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ORIGINAL ARTICLE

On the complexation of Trolox with methyl-β-cyclodextrin: characterization, molecular modelling and photostabilizing properties

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Abstract Exposure to UV radiations could reduce the efficiency of some antioxidants like Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble vitamin E analogue largely employed in cosmetic products. Accordingly, in this paper we examined the possibility of increasing the stability of Trolox towards UVB irradiation by its complexation with methyl-β-cyclodextrin. Formation of the inclusion complex was confirmed by solubility diagrams, differential scanning calorimetry (DSC), and diffusion study through hydrophilic membrane. The stability constants and docking results suggested that the complexation phenomenon was related to the pH of the medium. The photodegradation studies were carried out in different topical formulations (gel, O/W emulsion, and W/O/W emulsion) containing Trolox free or complexed with methyl-β-cyclodextrin. Results showed that in all the cases Trolox degraded following pseudo-zero order kinetics. Moreover, the host molecule increased Trolox photostability also in the presence of TiO_2, a physical sunscreen well-known as photocatalyzer.

Keywords Inclusion complex characterization · Methyl-β-cyclodextrin · Photostability · TiO_2 · Trolox

Introduction

Trolox is a hindered phenol with a para ether oxygen in a ring system. Because of its chromanol structure, which provides antioxidant activity [1] and the carboxyl group which effects moderate water-solubility, Trolox has advantages over other active antioxidant (e.g. vitamin E, vitamin C, natural thiols, etc…) [2]. Preliminary studies conducted in our laboratories [3] have shown that Trolox, upon UVB irradiation, could be oxidized to Trolox C quinone. Moreover it was found that titanium dioxide (TiO_2), a pigment employed as a pearl or a sunscreen agent in toiletry products [4], enhanced Trolox photoinduced oxidation [3].

In this regard, it could be advantageous to include Trolox in a carrier system to ensure adequate efficacy and stability for this active substance. Cyclodextrins are host molecules able to form inclusion complexes with a wide variety of guest molecules and to influence their photophysical and photochemical outcomes [5, 6]. In recent years modified cyclodextrins (e.g. hydroxypropyl-β-cyclodextrin, methyl-β-cyclodextrin) have attracted great attention as they show some advantages compared with native cyclodextrins [7]. Particularly methyl-β-cyclodextrin (Kleptose®Crysmeb; Me-β-CD) dissolves easily in water (unlike β-cyclodextrin), forms stable aqueous solutions and, unlike ionic derivatives, does not greatly increase osmotic strength of solution, simplifying formulation development. Moreover, according to the analytic specifications warranted from the supplier, the Kleptose®Crysmeb offers the advantage of a high biological tolerance.

Complexation with cyclodextrins is a valuable drug delivery strategy that requires a complete understanding of the topic as a whole before proceeding to practice. In silico tools such as docking strategies are routinely used in medicinal chemistry to predict and rank the structures arising from the association between a given ligand and a target receptor [8]. Since cyclodextrins can be seen as a peculiar class of receptors, a powerful in silico strategy tailored to the investigated system could also enable

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pharmaceutical scientists to improve the quality of their experimental results.

In the literature the use of various type of cyclodextrins as solubility enhancers to develop a validated assay for lipophilic antioxidants has been cited [9, 10]. Elsewhere the effectiveness of cyclodextrins to separate vitamins D and E has been claimed [11]. Nevertheless some authors discussed the application of cyclodextrins to stabilize food and cosmetic materials including coenzyme Q10, α-lipoic acid, retinol, α-tocopherol, menaquinone, farnesol, linoleic acid, unsaturated fatty acid-containing triglycerides and chlorophyll [12, 13]. The first purpose of this research was to prepare and characterize the inclusion complex between Trolox and methyl-β-cyclodextrin through different experimental and computational techniques. Secondly, it was investigated the effect of this inclusion on the photodegradation kinetics of the active molecule, in the absence and in the presence of TiO_2.

