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Chitosan cosmetic cream: stability and non-clinical studies

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Summary

Introduction: Chitosan has been used in various products for skin care, hair care, cleaning and other products. These not only provide health benefits, but also excellent cosmetic qualities and efficacy. **Objective**: To evaluate the stability of a 1.0 % chitosan cosmetic cream, as well as its non-clinical effects. **Methods**: Three pilot batches of the cosmetic formulation were prepared by the melting method. Physical, chemical and microbiological evaluation was performed for 365 days, under shelf life conditions. The studied parameters were organoleptic characteristics, pH, extensibility, degree of deacetylation and microbiological count. Non-clinical evaluations of the cream included dermal and ophthalmic irritability. The dermoregenerative effect was evaluated in a photoaging model in the skin of mice photosensitized with ultraviolet radiation. **Results**: The cream was stable for 12 months, packaged in high-density polyethylene bottles at room temperature, and by predictive analysis until to 24 months. The formulated semisolid is safe, being considered a system of low toxicity to the organism, with moderate dermoregenerative activity. **Conclusions**: The pilot batches maintained their stability for 12 months at 30 ± 2 °C and 70 % relative humidity. The 1.0 % chitosan cream is non-irritating dermally and ophthalmically and has a moderate dermoregenerative effect.

*Keywords***:** chitosan; cream; dermoregenerative effect, non-clinic study; stability

Resumen

Crema cosmética de quitosano: estudios de estabilidad y no-clínicos

Introducción: El quitosano se ha empleado en diversos productos para el cuidado de la piel, el cabello, de limpieza, entre otros. Los mismos no solo brindan beneficios para la salud, sino también excelentes cualidades cosméticas y eficacia. **Objetivo**: Evaluar la estabilidad de una crema cosmética de quitosano al 1,0 %, así como sus efectos no clínicos. **Métodos**: Se elaboraron tres lotes pilotos de la formulación cosmética por el método de fusión. Se realizó la evaluación física, química y microbiológica por 365 días, en condiciones de vida de estante. Los parámetros estudiados fueron las características organolépticas, pH, extensibilidad, grado de desacetilación y conteo microbiológico. Las evaluaciones no clínicas de la crema incluyeron la irritabilidad dérmica y oftálmica. El efecto dermorregenarador se evaluó en un modelo de fotoenvejecimiento en la piel de ratones fotosensibilizados con radiaciones ultravioletas. **Resultados**: La crema resultó estable durante 12 meses, envasada en frascos de polietileno de alta densidad y a temperatura ambiente, y por el análisis predictivo hasta 24 meses. El cosmético formulado es estable, seguro y de baja toxicidad al organismo, con actividad dermorregeneradora moderada. **Conclusiones**: Los lotes pilotos mantuvieron su estabilidad por 12 meses a 30 ± 2 °C y 70 % de humedad relativa. La crema de quitosano al 1,0 % no es irritante dérmico, ni oftálmico y tiene efecto dermorregenerador moderado.

Palabras claves: efecto dermorregenerador; estabilidad; estudios no clínicos; quitosano.

Resumo

Creme cosmético de quitosana: estabilidade e estudos não clínicos

Introdução: A quitosana tem sido utilizada em diversos produtos para cuidados com a pele, cabelos, limpeza, entre outros. Eles não só proporcionam benefícios à saúde, mas também excelentes qualidades cosméticas e eficácia. **Objetivo:** Avaliar a estabilidade de um creme cosmético de quitosana a 1,0 %, bem como seus efeitos não clínicos. **Métodos:** Três lotes piloto da formulação cosmética foram preparados pelo método de fusão. A avaliação física, química e microbiológica foi realizada durante 365 dias, nas condições de vida útil. Os parâmetros estudados foram características

