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Physicochemical Stability and Rheologic Properties of a Natural Hydrating and Exfoliating Formulation Beneficial for the Treatment of Skin Xeroses

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SUMMARY. **There are conditions in dermatology that cause severe cracking and flaking of the skin, representing a failure of normal desquamation. Several hygroscopic substances that affect the moisturization of the *stratum corneum* have been identified for the treatment of scaling disorders. Prominent among these are polyols and alpha hydroxy acids. We elaborated a new pharmaceutical product that combines the biomolecules glycerol and ammonium lactate. The aim of this study was to evaluate the physicochemical stability and rheologic properties of the new skin care preparation.**

RESUMEN. Existen condiciones en dermatología que causan agrietamiento severo y descamación de la piel, lo que representa una falla en la descamación normal. Varias sustancias higroscópicas que afectan a la hidratación de la capa córnea se han identificado para el tratamiento de los trastornos dérmicos. Entre ellos se destacan los polioles y los alfa-hidroxiácidos. Se elaboró un nuevo producto farmacéutico que combina biomoléculas tales como glicerol y lactato de amonio. El objetivo de este estudio fue evaluar la estabilidad físico-química y las propiedades reológicas de la nueva preparación para el cuidado de la piel.

INTRODUCTION

The skin undergoes a natural and continuous shedding process. Several layers are discernible at the microscopic level, the outermost being the *stratum corneum*. This layer is a heterogeneous structure made up of cells (corneocytes) enriched in proteins and embedded in a lipid matrix. Skin cells spontaneously mature, so that the corneous layer -that is normally organized and invisible to the naked eye- suffers desquamation. However, generally in xeroses, and particularly in ichthyoses (from the Greek *ichthys*, meaning fish), the normal desquamation process is altered because too many cells mature at once, or because they shed slowly, so that sticky scales are retained. In both instances, clinical signs include observable desquamation and hyperkeratosis, the latter implying the thickening of *stratum corneum* [1]. Several different types of xeroses are described in the medical literature. In these patients, the barrier function of the skin is impaired, loss of water is enhanced and, due to the ensuing dehydration, skin flexibility is lessened.

In general, there is no cure for these pathologies, but it is possible to alleviate the symptoms by treatments with products that combine two effects: moistening and scale removal, thus promoting the detachment of corneocytes [2]. Skin moisturizing involves the normal uptake of external humidity or the artificial application of various preparations. Moisturizing compounds of common pharmaceutical use include lactic acid, ammonium lactate, urea, and glycolic acid [3]. The alpha-hydroxy acid lactic acid (as ammonium lactate) acts as an effective natural moisturizing compound at low concentration. It helps to preserve the optimal water content of the *stratum corneum*, thus avoiding

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the loss of skin flexibility, contributing to reduce excessive keratinization and enhancing exfoliation [4].

Rawlings *et al.* [5] reviewed the available knowledge on the moisturizing effect on *stratum corneum* at the molecular level. Since the fifties, this phenomenon has been investigated by physical chemists, dermatologists, biologists and enzymologists. There is general agreement that this outermost skin layer fulfills a protective function, helping avoid water loss and maintaining a moistening ability essential for skin plasticity and exfoliation. At the biochemical level, the *stratum corneum* is composed of keratin and other specialized proteins, adhesive structures and enzymes, lipids and natural moisturizing factors. The latter are represented, among other molecules, by a mixture of amino acids and derivatives thereof, and by specific salts [6]. These factors are hygroscopic and highly soluble in water, therefore absorbing water from the atmosphere. In so doing, they help maintain an adequate level of liquid water, even when the skin outer layers become exposed to very low humidity. In particular cases such as psoriasis [7] and ichthyosis [8], a reduction or even a virtual absence of these factors occur, hence influencing the turnover of damaged surface corneocytes.

The influence of humidity on the water content of *stratum corneum* relates to its surface cell turnover. Because of this fact, hygroscopic compounds (glycerol or glycerol plus other polyols) are used as moisturizing and emollient agents for the *stratum corneum* to treat disorders of exfoliation [9,10]. The application of these substances in atopic dermatitis -yet another type of xerosis- has resulted in a significant improvement in the hydration status of *stratum corneum* and its function as barrier, relieving the symptoms of dry skin and intense pruritus, and lessening the decay of the outermost surface layer of the skin [11]. Alpha-hydroxy acids, *e.g.* lactic acid or its salts, at low concentration reduce the thickness of hyperkeratosic *stratum corneum* [12]. Histological and ultrastructural evidence point to the effect of this class of compounds on increasing collagen density [13], helping to maintain an optimal water content level [4] and also acting as antioxidant agents preventing the oxidative damage brought about by the exposure to ultraviolet radiation [14]. By virtue of the qualities brought together by both glycerol and ammonium lactate, these compounds have been successfully included in different proportions into pharmaceutical and cosmetic preparations intended for dry skin care [15,16].

