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Inclusion complexes of tretinoin with cyclodextrins

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Abstract

Complexes of tretinoin with β-cyclodextrin, hydroxypropyl β-cyclodextrin and dimethyl β-cyclodextrin were prepared by dissolving the products in suitable organo-aqueous solvents under nitrogen and sheltered from light and then stirred for 8 days. The solvents were then evaporated, the free tretinoin eliminated and the complexes dried. Characterization of the products was carried out by electron scanning microscopy, differential scanning calorimetry, IR spectrophotometry, X-ray analysis and ^{1}H-NMR study, which confirmed the existence of an inclusion compound but with weak affinity between tretinoin and the different cyclodextrins. Tretinoin solubility was dramatically enhanced by inclusion, especially in dimethyl β-cyclodextrin. However, in every case, the dissolved products dissociated more or less rapidly leading to reprecipitation of free tretinoin. © 1997 Elsevier Science B.V.

Keywords: Tretinoin; β-cyclodextrin; β-cyclodextrin derivatives; Inclusion complexes; Physicochemical analysis; Solubility; Dissolution

1. Introduction

In dermopharmacy, tretinoin has been the subject of growing interest during recent years. In fact, it can be used in the treatment of acne because of its keratolytic activity (Kligman et al., 1969). It also increases collagen synthesis and epidermal growth in the cicatrization process (Elias, 1986). More recently, improvement in photo-ageing was described (Weiss et al., 1988).

However, its topical use is limited due to several drawbacks: irritation power, high instability in the presence of air, light and heat and low water solubility. Its inclusion in a cyclodextrin could possibly improve all these characteristics.

Pitha reported the improvement in solubility of various retinoids by the use of α-or β-cyclodextrin, dimethyl β-or γ-cyclodextrin, trimethyl β-cy-

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clodextrin and hydroxypropyl β-cyclodextrin (Pitha et al., 1980; Pitha and Szente, 1983; Pitha, 1981, 1988). However, he did not separate the inclusion complexes. Frömming et al., 1988 prepared an inclusion complex of vitamin A acetate in β-cyclodextrin. Unfortunately, this did not result in a real increase in the stability of the product. Amdidouche et al. (1989) described the inclusion of retinoic acid in β-cyclodextrin in conditions preventing its degradation during the preparation process: stirring an isopropanol/water solution of β-cyclodextrin and retinoic acid at 6°C for 15 days.

The purpose of the present work is the preparation and isolation of inclusion complexes of retinoic acid in three different cyclodextrins: β-cyclodextrin, hydroxypropy β-cyclodextrin and dimethyl β-cyclodextrin.

2. Materials and method

2.1. Products

Tretinoin (retinoic acid) was provided by Produits Roche (France), β-cyclodextrin and hydroxypropyl β-cyclodextrin by Roquette Frères (France) and dimethyl β-cyclodextrin by Cyclolab (Budapest, Hungary).

All solvents used were of analytical grade. Water was obtained by bidistillation.

2.2. Complexes

Inclusion complexes were prepared using equimolecular solutions of tretinoin and cyclodextrin, in conditions leading to the best yield and to the most stable complexes.

2.2.1. Tretinoin/β-cyclodextrin

Cyclodextrin (173 mg) was dissolved in 30 ml of water and 46 mg of tretinoin in 80 ml of ethanol. The two solutions were mixed and subjected to nitrogen bubbling while sheltered from light. The mixture was cooled to 5°C and stirred for 8 days and the solution was then evaporated under vacuum. In order to eliminate free tretinoin, the resulting solid product was washed rapidly with isopropanol and filtered on a 0.45 μm cellulose acetate membrane and finally dried for 48 h in a desiccator.

2.2.2. Tretinoin/hydroxypropyl β-cyclodextrin

Hydroxypropyl β-cyclodextrin (200 mg) and 46 mg of tretinoin were dissolved in 60 ml of ethanol. The solution, sheltered from light, was subjected to nitrogen bubbling. After 8 days of strong agitation, the solution was evaporated under vacuum. As previously, the free tretinoin was removed by washing with isopropanol and filtration on a 0.45 μm cellulose acetate membrane and the product was then dried for 48 h in a desiccator.

2.2.3. Tretinoin/dimethyl β-cyclodextrin

Dimethyl β-cyclodextrin (200 mg) was dissolved in 5 ml of water and 46 mg of tretinoin in 50 ml of ethanol. The two solutions were mixed and subjected to nitrogen bubbling while sheltered from light. After 8 days of strong agitation, 10 ml of water was added in order to precipitate the tretinoin in excess. The product was filtered on a 0.45 μm cellulose acetate membrane and dried for 48 h in a desiccator.

2.3. Complex characterization

2.3.1. Tretinoin/cyclodextrin molar ratio

The determination of tretinoin contained in the products obtained was carried out by HPLC with UV spectrophotometric detection at 350 nm as described by Amdidouche et al. (1994).