organolépticas, pH, extensibilidade, grau de desacetilação e contagem microbiológica. As avaliações não clínicas do creme incluíram irritabilidade dérmica e oftálmica. O efeito dermorregenerativo foi avaliado em modelo de fotoenvelhecimento na pele de camundongos fotossensibilizados com radiação ultravioleta. **Resultados:** O creme manteve-se estável por 12 meses, acondicionado em frascos de polietileno de alta densidade e em temperatura ambiente, e por análise preditiva até 24 meses. O cosmético formulado é estável, seguro e de baixa toxicidade ao organismo, com moderada atividade dermorregenerativa. Conclusões: Os lotes piloto mantiveram sua estabilidade por 12 meses a 30 ± 2 °C e 70 % de umidade relativa. O creme de quitosana a 1,0% não é irritante dérmico ou oftálmico e tem efeito dermorregenerativo moderado.

Palavras-chave: efeito dermorregenerativo; estabilidade; estudos não clínicos; quitosana.

Introduction

Chitosan, obtained by deacetylation of chitin, is a polymer with a helix structure with reactive amino groups, which offers many possibilities for modification and ionic interactions. Being one of the few cationic polysaccharides, this gives it unique properties with respect to the rest of the polysaccharides, which are generally neutral or negatively charged [1].

It is characterized by being biodegradable, biocompatible and of low toxicity. It is attributed antibacterial, antifungal, surfactant, antitumor, mucoadhesive, healing, antioxidant and other properties [2, 3]. It has as other advantages that it is renewable and economically feasible.

Due to these properties, its field of use is very varied and includes the pharmaceutical, food, cosmetics, paper, textile, water treatment and other industries. Among the most important international markets are Japan, the United States, Europe and some Asian countries, mainly China and Korea [1].

Among the routes of administration of the biopolymer, the parenteral and oral routes (frequently as excipient) have been used, but the topical route (as an active pharmaceutical ingredient) is definitely the most represented. The most studied systems by this route are hydrogels, films, dressings, ointments, creams, among others [4-7].

It has been described that the wound-healing effect of chitosan is attributed to accelerating the infiltration of polymorphonuclear cells into the injury area, increasing the

effusion of dense forms of fibrin, activating migration and stimulating fibroblast proliferation in the wound, with the production of collagen [8-10].

Chitosan and its derivatives are included in skin and hair care products, oral care, perfumery, as well as dental care. They have been used to maintain skin moisture, treat acne, improve hair flexibility, tone the skin, and in tooth care products in toothpastes, tooth whiteners and chewing gums [1].

Other essential applications of chitosan are in hair care products such as shampoos, hairsprays and dyes. Also in the manufacture of face, hand and body creams; in cleaning products such as cleansing milks, tonics, facial peels, soaps and bath agents. It is also added in the production of color cosmetic products such as lipsticks, eye shadow makeup, nail polish and deodorizing products [11].

Among the essential advantages of cosmetics using chitosan are their efficacy and the adequate hydration they achieve in the skin. They provide, therefore, not only a health benefit, but also excellent cosmetic qualities due to their antimicrobial and antioxidant properties, especially against pathogenic fungi, as well as their stabilizing role in formulations [11].

Several works have been published on the obtaining, characterization, application of this biopolymer, derived from common lobster (*Panulirus aurgus*) chitin, and its salts [12-14], as well as the antioxidant activity of its solutions [15].

Most cosmetic skin care formulations are oil/water, water/oil or double emulsions. From a dermatological point of view, the water/oil emulsion is a better choice, as the lipid film formed on the skin favors the oil-soluble active ingredients. The emulsions oil/water are also more appreciated by the consumer as they are less greasy [16]. On the other hand, they can cause a cooling sensation due to water evaporation [17]. Basic formulations of creams with skin moisturizing function are typically composed of ingredients such as emollients, humectants, thickeners, emulsifiers, stabilizers, preservatives and neutralizers [18, 19].

The purpose of the stability study of cosmetic products is to provide information on their quality over a given time, under the influence of a variety of environmental factors such as temperature, light and humidity. Other factors are related with the product, like the physical and chemical properties of the active ingredient and excipients, the type of cosmetic product and its composition, the manufacturing processes and the nature and properties of the packaging used [20].

