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Stability studies on a steroidal drug/β-cyclodextrin coground mixture

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Summary

The aim of this study was to investigate the physico-chemical changes and the chemical stability of a steroidal drug, 6-methylenandrosta-1,4-diene-3,17-dione (FCE24304), an aromatase inhibitor, in the presence of β-cyclodextrins (β-cd) using a cogrinding procedure which enables amorphization. Results of stressed conditions stability tests are shown, with particular regard to the presence of moisture, and evaluated by HPLC, DSC and X-ray diffractometry techniques and correlated with the dissolution rate behavior. The transformation of the amorphous drug/β-cd system into a 1 : 2 mol/mol crystalline complex is indicated.

Introduction

One of the most interesting properties of cyclodextrins is their ability to improve the physico-chemical stability of some molecules in both solution and the solid state (Duchene et al., 1987), nevertheless, it has also been reported that, in the solid state the association of drugs with β-cyclodextrins sometimes fails to improve stability (Terada et al., 1983).

In some cases, amorphous drugs spontaneously crystallize to form inclusion compounds when exposed to high moisture conditions (Nozawa and Yamamoto, 1989).

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As described in a previous paper (Torricelli et al., 1990), we have recently studied 6-methylenandrosta-1,4-diene-3,17-dione (FCE24304)/β-cyclodextrin (β-cd) systems, and have improved the dissolution properties of the poorly water-soluble steroid. Among the preparation techniques used, particular attention was paid to the solid-state cogrinding procedure (Carli et al., 1987) which often allows amorphization. Amorphization, in the case of higher energetics, is very often accompanied by reduction in chemical stability or by physico-chemical changes, so that, before projecting a new pharmaceutical dosage form of the steroidal drug, it was very important to monitor the stability of the more soluble and rapidly dissolving system.

The aim of this work was to gather information about the chemical stability and physical changes of FCE24304 during the cogrinding procedure with

β-cd and to perform a stability study of the system evaluating the influence on the in vitro release of the drug.

Materials and Methods

Preparation

Pure crystallized 6-methylenandrosta-1,4-diene-3,17-dione (FCE24304, Farmitalia Carlo Erba, Chemical R&D; m.p. = 195 °C) and β-cyclodextrin as provided by the supplier (Spad Roquette, batch 409886) were used. Equimolar quantities of drug and β-cd (1 : 4.29 w/w ratio) were sieved, tumble mixed and then coground for 2 h in a laboratory high-energy mill (Giuliani, Torino), using porcelain balls as grinding media (no. 10 balls ∅ = 18 mm; no. 10 balls ∅ = 16 mm; no. 64 balls ∅ = 10 mm), the volumetric ratio between the powder to be ground and the grinding media being equal to 1 : 8. The powder obtained was sieved and tumble mixed.

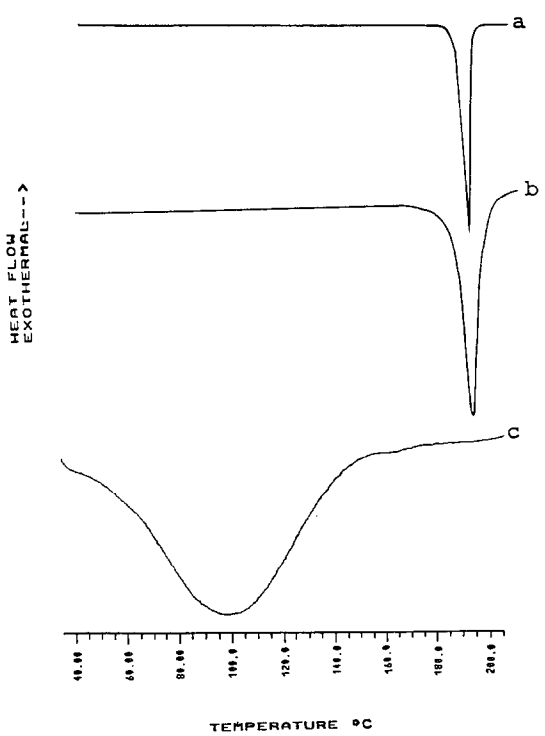

Fig. 1. DSC of (a) FCE24304 (parent drug); (b) FCE24304 after 4 h grinding; (c) FCE24304/β-cd coground system.

TABLE 1

Percentage of related substances by internal normalization of samples stored under different temperature and humidity conditions detected by HPLC

	Storage conditions	Initial	1 month	3 months
Parent drug	–	1.00		
	35 °C			1.23
	55 °C		2.23	
Coground system	–	0.92		
	35 °C		0.83	1.19
	55 °C		2.57	3.98
	25 °C + 80% R.H.			1.25
	25 °C + 90% R.H.			1.22
	35 °C + 80% R.H.			2.15

Storage conditions

At 35 and 55 °C samples of the coground system were sealed in vials (glass type I); under the other storage conditions (25 °C + 80% R.H.; 25 °C + 90% R.H.; 35 °C + 80% R.H.) the powder was spread on glass disks in order to have a higher surface area exposed to the moist atmosphere.

