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ORIGINAL ARTICLE

Photostability and solubility improvement of β-cyclodextrin-included tretinoin

Carla Caddeo · Maria Manconi · Donatella Valenti · Elena Pini · Chiara Sinico

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Abstract In this work, we investigated the influence of β-cyclodextrin on the photostability of tretinoin and compared the photo-chemical stability of tretinoin, either in methanol or complexed with β-cyclodextrin, when exposed both to UV and fluorescent light. The physico-chemical characterization of tretinoin-β-cyclodextrin complexes, prepared by the freeze-drying process, using different tretinoin:β-cyclodextrin molar ratios (1:1 and 1:3), was carried out in solution by phase solubility studies, ^{1}H-NMR spectroscopy, and in solid state by infrared spectroscopy (FT-IR); these analyses confirmed the existence of an inclusion compound. Solubility study results showed that tretinoin solubility was enhanced by inclusion in β-cyclodextrin as a function of increasing concentrations of β-cyclodextrin in aqueous solution at different pH values (i.e., 3.0, 5.5, and 7.0). Moreover, the complexation of the tretinoin with β-cyclodextrin effectively protected the photolabile drug and reduced the degradation of tretinoin induced by UV and fluorescent light, improving its photochemical stability in comparison with free drug in methanol. Indeed, dissolved tretinoin in methanol degraded very quickly and completely, while β-cyclodextrin-included tretinoin decomposition was delayed and, after 30 days under UV exposure, the percentage of remaining drug was about 20–25% (depending on the tretinoin concentration). The photodegradation of tretinoin in methanol under fluorescent light was slower: after 5 days of irradiation it reached a photostationary state and intact tretinoin remained constant (6.6%). In conclusion, the β-cyclodextrin complexation always led to a reduction of degradation, depending on the tretinoin:β-cyclodextrin molar ratio and on the drug concentration (0.2 mg/ml or 0.4 mg/ml).

Keywords β-Cyclodextrin · Inclusion complex · Phase solubility study · Photostability study · Tretinoin

C. Caddeo · M. Manconi · D. Valenti · C. Sinico (✉)
Dipartimento Farmaco Chimico Tecnologico, Università di Cagliari, Via Ospedale 72, 09124 Cagliari, Italy
e-mail: sinico@unica.it

E. Pini
Istituto di Chimica Organica "A. Marchesini", Università di Milano, Via Venezian 21, 20133 Milano, Italy

Introduction

As widely reported in literature, tretinoin or all-trans retinoic acid (TRA) is an effective drug used in the treatment of acne vulgaris, psoriasis, photo-aged skin, neoplasia and other skin disorders. In particular, well-known is its capability to increase epithelial cell turnover [1], collagen synthesis and epidermal growth in the cicatrization process, as well as its property to reduce sebum production [2]. The therapeutic use of TRA is limited to topical application, because several and severe side effects arise when systematically administered. However, unpleasant side effects, such as irritation of treated area in form of scaling, erythema, burning, and stinging [3], can also occur in the topical administration.

Moreover, it is well-known that TRA is susceptible to oxidation when exposed to air; also, it is thermally unstable and isomerizes in solution upon exposure to light. More specifically, Motto et al. [4] proved that the irradiation of a TRA solution leads to the formation of nine different isomers, the most abundant of which is isotretinoin: although

this isomer is active, its effects are very much reduced with respect to TRA. It is also known that a large amount of TRA, locally applied on skin surface, degrades when exposed to sunlight for 1–2 h [3]. The degradation products of a photolabile drug following light exposure (sunlight or artificial light, i.e. fluorescent light) can cause a loss of potency during storage or application [5, 6], resulting in photoreactions such as irritation and allergy [7, 8].

Therefore, as we already reported in previous works [9, 10], we focused our attention on the study of the TRA photostability, with the aim of finding topical delivery systems capable of protecting this photolabile drug and reducing the light induced decomposition. More precisely, we studied the photodegradation of TRA in methanol and in vesicular suspensions exposed both to UV and artificial light. A comparison work of our results showed that the incorporation of TRA in vesicles (niosomes and liposomes) reduces the photodecomposition process of the drug; we also found that the photoprotection offered by vesicles is dependent on their structure and composition [10].

Several researchers have demonstrated that such an improvement in terms of photoprotection can also be obtained by means of the inclusion of a photolabile drug in cyclodextrins [11–13].

