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Research paper

Improved photostability and reduced skin permeation of tretinoin: Development of a semisolid nanomedicine

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ABSTRACT

The aims of this work were to increase the photostability and to reduce the skin permeation of tretinoin through nanoencapsulation. Tretinoin is widely used in the topical treatment of various dermatological diseases such as acne, psoriasis, skin cancer, and photoaging. Tretinoin-loaded lipid-core polymeric nanocapsules were prepared by interfacial deposition of a preformed polymer. Carbopol hydrogels containing nanoencapsulated tretinoin presented a pH value of 6.08 ± 0.14, a drug content of 0.52 ± 0.01 mg g^{-1}, pseudoplastic rheological behavior, and higher spreadability than a marketed formulation. Hydrogels containing nanoencapsulated tretinoin demonstrated a lower photodegradation (24.17 ± 3.49%) than the formulation containing the non-encapsulated drug (68.64 ± 2.92%) after 8 h of ultraviolet A irradiation. The half-life of the former was seven times higher than the latter. There was a decrease in the skin permeability coefficient of the drug by nanoencapsulation, independently of the dosage form. The liquid suspension and the semisolid form provided K_p = 0.31 ± 0.15 and K_p = 0.33 ± 0.01 cm s^{-1}, respectively ($p \leqslant 0.05$), while the samples containing non-encapsulated tretinoin showed K_p = 1.80 ± 0.27 and K_p = 0.73 ± 0.12 cm s^{-1} for tretinoin solution and hydrogel, respectively. Lag time was increased two times by nanoencapsulation, meaning that the drug is retained for a longer time on the skin surface.

1. Introduction

Tretinoin (TTN), a natural retinoid, is the active form of a metabolic product of vitamin A. It is widely used in the topical treatment of various dermatological diseases such as acne, psoriasis, skin cancer and photoaging, regulating growth and differentiation of epithelial cell, sebum production, and collagen synthesis [1–3]. In the treatment of acne, TTN acts by increasing the turnover of follicular epithelial cells and by accelerating the shedding of corneocytes normalizing keratinization and facilitating comedolisys [4,5]. These effects are mediated by its interaction with a family of nuclear retinoic acid receptors [6]. Moreover, TTN may decrease the inflammatory component of acne through a decrease in the expression of toll-like receptor 2 and cytokine induction by *Propionibacterium acnes* [7].

Acne vulgaris is a multifactorial disease of the pilosebaceous follicles [8] affecting people of all ages, ranging from neonatal and child acne through adult acne vulgaris [9]. Current research indicates that the pathogenesis of acne involves four main processes: follicular hyperproliferation, excess sebum production, inflammation, and proliferation of *P. acnes* [10]. Active acne and its sequel, especially permanent scarring, may cause long-standing psychological or emotional harm in patients [11]. Thus, the effective treatment of acne is important to reduce the severity and the potential for recurrence of the disease, especially in adolescents and young people [8,12].

In the last 25 years, numerous topical and systemic drugs have been developed for the treatment of acne [13], and the selection of the appropriate therapeutic regimen depends on the classification type of acne and its severity grade [3]. Topical therapy (antibiotics, retinoids, benzoyl peroxide, keratolitycs, and alpha-hydroxy acids)

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is unavoidable in acne treatment and is mainly indicated in the mild to moderate acne. Systemic treatment (oral antibiotics and oral retinoids) is required in patients with moderate to severe acne, especially when acne scars start to occur. In more severe forms, a combined topical and systemic therapy is recommended [12,14,15].

Among all the topical treatments available, the most frequently prescribed products are antibiotics and retinoids [12]. Topical retinoids have been used in acne therapy since 1962, and the first substance to be studied was TTN [13]. Although TTN has considerable effectiveness in treatment after topical application, its use in dermatological formulations presents some problems such as skin irritation and high instability in the presence of air, light, and heat. Regarding its poor photostability, after 10 min of irradiation, in a tretinoin lotion applied topically, only 30% of the initial TTN concentration remains intact [16,17]. Moreover, its low solubility may limit its incorporation in a suitable aqueous vehicle. Products currently available have been prepared using co-solvents such as ethanol and propylene glycol.

Novel drug delivery strategies, as nanoparticles, have been proposed in optimizing the efficacy of many therapeutic agents by either modulating their physicochemical and biopharmaceutical properties or minimizing/eliminating the side effects associated with them, thus offering better patient compliance [15]. Polymeric nanoparticles are colloidal suspensions presenting diameters lower than 1 μm that can be classified as nanocapsules (NC) and nanospheres (NS), which can act as nanocarriers presenting vesicular and matrix-like structures, respectively [18]. These nanoparticles have a huge specific surface, which makes them suitable for interesting pharmaceutical and cosmetic applications, such as topical formulations of lipophilic encapsulated drugs for a homogeneous release [19].

