International Journal of Pharmaceutics, 54 (1989) 175–179
Elsevier

IJP 01853

Inclusion of retinoic acid in β-cyclodextrin

Didja Amdidouche [1], Henri Darrouzet [2], Dominique Duchêne [3] and Marie-Christine Poelman [1]

[1] *Département de Dermopharmacie, Faculté de Pharmacie, Université de Paris V, Paris (France),* [2] *Société Roquette Frères, Lestrem (France),* [3] *Laboratoire de Pharmacie Galénique et Biopharmacie, UA CNRS 1218, Faculté de Pharmacie, Université de Paris XI, Châtenay Malabry (France)*

(Received 6 December 1988)
(Modified version received 15 March 1989)
(Accepted 20 March 1989)

Key words: β-Cyclodextrin; Inclusion; Retinoic acid; Solubility

Summary

Retinoic acid is widely used in dermatology. Its use is, however, limited by a number of drawbacks, including its insolubility in aqueous vehicles. The inclusion of this compound in β-cyclodextrin would appear to reduce some of the drawbacks. The procedure adopted for the preparation of the inclusion was coprecipitation from a water/isopropanol equimolecular solution of β-cyclodextrin and retinoic acid, stirred for 16 days at low temperature (6 °C), and shielded from light. The inclusion is identified by scanning electron microscopy, circular dichroism, differential scanning calorimetry, and X-ray diffraction patterns. The aqueous solubility of retinoic acid, which is less than 0.5 mg/l for the pure product, increases to 12 mg/l for the physical mixture, and reaches 160 mg/l for the inclusion.

Introduction

The enhancement of the therapeutic interest of retinoic acid and of whole retinoids has been considerable during the past few years (Saurat, 1985).

In dermatology, they improve cicatrization by an increase in collagen synthesis. In the treatment of acne, the topical administration of retinoic acid has a keratolytic activity which contributes to the elimination of microcysts and comedones. Furthermore, it stimulates the follicular epithelium and free keratinocyte proliferation, which are then carried away by the sebum flow (Kligman et al., 1969). More recently, topical retinoic acid has been proposed for its favourable effect on epidermal growth and action on keratinization in the treatment of epidermal dysplasia and photo-ageing (Connor et al., 1986; Weiss et al., 1988).

Unfortunately, a number of drawback limits the use of this drug: its insolubility in aqueous medium, its poor stability in the presence of air and light, and, on the other hand, its local irritating reaction, and the low cutaneous tolerance it induces (Lehman et al., 1988).

For their part, cyclodextrins, and the most commonly-available, β-cyclodextrin, are well-known for their ability to form inclusion compounds with a large number of molecules, among which are found slightly hydrosoluble pharmaceutical active ingredients. The inclusion results in a

Correspondence: D. Duchêne, Laboratoire de Pharmacie Galénique et Biopharmacie, Faculté de Pharmacie, Université de Paris XI, Rue Jean Baptiste Clément, 92296 Châtenay Malabry Cédex, France.

hydrophilization of the host molecule with a concomitant improvement of its apparent solubility and dissolution rate (Duchêne et al., 1985a, 1987). Furthermore, there is generally an improvement in stability to air and light (Duchêne et al., 1985b, 1987).

The purpose of the work presented here was to prepare an inclusion of retinoic acid in β-cyclodextrin, despite the fact that the high instability to air and light of retinoic acid impedes the use of the grinding method, and, due to its extremely low solubility in many solvents, this considerably limits the possibility of using a coprecipitation method.

Materials and Methods

Materials

Retinoic acid, or 3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid (Scheme 1), was provided by Produits Roche (Paris, France) and used without any preliminary treatment.

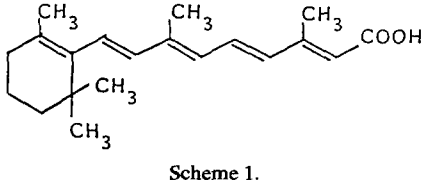
Its molecular weight is 300.44, and its melting point between 180 and 182°C. It appears in the form of thin yellow crystals, insoluble in water, slightly soluble in ethanol and chloroform, and soluble in isopropanol, dioxane, acetone, ether and boiling benzene. It is unstable in heat and light, and solutions are destabilized in the presence of oxidizing agents.