Chemical stability

The degradation products were detected by HPLC (SP8770 - Knauer detector, 245 nm; Partisphere 5 μm; water/acetonitrile, 60 : 40 v/v; 1 ml/min flux) and calculated by internal normalization.

X-ray diffraction measurements

X-ray powder diffractograms were obtained using a Siemens D500TT diffractometer, CuKα as radiation source, and the detector positron sensitive (PSPC).

DSC measurements

Thermal analyses were carried out using a Mettler TA3000 system equipped with a DSC20 cell under nitrogen flow (50 ml/min). The heating rate was 10 °C/min.

Dissolution studies

Dissolution rate tests were carried out at 37 °C, 150 rpm, 900 ml phosphate buffer pH 7.4 accord-

ing to USP XXII, no. 2 paddle method under sink conditions (maximum drug concentration 50 μg/ml). The drug concentration was detected by UV analysis (Philips PU8-7000) on filtered (Versapor 0.45 μm membranes) and diluted samples at 250 nm ($E^{1\%}_{1\,cm} = 496.5$).

Results and Discussion

Thermal analyses of FCE 24304/β-cd coground system show the total disappearance of the phenomenon of melting due to the drug. This indicates that there is a possibility of inclusion complex formation by grinding the mixture and, in any case, that the drug is amorphized using that technique. It is remarkable that the grinding efficiency on amorphization is improved by the addition of β-cd, since, as shown in Fig. 1, prolonged grinding (4 h) does not modify the physical state of the steroid. Such a phenomenon occurs without any damage to the chemical strength of FCE24304, as can be seen from Table 1 which also reports the quantity of degradation substances (percent of normalized area) after 1 and 3 months under various conditions of storage.

The stability study shows that a high moisture percentage generally does not influence the chemical stability of the steroidal drug, but at 35 °C + 80% R.H. the synergistic effect of humidity and temperature enhances chemical alteration. In a dry atmosphere, the coground system does not

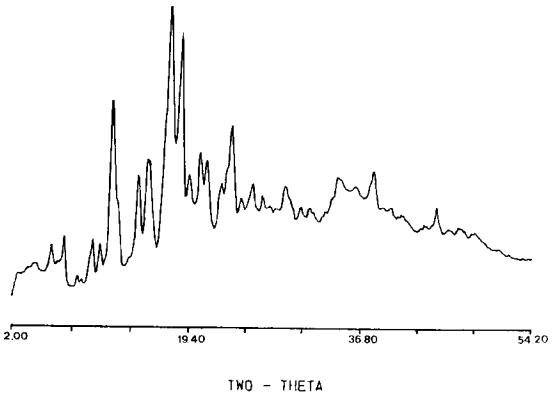

Fig. 3. X-ray diffractogram of FCE24304/β-cd 1:2 mol/mol kneaded system.

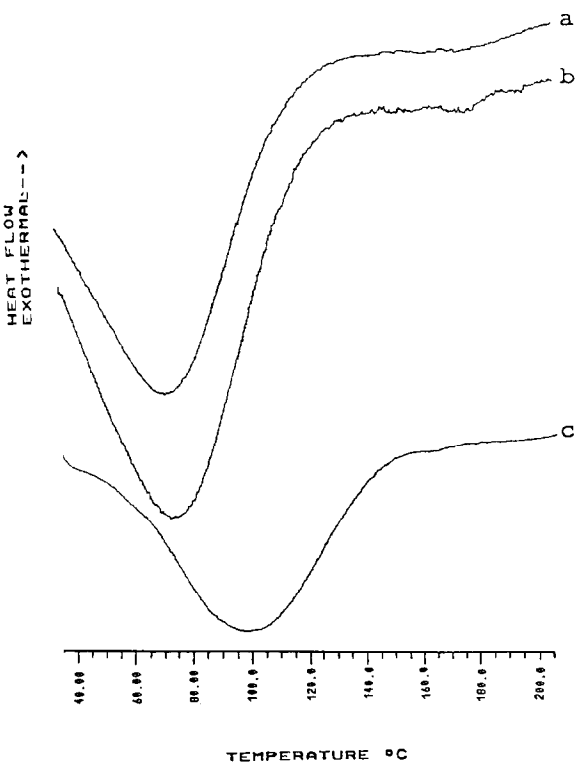

Fig. 2. DSC of FCE24304/β-cd coground systems: (a) 3 months at 55 °C storage; (b) 3 months at 35 °C storage; (c) initial.