Experimentals

Materials

Hydrochloric acid, sodium hydroxyde, sodium azide were purchased from Fluka (Milan, Italy), methanol from Carlo Erba (Milan, Italy); titanium dioxide (Degussa®P25) was a gift from Degussa Goldschmidt (Pandino, Cr, Italy). Hydroxyethylcellulose (Natrosol®MR, HEC), octyl octanoate (Tegosoft®EE), fluid paraffin and polyethylene (Pioner®PLW) were from A.C.E.F (Fiorenzuola d'Arda, Italy); imidazolidinyl urea (Gram®I) and methylchloroisothiazolinone/methylisothiazolinone (Kathon®CG) were supplied by Sinerga (Milan, Italy).

Cetearyl alcohol/cetearyl glucoside (Montanov®68) was a gift from Seppic (Paris, France),methyl glucose dioleate (Glucate®DO) was a gift from Amerchol (Milan, Italy). Xantan gum (Keltrol®T) was from Kelco (Milan, Italy). Saccharose monolaurate and saccharose monostearate were purchased from Biochim (Milan, Italy), polyglyceryl-2-sesquiisostearate (Hostacerin®DGI) from Clariant (Sulzbach, Germany). Sodium monohydrogen phosphate and sodium dihydrogen phosphate were Merck (Milan, Italy) products, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was supplied by Sigma Aldrich (Milan, Italy). Methyl-β-cyclodextrin (Kleptose®Crysmeb, Me-β-CD, MW 1191) was kindly gift from Roquette (Lestrem, France).

Apparatus

A Modulyo freeze dryer system (Edwards, West Sussex, UK) was used to prepare the inclusion complex of Trolox and Me-β-CD. The irradiation test was carried out in Pyrex® glass cells (5 ml volume) in a solar box equipped with a TL40/12RST40T12 UVB lamp (Philips, Milan, Italy). HPLC analysis was performed employing an apparatus (Shimadzu, Tokyo, Japan) consisting of a SPD-2A UV-Vis detector, a LC-6A pump unit control, a C-R3A chromatopac integrator and a RP-C18 column (80 × 4.6 mm; 5 μm). Trolox was eluted with a mixture of methanol/water/HCl (55/45/0.4) at a flow rate of 0.8 ml/min. The detection wavelength was 290 nm and the retention time was around 4.0 min. Thermograms were obtained by a DSC-7 power compensation (Perkin Elmer, Waltham, MA, USA). A 660/H transsonic cleaner (Elma, Singen, Germany) was employed to disperse TiO_2. A DLS stirrer (Velp Scientifica, Milan, Italy), a SL-2 (Silverson, Bucks, England) and a T25 basic Ultra-Turrax® (IKA, Staufen, Germany) homogenizers were employed to prepare emulsion and gel systems; a SM 16750 absorption simulator (Sartorius, Göttinghen, Germany) was used to prepare the multiple W/O/W emulsion. The measurements of pH were carried out with a microprocessor pHmeter (Hanna Instruments, Milan, Italy). A Servapor cellulose membrane (MWCO: 1000 Da; Serva, Milan, Italy), was used to study Trolox diffusion profiles.

Preparation of inclusion complex and physical mixture

Freeze drying technique [5] was used to prepare the inclusion complex of Trolox and Me-β-CD. A mixture of Trolox and cylodextrin (1:4 molar ratio) was prepared in water, at pH 3.8, and shaken for 24 h in the dark. After equilibration the suspension was filtered and freeze dried before re-dissolution in methanol to assess the active loading by HPLC. The average percentage of active loaded was around 2.0% w/w.

The physical mixture was prepared in the same molar ratio (1:4) by mixing appropriate amounts of the solid components in a glass mortar.

Solubility diagram and stability constant, at pH 4.0 and 5.8

Phase solubility studies were performed according to the Higuchi–Connors method [14].

