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MULTIPLE EMULSIONS OBTAINED BY A ONE-STEP METHOD - NIACINAMIDE RELEASE ASSAY

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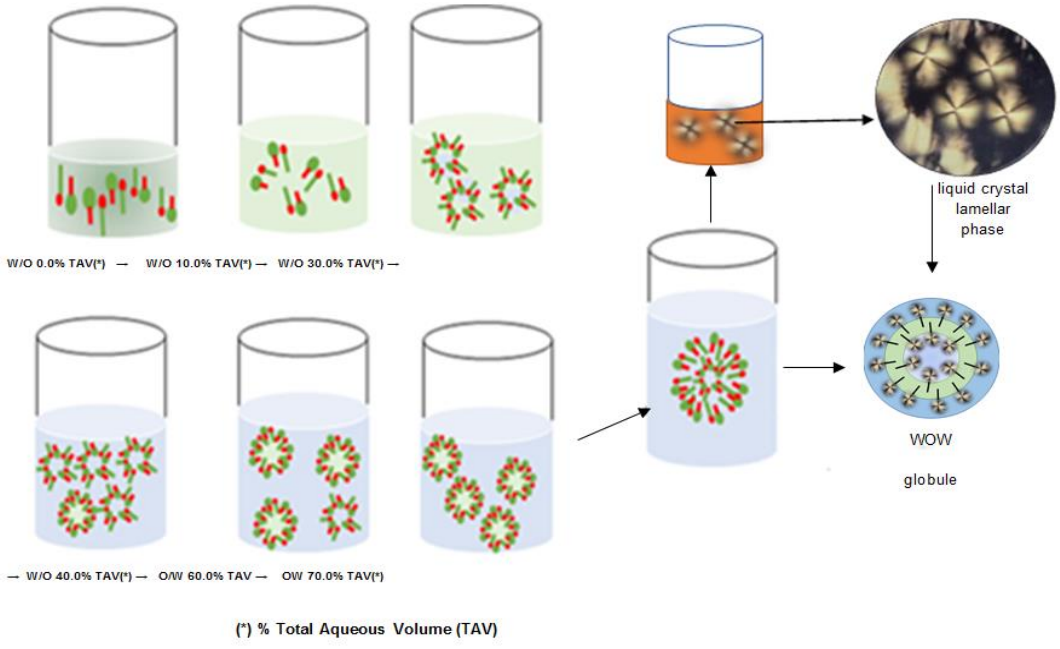
ABSTRACT

Multiple emulsions (ME) are used as alternatives in skin care products due to particular properties as: (a) ability to encapsulate cosmetics; (b) protection of the encapsulated substance from the oxidation process; (c) ability to encapsulate incompatible substances; (d) sustained release. Their use in cosmetics depends on the components of the formulation, parameters of the preparation process and studies release of the active in a safe and efficient way. ME cosmetic containing niacinamide was prepared by the one-step method and indicated for the protection against skin ageing. It was employed vegan surfactants and as oily phase the avocado oil and the sunflower oil. The niacinamide was used as active for studies of in vitro liberation. Multiple emulsions were developed by one step method by phase inversion using avocado or sunflower oils, which have antioxidant properties. It can be observed that niacinamide was encapsulated and released slowly from the W/O/W emulsions.

KEYWORDS: Multiple emulsions; one step method; avocado oil; sunflower oil; studies of stability and release of niacinamide.

GRAPHICAL ABSTRACT



INTRODUCTION

Cosmetic skincare products are made available to consumers in the form of emulsions and often contain different categories of active ingredients, which characterizes the versatility of use of these systems both in the cosmetic field and in different application domains.

Emulsions are widely used as vehicles in cosmetics due to their sensory characteristics and ease of practical application to the skin. Simple or primary emulsions consist of a heterogeneous two-phase system where two immiscible liquids (aqueous phase and oily phase) are dispersed by the presence of a surfactant. These surfactant molecules possess an amphiphilic characteristic, meaning that part of the chain is polar-hydrophilic and the other part is non-polar-lipophilic. This unique characteristic allows surfactant molecules to interact with both liquids- water and oils, thus reducing the surface tension between them. Emulsions are thermodynamically unstable systems and require energy from different sources, such as increased temperature and/or agitation. The most common types are called water-in-oil (W/O) or oil-in-water (O/W).

Multiple or complex emulsions are systems where both types of emulsion (W/O and O/W or O/W and W/O) exist simultaneously, and can therefore be of the W/O/W or O/W/O type.[1,2,3,4,5]

Multiple emulsions (MEs) have attracted great interest from the cosmetic, pharmaceutical, food, and chemical industries, mainly to optimize the prolonged release and transport of incompatible active ingredients in the different phases of their structure. They have been studied for application in different domains, such as food, chemistry, cosmetics, and pharmaceuticals, due to their particular properties, such as: (a) ability to encapsulate active ingredients; (b) protection of active ingredients against oxidation processes; (c) ability to encapsulate incompatible substances, with hydrophilic/lipophilic/hydrophilic characteristics, respectively, for W/O/W emulsions; (d) sustained release of the encapsulated active ingredient.

Generally, the process of obtaining multiple emulsions (ME) is carried out in two stages, with the redispersion of the primary emulsion in the dispersing phase, oily or aqueous, in the presence of one or two surfactants. However, this process is meticulous, time-consuming, has limiting factors, and can still produce unstable MEs, although it is the most studied and used method for the preparation of multiple emulsions.

