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Elaboration, characterization, and stability study of a sunscreen emulsion for use as a towelette application in pediatric photoprotection

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Synopsis

The World Health Organization (WHO) estimates that as much as 80% of the solar radiation that an adult receives throughout his/her life is received during the first 18 years (1). Skin protection against harmful solar radiation during this early stage of life is therefore a highly important factor in the prevention of future skin-related diseases. In this respect, recent developments in pediatric dermatology and cosmetic technology have led to remarkable improvements in child skin protection products. However, in spite of these scientific breakthroughs, many currently available commercial sunscreen formulations have not been well received by the general public, due to inadequate sensory properties, chemical instability, undesirable side effects, and low effectiveness. These disadvantages are not only attributable to the formulations themselves, active principle, and excipients, but also, to a large extent, galenic aspects.

The objective of this work was to develop and characterize a sunscreen emulsion for pediatric use, using a towelette as vehicle, to overcome problems of ineffectiveness and formulation instability, and to improve skin-sensory properties. The composition of the towelette, the emulsion, and the presentation format were selected on the basis of the differences between children's and adult skin.

In order to evaluate the chemical stability of the formulation, a study of the organoleptic, physicochemical, microbiological, and rheological characteristics was carried out at 4°, 25°, and 40°C over a period of 30 days. Tests were performed on both the sunscreen emulsion only and the same formulation impregnated within a towel, to test the influence the towel may have on the stability of the emulsion.

INTRODUCTION

Damage from ultraviolet radiation (UV) on human skin is dose-dependent and cumulative, and is related to the duration, frequency, and intensity of exposure to solar radiation. Sun causes dermal damage manifested by short- and long-term effects. In the short term, damage may cause burns and allergic reactions related to degranulation and the release of several mediators that play a crucial role in the inflammatory process, including histamine,

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prostaglandins, and cytokines. In the long term, UV radiation may cause erythematogenic and melanogenic harm, as well as immunosuppression and carcinogenesis (2–4), all of which have serious implications for human health. Protection against sun exposure is recommended at all ages, but should be especially encouraged for children and young people, given that a child's skin is considerably more sensitive to UV radiation than an adult's.

Given that skin possesses a memory effect, exposure to sun radiation during early life leads to cumulative and irreversible damage, which may later be manifested in adulthood (5). Moreover, increasing numbers of children are suffering from sun-related diseases such as photodermatosis (6), and when compared to adult skin, a child's skin presents histological and physiological differences that make it particularly sensitive to solar radiation (7). In addition, the dramatic increase in the incidence of skin cancer, especially malignant melanoma, highlights the need for new and improved prevention methods. In comparison with an adult's skin, an infant's skin is especially delicate, fine, and highly permeable. For this reason, a good dermatological sun-protection product must fulfill several critical requirements in terms of quality and non-aggressiveness (8). At an early stage of life, skin as a living tissue has not yet fully developed its own natural protection against actinic aggression (9). Exogenous photoprotection is therefore highly recommended (10). Given the importance of protection, both educational programs in prevention and new formulations for child skin care should be studied (11).

Sunscreens are photoprotective cosmetic products that can be designed to cover the dermatological needs of a determined segment of a population with specific requirements (12). In addition to the requirements of effectiveness, safety, and versatility (14), the type of sun protection required depends on individual skin characteristics and the purpose for which the product is to be used (13).

Characteristics such as softness, waterproofing, non-stickiness, ease of application, and a sun protection factor (SPF) of between 15 and 40 are especially desirable in formulations for children. On the other hand, compliance in use in adults is more dependent on the aesthetics of the product. Products with a lower SPF endowed with a lesser degree of waterproofing are more generally accepted, because they have less of an unpleasant, doughy, sticky feeling. For this reason, different levels of SPF are incorporated into a broad range of cosmetics for daily use.

Skin damage from ultraviolet radiation in early childhood must be avoided through improvements in photoprotective therapy, and research in this field should aim to produce new sunscreens and/or make already existing ones more effective. The objective of this work was to develop a topical sunscreen formulation for towelette application, as an alternative to the commercial semi-solid formulations available on the market, and to carry out a stability study of the emulsion used.