In this work we present a classical semi-solid formulation with moisturizing qualities, this is SGL: a starch glycerol ointment modified by the addition of 12% lactic acid/ammonium lactate (LAL). This ointment disperses easily, it is not oily and blends only biomolecules, natural constituents of our body and starch of plant origin. In addition, the preparation is environmentally acceptable and inexpensive, thus facilitating the continuity of long-term treatments without eliciting adverse effects. In particular, in this study we focused on the physicochemical stability and rheologic behavior of the orphan preparation.

MATERIALS AND METHODS

Instruments

The pH meter was model 720 by Beckman (USA), precise to → 0.001-0.005 pH units. The weighing scale was an OHAUS Precision Plus (USA), precise to 1 mg. The optical microscope was manufactured by UNITRON (Japan), equipped with a 10x objective lens by Ernst Leitz Wetzlar (Germany) and a 10x ocular lens. The rheometer was model AR-G2 by TA Instruments Ltd (United Kingdom), set with temperature controller and gap calibrator, 40 mm diameter cone-plate geometry, 2 degrees cone angle, and gap defined by truncation (55 μm).

Chemicals [17-20]

Corn starch (*Zea mays Linné*, Maizena Duryea®) was purchased to Unilever Bestfood of Argentina (Lot 00001 15014); glycerol (97% w/w purity, 1.25-1.26 g/mL density), was from Sanadrog (Argentina); lactic acid (85% w/w purity, 1.2 g/mL density), was purchased to E. Merck AG (Darmstadt, Germany) or Mallinckrodt Chemical Works (New York, Los Angeles, St Louis, USA); aqueous ammonia (30 % w/w purity, 0.892 g/mL density) was from Carlo Erba (Milano, Italy).

Lactic acid/ammonium lactate solution (LAL) preparation

Chemical formula: NH_4^+ $CH_3CHOHCOO^-$; molecular weight: 107.11. Lactic acid (50 mL, 85% w/w, density 1.2 g/mL, 0.566 moles) was poured into a beaker and partially neutralized with half equivalent of aqueous concentrated ammonia (18 mL, 30% w/w, density 0.892 g/mL, 0.283 moles). Given the exothermic nature

of this process, ammonia was added slowly with continuous stirring to the lactic acid put into a cooled water bath. Due to volume shrinking, the final lactic acid/ammonium lactate solution results 86% w/v.

Starch/glycerol (SG) and starch/glycerol/LAL (SGL) preparations

The SG preparation was modified as follows from the recipe described in Farmacopea Argentina, 6th edition [17]. Starch (20 g) was sifted and the powder was put into a glass beaker to which water (20 mL) was added. The aqueous suspension at RT should adopt a milky appearance. Then, glycerol (128 mL) is slowly poured and mixed well until homogeneous quality. The mixture is heated at moderate temperature (never reaching the limit of 100-105 ºC), with continuous stirring until a translucent and homogeneous jelly is obtained. After cooling down the preparation, distilled water (20 mL) is added and mixed well. For the orphan formulation, the LAL solution (20 mL) is added instead of water.

Fiedler's method for pH measurement (adapted for hydrophilic ointment) [21]

Ointment (5-10 g) is laid into a beaker located in a water bath heated up to 70 ºC. Then, distilled water (30 mL, pH 7) at the same temperature is added with vigorous stirring to maintain homogeneity. The suspension is then filtered through paper and the pH is measured on the clear filtrate.

Determination of extensibility

Several samples were kept at RT and others were stored in the refrigerator (at 4 ºC) for the periods indicated in each case. A glass plate (20 × 20 cm area, 3 mm thick) is located upon a sheet of graph paper. A sample of ointment (500 mg) is then located in a central position. A separate top glass plate (18 × 12.5 cm area, 1.5 mm thick) is weighted and gently placed on top of the sample. The preparation will spread into a circular shape by virtue of the exerted pressured. After 1 min, the measurements of two perpendicular diameters (d) are recorded and averaged. An estimate of the circle surface (extensibility equals $\pi d^2/4$) is then calculated. This procedure is repeated for different successive weights applied (50, 100, 200, and 500 g) on top and at the center of the upper glass plate. Extensibility (in mm^2) is then plotted against the applied weight (in g).

