J Incl Phenom Macrocycl Chem (2007) 57:451–455
DOI 10.1007/s10847-006-9233-8

ORIGINAL ARTICLE

Effect of akyl-γ-cyclodextrins on the stability of retinol

S. Sapino · M. E. Carlotti · R. Cavalli · M. Trotta · F. Trotta · D. Vione

Received: 15 May 2006 / Accepted: 20 October 2006 / Published online: 23 January 2007

Abstract Inclusion complexation between retinol (RET) and two synthetic alkyl carbonates of γ-cyclodextrin (alkyl-γ-CD) derivatives, ethyl-γ-cyclodextrin (E-CD) and octyl-γ-cyclodextrin (O-CD), was investigated by means of different techniques. The complexes were characterized by differential scanning calorimetry (DSC). Phase solubility studies, according to the method of Higuchi and Connors [1] were used to evaluate the complexation in aqueous solution at room temperature. In the present study inclusion complexes of retinol with E-CD and with O-CD were prepared to prevent its rapid degradation. In order to investigate the behavior of retinol under UV light, test of irradiation was performed separately on samples prepared dispersing retinol (0.1% w/w) or an equivalent amount of retinol/alkyl-γ-CD respectively in hydroxyethylcellulose (HEC) gel and in an O/W emulsion. The stability over time of retinol was also investigated storing the samples at 40 °C. Moreover retinol permeation through porcine skin has been evaluated employing Franz cells [2]. Retinol solubility was increased in presence of cyclodextrins while DSC analysis suggest that this inclusion agents are able to interact with retinol. Data for skin accumulation in porcine ear skin showed that alkyl-CDs increase of approximatively 1,5-fold retinol skin accumulation. Studies on the stability showed that both the inclusion complexes considered are able to increase retinol stability to light exposure and also to heat.

Keywords Retinol · Ethylcarbonate of γ-cyclodextrin · Octylcarbonate of γ-cyclodextrin · Phase-solubility diagrams · DSC thermograms · Skin permeation

S. Sapino · M. E. Carlotti (✉) · R. Cavalli · M. Trotta
Dip. di Scienza e Tecnologia del Farmaco-Università degli Studi di Torino, via P. Giuria 9, 10125 Turin, Italy
e-mail: eugenia.carlotti@unito.it

F. Trotta
Dip. di Chimica IFM-Università degli Studi di Torino, via P. Giuria 7, 10125 Turin, Italy

D. Vione
Dip. di Chimica Analitica- Università degli Studi di Torino, via P. Giuria 5, 10125 Turin, Italy

Introduction

Retinoids are a large class of compounds that are important in modern therapy for dermatological treatment of wrinkled skin [3]. However their topical use is limited due to several drawbacks: high instability in presence of air, light and heat and low water solubility. The interaction of these active substances with inclusion agents such as cyclodextrins (CDs) could possibly improve all these characteristics. Some authors reported the improvement in solubility of various retinoids by the use of cyclodextrins [4, 5]. Nevertheless complexation with CD has been used to protect the guest molecules against oxidation, light-induced reaction and loss by evaporation [6]. On the other side the use of cyclodextrins to modulate the release of an active substance in topical application is widely reported in literature [7, 8]. Anyway there are relatively few reports [5, 9] on the effects of CD on retinoids photo-degradation and degradation over time.

In the present work the inclusion equilibrium involving RET and CD was investigated by means of solubility studies and differential scanning calorimetry

(DSC). As inclusion agents, two synthetic alkyl carbonates of γ-CD derivatives were employed: ethyl-γ-cyclodextrin (E-CD) and octyl-γ-cyclodextrin (O-CD). A series of stability studies were performed in order to evaluate the ability of these two different CD to improve RET stability. Permeation studies were also carried out in order to assess the amount of vitamin A permeated after 24 h into the porcine skin.