An excess of Trolox (20 mg in 25 ml of water) was added to a series of vials containing increasing amounts of Me-β-CD, at pH 4.0 and 5.8. The closed vials were shaken in the dark for 24 h at room temperature. After equilibration, each sample was centrifuged and supernatants were analyzed by HPLC to detect the concentration of Trolox. Two phase diagrams were constructed by plotting the total molar concentration of Trolox found in solution at pH 4.0 or 5.8 against the molar concentration of Me-β-CD added

to each system. The stability constant (K_{st}) was calculated from the initial rise of the curve according the following equation:

$$K_{st} = \text{slope}/S_0(1 - \text{slope}) \quad (1)$$

Where the slope is obtained from the least squares linear regression of the molar concentration of Trolox in solution versus the molar concentration of cyclodextrin in water and S_0 is the intrinsic solubility of Trolox (0.55 mM) in the absence of cyclodextrin. The previous equation (1) was applied by assuming a 1:1 stoichiometry for the inclusion complex.

Molecular modeling

3D structures

3D structures were found in the Cambridge Structural Database (CSD, version 5.27; data updates January 2006). The cyclodextrin structure (code IQOZIX) was checked with Mercury [15] saved in the Tripos mol2 format (necessary for subsequent calculations) and read in MOE [16] to delete co-crystallized water molecules, to add missing bonds and to define the methyl groups positions. Since Kleptose Crysmeb Exp has, on average, four methyls per native cyclodextrin molecule, the four methyl groups (correspondent carbon atoms are C14, C28, C42, and C49) with the highest occupancy percentage were retained, whereas the remaining three methyl groups (correspondent carbon atoms are C7, C21, and C35) were deleted. Finally hydrogens were added and their geometry was minimized under MMFF94x and GB-SA conditions (RMS gradient < 0.001Å). Trolox structure in its neutral form was simply downloaded from CSD (code DEWVOQ02) and checked with standard MOE tools. The anionic species was obtained removing the hydrogen atom from the carboxylic group and further minimization

Docking

MOE-Dock methology which consists of three steps (ligand conformational analysis, placement and scoring) was used [16]. For the initial systematic search, a random initial orientation was used, alpha triangle was the placement methodology and affinity dG scoring (a function that estimates the enthalpic contribution to the free energy of binding using a linear function) the chosen scoring function. The two docking runs (one for the neutral and one for the anionic species) were set to generate 100 poses which were collected in a database. The resulting complexes were minimised using GB-SA conditions and finally poses were ranked by their scoring function (the lowest, the best).

DSC studies

The samples were placed in conventional aluminium pans and then heated under nitrogen flow at a scanning speed of 10 °C min^{-1} from 20 to 200 °C. The weight of each sample (pure Trolox, Trolox/Me-β-CD complex and physical mixture) was such that it contained a constant amount of the active.

Trolox diffusion through hydrophilic membrane

The permeation behaviour of Trolox in the absence and presence of Me-β-CD was examined. An artificial cellulose membrane was utilized. As donor phase 25 ml of 0.1 mM Trolox, either alone or complexed with Me-β-CD, in aqueous solution was employed. The donor phase filled a hermetically closed cellulose bag that was fully immersed in 25 ml of receiving phase represented by water. The system, sheltered from light, was maintained under stirring (300 rpm) at 25 ± 0.1 °C for 3 h. At scheduled times of 30 min, an aliquot (0.2 ml) of the receiving phase was withdrawn and replaced with fresh water. The collected samples were analyzed by HPLC.

Photodegradation study

Sample preparation

In order to investigate the effect of both Me-β-CD and of TiO_2 on the photostability of Trolox (1.0×10^{-3} M) upon UV irradiation, the test was performed separately on pure Trolox and on the complex in the absence and in the presence of TiO_2 (0.025% w/w). The runs were carried out in a gel and in two different emulsions (an O/W emulsion and a W/O/W emulsion), at pH 5.8. The gel was prepared by simply dispersing the appropriate amount of HEC (2.0% w/w) in water heated to about 80 °C, mechanically stirring until it reached room temperature; the pH was properly adjusted to 5.8. The O/W emulsion was prepared by dispersing, under homogenization, the melted lipid phase (3.5 g of Montanov®68 and 20 g of Tegosoft® EE) in water (76.5 g) heated to 70 °C. After homogenization the emulsion was mechanically stirred until room temperature was reached; the pH was properly adjusted to 5.8. The W/O/W emulsion was prepared following a two steps method. Firstly, a primary W/O emulsion was prepared by adding water to the lipid phase consisting of Tegosoft EE®, Hostacerin DGI®, Glucate DO®, and Pioner PLW® upon homogenization. The prepared emulsion was kept under vigorous stirring for about 1 h.