2.3.2. Physicochemical characterization of the products

Raw materials, physical mixtures and complexes were subjected to a series of physicochemical analyses.

Scanning electron microscopy (Philips, France) was carried out on samples stuck on plots by silver glue, subjected to vacuum for 20 min and gold metallized. Differential scanning calorimetry (Dupont DSC 910, France) was carried out on 2 mg samples in open capsules. IR spectrophotometry (Perkin-Elmer 257, France) was carried out on potassium bromide tablets, from 4000 to 625 cm^{-1}. X-ray analysis (Philips PW1729, France)

was carried out using an X-ray generator equipped with copper anticathode Ka1 = 1.5405 Å, nickel filter, of 80 mA power and 50 kV.

2.3.3. Investigation of inclusions

In order to characterize the location of tretinoin inside the cyclodextrin cavities, an NMR study (Varian EM 390, 90 MHz, France) was carried out in deuterated water. However, due to the non- or low-water solubility of tretinoin and its complexes, it was not possible to study directly the products obtained as described above, but only to study the inclusion possibility by working on tretinoin sodium salt pure or in complex form. Tretinoin was dissolved in a mixture of 5 ml of water, alkalinized by sodium hydroxide to pH 9 and 60 ml of ethanol and stirred for 8 days. Afterwards, free tretinoin was precipitated by returning to pH 8 and filtered on a 0.45 μm cellulose acetate membrane. The solution was evaporated and the powder dried in a desiccator for 48 h.

2.3.4. Solubility and dissolution characteristics

One of the major interests in preparing inclusion complexes of tretinoin is to increase its water solubility. This was assessed on an excess of product in 5 ml of water, under nitrogen, sheltered from light and stirred during 24 h at 20 ± 2°C. The solution is then filtered on a 0.45 μm cellulose acetate membrane and dosed. The dissolution kinetics were also investigated, in non-sink conditions, using the European Pharmacopoeia paddle method, at 20 ± 2°C, stirring at 96 r/min. The tretinoin dissolved is dosed for 1 h. Dosages are carried out at 350 nm in UV, by comparison with a standard curve obtained from water/isopropanol tretinoin solutions. Therefore, all the dissolution samples were diluted with appropriate amounts of isopropanol before UV analysis.

2.3.5. Stability constant

When working on inclusion complexes, the stability constant is very often the key to the explanation of the various results obtained. The classic determination method using solubility diagrams, which requires calculations involving drug solubility, could be impaired by low and therefore approximative drug solubility values. As this is the case for tretinoin, the stability constant was also assessed by the chromatographic method.

The solubility method is that described by Higuchi and Connors (1965). It consists of adding constant excesses of guest to increasing concentrations of cyclodextrin solutions in water. After thermodynamic equilibrium is reached, changes in guest solubility (S) are plotted as a function of cyclodextrin (CD) concentration. Two types of solubility diagram can be obtained: A or B. A corresponds to the formation of a soluble inclusion complex and B to the formation of an inclusion complex with well-defined solubility. In the case of the formation of a 1:1 inclusion complex, the stability constant $K_{1:1}$ can be obtained from the slope S_1 and intercept S_0 of the initial straight line portion of the diagram:

$$K_{1:1} = (S_t - S_0)/S_0\{[CD]_t - (S_t - S_0)\} = \text{slope}_1/S_0(1 - \text{slope}_1) \quad (1)$$

In some cases, after formation of a 1:1 inclusion complex, the formation of a 1:2 complex occurs, which is characterized by a second straight line with a second slope. The stability constant $K_{1:2}$ is then obtained from the second slope S_2 and intercept S'_0 of the two lines:

$$K_{1:2} = \text{slope}_2/S'_0(1 - \text{slope}_2) \quad (2)$$

The chromatographic method is derived from that described by Uekama et al., 1978. The addition of a cyclodextrin to the mobile phase of liquid chromatography will decrease the retention time of a guest molecule (tretinoin) depending on the stability constant of the inclusion complex. The stability constant is obtained from the intercept of the curve of $\{[CD]_m/T'_0 - T_{obs}\}$ versus the cyclodextrin concentration in the mobile phase $[CD]_m$.

The present study was carried out on a Shimadzu apparatus (Japan). The mobile phase was methanol/water 75/30 and the pH was maintained at 2.3 allowing the existence of tretinoin in the molecular state. It was used after ultrasound degassing and filtration on 0.22 μm cellulose acetate membrane. Its flow rate was 1 ml/min. The stationary phase was Spherisorb ODS2-5 mm (SFCC, France) 150 × 46 mm^2.

The presence of an organic solvent (methanol) in the mobile phase was necessary to obtain the dissolution of tretinoic acid and its migration. Due to this, the stability constants obtained by the two methods are not exactly comparable, but, however, constitute helpful indications of the affinity of tretinoin for the cyclodextrin cavity.