Skin care formulations are often based on emulsions and gels because of their technological feasibility of controlling their viscosity, which provides appropriated characteristics for cutaneous application by the patient [20]. Generally, in case of the application of such formulations onto facial skin, it is difficult to predict and evaluate their effects for a significant period of time, because they are easily removed by wetting, movement, and contacting [21]. On the other hand, nanoparticles are suitable to accumulate in hair follicles, reaching deeper functional structures, where they can be stored for some days and therefore provide a reservoir effect [22].

Some works have been reported in the scientific literature on the association of TTN to nanocarriers like solid lipid nanoparticles, liposomes, and niosomes in order to prevent their photodegradation and improve its bioavailability and efficacy [17,23–28]. Our group recently reported its association to polymeric nanocapsules [29] and lipid-core nanocapsules [30]. In those works, we demonstrated a better photostability of TTN-loaded nanocapsules compared to the free methanolic solution. A positive influence of the polymer around the oil droplets was demonstrated, comparing the results obtained from nanocapsules and nanoemulsions [30]. A better physicochemical stability under storage was demonstrated by the nanoencapsulation of tretinoin in lipid-core nanocapsules compared to conventional polymeric nanocapsules [30]. In addition, Fachinetto and co-workers [31] showed an increase in the antiproliferative properties of nanoencapsulated TTN, carrying out a preliminary study using the *Allium cepa* root tip cell model. However, to date, there is no study on the development of dermatological semisolid formulations containing TTN-loaded lipid-core nanocapsules. The development of such semisolid dosage forms should be an interesting strategy to overcome some TTN limitations in terms of topical use as its poor aqueous solubility and poor photostability. Furthermore, the encapsulation of TTN can provide a slow release of drug, allowing a higher permanence of drug in the skin layers and a lower permeability to systemic circulation, which should increase its therapeutic activity and reduce its important side effects. Mandawadge and Patravale [28] showed a lesser skin irritancy, greater occlusivity, and slow drug release from a Carbopol hydrogel containing tretinoin-loaded solid lipid nanoparticles in comparison with a commercial product (cream). However, to the best of our knowledge, there is no report available in the scientific literature showing the increase in the photostability of TTN in a semisolid nanomedicine.

In this work, we report the development of a hydrogel containing TTN-loaded lipid-core polymeric nanocapsules, showing its adequate properties as a dermatological nanomedicine such as pH, spreadability, drug content, and rheological characteristics. Our goals were to demonstrate the potential increasing of TTN photostability in hydrogels, as well as reducing its skin permeation by means of nanoencapsulation. Skin permeation studies across human heat separated epidermis were carried out to evaluate differences in the permeation of tretinoin using our approach. Carbopol® Ultrez 10 NF, a highly water dispersible polymer, was chosen for the preparation of the hydrogels. Its ability to provide high viscosity at low concentration and compatibility with many active ingredients and excipients makes it a good candidate for the purpose of this work [32,33].

2. Materials and methods

2.1. Materials

Tretinoin (TTN) was a gift from Roche (Switzerland). Poly(ε-caprolactone) (PCL) and sorbitan monostearate (Span 60®) were purchased from Sigma–Aldrich (São Paulo, Brazil); caprylic/capric triglyceride mixture was delivered from Brasquim (Porto Alegre, Brazil); polysorbate 80 (Tween 80®) was supplied by Henrifarma (São Paulo, Brazil) and acetone from Vetec (Rio de Janeiro, Brazil). Carbopol® Ultrez 10 NF and triethanolamine were obtained from DEG (São Paulo, Brazil), and imidazolidinyl urea (Germall 115®) was acquired from Alpha Química (São Paulo, Brazil). HPLC grade methanol was acquired from Tedia (São Paulo, Brazil). All chemicals and solvents presented pharmaceutical or HPLC grades.

2.2. Preparation and characterization of lipid-core nanocapsules

Lipid-core nanocapsule suspensions were prepared ($n = 3$) by the interfacial deposition of the preformed polymer method [34]; 250 mg of polymer (PCL), 191.5 mg of sorbitan monostearate, 0.82 mL caprylic/capric triglyceride mixture, and 12.5 mg of TTN were dissolved in 67 mL of acetone. This organic solution was added into 134 mL of an aqueous phase containing 191.5 mg of polysorbate 80 under moderate magnetic stirring. Magnetic stirring was maintained for 10 min. Then, acetone was removed, and the aqueous phase concentrated by evaporation at 40 °C under reduced pressure obtaining a final volume of 25 mL [30]. This formulation containing TTN (0.5 mg mL^{-1}) was named TTN-LCNC. Blank formulation (B-LCNC) was prepared following the same procedure, but not incorporating the drug. Formulations were prepared in triplicate and stored under protection from light and at room temperature.