Secondly, the external aqueous phase was prepared as follows: saccharose stearate, saccharose laurate and Keltrol T® were dispersed under T25 basic Ultra-Turrax® (IKA, Staufen, Germany) in water, then the preservatives (Kathon CG® and Gram 1®) were added.

Table 1 Percent composition of W/O/W emulsion containing Trolox in the presence of TiO_2

Components	Percentage (% w/w)
W/O emulsion	
Tegosoft EE®	17.94
Hostacerin DGI®	2.10
Glucate DO®	2.90
Pioner PLW®	6.97
Trolox	0.07
Water	as to 100
W/O/W emulsion	
W/O emulsion	36.53
Saccharose stearate	0.62
Saccharose laurate	1.40
Keltrol T®	0.50
Kathon CG®	0.014
Kemipur 100®	0.028
Glucose	0.007
TiO_2	0.025
Water	as to 100

By means of absorption simulator, the primary W/O emulsion was dropped, within 1 h, into the aqueous external phase upon stirring. The multiple emulsion was kept under stirring for 1 h and the experiments of irradiation were performed after 24 h. Trolox was introduced into the inner phase of the primary W/O emulsion while TiO_2 was introduced by simply dispersion in the external phase.

The percent composition of the multiple emulsion containing both Trolox and TiO_2 is listed in Table 1.

Irradiation runs

All the samples (10 ml) were placed at 10 cm from the UVB lamp, in closed Pyrex® cells upon magnetic stirring and at scheduled times of 30 min, the amount of non-degraded Trolox was detected by HPLC after proper dilution with methanol. The radiation intensity of the lamp, measured by means of a CO.FO.ME.GRA multimeter, was 2.6×10^{-4} W/cm^2 for UVB radiations; this experiment can be considered an accelerated test of stability.

Results and discussion

Solubility diagram and stability constant, at pH 4.0 and 5.8

A quantitative investigation on the inclusion complexation of Trolox in Me-β-CD was performed according to Higuchi and Connors method [14]. The solubility diagrams of Trolox against increasing concentration of Me-β-CD concentration, at pH 4.0 and 5.8 are reported in Fig. 1

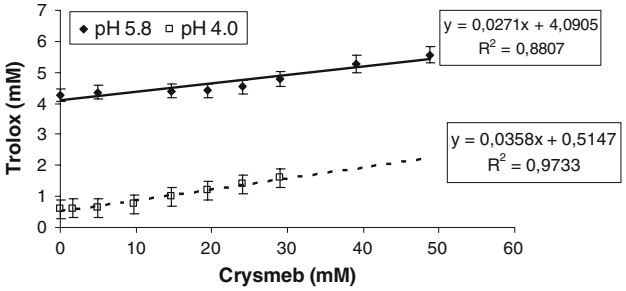

Fig. 1 Initial rise of the phase solubility diagrams for Trolox at different Me-β-CD concentrations, at pH 4.0 and 5.8

Table 2 Stability constant (K_{st}) values for Trolox/Me-β-CD at different pH of the medium

pH	K_{st} (M^{-1})
4.0	61.8 (±2.8)
5.8	6.5 (±1.2)

It was observed that the solubility of Trolox increased as Me-β-CD increased, displaying an A-type phase solubility diagram. The stability constants (K_{st}), calculated as previously described, are reported in Table 2

It can be noted that the constant K_{st} decreased with increasing the pH of the medium, as summarized in Table 1. Accordingly, it can be stated that the complexation phenomena was really influenced by protonation-deprotonation equilibrium of Trolox carboxylic group. Particularly, our hypothesis is that passing from lower to higher pH value, Trolox assumed a negative charge that can prevent the formation of the complex. On the contrary by decreasing the pH of the medium Trolox became more lipophilic and thus its affinity for the cavity of cyclodextrin increased.