3. Results and discussion

3.1. Tretinoin/cyclodextrin molar ratio

Chromatographic dosage of the tretinoin present in the products prepared indicated that tretinoin/cyclodextrin molar ratios were 1:5 for the tretinoin/dimethyl β-cyclodextrin complex and 1:2 for tretinoin/hydroxypropyl β-cyclodextrin and tretinoin/dimethyl β-cyclodextrin. It should be pointed out that in each case the excess of cyclodextrin was not eliminated from the final product.

3.2. Physicochemical characterization

3.2.1. Scanning electron microscopy

Scanning electron microscopy (Fig. 1) showed that the preparation methods led to new compounds. In fact, tretinoin (Fig. 1a) appears as long rectangular-shaped crystals, β-cyclodextrin (Fig. 1b) is in the form of larger tabular crystals, hydroxypropyl β-cyclodextrin (Fig. 1c) is amorphous and dimethyl β-cyclodextrin (Fig. 1d) is bulky. All three complexes obtained are very different from the mother products: tretinoin/β-cyclodextrin (Fig. 1e) is a more or less sticky amorphous powder, tretinoin/hydroxypropyl β-cyclodextrin (Fig. 1f) is in globular form and tretinoin/dimethyl β-cyclodextrin (Fig. 1g) is in the form of very well-defined lamellae.

3.2.2. Differential scanning calorimetry

All the cyclodextrins investigated (Fig. 2b, c and d) present a water loss, at around 85°C for β-cyclodextrin, 54°C for hydroxypropyl β-cyclodextrin and 36°C for dimethyl β-cyclodextrin. Furthermore, a characteristic thermal accident was observed at 212°C for β-cyclodextrin and, for all the cyclodextrins, fusion/decomposition occurred around and above 250°C. Tretinoin itself melted at 175°C (Fig. 2a). All the physical mixtures which are just the superposition of their own constituents are not reported. For all the complexes (Fig. 2e, f and g), disappearance of the melting peak of tretinoin was observed associated to a smaller peak for water loss of the mother cyclodextrin. These results are in favour of the existence of an inclusion complex associated to free cyclodextrin (presence of water), this not having been eliminated in the preparation process.

3.2.3. IR spectra

Tretinoin (Fig. 3a) is characterized by peaks appearing between 1700 and 1500 cm^{-1}, which cannot be confused with cyclodextrin peaks (Fig. 3b, c and d) around 1200–1000 cm^{-1}. In the physical mixtures, the spectra are the superposition of those of the pure products with attenuation of the tretinoin peak. For the complexes, the tretinoin peak mostly disappears and the excess of free cyclodextrin is still visible whatever the type of cyclodextrin (Fig. 3e, f and g).

3.2.4. X-rays

Tretinoin, β-cyclodextrin and dimethyl β-cyclodextrin presented well defined crystalline X-ray patterns (Fig. 4a, b, c and d). Patterns of physical mixtures are the superposition of patterns of pure products, but patterns of complexes are all amorphous, whatever the type of the mother cyclodextrin. The results obtained with dimethyl β-cyclodextrin are reported in Fig. 4e and f as examples.

This result can be explained by the preparation method in which evaporation under vacuum can reduce the crystallization possibility. Furthermore, the excess of free cyclodextrins in all the complexes prepared can prevent the mixture from achieving good crystallization. Moreover, this highlights the fact that scanning electron microscopy does not lead to the conclusion of different crystallization forms but just to different solid states.

