Optimization, physical-chemical evaluation and healing activity of chitosan ointment

[Optimización, evaluación físico-química y actividad cicatrizante del ungüento de quitosana]

Dania Pérez[1], Nilia de la Paz[2], Mirna Fernández[1*], Niurys Mantilla[2], Mariela Peña[1], Addis Menéndez[2]

[1]Instituto de Farmacia y Alimentos (IFAL). Universidad de La Habana. Calle 23 No. 21425 e/ 214 y 222. La Habana. Cuba.
[2]Centro de Investigación y Desarrollo de Medicamentos (CIDEM). Ave. 26 # 1605 e/ Puentes Grandes y Boyeros. La Habana, Cuba.
*E-mail: mirnafc@ifal.uh.cu

Abstract

Context: Chitosan has received attention as a functional, sustainably renewable, nontoxic and biodegradable biopolymer for pharmaceutical applications such as healing agent.

Aims: To design a semisolid pharmaceutical form (ointment), employing 1% chitosan as an active pharmaceutical ingredient.

Methods: The formulation was carried out through D-optimal mixing design, with a linear model. Variation components evaluated were the concentration of sodium hydroxide, water and anhydrous lanolin. Variables included those of response pH and extensibility. Three batches were produced, and the physical and chemical stability of the ointment was assessed through stress and shelf-life tests for 24 months. Physical-chemical parameters studied included organoleptic characteristics, chitosan content, rheological behavior, apparent viscosity, pH and extensibility. Wound healing activity was also tested for burns damage model in rats**.**

Results: The mixture design showed that the best formulation was the one containing 0.12% sodium hydroxide, 20.0% anhydrous lanolin and 40.0% water, with the mechanical-structural behavior characteristics of a semisolid product. This product had healing effects and showed adequate physical chemical stability during the time under study.

Conclusions: The results of this study suggest that 1% chitosan ointment design favors scarring in the second-degree burn damage model in rats skin applied for 14 days at the rate of daily administration.

Keywords: chitosan; D-optimal mixing design; healing effect; ointment.

Resumen

Contexto: La quitosana ha recibido gran atención al ser un biopolímero funcional, biodegradable, renovable y no tóxico con múltiples aplicaciones farmacéuticas, entre ellas como agente cicatrizante.

Objetivos: Diseñar una forma farmacéutica semisólida (ungüento), empleando quitosana al 1% como ingrediente farmacéutico activo.

Métodos: El diseño de formulación se llevó a cabo a través de un diseño de mezcla *D-optimal*, modelo lineal, evaluándose como componentes de variación la concentración de hidróxido de sodio, agua y lanolina anhidra y como variables respuesta el pH y la extensibilidad. Se elaboraron tres lotes y se evaluó la estabilidad la estabilidad física y química de los mismos durante 24 meses. Como parámetros fisicoquímicos se estudiaron las características organolépticas, concentración de quitosana, comportamiento reológico, viscosidad aparente, pH y extensibilidad. La actividad cicatrizante se evaluó mediante el modelo de daño por quemaduras en ratas.

Resultados: El diseño de mezclas arrojó que la mejor formulación resultó ser la que contenía 0,12% de hidróxido de sodio, 20,0% de lanolina anhidra y 40,0% de agua, coincidiendo con la formulación 6 del diseño, con un comportamiento mecánico-estructural característico de un producto semisólido, mostrando efecto cicatrizante y una adecuada estabilidad durante el tiempo de estudio.

Conclusiones: Los resultados de este estudio sugieren que el ungüento de quitosana al 1% diseñado favorece la cicatrización en quemaduras de segundo grado, al ser administrado en ratas durante 14 días.

Palabras Clave: diseño de mezcla D-optimal; efecto cicatrizante; quitosana; ungüento.

INTRODUCTION

In 1970, Prudden and collaborators were the first to publish evidence of the ability of chitosan to help expedite wound healing. From that moment on, chitosan became a focus of great attention. In addition to its hemostatic, antibacterial and antifungal properties, this ability guarantees better scarring, as it prevents the proliferation of microorganisms and avoids hemorrhagic events. There are reports of the use of this biopolymer as a wound-healing agent in different pharmaceutical forms, such as ointments, gels, hydrogels and dressings (García and Roca, 2008; Nascimento et al., 2009; Shinde et al., 2011; Velazco et al., 2012; Martínez Sánchez et al., 2014; Szymánska and Winnicka, 2015; El-Kased et al., 2017). Besides, it has been used in different forms of filament, powder granules, sponge and a composite with cotton or polyester (Mazher Ahmed et al., 2015).

Chitosan also stands out because of its healing action on wounds and burns, further promoting recovery from ulcers and other types of injuries. It has been suggested that it exerts this action by activating neutrophils and macrophages, and through the migration of nuclear polymorph and mononuclear cells, thus speeding up the regeneration of connective tissue and angiogenesis (Lemus Centes, 2007; García and Roca, 2008, Velazco et al., 2012). Due to their biocompatibility, ability to absorb exudates, enhancing the epithelization process and thus possess an accelerating effect on wound healing progress and film forming properties, chitosan products are good candidates for burn and wound management (Alsarra, 2009; Szymánska and Winnicka, 2015; El-Kased et al., 2017).

There has been an increase in research aimed at obtaining products for dermatological use with applications in tissue regeneration, wound closure and/or resurfacing. The objective of wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort and scarring to the patient (Wadood Khan et al., 2013). For the treatment of chronic wounds, ointments are a common pharmaceutical choice. Ointments avoid the loss of water, thanks to their occlusive capacity, thus achieving a moisturizing and protective effect on the affected area. They can be used to remove scabs and flakes, and to improve dry and cracked skin, due to their emollient action (García and Roca, 2008).