β-Cyclodextrin, or cyclo-heptaamylose, was provided by Roquette Frères (Lestrem, France) in a purity of at least 99%, and employed without particular purification.

The solvents employed were analytical grade.

Preparation of the inclusion

The inclusion was prepared by the coprecipitation method, using an organo-aqueous solution of retinoic acid and β-cyclodextrin.



Scheme 1.

The solvents investigated were water-miscible organic solvents capable of dissolving the retinoic acid and giving a clear solution when added to an aqueous solution of cyclodextrin. They were acetone, dioxane and isopropanol.

The procedure consisted in mixing an aqueous solution of β-cyclodextrin with an organic solution of retinoic acid, in order to obtain equimolecular amounts of the two products. The concentrations of the two solutions were calculated in order to avoid the extemporaneous precipitation of one or other of the components at the time of mixing.

Acetone did not lead to a clear solution. Dioxane led to the formation of an inclusion compound after 12 h of stirring, but unfortunately retinoic acid was progressively degraded with this solvent. For these reasons, the two solvents were not used subsequently.

Only isopropanol was retained. The formation of the inclusion is very slow. Hence, to avoid any degradation of retinoic acid, stirring must be carried out shielded from light and at low temperature (6°C). These specific conditions led to the use of very dilute solutions, especially for β-cyclodextrin.

The following protocol was established. Two parts of an isopropanolic solution of retinoic acid ($4 \cdot 10^{-3}$ M) were added, whilst stirring, to one part of an aqueous solution of β-cyclodextrin ($8 \cdot 10^{-3}$ M). The solution obtained was then brought to 6°C and stirred.

Under these conditions, spontaneous precipitation of the inclusion does not occur. The inclusion formed must therefore be isolated by evaporation of the solvent in a rotatory vacuum evaporator. In order to eliminate the unstable and irritating free retinoic acid possibly present in the coprecipitate, it is rapidly washed with isopropanol.

The presence and quantitative determination of retinoic acid in the product were evaluated by ultraviolet spectrophotometry (Perkin Elmer 550). The solvent used was DMSO, which is a solvent for both retinoic acid and β-cyclodextrin. The maximal absorption of retinoic acid in DMSO is 360 nm (Fig. 1), and cyclodextrin has no UV absorption.

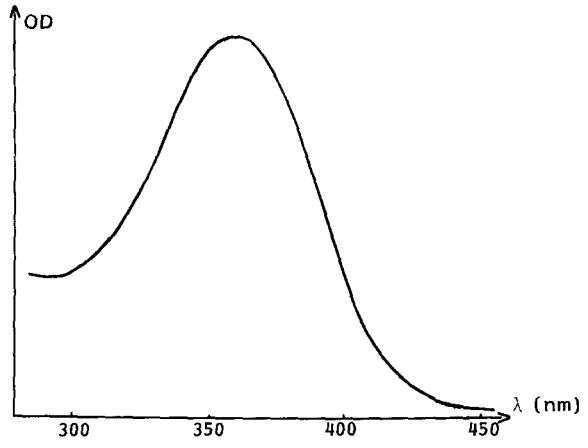


Fig. 1. Maximum ultraviolet absorption of retinoic acid in DMSO.

After stirring for 16 days, only 20% of the initial retinoic acid is included in the β-cyclodextrin.

Examination of the inclusion

The microscopic aspect of the raw materials isolated was compared with that of the product obtained by coprecipitation/evaporation by examination under the scanning electron microscope (Cameca).

The reality of the inclusion was confirmed by subjecting the various products to analysis by circular dichroism (Jobin Yvon III), differential scanning calorimetry (in open capsule, to allow the evaporation of the crystallization water of the β-cyclodextrin) (Du Pont 990), and X-ray diffractometry (Philips PW 1840 equipped with a copper anticathode).

The aqueous solubility of retinoic acid in various forms was determined in distilled water (pH 5.84–5.90) by dispersing a quantity of product corresponding to an excess amount of retinoic acid, or 5 mg, in 8 ml of distilled water, kept at 6 °C, shielded from light, and stirred for 68 h. 1 ml of the filtered solution is then added to 1 ml of isopropanol, and the quantity of retinoic acid dissolved is determined by UV absorption at 344 nm (Perkin Elmer 550).