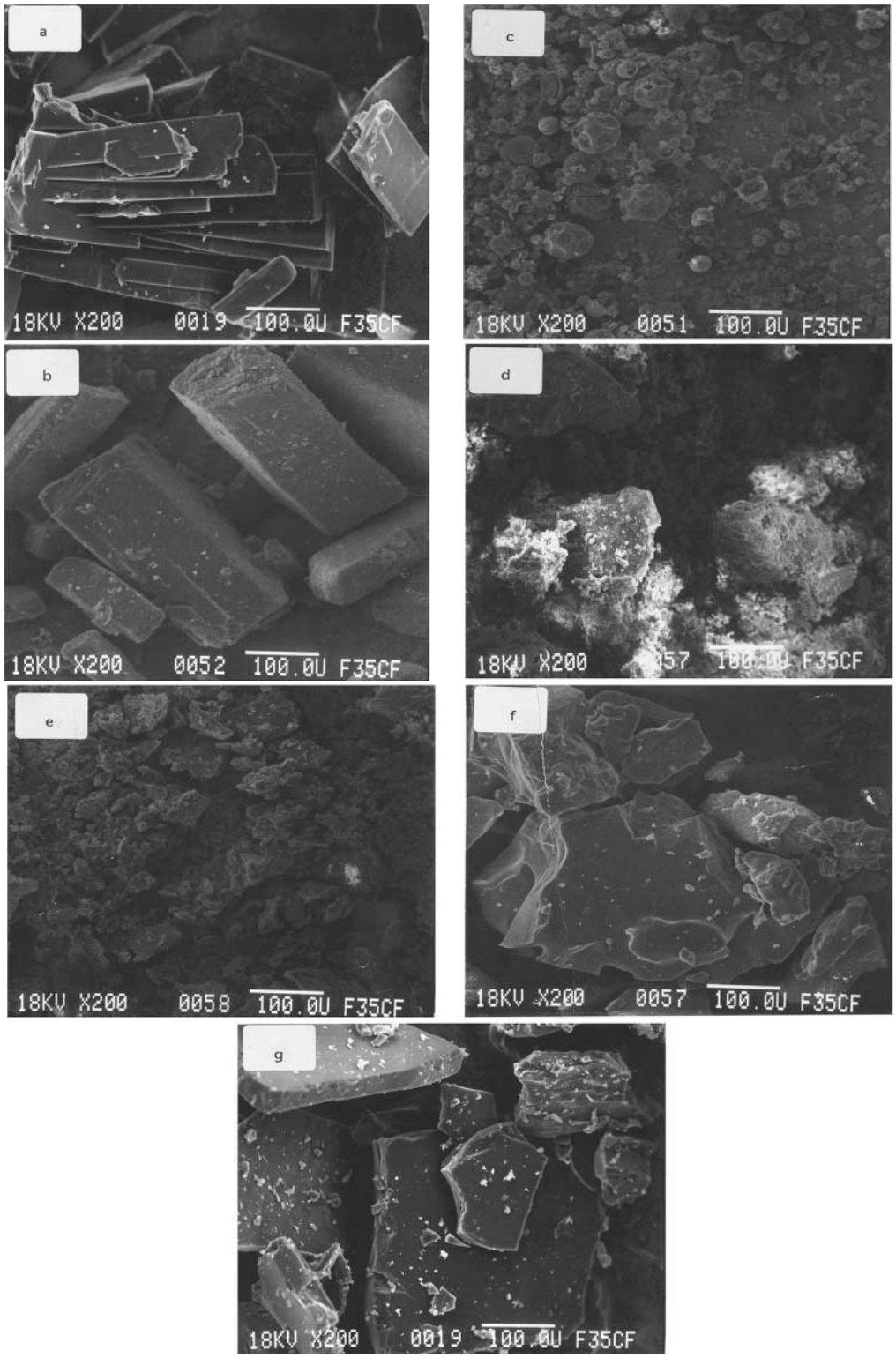

Fig. 1. Scanning electron microscopy photographs of (a) tretinoin; (b) β-cyclodextrin; (c) hydroxypropyl β-cyclodextrin; (d) dimethyl β-cyclodextrin; (e) tretinoin/β-cyclodextrin complex; (f) tretinoin/hydroxypropyl β-cyclodextrin complex; (g) tretinoin/dimethyl β-cyclodextrin complex.