Cyclodextrins (CDs) are cyclic oligosaccharides consisting of six (α-CDs), seven (β-CDs), eight (γ-CDs) α-1,4-linked D-glucopyranose units, with a hydrophilic outer surface and a hydrophobic central cavity of fixed size and shape into which non polar molecules can be accommodated [14, 15]. This inclusion complexation has been shown to improve, as well as the stability of labile compounds, the aqueous solubility and dissolution rate of poorly water-soluble drugs, like TRA [16–19]. Moreover, improved efficacy and tolerability of TRA-β-CD complexes in the treatment of acne vulgaris was demonstrated in the work of Anadolu et al. [20]: in particular, topical use of TRA-β-CD formulations does not significantly reduce sebum secretion but may help to preserve optimum epidermal moisture content.

The present work deals with the preparation and the characterization of the inclusion complex between TRA and β-cyclodextrin. The TRA-β-CD complexes (prepared by the freeze-drying process) were characterized by ^{1}H nuclear magnetic resonance spectroscopy (^{1}H-NMR) and by infrared spectroscopy (FT-IR), confirming the formation of the inclusion complexes. We also performed phase solubility studies, demonstrating that β-CD complexation actually enhances the TRA solubility. Moreover, we studied the effect of this complexation on the photochemical stability of TRA. Data from photodegradation studies confirmed that decomposition of cyclodextrin-included TRA by UV and fluorescent light irradiation is delayed with respect to free drug in methanol.

Experimental

Materials

β-cyclodextrin was kindly supplied by Roquette Co., France. Phosphate buffer (PBS) pH 7 was obtained from Carlo Erba Reagents (Rodano, Italy). Trans retinoic acid and all the other products were of analytical grade and were purchased from Sigma Aldrich, Milano, Italy.

Preparation of solid binary systems

TRA-β-cyclodextrin binary systems were prepared at 1:1 and 1:3 molar ratio, by means of a freeze-drying procedure [21, 22]. To prepare the freeze-drying mixture the required stoichiometric amounts of TRA and β-CDs were accurately weighed and dispersed in 2 ml of water until semisolid systems were obtained and then stored in a nitrogen atmosphere for 12 h. Next, products were dissolved in 400 ml of distilled water under mechanical stirring for 120 min at 40–50°C. After filtration, the filtrate was frozen at –15/–20°C and then freeze-dried for 24 h at –70°C and 60 mm Hg, using a Freeze-Dryer Criotecnica MMCOTA Company (Rome, Italy).

All binary systems were prepared under yellow light and kept in the dark at all times.

Infrared spectroscopy

FT-IR spectra of TRA, β-cyclodextrin, corresponding inclusion compound and physical mixture (1:1) obtained by mixing equimolar amounts of the pure compounds, were recorded by using a Perkin Elmer FT-IR Spectrometer Spectrum One in a spectral region between 4000 and 450 cm^{-1}. Samples were mixed in a mortar with KBr (1:100) and pressed in a hydraulic press (14 tons) to small tablets, which were then placed in the infrared beam.

Nuclear Magnetic Resonance studies

Proton NMR spectra of inclusion complexes, β-cyclodextrin and TRA were taken on a Bruker Avance 500 operating at 500 MHz. Chemical shifts were expressed as ppm (δ). Samples of about 5 mg were dissolved in 0.7 ml D_2O.

HPLC method

TRA and isotretinoin were determined at 353 nm using a liquid chromatograph Alliance 2690 (Waters), equipped with a photodiode array detector and a computer integrating apparatus (Millennium 32). The column was a Lichrospher® 100 RP 18 column (60 Å, 5 μm, Waters). The mobile phase was a mixture of acetonitrile, water and

acetic acid (90:9.7:0.3, v/v), delivered at a flow rate of 1.2 ml/min.

Phase solubility studies

Phase solubility studies were carried out according to the method described by Higuchi and Connors [23]. In particular, in solutions at pH 7.0, 5.5, and 3.0 (phosphate buffer solution, pH 7.0; bidistilled water, pH 5.5; bidistilled water with HCl, pH 3.0), 5 mg (0.016 mmoles) of TRA were added to 5 ml of aqueous solutions containing different concentrations of β-CD (ranging from 0 to 16 mM): this quantity of TRA exceeds its solubility throughout the whole experiment. Suspensions were vigorously stirred at 25 ± 1°C for 7 days. When equilibrium was reached (7 days), suspensions were centrifugated (Mikro 200, Hettich Zentrifugen, Germany) and the supernatant was withdrawn and analyzed by UV spectrophotometer (U-2000, HITACHI) set at 700 nm. We used turbidimetry as a detection method for the concentration of particles suspended in a solution which scatters the incoming light: the more turbid the solution, the more incoming light will be absorbed. Therefore, each suspension was centrifugated until the UV-absorbance decreased to zero. Subsequently, samples (100 µl) suitably diluted with methanol (1:20) were analyzed, for quantitative determination of TRA, by high performance liquid chromatography (HPLC), according to the conditions previously reported. The stock standard solution of TRA (1 mg/ml) was prepared by dissolving the drug in methanol and storing it at 4°C. A standard calibration curve (peak area of TRA versus known drug concentration) was built up by using standard solutions (10–100 µg/ml) prepared by dilution of the stock standard solution with the mobile phase. Calibration graphs were plotted according to the linear regression analysis, which gave a correlation coefficient value (R^2) of 0.999. Sample preparation and analyses were carried out at room temperature.

