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Original Article

Prediction of the physical stability and quality of O/W cosmetic emulsions using full factorial design

[Predicción de la estabilidad física y calidad de emulsiones cosméticas O/W mediante diseño factorial completo]

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Abstract

Context: Full factorial design is effective in predicting the properties optimization and processing conditions of cosmetic emulsions. However, in most factorial designs, the technological quality and accelerated stability tests of emulsions are not included together as dependent variables.

Aims: To predict the technological quality and physical stability of model cosmetic emulsions from the combination of formulation and processing factors using a 2^3 full factorial design.

Methods: A 2^3 full factorial design with 95 % of confidence was generated in order to evaluate the influence of critical factors during the manufacture of cosmetic emulsions on their quality and physical stability. Emulsifier, concentration of stearic acid (formulation factors) and type of cooling (processing factor) were the independent variables, whereas spreadability, bulk density and pH were dependent variables for technological quality and centrifugation, heating-cooling and freeze-thaw cycles were dependent variables for physical stability of the emulsions. All cosmetic emulsions were prepared with a low stirring speed of 100 rpm.

Results: The formulation containing 1% w/w sodium lauryl sulfate, 1.05% w/w stearic acid and prepared with continuous cooling, did not show indicative changes of physical instability by visual inspection after accelerated physical stability tests. These results were confirmed with emulsions stored for 12 and 24 months using real storage conditions. However, formulations containing an emulsifier blend composition (Tween 80: Sodium lauryl sulfate, 2:1 w/w), showed signs of creaming after these tests.

Conclusions: Sodium lauryl sulfate at 1 % w/w, increased the physical stability of the emulsions by increasing their consistency and by other possible mechanisms. Real storage conditions confirmed the stability prediction performed using the combination of accelerated stability tests (centrifugation/cooling-heating/freeze-thaw cycles), aided by the purposed full factorial design.

Keywords: cosmetic emulsion; creaming; emulsifier; full factorial design; sodium lauryl sulfate.

Resumen

Contexto: El diseño factorial completo es eficaz para predecir la optimización de las propiedades y las condiciones de proceso de las emulsiones cosméticas. Sin embargo, en la mayoría de diseños factoriales no se incluyen las pruebas de calidad tecnológica y estabilidad acelerada de las emulsiones, ambas como variables dependientes.

Objetivos: Predecir la calidad tecnológica y la estabilidad física de emulsiones cosméticas modelo a partir de la combinación de factores operacionales y de formulación utilizando un diseño factorial completo 2^3.

Métodos: Se realizó un diseño factorial completo 2^3 con un 95% de confianza para evaluar la influencia de factores críticos durante la elaboración de emulsiones cosméticas en su calidad y estabilidad física. Variables independientes: emulsionante, concentración de ácido esteárico (factores de formulación) y tipo de enfriamiento (factor de proceso). Variables dependientes: extensibilidad, densidad aparente y pH para la calidad tecnológica, y la centrifugación, ciclos frío-calor y ciclos congelación-descongelación fueron las variables dependientes para la estabilidad física de las emulsiones. Todas las emulsiones cosméticas se elaboraron con una velocidad de agitación de 100 rpm.

Resultados: La formulación que contenía lauril sulfato de sodio al 1% m/m, ácido esteárico al 1.05% m/m y con un enfriamiento continuo, no mostró cambios indicativos de inestabilidad física a simple vista, tras las pruebas de estabilidad física acelerada. Estos resultados se confirmaron a los 12 y 24 meses de almacenadas las emulsiones en condiciones reales de almacenamiento. Sin embargo, las formulaciones que contenían la mezcla de emulsionante (Tween 80: lauril sulfato de sodio, 2:1 m/m), mostraron presencia de cremado tras estas pruebas.

Conclusiones: La presencia de lauril sulfato de sodio al 1% m/v incrementó la estabilidad física de las emulsiones aumentando su consistencia, y también por otros mecanismos. Las condiciones reales de almacenamiento confirmaron la predicción de la estabilidad mediante la combinación de las pruebas de estabilidad aceleradas (centrifugación/ciclos frío calor/ciclos congelación-descongelación), lo que se logró mediante el diseño factorial completo.

Palabras Clave: cremado; diseño factorial completo; emulsión cosmética; emulsionante; lauril sulfato de sodio.

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INTRODUCTION

Optimization techniques are widely used in cosmetics. Recently, cosmetic formulations have employed experimental design techniques as an optimization alternative to find the best combination of formulation and processing variables (Kovács et al., 2016; Filipovic et al., 2017; Vasiljevic et al., 2017; Djiobie Tchienou et al., 2018). Full factorial design is a particularly useful technique, which provides lots of information especially when the number of factors is minor. That is why several cosmetic formulations apply this tool because it is effective in product properties optimization and processing conditions, so that the best emulsion will be obtained (Górecki et al., 2015). Górecki et al. (2015) reported the use of a 2^3 full factorial design to obtain the optimal formulation composition of an oiling bath cosmetic formulation.

Emulsions are thermodynamically unstable systems, consisting of at least one immiscible liquid intimately dispersed in another in the form of droplets, so an emulsifier must be added to make possible the formation of the emulsion and its long-term stability (Djiobie Tchienou et al., 2018). All emulsions will break down over time by different processes such as creaming, flocculation, coalescence and phase inversion. In order to predict this, accelerated physical stability is determined by several tests as centrifugation, cooling-heating and freeze-thaw cycles (Restu et al., 2015).

In most studies researchers use experimental design techniques in order to evaluate the influence from formulation and processing variables on response variables relating to the quality of the freshly prepared emulsion. Response variables used offer criteria of technological quality and accelerated stability tests such as centrifugation, cooling-heating and freeze-thaw cycles, which are usually applied to the optimized emulsion, but they are not included as dependent variables in most factorial designs (Almeida et al., 2015; Cekić et al., 2015; Avish and Swaroop, 2018). It is difficult to obtain emulsions with good technological quality but once this goal is achieved, it is even more difficult to maintain their physical stability for a reasonable period. In order to predict these stability problems, accelerated stability tests are applied to estimate short-term physical stability. Therefore, the aim of the present work is to predict the technological quality and physical stability of model cosmetic emulsions from the combination of formulation and processing factors using a 2^3 full factorial design.