Molecular modelling

The growing impact of computational strategies in pharmaceutical sciences is witnessed by the increasing number of papers reporting results of docking studies [17–19]

Docking is a computational strategy widely used in many scientific disciplines (bioinformatics, medicinal chemistry, etc.) that attempts to find the "best" matching between two molecules: a receptor and a ligand [20]. In this study, we consider cyclodextrin as the receptor and Trolox as the ligand. Before discussing docking results, some considerations about the 3D structures of both receptor and ligand are required. Both of them were found in the Cambridge Structural Database, as reported in the Experimental Section. The use of crystallographic structures is a very

common practice in molecular modelling, but flexibility must be taken into consideration. Here, we used a semi-flexible docking: the ligand, is considered flexible, whereas the receptor is regarded as rigid. With this approach the conformational freedom of the ligand was thus explicitly treated. On the other side, the flexibility of the receptor was taken into consideration by minimizing the complexes before calculating the score of each pose.

Docking results support the experimental evidences described above.

Firstly, numerical docking data show that neutral Trolox forms with the investigated cyclodextrin inclusion complexes more stable than those formed by the anionic species. In fact, the best pose of the protonated Trolox has a scoring function lower (= more stable) than the correspondent of the anion (−4.023 and −3.680 kcal/mol, respectively). The difference of about 0.4 kcal/mol in scoring values is significant in this kind of studies to show a difference in affinity between two species. However, to give an individual significance to the values, a general validation of the scoring function on a wider series of cyclodextrin complexes should be performed. Work along these lines is in progress in our laboratories but it goes beyond to the aim of the paper.

Secondly, graphical docking results rationalize the larger stability of the inclusion complex formed by the neutral Trolox compared with the one formed by the anion. Figure 2 shows the best pose of neutral Trolox (a) and the best pose of anionic Trolox (b) with the cyclodextrin (in blue). In the neutral species the hydroxyl group (the preferred degradation site as reported elsewhere [3, 9]) is protected in the CD cavity whereas in the anionic species the hydroxyl group is almost outside the CD cavity and thus more exposed to the environment.

DSC studies

The DSC thermograms gave further information about the interaction between Trolox and Me-β-CD. As shown in Fig. 3, the thermogram of pure Trolox displayed one endothermic peak around 195 °C while the thermogram of the complex Trolox/Me-β-CD did not show any peak indicating a certain interaction between the two components occurred. The melting peak of Trolox disappeared since the crystalline active molecule was enclosed into the cavity of the cyclodextrin. In the thermogram of the physical mixture the shape and the shift of the peak indicated the existence of a new solid phase in which the Trolox was dispersed with a lower cristallinity than the pure Trolox.

Trolox diffusion through hydrophilic membrane

Trolox and Me-β-CD association was also studied by evaluating the diffusion through an artificial membrane with a cut off of 1000 Da. The choice of this barrier was justified by the structural characteristic of the drug: this membrane allows the permeation of Trolox (MW = 250.29) but it is impermeable to the host molecule. The purpose of the present study was to investigate the effect of cyclodextrin on the permeability of the active through the membrane to further confirm its encapsulation in