Experimental

Material

All-*trans*-retinol or vitamin A ($C_{20}H_{30}O$, FW 286,45) was purchased from Sigma-Aldrich. Tegosoft®EE (*octyl octanoate*) and Natrosol®MR (*hydroxy ethyl cellulose*) were supplied by A.C.E.F., TWEEN®20 (*Polysorbitan*) by Fluka while Montanov®68 (*Cetearyl alcohol/Cetearyl glucoside*) was a kind gift from Seppic. Ethyl carbonate of γ-cyclodextrin (E-CD) and octyl carbonate of γ-cyclodextrin (O-CD) were prepared in our laboratories according the following procedure. Briefly the selected alcohol was activated by reaction with excess of carbonyldiimidazole in alcohol free chloroform. In the second step the imidazolyl derivative was allowed to react with anhydrous γ-CD in anhydrous pyridine at 80 °C for 4 h. Once the reaction was over, the residual precipitate was filtered off and distilled water was added to the organic solution. The solid was recovered by filtration, washed many times with water and then liophylized [10]. All other solvents were of analytical grade and were used without further treatments.

Apparatus

A Modulyo Edwards Freeze Dryer Systems was employed to prepare inclusion complexes of retinol and cyclodextrins. Irradiation tests were carried out in Pyrex glass cells (5 ml solutions) under a solarbox equipped with a TL40/12 RST40T12 UVB lamp (Philips). Absorbance spectra were recorded by a Perkin-Elmer Lambda 2 UV-VIS Spectrophotometer. HPLC was performed using a Shimadzu HPLC system consisting of UV detector SP-2A, a pump unit control LC6A and a C-R3A chromatopac integrator. The chromatographic conditions were as follows: retinol absorbance was measured at $\lambda = 325$ nm; injection volume 20 μl, isocratic flow rate at 0.8 ml min^{-1}. The mobile phase used consisted of a mixture methanol/water (90/10 v/v). DSC measurements were performed by a power compensation DSC-7 (Perkin Elmer).

Preparation of inclusion complexes

Freeze drying technique was used to prepare inclusion complexes of retinol and the two alkyl carbonates of γ-cyclodextrin, E-CD and O-CD, in 1:1 molar ratio. The CD/retinol mixtures were prepared in water/ethanol (95/5 v/v), stirred for 24 h then centrifuged. Supernatants were freeze-dried before re-dissolution in methanol to assess the active loading by HPLC analysis.

Physical mixtures were prepared by mixing appropriate amounts of solid components (molar ratio 1:1) in a glass mortar.

Solubility diagram and stability constant

Phase solubility studies were performed according to Higuchi–Connors method [1]. An excess of retinol was added to a series of solutions containing increasing amounts of a cyclodextrin (E-CD or O-CD) in a mixture water/ethanol (95:5 v/v). The closed vials were shaken for 24 h on a mechanical stirrer at room temperature. After equilibration, samples were centrifuged and analyzed by UV-VIS spectrophotometer ($\lambda = 325$ nm).

Stability constants (k_{st}) were calculated from the initial portion of the solubility diagrams, using the following formula:

$$k_{st} = \frac{\text{slope}}{S_0(1 - \text{slope})}$$

were the slope is obtained from the least squares linear regression of the molar concentrations of retinol in solution versus the molar concentration of cyclodextrin in the solvent and S_0 is the intrinsic solubility retinol (0.68 mM) in the absence of cyclodextrin.

Complexes characterization

DSC measurements were performed separately on retinol, on the physical mixtures and on the complexes. All samples accurately weighed (1 mg of RET or its equivalent) were placed in aluminum pans, before being heating under nitrogen flow at a scanning rate of 10 °C min^{-1}.

Photo-stability of retinol (Irradiation test)

In order to investigate the behavior of retinol under UV light irradiation, the test was performed separately on samples prepared dispersing retinol (0.1% w/w) or an equivalent amount of retinol-alkyl-γ-CD respectively in HEC (2% w/w) gel and in an O/W emulsion

(3% p/p Montanov®68, 20% p/p Tegosoft®EE 77% p/p water).