Determination of rheologic behavior

Rheograms were obtained employing a state-of-the-art low-torque performance rheometer (model AR-G2, TA Instruments Ltd, UK), using a 40 mm diameter 2° cone and plate measuring system. Shear rate spanned the range 0.1-200 s^{-1}. The cone-plate separation (gap) was 55 μm. Temperature was kept fixed at 23 or 37 °C with the Peltier device connected to a recirculating water bath (JULABO, model ACW 100, JULABO GmbH, Seelbach, Germany).

Model fitting to the recorded data obeyed the Herschel-Bulkley formalism. This treatment associates a threshold value (yield stress τ_o) to the power law given by the constitutive equation $\tau = \tau_o + K\gamma^n$. In this expression, τ stands for the shear stress, τ_o represents the initial stress necessary to initiate the flow, γ is the shear rate, and K and n are the consistency and flow indexes, respectively. The latter parameters were derived from the power law after linear regression analysis of the data plotted as log τ versus log γ.

RESULTS AND DISCUSSION

To certify the best quality of the preparation, the SGL ointment was made according to good manufacturing practices (GMP) for medicinal products. These ensure the traceability of starting materials, the design of the formulation, the satisfactory manufacture according to pharmaceutical technology, the proper quality control of products, and the cleanliness and safety of the equipments and workplace.

Physicochemical characteristics

Observation of organoleptic properties, appearance and adhesiveness

Based on laboratory expert criteria, these were carried out daily for a period of 21 days. These tests allowed us to detect qualitative signs of chemical instability or any alteration that could compromise the system behavior. SGL demonstrated homogeneous and translucent quality and a slightly yellowish color. It was consistently odorless and did not develop unpleasant smell over time. All along the full time span of the analysis, its texture proved to be smooth, soft, hygroscopic and uniform, showing no sign of

presence of any solid material. SGL demonstrated a softer and smoother character than SG and a more adhesive quality. No modification was observed along the storage period assayed, either for samples kept at RT or for those maintained at 4 °C (**Table 1**). The remaining properties of SGL were comparable to those shown by the SG preparation treated under identical conditions.

Storage	Microscopy	Physical aspect and color	Odor	pH
48 h	homogeneous	fluid, translucent, shiny	odorless	3.99
21 days	no alterations	unchanged	odorless	4.00
54 days	no alterations	translucent, slightly opaque, showing some signs of dehydration	odorless	4.00

Table 1. Organoleptic properties and pH of the SGL ointment as a function of time after preparation. Data shown correspond to different time periods of conservation at RT.

Solubility

SG and SGL are fully miscible in water in different proportions. In both cases, water addition under stirring yields a whitish opaque colloidal dispersion.

Identification of starch

To a sample of each ointment (0.5 g) dispersed in water (10 mL), iodized alcohol solution (0.05 mL) was added. Instantaneous development of a dark blue color is observed, as the result of the formation of the reversible adduct of molecular iodine inside the helical structure of the amylose component of starch. This test was repeated at each time assayed yielding always the same result.

Weight loss

No significant change was observed in the weight of samples of each preparation stored for 15 and 21 days in closed containers (plastic jars).

Homogeneity

To form a thin film, samples of each ointment (200 mg) were spread between two microscope slides. Then, visual inspection with the naked eye and under the microscope was carried out. In no case (i) clusters or clumps, or (ii) bubbles of trapped gas within the matrix were observed, but fine and uniformly dispersed starch granules. In addition, along the time periods assayed neither phase separation nor ooze exuding from samples stored in closed plastic jars was ever observed.

Determination of extensibility

This property was examined for SGL immediately after preparation and after a period of storage (48 h) at different temperatures (RT or 4 ºC). SG represented the control sample used as reference. Fig. 1 illustrates the extensibility behavior as a function of applied weight. Clearly, the preparation including ammonium lactate demonstrated higher extensibility. On the other hand, no differences were revealed between the two ointments due to the temperature of storage. The same assay was carried out after 21 days (**Fig. 2**), indicating that SGL maintained its original behavior, regardless of storage temperature. The control SG sample also kept its original extensibility. This study also included a sample of SGL kept at RT for almost two months (filled triangles in **Fig. 2**). Here, extensibility experienced some decrease, but this reached a magnitude close to that observed for the control SG sample aged for 21 days.