Data obtained by HPLC represented the mean values of three separate determinations.

Phase solubility diagrams were plotted and the apparent stability constants (Kc) were calculated according to the following equation:

$$Kc = \alpha/S_0(1 - \alpha)$$

where S_0 represents the solubility of TRA and α represents the slope of the straight line.

Photodegradation studies

UV irradiation

The degradation of TRA was studied using a UV lamp set at 366 nm (Min UVIS, Desaga, GmbH, Germany). The TRA methanolic solution and the TRA-β-CD complexes aqueous solution (2 ml in a glass flask) were maintained at room temperature and exposed to UV irradiation from a 30W lamp (366 nm) for 1 h at a fixed distance of 10 cm. At regular time intervals, samples were first stirred and then 50 µl aliquots of the dispersions were removed and diluted with methanol (1:20) in order to quantify the TRA concentration.

Experiments were carried out on samples with different initial TRA concentrations (i.e. 0.2 mg/ml and 0.4 mg/ml). Samples were taken every ten minutes for 1 h and then the analysis was extended to 30 days, because of the slow photolysis, and samples were taken every day. All experiments were carried out at 25 ± 1°C.

Fluorescent light irradiation

Stability testing in which the formulations were exposed to artificial light was also carried out. Samples (2 ml in a glass flask) were kept at room temperature and irradiated with the fluorescent light, at first for 24 h and then the exposure was extended to 30 days. Six continuously illuminated fluorescent tubes (58 W) were used. The overall light source intensity was 460 Lux. At regular time intervals (every 10 min for 1 h and then after 1, 2, 4, 6, 8, 24 h irradiation) samples were stirred and then 50 µl aliquots of the dispersions were removed and diluted with methanol (1:20) in order to quantify the TRA concentration.

Statistical analysis of data

Data analysis was carried out with the software package Microsoft Excel, version 2003. Results are expressed as mean ± standard error (3 independent samples). Statistically significant difference was determined using the Student's *t*-test and analysis of variance (Anova) with $p = 0.05$ as a minimal level of significance.

Results and discussion

Infrared spectroscopy

The infrared analysis of inclusion compounds was difficult because of the strong host absorption overlapping with the bands of the guest. However, some differences can be highlighted.

Figure 1 shows infrared spectra of TRA, β-cyclodextrin, corresponding inclusion compound and physical mixture in the most significant spectral regions.

In particular, in the range of 2000–4000 cm^{-1}, TRA (b) shows the absorption bands system due to Csp^3-H symmetric and asymmetric stretchings. In this region, β-cyclodextrin (d), beside the intense broad band of the

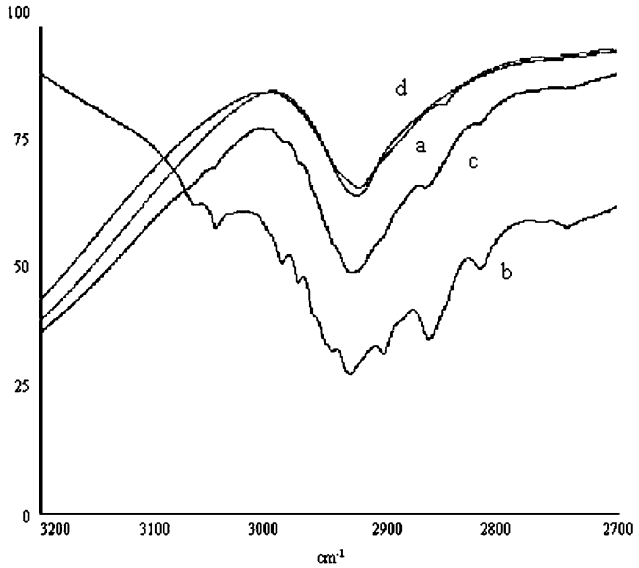

Fig. 1 IR spectra of tretinoin (**a**), β-CD (**b**), physical mixture (**c**) and inclusion complex (**d**)

associated OH, presents another broad band at 2926 cm^{-1} due to C–H vibrations. The physical mixture infrared analysis (c), as reported by Montassier et al. [24], is the superposition of the pure products with the attenuation of the TRA signals.

In the complex spectrum (a), we found the broad band of the host at 2926 cm^{-1}, and a new band detectable at 2851 cm^{-1}: this is clear sign of the TRA C-H stretching shifted by the interaction with cyclodextrin.