As a pharmaceutical format, the use of towels as a solid support for sunscreen formulae presents a number of advantages, which include: ease of application, increased stability, a reduction of side effects caused by solar filter penetration, and an increase in efficiency and safety. In addition, the towelette product is more easily conserved due to its single- dose format, does not produce a sticky feeling or generate residues, is easily transported, and is convenient to use.

Two tests were carried out to compare the performance of the towelette as a new form of topical application: one on the sunscreen formulation supported within an emulsion, and another on the impregnated towel formulation. Both were characterized for organoleptic

characteristics, dry matter, pH, microbiological contamination, density, and rheological properties. In parallel, a stability study was carried out on both formats at temperatures ranging from 4° to 40°C under light-protected conditions, in order to assess what effect the use of the towelette as a support vehicle may have on the stability of the emulsion. Stability was determined through centrifugation.

Over a 30-day assay period, samples were taken on day 0, and at 1-, 15- and 30-day intervals. Determinations for samples from both vehicles were performed in triplicate.

MATERIAL AND METHODS

MATERIALS

As emulsion components, Neo Heliopan AV® (ethylhexyl methoxycinnamate), Neo Heliopan MBC® (4-methylbenzylidene camphor), Neo Heliopan Hydro® (phenylbenzimidazole sulfonic acid), and Neo Heliopan 357® (butyl methoxydibenzoyl methane) were obtained from Symrise Ibérica (Barcelona, Spain). Tioveil AQ-N® (aqua, titanium dioxide, alumina, silica, sodium polyacrylate, and methylparaben sodium) and triethanolamine (triethanolamine 99%), were obtained from Comercial Química Massó (Barcelona). Arlamol HD® (isohexadecane), was received from Uniquema (Barcelona), and Tefose 2000 (Peg-6 stearate, ceteth-20, steareth-20) was from Gateffose (Madrid, Spain). Other components were Alliant OPT® (acrylates/C12-22 alkylmethacrylate copolymer) from Inquiaroma (Barcelona), and vitamin E acetate (tocopheryl acetate), methylparaben, ethylparaben, propylparaben, butylparaben, and chorphenesin from Impex Química (Madrid). Mineral oil fluid (paraffinum liquidum) was obtained from Quimidroga (Madrid), and alcohol benzoate C12–C15 (benzoatyl alcohol), cetostearyl alcohol 25.OE (ceteareth-25), and Dub Diol® (methylpropanediol) were obtained from Campi & Jove (Madrid). Dow Corning 200® (dimethicone), from Safic Alcan (Barcelona), propolis hydroglycolic extract (propolis cera), from Biogründl (Madrid), Perfume Green Coco® (perfume), from GmbH & Co (Paris, France), and deionized distilled water (aqua), from Interapothek (Murcia, Spain), were additional emulsion components.

We believe that an optimal sunscreen formulation depends not only on the total active filter content intended to prevent or mitigate harm from solar radiation, but also on other complimentary components. For this reason, hydroglycolic extract of propolis was included, due to its anti-inflammatory and immunoregulatory activity (15). This active ingredient is also believed to have a protective effect on skin (16).

A non-woven material was chosen as the physical support for the formulation (17), composed of directly ordered or randomly arranged sheets or membranes, forming fibers connected through friction, and/or cohesion and/or accession, thus excluding paper or textiles, welded or joined through thread tying. The fibers may be either natural or synthetic (18). A viscose non-woven product with a thickness of 50 g/m^2 (LIDRO™, supplied by Jacob Holm Industries, Soultz, France) was chosen for its composition and weight (mesh size), and in particular for its smoothness and biodegradability. Unlike synthetic fibers, such as polyester and polypropylene, this material decomposes rapidly (19).

A sheet of micronized black polyethylene complex, composed of PET (polyethylene terephthalate), 12μ/coext, and PE (polyethylene), BCO/black 50 g, from Amcor (Madrid) was chosen as packaging material.

