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SHORT COMMUNICATION

Effect of tretinoin inclusion in dimethyl-beta-cyclodextrins on release rate from a hydrogel formulation

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Abstract The aim of this work was to study the release, permeation and skin retention profiles of 0.05% tretinoin hydrogel formulations in which tretinoin was in free form or complexed with dimethyl-beta-cyclodextrin in a stoichiometry of 1:4. Theoretically, this complexation will mainly allow to: overcome drug's low water solubility and low stability; enhance the drug permeation by promoting skin absorption and alleviate drug inducing local irritation. In vitro release, permeation and skin retention tests were performed in both formulations in order to compare the main advantages of this complexation. The influence of the thermodynamic activity on the drug release profile was also investigated. This study proved that tretinoin inclusion complexes formulation with excess of cyclodextrins had better release profile than the free tretinoin formulation. It was concluded that in this study, thermodynamic activity was not the driving force for the release rate improvement observed with cyclodextrins. Probably, this improvement was due to the increased availability of tretinoin near the membrane surface. In fact, the percentage of total drug that had been retained in the skin was 0.41 ± 0.08% for complexed tretinoin gel and 0.17 ± 0.04% for the free tretinoin gel.

Keywords Tretinoin · Dimethyl-beta-cyclodextrin · In vitro drug release/permeation and skin retention · Thermodynamic activity

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Introduction

Acne *vulgaris* is a pathological dysfunction in the sebaceous follicles. It has been accepted that its pathogenesis is multifactorial, with abnormal follicular differentiation and increased cornification, abnormal activity of the sebaceous gland and bacterial hyper-colonization, as well as inflammation and immune reaction [1].

Retinoids are considered the first line treatment for acne and they act by binding to specific receptors (Fig. 1): RXR (retinoid X receptors) and RAR (retinoic acid receptors) which is composed of three isoforms: RARα, RARβ, and RARγ. The RARγ are localized in epidermis. These receptors are ligand-dependent transcriptional factors that belong to the steroid-thyroid hormone family. The interaction with those receptors results in comedolytic and anti-inflammatory actions which directly affect the evolution of acne [2–4].

Tretinoin, the generic name for retinoic acid and commonly referred to as vitamin A acid or all-trans retinoic acid (ATRA), is the active form of a metabolic product of vitamin A. It belongs to the first generation of retinoids. Tretinoin has a high affinity for all nuclear RARs and for the cytosolic skin binding proteins during cellular transportation and by interacting with epidermal receptors it results on the (1) increasing of the turnover of follicular epithelial cells, (2) accelerating the shedding of corneocytes, (3) normalizing keratinization, which leads to (4) drainage of comedones and inhibition of new comedone formation [5, 6].

For more than 15 years tretinoin has been a core in the topical treatment of uncomplicated acne *vulgaris*. However, unpleasant side effects, such as irritation of the treated area, erythema, burning, and stinging, can occur after topical administration. Moreover, it is well-known that tretinoin is

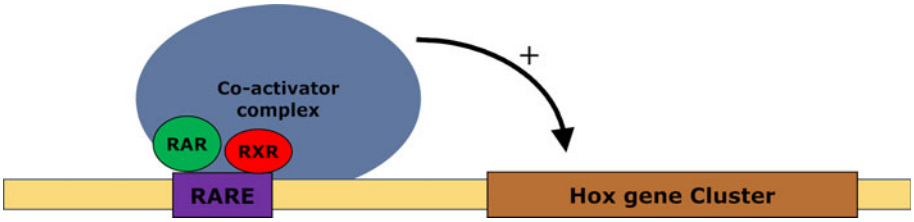

Fig. 1 Transcription regulation by tretinoin [4]

susceptible to oxidation when exposed to air; also, it is thermally unstable and isomerizes in solution upon exposure to light. More specifically, the irradiation of a tretinoin solution leads to the formation of nine different isomers, the most abundant of which being isotretinoin [7]. It should also be mentioned that tretinoin's poor water solubility may lead to technical drawbacks and compromise its action on skin when formulated in hydrogels formulations [8].