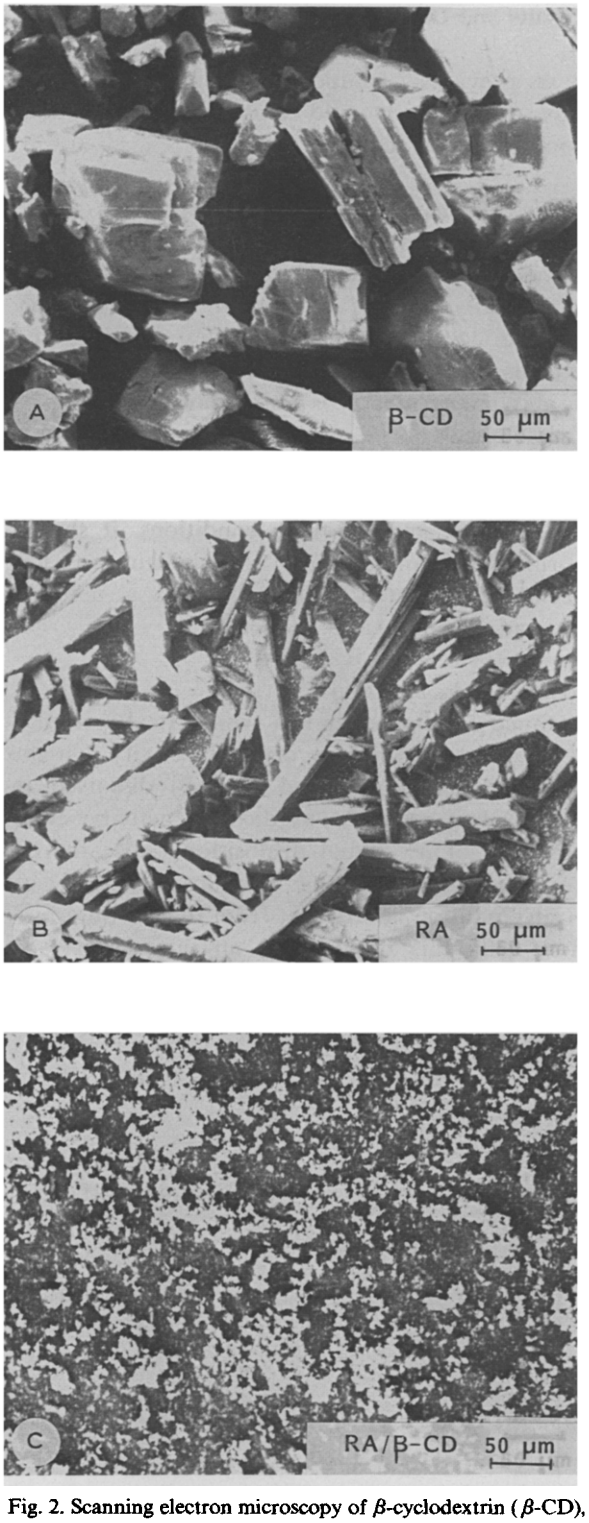


Fig. 2. Scanning electron microscopy of β-cyclodextrin (β-CD), retinoic acid (RA), and coprecipitated inclusion (RA/β-CD).

Results and Discussion

As previously mentioned, isopropanol was the only solvent which could be used without degradation of retinoic acid.

Microscopic aspect

Analysis by the scanning electron microscope revealed that, whereas β-cyclodextrin crystallizes in a relatively large polyhedral form (Fig. 2A), retinoic acid appears as needles or elongated crystals (B). The product obtained after evaporation is amorphous (C). This amorphous form is characteristic of the product obtained here, and is not the consequence of the evaporation process, because it has been very slow due to the low temperature, and, in these conditions, it should normally not impede any crystallization, as has been demonstrated previously with a dermocorticoid (Glomot et al., 1988).