Before the preparation of hydrogels, particle sizes and polydispersity ($n = 3$) were estimated by photon correlation spectroscopy (PCS) after adequate dilution of an aliquot of the formulation in purified water (Zetasizer Nanoseries, Malvern Instruments, Worcestershire, UK). Zeta potentials were measured using the same instrument at 25 °C, after dilution of the samples in 10 mmol L^{-1} NaCl aqueous solution. The drug was assayed by LC after extraction of TTN in methanol (30 min), centrifugation for

5 min, and filtration through a regenerated cellulose membrane (Sartorius Biolab Products 0.45 μm, Goettingen, Germany). LC system consisting of a Shimadzu instrument (LC-10AVP Pump, UV–VIS SPD-10AVP Module, Class-VP Software, Shimadzu, Tokyo, Japan), equipped with a Gemini RP-18 column (150 mm × 4.60 mm, 5 μm, Phenomenex, Torrance, USA). The mobile phase was composed of 85% methanol, 15% Milli-Q water, and 1% glacial acetic acid and pumped at a flow rate of 1 mL min^{-1}. The injected volume was 20 μL with UV detection at 342 nm (room temperature).

2.3. Preparation and characterization of hydrogels containing tretinoin-loaded nanocapsules

Carbopol® Ultrez 10 NF (polymer of acrylic acid) at 0.5% (w/w) was dispersed in the TTN-LCNC resulting in a concentration of 0.05% (w/w) of the drug. The polymeric dispersion was neutralized with triethanolamine (0.2%, w/w) to a suitable pH (~6.0) to obtain a proper consistency of the hydrogel (HG-TTN-LCNC) and to be applied onto the skin. Imidazolidinyl urea was added as a preservative (0.6%, w/w). Based on these same criteria, placebo hydrogels using blank nanocapsules (HG-B-LCNC) as well as a hydrogel base using distilled water instead of the nanocapsule suspension (HG-B) were prepared. All formulations were prepared in triplicate and stored protected from light and at room temperature (25 ± 4 °C).

The physicochemical characterization of hydrogels was carried out by means of drug content (by a validated LC method), pH, viscosity, and spreadability. pH was measured using a calibrated pH meter (MPA-210 Model, MS-Tecnopon, São Paulo, Brazil) after dilution of the samples in water (10% w/v).

TTN assay in samples was done by LC after its extraction with methanol according to the conditions described in item 2.2. Approximately 1.0 g of each formulation was accurately weighed and transferred to a 50-mL volumetric flask. Methanol was added, and the flask was subjected to ultrasound for 30 min after the sample was centrifuged (15 min, 2050*g*), filtered through a paper filter (Quantitative filter, JP41 – 28 μm, J. Prolab, São José dos Pinhais, Brazil) and through a regenerated cellulose membrane (Sartorius, 0.45 μm). The method for assay TTN in hydrogels proved to be linear (r = 0.9999) in a concentration range of 2.5–20.0 μg mL^{-1} and precise [RSD = 1.92% and 1.84% for repeatability (intra-day precision) and intermediate precision (inter-day precision), respectively]. The method was accurate with percentages of recovery of 98 ± 2%. LC method was suitable to resolve TTN and its main degradation product, isotretinoin (Fig. 1).

The rheological characteristics of the semisolid formulations were determined using a rotational viscometer (LVDV-II + PRO Digital Viscosimeter, Brookfield Instruments, UK), spindle SC4-25. The analysis was carried out at 25 ± 1 °C. Rheograms were analyzed to the best fit using Binghan, Casson, Ostwald, and Herschel–Bulkley models [35] (Eq. (1)–(4)) in order to determine the rheological behavior, consistency indices, and plasticity of the formulations.

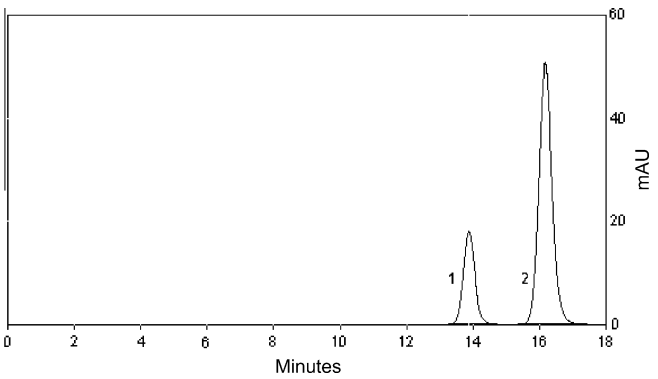

Fig. 1. Chromatogram showing the separation of tretinoin (2) and its major degradation product, the isomer, isotretinoin (1).

$$\tau = \tau_o + \eta\dot{\gamma} \quad (1)$$

$$\tau^{0.5} = \tau_o^{0.5} + \eta^{0.5}\dot{\gamma}^{0.5} \quad (2)$$

$$\tau = \kappa\dot{\gamma}^n \quad (3)$$

$$\tau = \tau_o + \kappa\dot{\gamma}^n \quad (4)$$

The τ_o is the yield stress, η is the viscosity, n is the index of flow, K is the index of consistency, τ is the shear stress, and $\dot{\gamma}$ is the shear rate [35].