Complexation with cyclodextrins (CDs) is one approach that has been studied to overcome the low solubility and stability of tretinoin. In an aqueous solution the slightly apolar CD cavity is occupied by water molecules which are energetically unfavoured (polar-apolar interaction) and therefore can be substituted by appropriate guest molecules which are less polar than water. The driving force for complex formation is the substitution of the high-enthalpy water molecules by an appropriate guest molecule [9]. By increasing drug availability at the surface of the biological barrier, CDs may act as permeation enhancers [10, 11]. In general, CD molecules do not penetrate biological membranes but act as penetration enhancers by assuring high concentration of dissolved drug at the membrane surface. However, the permeability will decrease if cyclodextrin is added in excess of the concentration needed to solvate the drug. By caging the guest molecules inside the lipophilic cavity, CD acts as a drug carrier that delivers the drug molecule through the aqueous exterior of the membrane [12, 13].

The drug thermodynamic activity in the formulation vehicle as well as its skin/vehicle partition coefficient can significantly affect the CD induced changes in drug permeability. While enhancing apparent drug solubility, the CDs also increase its thermodynamic activity in the vehicle, promoting its permeation and, consequently, its action. In spite of the partition coefficient (e.g., a hydrophobic drug, could be decreased by the hydrophilic CD complexation), the drug solubility will increase and its thermodynamic activity in the vehicle may lead to the permeability increase. 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 [11] that the free CDs permeation 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.

An important characteristic of the use of beta-CDs for anti-acne treatment drifts from the fact that 2,6-dimethyl-beta-cyclodextrin (DM-β-CD) may incorporate the polyunsaturated fatty acids from the skin, increasing the drug permeation and, leading indirectly to the reduction of the infections incidence and cutaneous inflammations [11].

In previous studies, we have demonstrated that it is possible to complex tretinoin with DM-β-CD by different techniques, obtaining a relatively high complexation constant and higher drug solubility. Such successful results could lead to new perspectives of a gel formulation for topical application. In fact, we have already obtained a microbiological and physically stable gel formulation during 1 month [3]. However, the chemical stability was less encouraging, leading us to consider the extemporaneous gel preparation.

The aim of this work was to study minutely the tretinoin release/permeation profiles and skin retention from the free and complexed form gels, and also to determine the influence of thermodynamic activity equalization on these profiles, which in theory is the driving force for the delivery of substances, i.e., the drug permeation from the vehicle and its penetration into the skin membrane [14]. In fact, there is a lack of knowledge on the comparison between release/permeation profiles of different formulations with and without thermodynamic activity equalization.

Materials and methods

Materials

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

Methods

Complex preparation

Dimethyl-β-cyclodextrin (DM-β-CD) (5330 mg) was dissolved in purified water (40 ml) and tretinoin (368 mg) in ethanol (400 ml). The two solutions were mixed in a closed dark glass recipient and stirred with a magnetic stirrer at about 500–700 rpm during 8 days.

Gel formulation

The hydrogels were formulated according to previous work [3]: Glycerine (15 g) and potassium sorbate (0.15 g) were added to purified water (qs ad 100 g) with the tretinoin (either free form (FT) or complexed form (IC)) followed by the addition of hydroxypropylmethylcellulose (HPMC) (1.5 g) under stirring until the swelling was completed. Both, DM-β-CD: tretinoin complex and free tretinoin hydrogel formulations were kept under stirring for 48 h at room temperature protected from light.

HPLC instrumentation and conditions

Tretinoin quantification was performed by HPLC (Hewlett Packard system with a Lichrocart® 250-4, RP18, 5 μm column) under the following conditions: 50 μL injection volume; flow-rate of 1.0 mL/min; run time of 20 min and the mobile phase was composed of 0.01% trifluoroacetic acid solution: acetonitrile (15:85) (method adapted from Tashtoush et al.) [15]. The hydrogels samples were solubilised in acetonitrile and centrifuged at 10,000 rpm.

In vitro release studies

In vitro release profile was determined using vertical Franz diffusion cells with a diffusion area of 0.95 cm^2. A 0.25 g sample of gel was spread over the donor side of the membrane (Tuffryn® 25 mm, 0.45 μm). Tuffryn membranes were soaked for thirty minutes in isopropyl myristate, a surfactant that is representative of skin lipids. The receptor phase, a mixture of saline phosphate buffer (pH 7.4) and 0.1% TAGAT CH 40®, was kept at 37 °C and the volume was accurately determined for each cell in each experiment. TAGAT was used to solubilise tretinoin in the receptor solution. At pre-determined times (1.0, 2.0, 3.0 and 4.0 h), samples were collected and the same volume was replaced with fresh solution. The tretinoin amount in the receptor phase was quantified by HPLC. Data was expressed as cumulative amount of tretinoin released and diffused through the membrane filter, considering the total amount of drug applied in each formulation (gels with free tretinoin and with complexed tretinoin).