MATERIAL AND METHODS

Materials

Sodium lauryl sulfate (SLS) was obtained from Tames Trading, Spain. Polysorbate 80 (Tween 80), sodium chloride and hydrochloric acid from Panreac, Spain. The Provincial Laboratory of Natural Products (Santa Clara, Cuba) supplied the *Aloe vera* hydroalcoholic extract. All other reagents used in this study were of reagent quality (Yangling, China).

Quality controls carried out on the *Aloe vera* extract informed by the producer included: Organoleptic characteristics as transparent liquid with characteristic odor and color, pH 5.1, alcoholic content of 63% v/v, and density of 0.9074 g/cm^3.

Preparation of *Aloe vera* extract by maceration

The gel from the leaves was cut into small pieces and 15 g was weighed in a digital balance (Boeco, Alemania). Subsequently, 15 mL of distilled water along with 70 mL of ethanol, which had been measured in a graded cylinder was added to *Aloe vera* gel. It was kept in maceration during seven days for 24 h at room temperature.

The leaves of *Aloe vera* (L.) Burm.f. (*Xanthorrhoeaceae*) were collected from a seed farm in the municipality of Santa Clara (GPS coordinates: 22.441548, -79.965827), belonging to the ministry of agriculture. The sample of the species was identified by Dr. Idelfonso Castañeda Noa, professor of the faculty of Biology and specialist in Plant Taxonomy of the UCLV Botanical Garden. Specimens of the species were compared with the sample identified in the ULV herbarium located in the

Botanical Garden of the mentioned institution, with number 11400.

Formulation optimization

A full two-level, three-factors (2^3) factorial experimental design was carried out, in triplicate (Argenta et al., 2014). The components used in the cosmetic emulsions are described in Table 1. Emulsifier, concentration of stearic acid (SA) and type of cooling (TC) were selected as independent variables. Table 2 summarizes the experimental runs, their factors combination and the translation of the coded levels to the experimental units used in this study (Table 2). Eight formulations were prepared due to the combination of the independent variables (Table 3). Spreadability, bulk density, pH, centrifugation, cooling-heating and freeze-thaw cycles tests were considered as the response variables (dependent variables). The order for the preparation of the emulsions was randomized, based on the experimental design.

Table 1. Model cosmetic emulsion composition.

No	Ingredients	Quantity for 100 g
	Oil phase	
1	Solid petrolatum	5.225 (%, w/w)
2	Stearic acid	**0.500 or 1.050 (%, w/w)**
3	Stearyl alcohol	2.116 (%, w/w)
	Water phase	
4	Propylene glycol	1.0083 (%, v/w)
5	SLS	**0.500 or 1.000 (%, w/w)**
6	Tween 80	1.000 (%, v/w)
7	Sodium benzoate	0.583 (%, w/w)
8	Sodium chloride	0.500 (%, w/w)
9	Hydrochloric acid (5 %)	1.000 (%, v/w)
10	*Aloe vera* extract	1.025 (%, v/w)
11	Perfume	0.208 (%, v/w)
12	Distilled water	86.033 (%, v/w)

For all the experiments stirring speed was set at 100 rpm and the type of cooling (TC) was continuous or at intervals, according to the experiment. SLS: Sodium lauryl sulfate.

Table 2. Experimental runs for the formulations of *Aloe vera* cosmetic emulsion used in the study with coded values.

Independent variables	Name	Unit	Levels	
			Low (-)	High (+)
X_1	Emulsifier	%, w/v	SLS 1 %, w/w	Tween 80 1% v/w + SLS 0.5% w/w
X_2	SA	%, w/w	0.5%, w/w	1.05%, w/w
X_3	TC	-	At intervals	Continuous

SA: Stearic acid; TC: Type of cooling; SLS: Sodium lauryl sulfate.

Table 3. Description of eight formulations generated according to the 2^3 full factorial design.

Formulation	Factor		
	Emulsifier	**SA**	**TC**
F1	Tween 1%+ SLS 0.5%	1.05%	Continuous
F2	SLS 1%	0.50%	At intervals
F3	Tween 1%+ SLS 0.5%	1.05%	At intervals
F4	Tween 1%+ SLS 0.5%	0.50%	Continuous
F5	SLS 1%	1.05%	Continuous
F6	SLS 1%	1.05%	At intervals
F7	SLS 1%	0.50%	Continuous
F8	Tween 1%+ SLS 0.5%	0.50%	At intervals

SA: Stearic acid; TC: Type of cooling; SLS: Sodium lauryl sulfate.

Preparation of cosmetic emulsion model

Oil in water emulsion was prepared by initially melting SA in a 500 mL beaker in a temperature-controlled water bath (MLW, Alemania) at 75°C and to the molten mass added stearyl alcohol and solid petrolatum. Aqueous phase along with SLS, NaCl, propylene glycol and sodium benzoate heated at the same temperature as oil phase. Previous to the incorporation to the aqueous phase, a gel was formed when 0.5 g NaCl added to 0.7 mL distilled water to form a solution, and which was added then to SLS. Those formulations where an emulsifier blend was used, were composed by Tween 80: SLS (2:1) at 1.5 % at w/w total concentration. Aqueous phase was heated at the same temperature and then added to the oil phase, electric agitator (IKA, United States), with continuous stirring (100 rpm) for 15 min. Then, emulsions were cooled to 40°C continuously or at specific intervals of 10 min with agitation. Perfume, hydrochloric acid and *Aloe vera* extract were added when the temperature downs at 40°C (Dănilă et al., 2019).