The evaluation of spreadability (S_i) was performed at room temperature and using the parallel plate method with known weights [36]. The sample was introduced in the central 1-cm-diameter hole of a mold glass plate. The mold plate was carefully removed, and the sample was pressed subsequently with glass plates of known weights, with intervals of 1 min between each plate. Spreading areas reached by samples between every addition of a glass plate were measured in millimeters in two perpendicular directions considering the center of the plate. Results were expressed in terms of the spreading area as a function of the applied mass. The spreading area was plotted against the plate weights to obtain the spreading profiles. The spreadability factor (S_f) was also calculated and represents if spread of a formulation is able to expand on a smooth horizontal surface when a gram of weight is added on it, under the conditions described in the methodology above. The following equation (Eq. (5)) is used to calculate the spreadability factor [37]:

$$S_f = \frac{A}{W} \quad (5)$$

in which S_f ($mm^2\ g^{-1}$) is the spreadability factor resulting from the ratio between (A) the maximum spread area (mm^2) after the addition of the sequence of weights used in the experiment and (W) the total weight added (g).

2.4. Photodegradation studies

The photodegradation studies of TTN in the formulation (HG-TTN-LCNC) were carried out in triplicate using an UVA lamp (Fluorescent Blacklight blue lamps, 30 W, Ecolume). In order to establish an adequate comparison, the photodegradation of TTN in a marketed hydrogel (HG-TTN-M) was also studied (VITANOL-A, lote 10,05,067, Stiefel, São Paulo, Brazil). Samples were introduced in UV-transparent disposable cuvettes (exactly weighed: 1 g) and exposed to the UVA light in a closed chamber. Individual cuvettes were used for each time interval. At prefixed time (0, 2, 4, 6, and 8 h), one cuvette was withdrawn, and the amount of remaining TTN was assayed by LC after an extraction with methanol, according to the experimental described in Section 2.2. In order to ensure that the degradation of TTN was due the UVA light, the amount of remaining TTN was evaluated in parallel from a cuvette containing the same sample completely covered by aluminum foil. The results were analyzed to establish the kinetic order of degradation of TTN in formulations. The kinetic order of degradation was calculated for each sample using the graphic method. Zero-, first-, and second-order graphs were drawn by plotting tretinoin remaining (μg mL^{-1}) versus time, ln [tretinoin remaining (μg mL^{-1})] versus time, and 1/[tretinoin remaining (μg mL^{-1})] versus time. The correlation coefficient was calculated (Microsoft Excel 2007). The best correlation coefficient (linearity) was considered to establish the kinetic order and to calculate the respective degradation constants (k) and half-life times [38,39].

2.5. Human skin permeation experiments

The permeation of the formulations through human skin was studied by means of static Franz diffusion cells (15 mm diameter, type 4G-O1-00-20, Perme Gear, Riegelsville, PA, USA) using human heat separated epidermis (HHSE) as diffusion membrane. The purpose of this study was to evaluate the influence of nanoencapsulation of this drug on its skin permeation profile. In this case, the use of the marketed hydrogel was discarded due to the presence of many other excipients which could influence the permeability of TTN. So we prepared a hydrogel containing non-encapsulated tretinoin using Carbopol® Ultrez NF 10 at 0.5% (w/w) (HG-TTN). An hydroalcoholic solution of tretinoin (50:50 ethanol:water v/v) was used to prepare these hydrogels.

2.5.1. Preparation of HHSE

Abdominal human skin samples were obtained from the Plastic Surgery Department of the Karitas Hospital (Lebach, Germany) and was approved by the Ethical Commission of Department of Plastic and Hand Surgery, Caritaskrankenhaus, Lebach, Germany. Samples of the same skin donor (female, 38 years old) were used for all experiments to avoid interindividual differences. The skin was prepared for storage within the first 3 h after excision, by removing all subcutaneous fatty tissue, and kept frozen in PE-bags (−26 °C) until use. For further details, see Wagner et al. [40]. HHSE was prepared by the method described by Kligmann [41]. Briefly, 25-mm-diameter disks are punched out from the frozen skin piece cleaned with water and cotton and let thaw. The disks are submerged in a 60 °C water bath for exactly 90 s. The epidermis can be then easily removed from the dermis by pulling it with forceps.