Solubility of tretinoin in the receptor phase was previously determined to ensure that sink conditions were maintained throughout the study. It was added an excess amount of tretinoin to the receptor phase, keeping the oversaturated mixture under stirring (1000 rpm) for 48 h protected from light. An aliquot was centrifuged at 10,000 rpm for 5 min using a microcentrifuge (SIGMA 112, B. Braun Biotech International). An aliquot of the supernatant was filtered and injected into the HPLC system to determine the solubility limit of tretinoin in the receptor phase. The term *sink conditions* is defined as the volume of medium at least greater than three times that required to form a saturated solution of a drug substance [16]. In this study, the sink conditions were considered valid for tretinoin concentrations below 10% of its solubility in the receptor phase.

Equalization of thermodynamic activity

Thermodynamic activity (TA) was estimated using the simplified relationship between the concentration and the solubility of tretinoin in the hydrogel formulation (TA $\approx$ Concentration/Solubility). In order to determine the solubility of tretinoin in the hydrogels, an excess amount of tretinoin was added to a sample (0.5 g) of each formulation (free tretinoin and complexed tretinoin). The saturation point was detected visually. The oversaturated mixtures were kept under stirring (1000 rpm) for 48 h protected from light and then the tretinoin was extracted and quantified by HPLC. A new formulation was prepared having the same thermodynamic activity as the complexed tretinoin formulation. The in vitro release profile of the new gel was determined as described before.

Statistical analysis

The results are expressed as mean $\pm$ standard deviation and all of these experiments were statistically analyzed by analysis of variance (ANOVA—two factors with replication). The differences were considered statistically significant when $P < 0.05$.

Permeation and skin retention study

The in vitro permeation and skin retention profiles were determined by using vertical Franz diffusion cells with a diffusion area of 1.76 cm^2. A 0.3 g sample of gel was spread over the skin. Fresh pig ears, which have not gone through the scalding procedure, were used for this study. The skin was removed and then sliced by using a dermatome. The receptor phase was a mixture of saline phosphate buffer (pH 7.4), 0.5% Volpo N20®, 5% ethanol and 0.5% ascorbic acid. After 24 h, the tretinoin amount in the

donor and receptor phase were quantified by HPLC. Skin retention data was expressed as the amount of tretinoin retained in the full skin, considering the total amount of drug applied in each formulation (gel with free tretinoin and with complexed tretinoin).

Results and discussion

In vitro release studies with synthetic membranes of the formulations with non-equalized thermodynamic activity

In vitro release studies were performed for four consecutive days (three samples of each gel per day) and the results are represented in Figs. 2, 3. As showed in Fig. 2, the 2nd hour of the first day results are considerably different from the others and for this reason they were excluded from the overall results in Fig. 3. In fact, it was considered this mean value as an outlier.

Sink conditions were just maintained until the 4th hour of the study. This fact is important as it ensures that the drug diffusion rate into the receptor fluid does not become the rate-limiting step. The complexed tretinoin gel had a higher diffusion through the synthetic membrane than the free tretinoin gel. The results confirm that CDs can act as permeation enhancers. In fact, the relatively large sizes of the molecules do not make possible complexes to diffuse through the membrane. Therefore, CDs act by keeping tretinoin molecules at the surface of the synthetic membrane (Fig. 4), which leads to an increase of the tretinoin release rate by the higher availability of the drug at the membrane surface [10]. In general, permeation through membranes involves drug dissolution in the vehicle, diffusion of the solubilised drug from the swollen polymer matrix and finally, drug permeation through the membrane. All three processes make a contribution to the overall diffusion rate [17]. The results showed that complexation increased the overall tretinoin diffusion by increasing the amount of diffusible species in the donor phase by enhancing drug solubility. Although the complex could not penetrate, tretinoin in the inclusion complexes was in a dynamic equilibrium with free drug, thus continuously supplying more tretinoin molecules to permeate the