Production of natural alternatives is being prioritized in order to minimize sensitization and resistance reactions, as well as the expense for the pharmaceutical sector arising from the design, formulation and development of synthetic medicines (Ayukekbong et al., 2017).

The main aim of this study is focused in the design and optimization of a semisolid pharmaceutical formulation employing chitosan as an active pharmaceutical ingredient. The formulations were carried out through D-optimal mixing design and evaluated in terms of pH and extensibility. Physicochemical and stability studies of the optimized formulation and wound healing activity were also carried out over time.

MATERIAL AND METHODS

Chemical products and reagents

Chitosan (CH), derived from lobster chitin obtained from the specie *Panulirus argus,* with a deacetylation degree (DD) of 79.90%, prepared on an industrial scale in a Cuban factory. DD was determined using a previously-validated potentiometric method (de la Paz et al., 2012). Solid petrolatum was purchased from Dilube S.A. (Spain). Acetic acid and sodium hydroxide (Panreac, Spain) and anhydrous lanolin (Sauco S.A., Spain).

Design and optimization of semisolid formulation (ointment) containing chitosan

A D-optimal mixture experimental statistical design, adjusted to a linear model, was developed using version 6.0.1 of the Design-Expert (DX-6) software (Stat-Ease, Inc., Minneapolis, United States). In the study, substance concentrations were analyzed as independent variables: sodium hydroxide (0.00-0.12%), anhydrous lanolin (15.00 - 25.00%) and deionized water (35.00 - 45.00%), for a

total mixture formulation of 60.12%. Table 1 shows the design matrix used. Chitosan (1%) was added as active pharmaceutical ingredient.

The fusion method was used for the purpose of preparing the formulations. An electric stirrer was used as agitation system (Heidolh RZ R1, Germany). A total of 300 g for each variant was prepared and packed in polyethylene bottles. The formulation was prepared in a randomized order. All eight formulations were evaluated, periodically, according to organoleptic characteristics, pH and extensibility, for a period of 35 days. The dependent variables were aqueous phase pH before emulsification and extensibility, measured after five weeks of developing the design variants.

DX-6 statistical software was used to optimize the design. The following criteria were used in the analysis: independent variables were kept within the limits studied, except for anhydrous lanolin, which was restricted to a 20 – 25% interval. Lower limits were established for dependent variables: 17.0 cm^2 for extensibility, and 6.5 for pH. Regarding the level of importance, pH was given a maximum value (5) and the remaining variables were kept at the mean value (3). The variant with the maximum desirability index was considered the optimal formulation.

Preparation of optimized formulation

Based on the optimal formulation according to the design, three batches of 10 kg each were produced (L-16001, L-16002, L-16003). To this end, the fusion method was used. Final homogenization of the product was carried out in a colloid mill (Probts and Class, Germany). Packaging was done semi-automatically, using a model AR403 filler (ERWEKA, Germany). Packaging was carried out in two different containers, for each batch: vials of low density polyethylene (F3), liner and cap of high density polyethylene (T3) with tamper-proof seal, nominal capacity of 30.0 g (Frasplast, Cuba), and aluminum blind collapsible tubes, with interior lacquer, with plastic lids and perforator, nominal capacity of 25.0 g (Jurcal, Spain).

Physical-chemical and technological analysis of optimized ointment

The organoleptic properties including general appearance, consistence, homogeneity of the formulation, absence of phase separation, instabilities of color, coalescence or exudation and creaming were evaluated. The presence of grit and consistency were assessed through touch.

Viscosity measurements were made in a rheometer (Anton Paar, MCR 302, Germany), equipped with cone-plate geometry (CP25). Plate diameter was 24.980 mm, angle 1.01°, truncation 49 μm, measurement position 0.049 mm, and sample volume of 0.07 mL. Measurements were made at a temperature of 20 ± 0.01°C, with a velocity gradient of 0 - 50 - 0 s^{-1}, for 6.2 minutes. Data and graphics obtained were processed using the Rheo-Plus software, version 3.6x. The following rheological parameters were determined: yield value (σ_0), hysteresis area (A), apparent viscosity (η) at 20 s^{-1}.

To extensibility tests, 2 g of semisolid were weighed (Sartorius, EnTRIS 6202-1S, Germany) and deposited on the center of the glass base plate, and a second glass plate (292 g) was homogeneously disposed onto the sample. After 5 minutes, the area of the formulation spread between both plates was measured. Average value and standard deviation resulting from three determinations per sample were reported.

pH measurements were determined using a pH meter (Mettler Toledo, Seven Compact TM S220, Switzerland), which was calibrated previously with buffer solution at 4.01 and a 7.00. For the formulation analysis, a sample of approximately 25 mL was transferred to a 50 mL beaker. Three replicates were made for each sample and average value and standard deviation were reported.

Stability study

Preparations were stored at room temperature (30 ± 2°C) and refrigeration (5 ± 2°C) evaluating shelf life behavior for 24 months.

In vivo wound healing activity

Male Wistar rats were used, from 200 to 250 g of body weight from the National Center for the Production of Laboratory Animals (CENPALAB, Cuba). The animals were adapted for 7 days to the laboratory conditions, in acclimated rooms with controlled temperature of 22 ± 2°C, light-dark cycles 12 x 12 hours, bed with chip and change every 48 hours. Experiments were conducted in accordance with the Guiding Principles in the Use of Animals in Toxicology (Guide for the Care and Use of Laboratory Animals, 2010). The experimental protocols were approved by the Institutional Ethical Committee.

