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ORIGINAL ARTICLE

Formulation studies on a topical gel of tretinoin–dimethyl-beta-cyclodextrin complex

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Abstract The aim of this work was to develop and characterize a 0.05% tretinoin hydrogel formulations in which tretinoin is free or complexed with dimethyl-beta-cyclodextrin in order to compare the main advantages of its complexation. Theoretically, the complexation will mainly allow to: overcome drug low solubility in water and low stability; enhance the drug release by promoting skin absorption and alleviate of drug inducing local irritation. The hydrogels prepared were both microbiological and physically stable during 30 days. However, the chemical stability was less encouraging. The complexed tretinoin gel had also a higher releasing profile than the free tretinoin gel. This study has demonstrated that it is possible to obtain a microbiological and physically stable gel formulation with good releasing profile.

Keywords Dimethyl-beta-cyclodextrin · Tretinoin · Gel physical characterization · Gel chemical characterization · In vitro release study

Introduction

The retinoic acid receptors are α, β and γ-RAR and RXR and the CRABP are the citosolic skin binding proteins, which lead to an anti-inflammatory and comedolytic action. Retinoids are considered the first line treatment for acne, being also a maintenance therapy. These drugs cause the desobstruction of the pores, preventing the formation of white spots and still present the benefit of decreasing the first signals of cutaneous aging, being therefore an essential treatment for acne in adults. However, the retinoids can irritate the skin making it more sensitive to solar expositor. A recent study with the retinoic acid/ β-CD complex shows a significant increase of the effectiveness and tolerance to the acne *vulgaris* treatment, which will also increase the patients' compliance to this treatment [1]. It is usual the topical antibiotic combination with retinoids to treat comedogenesis, bacterial growth and inflammation. This combination also increases the effectiveness and tolerance to the treatment.

The transdermal permeation and systemic bioavailability of topical retinoids are not yet completely clarified, thus, there isn't a consensus about the use of topical retinoids during the pregnancy [2].

The formulations may contain 0.025–0.1% of drug on topical creams and 0.01–0.025% on gels. However, there has been emerging new sustaining release systems in which the active substance is vehiculated in microsponges or polymers in order to remain at the stratum corneum and thus promote comedolisis and modulation of the keratinocytes proliferation. The side effects include redness, descamation, dryness, and burning tingle [3].

The main applications of cyclodextrins (CDs) in drug topical administration could be: enhancement of drug release and/or permeation; drug stabilization in formulation; reduction of the adverse effects, mainly the cutaneous irritation due to the lower quantity of free drug fraction and dose reduction to reach the therapeutic effect; sustaining of drug release from vehicle; alteration of drug bioconversion in the viable skin; formulation of incompatible compounds; fixation of volatile compounds and improvement of the organoleptic properties; quantity reduction or absence of surfactants agents in emulsions [4–11].

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The aim of this work is to develop and characterize a 0.05% tretinoin hydrogel formulations in which tretinoin is free or complexed with 2,6-dimethyl-beta-CD (DM-β-CD) in order to compare the main advantages of its complexation. The tretinoin complexation with DM-β-CD will also enhance the drug release by promoting skin permeation and alleviation of drug induced local irritation by lowering the extent of free drug resulting from inclusion equilibrium and [12] by the emollient and refreshing effect of the hydrogel formulation.

Materials and methods

Materials

Tretinoin was purchased from Fagron (Spain) and DM-β-CD (degree of substitution: 1.8) was a generous gift from Wacker (Germany). Hydroxypropylmethylcellulose, glycerine and potassium sorbate were purchased from Fluka, José M. Vaz Pereira (Portugal) and Sigma Aldrich Corp., respectively. All other reagents were of analytical reagent grade.

Methods

Gel formulations and stability studies

The hydrogels were formulated according to Table 1.

Glycerine (35 g) and potassium sorbate (0.15 g) were added to the hydro alcoholic solution with the complex or tretinoin followed by hydroxypropyl methylcelullose (1.5 g) and purified water. These were stirred well until the swelling was completed.