METHODS

Preparation of the sunscreen emulsion. The formulation was developed as a waterproof O/W emulsion, appropriate for children's skin and produced with the aim of achieving high-level protection against UVA-UVB radiation. The composition of each phase is detailed in Table I. At each stage of preparation, the quantity of every component was determined by weight. Oil and water were added separately after previous heating to 80°C, taking care to ensure all components had fused after each addition. Phase N was prepared, obtaining a transparent solution with a pH of between 7.00 and 7.50, and subsequently added to phase A. Shaking was used to ensure homogeneity, with the final emulsion obtained after adding the water phase to the oil phase. At 40°C, T phase was added, followed by C and P phases at temperatures of between 30° and 35°C respectively. The system was then stabilized through further moderate shaking until cooling to room temperature. The formulation was then shaken for a further 20 minutes, after which 5.25 ml of emulsion was introduced into each envelope containing a towel (T) and 100 ml sealed in sterile containers (E).

Table I
Description of the Emulsion

Phases	Components	%
Oil phase (O)	4-Methylbenzylidene camphor (Neo Heliopan MBC®)	4%
	Ethylhexyl methoxycinnamate (Neo Heliopan AV®)	9%
	Butyl methoxydibenzoyl methane (Neo Heliopan 357®)	2%
	Peg-6 stearate, ceteth-20, steareth-20 (Tefose 2000®)	8%
	Isohexadecane (Arlamol HD®)	14%
	Cetostearyl alcohol 25O.E	2%
	Alcohol benzoate C12-C-15	3%
	Mineral oil fluid	1%
	Vitamin E acetate	0.1%
Aqueous phase (A)	Deionized distilled water	q s 100
	Dimethicone (Dow Corning 200®)	1.5%
	Methylparaben	0.7%
	Ethylparaben	0.7%
	Propylparaben	0.7%
	Butylparaben	0.05%
Phase N	Deionized distilled water	2%
	Phenylbenzimidazole sulfonic acid (Neo Heliopan Hydro®)	4%
	Triethanolamine	2.4%
Phase T	Aqua, titanium dioxide, alumina, silica, sodium polyacrylate (Tioveil aq-N®)	6%
	Acrylates/C12-22 alkylmethacrylate copolymer (Alliant OPT®)	2%
Phase C	Chorphenesin	0.15%
	Methyl propanediol (Dub Diol®)	1%
Phase P	Propolis extract hydroglycolic	5%
	Perfume green coco	0.26%

Calculation of the solar protection factor. In order to determine the effectiveness of the formulation in terms of SPF, ten volunteers were chosen to take part in the study. The source of radiation was set to a constant spectral emission within the UV region, and appropriately filtered to avoid cutaneous harm, in accordance with COLIPA recommendations for the spectral quality of UV radiation (20). The device used was a solar malingerer Multiport®, with an emission spectrum ranging between 290 and 400 nm.

Given the variability of the skin's reactivity to UV radiation, randomly selected areas of the back of each volunteer were treated with one impregnated towel prior to radiation exposure. The MED evaluation of protected skin (MED_p) and unprotected skin (MED_n) was carried out visually and simultaneously under a perfect standard source of diurnal light:

- MED_p was considered as the quantity of radiant energy required to cause non-ambiguous redness, with defined edges, equivalent to that required for the MED_n area, evaluated by visual comparison after 16 or 24 hours of exposure.
- MED_n was considered as the quantity of radiant energy required to produce the minor visual redness obtained through MED_p under a perfect standard source of diurnal light.

Impregnated towelette preparation. The volume of formulation required for the impregnation of the towelettes was determined after a series of trials and from the experience gained from the validation of different formulations in previous studies. The total volume of formulation was therefore calculated according to the dimensions, weight, and total number of towels used in this trial. The dimensions of the towels used in this study were 200×175 mm, and the quantity of formulation required was calculated according to the following parameters:

(a) VOL (sunscreen emulsion volume necessary to impregnate the towel)

(b) G (non-woven weight)

(c) D (towel dimensions, width and length)

(d) C.I. (coefficient of impregnation as stipulated by COLIPA (21), for the purpose of obtaining a homogeneously moistened surface for correct application. This coefficient is experimentally calculated and is dependent on the viscosity of the fluid)

(e) N (number of towels contained in the package to be impregnated)

Organoleptic characteristics. Organoleptic characteristics were classified according to the following terminology: thick, hard, creamy, smooth, soft, dry, thin, spreadable, cool, and warm (22). The sunscreen emulsion and the towel were subjected to the sensory analyses of color, odor, texture, consistency, and external appearance (exudate), at 0 h, just after preparation, and 24 h after preparation. The same analyses were carried out after storage at all selected temperatures and time intervals, with determinations for both samples (T and E) performed in triplicate.