The stability of a cosmetic is the property of the product, preserved in a specific container, to retain within a period of time, and during its shelf life, the properties it had

at the time it was manufactured by a standardized procedure. This includes chemical, physical, microbiological, toxicological and functionality evaluation [20].

Considering the need to deepen the properties of the biopolymer derived from lobster chitin (*Panulirus aurgus*), which allows the development of safe pharmaceutical and cosmetic products, the stability of a 1.0 % chitosan cosmetic cream was evaluated in the present work, as well as its non-clinical effects (dermal and ophthalmic irritability and dermoregenerative effect).

Material and y methods

Chemical products and reagents

Chitosan, supplied by the Natural and Synthetic Products Production Plant (CIDEM, Cuba), batch 11002 with 9.2 % moisture, 0.8 % sulfated ash, 0.4 % insoluble material, 78.5 % degree of deacetylation and 310 000 g/mol molecular mass, was used as active ingredient [13]. The following were used as excipients in the formulation: isopropyl myristate (Henkel, Germany), glacial acetic acid and sodium hydroxide (Panreac, Spain), propylene glycol (BASF We Create, Germany), cetyl alcohol (Ecogreen, Malaysia), stearic acid (Renichem S.L., Malaysia), sodium benzoate (Panreac, Spain) and floral fragrance (Robertet, France).

Pilot-scale production of the cream

Based on the optimized formulation [21], three 10.0 kg batches identified as L-21001, L-21002, L-21003 were prepared, packaged in bottles (F-14), high-density polyethylene caps (T-14) and low-density polyethylene liner (L-14) with tamper-evident seal, nominal capacity of 250 mL (Frasplast, Cuba).

The composition of the batches of cream was similar, and the components were: sodium hydroxide (0.60 %), cetyl alcohol (4.50 %) and stearic acid (7.40 %). It was established that stearic acid should be maximum and cetyl alcohol minimum, thus achieving the best organoleptic characteristics. Based on the results obtained in the formulation and design stage, the numerical optimization of the formulation was developed [21].

The final homogenization of the product was carried out in a colloidal mill (Probts and Class, Germany). Packaging was carried out semi-automatically, using a filling machine model AR/403 (ERWEKA, Germany).

Cream evaluation

The batches were periodically evaluated according to organoleptic characteristics, pH, extensibility and degree of deacetylation. The methodologies used for each test are described below:

Organoleptic characteristics: appearance, color and odor were determined. Samples were observed for visible modifications and instabilities like: coalescence, creaming and presence of lumps. The existence of sandiness was evaluated by touch. Acceptance criteria: Semisolid product of uniform appearance, odor and characteristic color.

Area of extensibility: 2.0 g of the semisolid was weighed on an analytical balance (SARTORIUS model ENTRIS 6202-1S, Germany) and placed in the center of a glass plate with a millimeter paper adhered to the bottom, so that the point of application of the semisolid coincided with the axes of the coordinates drawn on it. Subsequently, a 292 g glass plate was homogeneously placed on the semisolid. After 5 min, the radii corresponding to the circle formed by the sample, determined from the point of application in four directions, were measured. The total area of extension of sample was calculated reporting the average value and the standard deviation, resulting from three determinations per sample. Acceptance criteria: 25.50 - 36.20 cm^2.

Determination of pH: A Seven Compact TM S220 pH meter (Mettler Toledo, Switzerland) calibrated with buffer solutions of pH 4.01 and 7.00, respectively was used. Approximately 20.0 g of sample was transferred to a 50 mL beaker. Three replicates were performed for each sample and the mean value and standard deviation were reported. Acceptance criteria: 6.50 - 7.25.