Reality of the inclusion

Since β-cyclodextrin is a symmetrical molecule, it does not present any dichroic activity. On the other hand, retinoic acid (Fig. 3) dissolved in DMSO has a significant dichroic activity, characteristic of its spatial disposition. Since the dichroic activity of the product obtained by coprecipitation is different from that of pure retinoic acid, this reveals that an interaction took place between retinoic acid and β-cyclodextrin,

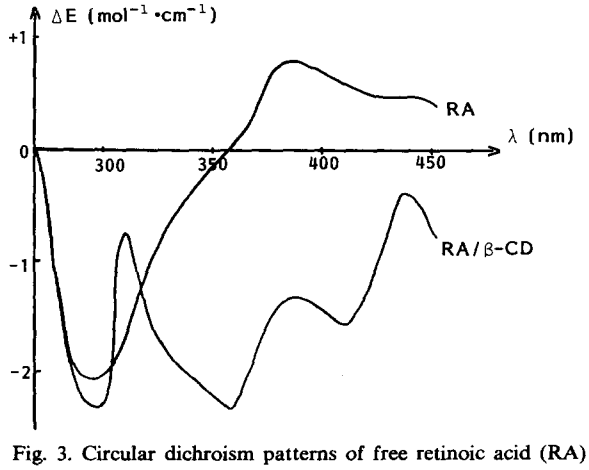


Fig. 3. Circular dichroism patterns of free retinoic acid (RA) and retinoic acid included in β-cyclodextrin (RA/β-CD).

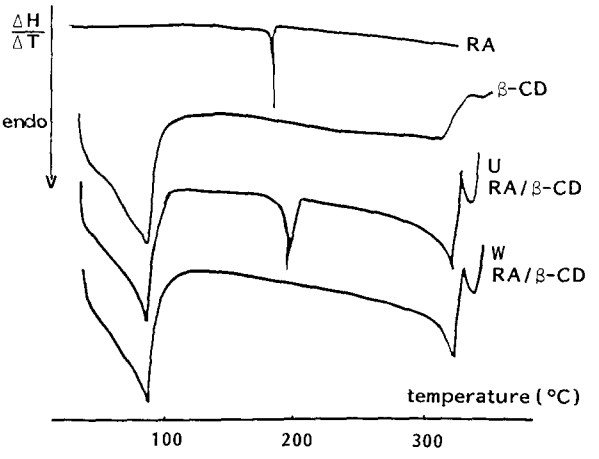


Fig. 4. Differential scanning calorimetry curves of free retinoic acid (RA), β-cyclodextrin (β-CD), unwashed coprecipitate (U RA/β-CD) and washed coprecipitate (W RA/β-CD).

modifying its spatial disposition and corresponding probably to an inclusion.

The differential scanning calorimetry curves of the raw materials (retinoic acid and β-cyclodextrin) compared with those obtained by coprecipitation before and after washing (Fig. 4) confirm not only an interaction between retinoic acid and β-cyclodextrin, but a real inclusion. In fact, the characteristic thermal accident of retinoic acid, corresponding to its melting point at 180 °C, does not appear in the washed coprecipitate, but is found at a higher temperature (282 °C) before the browning of β-cyclodextrin, which occurs between 300 and 350 °C. For the unwashed coprecipitate, the fusion peak of the free retinoic acid (at 180 °C) appears simultaneously with that of the retinoic acid coprecipitated with β-cyclodextrin (at 282 °C). This displacement of the fusion peak of retinoic acid confirms that coprecipitation leads to an inclusion: retinoic acid is protected by the β-cyclodextrin ring molecule. It should be noted that the water molecules of β-cyclodextrin do not disappear in the coprecipitate inclusion obtained. This can be explained partly by the fact that the coprecipitate has been washed with isopropanol in order to eliminate the free retinoic acid, and has not been washed with water to eliminate the free cyclodextrin whose quantity is unknown. In fact, after the UV spectrophotometric determination,