2.5.2. Franz diffusion cells set-up

The hydrogel formulations (HG-TTN-LCNC and HG-TTN) were applied first to the donor compartment. It was previously filled with a rubber insert covered with aluminum foil, leaving a 2-mm thickness space for placing the formulation. Then, the formulation was applied and weighed. The HHSE disk was placed over the formulation, with the *Stratum corneum* side touching it. The donor compartment was then placed underneath the HHSE, fixed with a clamp, and filled with 12 mL ethanol/phosphate buffer solution (1 mM) pH 7.4 (50:50, v:v) to assure sink conditions, according to Manconi and co-workers [26]. In order to obtain a better understanding of the effect of the TTN nanoencapsulation on its skin permeation, experiments were also carried out with the TTN-LCNC and a tretinoin solution at 0.02% (in water:ethanol 50:50 w/w) without any addition of a thickening agent. The concentration of TTN in the solution was lower than in the TTN-LCNC due to its low solubility in the vehicle. In this case, the receptor compartment was firstly filled with the receptor solution, afterward the HHSE was applied onto it with the *Stratum corneum* side facing up and avoiding air bubble formation. The donor compartment was fixed over the HHSE by means of clamps. Then, 500 μL of formulation were applied to the donor compartment. Franz cells were sealed with Parafilm® and aluminum foil to avoid evaporation of solvents. The experimental set-up was kept under 32 ± 2 °C, which corresponds to the physiologic temperature of the *Stratum corneum*. The receptor compartment was stirred continuously at 500 rpm, and the experiments were performed under infinite dose conditions. Sampling was performed over 30 h. The same volume (0.4 mL) was refilled with fresh ethanol/buffer solution. At least four replicates were used for each experimental condition. TTN concentration was analyzed by LC according to the conditions described in Section 2.2. No interference of any substance of the HHSE was observed in the chromatographic run, as evaluated in a specificity test.

2.5.3. Data plot and parameter calculations

The cumulative amounts of TTN per diffusion area were calculated for each time point and plotted versus the sampling time points. The permeation parameters were calculated from the linear part of the plot, which corresponds to the steady state, being the flux (J), the slope, the lag time (t_L) and the x-axis intercept calculated by linear regression of these data points. The permeability coefficient (K_p) was obtained dividing the flux by the drug concentration in the donor compartment. Results were discussed in terms of K_p, which is a parameter independent of the applied dose. The decrease ratio (DR) of TTN permeation has been calculated by relating the obtained K_p for each formulation with the obtained K_p of free TTN solution.

2.6. Statistical analysis

All formulations were prepared and analyzed at least in triplicate. Results are expressed as mean ± SD (standard deviation). One-way analysis of variance (ANOVA) was employed for comparison of the experimental data, at a significance level of 5%.

3. Results and discussion

In our previous work, we reported the preparation of TTN-loaded lipid-core nanocapsules presenting a encapsulation efficiency higher than 99.9%, neutral pH values (6.7–7.2), z-average diameters between 200 and 250 nm and polydispersity index lower than 0.25 [30]. These formulations showed a higher photostability under UVA and UVC light if compared to the free methanolic TTN solution. Analyzing these previous results, we hypothesized the increasing of TTN photostability in dermatological formulations as well as the development of TTN aqueous semisolid dosage forms without the use of organic co-solvents using these TTN-loaded lipid-core nanocapsules. Therefore, these nanostructured suspensions were formulated in Carbopol® Ultrez NF 10 hydrogels.

The preparation of hydrogels was done using TTN-LCNC ($n = 3$) instead of water during the step of polymer dispersion. TTN-LCNC showed particle size and zeta potential similar to those reported in our previous work [30]. TTN was assayed in nanocapsules before the preparation of hydrogels. All batches showed drug content in the range of 95–105% of the theoretical content (0.5 mg mL^{-1}).

HG-TTN-LCNC showed a pH value of 6.08 ± 0.14 and a spreadability factor of 2.48 ± 0.14 $mm^2\ g^{-1}$ (Table 1). Drug content was near (0.52 ± 0.01 mg g^{-1}) to the expected value (0.50 mg g^{-1}). As can be observed in Table 1, neither the presence of TTN-LCNC nor the presence of B-LCNC affected the pH of the formulations. On the other hand, the presence of the nanocapsules (HG-TTN-LCNC and HG-B-LCNC) tends to decrease the spreadability factor of formulations compared to the hydrogel without them (HG-B), as previously reported by Marchiori and co-workers [37] for hydrogels containing dexamethasone-loaded nanocapsules. However, although the HG-TTN-LCNC presented a lower spreadability compared to the HG-B (ANOVA, $p \leqslant 0.05$), its value is higher than that

Table 1
Physicochemical characteristics of hydrogels containing TTN-loaded lipid-core nanocapsules (HG-TTN-LCNC), blank lipid-core nanocapsules (HG-B-LCNC), and hydrogels base (HG-B).