To prepare these systems, it is necessary to study the components of the formulation, as well as analyze the parameters of the preparation process and the consequent release of the active ingredient(s) in a safe and effective manner. The stability of primary and multiple emulsions is essentially due to the choice of surfactants and their respective Hydrophilic-Lipophilic Balance (HLB) values. The HLB value of a surfactant is an important property in the emulsification process, as it determines the type of emulsion to be produced, that is, it allows predicting the type of behavior of the compound.[6]

Multiple emulsions, also called complex emulsions, considered as "emulsions of emulsions," are polydisperse structures in which each oil/water (O/W) and water/oil (W/O) emulsion coexists in a single system. Thus, a first emulsion is formed and then dispersed as tiny globules in another phase, known as the continuous phase.[7]

Multiple emulsions can also be produced by the one-step method, which can occur, for example, with non-ionic emulsifiers. The one-step emulsification process is feasible, but rarely used due to the low reproducibility and lack of long-term stability of multiple emulsions.

The method is based on well-designed surfactant systems capable of spontaneously generating multiple emulsions when the phases are combined under certain conditions. The correct hydrophilic-lipophilic balance is crucial, generally involving specific polymeric emulsifiers or combinations of surfactants capable of stabilizing several interfaces in parallel [6, 8]

In phase inversion technique or single-step technique, the increase in the dispersed phase volume can lead to the formation multiple emulsions by increasing the phase-volume ratio. It involves the addition of aqueous phase containing the hydrophilic emulsifier to an oil phase containing of liquid paraffin and lipophilic emulsifier.[9]

The effects of some composition variables on the development of multiple emulsions by one-step method were evaluated and their morphology characterized by Morais, JM *et al*.[10] The formulations that remained stable during the period of the test were submitted to centrifugation and thermal stress tests. The stability and the morphology of multiple droplets were affected not only by the type and concentration of the surfactants employed, but also by the water/oil ratios used. The results suggest that the formation of multiple droplets could involve a combination of transitional and catastrophic phase inversions. The results provide improved knowledge about the one-step emulsification method, a simplified process to prepare multiple emulsions when compared to the two-steps method.

Avocado oil

Avocado oil is rich in fatty acids as oleic, palmitic, linoleic, palmitoleic acid, as well as other unsaturated acids such as linolenic and arachidic, it also contains antioxidants (vitamin E), beta carotene, vitamin D, potassium, lecithin, and many other nutrients that can nourish and moisturize the skin. Additionally, macro elements (Ca, Mg, and Na) and microelements (Fe, Zn, Cu, and Mn) were determined in the mineral profile. Based on its probable chemical composition, it is

recommended in cosmetic formulations for skin care because it moisturizes and nourishes, relieves inflammation from psoriasis and eczema, prevents and treats acne; accelerates wound healing, treats sunburned skin, reduces signs of aging, improves nail health and keeps surrounding skin soft, and can reduce breakage and improve scalp health. Avocado oil is generally considered non-comedogenic and does not clog skin pores.[11,12,13]

Sunflower oil

Sunflower oil is a low-cost, natural alternative containing lipids with a composition similar to those of the stratum corneum. Applied topically, sunflower oil helps reduce transepidermal water loss (TEWL), keeping the skin hydrated and contributing to the integrity of the outer layer. It has moisturizing and nourishing properties, as well as a tissue-repairing effect (skin healing). Due to its high vitamin E content, it exhibits antioxidant activity.

It is gentle and quickly absorbed by the skin, making it suitable for all skin types. It softens and smoothes dry and rough skin, as well as protecting it from future damage. Sunflower oil acts as a moisturizer in the treatment of skin conditions such as xerosis and atopic dermatitis. It is a non-comedogenic vehicle (it has high absorption and does not clog pores). Sunflower oil contains an exceptionally high amount (70 to 80%) of linoleic acid and can help maintain and restore the skin's natural moisture barrier. The fatty acid composition of sunflower contains essential polyunsaturated fatty acids (PUFAs), such as linoleic and alpha-linolenic acids, which aid in the normal protection of the epidermis.[14,15]

Niacinamide

Niacinamide (the active form of vitamin B3) is recognized for its significant effects on the skin, such as protecting the skin barrier, rejuvenating, brightening, and also treating dermatitis and acne vulgaris. It acts by maintaining the skin barrier intact, reducing transepidermal water loss (TEWL) with topical application. It is considered safe and can be incorporated into skin care products, alone or in combination with other active ingredients, in the range of 0.001 to 0.5% in body creams, makeup removers, shampoos, and in products to minimize wrinkles, in concentrations between 1.0 and 5.0%, in products labeled anti-aging.[12]

It has skin lightening and anti-pigmentation effects and is indicated in topical creams for skin lightening and correction of dark spots. Due to its anti-inflammatory action when applied topically, it can relieve skin imperfections and acne and act as an antibacterial agent in vivo against *E. coli* and *S. aureus*.[16]

OBJECTIVES

The aim of this research was to obtain multiple emulsions using a single-step formation method, utilizing natural oils and niacinamide as active ingredients. The specific objectives were: to study the influence of surfactant concentration and the percentages of aqueous and oily phases on the formation of multiple emulsions, as well as to study the *in vitro* release of niacinamide by dialysis.