Samples were placed at 10 cm from UVA or UVB lamp under stirring and at scheduled times retinol concentration was detected spectrophotometrically. The radiation intensity of the lamps, measured by a CO.FO.ME.GRA multimeter resulted to be 8.9×10^{-4} W/cm^2 for UVA lamp and 2.6×10^{-4} W/cm^2 for UVB one. These experiments can be considered accelerated test of stability and allowed to understand photo-degradation processes.

Thermo-stability of retinol

Samples, whose composition is the same used for irradiation test, were stored in an oven at 40°C over a period of 10 days. At scheduled times retinol concentration was measured spectrophotometrically.

Retinol permeation and accumulation in porcine skin

This study, performed on porcine skin allowed to determine the amount of retinol which penetrate into the skin. The study was performed employing Franz-type cells [2]: the donor phase consisted of 0.1% (w/w) retinol in 20% Tween®20 or an equivalent amount of retinol-alkyl-γ-CD in water. The receptor phase was a 0.5% (w/v) SDS water solution. For each experiment a different skin batch was used and on each cell, exactly 1 ml of sample was deposited. After 24 h the cells were dismantled, the excess sample on the surface was removed and the skins were cut up and transferred in 5 ml methanol. After 2 h agitation the suspension were filtered and assayed by HPLC to determine retinol skin accumulation.

Results and discussion

Solubility diagram and stability constant

The results of solubility studies of RET in alkyl-γ-CD solutions are presented in Fig. 1. The solubility diagram is of the Bs-type, indicating limited solubility of the complex in the mixture water/ethanol (95:5 v/v). The solubility of the RET-alkyl-γ-CD complexes is limited to about 2.5 mM. The complexation constants (k_s) were calculated on the linear portion of the solubility diagram, assuming a 1:1 stoichiometry (see Table 1).

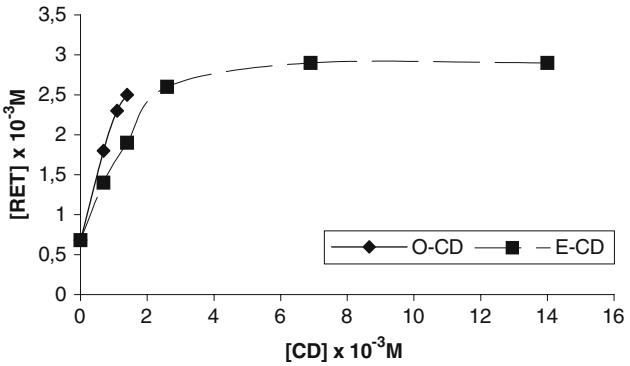

Fig. 1 Solubility of retinol in a mixture water/ethanol (95:5 v/v) containing increasing amount of alkyl-γ-CD, at ambient temperature

Complexes characterization

The inclusion of a guest in a CD induces modification of various chemical and physical properties on the former. The measurements of those changes are the basis of the methods used to confirm the complex formation. DSC evidences inclusion in a CD by the modification of the guest's molecule endothermic peak. The endothermic peak (50–55 °C) can be observed in the physical mixtures, but it is absent in the complexes. Figure 2 shows the endothermic curves of retinol, E-CD, O-CD and of RET:alkyl-γ-CD physical mixtures and solid complexes, both at a 1:1 ratio.

It is evident that there is an endothermic signal around 50 °C on the physical mixtures which correspond to retinol melting point that does not appear in the thermograms of the complexes. These results indicated that intermolecular host-guest complexation occurred.

Stability studies

In these studies the photo- and thermo-stability of RET was examined in gel (HEC 2%) and in O/W emulsion. A control was carried out dispersing RET, in the absence of cyclodextrin. Irradiation test showed that both E-CD and O-CD delayed the photo-degra-

Table 1 Stability constants for the complexation of retinol with the studied cyclodextrins. S_0 is the solubility of retinol without cyclodextrin

