Formulation and Evaluation of a Nanogel System for the Skin Delivery of Hyaluronidase

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INTRODUCTION

Microemulsions and nanoemulsions are **new topical delivery systems** for the treatment of minor skin diseases. The delivery of active ingredients in these vehicles leads to obtain "**cosmeceutical**" products [1-2] characterized by a biological activity focused on a peculiar dermatological aspect. Nanoemulsions can be considered "cosmeceutical" carrier because they promote active ingredient permeation without affecting the structure of the skin [3-4-5]. In cosmetic applications, nanoemulsions are used in fluid and semisolid forms like spray and gel. Nanogels are [6] homogeneous, isotropic and transparent systems; they are thermodynamically stable dispersions, consisting of two immiscible liquids stabilized by a surfactant that reduces the interfacial tension. They [7-8] play an important role as vehicles because of the **small droplet size**, the **long-term stability** and the enhanced penetration. Because of the **powerful permeation skill**, they also improve the beneficial efficacy of active ingredients, allowing the use of a minor part of vehicle and avoiding high skin irritation.

Hyaluronidase is a glycoprotein widely employed in medicine thanks to the well known properties as "tissue diffusion enhancer". It has been demonstrated that hyaluronidase can permeate the epidermal culture system or human skin to a significant degree, depleting HA and decreasing CD44 expression [9].

The ability of hyaluronidase to permeate the stratum corneum suggests that topical application may, additionally, be useful as a clinical modality.

OBJECTIVE

The development of a new topical semisolid nano-dispersion based on **a vegetable-derived O/W nano-emulsifiers** called **Nanocream**® (*Potassium Lauroyl Oat Amino Acids, Palm Glycerides, Capryloyl Glicin)*, formed by a blend of vegetable lipoproteins and glycerides of fatty acids and a fat-aminoacid, easy to prepare, at low energy levels, and without the addition of any other surfactants.

The *in vivo* evaluation of a nano-sized emulsion gel containing **hyaluronidase** as topical treatment for the most common side effects occurring after sclerotherapy.

EXPERIMENTAL METHODS

Preparation of nanogels The oily phase was prepared by mixing Nanocream® in the oils (dicaprylyl ether, octyl isonanoate) with a blade impeller (150-250 rpm) for 10-12 minutes and started heating until all components formed a clear phase. The water, pre-heated at the same temperature, were added very slowly into the oily phase until a viscous lipogel was obtained. The formation of bluish coloration (Tyndall colour) typical of the nanosize droplets must be obtained. Nanogels were prepared with different ratios between aqueous phase and oily phase, keeping fixed the ratio between oils and emulsifiers (dicaprylyl ether:octyl isonanoate:Nanocream® = 8:2:10).

In vivo evaluation

20 patients affected by leg telangectasia were submitted to multiple sclerotherapy sessions with Purified Sodium Tetradecyl Sulphate 0,2% w/w (FibroveinTM). The patients were enrolled in this clinical protocol according to the following requirements:

Patients were not treated with sclerothepy in the previous 12 months, were not in currency with any pharmacological therapy except sporadic FANS assumption, were not affected by any dismetabolic, auto-immune or thrombo-embolic diseases, were excluded any previous allergic or sensitizing reaction to drugs, cosmetics and different substances.

Clinical protocol: Patients were treated with 3 sclerotherapy sessions over 4 weeks to treat small reticular veins (average 6-8 veins for leg) and telangectasia in both legs. After sessions, 6 patients showed a severe ferric hyperpigmentation, 9 showed skin irritation and erythema, 3 showed ecchymosis in association with ferric pigments deposition occurred.

Nanogel treatment: all the patients were treated 3 times a day for two weeks with nano-lipogel containing 350 I.U.(1% w/w) of pure Hyaluronidase (Sigma-Aldrich).

RESULTS AND DISCUSSION

FORMULATION

Nanogels were obtained by adding slightly different amounts of water to the oily phase: the viscosity trends, in function of stress, of the nanosystems prepared with increasing water percentage **28.6**, **33.3**, **36.5**, **37.5**, **39.4**, **41.9**, and **44.4** are shown in **Figure 1**. All the products show ed a pseudoplastic behavior (the viscosity decreases with the applied stress) but the viscosity dramatically decreased for water content upper to 40% w/w .

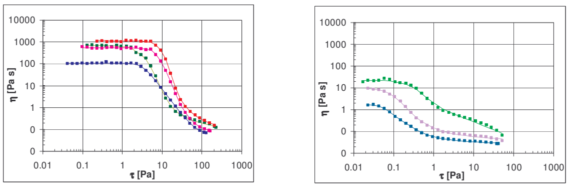

Fig.1 *Nanogel viscosity trends in function of stress (% water **28.6**, **33.3**, **36.5**, **37.5**, **39.4**, **41.9**, and **44.4**)*

The rheological properties of nanogel systems were registered in function of the water concentration: by increasing the aqueous phase, the structural features of the materials develop getting to a maximum, around 35.5%, after which the progressive dilution of the nanogel ends to the formation of the fluid nanoemulsion.

The rheological changes, typical of a gel-sol transition [10], were demonstrated by measuring the structural properties of the material, at different shear conditions, and calculating the correspondent parameter trends in function of the water percentage (zero shear viscosity, critical strain, elastic modulus) (**Figure 2**)

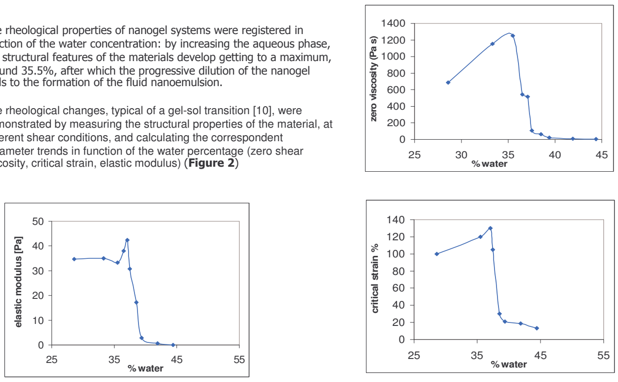

Fig.2 *Nanogel structural parameters vs water concentration.*

The nanogel system prepared with 35.5% water and added with hayluronidase was a stable formulation with a jelly consistency and a transparent appearance.