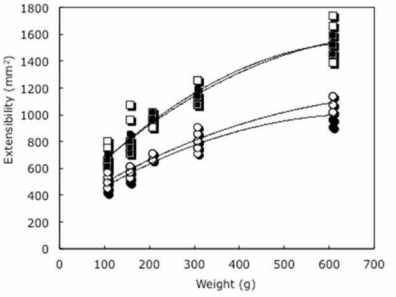

Figure 1. Starting extensibility of the ointments as a function of added weight. All assays were run at RT. The SGL (squares) or SG preparations (circles) were tested fresh (open symbols) or after 48 h of storage at 4 ºC (filled symbols).

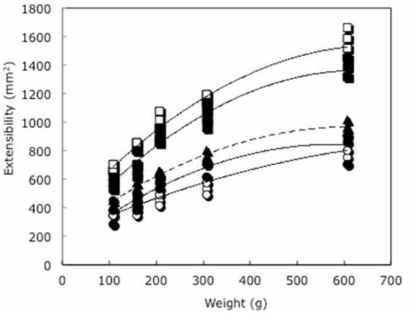

Figure 2. Extensibility of the ointments as a function of

added weight and time after preparation. All assays were run at RT. The SGL (squares) or SG preparations (circles) were tested fresh (open symbols) or after storage for 21 days at 4 ºC (filled symbols). The SGL preparation was also assayed after storage for 54 days at RT (filled triangles, dashed line).

To facilitate the comparison of SGL along time, **Fig. 3** shows the extensibility behavior of samples of this ointment kept at RT: the first was recently prepared and a second one was stored for 21 days before being assayed. This treatment corresponds to the usual mode and time of storage in patient care. As a result, it was proven that SGL is able to maintain the original physicochemical features.

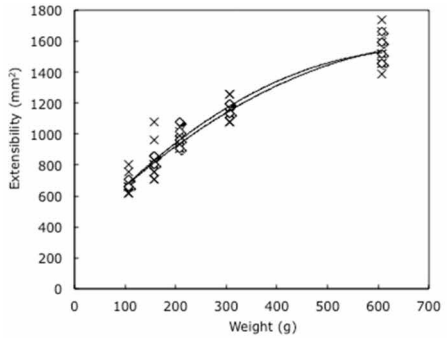

Figure 3. Extensibility of the SGL ointment as a function of time after preparation. Data shown correspond to 48 h (crosses) or 21 days (open diamonds) of storage at RT.

Assessment of rheologic behavior

Rheology studies the flow and deformation of matter in liquids and soft solids. Rheologic behavior responsible for the adequate flow of material is of paramount importance for products intended for topical application. From the perspective of pharmaceutical technology, rheologic behavior is fundamental for the effectiveness of the therapeutic action and cosmetic function.

Two parameters are evaluated in rheologic assays: (i) *shear stress* (τ), defined as the coplanar force needed to move a defined area (force per unit area, in Newton m $^{-2}$, or Pascal), and (ii) shear rate (γ), defined as the gradient of velocity of a fluid placed between stationary and moving plates (measured as the difference in velocity relative to the distance of separation between plates, in s^{-1}). Viscosity (η) is in turn defined as the ratio between the two above parameters ($\eta=\tau/\gamma$, in Pascal s). For Newtonian fluids, viscosity is constant at a given temperature. However, for non-Newtonian fluids, the ratio depends on the range of the gradient of velocity assayed. In these cases, mathematical models were developed that describe the relationship between shear stress and shear rate. This property is to be characterized in this fashion for a preparation intended to fulfill a specific therapeutic function.

Definitely, the SGL and SG ointments exhibit clear non-Newtonian behavior. The rheologic assays were carried out both at RT and at physiological temperature (37 °C), because it is imperative to evaluate the consistency of the preparation at the particular place where it is applied in the body. It is also fitting to know the advantages of the new preparation (SGL) by comparison with that used for a long time in pharmaceutical practice (SG). **Fig. 4** displays the rheologic behavior of samples of SGL kept at RT for 21 and 54 days, together with a third sample of SGL stored in the refrigerator (4 °C) for 21 days and a control sample of SG kept at RT for 21 days. Results from assays run at 23 or 37 °C are presented in **Figs. 4A** and **4B**, respectively.