Degree of deacetylation: For the determination of the content of amino groups, 5.0 g of the cream sample was weighed, transferred to a plastic tube, added 5 mL of chloroform (Sigma-Aldrich, Germany) and subjected to vigorous vortexing (SeouLin, Korea). Then 25 mL of 0.3 N hydrochloric acid (37 %, Merck, Germany) was added and shaken in a sieve for 1 h. Subsequently, it was centrifuged (SORVALL RC 3B PLUS, USA) at 4000 s^{-1} for 15 min at 30 °C. Then, 20 mL of aqueous phase was extracted and 10 mL of purified water was added. It was titrated (Mettler Toledo, Switzerland) with 0.3 mol/L sodium hydroxide (Merck, Germany) slowly and with continuous stirring. The degree of deacetylation was determined using the following equations:

$$MQC = Pm / 100$$
$$\%NH_2 = 16.1(Y - X) / (MQC \cdot f)$$

Where: MQC: chitosan in cream sample weighed (mg); Pm: cream weighed (mg); 100: mathematical factor for calculation; $\%NH_2$: percentage of free amino groups; 16.1: milliequivalent value between hydrochloric acid and chitosan; X: major inflection point (mL); Y: minor inflection point (mL); f: factor of sodium hydroxide dissolution.

To calculate the degree of deacetylation/g of the cream was carried out using the following formula: Degree of deacetylation (%) = ($\%NH_2 \cdot 100$) / 9. Acceptance criteria: 70-95 %.

Stability of pilot batches

Pilot batches were stored at 30 ± 2 °C and 70 % relative humidity, for 12 months. The parameters described above were periodically determined at initial, 1, 3, 6 and 12 months [20]. The stability of the product was predicted from the results of the variables pH, extensibility and degree of deacetylation using the Statagraphics® Centurion 19 program (USA, 2022).

Microbiological stability was determined through the microbial limit test for non-sterile products, at initial and at 12 months [22]. Acceptance criteria: total aerobic microorganism count (bacterial count, BC) $<10^2$ cfu/g, combined total count of filamentous fungi and yeasts (FC and Y) <10 cfu/g, absence of *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Non-clinical studies

Dermal and ophthalmic irritancy tests were performed on the cream formulation at the Department of Pharmacological and Toxicological Research (CIDEM, Cuba). Male New Zealand albino rabbits of 1.5 to 2.0 kg body weight with lot number 0516, from the National Center for the Production of Laboratory Animals (CENPALAB, Cuba), were used and were kept during the assay in a room with controlled temperature of 22 ± 2 °C and 12/12 h light-dark cycle. Feeding consisted of standard diet for rabbits CMO 1400 from CENPALAB and acidulated water on demand.

Dermal irritancy

The rabbits, 24 h before the start of the trial, were depilated and shaved on the dorsal area of the back at a sufficient distance from the spine to proceed with the application and observation of the test sites. Three rabbits with intact skin (three sites per product) were selected and 0.5 g of the cream was applied to the skin for 4 h. The application sites were covered with 2.5 cm^2 gauze patches, which were attached to the skin with

hypoallergenic tape. At the end of this period, the patches were removed and once the application site was marked, the remnants of the test substance were removed using sterile water [23].

Observations were made at 0.5, 1, 1, 24, 48 and 72 h after patch removal. Skin reactions for erythema and edema were established according to the Evaluation System for Skin Irritation [24]. Only observations made at 24, 48 and 72 h after application were taken into account to calculate the Primary Irritation Index, which was compared with the Response Classification System for dermal irritation testing to give the test results and classify the test substance [25].

Ophthalmic irritancy

Three rabbits were taken and subjected, in the 24 h prior to the assay, to a rigorous examination of their ocular structures (cornea, iris and conjunctiva) to rule out the presence of any underlying damage. On the day of the trial, 0.1 g of the cream was instilled in the conjunctival sac fundus of the right eye; the left eye was taken as a control. Both eyelids were closed for 15 seconds to ensure maximum contact of the test substance with the ocular structures. Both eyes were examined at approximately 1, 24, 48 and 72 h after the initial application in order to detect damage to the mentioned ocular structures. Observations were made with white light to detect the presence of erythema, edema and abnormal secretions, in addition to the reaction of the iris to light, followed by examination of the cornea by fluorescein staining and observation with UV lamp.