CD type	$k_{1:1}$ [M^{-1}]
E-CD	3260
O-CD	5976

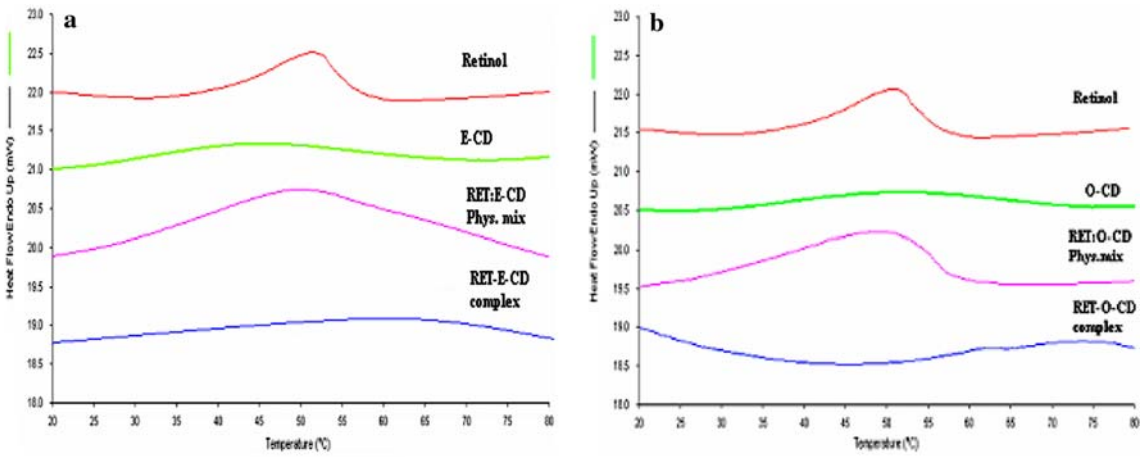

Fig. 2 ndothermic curves obtained by DSC analysis employing (**a**) E-CD or (**b**) O-CD

dation of RET (Figs. 3, 4), thatin all cases studied followed a pseudo-first order kinetics.

We also noticed that the curves of photo-degradation of RET in HEC gel resulted slightly different from that obtained in O/W emulsion. The different polarity of the media and the interaction with the CD could explain those differences.

The general equation describing RET time evolution under UVB light is $C_t = C_0 e^{-kt}$, where C_0 is the initial RET concentration, C_t the concentration at time t and k the degradation rate constant. The degradation constants were calculated using the following equations: ln $C_t/C_0 = -kt$ and the values were displayed in Table 2.

The thermo-degradation of RET alone and complexed with cyclodextrins was determined. Data, revealed that the thermo-stability of the vitamin was significantly increased by the complexation. After 9 days in oven at 40 °C the loss of RET in HEC gel was 93% while under the same experimental conditions the alkyl-γ-CD offered significant protection over the test period reducing the active loss to about 40% of the initial concentration.

Retinol permeation and accumulation in porcine skin

Table 3 shows the amount of RET accumulated into the skin in the presence and in the absence of the cyclodextrin derivatives. Our present data demonstrated that E-CD and O-CD were effective in enhancing the skin absorption of the vitamin A. Moreover RET was proven not to reach the circulation, in fact it was not found in the receptor phase.

The mechanism whereby the skin accumulation of RET was improved is not clearly understood. Generally penetration enhancers acts via one of the following mechanisms: they increase membrane fluidity, inhibit enzyme activity, reduces elasticity, open up tight junctions or they solubilised the drug. Cyclodextrins have shown to solubilise specific membrane lipids from

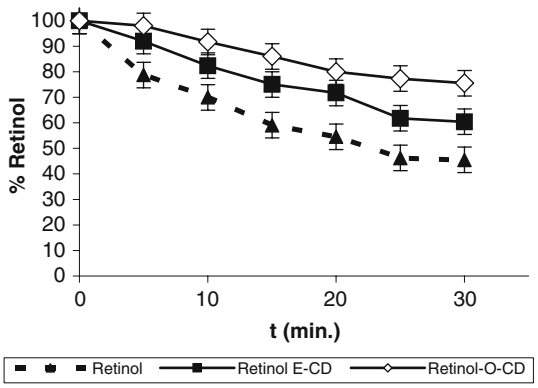

Fig. 3 Photo-degradation curves, under UVB irradiation, of RET, RET-E-CD and RET-O-CD in HEC (2%) gel