Moreover, (figures not reported) we found that, in accordance with this evidence, in the C=O and C=C stretching region the inclusion complex (a), in addition to the broad band of O–H bending (1644 cm^{-1}) typical of cyclodextrin, shows a shoulder at 1691 cm^{-1} imputable to the C=O TRA stretching. Furthermore, as a consequence of the interaction with TRA, the cyclodextrin band in the complex shifts from 1259 cm^{-1} to 1245 cm^{-1}. At this absorption frequency, physical mixture shows an intense band typical of TRA.

Nuclear Magnetic Resonance studies

NMR spectroscopy has become one of the most important methods for structural elucidation of inclusion complexes.

^{1}H-NMR spectra of β-cyclodextrin (a) and TRA-β-CD inclusion compounds (b) are reported in Fig. 2. Since mere shifts of protons and no new signals were observed, it is

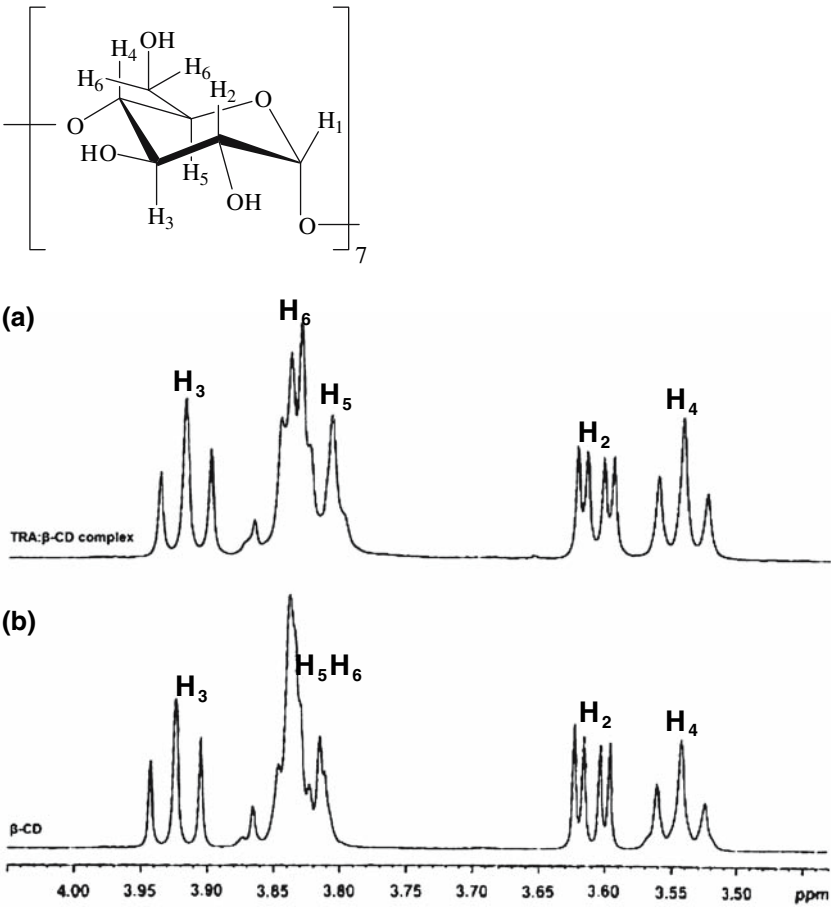

Fig. 2 NMR spectra of β-CD (**a**) and tretinoin: β-CD inclusion complex (**b**). Chemical shift displacements ($\Delta\delta$, ppm): H_3 $\Delta\delta$–0.006; H_5 $\Delta\delta$–0.008; H_2 $\Delta\delta$–0.001; H_4 $\Delta\delta$ + 0.001

justified to assume that, under the working conditions, complexation-decomplexation is a dynamic process with the same time scale as NMR [25].

The difference of the hydrogen chemical shift values concerning β-cyclodextrin in free and complexed state indicates the penetration of TRA into the cyclodextrin cavity and hence its complexation. Indeed, the protons located outside the cyclodextrin cavity, H_1, H_2, show a low upfield shift [26]. H_6 proton signal, which in free cyclodextrin overlaps with H_5, becomes more visible because of the inclusion, while the protons located inside the cavity, H_3 and H_5, are appreciably shifted.

Phase solubility studies

Fig. 3 presents the phase solubility plots for TRA and increasing concentrations of β-cyclodextrins in solutions at different pH values (ie, 3.0, 5.5, and 7.0) at 25°C.

As can be seen, it shows A_L type solubility diagrams as the TRA solubility increases with increasing β-CD concentrations, according to the classification established by Higuchi and Connors [23].