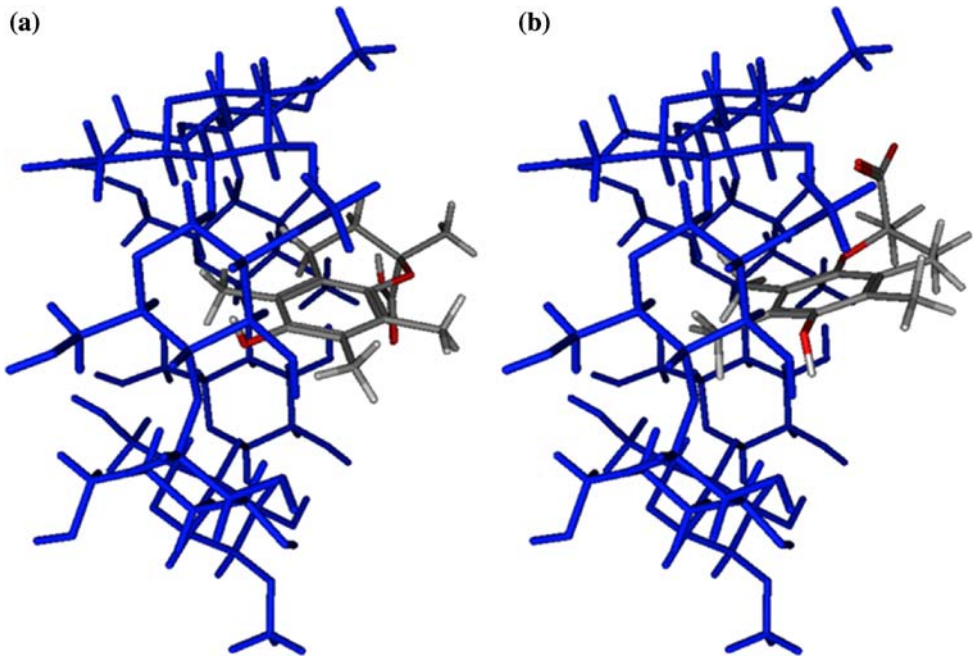

Fig. 2 Graphical docking results with the cyclodextrin molecule (in blue) in the same orientation. (**a**) the best pose of neutral Trolox and (**b**) the best pose of anionic Trolox

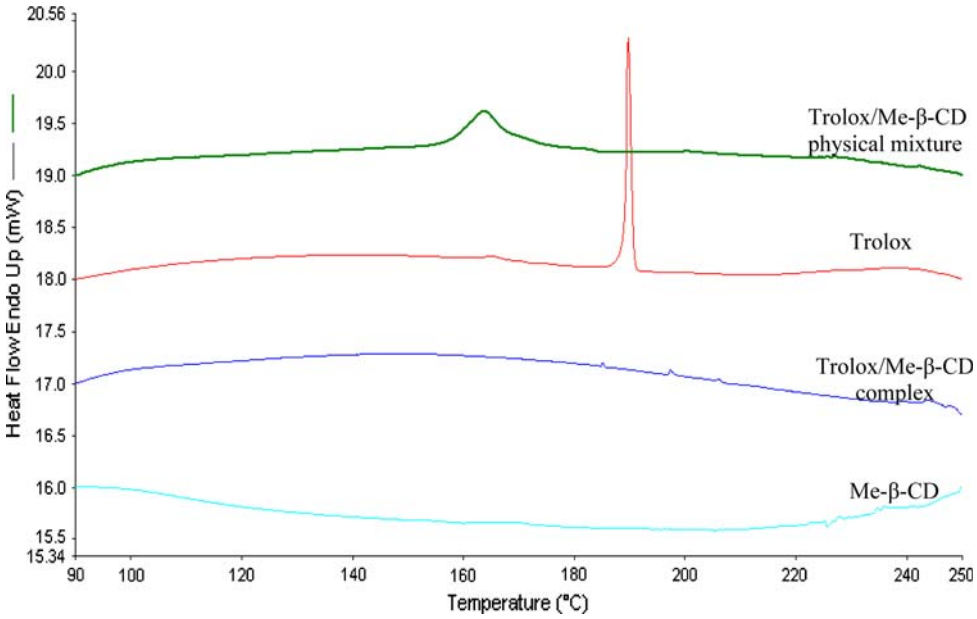

Fig. 3 DSC thermograms of Trolox, Trolox/Me-β-CD (1:4 mol) complex, Trolox/Me-β-CD (1:4 mol) physical mixture and Me-β-CD

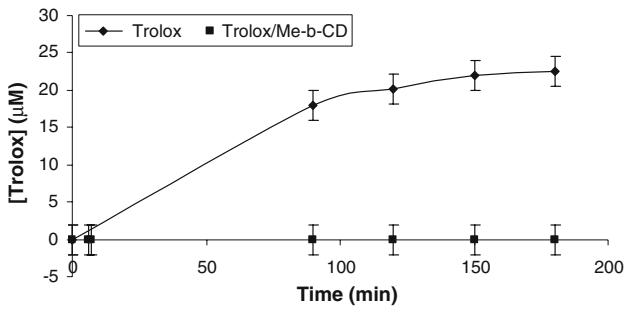

Fig. 4 Diffusion profile of Trolox, pure or included in Me-β-CD, through hydrophilic membrane

Me-β-CD. A plot of Trolox permeation in the absence and in the presence of Me-β-CD is shown in Fig. 4

The diffusion profiles evidence that pure Trolox diffused from the donor to the receiving phase, on the contrary in the presence of Me-β-CD it did not permeate through the membrane confirming the formation of the inclusion complex.

