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Improvement in availability and stability of a dermocorticoid by inclusion in β-cyclodextrin

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Summary

Tixocortol 17-butyrate 21-propionate is a dermocorticoid with anti-inflammatory properties. However, formulation problems result from its insolubility in water, its very low bioavailability and its instability. Inclusion in β-cyclodextrin is a means of resolving this problem. Inclusions are obtained from an equimolecular hydroacetonic solution of TBP and β-cyclodextrin, either by spontaneous precipitation or by evaporation at 60 °C. The existence of genuine inclusion is confirmed by scanning electron microscopy, but more precisely by differential thermal analysis and infrared spectrophotometry. TBP from inclusions is 5–7 times more water-soluble than pure TBP. TBP and its inclusion obtained by evaporation are incorporated in two ointment bases: vaseline and an o/w emulsion. In vitro release is studied with Franz cells, isopropyl myristate being the acceptor phase. After 3 h at 34 °C, the release of TBP is always significantly improved in the form of the inclusion; approximately 2 times from the emulsion or from the vaseline base. After 30 days storage at 40 °C, a loss of 40–50% of the TBP content is observed when incorporated pure in one or other of these bases, and it is barely 5% when incorporated in the inclusion form.

Introduction

Many investigations have been conducted on inclusions in cyclodextrins and their methylated derivatives, in order to improve the stability and/or solubility and bioavailability of the pharmaceutically active ingredients (Duchêne et al., 1986, 1987; Uekama and Irie, 1987). In general, however, they are primarily concerned with oral administration. Yet today the administration of drugs by other routes, particularly transdermal, is the focus of considerable research. Le Gall and Poelman (1986) have pointed out the role of formulation on the bioavailability of hydrocortisone acetate dermatological preparations. So far few investigations have dealt with the topic administration of the active ingredient included in cyclodextrins and their derivatives.

Otagiri et al. (1984) and Uekama et al. (1985) pointed out the value of including betamethasone

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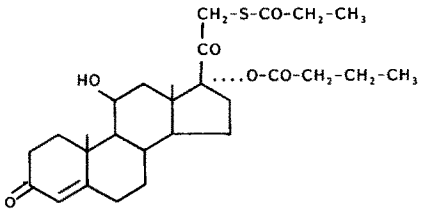

Scheme 1. Tixocortol 17-butyrate 21-propionate.

and beclomethasone dipropionate in β- and/or γ-cyclodextrin for in vitro release and percutaneous absorption. In contrast, Okamoto et al. (1986) described a slowing of the percutaneous absorption of butylparaben and of indomethacin included in β-cyclodextrin or in di-*O*-methyl/β-cyclodextrin and presented in the form of aqueous solutions or suspensions. However, these authors also show the favourable role of the inclusion of sulfanilic acid. They attribute these variable results to the different values of the partition coefficient of the substances investigated, in favour of the skin and the vehicle. It also appears that the stability constant cannot be ignored, as pointed out by Irie and Uekama (1985), analyzing the effect of inclusion in α-, β- and γ-cyclodextrins on the photosensitizing effect of chlorpromazine.

The work presented here was intended to try to improve the pharmaco-technical characteristics of tixocortol 17-butyrate 21-propionate (TBP) (Scheme 1), a corticosteroid administered by the topic route. In fact, this anti-inflammatory exhibits a virtual insolubility in water and extremely low bioavailability which, added to its instability, make its formulation very problematic.

Materials and Methods

Materials

TBP is produced by Jouveinal Laboratoires and is used without preliminary treatment. Its molecular weight is 504.68. Its solubilities are (g/100 g): benzylic alcohol > 9, ethanol = 3, isopropyl myristate = 0.3, Span 80 = 0.4, ethyl oleate = 0.03.

The β-cyclodextrin was supplied by Société Roquette Frères at a purity of at least 98%, and was employed without further purification.