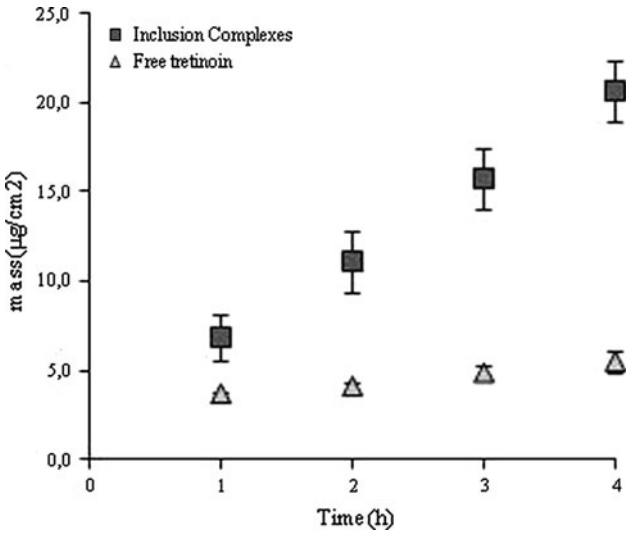

Fig. 3 Mean released mass ± standard deviations of free tretinoin gel and complexed tretinoin gel through synthetic membranes over 4 h of 4 days, in sink conditions

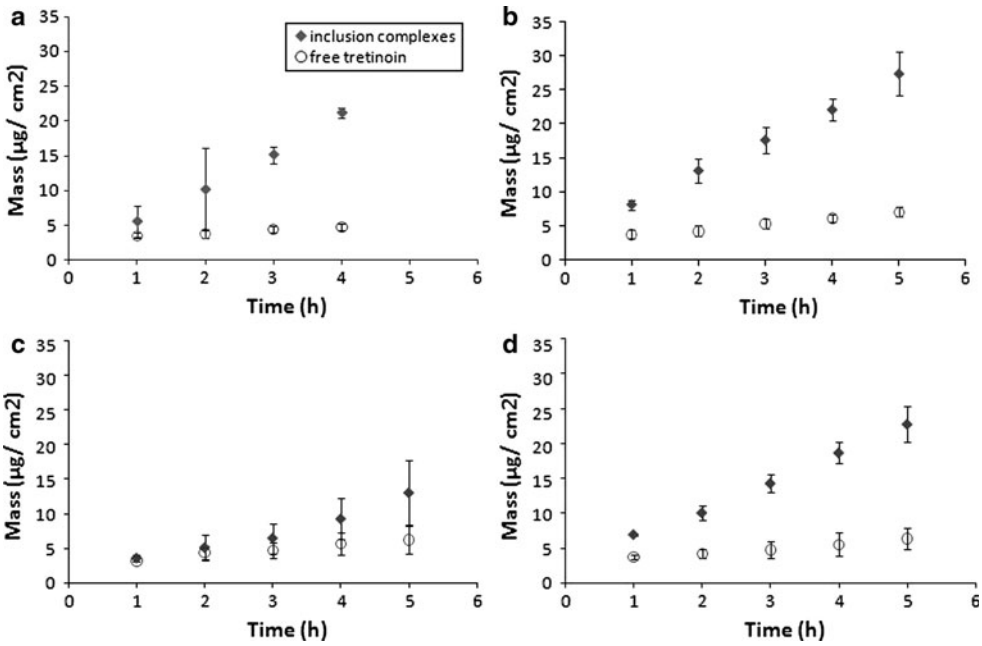

Fig. 2 Mean released mass ± standard deviations of tretinoin from free tretinoin (*open circle*) and inclusion complexes (*filled diamond*) gels through synthetic membranes. Results are presented per day (**a** day 1; **b** day 2; **c** day 3; **d** day 4) and over 4 h ($n = 3$ per day)

membrane. Therefore, DM-β-CD complexation increased tretinoin concentration gradient over the membrane and this resulted in an increased flux.

The statistical analysis of these results confirms that the complexed and free tretinoin gels release rates were significantly different from each other ($P < 0.05$).

In vitro release studies with synthetic membranes of the formulations with equalized thermodynamic activity

The results of in vitro release studies of the gels with equalized thermodynamic activity (TA) are shown in Fig. 5. It should be mentioned that the difference between Figs. 3 and 5 resides only in the results of the free tretinoin gel because the equalization of thermodynamic activity was performed by adding more tretinoin to one gel than the other (complexed tretinoin gel) which remained with the same amount of tretinoin.

By equalizing the thermodynamic activity of both gels, this factor had no longer an influence on the release rates of the two gels. Therefore, these results show that, although the release rate of free tretinoin increased compared to the non-equalized TA free tretinoin gel (as it can be seen in Fig. 6), the complexed tretinoin gel still has an higher release rate (Fig. 5). This indicates that the role of CDs in release/permeation is not primarily related to the thermodynamic activity variation.