The ocular reactions observed were evaluated according to the scale for classifying ocular lesions. Once both eyes were examined during the established hours, the Ocular Irritation Index was determined [24, 25].

Determination of the dermoregenerative effect

To test the dermoregenerative effect, chitosan creams were tested at concentrations of 0.25 %, 0.5 % and 1.0 % (w/w), in a model of photoaging in the skin of mice photosensitized with ultraviolet radiation (UVB), supported by standard histological techniques [26].

Male Balb/C mice of 20 to 25 g body weight, batch number 1811, from CENPALAB (Cuba) were used. These were maintained during the trial in a room with controlled temperature of 22 ± 2 °C and 12/12 h light-dark cycle. Feeding consisted of standard diet for rodents CMO 1000 from CENPALAB and acidified water on demand. The

animals remained in quarantine for seven days, before starting the trial, for their adaptation to the laboratory conditions. Five groups of five animals were formed: I: control without radiation, II: control radiation, III: treated low dose (0.25 %), IV: treated medium dose (0.5 %), V: treated high dose (1.0 %), VI-treated placebo.

All animals, except the controls (group I), were subjected to UVB radiation using a lamp (Spectroline®, USA), at a wavelength of 312 nm. The radiation was directed to the dorsal area of the animal at a distance of 15 cm, for 30 min. Immediately after the radiation, the respective treatments were applied, which were repeated every 24 h for a period of six days. Each substance was applied to the skin of the dorsal area in an amount of 0.1 g spread over the skin surface [27].

Daily, before each application, the appearance of the skin was evaluated to detect the presence of erythema, bedsores and skin wrinkling, using a scale from zero to four, according to the degree of damage observed. At the end of the treatment, 24 h after the last administration, the animals were sacrificed by cervical traction and the skin was removed from the dorsal area for histopathological analysis. Skin samples were processed by traditional histological procedures. Aspects such as the appearance or not of an inflammatory infiltrate, presence or not of hyperkeratosis and acanthosis, disposition of the elastic and collagen fibers and other alterations that could appear, characteristics of photoaging, as well as their possible recovery or not due to the use of the cosmetic under study were analyzed [28].

Results and discussion

Stability of the optimized formulation at pilot scale

The composition of the three batches was similar, during the elaboration process of those no difficulties were manifested, obtaining a homogeneous product, which demonstrated the reproducibility of the developed methodology.

Table 1 shows the average results of the analyses carried out on the pilot batches during 12 months, under shelf-life conditions. In all cases, the criteria established in the quality specifications of the finished product were met [21].

The organoleptic properties of the three batches remained unchanged during the study period. The preparation presented a white color, floral odor, a homogeneous appearance with brightness, free of lumps and sandiness.

Table 1. Stability of pilot batches

Parameter	Batch	Time (month)					Acceptance criteria
		Initial	1	3	6	12	
Organoleptic characteristics	21001	Accepted	Accepted	Accepted	Accepted	Accepted	Semisolid product of uniform appearance, odor and characteristic color
	21002	Accepted	Accepted	Accepted	Accepted	Accepted	
	21003	Accepted	Accepted	Accepted	Accepted	Accepted	
pH	21001	6.93/0.10	6.86/0.08	6.86/0.08	6.81/0.10	6.79/0.05	6.50 – 7.25
	21002	6.95/0.05	6.90/0.10	6.87/0.08	6.86/0.04	6.81/0.05	
	21003	6.91/0.07	6.87/0.08	6.85/0.09	6.83/0.07	6.80/0.05	
Extensibility (cm^2)	21001	31.12/1.81	29.55/0.11	30.25/3.32	29.68/4.81	27.70/2.82	25.50 – 36.20
	21002	30.21/1.95	30.53/1.50	28.80/2.84	29.59/2.92	29.00/3.91	
	21003	31.68/2.81	30.34/5.05	29.59/2.92	30.21/1.95	29.59/2.92	
Degree of deacetylation (%)	21001	78.20/2.30	78.10/2.55	78.14/2.14	78.10/2.24	78.00/2.54	70 – 95
	21002	77.80/2.35	77.90/2.60	77.76/2.55	77.50/2.10	77.40/2.75	
	21003	78.00/2.55	77.95/2.32	77.85/2.75	77.65/2.56	77.00/2.85	
Microbiological test	21001	Accepted	-	-	-	Accepted	BC: 102 cfu/g; FC and Y: 10 cfu/g; Absence of *P. aeruginosa* and *S. aureus*
	21002	Accepted	-	-	-	Accepted	
	21003	Accepted	-	-	-	Accepted	