Initially, the rats were anesthetized by intraperitoneal (i.p.) injection of sodium thiopental (50 mg/kg) using a volume factor of 0.001 mL/g and the dorsal area of each was shaved and depilated with depilatory cream. Subsequently, the skin was disinfected using an ethanol solution 70%. It then proceeded to induce the injuries to all the animals. A metal device of 1 cm diameter was used to induce burns. It was immersed in boiling water (100°C) and after equilibration the temperature for 5 minutes was placed without pressure on the skin of the animal in the dorsal area for 20 seconds, with the aim of achieving a second-degree burn. Each animal was randomly distributed in four groups: 1) control, 2) placebo, 3) chitosan ointment and 4) silver sulfadiazine as a reference compound. It was administered 0.030 g per day of each formulation for 14 days. The condition of treated injuries was checked on a daily basis. As a macro-scopic parameter for the healing effect, the contraction of the wound area was controlled throughout the study. The results are presented as a percentage of decrease in the area (wound contraction) in relation to the basal value (Alemdaroglu et al., 2006; Rojas et al., 2011).

RESULTS AND DISCUSSION

Organoleptic characteristics of the formulations remained unchanged during the period under analysis, with the exception of formulations 2 and 8 (both of equal composition) (Table 1) in which the presence of exudates was observed 30 days after preparation. This may have been caused by the concentration ratio of anhydrous lanolin and water (15/45). Anhydrous lanolin is capable of emulsifying up to twice its weight in water (Rowe et al., 2009). The other formulations were found to be stable in structure; they keep their clear and uniform appearance.

Table 2 summarizes the results obtained during the physical-chemical and technological evaluation of the preparations obtained in the five weeks of the trial. It is accepted that skin pH ranges between 5 and 6, under normal conditions. Hence, a topical formulation should have a pH near that range to avoid irritability and problems of tolerance (Acosta et al., 2015). Determining pH is necessary to as certain that the registered value is compatible with the formulation components and the application site, in order to avoid irritation (Marquele-Oliveira et al., 2007).

Table 1. Formulation design of the ointment.

Random order	Assay	Sodium hydroxide (%)	Anhydrous lanolin (%)	Deionized water (%)
5	1	0.03	17.57	42.52
7	2	0.12	15.00	45.00
3	3	0.00	15.12	45.00
4	4	0.06	20.03	40.03
8	5	0.12	25.00	35.00
1	6	0.12	20.00	40.00
2	7	0.09	22.51	37.52
6	8	0.12	15.00	45.00

Table 2. Technological evaluation of the formulations.

Assay	pH			Extensibility area (cm^2)			
	Aqueous phase	0 days	35 days	0 days	7 days	21 days	35 days
1	5.64 ± 0.02	5.84 ± 0.01	5.94 ± 0.10	20.85 ± 0.41	17.79 ± 0.75	16.09 ± 0.50	17.54 ± 0.52
2	6.69 ± 0.01	7.78 ± 0.03	9.42 ± 0.03	24.91 ± 2.92	19.65 ± 1.57	18.49 ± 0.38	19.49 ± 1.72
3	5.34 ± 0.01	5.53 ± 0.02	5.50 ± 0.01	20.96 ± 0.25	20.85 ± 0.41	19.84 ± 1.03	18.55 ± 0.51
4	5.93 ± 0.03	6.61 ± 0.01	6.51 ± 0.09	20.65 ± 1.37	18.09 ± 0.30	18.80 ± 0.22	20.47 ± 0.40
5	6.66 ± 0.01	6.73 ± 0.04	6.20 ± 0.25	15.76 ± 0.14	16.09 ± 0.22	15.76 ± 0.56	14.76 ± 0.37
6	6.66 ± 0.00	6.87 ± 0.01	6.78 ± 0.09	20.70 ± 0.61	18.13 ± 0.47	18.84 ± 0.09	19.67 ± 0.79
7	6.29 ± 0.01	6.85 ± 0.01	6.80 ± 0.09	17.05 ± 0.50	19.06 ± 1.01	17.05 ± 0.60	16.10 ± 0.50
8	6.69 ± 0.01	6.76 ± 0.02	7.82 ± 0.02	22.55 ± 1.00	21.90 ± 1.49	20.12 ± 0.26	19.86 ± 1.82

Data represent mean ± SD of n=3.

The pH obtained in the experiments proved to be variable, in the 5.50 to 6.80 range (Table 2). The pH values were conditioned mainly by the NaOH present in the formulations. Keeping in mind that the product is intended to be applied on damaged areas of the skin, where pH values close to neutrality are preferred, some formulations showed a very low pH. In particular, formulations 2 and 8 showed an abrupt increase in pH at the end of the assay. This might be associated with a type of physical instability that was observed—i.e., the presence of exudates, or expulsion of water in the preparation. This instability also occurred in formulation 3, but to a lesser extent. In terms of time, little variation in pH was noted for the rest of the formulations. This shows the stability of this parameter.

One of the criteria for a topical formulation is that it should possess good extensibility and spreadability (Wadood et al., 2013; Acosta et al., 2015). Extensibility is the term expressed to denote the area occupied by a given amount of sample to be subjected to a fixed pressure between two glass plates.

Extensibility showed variations among the different experiments carried out, which was related to the composition of each formulation (Table 2). Formulations 5 and 7 showed the lowest values throughout the study. The composition of these preparations involves the least amount of water (35.0% and 37.5%, respectively), which can lead to

obtaining greater consistency in the preparations. However, it should be noted that all the formulations showed positive results in this parameter.