MATERIAL AND METHODS

1. Material (International Nomenclature of Cosmetics Ingredients -INCI)

Oil Phase

Persea gratissima (Avocado) Oil- Avocado oil. Destilaria Bauru SP. Brazil

Helianthus annus (Sunflower) seed oil Sunflower oil- Bunge Brazil

Surfactants

Sorbitan monooleate (Span®80- SP80)- Croda do Brazil- HLB value= 4.3

Lauroyl/ Myristoyl Methyl Glucamide (Glucotain® Flex- GTF)- Clariant. HLB value= 11.5

Capryloyl/ Caproyl Methyl Glucamide (and) Lauroyl/Myristoyl Methyl Glucamide **(**Glucotain® Plus- GTP)- Clariant. HLB value= 12.0

Capryloyl/ Caproyl Methyl Glucamide (Glucotain® Clear- GTC) Clariant. HLB value= 13.0

Aqueous phase: Purified water

Active: Niacinamide (Innovasell. SP- Brazil

2. METHODS

2.1. Determination of the required HLB value for avocado and sunflower oils

It was carried out following these steps: (a) Microscopic analysis of the surfactant mixture by polarized light microscopy; (b) Formulations with avocado or sunflower oil were prepared with appropriate amounts of surfactants.

The required HLB value for the oils was calculated using the following equations*:*

$$A+ B = 100$$

$$HLB_A \times 0.01.A + HLB_B \times 0.01.B = HLB_R$$

where:

A = percentage of hydrophilic surfactant; B = percentage of lipophilic surfactant; HLBA = Hydrophilic-Lipophilic Balance of A; HLBB = Hydrophilic-Lipophilic Balance of B; HLBR = Hydrophilic-Lipophilic Balance resulting from or required for the oil phase.

2.2. Preparation of dispersions by the ternary diagram method

After determining the necessary HLB value for avocado oil and sunflower oil, a ternary diagram was constructed for each oil. The formulations were prepared as follows:

Phase Inversion Temperature (PIT) Method

Different formulations were prepared according to the method reported by Morais et al.[10]: a mixture 5.0% (w/w) of hydrophilic surfactant +SP80 was used and solubilized in the oil phase. Then, the oil and aqueous phases were heated separately to 70 ± 5°C in a water bath. The aqueous phase was slowly added to the oil

phase under continuous stirring at 600 ± 10 rpm (RW 20 digital – IKA®) for better mixing and uniform distribution of the emulsion components. Niacinamide was initially dissolved in part of the volume of the aqueous phase and then kept under stirring until the temperature reached 25± 2°C. Subsequently, a multiple emulsion was prepared under stirring and rapidly cooled in an ice bath until it reached a temperature of 25± 2°C.[17,18,19]

2.3. Preliminary Stability Assessment

After 24 hours of preparation, the emulsions were subjected to macroscopic evaluation, microscopic analysis (Olympus BX50 microscope), and centrifugation tests (Centrilab CE80 model). Subsequently, they were selected for further research by determining pH, electrical conductivity, and centrifugation values before, during, and after thermal stress tests and freeze-thaw cycles. All determinations were performed in triplicate.

2.3.1. Determination of pH value

A digital pH meter (Digimed Model DM 22 Digicron Analytical) was used. The sample was diluted in purified water (1:10 w/v), homogenized at a temperature of 25± 5ºC (in triplicate).[20, 21]

2.3.2. Freeze-thaw cycle

The samples were subjected to 4± 2°C for 24 hours and then to a temperature of 45± 5°C also for 24 hours. In this way, one cycle was completed, with seven cycles being carried out, and the emulsions were evaluated by centrifugation, determination of pH and electrical conductivity values.[21]

2.3.3. Thermal Stress

The samples were subjected to heating in an ultrathermostatic bath (Art Lab- Nova Técnica) in the temperature range of 40 to 85± 2°C, with the temperature increased in increments of 5 °C and maintained at that temperature for 30 minutes. After 30 minutes at each temperature, the samples were macroscopically analyzed for signs of instability.[22]

2.3.4. Determination of Electrical Conductivity

The electrical conductivity of freshly purified water, was used as a reference, was determined (Digimed model DM-32 Digicron Analytical). The electrical conductivity of O/W emulsions resulted in 25 ± 2°C and was evaluated in triplicate.[21]

2.4. Accelerated Stability Assessment

Samples (in triplicate) considered stable through preliminary stability tests were subjected to accelerated aging at 4± 2°C, 25± 5°C and 45± 5°C. After 120 days, they were evaluated in relation to macroscopic appearance and physicochemical properties. Samples were initially evaluated at time zero, 24 hours and on days 7, 15, 30, 60, 90 and 120.[21]

2.5. Niacinamide Release by Dialysis Method

The study was conducted in a dialysis cell (Figure 1) using a cellulose membrane and carried out in different stages: a) determination of the niacinamide standard curve; b) determination of the amount of niacinamide to be used in the formulation:

a) Determination of the niacinamide absorption peak - was determined from niacinamide in aqueous solution in the range of 190 to 350 nm;

b) It was necessary to plot a standard analytical curve from an aqueous solution (in triplicate) containing 0.175g of niacinamide (recommended value) stirred in a water bath. Samples (2.0 mL) were collected from the receiving medium at different times: 30 (t1), 60 (t2), 120 (t3) and 240 (t4) minutes;

c) study of niacinamide release from an aqueous solution containing niacinamide;

d) study of niacinamide release from multiple emulsions obtained at point 36 - the same concentration of niacinamide was added to the aqueous phase and subjected to a release study by dialysis. The concentration of niacinamide released was determined by UV spectrophotometry.