Dry matter. A thermobalance, Sartorius model 150C-000230V1 MA, was used at a temperature of 105°C to determine the quantity of water present in both emulsion and towel formulations, in order to assess chemical stability.

Density. Density was determined at 20°C with a 100-ml graduated glass cylinder and a METAS hydrometer with a nominal range of between 0.900 and 1.000 and a minimal division of 0.002 g/m^3.

pH. In order to detect any possible variations in pH, values were measured in both formulations using an mv/pH meter, Crison model digit 501, at the determined intervals throughout the entire storage period.

Microbiological control. An impregnated towel was introduced into an inactivator medium of preservatives (MIP) in a laminar flow cabinet (Testalr model BIO IIA). After incubation for 30 minutes, 1 ml of MIP was planted onto both a Triptona soy agar (TSA) medium plate, for the detection of aerobic bacteria, and a Sabouraud's medium plate, for the detection of fungi and yeasts. The plates were incubated at 37°C for 48 hours and at 22°C for five days, respectively (23). A negative control was performed by planting 1 ml of sterile peptone water and 1 ml of inactivation medium (MIP) in each of the culture media. In the case of the samples from the emulsion, the same technique was carried out by adding the emulsion to the inactivation medium (MIP).

Rheological characteristics. The rheological properties of the formulations were studied in terms of viscosity, a parameter closely related to stability (24). Assays were run at increasing shear rates in a Brookfield DV II+ viscosimeter (Brookfield Engineering Laboratories, Stoughton, MA).

Stability. To determine the effect of temperature on the stability of the samples, tests were carried out at 4° and 25°C. The formulae were also tested in a closed container, introduced into a Friocell 111 oven at 40°C throughout the entire test period. The samples were then subjected to a centrifugal testing using a microcentrifuge (Kendro, Heraeus, model peak 17, at 3.500 rpm) for ten minutes, to ascertain whether any separation of the two phases of the emulsion had taken place.

RESULTS AND DISCUSSION

The results are expressed as the mean and standard deviation of three determinations of samples from each formulation, at each temperature and storage time interval. All the results were compared using variance analysis (ANOVA) for a 95% confidence level to detect any significant differences.

Triplicate analysis commenced at time 0 and continued at each time interval until finalization of the storage period, a procedure that indicates whether any modification of the formulae occurs over time and at determined temperatures. The results are presented in Table II.

CALCULATION OF SOLAR PROTECTION FACTOR (SPF)

Final SPF was determined from the average of the values for individual SPF (SPF_i) for the ten volunteers (n) included in this study. The individual value of the SPF of the emulsion was defined as the ratio of the MED_p and MED_n on the same volunteer:

$$SPF = \frac{\sum SPF_i}{n}$$

The results obtained from the analysis of the emulsion proposed in this work reached an SPF of 30, classified as a high protection value according to guidelines published in 2006 (2006/647/EC) (25).

Table II
Towlette Test and Emulsion at Time 0

Analysis	Specification	Results	
		T0	E0
Organoleptic characteristics	Milky white emulsion; coconut smell	A	A
Dry matter	24.0–29% (emulsion); 3.5–4.2% (towelette)	3.9%	27.30%
Density	0.985–0.995 g/m^3	NA	0.988
pH	7.2–7.5	7.24	7.23
Microbiology control	>100 UFC/g	1	2
Rheological characteristics	280–350cps	NA	298
Stability in centrifuge	Without alteration	NA	A
Stability in oven	Without alteration	A	A

A: agreed. NA: did not agree. NA: not applicable.