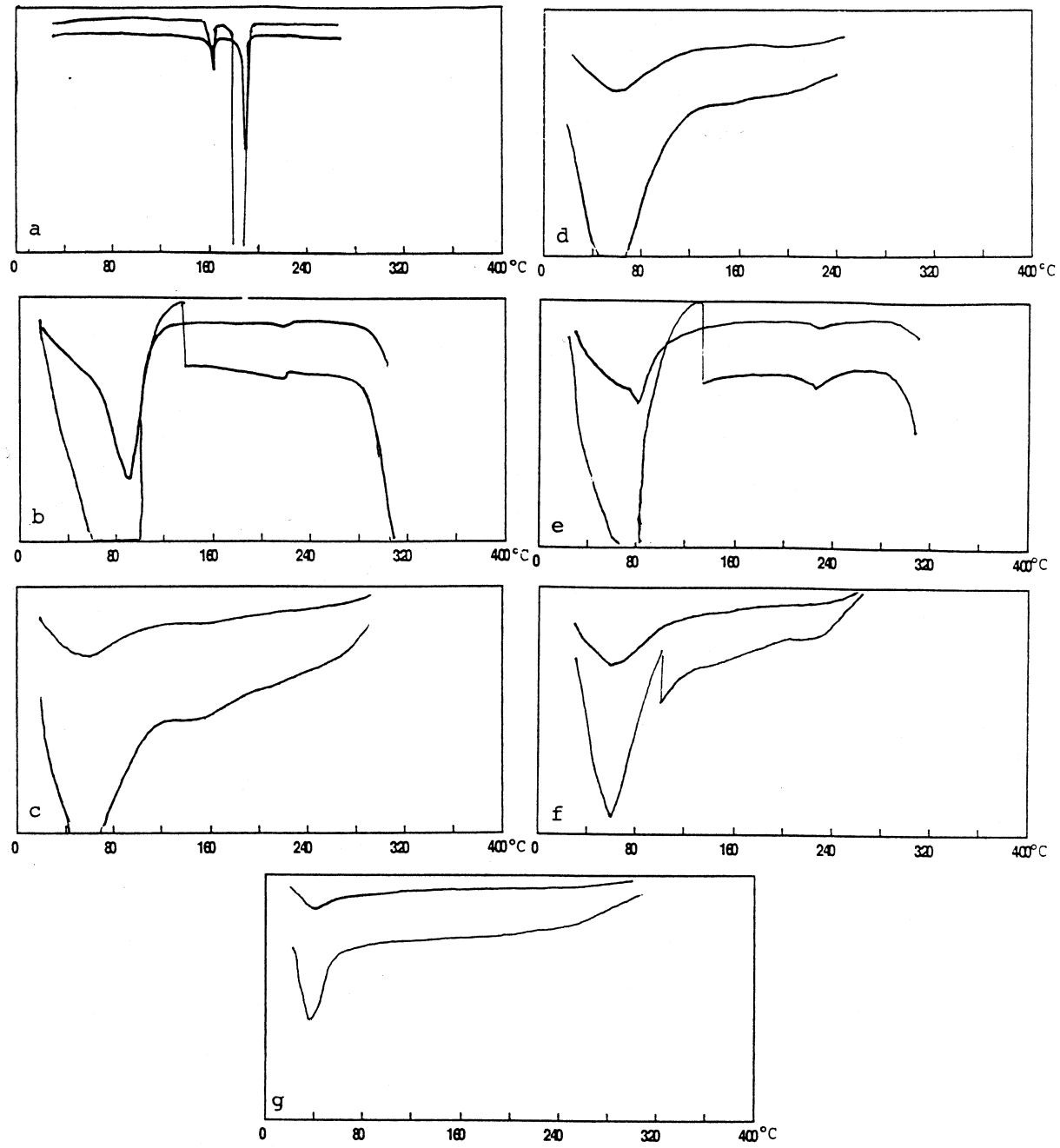

Fig. 2. Differential scanning calorimetry patterns of (a) tretinoin; (b) β-cyclodextrin; (c) hydroxypropyl β-cyclodextrin; (d) dimethyl β-cyclodextrin; (e) tretinoin/β-cyclodextrin complex; (f) tretinoin/hydroxypropyl β-cyclodextrin complex; (g) tretinoin/dimethyl β-cyclodextrin complex.