(X/DS) Mean/Standard Deviation: n=3; BC: total count of aerobic microorganisms, FC and Y: combined total count of filamentous fungi and yeasts.

The pH of a cosmetic formulation in the form of an emulsion is of vital importance because it can influence its stability, causing its breakdown, affecting the efficacy of the preservatives, as well as the chemical stability of other components of the formulation. The pilot batches maintained a pH between 6.50 and 7.25 during the study period, with no significant differences ($p > 0.05$) between the reported values. No significant variations were observed between batches or during the study period, evidencing the stability of the formulation, which allows guaranteeing the safety of the cosmetic.

The values of the extensibility area remained in accordance with the characteristics reported during the optimization of the formulation [21]. All the above evidenced the reproducibility of the formulation and of the elaboration process developed, under the conditions used.

The extensibility of the batches showed a general tendency to decrease as storage time progressed, an expected result, considering the effect of temperature on the viscosity of semisolids. It is known that semisolids experience a structuring of the system during storage time, which implies an increase in consistency causing a decrease in the extensibility of the product [12].

Regarding the percentage of the degree of deacetylation, the values showed a tendency to decrease with time, but within the established range [21], which shows that the chitosan in the cream maintains this property during the 12 months of study.

The results of the microbiological study satisfactorily met the established limits, which demonstrate the use of quality raw materials and compliance with Good Manufacturing Practices.

The results obtained as a whole allow affirming that the 1.0 % chitosan cream maintains its physical, chemical and microbiological stability during 12 months, packed in high-density polyethylene bottles and stored at 30 ± 2 °C and 70 % relative humidity, therefore these results approve the reproducibility of the three batches.

Statistical analysis of the experimental values of pH, extensibility and degree of deacetylation demonstrated their fit to linear models (Table 2), with probability values less than 0.05. The variation of the three parameters was linearly dependent with time, with very low degradation rates, which explains the low correlation coefficients. These models were used to predict the stability by extrapolation of the three batches of the cosmetic cream, proving that it can have a stability of approximately 24 months (Figure 1).

Table 2. Analysis of mathematical models for the prediction of cream stability by extrapolation.

Parameter	Model	Fit (p)	Correlation coefficient (r^2)
pH	Linear	0.000	77.50
Degree of deacetylation	Linear	0.0115	39.40
Extensibility	Linear	0.0039	48.61