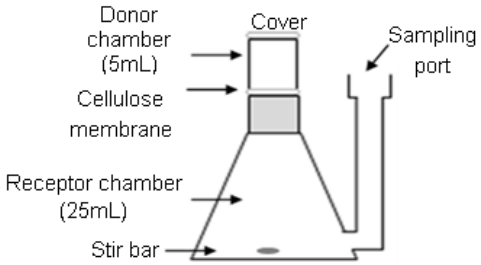
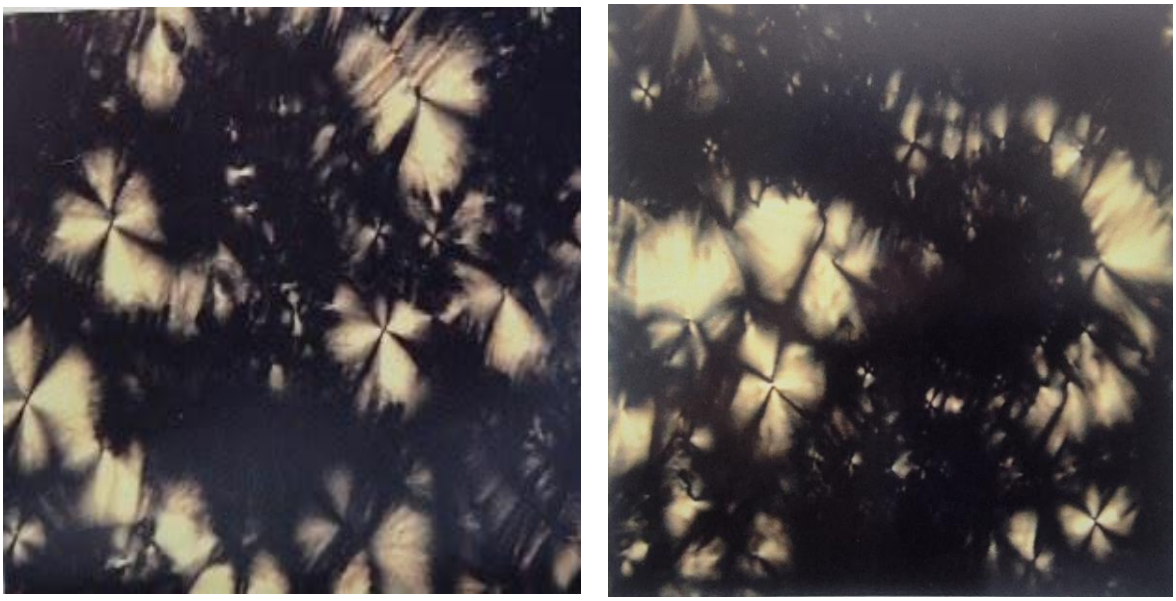


Figure 1: Dialysis cell diagram.

3. RESULTS AND DISCUSSION

3.1. Microscopic analysis of surfactant mixtures and determination of HLB value

Surfactant mixtures were prepared according to Table 1, with HLB values ranging from 5.0 to 12.0. The SP80 + GTC surfactant mixture, with an HLB value of 8.0, differs from the others by the presence of liquid crystals identified microscopically (200X) (Figure 2)- and by the absence of phase separation in the other surfactant mixtures.



Lamellar and median crystalline phases
Figure 2: Mixture of SP80 + GTC with HLB value = 8.0.

3.2. Preparation of Dispersions by the Ternary Diagram Method

The development of the dispersed system was carried out through the execution of the ternary phase diagram, which macroscopically shows the appearance of each formulation as a function of the amounts of oil, water, and surfactants. In this way, the number 36 cosmetic formulation on ternary diagram was identified and differentiated macroscopically, remaining stable without phase separation after 48 hours of preparation. In the preparation of the ternary diagram, purified water, avocado or sunflower oil, and surfactants (SP80 + GTC) with an HLB value of 8.0, were used, in varying concentrations from 10.0% to 10.0%. According to Maciel, NR et al.[4] and Dănilă, E., et al.[22] qualitatively identical and quantitatively different simple emulsions (O/W and W/O) and multiple emulsions (W/O/W) can be identified, the latter being observed and identified under a microscope in this study. The presence of multiple emulsions at point 36 (80.0% purified water, 10.0% oil, and 10.0% surfactant) of the ternary diagram for avocado oil (Figure 3A) and sunflower oil (Figure 3B), and with the SP80+GTC surfactant mixture with an HLB value of 8.0, was identified by microscopic analysis.

Table 1: Mixtures of surfactants (SP80+GTC) with HLB values ranging from 5.0 to 12.0.

HLB A= 4.3												
HLB B1= 11.5					HLB B2= 12.0				HLB B3= 13.0			
HLB F↓	A (%)	B1 (%)	A (g)	B1 (g)	A (%)	B2 (%)	A (g)	B2 (g)	A (%)	B3 (%)	A (g)	B3 (g)
5.0	90.28	9.72	4.51	0.49	90.91	9.09	4.55	0.45	91.95	8.05	4.60	0.40
6.0	76.39	23.61	3.82	1.18	77.92	22.08	3.90	1.10	80.46	19.54	4.02	0.98
7.0	62.50	37.50	3.13	1.88	64.94	35.06	3.25	1.75	68.97	31.03	3.45	1.55
8.0	48.61	51.39	2.43	2.57	51.95	48.05	2.60	2.40	*57.47*	*42.53*	*2.87*	*2.13*
9.0	34.72	65.28	1.74	3.26	38.96	61.04	1.95	3.05	45.98	54.02	2.30	2.70
10.0	20.83	79.17	1.04	3,96	25.97	74.03	1.30	3.70	34.48	65.52	1.72	3.28
11.0	6.94	93.06	0.35	4.65	12.99	87.01	0.65	4.35	22.99	77.01	1.15	3.85
11.5	0.00	100.00	0.00	5.00	-	-	-	-	-	-	-	-
12.0	-	-	-	-	0.00	100.00	0.00	5.00	11.49	88.51	0.57	4.43
13.0	-	-	-	-	-	-	-	-	0.00	100.00	0.00	5.00