Criteria for the selection of cosmetic emulsion model

Cosmetic emulsion model must show no visible signs of physical instability (creaming, phase separation, coalescence). Initial formulation development was separated in two batches of 100 g and any formulation that shows signs of physical instability immediately and/or after 24 h of storage at room temperature (30°C and 70% RH) was considered unsuitable and therefore not used for further investigation. Physical instability was evaluated by visual inspection immediately after manufacture and then 24 h later. Fresh formulations were stored for 48 h at 30°C/70%HR and then analyzed.

Evaluation of model cosmetic emulsion

All the manufactured formulations were evaluated through quality control tests and physical stability tests. All tests were carried out in triplicate.

Organoleptic properties

Color, odor, appearance, brightness, homogeneity and presence of lumps were determined for each formulation, according to Cuban Standard 1085 (2015) for cosmetic tests.

Type of emulsions

Dilution test

The emulsion (0.5 g) was dispersed in 50 mL of distilled water. Type of emulsion was determined by the following criteria: It is a direct emulsion, oil/water (O/W), if after dilution the preparation becomes milky. It is a reverse emulsion, water/oil (W/O) if it does not allow dilution: the water sep-

arates as a layer on the emulsion (Altuntas and Yener, 2015.)

Spreadability test

The emulsion (2 g) was placed within a circumference of 1 cm diameter pre-marked on a glass plate (Proaño et al., 2020). Then, a second glass was placed over with a weight of 467.8 g and allowed to rest for five min. Spreadability refers to the area covered by a fixed amount of cream sample after the uniform spread of sample on the glass slide. The average of three readings was recorded. The spreadability (S) can be calculated using the following equation [1]:

$$S = A = \pi\,(\bar{r}/8)^2 \quad [1]$$

Where:

S = Spreadability (cm^2); A =Area of the formed circumference (cm^2); π = Constant (3.1416); r = Radius of the circumference formed by the emulsion (cm).

Bulk density

The bulk density determination was performed according to the procedure established in Cuban Standard 1086 (2015), which indicates the test method for the determination of bulk density in semisolid cosmetics.

A dry and clean measuring cylinder was weight, and 10 g of the emulsion were added slowly and constantly to avoid occluded air. The specimen was tapped ten times gently on a folded towel to eliminate possible incorporation of air. Then, the sample was left to rest for ten min and completed volume up to 10 mL. Finally, the measuring cylinder with the sample was weight. The density (D) can be calculated using the following equation [2]:

$$D = \frac{W1 - W0}{V} \quad [2]$$

Where:

D = Density (g/mL); W_0= Weight of measuring cylinder (g); W_1 = Weight of measuring cylinder with the sample (g); V = Volume of measuring cylinder (mL).

pH determination

The pH determination was performed according to the procedure established in Cuban Standard 836 (2011) that indicates the test method for the determination of pH in cosmetics. Briefly, approximately 1 g of product was dispersed in 10 mL of distilled water and the pH was measured in a digital pH meter (pH 300, HANNA, Romania) previously calibrated at 28°C.

Accelerated stability tests

All formulations obtained through experimental design were subjected to centrifugation test, cooling-heating and freeze-thaw cycles. Physical stability was determined using the equation [3] (Badawi and El-Khordagui, 2014).

$$EPE = \frac{VUP}{TEV} \quad [3]$$

Where:

EPE: Emulsion phase stability; VUP: Volume of upper phase (cm^3); TEV: Total emulsion volume (cm^3).

Centrifugation test

The emulsion resistance to external factors was evaluated via centrifugation test, which allowed to assess the stability of the emulsion by the influence of the centrifugal force. Centrifugation assay was carried out according to ANVISA specifications for cosmetic products (Brasil, 2004). To perform the test, 10 g of each sample was placed in centrifugal tubes and centrifuged at 3000 rpm for 30 min with a centrifuge diameter of 8.5 mm (Yingtai TG 16, China).

Cooling-heating and Freeze-thaw cycles

Cooling-heating cycles: Two grams of each emulsion in a test tube were kept in a refrigerator (4°C) changing to the oven (40°C), every 24 h for eight days (Brasil, 2004).

Freeze-thaw cycles: Four cycles between freezer (-5°C) and oven (40°C) were performed every 24 h, using the same methodology as in the cooling-heating cycle.

A satisfactory physical stability is considered when no signs of instability already mentioned are detected at the end of these tests.

Physical stability using real storage conditions

Three batches of 200 g each were prepared and packed in 235 mL amber glass bottles with black Bakelite lids. Physical stability of cosmetic emulsions was evaluated 24 months after manufacture in real conditions at 30°C and 70% RH (González and Mazorra, 2007). Organoleptic properties, phase separation or another sign of instability were observed.

Statistical analysis

Statistical analysis was performed using the software package Statgraphics Centurion version 15.2.14. Fisher's least significant difference (LSD) procedure was used to discriminate among the means. The level of significance was defined as $p<0.05$.

RESULTS AND DISCUSSION

Organoleptic properties and type of emulsion

All formulations freshly prepared have a shiny white color. Their appearance is homogeneous, and no lumps were detected after 24 and 48 h. They offer smoothness to the touch. After dilution, emulsions presented a milky aspect and they were completely washed, so they were classified as direct emulsions (O/W).