Preparation of inclusions

The inclusions were prepared from equimolecular hydroacetonic solutions of β-cyclodextrin and TBP. Two processes are available for this: (a) at ambient temperature with agitation in a stoppered bottle for 2 days and spontaneous precipitation; (b) or with evaporation and agitation at 60 °C.

Determination of the physicochemical properties of the products

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

The reality of the inclusion was confirmed by subjecting the raw materials, the simple physical mixture and the coprecipitated products to differential thermal analysis and infrared spectrophotometry. The DTA analysis was performed using a Perkin Elmer instrument connected to a plotting table. The samples were analyzed in an open capsule to allow the evaporation of the water lost by the cyclodextrin. Determinations were carried out on 3 mg of TBP, or on the corresponding quantities. The different products were heated from 27 to 230 °C at a rate of 8 °C/min.

For the infrared analysis, the products were dry-compressed in a KBr pellet using a Perkin Elmer hand pelletizer. The spectra were obtained on a Perkin Elmer 257.

The aqueous solubility of TBP and the inclusions was determined in distilled water (pH 5.85–5.90). This was done by dispersing an excess amount of TBP, or 50 mg, in 100 ml of water, kept at 25 °C with agitation and sheltered from light for 24 h. The determinations were carried out by HPLC on centrifuged 2 ml samples, and diluted to 1 : 2 in methanol.

Solubility in propylene glycol was determined in the same way, but by using 150 mg of TBP for 10 ml of solvent.

Preparation of dermal forms

The bases selected for the incorporation of TBP and of the inclusion were of two types: a very simple fatty base, vaseline, and a washable o/w emulsified base, corresponding more closely to the properties of current ointments. The formula of

the emulsion was selected for its organoleptic properties (viscosity and spreading power) and for its stability at 50 °C and in centrifuging. The following formula was adopted:

mineral oil	20 g
polysorbate 60	4 g
sorbitan stearate	6 g
cetyl alcohol	1.5 g
stearyl alcohol	1.5 g
glycerin	7 g
water	60 g

Like the emulsion, the fatty ointment contains 0.05% of TBP, pure or in the form of an inclusion. Preparations were also made in which TBP and β-cyclodextrin were incorporated separately.

The products were incorporated in vaseline by simple blending in the melted product. For the emulsion, the TBP or the inclusion was dispersed in the oily phase. The emulsion was prepared by phase inversion by incorporating the aqueous phase at 70 °C with agitation in the oily phase raised to the same temperature. Agitation was continued to cooling. When the TBP was incorporated separately from β-cyclodextrin, the TBP was dispersed in the oily phase, whereas the cyclodextrin was dissolved in the aqueous phase.

Pharmacotechnical analysis of dermal forms

The in vitro release of TBP from the different ointments was analyzed by means of Franz cells equipped with Silastic membranes (Dow Corning), and the acceptor phase consisted of isopropyl myristate. The unit was kept at 34 °C.

This system was selected for the following reasons. Franz cells enable the entire acceptor phase to be in contact with the membrane, the diffusion system is stationary, and homogenization of the acceptor phase is achieved by magnetic agitation. In these cells, the volume of the acceptor phase is 4 ml, the contact area between the preparation and the membrane is 1 cm^2, and the amount of ointment deposited is about 3 g, accurately weighed. The Silastic membrane (polydimethylsiloxane) is a non-porous membrane in which TBP must dissolve and diffuse to pass through it. Isopropyl myristate was selected because it is considered a good model of the partly lipophilic character of the skin. The solubility of TBP in this solvent is 0.29% (w/w). The temperature of 34 °C can be considered as the outer temperature of the skin.

The amount of TBP released was determined at 30-min intervals, during 3 h, on 1 ml samples of the acceptor phase, replaced by 1 ml of fresh medium. Determination was carried out by HPLC.

The stability of the included TBP was compared with that of TBP for the two formulations examined, vaseline and emulsion. This was done by keeping the ointments in the oven at 40 °C and the determinations were carried out after 4, 8, 16, 24 and 32 days of storage.