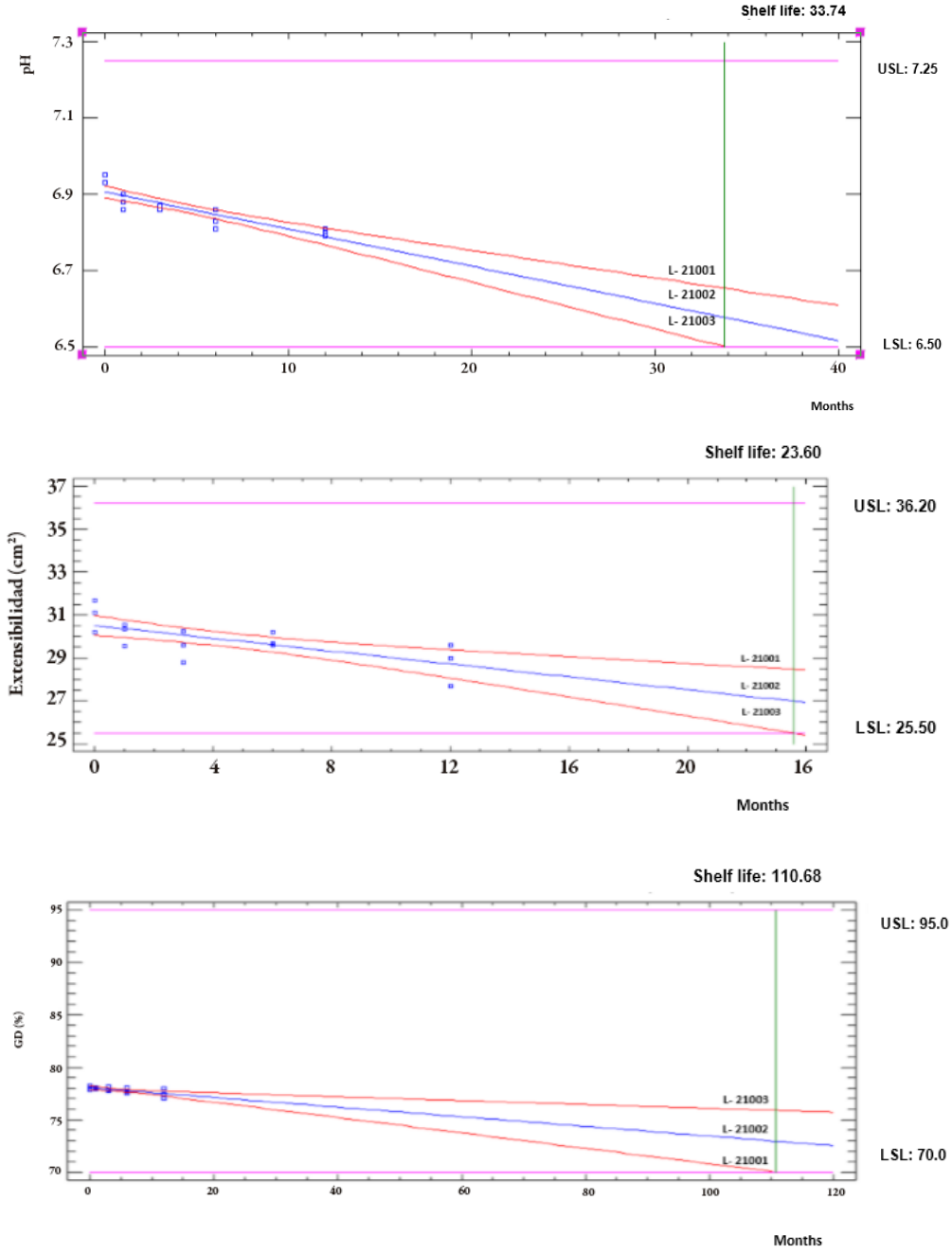

Figure 1. Extrapolation prediction of cream stability (Batch: 21001, 21002, 21003), using data of pH, extensibility and degree of deacetylation (DD).

Non-clinical evaluations

Dermal and ophthalmic irritancy tests were performed to determine the possible effects of the cream, taking into account that the product is generally intended for facial use.

During the evaluation of dermal irritability, erythema was observed in some animals. These animals did not show alterations in their behavior or weight. The irritability index was 0.32 points, which classifies the product evaluated as non-irritating.

Regarding ophthalmic irritability, during the first hour after the application of the product, there were erythematous and edematous reactions and secretions in the conjunctiva of the treated animals, but not in the iris. The cornea was affected during the first 24 h and from the clinical point of view there were no alterations in the experimental animals. From these results, the irritability index was calculated, reaching a value of 8.33 points, which allowed classifying the 1.0 % chitosan cream as non-irritant.

Considering these results, it was demonstrated that the formulated semisolid is a product that offers good safety, since it is considered a system of low toxicity to the organism.