Formulation	Drug content (mg mL^{-1})	pH	Spreadability factor ($mm^2\ g^{-1}$)
HG-TTN-LCNC	0.52 ± 0.01	6.08 ± 0.14	2.48 ± 0.12
HG-B-LCNC	–	6.16 ± 0.03	2.30 ± 0.18
HG-B	–	6.26 ± 0.06	3.13 ± 0.37

– not determined.

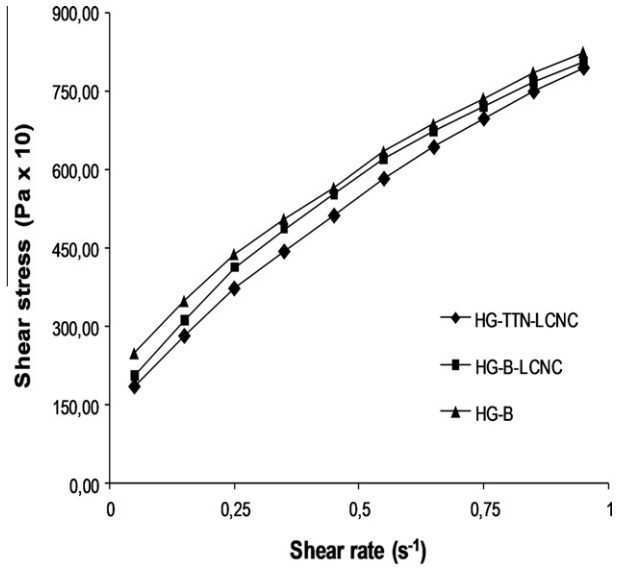

Fig. 2. Rheograms of hydrogels containing TTN-loaded lipid-core nanocapsules (HG-TTN-LCNC), blank lipid-core nanocapsules (HG-B-LCNC), and hydrogels base (HG-B).

presented by the marketed hydroalcoholic gel (spreadability factor = 1.68 $mm^2\ g^{-1}$).

Regarding the rheological analysis, all hydrogels showed non-Newtonian behavior, as depicted in Fig. 2. There was no linear correlation between shear stress (Pa) and shear rate (s^{-1}) [42,43]. Non-Newtonian systems can be represented by three general types such as plastic (it needs to exceed a yield value to allow formulation begin to flow), pseudoplastic (the viscosity decreases with increasing shear rate), and dilatant (the viscosity increases with increasing shear rate) [44]. Based on this, all formulations seem to show pseudoplastic behavior regardless of the presence of lipid-core nanocapsules. This rheological behavior is preferred for dermatological dosage form because the formulation flow resistance is low when it is applied under medium to high shear conditions [45].

In order to establish the index flow (n) in different non-Newtonian systems [35], the rheograms can be analyzed using different models such as Binghan, Casson, Ostwald, and Herschel–Bulkley [45]. Our results for HG-TTN-LCNC, HG-B-LCNC, and HG-B fit better to the Herschel–Bulkley model (Tables 2 and 3), in accordance with the previous report by Marchiori and co-workers [37]. Considering this model, the consistency indices and plasticity were similar for all formulations (HG-TTN-LCNC, HG-B-LCNC and HG-B), in spite of the presence of the lipid-core nanocapsules or TTN (ANOVA, $p \leqslant 0.05$). Furthermore, the flow index below 1 indicates a pseudoplastic behavior for all formulations, in accordance with our previous suggestion.

Up to here, we demonstrated the feasibility to prepare aqueous semisolid formulations containing TTN with adequate characteristics for dermatological administration, without the use of alcoholic co-solvents. The presence of intact nanostructures in similar Carbopol hydrogels after the redispersion of polymeric nanocapsules was previously demonstrated by transmission electron microscopy [37]. In the next part of our work, we should prove our initial hypothesis of increasing the photostability of TTN not only by its nanoencapsulation in liquid suspensions as previously demonstrated [30] but also after its incorporation in a semisolid dermatological formulation.

Table 3
Flow index (n) and consistency index (K) of hydrogel containing TTN-loaded lipid-core nanocapsules (HG-TTN-LCNC), blank lipid-core nanocapsules (HG-B-LCNC), and the hydrogel base (HG-B).

Formulation	n[a]	K (Pa S^n)[a]
HG-TTN-LCNC	0.72 ± 0.19	74,894 ± 20,456
HG-B-LCNC	0.74 ± 0.28	71,129 ± 15,092
HG-B	0.63 ± 0.09	71,829 ± 7530

[a] According to the Herschel–Bulkley model.