During the 35 days of evaluation, the formulations showed a tendency to decrease extensibility due to a contraction of the system caused by the restructuring of semisolid preparations in the first days after the product was prepared (Delgado et al., 1997; Suárez et al., 2007). In this particular case, there is a tendency for values to stabilize in the last determinations. This is typical of emulsified semisolid systems, such as the preparation involved in this study.

In the case of pH, a better adjustment was achieved when analyzing the inverse of the square root of the values reached in this property. Table 3 shows the results obtained during the study of the best-fit model. According to the results achieved, only the linear model was significant, with a probability of less than 0.05, for 95% confidence. In addition, the linear model showed a non-significant loss of adjustment, a high coefficient of determination and the lowest value of PRESS. Some of the points studied had residues of 0.0, demonstrating the good fit of the system.

Fig. 1 summarizes the behavior of diagnostic charts that were prepared in order to verify the design adjustment. As shown, waste tends to follow a straight line near the theoretical distribution (Fig. 1A). Therefore, it can be said that waste follows a normal distribution. On the other hand,

waste also showed randomness and independence when represented graphically against the assays, as well as against independent variables (water concentration), and a random distribution of values (Fig. 1B-C). The rest of the components (anhydrous lanolin and sodium hydroxide) showed a similar behavior in terms of the randomness of the waste.

The pH trace graph is shown in Fig. 2. It indicates a negative effect of concentration of anhydrous lanolin and water in the pH. The opposite occurs for sodium hydroxide, where small changes in concentration affect considerably the value of this parameter. This is to be expected, considering that this is a strong base. Coefficients observed in this equation corroborate what was observed in the trace chart. Components such as anhydrous lanolin and water have a negative though barely noticeable effect on pH. However, for sodium hydroxide, the effect is remarkably positive despite the small range of concentrations evaluated.

In terms of extensibility, it was possible to obtain a good adjustment without transforming the variable. This also involved a linear model that complied with all parameters, as shown in Table 3.

Fig. 1 illustrates diagnostic graphs for waste in the analysis of the extensibility variable. As in the previous variable, normality, randomness and independence of the waste were achieved.

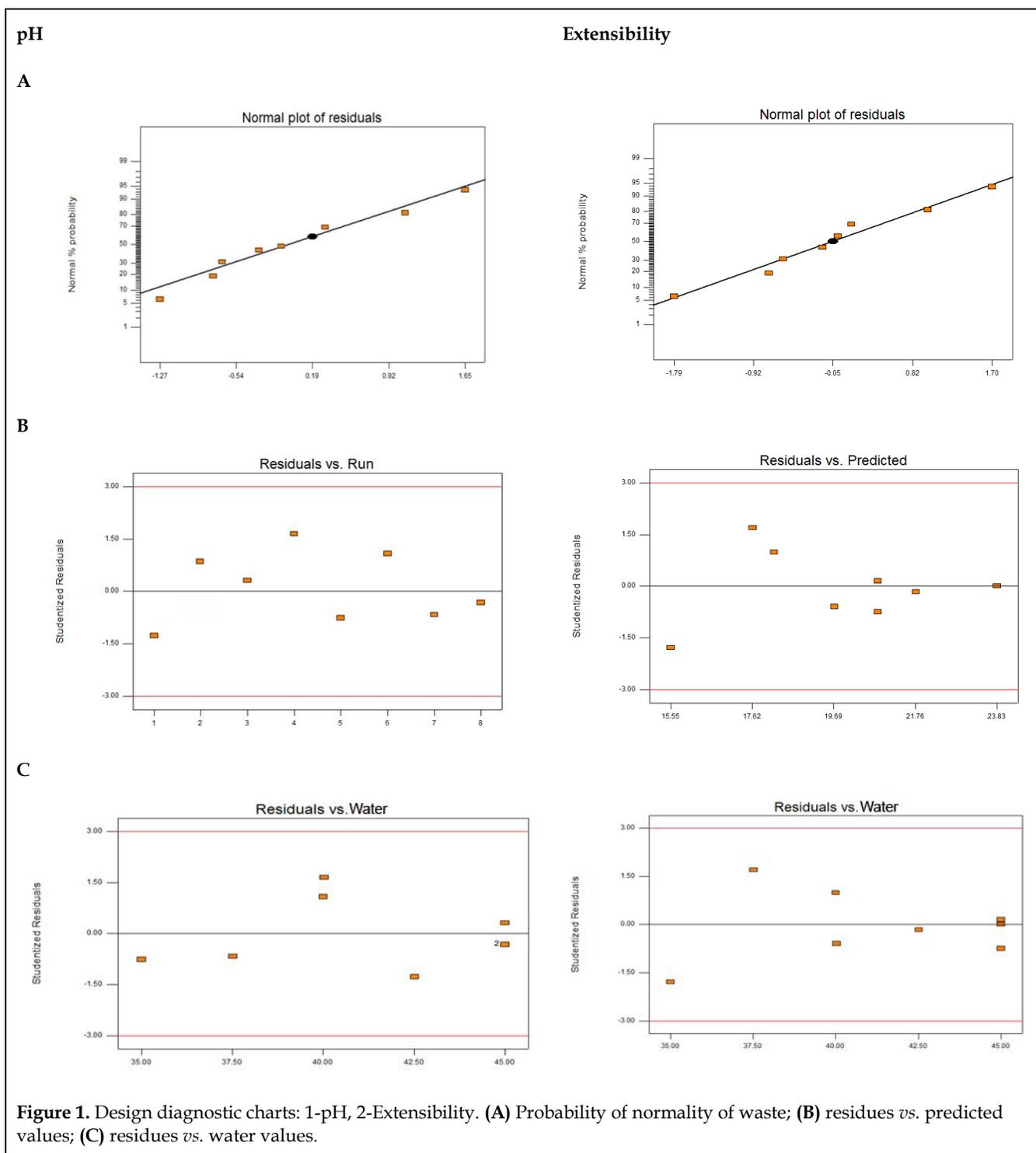The trace graph is useful for analyzing the behavior al trend of each independent variable sepa-rately when other independent variables are in the central position. As observed on Fig. 2, an increasing concentration of water favors a greater degree of extensibility of the semisolid, which could be attributed to the lesser consistency and/or oiliness of the preparation. On the other hand, anhydrous lanolin does not bring about major changes in this parameter by varying its concentration. Meanwhile, sodium hydroxide does cause a significant decrease in extensibility, due to its increased concentration. This behavior may be related to the possibility that chitosan might precipitate, depending on the pH of the medium. It is thus proposed that this polymer might be insoluble in neutral or alkaline media (Skaugrud, 1991; de la Paz et al., 2017). The effect caused by independent variables in the extensibility of the formulations is summarized, according to the design, in the equation (Fig. 2).