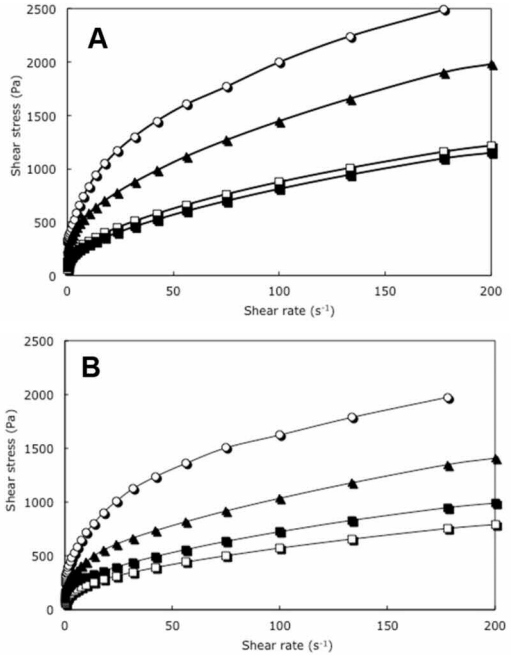

Figure 4. Rheograms of SGL and SG ointments. SGL ointment kept for 21 (open squares) or 54 days (filled triangles) at RT, or stored for 21 days at 4 ºC (filled squares). For comparison, data is shown corresponding to the SG ointment kept for 21 days at RT (open circles). Panels A and B correspond to assays run at 23 or 37 °C, respectively.

From an overall comparison, one can conclude that all preparations proved to be more effective for

their application at 37 °C. However, the best behavior corresponds to SGL. In a topical ointment, the consistency parameter (K) should be optimized to facilitate its application. **Table 2** summarizes the characteristic values derived from each curve corresponding to the SGL ointment and the control SG sample, kept at RT or 4 °C. SGL is more effective as regards its extensibility, as demonstrated by the lower values of K observed by comparison with those measured for SG. In addition, this condition is also maintained if the ointment is kept in the refrigerator. The aged preparation of SGL acquires higher consistency, but even so, it results more effective than SG. It was shown that both ointments display non-Newtonian behavior with pseudo-plastic features, *i.e.* the consistency parameter decreases upon an increase in distortion. In addition, the performance can also be described as tixotropic (from the ancient Greek *thixis*: touch and *tropos*: mutable), *i.e.* consistency decreases with time for a given shear rate applied (results not shown). The combined effect of these two qualities promotes the spreading of the preparation on the skin.

Ointment	Temperature (°C)	K (Pa s^n)	n (adimensional)	τo (Pa)
SGL 21d RT	23	78.6	0.50	73.2
SGL 54d RT	23	118.9	0.51	190.7
SGL 21d 4	23	52.2	0.57	98.9
SG 21d RT	23	269.7	0.41	148.9
SGL 21d RT	37	57.0	0.48	54.6
SGL 54d RT	37	119.6	0.45	107.4
SGL 21d 4	37	57.8	0.51	113.3
SG 21d RT	37	274.5	0.37	99.8

Table 2. Parameters derived from the rheologic assay of the ointments. After fitting the model to data in **Fig. 4**, rheologic parameters are shown corresponding to samples of the SGL or SG ointments assayed at RT (23 °C) or at physiological temperature (37 °C). Storage conditions comprise 21 or 54 days (d) at RT or at 4 ºC 4.

Measurement of density

After measuring a given volume of ointment in a graduated cylinder and weighing the content in a precision balance, density of the preparations was determined. SGL yielded a value of 1.297 g/mL.

Measurement of pH

Application of Fiedler's method with modifications (see Methods) on samples of SGL after 48 h or 21 days after preparation resulted in pH values of 3.99 and 4.00, respectively, fully coherent with the elevated pH buffering capacity conferred on the preparation by the high concentration of LAL. The consistency of this result ensures the minimization of chemical decomposition -generally hydrolytic- that could compromise the stability of the preparation. Particularly in the case in question, acidity of the ointment contributes positively to its preservation. The great majority of microorganisms display optimal growth at pHs close to neutrality, whereas only a few germs develop below pH 4.0. In this latter condition, bacteria result more sensitive than fungi or yeast toward an acid milieu. Finally, after 54 days of preparation, a pH determination on a sample of SGL also failed to reveal any significant change of this parameter (**Table 1**).