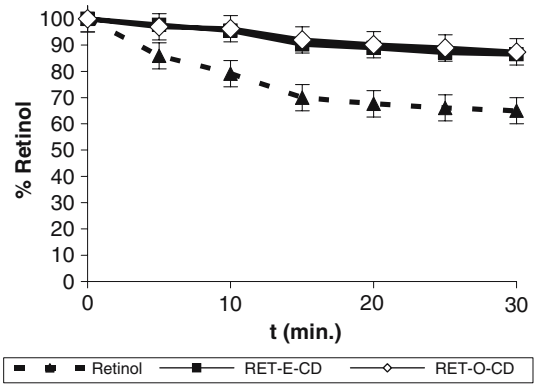

Fig. 4 Photo-degradation curves, under UVB irradiation, of RET, RET-E-CD and RET-O-CD in O/W emulsion

Table 2 Photo-degradation rate constants (k_{RET}) of RET, free or complexed with alkyl-γ-CD

	k_{RET} (min^{-1}) in HEC gel	k_{RET} (min^{-1}) in O/W emulsion
RET free	–0.0266	–0.0158
RET-E-CD	–0.0178	–0.0048
RET-O-CD	–0.0104	–0.0051

Table 3 Effect of alkyl-γ-CD on skin accumulation of retinol in ear porcine skin

Retinol in TWEEN®20	RET-E-CD in water	RET-O-CDin water
0.0010 ± 0.2 mg/cm^2	0.0017 ± 0.2 mg/cm^2	0.0041 ± 0.2 mg/cm^2

human erythrocytes through the formation of inclusion complexes, leading to an increase in membrane permeability [9].

Conclusion

Cyclodextrin derivatives, considered in this present work (E-CD and O-CD), allowed to increase the stability and the water-solubility of retinol. By DSC studies useful information about the interaction of the guest molecule with the inclusion agents were obtained. The results of permeation study indicated that the complexation with alkyl-γ-CD increases the amount of vitamin A that accumulates into the skin improving its effectiveness. In conclusion the alkyl-γ-CD studied can be considered suitable cosmetic carrier systems for very unstable molecules like vitamin A.

References

1. Higuchi, T., Connors, K.A.: Phase solubility techniques. Adv. Anal. Chem. Instrum. **4**, 117–212 (1965)
2. Franz, T.J.: The percutaneous absorption on the relevance of in vitro data. J. Invest. Dermatol. **64**, 190–195 (1975)
3. Guénin, E.P., Zatz, J.L.: Skin permeation of retinyl palmitate from vescicles. J. Soc. Cosmet. Chem. **46**, 261–270 (1995)
4. Fromming, K.H., Geider, R., Mehnert, W.: Inclusion compound of cyclodextrin and vitamin A acetate. Acta Pharm. Technol. **34**, 152–155 (1988)
5. Montassier, P., Duchêne, D., Poelman, M.C.: Inclusion complexes of tretinoin with cyclodextrins, Int. J. Pharm. **153**, 199–209 (1997)
6. Martin, Del Valle, E.M.: Cyclodextrins and their uses: a review. Process Biochem. **39**, 1033–1046 (2004)
7. Loftsson, T., Masson, M.: Cyclodextrin in topical drug formulations: theory, practice. Int. J. Pharm. **225**, 15–30 (2001)
8. Matsuda, H., Arima, H.: Cyclodextrins in transdermal and rectal delivery. Adv. Drug Del. Rev. **36**, 81–99 (1999)
9. Semenova, E.M., Cooper, A., Wilson, C.G., Converse, C.A.: Stabilization of all-trans-retinol by cyclodextrins: a comparative study using HPLC and fluorescence spectroscopy. J. Incl. Phenom. Macrocyclic Chem. **44**, 155–158 (2002)
10. Trotta, F., Cavalli, R., Trotta, M.: Investigation of haemolytic and complexation properties of γ-cyclodextrin carbonate derivatives. J. Incl. Phenom. Macrocyclic Chem. **44**, 345–346 (2002)