Legend: (A) *Sorbitan monooleate (Span®80- SP80) = HLB value= 4.3; (B1) Lauroyl/Myristoyl Methyl Glucamide (Glucotain® Flex- GTF) HLB value= 11.5 (B2) Capryloyl/Caproyl Methyl Glucamide (and) Lauroyl/Myristoyl Methyl Glucamide (Glucotain® Plus- GTP) HLB value= 12.0; (B3) Capryloyl/Caproyl Methyl Glucamide (Glucotain® Clear- GTC) HLB value= 13.0.*

(A) Emulsion with avocado oil **(B) Emulsion with sunflower oil**

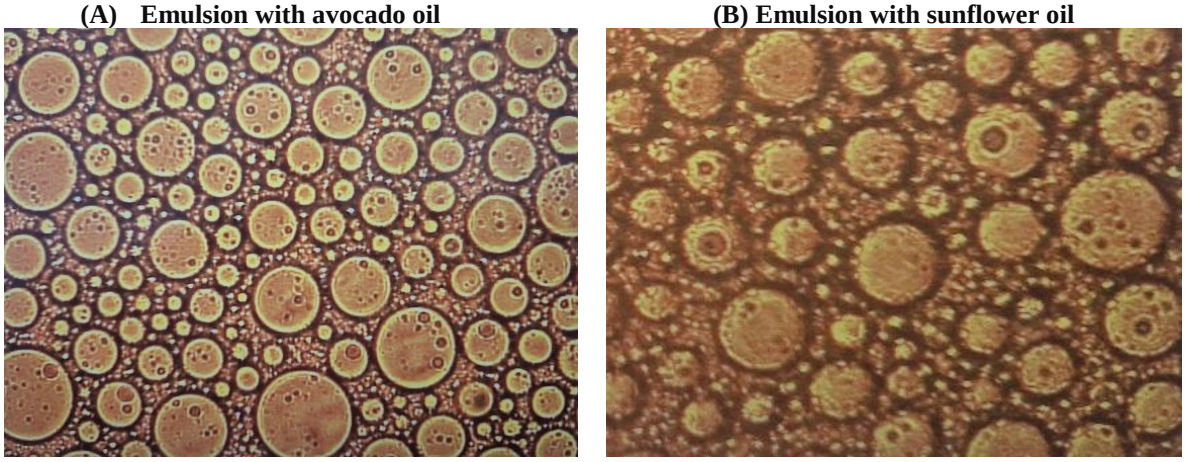


Figure 3: Multiple emulsions obtained in a single step with avocado oil at point 36 (A) and sunflower oil at point 36 (B).

3.3. One-step method

The development of W/O/W systems was first reported during the phase inversion process of a W/O emulsion to an O/W emulsion (Figure 4). Unlike other researchers, in this study, purified and heated water (75± 2°C) was added to the oil phase containing both hydrophilic and lipophilic surfactants, under constant stirring at 600 rpm until room temperature was reached (25± 2°C). The phase inversion mechanism can occur in different steps: W/O emulsion→ lamellar and median crystalline phase structure formation→ O/W emulsion surrounded by micelles→ W/O/W emulsions (Figure 5). W/O/W phase inversion occurs if the total HLB value of the surfactants in the system approaches the required HLB value of the oil.[23,24]

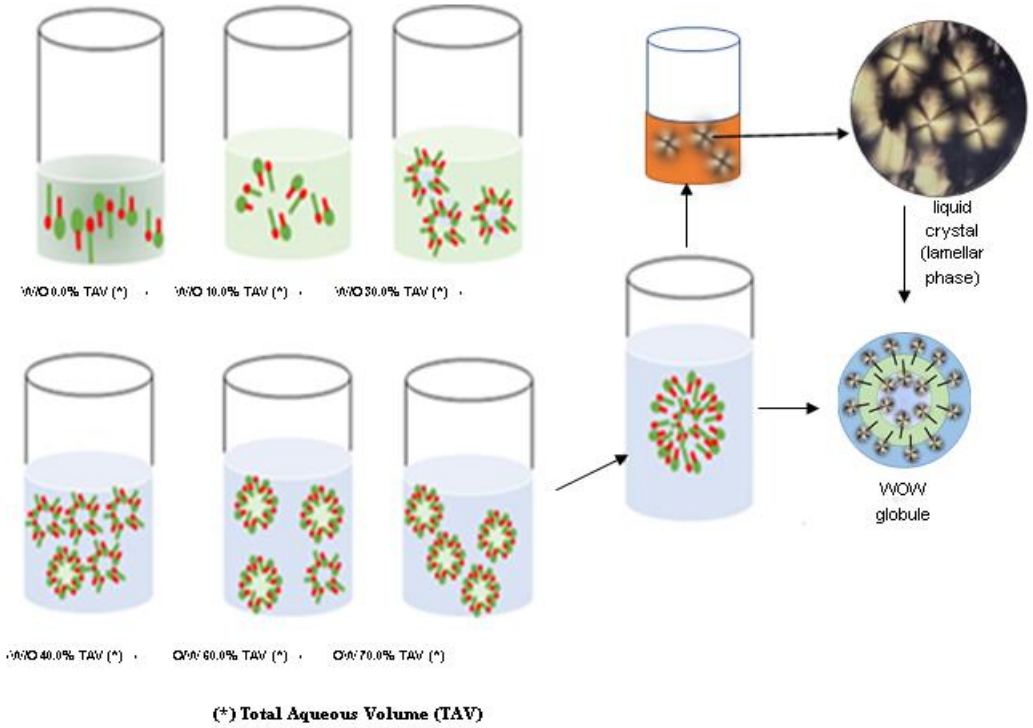


Figure 4: W/O/W formation scheme by one step- phase inversion method.