Formulation optimization and factor influence from factorial design

Alkyl sulfate group (SLS) is an anionic emulsifier widely used in cosmetics and has been employed in numerous types of cosmetics such as baby shampoos, bath products, eye makeup, hair conditioners, hand and body care preparations, moisturizers, skin care preparations (Sheskey et al., 2017). SLS forms self-emulsifying bases with fatty alcohols in a concentration between 0.5 – 2.5% (Sheskey et al., 2017). SLS also can form molecular complexes with long chain fatty alcohols (e.g. stearyl alcohol), which contributes remarkably to the consistency and absorption properties of the preparation. The addition of salts like sodium chloride can improve the rheological properties of alkyl sulfates surfactants and viscosity of the medium. Low levels of added sodium chloride to alkyl sulfates preparations form a solution with spherical micelles and Newtonian behavior. The micelles transform in wormlike micelles and increase the formulation viscosity (Cornwell, 2018). The formation of molecular complexes, added to the effect of electrolytes in increasing the consistency of preparations containing SLS, can lead to a higher degree of consistency and better physical stability, with a lower requirement of emulsifier and oil phase (Limbu et al., 2014). Also, in a matter of safety, anionic emulsifiers such as SLS can cause eye and skin irritation in some people, as well as other surfactants. In this sense, Cosmetic Ingredient Review (CIR), an independent organization of the cosmetic industry of the United States, considers that it is a safe ingredient in its current uses in cosmetic products (Panel, 2008). However, irritation is quite common when SLS is used in concentrations higher than 2% w/v (Sheskey et al., 2017). In products intended for prolonged contact with the skin, their concentrations should not exceed 1% w/v (Bondi et al., 2015). These safety aspects were considered for the inclusion of polysorbate 80 (Tween 80) as emulsifier in the formulations, as well as other technological and/or formulation factors.

In order to avoid high pH values out of the skin physiological range, a hydrochloric acid solution (HCl 5%, w/w) was added to the formulations. This ingredient is used as an acidifying agent, generally as dilute acid in 1% v/v as maximum allowable concentration (Sheskey et al., 2017). The use of sodium benzoate also contributes to maintain the pH of the skin. Sodium benzoate is the sodium salt of benzoic acid (organic acid), with antiseptic properties and is generally used as a preservative for foods, pharmaceuticals and cosmetics, since it eliminates most yeasts, bacteria and fungi (Halla et al., 2018). Sodium benzoate is effective in the undissociated form, but according to its dissociation constant pK_a, more acidic conditions are required

and a maximum pH 5 is recommended (Boukarim et al., 2009).

A general matrix for the statistical factorial design, containing the obtained formulations, independent variables at various levels and the observed responses is shown in Table 4.

Effect of factors on spreadability (SP)

As shown in Table 4, the spreadability values were found to be in the range of 149 to 207 cm^2. Emulsifier (X_1) and concentration of SA (X_2) are the main factors influencing on spreadability (Fig. 1A). The regression equation [4] for SP is shown below.

$$SP = 180.611 + 14.9943*X_1 - 7.87464*X_2 \quad [4]$$

Fig. 1B shows LSD Fisher intervals for SP when X_1 is the main factor. It should be noted SP was increased when X_1 was at a high level: Tween 80 1% v/w + LSS 0.5% w/w. Formulations F1, F3, F4 and F8 are in agree with these results. This behaviour could be related to the low concentration of SLS (0.5%, w/w), used in this case. SLS contributes to the consistency of the emulsions by two mechanisms: (1) Molecular complexes formation with high molecular weight alcohols and (2) by adding electrolytes such as sodium chloride (Dickinson and Ritzoulis, 2000). Both contribute to decrease the spreadability values. Thus, formulations from factorial design with the lowest concentration must show less consistency, and so spreadability values will be higher. Ekott and Akpabio (2010) report that SLS acts forming films around the water drops in oil/water interfaces and therefore it presents a thin film stabilization mechanism. The formation of rigid and viscoelastic films increases the stability of the emulsion by increasing interfacial viscosity and decreasing interfacial tension. Consequently, interfacial barrier prevents coalescence of the dispersed water droplets. SLS, an anionic surfactant, stabilizes emulsions by increasing the electrostatic repulsion between droplets, the Marangoni-Gibbs effect and the already mentioned mechanism of surfactant adsorption and interfacial film (Akbari and Nour, 2018). When the concentration of emulsifier is low (0.5%, w/w), the ability of covering the droplets is low as well, thereby the droplets are likely to coalesce with their neighbors and form larger droplets.

Table 4. Influence of factors on the response variables from factorial design.

Formulation	Independent variables			Response variables (mean ± SD)				
	X_1	X_2	X_3	Bulk density (g/mL)	Spreadability (cm^2)	pH	Phase stability	
							Centrifugation	Freeze/thaw cycles
F1	+	+	+	0.99 ± 0.02	188.01 ± 16.93	5.18 ± 0.05	0.69 ± 0.28	0.96 ± 1.24
F2	-	-	-	0.97 ± 0.04	179.55 ± 12.25	5.12 ± 0.02	1.00 ± 0.53	0.95 ± 0.29
F3	+	+	-	1.05 ± 0.08	196.09 ± 25.22	5.17 ± 0.09	0.66 ± 0.85	0.71 ± 1.52
F4	+	-	+	1.12 ± 0.06	207.25 ± 19.39	5.18 ± 0.06	0.73 ± 0.54	0.79 ± 1.08
F5	-	+	+	0.94 ± 0.02	157.69 ± 6.81	5.28 ± 0.41	1.00 ± 0.29	1.00 ± 0.17
F6	-	+	-	0.98 ± 0.01	149.15 ± 8.96	5.14 ± 0.02	1.00 ± 0.34	0.97 ± 0.17
F7	-	-	+	0.99 ± 0.06	176.09 ± 23.50	5.33 ± 0.07	1.00 ± 0,81	0.87 ± 0.00
F8	+	-	-	0.97 ± 0.02	191.06 ± 35.87	5.16 ± 0.07	0.67 ± 1,06	0.68 ± 1.44

X_1: Emulsifier: - (SLS 1%, w/w), + (Tween 80 1%, v/w +SLS 0.5%, w/w), X_2: Concentration of SA: - (0.5%, w/w), + (1.05%, w/w), X_3: Type of cooling: - (At intervals), + (Continuous), SD: Standard deviation. Phase separation was used as a measure of accelerated physical stability of the emulsions in both tests.