Three gel batches were produced and stored at room temperature and 40 °C (75% relative humidity). The samples were collected at predetermined time points to evaluate their physical stability (such as organoleptic properties and rheology—Brookfield DV-II RV) and microbiological examination of non-sterile products according to the European Pharmacopeia 6.0 (2.6.12/13—topical formulations) [14]. The rheology data were analyzed using a factorial ANOVA (temperature, batch) under repeated observations (days). These data were previously logaritmized to ensure the normality whenever there were any significant statistic differences. Chemical stability was performed by HPLC (Hewlett Packard system with a Lichrocart® 250-4, RP18 (5 µm) column) under the following conditions: 50 µL injection volume; flux of 1 mL/min; retention time of 20 min and the mobile phase consisted of 0.01% trifluoroacetic acid : acetonitrile (15:85) (method adapted from [15]). The pH was measured by Metrohm 744 pH meter (with calibrated glass electrode).

In vitro release studies

In vitro permeation profile was determined using vertical Franz diffusion cells with a diffusion area of 0.95 cm^2. A 0.1 g sample of gel was spread over the donor side of the membrane (Tuffry® 25 mm, 0.45 µm). The receptor phase contained a mixture of phosphate buffer and 0.1% TAGAT CH 40. TAGAT was used to solubilise tretinoin in the receptor solution maintained at 37 °C. At pre-determined times, several samples were collected and the same volume replaced with fresh solution. The tretinoin in the receptor phase was analysed by HPLC using an UV detector ($\lambda = 342$ nm). The data was expressed in cumulative amount of tretinoin permeated through membrane filter, considering the total amount of drug applied in each formulation (gel with free tretinoin and with complexed tretinoin).

Results and discussion

Gel formulation

Considering that the hydrophilic CDs promote the release of lipophilic drugs from hydrophilic aqueous vehicles, it was formulated as a hydrogel. A cellulose derivative was used as a viscosity-increasing agent because it is non-ionic and it does not interfere with the pH of the final

Table 1 Tretinoin—DM-β-CD complex and free tretinoin hydrogel formulations [13]

Ingredients	Amount	
Tretinoin	267 mg of complex (1:4 stoichiometry) in 60 mL of hydro alcoholic solution (1 water:10 alcohol)	0.05% tretinoin (equivalent free form) in 60 mL of hydro alcoholic solution
DM-β-CD		–
Hydroxypropylmethylcellulose	1.5%	1.5%
Glycerine	15%	15%
Potassium sorbate	0.15%	0.15%
Purified water	qs 100%	qs 100%

formulation. Moreover, aqueous solutions are comparatively enzyme-resistant, providing good viscosity stability during long-term storage. The gels are easily removed when washing, but tend to be dehydrated, losing their original texture. To avoid such situation, a hygroscopic agent such as glycerine was added. Compared to propylene glycol, this humectant is less irritating to the skin. The preservative chosen was the potassium sorbate because it presents less toxicity in relation to parabens and has antibacterial and antifungal activity at pH < 6; besides, potassium sorbate is still stable and soluble in water [13].

The tretinoin may suffer isomerisation in the presence of atmospheric oxygen, light and high temperature. Therefore, tretinoin is an agent that is extremely sensitive to oxidation partially due to its high degree of unsaturation [16]. It was excluded an antioxidant, even the drug was included in DM-β-CD, to analyse the impact of complexation in reducing the oxidation drug in future works. A previous study has demonstrated that it is possible to complex tretinoin with DM-β-CD by different techniques (DSC, NMR, FTIR, X-ray diffraction, molecular modelling) [17] and Raman spectroscopy (unpublished data) obtaining a relatively high complexation constant and higher drug solubility.

Gel stability

Physical stability: organoleptic properties

After preparing the gels, both odour and colour (yellow) were consistent with the drug included. The gels also had a uniform appearance and an appropriate consistency ("no drip"), but easy to spread over the skin. Over time it appeared that there was no significant variation of its organoleptic properties in the different batches. However, it is noteworthy that the gels stored in the oven have become more fluid, as it can be seen in the rheological analysis.