All formulation fluxes are represented in Fig. 7. Theoretically, according to Modelling Enhancer Activity calculations (SPSS program), the tretinoin flux would be about 0.9 ($\mu g/cm^2$)/h which is similar to the obtained values (0.6–1.3 ($\mu g/cm^2$)/h) for free form.

The statistical analysis of the results and the comparison with the previous results confirms that the release rates of free tretinoin gels with equalized TA and with non-equalized TA are significantly different from each other ($P < 0.05$). However, the difference between the release rates of the two free tretinoin gels (with equalized and non-equalized TA) is much smaller than the difference between the release rates of the complexed gel and the free tretinoin gel with equalized TA. This means that in this case, the main driving force for diffusion of tretinoin when complexed with CDs is not the thermodynamic activity.

Thermodynamic activity is strictly related to the drug solubility in the vehicle. In theory, CDs solubilise water-insoluble drugs like tretinoin and deliver the drug molecules at the barrier surface. Thus, drug delivery from CDs in an aqueous environment depends not only on the solubility of tretinoin in the vehicle (i.e. the thermodynamic activity), but also on other factors, like the binding properties to the vehicle, the partition coefficient between the

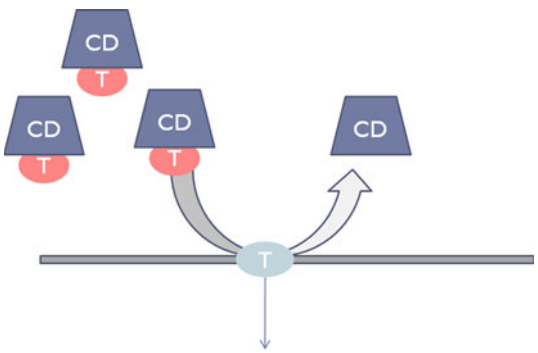

Fig. 4 Schematic representation of increased tretinoin concentration gradient over the membrane surface by complexation

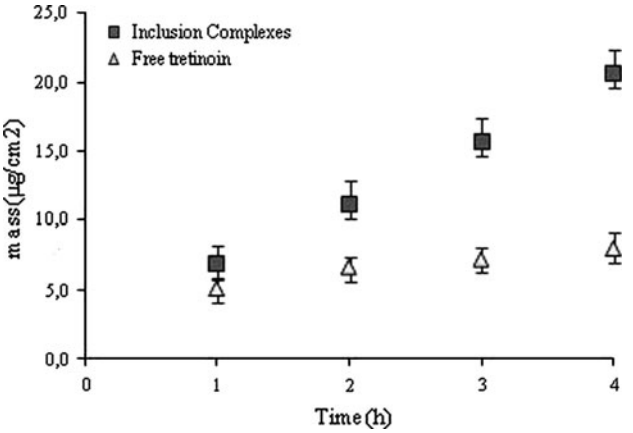

Fig. 5 Mean released mass ± standard deviations of complexed tretinoin gel and free tretinoin gel with equalized Thermodynamic Activity (TA) through synthetic membranes over 4 h, in sink conditions ($n = 12$ for the free tretinoin gel and $n = 9$ for the complexed tretinoin gel)

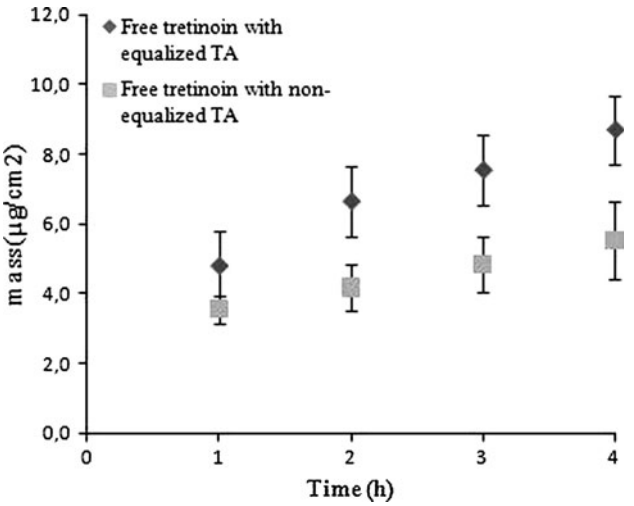

Fig. 6 Mean released mass ± standard deviations of free tretinoin gel with non-equalized Thermodynamic Activity (TA) and free tretinoin gel with equalized TA through synthetic membranes over 4 h in sink conditions ($n = 12$ for the free tretinoin gel with equalized TA and $n = 9$ for the free tretinoin gel with non-equalized TA)