PREPARATION OF THE TOWELETTES

A coefficient of impregnation (C.I.) of 3 was chosen for our formulation, with a viscosity of less than 350 cps. The different permeation coefficients were calculated from experimental determinations using as a standard the commercial towelettes currently available on the market. The volume required to moisten each individual towelette was calculated as follows:

$$\mathrm{VOL\,(ml)}: 50\ \mathrm{g/m^2} \times 0.2\mathrm{m} \times 0.175 \times 3\mathrm{ml/g} \times 1 = 5.25\ \mathrm{ml}$$

ORGANOLEPTIC CHARACTERISTICS

After macroscopic observations of the preparations, the emulsion was found to be creamy to the touch, and was easily spread but felt slightly sticky after application to the skin. On the other hand, the towelette preparation was found to be homogenously impregnated and easy to apply, and it left a pleasant soft feeling on the skin. These results were constant at each time and temperature interval, with no significant changes in organoleptic characteristics, texture, or appearance.

After heat treatment at 40°C, there was an increase in the consistency of the emulsion, with the formation of a thin solid layer on the surface of its airtight container. However, no modifications were observed in the towelettes contained in the single-dose sachets.

DRY MATTER

The definition of the intervals was carried out in the laboratory for the three consecutive, identical, batches from the emulsion and towelette formulations. After each triplicate analysis per formulation, average values for the results and standard deviation were calculated, resulting in an interval range of 24–29% for the emulsion and 3.5–4.2% for the towel. This differences in interval range may be attributed to the non-woven structure of the towlette, which limits loss of moisture and therefore improves stability. The results are shown in Table III.

As shown by Figures 1 and 2, the results of the dry matter analysis do not show any significant variability between the emulsion and towel formulations. Moreover, this

Table III
Results for Dry Matter Analysis of the Emulsion and the Towlette Formulations

	Dry matter emulsion		Dry matter towelette	
Batch	Mean	± Standard dev.	Mean	± Standard dev.
1	24.90%	0.00577	3.70%	0.06110
2	28.40%	0.02517	4.02%	0.06028
3	26.80%	0.03055	3.79%	0.09504

parameter not only remains constant, but framed within the above predefined interval, ranging between 25.62% and 27.11% for the emulsion and 3.75% and 3.90% for the towelette (see Figures 1 and 2).

DENSITY

The parameter of density determines the consistency of the emulsion and must be maintained in a state of fluidity in order to impregnate the towel through capillary action. The density values for an emulsion must fall within the 0.985–0.995 g/m^3 range, a value that

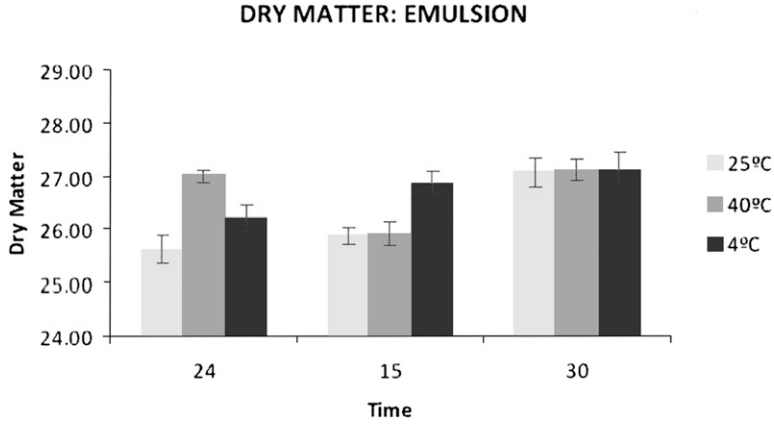

Figure 1. Evolution of dry matter results for the emulsion.

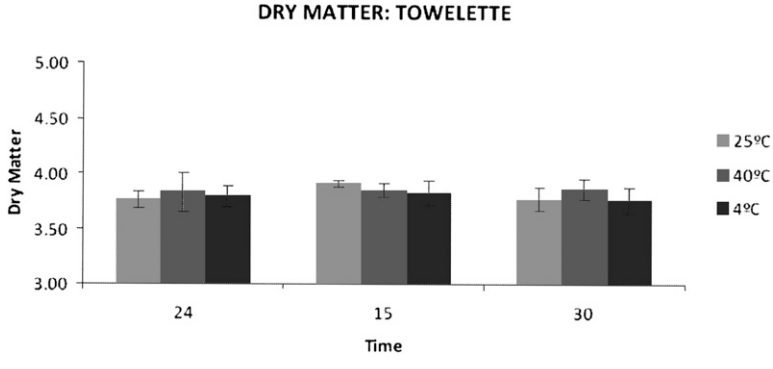

Figure 2. Evolution of dry matter results for the towelette preparation.

is lower than the density of water. This interval was previously established in the laboratory form the production and testing of three consecutive and identical batches of the emulsion. The values obtained were between 0.986 g/m^3 ± 0.00153 and 0.993 g/m^3 ± 0.00306. Figure 3 shows no changes in density over time or temperature variability, remaining constant at values between 0.988 and 0.992 g/m^3.