Dermoregenerative effect of the chitosan cream

According to macroscopic observations, in group I (control without radiation) there were no alterations, serving as a comparison of the normal structures of the skin. In groups II (radiation control) and VI (placebo treated) the characteristic alterations of photoaging appeared: erythema, wrinkling of the skin and crustal-desquamative lesions. In groups III (chitosan 0.25 %), IV (chitosan 0.50 %) and V (chitosan 1.0 %), damage to dermal and epidermal structures was observed, as well as recovery from post-treatment damage.

According to microscopic observations, there were characteristic alterations of photoaging, with marked significance in the disorganization of the collagen fiber in the control group (II) and in the group treated with placebo (VI). In groups III (chitosan 0.25 %) and group IV (chitosan 0.50 %), photoaging alterations were observed, but less marked than in groups II and VI, after treatment, and average grading values of 2 were reached for both cases (Table 3), indicating that the 0.25 and 0.50 % chitosan creams have a low dermoregenerative effect.

According to the average values of graduation of histological damage observed in the skin sections of the different groups, after treatment, the recovery is appreciated in group V, treated with 1.0 % chitosan cream, reaching an average graduation value of 1 (Table 3), which indicates a moderate dermoregenerative effect.

Table 3. Grading values in the evaluation of histological damage.

Animal number	Histological scoring in the animal groups					
	I	II	III	IV	V	VI
I	0	3	1	1	1	3
II	0	3	2	2	1	3
III	0	3	3	2	1	3
IV	0	3	2	2	1	3
VI	0	3	2	3	1	3
Average graduation	0	3	2	2	1	3

Histological score: (0) normal; (1) mild; (2) moderate; (3) severe.
I: control without radiation, II: control radiation, III: treated low dose (0.25 %), IV: treated medium dose (0.50 %), V: treated high dose (1.0 %), VI: treated placebo.

The average results and standard deviation in each group of animals, showed that there are no significant differences between the groups treated with 0.25 % and 0.5 % chitosan cream, with respect to the irradiated control group, however, between the placebo and irradiated groups and the 1.0% chitosan cream group, significant differences were found (Figure 2).

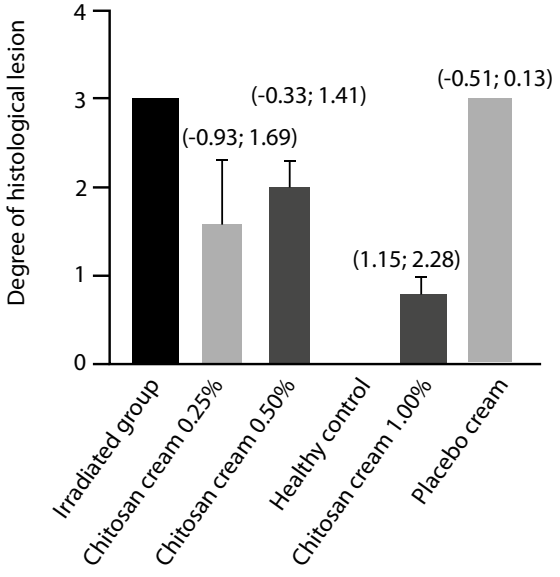

Figure 2. Microscopic evaluation of the skin of irradiated animals treated with 0.25 %, 0.50 %, 1.0 % and placebo chitosan cream. The confidence interval (σ =95 %) is shown for each of the treated groups with respect to the irradiated group.

The dermoregenerative activity of the cosmetic cream was evaluated in the photodamaged skin of mice by histological sections of the different skin layers. The skin condition was good for 100 % of group I (mice depilated without irradiation) and group V (mice depilated, irradiated and treated with 1.0 % chitosan cream). The recovery of the normal properties of the epidermis and dermis, previously damaged by radiation, was verified, particularly in the arrangement and organization of the collagen fibers. Chitosan proved to be the compound responsible for the dermoregenerative effect after application of the cream.

Conclusions

The pilot batches of the formulation maintained their stability for 12 months, packaged in high-density polyethylene bottles at room temperature. The 1.0 % chitosan cream is non-irritating dermally and ophthalmically and has a moderate dermoregenerative effect.

Acknowledgments

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Conflict of interest

All authors report that they do not have any conflicts of interest.

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