The foregoing is confirmed with respect to the effect of components on extensibility, as observed in the trace chart.

The numerical optimization of the formulation was developed based on the previous results. Of the three components that were varied in the design, a decision was made to put a lower limit on anhydrous lanolin, due to physical instabilities observed during the study, in which the content was less than 20%. The remaining independent variables were kept within the value range that was studied in the design, with an importance of 3.

Table 3. Analysis of mathematical models for pH and extensibility.

Variable	Model	Fit (p)	Loss of fit (p)	r^2	PRESS
pH	Lineal	0.0206	0.1879	0.99	0.0001
	Quadratic	0.7674	0.1268	0.67	1.3510
	Special cubic	0.2272	0.9155	0.34	9.4500
Extensibility	Lineal	0.0312	0.2176	0.95	0.083
	Quadratic	0.1967	0.1268	0.59	11.39
	Special cubic	0.5198	0.8632	0.37	+

pH   **Extensibility**

A

B

C

Figure 1. Design diagnostic charts: 1-pH, 2-Extensibility. **(A)** Probability of normality of waste; **(B)** residues *vs.* predicted values; **(C)** residues *vs.* water values.
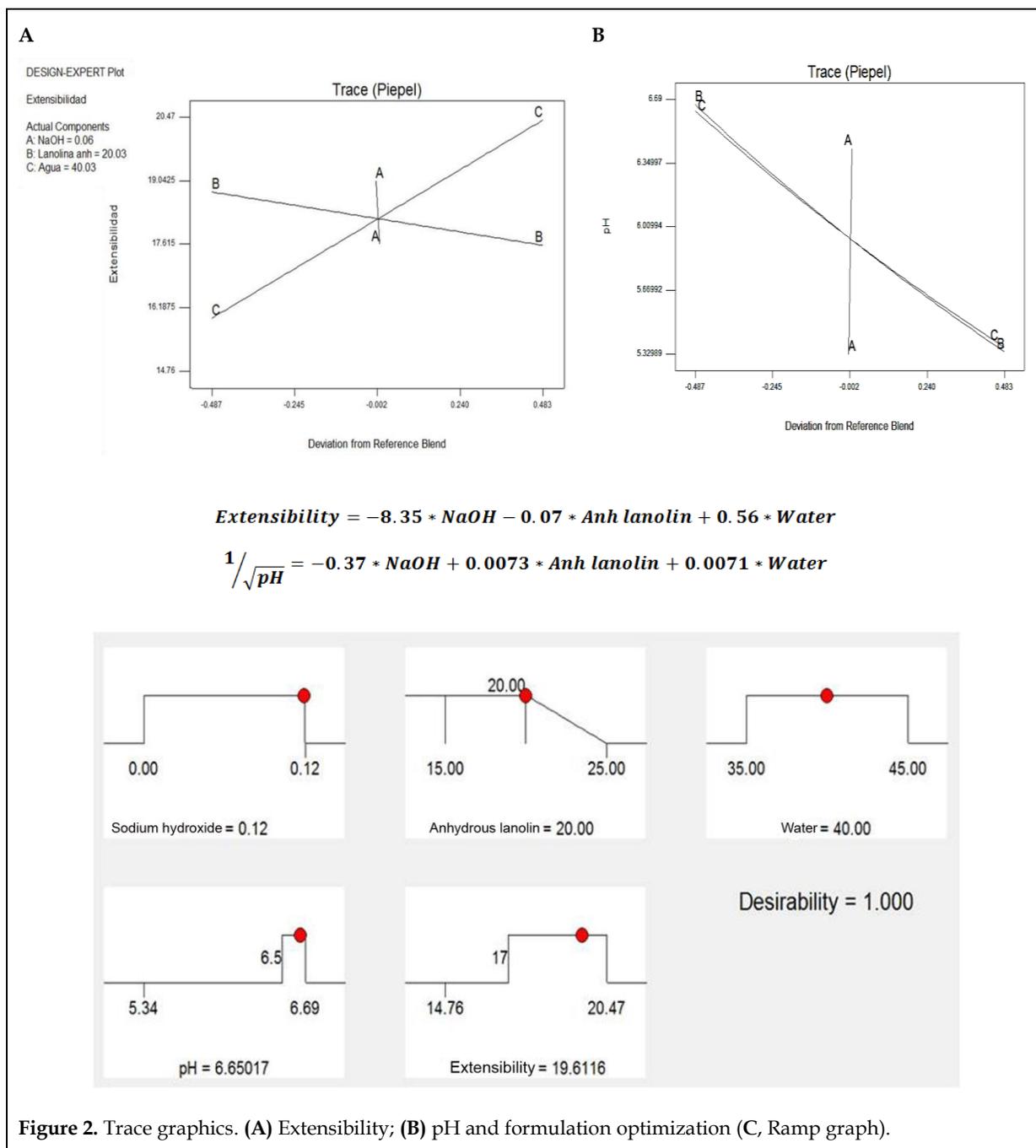
Figure 2. Trace graphics. **(A)** Extensibility; **(B)** pH and formulation optimization (**C**, Ramp graph).