PH

Analysis of the pH of the emulsion gave values ranging from 7.233 to 7.253, and 7.223 to 7.247 for the towelette preparation. These values suggest that both formulations are suitable not only from a physiological, but also from technological point of view. The Neo Heliopan Hydro used in this study is a crystalline powder in acid-insoluble form, but on addition of neutralizing bases, such as triethanolamine, it becomes water-soluble. Consequently, the pH range must be kept within the range of 7.2 to 7.5 in order to maintain hydrosolubility. The evolution of pH values versus time and temperature did not present significant differences in any sample, and thus both towel and emulsion formulations were deemed suitable for topical application.

MICROBIOLOGICAL CONTROL

Figures 4 and 5 show that both formulations under study met the requirements dictated by the RFE (<100 UCF). In the emulsion samples stored at 40°C from day 15, an increase in the number of CFUs was observed, but this increase was significantly lower in samples obtained from the towelette preparation.

RHEOLOGICAL CHARACTERISTICS

Rheological assays to measure viscosity under different storage conditions and at different times indicated that both formulations showed a pseudoplastic behavior. During the time and conditions applied, the viscosity of the sample was maintained at values between 290,087 and 316,897 cps. Viscosity was determined in cps at a speed of 5 rpm. The results obtained were the average of the three independent determinations.

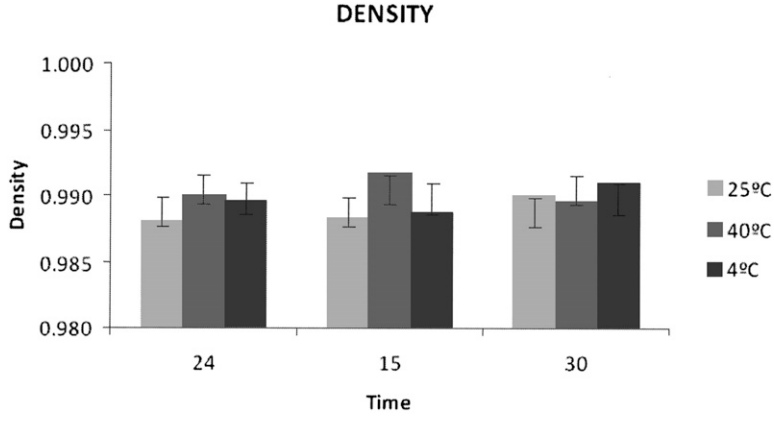

Figure 3. Evolution of density readings for the emulsion.

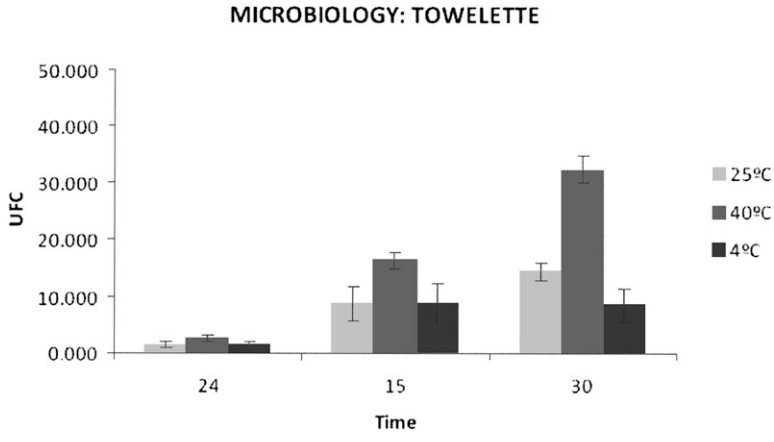

Figure 4. Microbiological results for the towelette preparation.