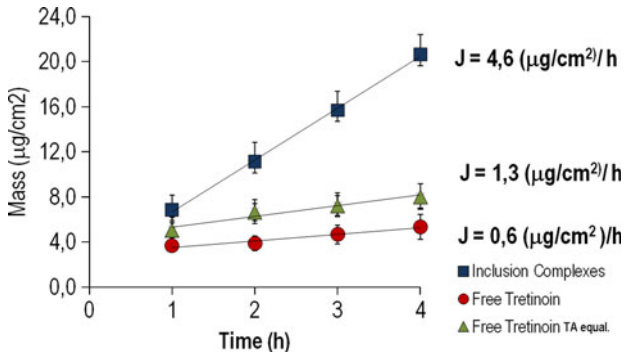

Fig. 7 Fluxes (J) of all tretinoin formulations: inclusion form, free form and free form with Equalized Thermodynamic Activity, respectively

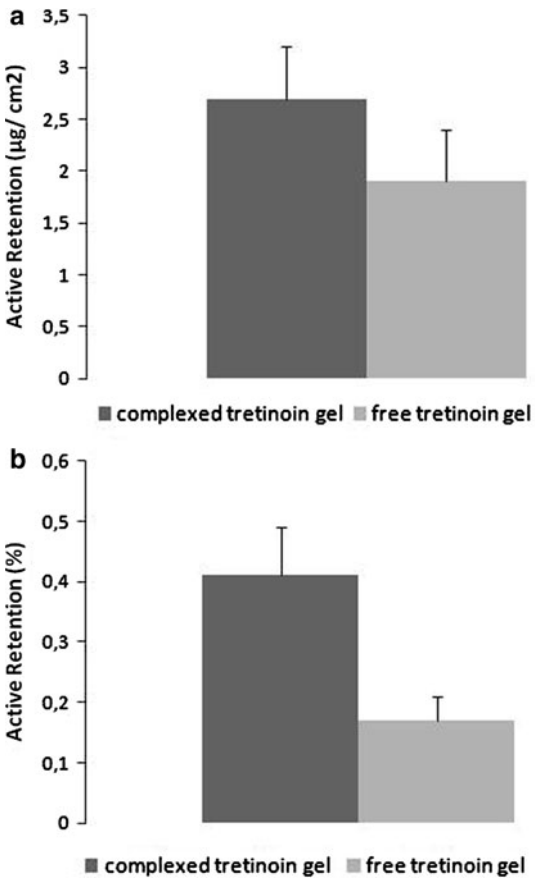

Fig. 8 Cutaneous retention of complexed tretinoin gel and free tretinoin gel after 24 h of study (Mean values ± SD, $n = 6$ for each formulation). **a** Values of the amount of retained active substance per cutaneous permeating area; **b** Values of the amount of retained active in relation to the total active contained in the sample applied in the study

vehicle and the membrane, the affinity of CDs to the membrane and the interaction between CDs and some components of the membrane.

In vitro permeation and skin retention study of formulations with equalized thermodynamic activity

The results of the tretinoin skin retention (Fig. 8) show that the samples of complexed tretinoin gel and free tretinoin gel had a skin retention of 2.7 ± 0.5 and 1.9 ± 0.5 µg/cm^2, respectively. The percentage of total drug that had been retained in the skin was 0.41 ± 0.08% for complexed tretinoin gel and 0.17 ± 0.04% for the free tretinoin gel. In fact, it is well known that CDs can extract lipids, such as cholesterol, from the skin barrier leading to an increase of the drugs permeability [11, 18].

However, it should be noted that after 24 h, it was not detected any drug amount in the receptor phase. Thus, it seems that these formulations have a dermal instead of a transdermal effect.

Similar results were obtained for isotretinoin in another study*:* in vitro tape stripping in human and pig skin showed that no isotretinoin reaches the receptor compartment for both formulations up to 8 h [19].

Conclusion

This study has demonstrated that the inclusion of tretinoin in DM-β-CDs has enhanced the release rate from hydrogel formulations which is important for in vivo skin absorption and further interaction with retinoid receptors. This is likely to be related with the tretinoin availability near the membrane surface. This study has also demonstrated that thermodynamic activity is not the driving force for the improvement of the tretinoin permeation rate by CDs.

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