Table 1 summarizes the TRA solubility, slopes, stability constants and correlation coefficients of the phase solubility diagrams (as a function of pH value). Results evidenced that the TRA solubility is enhanced (up to 4 times at higher β-CD concentration, pH 7.0, Fig. 3) by the β-CD inclusion complexation. Moreover, since the slope of the diagrams is less than 1, the complex stoichiometry was assumed to be 1:1, at different studied pH values [17, 28]. Table 1 also shows that the Kc values increase as the pH increases, as a consequence of the lipophilic character of the drug, which is a weak carboxylic acid (pK_a = 7.85) partially dissociated at pH 5.5 and 7.0. Therefore, while at pH 3.0 the TRA solubility enhancement is attributable to the β-CD solubilizing ability, at pH 5.5 and 7.0 it is due to the combined effects of ionization and β-CD complexation.

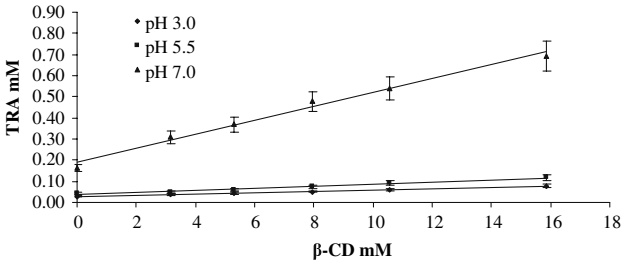

Fig. 3 Combined effects of pH and β-CD concentration on the total solubility of tretinoin at 25°C (mean ± SD, n = 3, vertical bar)

Table 1 Solubility of tretinoin (S_0), slope (α), stability constant (Kc), and correlation coefficient (R^2)

Medium	S_0 ± SD ($\times 10^{-3}$M)	α	Kc (M^{-1})	R^2
pH 3.0	0.0282 ± 0.0022	0.0031	110.28	0.9951
pH 5.5	0.0384 ± 0.0031	0.0049	128.27	0.9814
pH 7.0	0.1917 ± 0.018	0.0328	176.90	0.9885

Noy [29] reported that the apparent pKa of TRA is affected by the concentration of the acid. At physiological pH and concentration higher than CMC TRA self-associates by hydrophobic interactions between the rings of TRA molecules, resulting in the formation of negatively charged micelles that raise the pKa by ~ 2 units. Moreover, Noy refer that this anionic form of TRA is soluble at the above-mentioned concentration. Therefore, as the TRA concentration in our study is considerably higher than the CMC, it is justified to claim that the observed TRA solubility improvement at pH 7.0 can be due to the micelles formation, in addition to the combined effects of the ionization and β-CD complexation.

We point out that, in the literature (for instance, see [24, 30]), various authors found higher values for the stability constants together with a relevant enhancement of the solubility: however, experimental conditions (in particular, dissolution medium or preparation method) were different. Therefore, we stress the fact that all findings concerning these parameters have to be considered only in connection with their specific experimental setting.

Photodegradation studies

The degradation of TRA was followed by HPLC measurements of the remaining concentration of drug in a methanolic solution first, and then in the TRA-β-CD complexes in aqueous solution under both UV and fluorescent light irradiation.

TRA was dissolved in methanol because of its extremely low water solubility.

UV irradiation

The percentage of free and β-CD-complexed TRA concentrations (obtained by HPLC analyses) were plotted as a function of time, as shown in Fig. 4a, b.

The TRA decomposition was very fast in methanol. Indeed, after 1 h of UV light exposure (Fig. 4a), the more diluted methanolic solution of TRA (0.2 mg/ml) showed the higher loss (70%) of the initial TRA concentration, while in the more concentrated one the percentage of left intact TRA was about 58% (Table 2).

The irradiation was carried out for 30 days, but after just 7 days, TRA was completely degraded (Fig. 4b).

The TRA degradation rate significantly decreased when the TRA was complexed with β-CD.