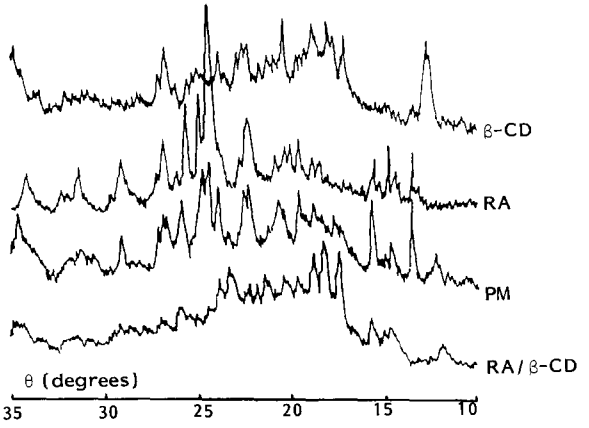


Fig. 5. X-ray diffractometry patterns of β-cyclodextrin (β-CD), free retinoic acid (RA), physical mixture of retinoic acid and β-cyclodextrin (PM), and coprecipitated inclusion of retinoic acid in β-cyclodextrin (RA/β-CD).

the amount of retinoic acid in the dry compound is 20%, which corresponds to 1 molecule of retinoic acid for 4 molecules of β-cyclodextrin. This does not mean that there are 3 cyclodextrin molecules in excess, because we do not know if the long hydrocarbon chain of retinoic acid retains, more or less, some cyclodextrin molecules.

The X-ray diffractometry pattern (Fig. 5) of the physical mixture of retinoic acid and β-cyclodextrin is approximately the superposition of the patterns of the raw materials. On the other hand, the coprecipitate product has a completely different pattern in which it is no longer possible to distinguish the characteristic peaks of retinoic acid, thus confirming the existence of a new compound. Some peaks of free β-cyclodextrin appear around 18 °C. Furthermore, the amorphous character of the inclusion is obvious, compared with the physical mixture.

Aqueous solubility

If further proof were needed, the aqueous solubility of retinoic acid from the inclusion compound, compared with that from the physical mixture, confirmed the reality of the inclusion. Raw retinoic acid is almost insoluble in water (less than 0.5 mg/l). Its solubility from the physical mixture increases to 12 mg/l, and from the inclusion compound it reaches 160 mg/l.

Conclusion

The feasibility of preparing an inclusion compound of retinoic acid in β-cyclodextrin by a coprecipitation method has been demonstrated. However, due to the low solubility of retinoic acid in various water-miscible solvents, and to its great instability to air and light, the procedure is rather difficult to bring into operation. Nevertheless, the new compound presenting a highly increased solubility seems to be of great interest for dermatological applications, as already proved by preliminary in vivo studies.

References

Connor, M.J., Ashton, R.E. and Lowe, N.J., A comparative study of the induction of epidermal hyperplasia by natural and synthetic retinoids. *J. Pharmacol. Exp. Ther.*, 237 (1986) 31–35.

Duchêne, D., Debruères, B. and Vaution, C., Improvement of drug stability by cyclodextrin inclusion complexation. *STP Pharma*, 1 (1985a) 37–43.

Duchêne, D., Glomot, F. and Vaution, C., Pharmaceutical applications of cyclodextrins. In D. Duchêne (Ed.), *Cyclodextrins and their Industrial Uses*, Editions de Santé, Paris, 1987.

Duchêne, D., Vaution, C. and Glomot, F., Amélioration de la dissolution et de la biodisponibilité des principes actifs par inclusion dans les cyclodextrines. *STP Pharma*, 1 (1985b) 323–332.

Glomot, F., Benkerrour, L., Duchêne, D. and Poelman, M.C., Improvement in availability and stability of a dermocorticoid by inclusion in β-cyclodextrin. *Int. J. Pharm.*, 46 (1988) 49–55.

Kligman, A.M., Fulton, J.E. and Plewig, G., Topical vitamin A acid in acne vulgaris. *Arch. Dermatol.*, 99 (1969) 469–476.

Lehman, P.A., Slattery, J.T. and Franz, T.J., Percutaneous absorption of retinoids: influence of vehicle, light exposure and dose. *J. Invest. Dermatol.*, 91 (1988) 56–61.

Saurat, J.H., *Retinoids, New Trends in Research and Therapy*, Karger, Basel, 1985.

Weiss, J.S., Ellis, C.N., Headington, J.T., Tincoff, T., Hamilton, T.A. and Voorhees, J.J., Topical tretinoin improves photo-aged skin; a double blind vehicle-controlled study. *J. Am. Med. Assoc.*, 259 (1988) 527–532.