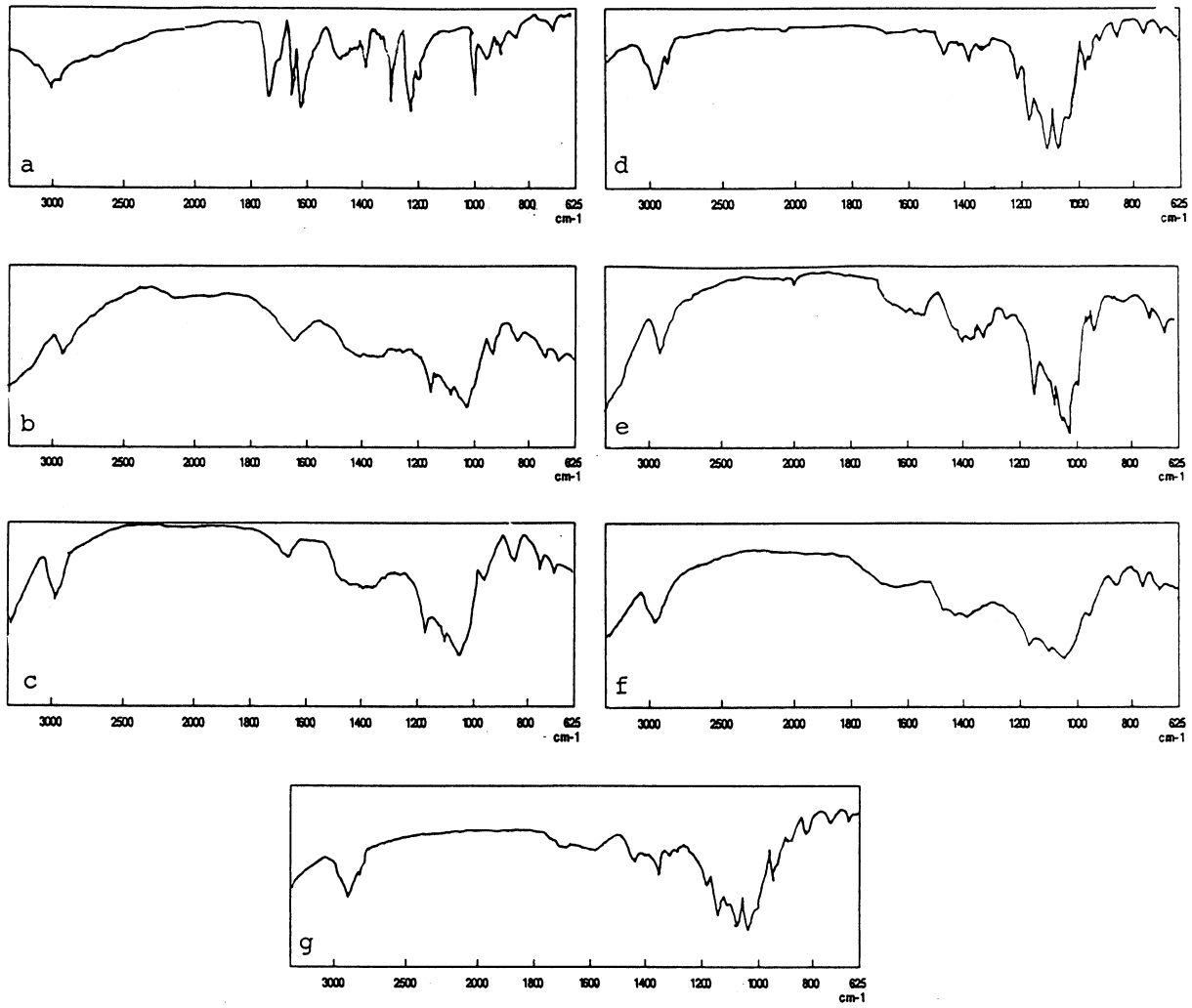

Fig. 3. IR spectra of (a) tretinoin; (b) β-cyclodextrin; (c) hydroxypropyl β-cyclodextrin; (d) dimethyl β-cyclodextrin; (e) tretinoin/β-cyclodextrin complex; (f) tretinoin/hydroxypropyl β-cyclodextrin complex; (g) tretinoin/dimethyl β-cyclodextrin complex.

3.2.5. ^{1}H-NMR study

The objective of the ^{1}H-NMR study was to demonstrate a possible displacement of cyclodextrin protons located inside the cavity (namely H3, H5 and H6) resulting from tretinoin inclusion and interaction.

However, results obtained (not presented here) showed only weak displacements, but some clear modifications were observed such as duplication of peaks.

These results prove the existence of weak interaction between tretinoin and cyclodextrins and the formation of inclusion complexes in the products obtained.

3.3. Solubility and dissolution

Tretinoin water solubility, which is almost nil ($\approx 8 \times 10^{-3}$ mg/100 ml), is dramatically improved by complexation and it increases from β-cyclodextrin (2.7×10^3 mg/100 ml) to hydrox-