We also proposed that extensibility and pH have minimum values of 17.0 cm² and 6.5, respectively. Maximum importance was given to pH (5), and extensibility an average value (3). As a result, optimal preparation was obtained, with a desirable range equal to 1 (Fig. 2C), which indicated that there was a 100% probability of obtaining a formulation with positive results. Its composition must

be 40% water, 20% anhydrous lanolin and 0.12% sodium hydroxide, which coincides with formulation 6, which guarantees the effectiveness of the developed design.

No difficulties were encountered during the preparation of the three batches. A homogeneous and lump-free product was obtained, which

proved the reproducibility and consistency of the chosen methodology. Table 4 shows the technological evaluation results obtained for freshly-made batches of the optimized preparation.

All three batches showed similar results with respect to pH value, according to the characteristics observed in the preparation during the design stage. However, the extensibility area showed a slight decrease, compared to the value of extensibility as a variable response obtained regarding the design for optimal formulation (19.61 cm²). This could be associated with the use of the colloid mill for the final homogenization of the product. The formulation also complied with the criterion established in the design, higher than 17.00 cm² (Fig. 2). As shown in Table 4, values were reproducible in each of the batches, matching values reported for this type of products (Suárez et al., 2007). All of the above evidenced the reproducibility of the proposed formulation and the elaboration process.

Fig. 3 shows flow curves obtained for semisolid formulations, three days after elaboration, to guarantee the structuring of the system. The formulated ointment behaved as a non-Newtonian fluid, with a variation in viscosity depending on the velocity gradient (Braun and Rosen, 2000). This behavior is in correspondence with the dispersed chemical-physical system that was developed (water/oil emulsion).

The graphs show that a minimum cutting effort value (τ_0) is required to start the flow, which oscillates between 770 and 840 Pa, approximately, for the three batches. The preparation flows with relative ease from that value. On the other hand, as there is an increase in speed, there is a noticeable decrease in the apparent viscosity of the system. This behavior is in keeping with general plastic systems, such as Herschel-Bulkley (Braun and Rosen, 2000; Ramírez Navas, 2006).

In addition, when returning the system to the initial position, Fig. 3 (green curve) shows apparent viscosity values lower than those reached in the initial analysis (blue curve). This creates a hysteresis loop, so it can be said that the ointment showed thixotropy. This is useful in semisolid products, since viscosity decreases quickly under an effort. This facilitates its application and redispersion, as viscosity recovers more slowly. Thixotropy is desirable in topical formulations because it helps to maintain the suspending components stability; moreover, it can influence the active substances release to the skin due to the structural disarrangement of the system, where the active substances diffusion is facilitated (Acosta et al., 2015).

Fig. 4 shows rheological parameters in the time determined from the statistical analysis and adjustments to the model of the corresponding flow curves. Extensibility area determinations are shown also in Fig. 4. Results were compared for each test performed with respect to initial time, considering the results per batch as replicas in each condition. Extensibility decreases with ageing of the samples, due to the restructuring of the semisolids.

A comparison was also made of values obtained for the two types of packaging. Significant differences were observed, which could be attributed to loss of water by evaporation in plastic containers. Nevertheless, there were no significant differences among lots for the same condition, according to Duncan's multiple ranges (p>0.05).

These results are in accordance with those observed in the evaluation of appearance and psycho-chemical properties, showed that the preparation possesses an adequate physical stability, as well as reproducible result in the three batches.

Evaluation shows that the product maintained its rheological behavior as a non-Newtonian fluid, of the Herschel-Bulkley plastic type with thixotropy. Rheological properties of the ointment were not affected by the type of container used. Fig. 5 shows the results of the analysis of the contraction of the wounds. As can be seen, the group to which the ointment was applied with chitosan showed an increase in the speed of healing, which is evidence from the seventh day from which these differences reach levels of significance statistics in the increased contraction of the wound similar to the conclusion of the treatment.

The results of this study show that 1% chitosan ointment, derived from lobster chitin obtained from the specie *Panulirus argus,* favors scarring in the model of burn damage in rat skin.