Photodegradation study

Under UVB irradiation Trolox evolves to Trolox C quinone, an oxidation product typical of peroxidation reactions suggesting that oxygen is a partner of reaction in the photodegradation pathway. In our previous paper [3] it was found that less polar environments (water/ethanol mixtures, micellar solutions) are able to inhibit the charge transfer reactions that cause Trolox photoxidation. In this regard, we also examined the influence of the medium on the photodegradation phenomena by extending this study to different Trolox-containing formulations: gel, O/W emulsion and W/O/W emulsion. Irradiations runs were carried out separately on Trolox and on Trolox/Me-β-CD to evaluate the protective effect of the cyclodextrin on the photooxidation rate of the active. Moreover this test was performed in the absence and in the presence of TiO_2 to assess its possible catalytic effect on the photodegradation process. The final pH of the considered systems was 5.8 for the gel and for the O/W emulsion and 4.0 or 5.8 for the outer phase of the W/O/W emulsion. In all the media Trolox photodegraded following a pseudo-zero order kinetic, probably by a mechanism of photo-oxidation.

The first medium considered in the present study was HEC gel: Fig. 5 displays the photodegradation trends of Trolox, pure and complexed with Me-β-CD; in Table 3 the corresponding kinetic constants are reported.

Table 3 shows that in HEC gel Me-β-CD exerted a protective effect against Trolox photodegradation decreasing its rate of photolysis, in fact the kinetic constants of the complex are lower than those of pure active.

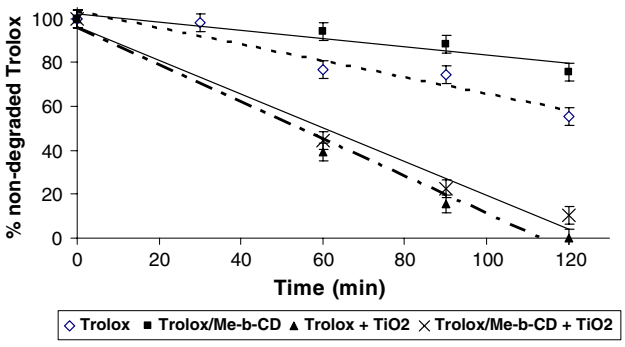

Fig. 5 Time evolution of Trolox (1.0×10^{-3} M) in HEC gel, pure and complexed with Me-β-CD, in the absence and in the presence of TiO_2, at pH 5.8

Table 3 Kinetic constants of Trolox (1.0×10^{-3} M) degradation in HEC gel upon UVB irradiation, in the absence and in the presence of TiO_2, at pH 5.8

Samples	Trolox $k \times 10^7$ ($M\ s^{-1}$)	Trolox/Me-β-CD $k \times 10^7$ ($M\ s^{-1}$)
Without TiO_2	0.58 (±0.04)	0.32 (±0.02)
With TiO_2	1.22 (±0.02)	1.10 (±0.02)

Table 4 Kinetic constants of Trolox (1.0×10^{-3} M) degradation in O/W emulsion upon UVB irradiation, in the absence and in the presence of TiO_2, at pH 5.8

Samples	Trolox $k \times 10^7$ ($M\ s^{-1}$)	Trolox/Me-β-CD $k \times 10^7$ ($M\ s^{-1}$)
Without TiO_2	0.19 (±0.02)	0.10 (±0.01)
With TiO_2	0.29 (±0.03)	0.18 (±0.02)

Moreover it can be noted that in both the case the presence of TiO_2 increased the rates of Trolox photodegradation indicating that the oxide can act as photocatalyzer towards both pure Trolox and complexed Trolox. Globally, these results suggest that the complexation with Me-β-CD conferred only a moderate protective effect probably due to the rather low stability constant values. A similar result was already observed with the complex Trolox/HP-β-CD [21].