Results and Discussion

Physicochemical analysis of inclusions

Analysis by the scanning electron microscope revealed the crystallization of β-cyclodextrin in polyhedral form with relatively large dimensions, about 1 mm (Fig. 1A), whereas TBP is substantially amorphous (Fig. 1B). The products obtained by coprecipitation occur in the form of large needles for the product obtained without evaporation (Fig. 1C), and much finer for the evaporated product (Fig. 1D). This observation, while revealing a clear difference in crystallization after coprecipitation, is inadequate to conclude in a genuine inclusion, but nevertheless helps to assess the existence of a single component in the preparations obtained.

Differential thermal analysis was much more informative (Fig. 2). β-Cyclodextrin exhibits a very broad thermal rise around 67 ± 5 °C, corresponding to a release of water. Its degradation, which takes place at 300 °C (caramelization) does not appear on the recording. TBP exhibits a thin fusion peak at 139 °C. The physical mixture produces a recording that is more or less the superposition of the recordings of cyclodextrin and TBP. On the other hand, for the products obtained by coprecipitation, the recordings are rather similar and are distinguished by the disappearance of the rise due to the loss of water from the cyclodextrin, the disappearance of the TBP fusion peak, and the appearance of a new characteristic peak at 144 °C. TBP does not display polymor-