This polysaccharide has an antimicrobial and immunology activity against bacteria and fungi, accelerating healing. Concentrations such as the one used in this work or below are able to show healing in this or another type of damage, reducing the time of tissue regeneration by more than 50% and thus the healing time (Baltodano et al., 2009, Fráguas et al., 2015). Lysosomes are activated by

damaged cells and stimulated by chitosan when it is in contact with the wound. The stimulation of the lysosomes occurs by the presence of three consecutive acetyl groups located in the structure of the chitosan, allowing the identification and degradation of the same forming oligomers that expose the amino and acetyl group that act as antiseptics. When the lysosomes are activated by chitosan, the activation of glycosaminoglycans is induced, which are bound to proteoglycans forming adhesion proteins and responsible for the formation of fibrils and collagen bonds in an orderly manner (Martinez Sanchez et al., 2014).
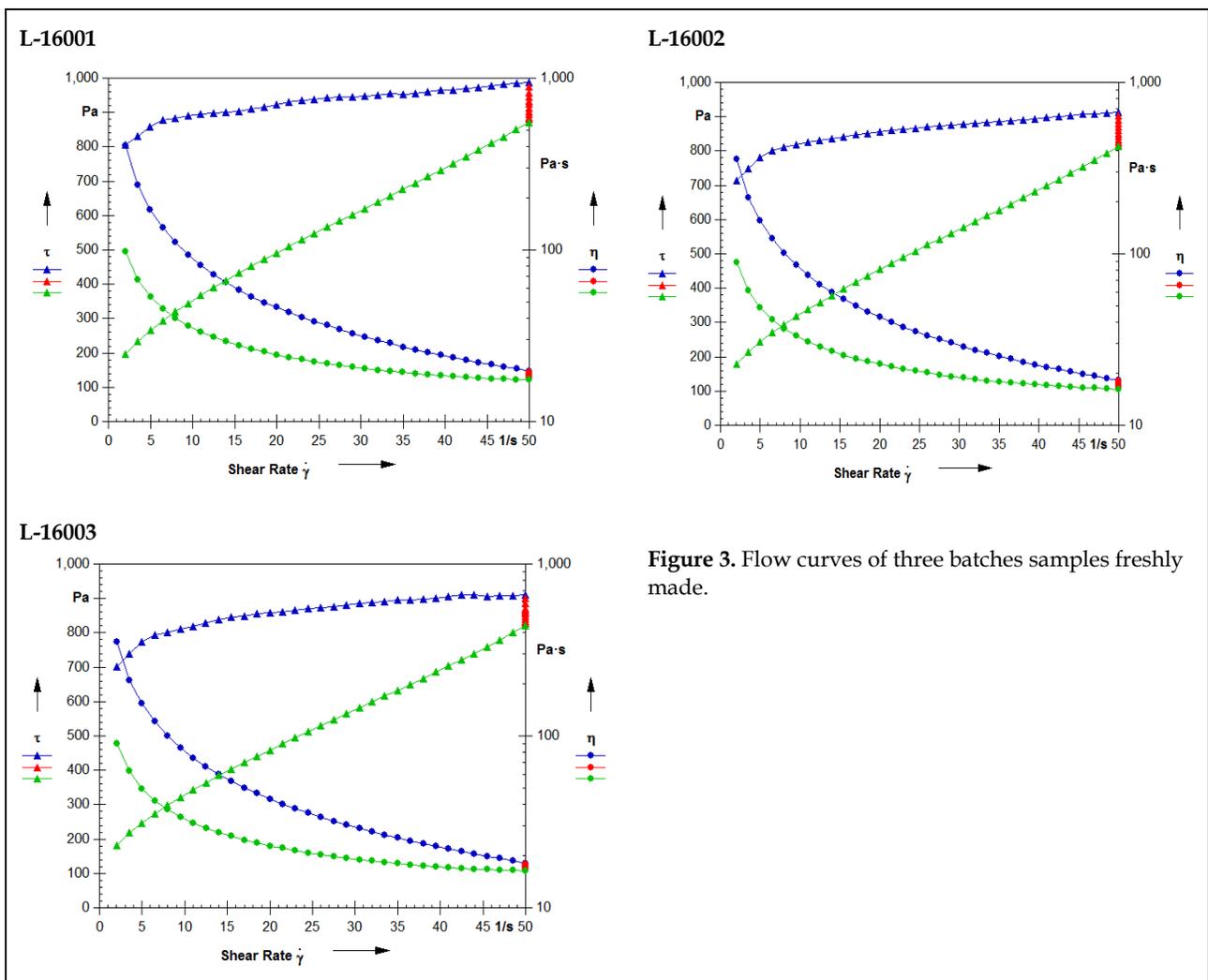