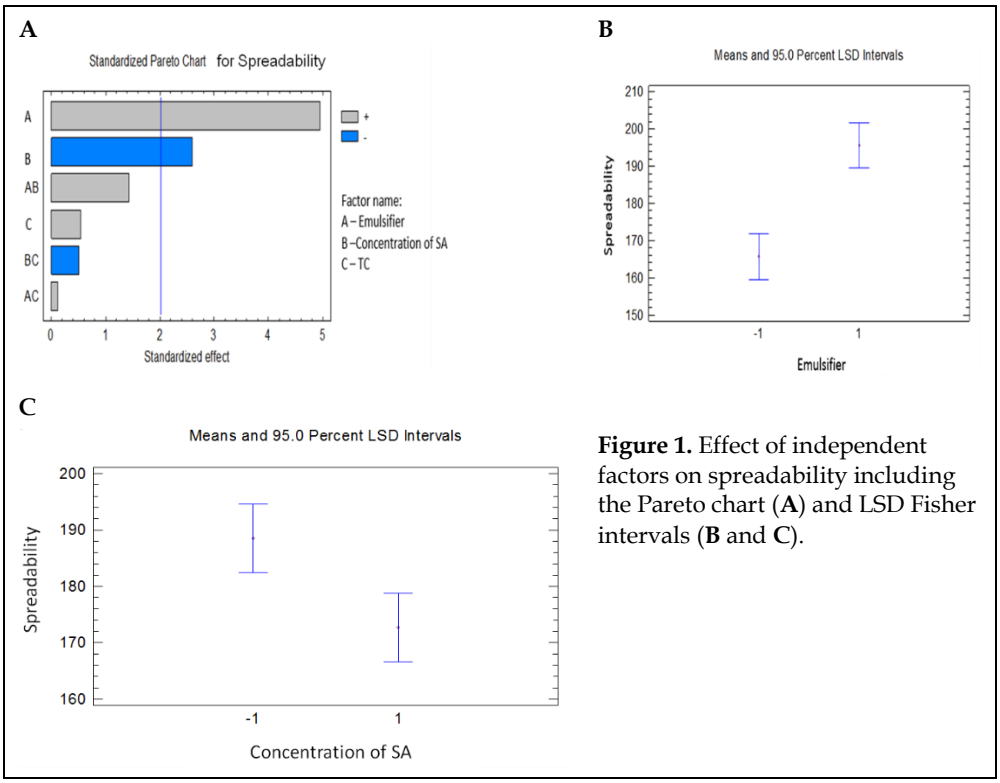

Figure 1. Effect of independent factors on spreadability including the Pareto chart (**A**) and LSD Fisher intervals (**B** and **C**).

In the present study, SLS 0.5% w/w was employed in formulations F1, F3, F4 and F8, in a surfactant blend: Tween 80: SLS (2:1) at 1.5% w/w total concentration. In particular, non-ionic surfactants like polysorbates are used in combination with hydrophilic emulsifiers in oil-in-water emulsions in a concentration of 1 - 10%, as emulsifying agent (Sheskey et al., 2017). In the present study, Tween 80 was used as emulsifier at 1% v/w concentration in formulations with low quantity (0.5%, w/w) of SLS. Tween 80 stabilizes the emulsion by decreasing interfacial tension and it is a very good steric stabilizer (Akbari and Nour, 2018). Nevertheless, it does not stabilize the system by all the stabilization mechanisms of SLS described before. This behavior may lead to a lower viscosity of the system, so the emulsions will be less stable. Considering that all stabilization mechanisms of SLS could be acting at higher concentrations, we proposed the substitution of the emulsifier blend for SLS at a concentration of 1% w/w.

Effect of factors on pH

All formulations showed pH values in a range of 5.11 - 5.32 (Table 4), which represented a slightly acid acceptable value for cosmetic preparations. Fig. 2A (Pareto Chart) shows the effect of factors on pH, where the interaction X_1X_2 and X_2X_3 are the main factors influencing on the response variable, with p values of 0.0272 and 0.0078 respectively. The regression equations [5 and 6] for pH are shown below.

$$pH = 5.16969 + 0.0034375*X_1 - 0.0259375*X_2 + 0.0290625*X_1*X_2 \quad [5]$$

$$pH = 5.16969 - 0.0259375*X_2 + 0.0290625*X_1*X_2 - 0.0359375*X_2*X_3 \quad [6]$$

Fig. 2B showed pH values were increased when emulsifier was at a low level (SLS 1%, w/v) and concentration of SA was changing from high to low level. The interaction of both factors also increased the pH values (Fig. 2B-C). This is typical to expect, since SLS is an alkaline salt that exhibits a pH in a range of 7.0 – 9.5 (for a 1% w/v aqueous

solution) and it is freely soluble in water (Sheskey et al., 2017). Hence, small amounts of the salt are sufficient to raise the pH of semi-solid preparations. However, SA is a saturated long-chain fatty acid practically insoluble in water (Sheskey et al., 2017), so pH values would be influenced mainly by the SLS concentration, in this case, 1% w/w.

Effect of factors on bulk density (BD)

Bulk density values ranging from 0.94 to 1.12 g/mL, exhibited little variation among them. These results are illustrated on Table 4. Fig. 3 shows the effect of the interaction (according to LSD Fisher intervals) between X_2X_3, which is the main interaction influencing on the response variable ($p = 0.0010$). The regression equation [7] for BD is shown below.

$$BD = 1.00333 - 0.0126531*X_2 + 0.00854688*X_3 - 0.0347594*X_2*X_3 \quad [7]$$

Bulk density is a property that is commonly used in the quality control of semi-solid cosmetic and pharmaceutical preparations, and gives a measure of the ratio between the mass of the components and the volume they occupy (Peredo-Luna et al., 2017). All the emulsions with a continuous TC reached higher BD values regardless the SA concentration. This property can be influenced mainly by the presence of entrapped air in the formulation. However, all the emulsions had suitable BD values (Peredo-Luna et al., 2017) and did not presented significant differences ($p>0.05$) among them.