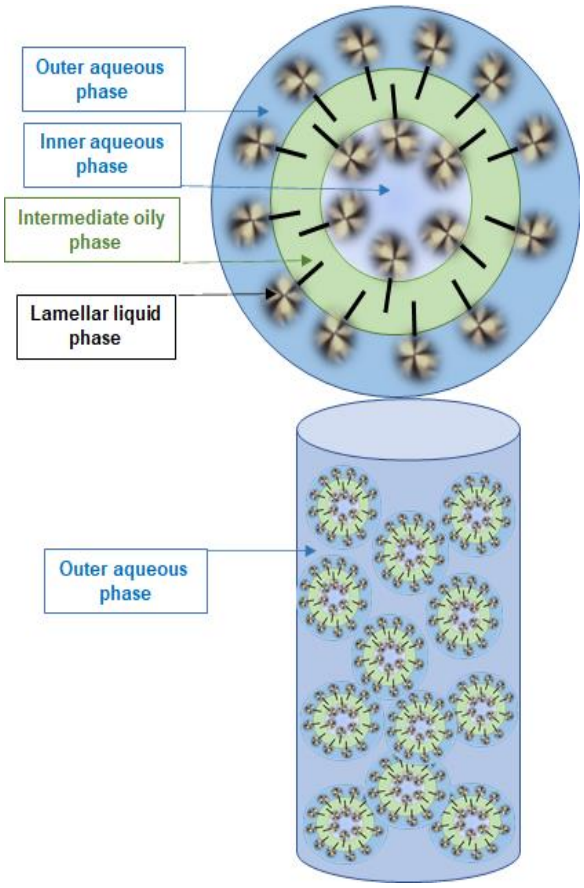


Figure 5: W/O/W globule scheme with liquid crystal-lamellar phase.

The phase inversion process occurs due to the gradual increase in the volume of the added aqueous phase and, also due to the solubility of the surfactants, which varies with increasing temperature, defined as the phase inversion temperature. Therefore, this requires greater dedication to developing preliminary formulations for choosing the ideal surfactant system, as well as the conditions (temperature, agitation, agitation time) employed in the process.

Transitional inversion occurs when there is a change in the surfactant's affinity for the two phases, induced by factors such as temperature and the hydrophilic-lipophilic equilibrium (HLB) value. The correct hydrophilic-lipophilic balance is crucial, generally involving specific emulsifiers or combinations of surfactants capable of stabilizing several interfaces in parallel. The stable surfactant system exhibits some distinct properties, such as strong solubilizing power and ultra-low interfacial tension.[17,23,24,25]

For the phase inversion phase method is necessary a well-designed surfactant system capable of spontaneously generating multiple emulsions when the phases are combined under certain conditions.[8,24,25]

A W/O/W multiple emulsion containing d-biotin [26] was prepared by the one-step method, but demonstrating a high polydispersity index and, with aging, the droplet size increases over a longer storage period, showing the fragile stability of this method.

3.4. Variation in the volume of purified water and the amount of oil in the formulation

3.4.1. Concentration of the volume of purified water

From the main point 36, derived formulations were prepared, maintaining the concentration of surfactants (SP80+GTC -HLB value = 8.0) and oil (avocado or sunflower), but varying the concentration of purified water by 3.0 mL, to observe its influence on the formation of multiple globules (Table 2).

Table 2: Variation in the volume of purified water (3.0 mL).

Formula nr↓	*Purified water (mL)*	Oil (AO/SO) (w/w)	Surfactant (GTC+ SP80) w/w)	ME (+presence; - absent)
36	*80.0*	*10.0*	*10.0*	*+(AO/SO)*
36.1	*17.0*	2.5	2.5	+(AO/SO)
36.2	*14.0*	2.5	2.5	+ (AO/SO)
36.3	*10.0*	2.5	2.5	+ (AO/SO)
36.4	*8.0*	2.5	2.5	+(SO)
36.5	*5.0*	2.5	2.5	-

Legend- ME- Multiple Emulsions, AO- Avocado Oil; SO- Sunflower Oil

Microscopic analysis identified the presence of multiple emulsions at the points named as points 36.1, 36.2, and 36.3 for avocado oil and for sunflower oil at points 36.1, 36.2, 36.3, and 36.4.

3.4.2. Concentration of the amount (g) of oil

From the main point 36, derived formulations were prepared, maintaining the surfactant concentration (SP80+GTC- HLB value = 8.0) and purified water, but varying the oil concentration (avocado or sunflower) by 0.5g, to observe its influence on the formation of multiple globules (Table 3).

Table 3: Variation in the amount of oil (0.5g).