After UVA exposure, HG-TTN-LCNC showed a TTN degradation of 24.17 ± 3.49% after 8 h, while the HG-TTN-M showed a 2.8-fold higher degradation of 68.64 ± 2.92% after 8 h. Results from both formulations fit to the second-order kinetic model (r = 0.9365 and 0.9918 for HG-TTN-NC and HG-TTN-M, respectively). The half-life values were 3.80 ± 0.42 h for HG-TTN-M and 26.60 ± 9.75 h for HG-TTN-LCNC (Table 4), confirming our initial hypothesis and showing the potential of lipid-core nanocapsules to improve TTN photostability even after their incorporation in semisolid formulations. Furthermore, TTN photostability in hydrogels was higher than that obtained for nanocapsule suspensions [30], where the nanoencapsulated drug degraded 27.09 ± 3.73% after 1 h of exposure to UVA radiation and the non-encapsulated drug (in methanolic solution) showed a degradation of 58.46 ± 2.33% after the same exposure time.

Tashtoush and co-workers [46] showed that UVA radiation is the main responsible factor for the photodegradation of TTN. Considering that UVA penetrates deeply into the skin, they suggest that photodegradation of TTN may contribute to the photosensitivity associated with TTN therapy. In this context, our results showed that the incorporation of TTN-LCNC in dermatological medicines could increase the drug residence and its availability in the skin. In this way, we could suggest that this approach can reduce the skin photosensitivity caused by topical treatment with TTN in conventional formulations.

In the last part of our work, we evaluated the effect of TTN nanoencapsulation on its skin permeation, using Franz diffusion cell technique. The cumulative amounts of drug diffused through HHSE at the different sampling intervals are shown in Figs. 3A

Table 4
Results from photodegradation studies[a] under UVA exposition of hydrogels containing TTN-loaded lipid-core nanocapsules (HG-TTN-LCNC) and a marketed gel containing tretinoin (HG-TTN-M).

Sample	$t_{½}$ (h)	Kinetic order	$k \times 10^6$ (L mol^{-1} h^{-1})	r
HG-TTN-LCNC	26.6 ± 9.75	Second	1.0 ± 0.6	0.9365
HG-TTN-M	3.8 ± 0.42	Second	7.0 ± 0.6	0.9918

[a] Calculated using the graphic method (Microsoft Excel 2007). The best correlation coefficient (linearity) was considered to establish the kinetic order.

Table 2
Regression coefficient (r) for various flow models in shear rate–shear stress curve obtained for hydrogels containing TTN-loaded lipid-core nanocapsules (HG-TTN-LCNC), blank lipid-core nanocapsules (HG-B-LCNC), and hydrogels base (HG-B).

Formulation	Bingham	Casson	Ostwald	Herschel–Bulkley
HG-TTN-LCNC	0.955 ± 0.003	0.988 ± 0.004	0.958 ± 0.003	0.999 ± 0.001
HG-B-LCNC	0.943 ± 0.004	0.982 ± 0.008	0.961 ± 0.003	0.999 ± 0.002
HG-B	0.955 ± 0.001	0.992 ± 0.002	0.966 ± 0.001	0.999 ± 0.001

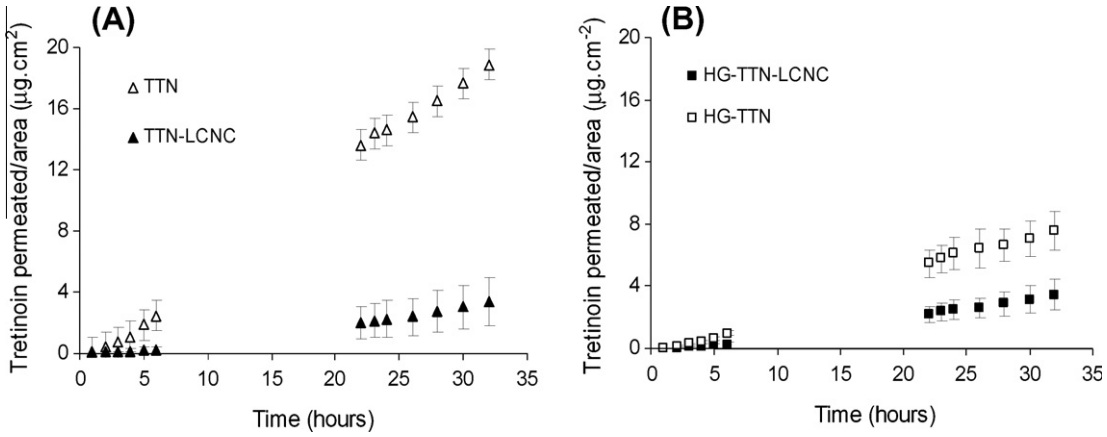

Fig. 3. Permeation profile of TTN (μg cm^{-2}) from (A) liquid formulations: tretinoin solution (TTN) and tretinoin-loaded lipid-core nanocapsules (TTN-LCNC) and (B) semisolid formulations: hydrogel containing non-encapsulated tretinoin (HG-TTN) and hydrogel containing tretinoin-loaded lipid-core nanocapsules (HG-TTN-LCNC).