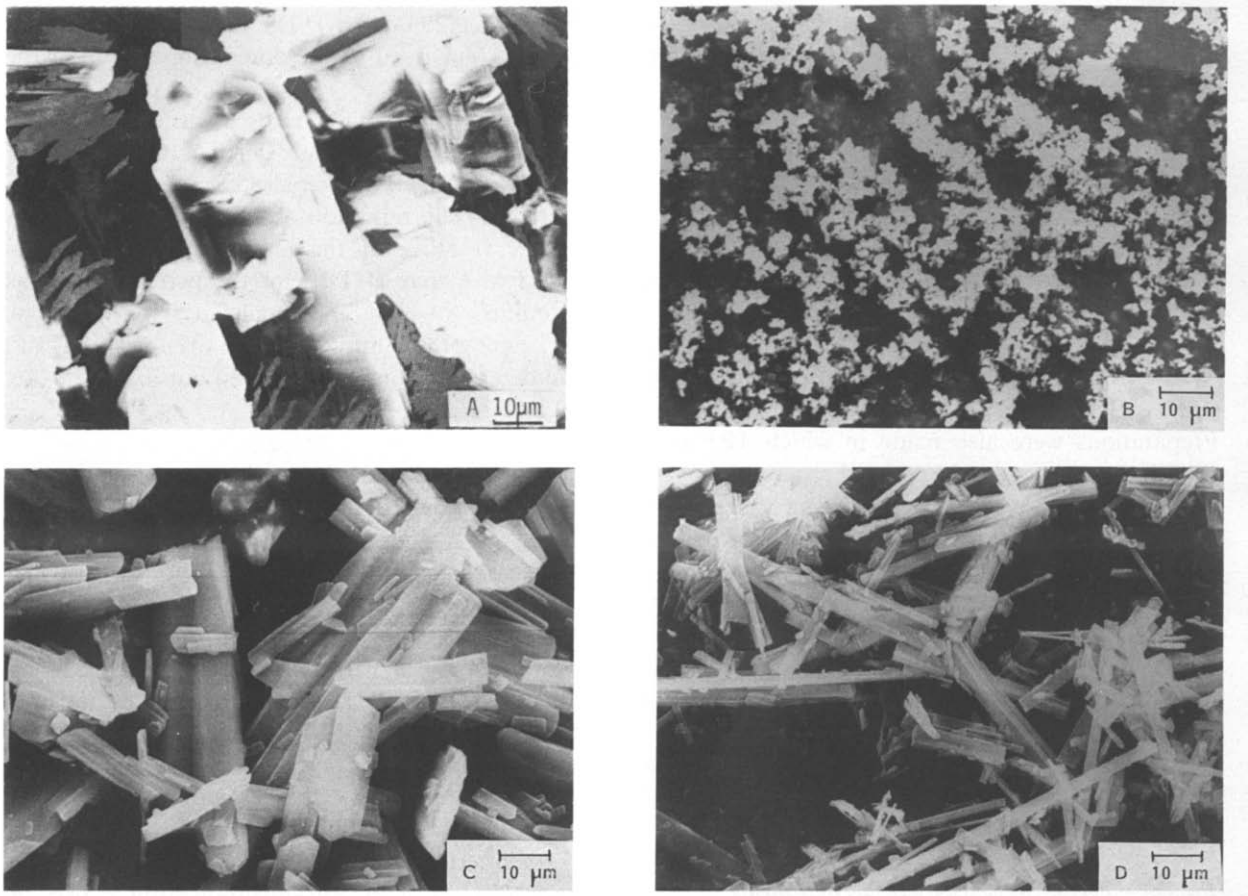

Fig. 1. Scanning electron microscopy. A: β-Cyclodextrin, B: TBP, C: β-cyclodextrin/TBP coprecipitate, without evaporation. D: β-cyclodextrin/TBP coprecipitate, with evaporation.

phism, and it was confirmed that the recrystallization of TBP in organic solvent did not lead to any changes in its thermal behaviour. Thus, the 5 °C shift between the thermal rises of TBP and of the products obtained by coprecipitation proves that some interaction exists between TBP and β-cyclodextrin, corresponding to an inclusion with weak physical bonds. The existence of this new compound is also confirmed by the disappearance of the water loss peak of cyclodextrin.

Infrared spectrophotometry confirmed these results (Fig. 3). In fact, TBP is characterized in particular by 3 peaks between 1600 and 1800 cm^{-1}, corresponding to its different functional groups. For cyclodextrin, the spectrum shows only the vibrations of free OH between 3500 and 3300 cm^{-1} and those of bound OH at 2900 cm^{-1}. The spectrum of the physical mixture is the superposition of the spectra of the pure compounds, whereas, for the product obtained by coprecipitation with evaporation, a change in the peaks of the carbonyl groups of TBP appears, as if they were bound. This spectrum appears to confirm a blocking of the TBP molecule in the cyclodextrin cavity, characteristic of an inclusion. Moreover, no new peak appears, so that no chemical bonds were created in the compound formed. Note that the spectrum of the inclusion obtained without