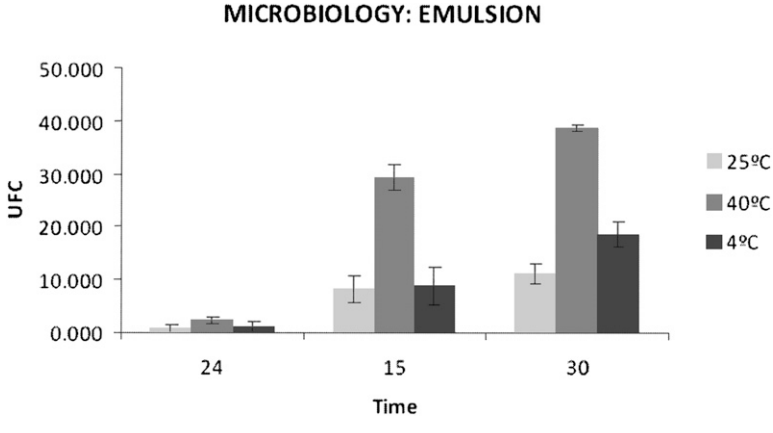

Figure 5. Microbiological results for the emulsion.

A direct relationship between temperature and viscosity has been observed in other studies, in which viscosity decreases with increasing temperature and vice versa (26). In our case, reference values obtained after 24 hours were 290 cps, 298 cps, and 303 cps at 4°C, 25°C and 40°C, respectively (Figure 6).

Slight time-dependent variations in viscosity were observed, which were more pronounced just after preparation, but they gradually stabilized over time. However, changes were more evident in samples observed at 25° and 40°C. The slight variations that were observed at 4°C, could be attributed to an internal rearrangement of the formulation, and for this reason, storage temperatures of between 4° and 8°C are recommended.

STABILITY

The three emulsion samples were subjected to a centrifugal speed of 3500 rpm for ten minutes. No phase separation was detected at any of the predetermined time intervals, showing that the emulsion possessed good physical stability.

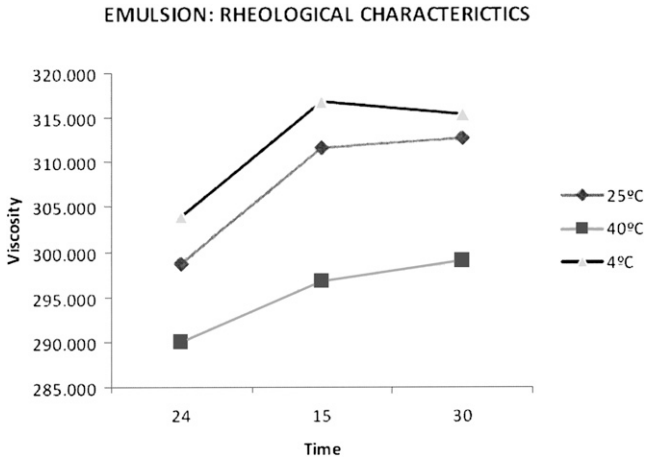

Figure 6. Test results for the rheological properties of the emulsion.

Centrifugal analysis of the influence of temperature (4°C, 25°C) and time (0, 1, 15, and 30 days) on the stability of both the emulsion and towelette preparations showed no influence on the physical, chemical, or microbiological parameters, even in extreme conditions (40°C). However, the results for viscosity showed slight but insignificant decreases at 40°C. At this temperature, microbiological analysis also produced better results for the towelette formulation than for the emulsion.

CONCLUSIONS

These preliminary tests for density, dry matter, viscosity, pH, etc, demonstrate the suitability and uniformity of this sunscreen emulsion, and its adequate state of fluidity in the impregnated towel formulation. It presents a pseudoplastic behavior and a stable pH, while at the same time it is not subject to significant rheological or organoleptic changes, neither in the case of the emulsion nor as an impregnated towel formulation. Both formulations meet legal specifications for microbiological stability and remain unaltered under the conditions studied in this work. The sunscreen emulsion used in the towels selected for this study remained stable over time and under the conditions tested. The advantages presented by this presentation format make it an interesting proposal for future sunscreen products.

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