and 3B. For all samples, steady state is achieved as can be deduced by the linear portion of the graphs. The calculated parameters are summarized in Table 5. The permeability of TTN from the hydroethanolic solution was very effective, as expected (K_p = 1.80 ± 0.27 μg cm^{-2} h^{-1}). Formulating non-encapsulated TTN in a hydrogel decreases its permeability (K_p = 0.73 ± 0.12 μg cm^{-2} h^{-1}) compared to the drug solution (K_p = 1.80 ± 0.27 μg cm^{-2} h^{-1}). For both formulations, the lag time was the same. Independently of the dosage form used, there was a decrease in the permeability coefficient of the drug by nanoencapsulation (K_p = 0.31 ± 0.15 and K_p = 0.33 ± 0.01 μg cm^{-2} h^{-1} for TTN-LCNC and HG-TTN-LCNC, respectively) ($p \leqslant 0.05$) compared to the samples containing non-encapsulated drug. Furthermore, the lag time was increased two times meaning that TTN is retained for a longer time on the skin surface. In summary, the use of a thickening agent like Carbopol and subsequently the higher viscosity of hydrogels did not interfere in the skin permeation profile of nanoencapsuled TTN, as observed comparing the values of drug flux, lag time, permeability coefficient, and decrease ratio for both formulations – TTN-LCNC and HG-TTN-LCNC ($p > 0.05$, Table 5). These results show that nanoencapsulation is the controlling step of the drug delivery in this case. As some authors reported the influence of the pH of Carbopol gels in the TTN permeation across the skin [21], pH of all formulations was adjusted to similar values (between 6.0 and 6.3).

Some other efforts have been reported to modulate the skin permeation profile of TTN using nanostructured materials. For liposomal formulations, TTN dermal delivery is affected by vesicle composition, morphology, surface charge, and particle size [26]. Manconi and co-workers [27] showed that TTN cutaneous delivery is strongly affected by the vesicle composition of niosomes and the thermodynamic activity of the drug. According to the authors, very hydrophilic surfactants improved the diffusion of TTN across pig skin. Mandawagle and Patrava [28] showed a considerable less skin permeation after the application of TTN-loaded solid lipid nanoparticles-based gels compared to a marketed formulation (cream). Considering these previous studies, our present work presents an alternative to increase the depot formation of TTN in the skin layers by its encapsulation in lipid-core polymeric nanocapsules with the advantage of an additional increase in its photostability.

Table 5
Permeation parameters of TTN through HHSE from the different samples (tretinoin solution – TTN; tretinoin-loaded lipid-core nanocapsules – TTN-LCNC; hydrogel containing tretinoin-loaded lipid-core nanocapsules – HG-TTN-LCNC; and hydrogel containing non-encapsulated tretinoin – HG-TTN).

Sample	J (μg cm^{-2} h^{-1})	Lag time (h)	$K_p \times 10^6$ (cm s^{-1})	DR
TTN	1.30 ± 0.20	1.70 ± 0.20	1.80 ± 0.27	
TTN-LCNC	0.56 ± 0.30	3.59 ± 1.40	0.31 ± 0.15	5.83
HG-TTN-LCNC	0.60 ± 0.15	3.75 ± 1.25	0.33 ± 0.01	5.45
HG-TTN	1.33 ± 0.20	1.82 ± 0.20	0.73 ± 0.12	2.47

4. Conclusion

Hydrogels containing tretinoin-loaded lipid-core nanocapsules were prepared without using alcoholic co-solvents, showing adequate drug content and pH, pseudoplastic behavior, and better spreadability compared to a currently marketed formulation. Our report shows that even after their incorporation in hydrogels, polymeric nanocapsules are able to protect nanoencapsulated TTN against UVA radiation, being an important alternative to overcome its main pharmacotechnical limitation. Furthermore, skin permeation studies showed that our strategy was able to control the skin permeability of tretinoin. The novelty of our strategy was showed by its combining advantages of increasing the tretinoin photostability and its higher retention on the skin surface compared to formulations containing non-encapsulated drug. Therefore, we can highlight that our approach represents a useful tool in the development of topical nanomedicines containing tretinoin for the treatment of skin disorders.

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