Physical stability: Rheology

The tretinoin hydrogel appeared to have a pseudoplastic flow (Fig. 1). In fact, this flow type is typical of fluids that contain macromolecules linked in a non rigid structure that forms a flexible lattice. When the fluid is subjected to a shearing stress, the molecules orientate in the flow direction as well as a strain of the lattice. Consequently, there is no proportionality between shearing stress and shear rate and the viscosity decreases with the shearing stress applied [18].

There were no significant statistic differences between batches (F2, 255 = 0.82, p = 0.440) and there was no interaction between batches and its storage temperature (F2, 255 = 0.53, p = 0.587). As expected, there were significant differences between the two storage temperatures (F1, 255 = 10.96, p = 0.001): the viscosity of the batches stored at room temperature was higher than those stored at 40 °C. The rheological characteristics varied over time (F3, 255 = 2.82, p = 0.040) and the data revealed the existence of differences between the 1st and 30th day (in general, with higher values for the 30th day).

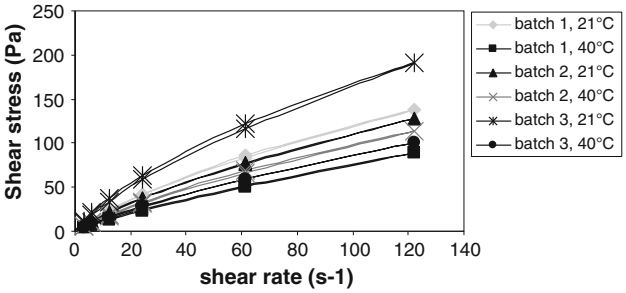

Fig. 1 Rheograms of the three tretinoin hydrogel batches stored at room temperature and in the oven after 30 days

Microbiological stability

The tretinoin hydrogel appeared to be microbiologically stable at least during 30 days. No colony forming units (CFU/g) were observed during the microbiological control of the tretinoin hydrogel. The results obtained are within the limits of European Pharmacopeia 6.0 (class 2):

- Total Viable Aerobic Count $<10^2$/g
- Enterobacteria and other gram-negative $<10^2$ /g
- Absence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* pour gram of product 3.2.

Chemical stability

The complexed tretinoin gel was not as stable as expected comparing to the free tretinoin gel, especially after 7th day (Fig. 2). In fact, it is quite difficult to establish the same extraction procedure to both gels according to different solubilities of tretinoin forms. Therefore, it is convenient to add hydrophilic antioxidants [16] and also CD in excess in order to obtain the assay required limits ($\approx$90–110%) over time. In addition the humectant (glycerine) could be present in a higher concentration (about 35%) according to some higher values (not shown) obtained when the gels were kept at 40 °C in closed containers. Another hypothesis is to incorporate the complex in deformable liposomes.

pH measurement

The results are represented in Table 2. The pH obtained is suitable for topical administration and there are no significant differences between both gels.

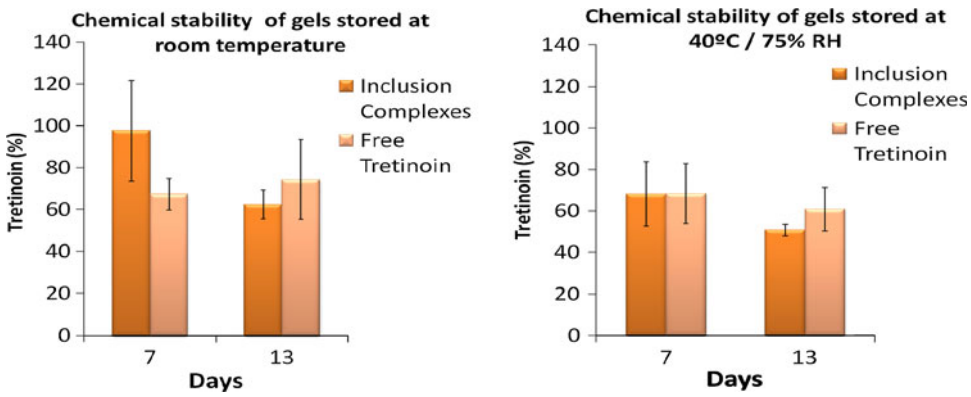

Fig. 2 Mean assay values of free and complexed tretinoin hydrogels batches stored at about 21 and 40 °C, respectively during about 15 days ($n = 3$)