The second formulation employed in this study was an O/W emulsion prepared using Montanov®68 as emulsifier: the kinetic constants of Trolox photodegradation are reported in Table 4.

In O/W emulsion the rates of Trolox photodegradation were lower (Table 4) than in HEC gel (Table 3). Our hypothesis is that, when Trolox was in the internal hydrophobic phase of the emulsion, it experienced an apolar environment that should inhibit the photooxidative reactions. Anyway the inclusion of TiO_2 in emulsion increased the rate of photodegradation of Trolox even when it was complexed with cyclodextrin, displaying a certain photocatalytic activity.

Finally, the photodegradation study was extended to a Trolox-containing W/O/W emulsion. Table 5 reports the photodegradation rate constants of Trolox, free or complexed, entrapped into the inner phase of the multiple system, in the absence and in the presence of TiO_2, introduced in the outer phase of the emulsion at two different pH.

Similarly to the previous formulations also in multiple emulsion the kinetic constants related to the complex are lower than those related to the pure active (Table 5) suggesting a certain protective effect of Me-β-CD towards the photodegradation of Trolox.

Moreover, the influence of the pH of the outer phase on Trolox degradation rate was almost negligible, as expected since the active was enclosed into the inner phase (= pH 3.0). This finding demonstrated on one side the multiplicity of the emulsion and on the other side that this multi-phase structure was stable even after pH changes and UVB irradiation.

Table 5 Photodegradation kinetic constants of Trolox (1.0×10^{-3} M) introduced pure or complexed in W/O/W emulsion, in the absence and in the presence of TiO_2, at external pH of 4.0 or 5.8

Samples	Trolox $k \times 10^7$ ($M\ s^{-1}$)	Trolox/Me-β-CD $k \times 10^7$ ($M\ s^{-1}$)
pH 4.0, without TiO_2	0.52 (±0.05)	0.40 (±0.03)
pH 4.0, with TiO_2	0.48 (±0.04)	0.39 (±0.04)
pH 5.8, without TiO_2	0.55 (±0.06)	0.48 (±0.03)
pH 5.8, with TiO_2	0.45 (±0.03)	0.13 (±0.01)

Interestingly, it is possible to note that unlike in gel and in O/W emulsion, in W/O/W emulsion the kinetic constants of photodegradation were lower in the presence than in the absence of TiO_2 especially at pH 5.8. Accordingly, TiO_2 did not act as catalyser but it rather decreased the rates of Trolox photodegradation displaying its own sunscreen efficiency and suggesting that Trolox was really included into the inner phase of the multiple emulsion. In fact our hypothesis is that since TiO_2 was included into the outer phase of the multiple emulsion, its interaction with Trolox was prevented by the presence of a hydrophobic barrier (the lipid phase) interposed between the two aqueous phases. In conclusion it can be stated that the multiple system allows to introduce at the same time Trolox and TiO_2 minimizing their interaction and hence avoiding the photocatalytic activity of the oxide.

Conclusions

In the present study the inclusion complex between Trolox and Me-β-CD was prepared. The computational study suggested the formation of the complex. Differential scanning calorimetry, solubility diagrams and diffusion profiles were employed to characterize the complexation phenomenon. The photostability studies revealed that Me-β-CD was quite protective towards UVB-induced degradation of Trolox, also in the presence of TiO_2. This finding was revealed in all the considered formulations by the analysis of the variations of the kinetic constants. It was also found that Trolox displayed greater stability in the biphasic systems (O/W and W/O/W emulsions) than in gel. On the other hand, it was observed that TiO_2 acted as photocatalyser in HEC gel and in O/W emulsion whereas in W/O/W emulsion it worked as sunscreen agent. Accordingly, the inclusion of Trolox in Me-β-CD could facilitate the production of formulations rather stable both towards the direct photolysis and the TiO_2-induced photocatalysis of the active.

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