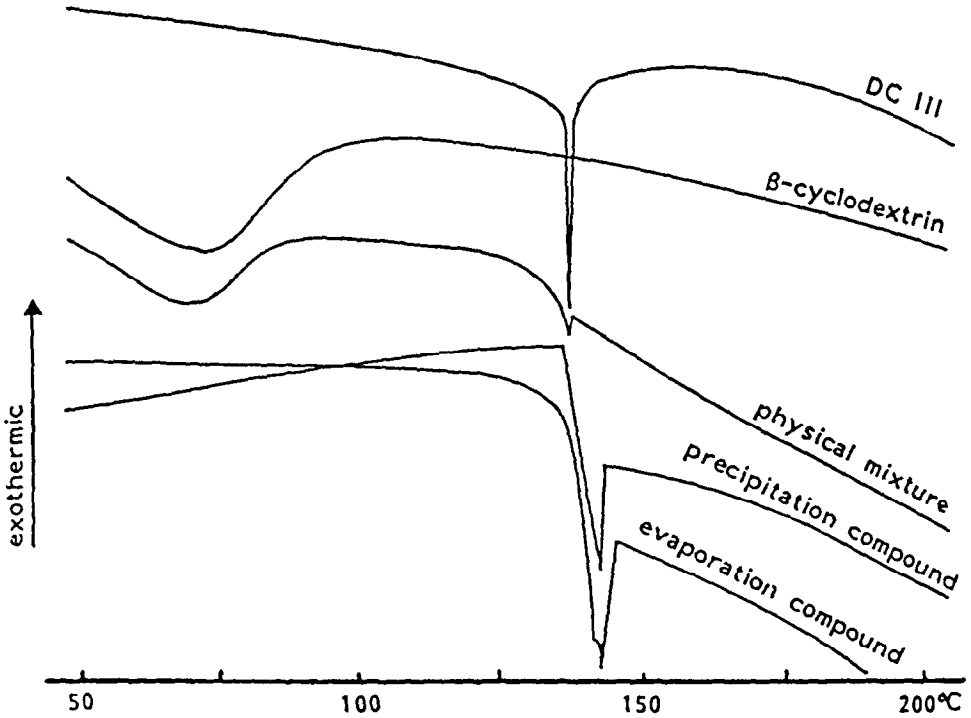

Fig. 2. Differential thermal analysis of pure TBP and β-cyclodextrin, their physical mixture and inclusion compounds.

evaporation is identical to that of the inclusion obtained with evaporation, the only one shown here.

The solubilities of TBP in water and in propylene glycol are significantly increased by inclusion in β-cyclodextrin (Table 1). Aqueous solubil-

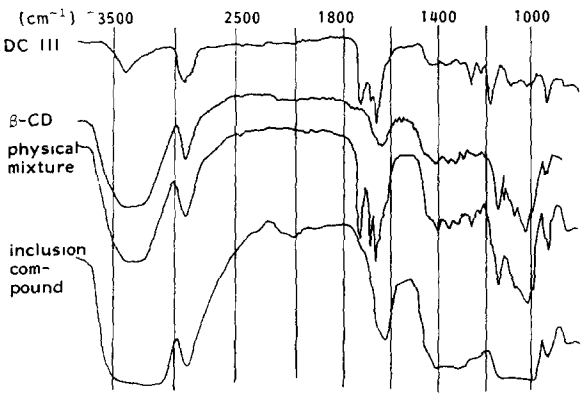

Fig. 3. Infrared spectroscopy of pure TBP and β-cyclodextrin, their physical mixture and inclusion compound (coprecipitate with evaporation).

TABLE 1

Solubility of TBP and of its inclusion compounds in water and in propylene glycol

Product	Solubility at 20 °C (mg/100 ml)	
	Water	Propylene glycol
pure TBP	0.30	317.5
precipitated inclusion	1.58	604.0
evaporated inclusion	2.03	846.5

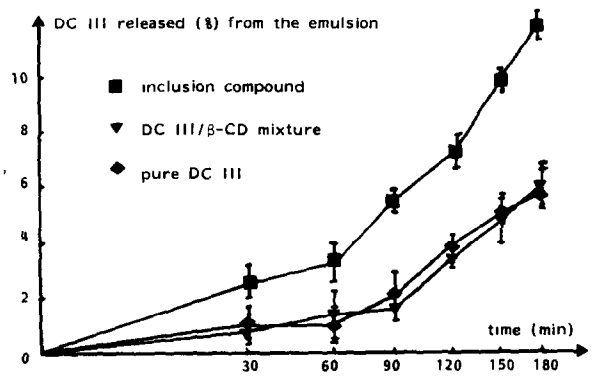

Fig. 4. In vitro release of TBP from the emulsion.

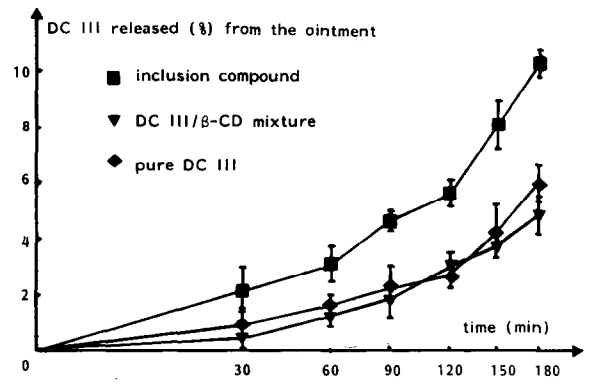

Fig. 5. In vitro release of TBP from the vaseline.