Table 2 pH measurement results

Drug gel	T ≈ 21 °C	T = 40°C	Complex gel	T ≈ 21 °C	T = 40°C
1st day	6.87 ± 0.16	–	1st day	6.90 ± 0.03	–
30th day	6.61 ± 0.01	6.56 ± 0.02	30th day	6.37 ± 0.03	6.31 ± 0.01

$n = 3$, pH average ± standard deviation

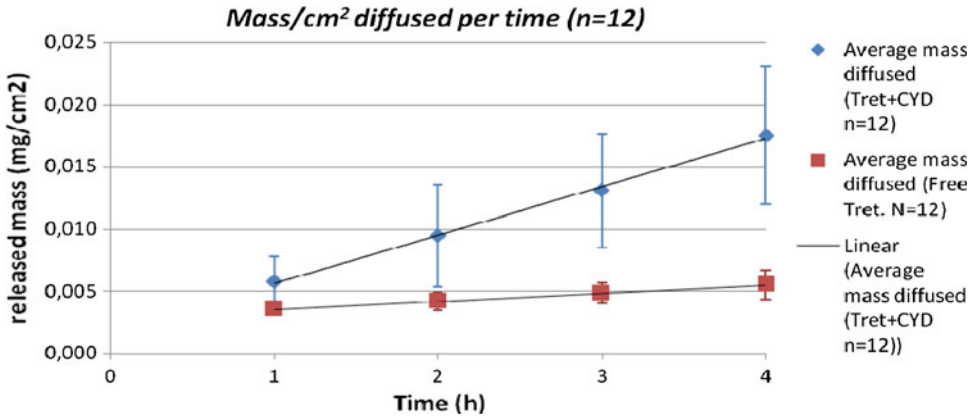

Fig. 3 Released mass of free tretinoin gel and complexed tretinoin gel through synthetic membranes over 4 h (in sink conditions)

As mentioned before, the pH decreased with temperature and storage time which could be associated with the chemical degradation of the formulations.

Gel in vitro release study

An important characteristic of the use of β-CDs as acne treatment stems from the fact that 2,6-dimethyl-beta-cyclodextrin (DM-β-CD) may incorporate the poly-unsaturated fatty acids from the skin, increasing the drug permeation and leading indirectly to the reduction of the infections incidence and cutaneous inflammations [6].

The results of in vitro release studies are presented in Fig. 3. The complexed tretinoin gel released more tretinoin through synthetic membranes than the free tretinoin gel mainly because of its higher solubility and to the dissociation of dissolved inclusion compounds leading to free molecules of tretinoin able to diffuse through the membrane. The release of tretinoin is related not only to the stability constant of the inclusion (Ks ≈ 13 600 M^{-1}) according to Higuchi and Connors (1965) method [19], but also to the binding properties of the inclusion compounds to the vehicle and the partition coefficient between the vehicle and the membrane [20].

The drug solubilisation in complex form is higher and cyclodextrins may contribute to increase the drug availability on the membrane surface and probably exert a driving force towards greater membrane/ skin permeation. The free drug fraction on the skin depends on its dissolution rate; relative magnitude of the complexation constant; competitors' presence at the absorption site, the drug absorption rate constant, etc.

Though only insignificant amounts of CDs and of drug/CD complexes can penetrate into biological barriers because of their size and hydrophilicity, CDs may interact with some of the skin components. It was reported that the

free CDs released on complex dissociation, due to their ability to remove some membrane surface components, can modify the membrane transport properties and thus to facilitate drug absorption, especially water-soluble drugs [6].

Conclusion

This study has demonstrated that it is possible to obtain a microbiological and physically stable gel formulation with good releasing profile.

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