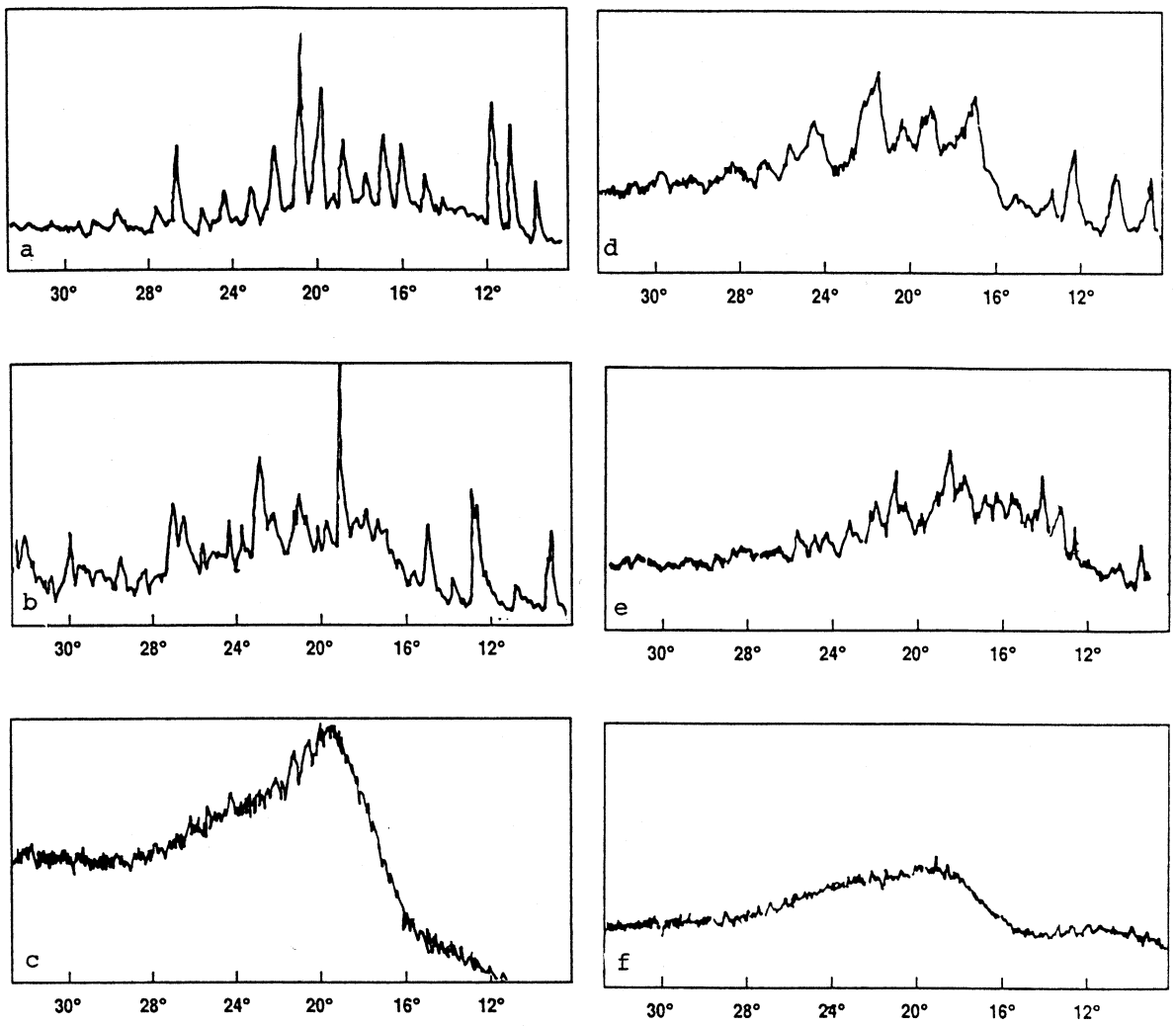

Fig. 4. X-ray spectra of (a) tretinoin; (b) β-cyclodextrin; (c) hydroxypropyl β-cyclodextrin; (d) dimethyl β-cyclodextrin; (e) physical mixture of tretinoin and dimethyl β-cyclodextrin; (f) tretinoin/dimethyl β-cyclodextrin complex.

ypropyl β-cyclodextrin (9.3×10^3 mg/100 ml) to dimethyl β-cyclodextrin (64×10^3 mg/100 ml). It is interesting to note that this increase does not follow the water solubility of the mother cyclodextrin, which increases in the order β-cyclodextrin < dimethyl β-cyclodextrin < hydroxypropyl β-cyclodextrin. There is probably a stronger interaction between tretinoin and dimethyl β-cyclodextrin than between tretinoin and hydroxypropyl β-cyclodextrin or β-cyclodextrin itself.

This conclusion is confirmed by the complex dissolution kinetics. In every case there is a very fast dissolution with a maximum increasing from β-cyclodextrin to hydroxypropyl β-cyclodextrin and dimethyl β-cyclodextrin complex (Fig. 5a, b and c). However, dissolution is followed by rapid reprecipitation of tretinoin, which occurs after some 10 (β-cyclodextrin) to 25 min (hydroxypropyl β-cyclodextrin and dimethyl β-cyclodextrin). This confirms the probable low stability constant of the complexes and especially

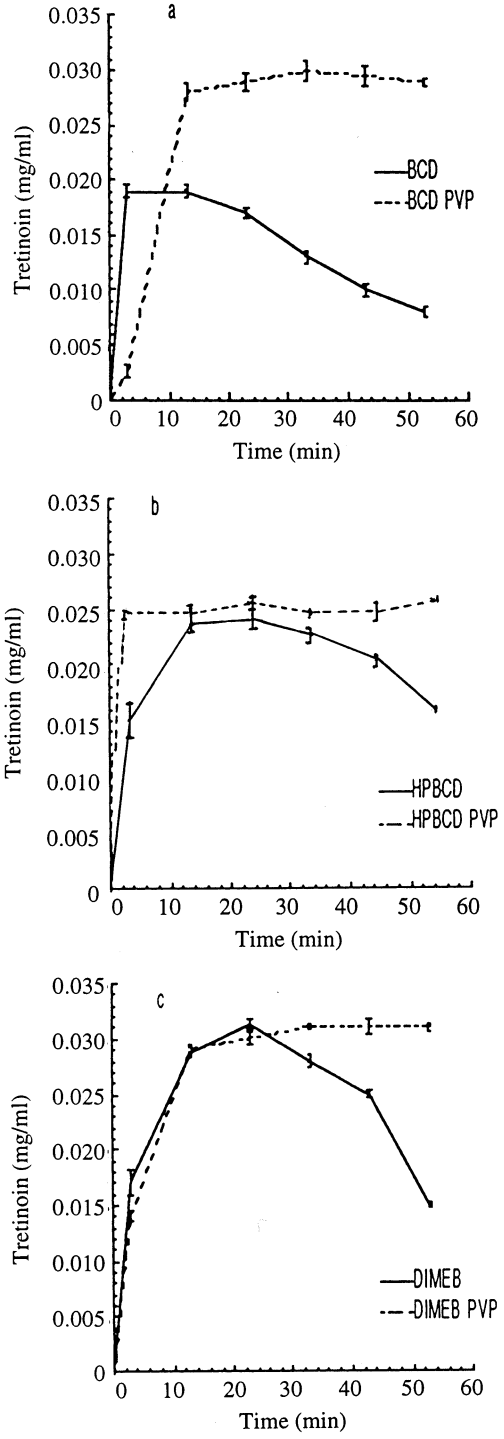

Fig. 5. Dissolution kinetics of (a) tretinoin/β-cyclodextrin complex; (b) tretinoin/hydroxypropyl β-cyclodextrin complex; (c) tretinoin/dimethyl β-cyclodextrin; (d) tretinoin/dimethyl β-cyclodextrin + PVP 20.