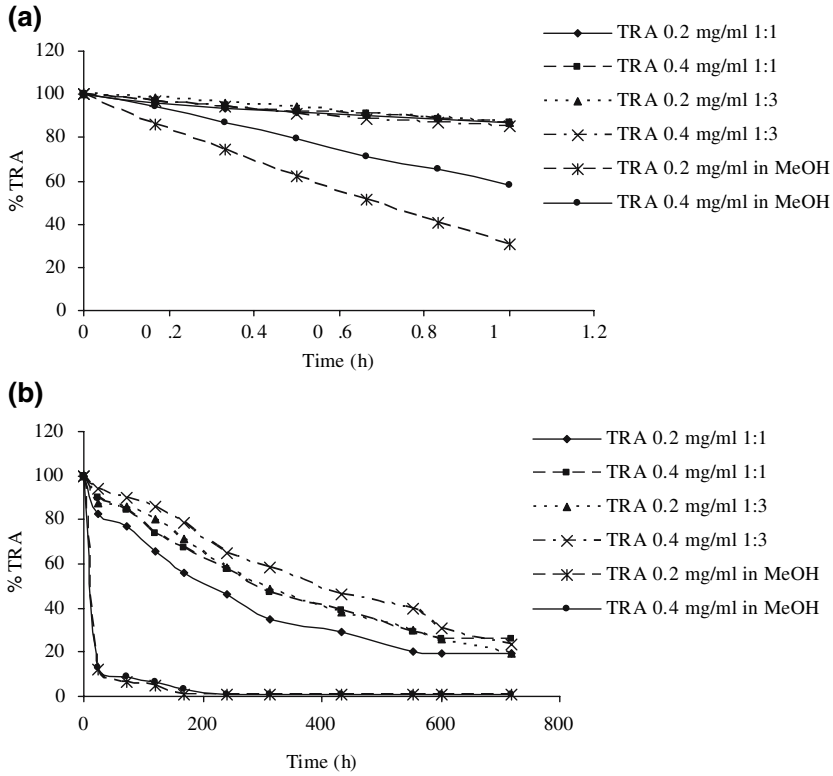

Fig. 4 Photodegradation of tretinoin (0.2 mg/ml and 0.4 mg/ml) in β-CD complex (1:1 and 1:3 molar ratio) and in methanol after 1 h (**a**) and 30 days (**b**) of UV irradiation (λ = 366 nm)

Table 2 Percent left drug from free and β-CD-complexed tretinoin exposed to UV light for 1 h and for 30 days

Sample	Molar ratio	TRA 0.2 mg/ml 1 h UV exposure % left	TRA 0.4 mg/ml 1 h UV exposure % left	TRA 0.2 mg/ml 30 days UV exposure % left	TRA 0.4 mg/ml 30 days UV exposure % left
Methanol	–	30.3 ± 2.7	58.3 ± 4.1	1.0 ± 02	1.0 ± 0.3
TRA-β-CD	1:1	87.0 ± 5.4	87.1 ± 6.0	19.7 ± 2.3	25.8 ± 2.1
TRA-β-CD	1:3	87.0 ± 6.2	85.3 ± 6.5	19.8 ± 1.8	23.2 ± 1.7

In fact, after 1 h of irradiation the tretinoin percentage loss was 13–15% (Fig. 4a), it remained constant until 24 h and after 30 days of irradiation (Fig. 4b) the left TRA was about 20% for TRA-β-CD complex (TRA 0.2 mg/ml) and 23–26% for TRA-β-CD complex (TRA 0.4 mg/ml), reaching a photostationary state (Table 2).

These results demonstrated the effectiveness of β-cyclodextrins complexation in improving the photostability of TRA, especially for the TRA-β-CD complex at 1:3 molar ratio, which was more efficient in the photoprotection of TRA, preventing and reducing the drug oxidation and isomerization processes.

Fluorescent light irradiation

The photodecomposition of both free and β-CD-complexed drug was also studied when exposed to fluorescent light. The results of this study, obtained by HPLC analyses and summarized in Fig. 5 and Table 3, showed that the TRA degradation rate under fluorescent light exposure was slower than under UV light exposure.

After 24 h of fluorescent light irradiation, the content of TRA in a methanolic solution was 40% and 42%, depending on the TRA concentration (respectively: 0.2 mg/ml–0.4 mg/ml).

This irradiation-induced decomposition was significantly reduced by inclusion of TRA into the β-cyclodextrin cavity. In fact, the amount of left intact TRA in the TRA-β-CD complex at 1:1 molar ratio after 1 h irradiation (data not shown) was still 100% and then, after 24 h irradiation, left intact TRA was about 91%. While the degradation of the TRA in the TRA-β-CD complex at 1:3 molar ratio started after 4 h irradiation (data not shown) and after 24 h irradiation the left intact TRA was 88–85%, depending on the TRA concentration (respectively: 0.2 mg/ml–0.4 mg/ml).