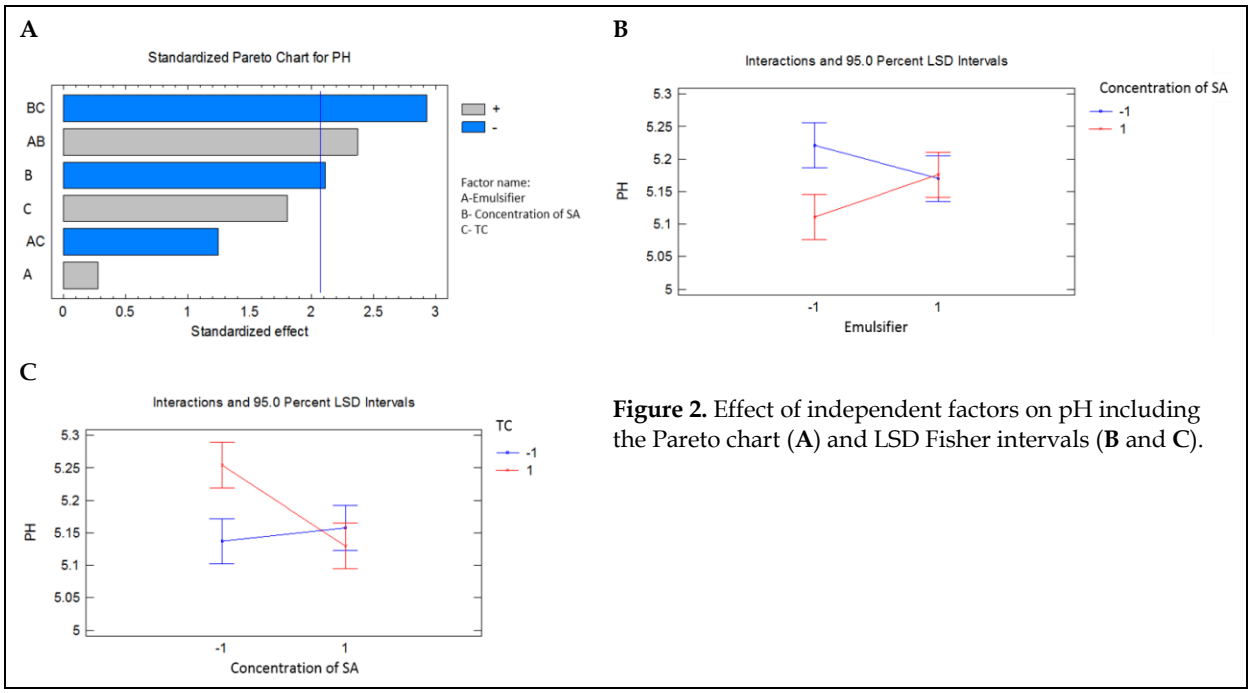

Figure 2. Effect of independent factors on pH including the Pareto chart (**A**) and LSD Fisher intervals (**B** and **C**).

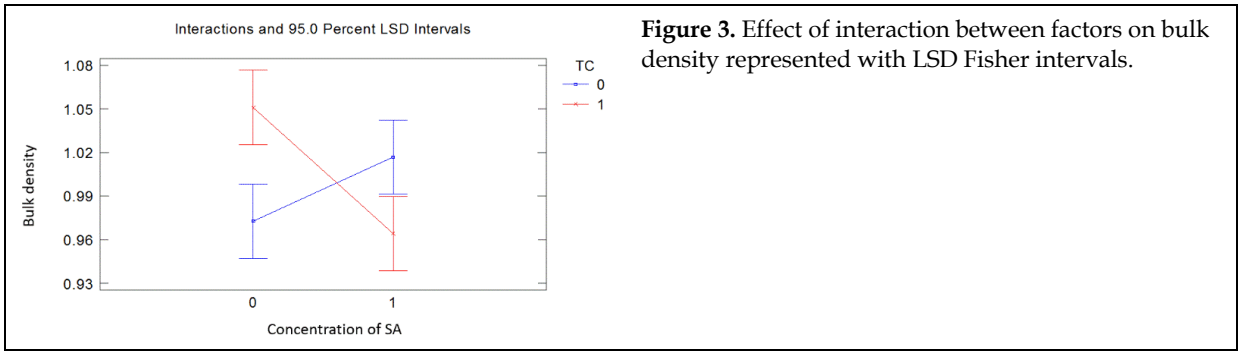

Figure 3. Effect of interaction between factors on bulk density represented with LSD Fisher intervals.

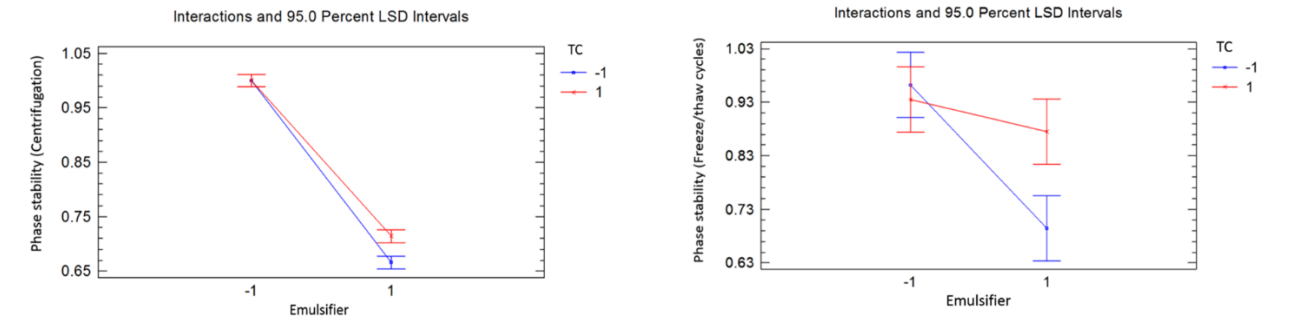

Figure 4. Main interaction effects between emulsifier and type of cooling (TC) on phase stability from centrifugation test **(A)** and phase stability from freeze/thaw cycling (**B**), represented with LSD Fisher intervals.