Formula nr↓	Purified water (mL)	*Oil (AO/SO) (w/w)*	Surfactant (GTC+ SP80) (w/w)	ME (+presence; - absent)
36	80	*10*	10	+(AO/SO)
36.1	20.0	*2.0*	2.5	+(AO/SO)
36.2	20.0	*1.5*	2.5	+ (AO/SO)
36.3	20.0	*1.0*	2.5	+ (AO/SO)
36.4	20.0	*0.5*	2.5	+(SO)

Legend- ME- multiple emulsions, AO- Avocado Oil; SO- Sunflower Oil

Microscopic analysis identified the presence of multiple emulsions at the point named as points 36.1; 36.2 and 36.3 for avocado oil and for sunflower oil in derived points 36.1; 36.2, 36.3 and 36.4.

3.5. Preliminary Stability Assessments

After microscopic analysis, samples with multiple globules were subjected to preliminary stability tests.

3.5.1. Determination of pH Value

The pH value of the purified water used in all formulations was 6.72, which is within the theoretical range considered standard for water (6.0 to 9.0). pH values were determined (26 ± 2 °C) for formulation 36 and for the derived formulations with variation in the volume (mL) of water (Table 4) or in the amount (g) of oil (Table 5).

Table 4: pH values for the formulations derived from point 36 with variation in the volume (mL) of purified water.

AO	1	2	3	Mean
36	5.36	5.25	4.82	5.14
36.1	6.15	5.11	4.82	5.39
36.2	5.74	5.31	5.28	5.44
36.3	5.57	5.35	5.20	5.37
SO	**1**	**2**	**3**	
36	5.46	5.34	5.47	5.42
36.1	5.42	5.47	5.37	5.42
36.2	5.61	5.59	5.49	5.56
36.3	5.55	5.38	5.51	5.48
36.4	5.55	5.59	5.58	5.57

Legend- AO- Avocado Oil; SO- Sunflower Oil

Table 5: pH values for formulas derived from point 36 with variation in the amount (g) of oil.

AO	1	2	3	Mean
36.1	4.89	4.89	4.70	4.82
36.2	4.74	4.96	5.01	4.91
36.3	4.95	4.93	4.71	4.86
SO	**1**	**2**	**3**	
36.1	5.61	5.60	5.50	5.57
36.2	5.45	5.52	5.52	5.49
36.3	5.54	5.50	5.53	5.52
36.4	5.76	5.32	5.52	5.43

Legend- AO- Avocado Oil; SO- Sunflower Oil

The pH value of the aqueous niacinamide solution (Table 6) used as the active ingredient in the formulation was determined at room temperature (26± 2ºC).

Table 6: pH values for aqueous niacinamide solution.

Sample	1	2	3	Mean
Niacinamide (aq. sol.)	5.02	4.85	4.92	4.93

3.5.2. Determination of electrical conductivity

The electrical conductivity value of the purified water was 1.512 (µs/cm), therefore falling within the theoretical range considered standard for water (1 to 50 µs/cm, at 25°C). The electrical conductivity values (µs/cm) were determined (26± 2°C) for formulation 36 and derived formulas (Tables 7 and 8).

3.5.3. Thermal Stress

The samples showed the presence of multiple globules and macroscopic stability up to a temperature of 70ºC. At higher temperatures, phase separation was observed, and absence of multiple globules were observed in the formulations for avocado and sunflower oils, at point 36.

3.5.4. Freeze-Thaw Cycle

The emulsions at point 36 and derived, with avocado or sunflower oil were subjected (in triplicate) to a temperature of 4± 2°C for 24 hours and then to a temperature of 45± 5°C also for 24 hours, thus completing the freeze-thaw cycle. After the first period of 24 hours at a temperature of 4± 2°C, the emulsions were observed to be stable and showed the presence of multiple globules.

3.6. Accelerated Stability Assessments

Samples were prepared at point 36 using avocado oil or sunflower oil and were subjected to variable temperature conditions (4± 2°C, 25± 5°C and 45± 5°C) and were evaluated (triplicate) in relation to their macroscopic and also physicochemical characteristics at different times: at time zero (1h), 24 hours and at the 7th, 14th and 30th days.

Emulsions subjected to temperatures of 4± 2°C and 25± 5°C proved to be stable and showed a large presence of multiple globules. However, a slight phase separation was observed from the 7th day onwards in emulsions subjected to 45± 5°C.

Table 7: Electrical conductivity values (µs/cm) for the formulations at point 36 and derived with variation in the amount of vegetable oil.

AO	1	2	3	Mean
36	98.10	95.70	94.46	96.08
36.1	92.37	91.60	92.46	92.14
36.2	95.55	94.93	95.96	95.37
36.3	90.32	91.61	92.50	91.48
SO	**1**	**2**	**3**	
36	82.90	87.79	89.86	86.85
36.1	45.82	45.92	46.53	48.09
36.2	69.72	69.78	68.21	69.23
36.3	74.00	74.46	73.87	74.11
36.4	63.54	64.39	64.43	64.12

Legend- AO- Avocado Oil; SO- Sunflower Oil

Table 8: Electrical conductivity values (µs/cm) for the formulations at point 36 and derived with variation in the volume of purified water.

AO	1	2	3	Mean
36	102.86	103.19	102.93	102.99
36.1	95.89	96.27	96.41	96.19
36.2	77.50	77.86	78.13	77.83
36.3	95.58	95.68	96.24	95.83
SO	**1**	**2**	**3**	
36	137.69	134.77	135.19	135.78
36.1	76.36	76.68	77.09	76.77
36.2	62.02	62.16	60.90	61.70
36.3	92.16	91.26	91.68	91.70
36.4	113.37	112.50	114.80	113.56

Legend- AO- Avocado Oil; SO- Sunflower Oil

4. Niacinamide Release Analysis by Dialysis

The absorption peak was identified at 260 nm. Figure 6 shows the standard analytical curve of niacinamide in aqueous solution (in triplicate) containing 0.175 g of niacinamide.