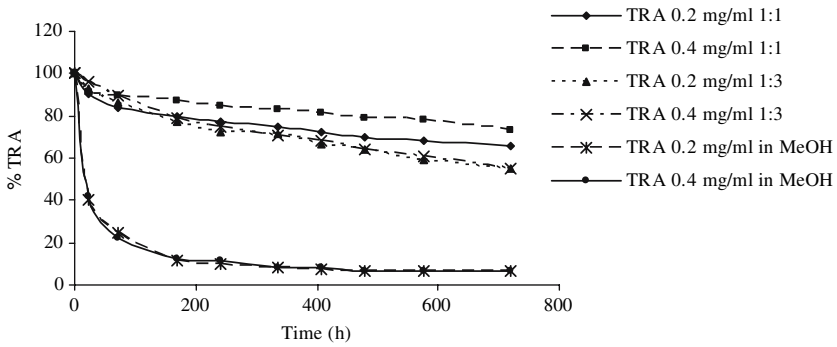

Fig. 5 Photodegradation of tretinoin (0.2 mg/ml and 0.4 mg/ml) in β-CD complex (1:1 and 1:3 molar ratio) and in methanol after 30 days of fluorescent light irradiation

Table 3 Percent left drug from free and β-CD-complexed tretinoin exposed to fluorescent light for 30 days

Sample	Molar ratio	TRA 0.2 mg/ml % left	TRA 0.4 mg/ml % left
Methanol	–	6.6 ± 0.8	6.6 ± 1.0
TRA- β-CD	1:1	66.0 ± 5.4	73.3 ± 6.1
TRA- β-CD	1:3	55.2 ± 6.7	54.9 ± 6.5

Because of the slow degradation, the samples were kept under irradiation for 30 days.

The TRA remaining in the methanolic solution, after 14 days irradiation, was about 10% and it decreased slowly until 30 days of study, reaching 6.6% (as shown in Table 3) for both the two different drug concentrations (0.2 mg/ml–0.4 mg/ml). As for the β-CD-complexed TRA, the degradation continued slowly reaching, after 30 days irradiation, 55% (of the initial TRA) for the TRA-β-CD complex at 1:3 molar ratio and 66–73% for the TRA-β-CD complex at 1:1 molar ratio, depending on the TRA concentration. Deviations from mean value in these measurements (not reported in the plots for the sake of clarity) are in the order of magnitude of ±10%: ANOVA analysis (with $p = 0.05$ as a minimal level of significance) showed that the differences between 1:3 and 1:1 molar ratios complexes are not statistically significant, while the increase of stability of both complexes in comparison to free TRA was confirmed.

In conclusion, our results show that, as a consequence of the host-guest interaction, β-cyclodextrins do protect TRA, reducing photo-degradation effects with respect to the free drug, as evidenced by the increase of left intact TRA after both UV and fluorescent light exposure.

References

1. Mandy, S.A., Thorne, E.G.: A comparison of the efficacy and safety of tretinoin cream 0.025% and 0.05%. Adv. Ther. **7**, 94–99 (1990).
2. Elias, P.M.: Epidermal effects of retinoids. Supramolecular observations and clinical implications. J. Am. Acad. Dermatol. **15**, 797–809 (1986).
3. Elbaum, D.J.: Comparison of the stability of topical isotretinoin and topical tretinoin and their efficacy in acne. J. Am. Acad. Dermatol. **19**, 486–491 (1988).
4. Motto, M.G., Facchine, K.L., Hamburg, P.F., Burinsky, D.J., Dunphy, R., Oyler, A.R., Cotter, M.L.: Separation and identification of retinoic acid photoisomers. J. Chromatogr. **481**, 255–262 (1989).
5. Connors, K., Amidon, G., Kennon, L.: Chemical Stability of Pharmaceuticals. John Wiley and Sons, New York (1979).
6. Carstensen, J.: Drug Stability: Principles and Practices, Marcel Dekker, New York (1990).
7. Deflandre, A., Lang, G.: Photostability assessment of sunscreens. Benzylidene camphor anddibenzoylmethane derivatives. Int. J. Cosmet. Sci. **10**, 53–62 (1988).
8. Dromgoole, S.H., Maibach, H.I.: Sunscreening agent intolerance: contact and photocontact sensitization and contact urticaria. J. Am. Acad. Dermatol. **22**, 1068–1078 (1990).
9. Manconi, M., Sinico, C., Valenti, D., Loy, G., Fadda, A.M.: Niosomes as carriers for tretinoin I. Preparation and properties. Int. J. Pharm. **234**, 237–248 (2002).
10. Manconi, M., Sinico, C., Valenti, D., Loy, G., Fadda, A.M.: Niosomes as carriers for tretinoin II. Influence of vesicular incorporation on tretinoin photostability. Int. J. Pharm. **260**, 261–272 (2003).
11. Loukas, Y.L., Jayasekera, P., Gregoriadis, G.: Characterization and photoprotection studies of amodel γ-cyclodextrin-included photolabile drug entrapped in liposomes incorporating lightabsorbers. J. Phys. Chem. **99**, 11035–11040 (1995).
12. Loftsson, T., Brewster, M.E.: Pharmaceutical applications of cyclodextrins. 1. Drug solubilazation and stabilization. J. Pharm. Sci. **85**, 1017–1025 (1996).
13. Andersen, F., Bundgaard, H.: Inclusion complexation of metronidazole benzoate with β-cyclodextrin and its depression of anhydrate-hydrate transition in aqueous suspensions. Int. J.Pharm. **19**, 189–197 (1984).
14. Loukas, Y.L., Vraka, V., Gregoriadis, G.: Fluorimetric studies of the formation of riboflavin-β-cyclodextrin inclusion complex: determination of the stability constant by use of a non-linear leastsquares model. J. Pharm. Pharmacol. **49**, 127–130 (1997).
15. Nalluri, B.N., Chowdary, K.P.R., Murthy, K.V.R., Hayman, A.R., Becket, G.: Physicochemical characterization and dissolution properties of nimesulide-cyclodextrin binary systems. AAPS Pharm. Sci. Tech. **4**(1), E2 (2003).
16. Scalia, S., Casolari, A., Iaconinoto, A., Simeoni, S.: Comparative studies of the influence of cyclodextrins on the stability of the sunscreen agent, 2-ethylhexyl-p-methoxycinnamate. J. Pharm. Biomed. Anal. **30**, 1181– (2002).