Effect of factors on centrifugation, cooling-heating and freeze-thaw cycling as response variables

Accelerated physical stability tests allow predicting long-term physical stability of emulsions. Table 4 shows the values for emulsion phase stability after centrifugation and freeze-thaw cycles test, respectively. No signs of instability were observed in the emulsions after cooling-heating cycles test; values of phase stability for all formulations were equal to one, results that were not included in Table 4. Fig. 4 shows the main interaction effects (according to LSD Fisher intervals) between factors on phase stability from centrifugation test (A) and phase stability from freeze/thaw cycling (B), showing p values of 0.0123 and 0.0334, respectively. The regression equations [8 and 9] for centrifugation (CF) and freeze/thaw cycling (FT) are shown below.

$$CF = 0.844991 - 0.155009*X_1 + 0.0121785*X_3 + 0.0121785*X_1*X_3 \quad [8]$$

$$FT = 0.860.03346689 - 0.0818478*X_1 + 0.03822*X_3 + 0.0517563*X_1*X_3 \quad [9]$$

Formulations F1, F3, F4 and F8 exhibited the lowest values of phase volume separation after centrifugation test (in the range of 0.69 - 0.73), resulting in creaming due to the presence of oil droplets on the top of emulsions (Molet-Rodríguez et al., 2018). Usually, creaming can be accelerated in a centrifuge machine. Creaming is related to the homogeneity of the particles, the size of the particles, and the viscosity of the system. Stokes' law explains the relationship between the viscosity and the velocity of creaming process (Sarker, 2013). In O/W dispersions, the viscosity of the system decreases when the velocity of this process is high. Usually, this behavior is expected due to the density of most oils, which is significantly lower (0.7 – 0.9 g/cm^3) than that of water (Sarker, 2013).

Although centrifugal tests are very widely used to predict emulsion shelf life, there are some others tests of greater importance in accelerated stability testing such as cyclic temperature stress tests. According to ANVISA guidelines for cosmetic products (Brasil, 2004), four cycles were performed during eight days in every test: Cooling-heating at 4 and 40°C every 24 h and freeze-thaw at -5°C and 40°C every 24 h. In the present work, most of emulsions showed a similar behavior after freeze-thaw cycles (-5°C and 40°C), compared to those after centrifugation test (Fig. 5). Freeze-thaw tests employ severe temperatures compared to cooling-heating tests, allowing to detect instability problems more quickly. Only F5 remained without phase volume separation after both tests.

Fig. 4 refers to the main interaction effects between factors on the emulsion phase stability after centrifugation and freeze-thaw cycles tests, respectively. It seems to be that emulsifier significantly influences on the emulsion's stability. In this sense, some authors have studied the influence of SLS concentration on oil-in-water emulsions stability. Krstonošić et al. (2012) employed SLS concentrations at 1, 3 and 5% w/w in macromolecular emulsifier-SLS mixtures to evaluate the presence of creaming in formulations with only macromolecular emulsifier and those with macromolecular emulsifier-SLS mixtures.

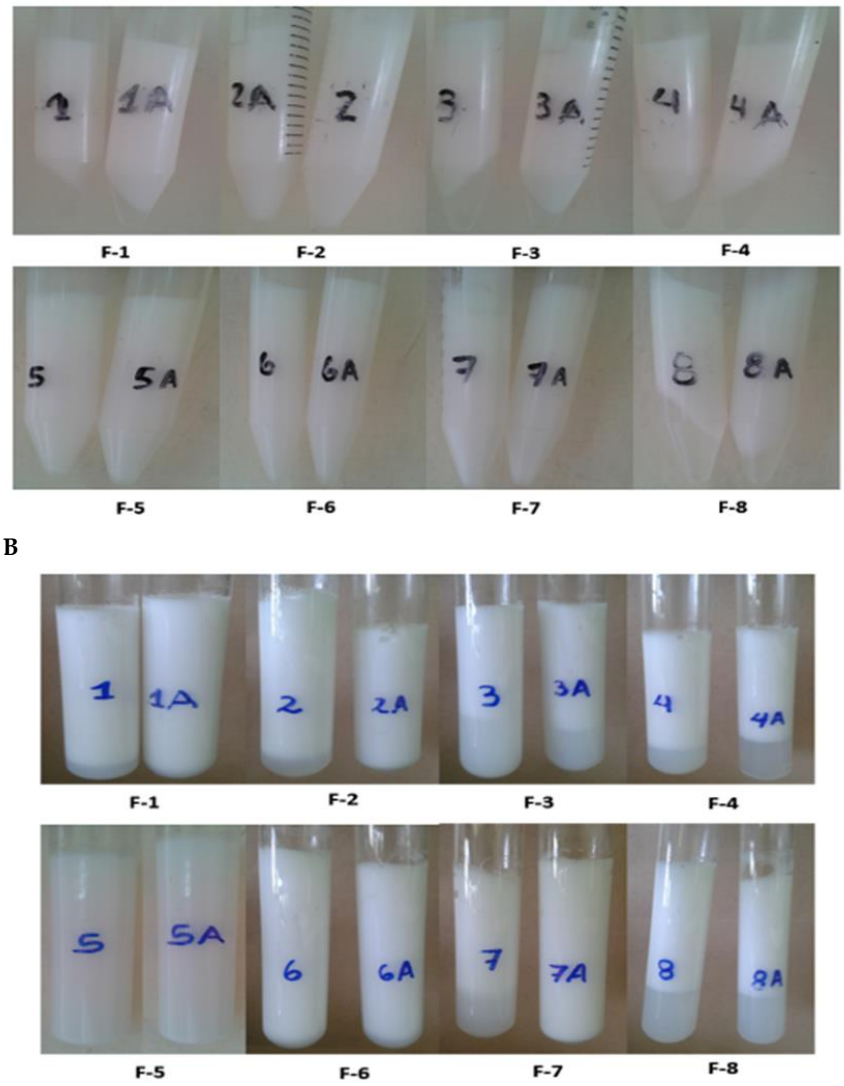

Figure 5. Photos of formulations subjected to accelerated stability conditions after centrifugation test **(A)** and freeze/thaw tests **(B)**.

