case study. *European Journal of Pharmaceutics and Biopharmaceutics*. 80(1), 164–175. <https://doi.org/10.1016/j.ejpb.2011.09.001>
- Larson, R. G., & Wei, Y. (2019). A review of thixotropy and its rheological modeling. *Journal of Rheology*. 63(3), 477–501. <https://doi.org/10.1122/1.5055031>
- Lerche, D., & Sobisch, T. (2011). Direct and accelerated characterization of formulation stability. *Journal of Dispersion Science and Technology*. 32(12), 1799–1811. <https://doi.org/10.1080/01932691.2011.616365>
- Lodén, M., & Wessman, C. (2001). The influence of a cream containing 20% glycerin and its vehicle on skin barrier properties. *International Journal of Cosmetic Science*. 23(2), 115–119. <https://doi.org/10.1046/j.1467-2494.2001.00060.x>
- Logger, J. G. M., Olydam, J. I., der Weng, W. W., & Erp, P. E. J. (2019). Noninvasive Skin Barrier Assessment: Multiparametric Approach and Pilot Study. *Cosmetics*. 6. <https://doi.org/10.3390/cosmetics6010020>
- Lonni, A. A. S. G.; Munhoz, V.M.; Lopes, G. C.; Longhini, R.; Pangoni, F. B. B.; Santos, R. S.; Junqueira, M. V.; Natali, M. R. M.; Leite-Mello, E. V. S.; Guimaraes, F. B.; Baesso, M. L.; Scarminio, Ieda S; Bruschi, M. L.; Mello, J. C. P. (2015). Development and characterization of multiple emulsions for controlled release of Trichilia catigua (Catuaba) extract. *Pharmaceutical Development and Technology*, 1-10. <http://dx.doi.org/10.3109/10837450.2015.1081611>
- Otto, A., Wiechers, J. W., Kelly, C. L., Dederen, J. C., Hadgraft, J., & Du Plessis, J. (2010). Effect of Emulsifiers and Their Liquid Crystalline Structures in Emulsions on Dermal and Transdermal Delivery of Hydroquinone, Salicylic Acid and Octadecenedioic Acid. *Skin Pharmacology and Physiology*. 23, 273-282. <https://doi.org/10.1159/000314702>
- PHARMACOPOEIA. (2019). *Brazilian Pharmacopeia*, v. 1. 6° ed. Brasília, Brazil.
- Prestes, P. S., Peres, D. D., de Freitas, A. Z., Consiglieri, V. O., Kaneko, T. M., Velasco, M. V., & Baby, A. R. (2016). Particle size and morphological characterization of cosmetic emulsified systems by optical coherence tomography (OCT). *Brazilian Journal of Pharmaceutical Sciences*. 52(2), 273–280. <https://doi.org/10.1590/S1984-82502016000200005>
- Ribeiro, A. S., Estanqueiro, M., Oliveira, M. B., & Lobo, J. M. S. (2015). Main Benefits and Applicability of Plant Extracts in Skin Care Products. *Cosmetics*. 2(2), 48-65. <https://doi.org/10.3390/cosmetics2020048>
- Rogiers, V. (2001). EEMCO Guidance for the Assessment of Transepidermal Water Loss in Cosmetic Sciences. *Skin Pharmacology and Applied Skin Physiology*. 14(2): 117–128. <https://doi.org/10.1159/000056341>
- Sánchez-Regaña, M., Llambí-Mateos F., Salleras-Redonnet, M., Iglesias Sancho, M., Collgros Totosaus, H., & Umbert-Millet, P. (2013). Compounding as a Current Therapeutic Option in Dermatology. *Actas Dermo-Sifiliográficas*. 104(9), 738–756. <https://doi.org/10.1016/j.ad.2012.03.007>
- Savic, S., Tamburic, S., Kovacevic, A., Daniels, R., & Müller-Goymann, C. (2008). Natural surfactant-based emulsion systems: The influence of common pharmaceutical excipients on colloidal structure and physical stability. *Journal of Dispersion Science and Technology*. 29(9), 1276–1287. <https://doi.org/10.1080/01932690701857558>
- Silva, A. C., Amaral, M. H., González-Mira, E., Santos, D., & Ferreira, D. (2012). Solid lipid nanoparticles (SLN) - based hydrogels as potential carriers for oral transmucosal delivery of Risperidone: Preparation and characterization studies. *Colloids and Surfaces B: Biointerfaces*. 93, 241–248. <https://doi.org/10.1016/j.colsurfb.2012.01.014>

Siska, B., Snejdrova, E., Machac, I., Dolecek, P., & Martiska, J. (2019). Contribution to the rheological testing of pharmaceutical semisolids. *Pharmaceutical Development and Technology*. 24(1), 80-88. <https://doi.org/10.1080/10837450.2018.1425432>

Surber C., & Smith E.W. (2005). The mystical effects of dermatological vehicles. *Dermatology*. 210(2), 157-68. <https://doi.org/10.1159/000082572>

Thiemann, A., & Petersen, W. (2016). Emulsifier system influences O/W emulsion preservation. *Personal Care*. 60-62.

Willenbacher, N., & Georgieva, K. Rheology of disperse systems. In: Bröckel, U., Meier, W., & Wagner, G. *Product Design and Engineering: Formulation of Gels and Pastes*. Weinheim: Wiley-VCH. (2013), p. 7-49.