Measurements performed in oscillatory flow conditions put out the presence of a mainly elastic structure, with elastic modulus G' less dependent on frequency and with absolute values typical of weak gel materials (**Figure 3**).

Figure 4 shows a temperature sweep test in which elastic and viscous moduli were measured at fixed stress and frequency (0.1Hz), within the linear viscoelastic region of the material, in function of temperature gradient (range 25-50°C, 0.5°C/min).

The microstructure of the nanogel system prepared less dependent on temperature, according to the existence of a coherent stuctural organization with a good stability in time [11].

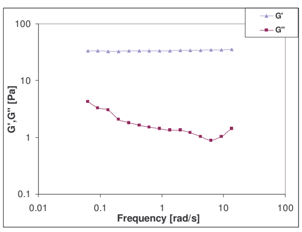

Fig.3 *Nanogel elastic G' and viscous G" moduli trends in function of frequency*

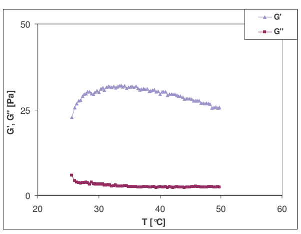

Fig.4 *Nanogel G', G" trends in function of temperature*

RHEOLOGICAL CHARACTERIZATION

Rheological measurements were performed at 25°C±2 with a stress controlled rheometer Haake RS100 equipped with a plate-plate sensor PP35, gap 0.5 mm. The rheological characterization of the systems was performed both in continuous and oscillatory flow conditions:

CONTINUOUS FLOW TESTS

CONTROLLED STRESS - function of stress, 1 cycle up-down in the appropriate stress range in 12 minutes

Controlled stress up curve data were fitted using the Carreau-Yasuda equation. The structural parameters viscosity at rest (η_0) and critical stress (τ_{crit}) were calculated.

$$\eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{\left(1 + (\dot{\gamma}\lambda)^2\right)^n}$$

OSCILLATORY FLOW TESTS

Oscillatory flow tests were performed in order to quantify the viscoelastic properties of each sample: G' elastic modulus, G" viscous modulus and δ the phase angle.

Stress sweep curve data were fitted using a **damping equation**.The complex viscosity ($\eta*$) and the critical strain $\gamma_{\%}$ were calculated

$$\gamma\%_{crit} = \left(\frac{0.05}{0.95*(b-a)}\right)^{\frac{1}{n}}$$

IN VIVO EVALUATION

Nanogel applications 4 of the 6 patients treated for hyperpigmentations showed a significative improvement in terms of skin darkness appearance and color fading, direct expression of the deep penetration of Hyaluronidase through the horny layer. In the other 2 patients there were no significant improvement in terms of hyperpigmentation reabsorption

(**Figure 5**).

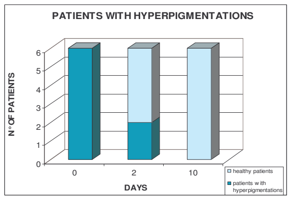

Fig.5 *Evaluation of nanogel application in patients with hyperpigmentations*

6 patients treated for skin irritation and erythema showed, under dermatological evaluation after only 2 days, a dramatic improvement of the skin irritation and bruise signs and same patients referred a sensible amelioration of the symptomatology. In the other 3 patients only a poor improvement in the signs and symptoms were achieved and in two cases corticosteroid emulsion was applied to reduce inflammatory signs and pain

(**Figure 6**).

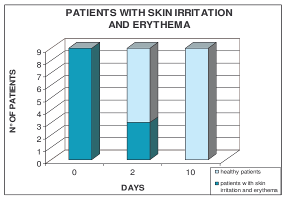

Fig.6 *Evaluation of nanogel application in patients with skin irritation and erythema*

All the patients in which ecchymosis occurred showed a significant improvement of the blood deposit appearance after only 4 applications (**Figure 7**).

A **complete resolution** was achieved in all the patients **after 10 days of application**. All the patients referred an high grade satisfaction in terms of application compliance and skin discomfort reduction.

No side effects were noted in course and after nanogel application.

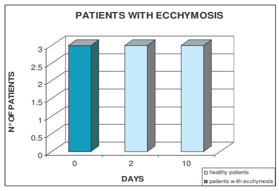

Fig.7 *Evaluation of nanogel application in patients with ecchymosis*

CONCLUSIONS

The concentrated system, **nano-lipogel**, is a well structured jelly material with viscoelastic properties consistent with a good spreadability, film forming properties and retention over the skin and the epithelial surfaces.

The nanoemulsion based gel containing hyaluronidase showed a very high grade effectiveness to improve skin conditions side effects after sclerotherapy sessions, aiming to the complete resolution of the most common and unpleasant side effects such as hyperpigmentations, bruising and skin irritation, ecchymosis in the most part of the patients treated. These evidences support the hypotesis that this innovative technical form is able to enhance absorption of hyaluronidase through the intact horny layer and induce a deep and quick diffusion of the molecule into the viable skin tissues.

The compliance of this novel topic nanogel was excellent in all the patients treated as far as its safety.

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