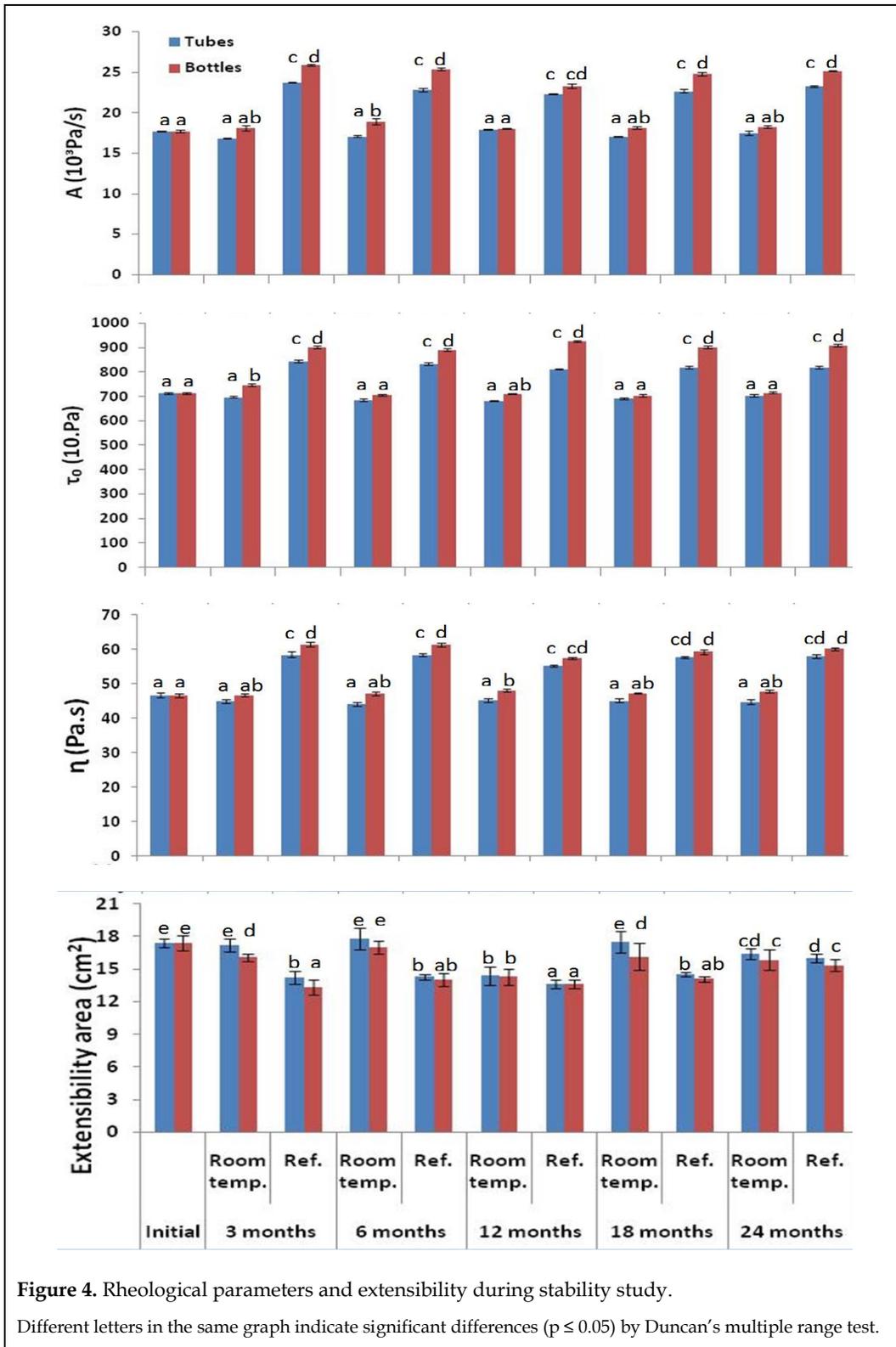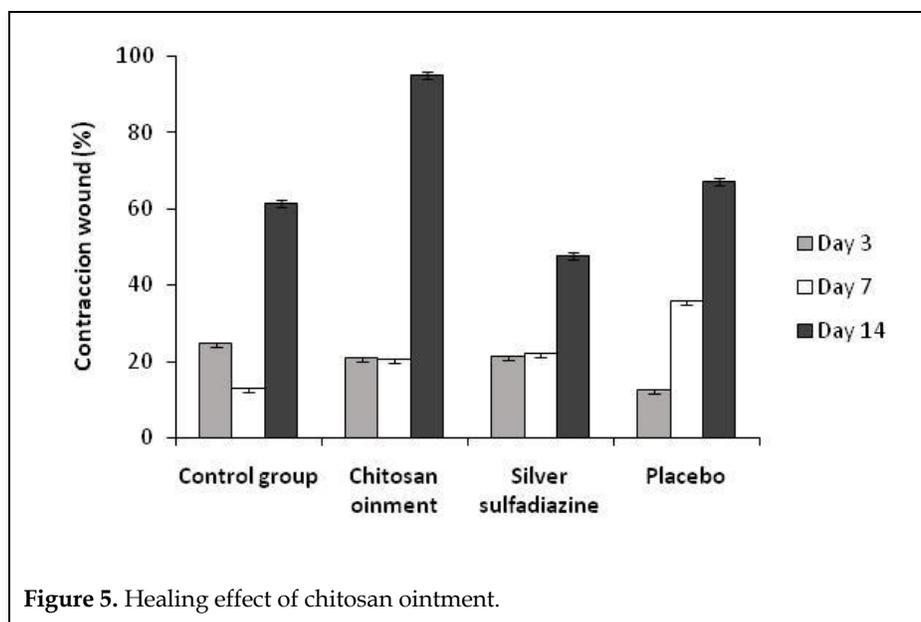**Figure 3.** Flow curves of three batches samples freshly made.

Figure 4. Rheological parameters and extensibility during stability study.

Different letters in the same graph indicate significant differences ($p \le 0.05$) by Duncan's multiple range test.

Figure 5. Healing effect of chitosan ointment.

CONCLUSIONS

The mixture design showed that the best formulation was the one containing 0.12% sodium hydroxide, 20.0% anhydrous lanolin and 40.0% water, with the mechanical-structural behavior characteristics of a semisolid product. This product had healing effects and showed adequate physical stability during the time under study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTION:

Contribution	Pérez D	de la Paz N	Fernández M	Mantilla N	Peña M	Menéndez A
Concepts or ideas	x	x	x			
Design	x	x				
Definition of intellectual content			x			
Literature search	x	x	x			
Experimental studies	x	x		x	x	x
Data acquisition	x	x		x	x	x
Data analysis	x	x		x	x	x
Statistical analysis	x	x				x
Manuscript preparation	x	x	x			
Manuscript editing			x			
Manuscript review	x	x	x	x	x	x