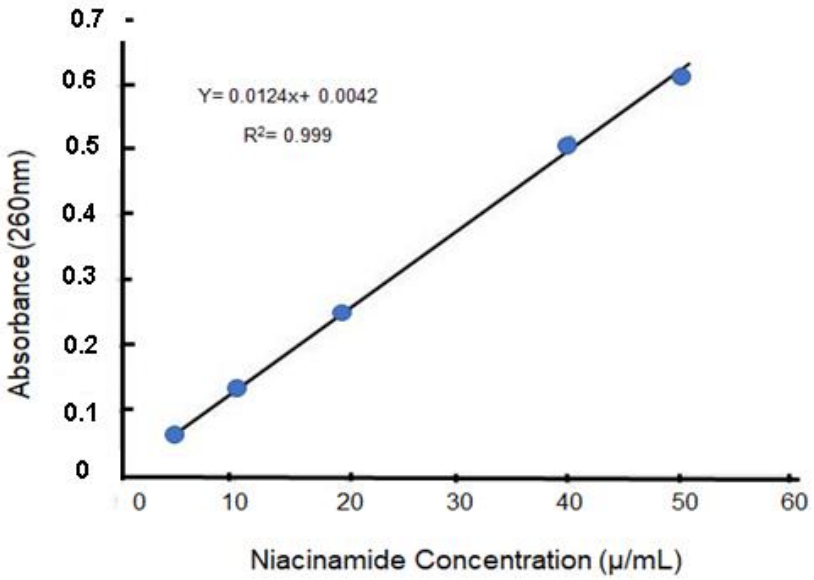


Figure 6: Standard analytical curve for niacinamide.

Niacinamide Release Analysis by Dialysis

Aqueous solutions of 15.0 mL containing 0.42% (0.06g) niacinamide was then prepared, and the samples were diluted to calculate the niacinamide concentration released at times t1, t2, t3 and t4 (Table 9).

Table 9: Determination of the amounts of niacinamide released from the aqueous solution.

Time (min.) Sample ↓	30 (t1)	60 (t2)	120 (t3)	240 (t4)
1	0.152	0.168	0.171	0.190
2	0.121	0.136	0.149	0.185
3	0.101	0.109	0.160	0.189
niacinamide released (µg/mL)	6.61	7.71	9.51	11.77
dilution factor (x5000) (µg/mL)	33050	38550	47550	58850
niacinamide released final (g/mL)	0.033	0.038	0.047	0.059

For multiple emulsions it was added 0.175g/mL of niacinamide in a 25mL in aqueous phase. The niacinamide liberation was showed in Table 10 for WOW AO and in Table 11 for WOW SO.

Table 10: Determination of the amounts of niacinamide released from WOW AO.

Time (min.) Sample ↓	30 (t1)	60 (t2)	120 (t3)	240 (t4)
1	0.090	0.124	0.258	0.376
2	0.102	0.159	0.239	0.345
3	0.098	0.135	0.205	0.358
niacinamide released (µg/mL)	4.40	7.84	15.48	25.61
dilution factor (x5000) (µg/mL)	22000	49362	77400	128050
niacinamide released final g/mL)	0.022	0.049	0.077	0.128

Considering that was added to W/O/W sample, at the end of the dialysis process, there was a release of 25.13% of niacinamide from the multiple emulsion with avocado oil (Table 10).

Table 11: Determination of the amounts of niacinamide released from WOW SO.

Time (min.) Sample ↓	**30 (t1)**	**60 (t2)**	**120 (t3)**	**240 (t4)**
1	0.128	0.256	0.289	0.312
2	0.178	0.266	0.300	0.315
3	0.157	0.234	0.296	0.342
niacinamide released (µg/mL)	9.05	16.93	20.40	22.96
dilution factor (x5000) (µg/mL)	45250	84650	102000	113300
niacinamide released final (g/mL)	0.045	0.084	0.102	0.113

Considering that 0.175g/mL was added to W/O/W sample, at the end of the dialysis process, there was a release of 22.18% of niacinamide from the multiple emulsion with sunflower oil.

Table 12: Comparison of the percentage of niacinamide released from different systems as a function of time.

Time (min)→ System ↓	**30 (t1)**	**60 (t2)**	**120 (t3)**	**240 (t4)**
Niacinamide Aq. Sol.	55.0	63.33	78.33	98.33
WOW AO	4.31	13.33	15.12	25.13
WOW SO	8.83	16.49	20.02	22.18

Legend: WOW AO= Multiple Emulsions with avocado oil; WOW SO= Multiple Emulsions with sunflower oil.

CONCLUSION

The main objective was to develop stable formulations for improved skin care. During development, it was observed that emulsification temperatures values applied was a necessary parameter to obtain in a single step multiple W/O/W. It was also observed the presence of lamellar crystalline phase at surfactant HLB values influenced the production of multiple globules. The use of the ternary or phase diagram enhanced the best choice of water/oil/surfactant concentrations used in the formulation of the multiple emulsions. The applied emulsification method allowed for the production of a

multiple system in a single step and determined the physicochemical characteristics of the resulting dispersed systems. W/O/W globules were identified by microscopy. After evaluating the influence of thermal stress and the addition of additives on the complex systems, it was found that multiple emulsions show a prolonged release for both emulsions based in sunflower and avocado oil. The multiple emulsions obtained can be considered stable in view of the evaluation and analysis methodologies employed.

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