Advantageous function of the composition and formulation of SGL

The SGL preparation exclusively contains natural non-toxic components: corn starch, glycerol (a product of carbohydrate fermentation or fat hydrolysis) and lactic acid (a final product of carbohydrate fermentation). Starch soothes and protects the skin, retains water by absorbing ambient moisture (hygroscopic function), and exerts simultaneously a moisturizing effect, because it opposes the changes in humidity [22]. This polymer imparts adequate texture and thickness to the ointment, so that it can be extended effortlessly over the affected surfaces, and is eliminated straightforwardly.

According to Choi *et al.* [23], endogenous glycerol influences the hydration of *stratum corneum* by two mechanisms: filtration from blood and input by the oil glands. Glycerol in the formulation of SGL is not absorbed, but it prevents skin dehydration due to a

chemical structure that allows this compound to retain water, *i.e.* due to its hydrotropic nature, it retards water evaporation.

As an emollient agent, ammonium lactate -the ammonium salt of an alpha-hydroxyacid- proves useful to combat xerosis and desquamation, given that it enhances moisturization and exfoliation of *stratum corneum*. In addition, due consideration should be given to the pH dependence of enzymes involved in the process of desquamation. In this scenario, an abnormal pH gradient might play a pathogenic role. Typically, pH is lower on the skin surface, but under certain conditions -such as ichthyosis vulgaris- pH is higher than normal [24]. In this fashion, the acidity of the SGL ointment stimulates peeling off of the skin. Concurrently, the SGL formulation improves the barrier function of the skin. In fact, the SGL components -used in different proportions- are considered the most effective for dry skin care [15].

SGL does not contain any preservative, fragrance or colorant, compounds that by their chemical structure might potentially give rise to hypersensitivity reactions, irritation or burning. In addition, at variance with several lotions SGL lacks volatile components that by contact with skin could cause desiccation. The very formulation of SGL at pH 4 renders it acidic enough so as to inhibit the development of many microorganisms without affecting the physicochemical stability of its components [22] or the integrity of the skin.

The hydrophilic ointment SGL is optimal for topical application and possesses good adhesiveness, this latter feature allowing it to stay on the surface of epidermis for a reasonable time before being eliminated by washing. Its physical properties are based upon its rheologic behavior of the pseudo-plastic type. These semi-solid ointments maintain their shape and adhere as a film whenever an external force is applied over them. In this way, they easily deform and flow, maximizing their capacity to spread. As a result, they can be distributed over ample body surfaces, showing the ability to absorb and maintain water for useful periods of time. SGL results little occlusive and it does not stain the skin or clothes in direct contact with it. Last but not least, another advantage of this preparation is its low cost, by comparison with emulsions, creams or lotions regularly expended to treat ichthyoses or other related conditions. Commercial dermatological products are usually much more expensive, a fact that prevents its use by patients who cannot afford them. In these cases, the requirement for frequent use is a main reason behind giving up the treatment.

CONCLUSIONS

Physicochemical assays allowed us to characterize the starch-glycerol-lactate (SGL) ointment as a stable preparation along the usual period of pharmaceutical use. This maintains its homogeneity and does not form exudates, keeping its organoleptic qualities and acidic pH, a main factor undoubtedly contributing to its preservation. SGL holds a good adherent power, creating a thin film upon topic application, thus enabling its easy spreading over large surfaces. SGL is endowed with a high capacity to absorb moisture, maintaining hydration over a long useful period. This ointment is not greasy, thus facilitating its ready elimination by washing with water, and its preparation is straightforward and inexpensive.

On the other hand, preliminary results indicate that SGL is well tolerated by patients and can be administered as many times as necessary. Because its constituents are exclusively non-toxic biomolecules, SGL is not expected to cause any adverse side-reaction, pointing to its safety for use with children, adults and elderly persons. A thorough field trial including a properly selected cohort of patients followed by a rigorous statistical study will be the matter of future efforts.

Because of all the reasons indicated above we strongly recommend the 12% ammonium lactate hydrophilic ointment SGL for the treatment of dermatological disorders characterized by abnormal skin hydration, such as atopic dermatitis, eczema, psoriasis, senile xerosis and ichthyosis.

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