tretinoin/β-cyclodextrin as could be concluded from the NMR study.

It is interesting to note that reprecipitation of tretinoin can be delayed by the addition of 1% of PVP 25 (Fig. 5e) which is well known as a complexing/dissolving agent (Bahal and Kostenbauder, 1964; Cho et al., 1971; Loftsson et al., 1994).

3.4. Stability constant

Previous results highlight the interest of using dimethyl β-cyclodextrin in the formulation of tretinoin hydrogel for dermal administration because of the high water solubility improvement obtained. However, a low stability constant being evoked, it was determined for this cyclodextrin derivative.

3.4.1. Solubility method

The solubility diagram of tretinoin in dimethyl β-cyclodextrin presented two linear segments (Fig. 6), characteristic of the formation of inclusion complexes of types 1:1 and 1:2, similar to that obtained by Brewster et al., 1988 with oestradiol and hydroxypropyl β-cyclodextrin. Corresponding stability constants were $K_{1:1} = 12\,265\ M^{-1}$ and $K_{1:2} = 89\ M^{-1}$.

The very high stability constant of the 1:1 complex is probably not to be taken into consideration: due to the low water solubility of tretinoin ($\approx 4 \times 10^{-4}$ mmole/ml) and to the difficulty in measuring its exact value, very significative errors can impair the calculation of $K_{1:1}$. On the other hand, the 89 M^{-1} stability constant of the 1:2 complex corresponds better to the data obtained in the physicochemical and dissolution studies.

3.4.2. Chromatographic method

Retention time of tretinoin (19 min) was decreased by dimethyl β-cyclodextrin and the stability constant, calculated from the graph of Fig. 7, was $K = 80\ M^{-1}$. This value, even if it is not comparable with that of $K_{1:1}$ obtained by the solubility method, is probably more realistic with respect to the dissolution behaviour of the complex.

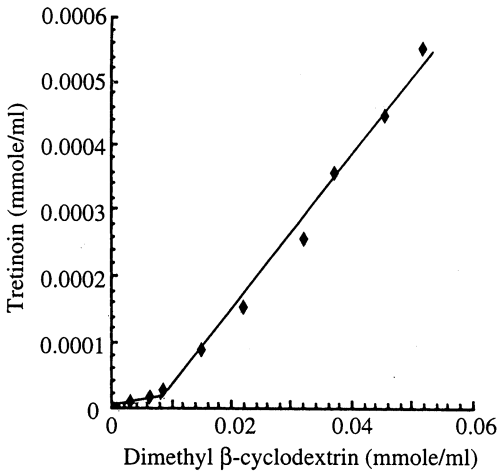

Fig. 6. Solubility diagram of tretinoin in dimethyl β-cyclodextrin.

4. Conclusion

Complexes of tretinoin with β-cyclodextrin, hydroxypropyl β-cyclodextrin and dimethyl β-cyclodextrin were prepared by the coprecipitation method. The products obtained have proved to be amorphous inclusion compounds in the presence of an excess of cyclodextrins. However the inclusion compounds have a low affinity constant as shown by reprecipitation of the dissolved products and as confirmed for the dimethyl β-cyclodextrin complex. Nevertheless, the increase in solubility is noticeable and can allow, in the future, preparation of hydrogels containing tretinoin amounts suitable for dermatological uses.

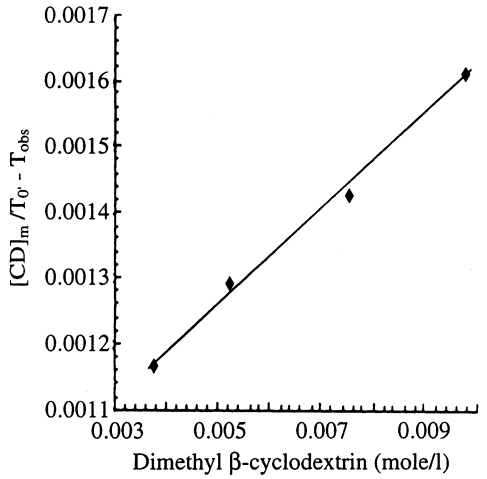

Fig. 7. HPLC determination of stability constant of tretinoin/dimethyl β-cyclodextrin.

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