17. Mukne, P., Nagarsenker, M.S.: Triamterene-β-cyclodextrin systems: preparation, characterization and in vivo evaluation. AAPS Pharm. Sci. Tech. **5**(1), article 19, E19 (2004).
18. Duchêne, D., Wouessidjewe, D.: Pharmaceutical uses of cyclodextrins and derivatives. Drug Dev. Ind. Pharm. **16**, 2487–2499 (1990).
19. Frömming, K.H., Szejtli, J.: Cyclodextrins in Pharmacy, Topics in Inclusion Science, Kluwer, Dordrecht (1994).
20. Anadolou, R.Y., Sen, T., Tarimci, N., Birol, A., Erdem, C.: Improved efficacy and tolerability of retinoic acid in acne vulgaris: a new topical formulation with cyclodextrin complex ψ. J. Eur. Acad. Dermatol. Venereol. **18**, 416–421 (2004).
21. Dollo, A.G., Le Corre, P., Chevanne, F., Le Verge, R.: Inclusion complexation of amide-typed local anaesthetics with beta-cyclodextrins and its derivatives. Int. J. Pharm. **131**, 219–228 (1996).
22. Barbato, F., Cappello, B., La Rotonda, M.I., Miro, A., Quaglia, F.: Diclofenac β-cyclodextrin binary systems: a study in solution and in the solid state. J. Incl. Phenom. **46**, 179–185 (2003).
23. Higuchi, T., Connors, K.A.: Phase-solubility techniques. Adv. Anal. Chem. Instrum. **4**, 117–212 (1965).
24. Montassier, P., Duchêne, D., Poelman, M.C.: Inclusion complexes of tretinoin with cyclodextrins. Int. J. Pharm. **153**, 199–209 (1997).
25. Djedaini, F., Lin, S.Z., Perly, B.: Nuclear magnetic resonance of cyclodextrins, derivatives and inclusion compounds. In: Duchêne, D (ed.) New trends in Cyclodextrin and Derivatives, pp. 217–246. Editions de Santè, Paris, (1991).
26. Pose-Vilarnovo, B., Perdomo-Lòpez, I., Echezarreta-Lopez, M., Schroth-Pardo, P., Estrada, E., Torres-Labandeira, J.J.: Eur. J. Pharm. Sci. **13**, 325–331 (2001).
27. Ficarra, R., Ficarra, P., Di Bella, M.R., Raneri, D., Tommasini, S., Calabro, M.L., Gamberini, M.C., Rustichelli, C.: Study of beta-blockers/beta-cyclodextrins inclusion complex by NMR, DSC, X-ray and SEM investigation.. J. Pharm. Biomed. Anal. **23**, 33–40 (2000).
28. Manca, M.L., Zaru, M., Ennas, G., Valenti, D., Sinico, C., Loy, G., Fadda, A.M.: Diclofenac-β-cyclodextrin binary systems: physicochemical characterization and in vitro dissolution and diffusion studies. AAPS Pharm. Sci. Tech. **6**(3), E464 (2005).
29. Noy, N.: The ionization behavior of retinoic acid in aqueous enviroments and bound to serum albumin. Biochim. Biophys. Acta. Biomembr. **1106**(1), 151–158 (1992).
30. Guo, Q.-X., Ren, T., Fang, Y.P., Liu, Y.-C.: Binding of vitamin A by β-cyclodextrin and heptakis(2,6-O-dimethyl)-β-cyclodextrin. J. Incl. Phenom. Molec. Recogn. Chem. 22, 251–256 (1995).