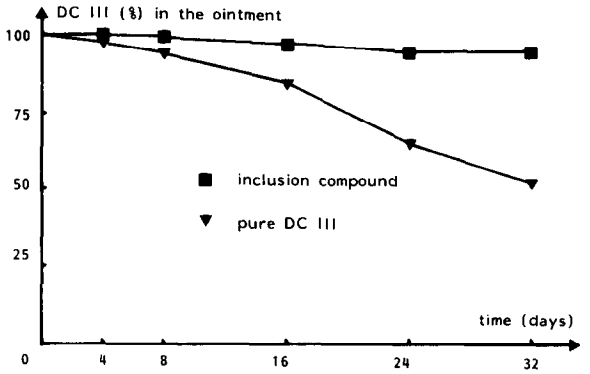

Fig. 7. Stability of TBP in the vaseline.

ity is increased by more than 500% from the inclusion obtained by spontaneous precipitation, and by about 700% from the inclusion obtained by evaporation. In propylene glycol, the coefficients are 200 and 250% respectively.

Due to these solubility results and the better yield of the preparation by evaporation, only the inclusion obtained by this process is analyzed in a formulation.

Analysis of dermal forms

The release of TBP from dermal forms (Figs. 4, 5) is always significantly improved in the form of the inclusion (approximately 2 times). After 3 h, release is 20% better from the emulsion than from the vaseline. It should be noted that, when TBP and β-cyclodextrin are incorporated separately in ointment bases, no improvement occurs in the release of TBP. This result confirms the observation made by Shankland and Johnson (1984) on the release of hydrocortisone from a cream.

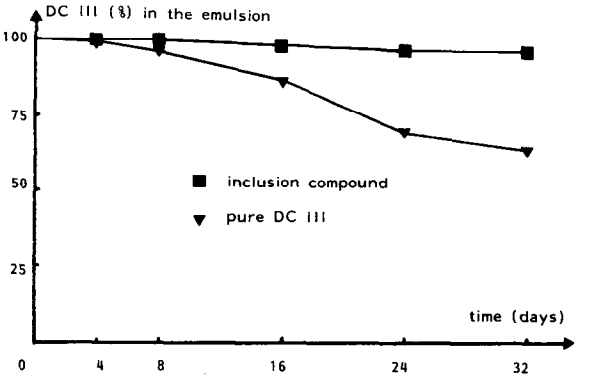

Fig. 6. Stability of TBP in the emulsion.

The stability analyses (Figs. 6 and 7) carried out at 40 °C reveal a clear increase in the stability of TBP when included in β-cyclodextrin. In fact, after one month, a loss of 40–50% of the TBP occurs when it is incorporated pure in the emulsion or in the vaseline. This loss is barely 5% from the inclusion.

Conclusions

The inclusion of tixocortol 17-butyrate 21-propionate, an anti-inflammatory corticosteroid, in β-cyclodextrin is easy to achieve by evaporation at 60 °C of an equimolecular hydroacetonic solution of the products. The process by evaporation produces a good total yield, and the various physical examinations reveal the existence of an inclusion with weak physical bonds and the absence of residues of the initial products.

The inclusion thus obtained offers a very sharp improvement in the in vitro availability and stability of tixocortol 17-butyrate 21-propionate. It also appears that the emulsified base is a better vehicle for inclusion than simple vaseline.

These preliminary results must be confirmed by tests of cutaneous penetration and in vivo availability. A more detailed analysis of the formulation of ointments should help to develop a formula that is perfectly acceptable from the therapeutic standpoint.

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