In the present study, an increase of emulsions stability occurs when SLS is used at concentration of 1% w/w, after centrifugation and freeze-thaw cycling (Fig. 4), contrarily to the results obtained by Krstonošić et al. (2012). The authors describe creaming phenomena for all formulations prepared with macromolecular emulsifier-SLS blend mainly when SLS concentration was increasing. However, they worked with a higher concentration range of SLS (1 - 5% w/w) and the concentration of 1% w/w (blend or neat SLS) presented the lower creaming behavior after 72 h.

Interestingly, Dickinson and Ritzoulis (2000) investigated the influence of SLS and sodium caseinate on oil-in-water emulsions stability, and they concluded that the addition of NaCl 2% w/w to an SLS 1% w/w- stabilized emulsion contributed to a fast destabilization. In our study, all formulations stabilized with SLS included lower concentrations of the electrolyte (NaCl 0.5% w/v), and signs of instability were not detected.

In this study, emulsions with the combination of Tween 80/SLS showed less consistency than formulations with SLS alone. Wu et al. (2016) investigated the influence of Tween 80 as emulsifier in an O/W emulsion to improve the stability by lowering the creaming rate and evaluated the physical characteristics according to droplet size, surface protein concentration, ζ-potential, and apparent viscosity of the emulsion. It has been re-

ported that formulations containing 1% v/w Tween 80 and 20% v/w oil showed nearly Newtonian properties with very low viscosity (Mao et al., 2014). Concentrations between 0.02 – 0.08% v/w reduced the apparent viscosity, and this is explained by the high concentration of Tween 80 on the surfactant-fat domains and its coverage on the oil surface, which decreased (Wu et al., 2016). Although the viscosity was higher at 0.02% v/w Tween 80, the droplets were destabilized by strong associations that tend to aggregate during shear behavior, resulting in high creaming rate at that concentration (Wu et al., 2016). In the present work, the highest creaming rate presented in formulations with 1% v/w Tween 80, could be caused by the low viscosity and also by the decreased ζ-potential and the droplet size increased by the presence of Tween 80 as emulsifier at very low concentrations, properties that were not evaluated in this study.

Selection of the best test in predicting the physical stability of emulsions

Accelerated stability tests are very useful because they provide criteria in terms of physical stability of emulsions in a relative short time. However, only studies under real storage conditions provide definitive results. In this sense, the results obtained from the centrifugation tests, cooling-heating and freeze-thaw cycles used for these purposes were compared with those obtained for emulsions stored at 30°C/70% RH for two years (Table 5). The accelerated method for physical stability herein used by which predicted results matched with those obtained for emulsions stored under real conditions was the centrifugation method. Also, preparations with good resistance to phase separation in the course of this test give a good idea about developing stable formulation (Mohsin and Akhtar, 2017). The centrifugation test predicted 100% of cases successfully, followed by freeze-thaw cycles with 63% of cases predicted and the cold-heat cycles only predicted 50% of cases. As part of the assessment made, regarding organoleptic properties after two years, the presence of grainy texture and lumps formation was observed in F1, F4 and F8, respectively. However, the greatest instability observed was phase separation in F3, F4 and F8, as previously predicted (Table 5).

The present study only considers the quality and physical stability of cosmetic emulsions. However, in order to have criteria as regards integral stability, those related to sensory, chemical and microbiological evaluation could be included as response variables.

Table 5. Comparison of the predicted results after accelerated physical stability tests with the real conditions of storage after 24 months of manufacture (30°C/70%RH).

Formulation	Centrifugation	Predicted		Real
		Cooling-heating cycles	Freeze-thaw cycles	
1	-	+	-	-1, -2
2	+	+	-	+
3	-	+	-	-3
4	-	+	-	-2, -3
5	+	+	+	+
6	+	+	-	+
7	+	+	-	+
8	-	+	-	-1, -3

(+): Stable; (-): Unstable or nonacceptable; (-1): Grainy texture; (-2): Formation of lumps; (-3): Phase separation.

CONCLUSIONS

In this study, the full factorial design technique allowed to obtain formulations with a desirable technological quality and accelerated physical stability, despite of the presence of a limited number of experiments and the simplest assays available in every laboratory. The results indicated that the presence of SLS 1% w/w alone stabilized better the emulsions than the combination of Tween 80 1% v/w + LSS 0.5% w/w. Spreadability values were higher with the combination, indicating that the lowest concentration of emulsifiers must show less consistency. The pH values increased with the presence of SLS 1% w/w that is an alkaline salt freely soluble in water capable to raise the pH with small amounts. The interaction between the TC and SA concentration positively influenced on the BD, reaching higher values with the use of a continuous TC, regardless the SA concentration. Cooling-heating cycles test presented no signs of instability after 48 h of manufacture. Formulations with the combination Tween 80/SLS showed signs of creaming after centrifugation and freeze-thaw cycles tests. The presence of 1% v/w Tween 80 conduced to very low viscosity and higher creaming rate of the emulsions. Properties like ζ-potential and droplet size could influence on these results. The test that best predicted physical stability of emulsions after 24 months of manufacture was centrifugation with a 100% of cases predicted.

The present study could apply not only in the cosmetic emulsions field but also in pharmaceutical, food industries or another science field. In cosmetics, it might include, also, the results of sensory evaluation as response variables.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution	Navarro-Pérez YM	Cedeño-Linares E	Norman-Montenegro O	Ruz-Sanjuan V	Mondeja-Rivera Y	Hernández-Monzón AM	González-Bedia MM
Concepts or ideas	x						x
Design	x				x		x
Definition of intellectual content	x				x		x
Literature search	x				x		x
Experimental studies	x	x				x	x
Data acquisition	x						x
Data analysis	x						x
Statistical analysis			x				
Manuscript preparation	x						x
Manuscript editing	x						x
Manuscript review	x	x	x	x	x	x	x

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