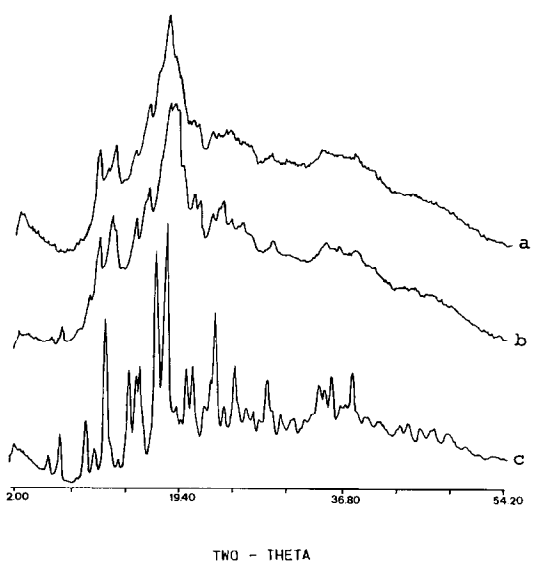

Fig. 4. X-ray diffractograms of FCE24304/β-cd coground system at 25 °C + 80% R.H.: (a) 15 days storage; (b) 1 month storage; (c) 3 months storage.

change in chemical strength at 35 °C, while at 55 °C degradation is evident and similar to that of the pure crystalline drug. From such a viewpoint, the protective effect of β-cd fails to reduce the slow degradation process induced by increasing temperature.

Samples stored at 35 and 55 °C were examined by DSC: they do not modify the thermal behavior (Fig. 2). On the other hand, after exposure to water vapor a new endothermal phenomenon was evident: a broad peak with a maximum at 183–184 °C. A possible explanation for the phenomenon of such low intensity could be the partial microcrystallization of the drug amorphized and dispersed in the macrocyclic carrier, induced by the moisturized atmosphere. Literature data (Lin et al., 1988) report marked and progressive crystallization of the active substance from drug/β-cd ground mixtures when stored at 40 °C and 75% R.H. and the lowering of the melting point might be correlated with crystal size reduction (Carli and Colombo, 1988). In fact, previous experiments (unpublished data) showed that on cogrinding FCE24304 mixed with other pharmaceutical excipients, the same extent of melting point lowering was obtained without any damage to the chemical strength or any change in the crystalline form of the drug. Nevertheless, such a hypothesis does not seem to be confirmed by optical microscopy analysis: in fact, the degree of crystallinity of the system, at the end of the stability program, is higher than that initially found, but it is impossible to attribute the loss in amorphicity exclusively to the drug. Results obtained by X-ray diffractographic investigation confirm the above: in Table 2 crystallinity variations are reported (the differences between the values obtained by DSC vs those evaluated by X-ray analysis are due to the fact that DSC quantifies only the remaining crystallinity of the drug, while X-ray analysis evaluates the crystallinity of both the drug and β-cd). A high degree of relative humidity plays an im-

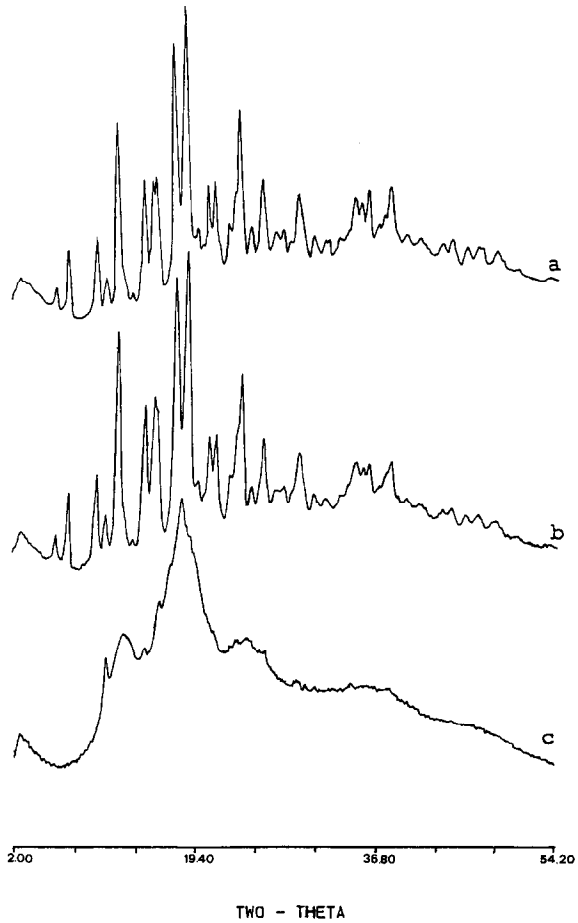

Fig. 5. X-ray diffractograms of FCE24304/β-cd coground system at 25 °C + 90% R.H.: (a) 3 months storage; (b) 1 month storage; (c) 15 days storage.

TABLE 2

Degree of crystallinity of samples stored under different temperature and humidity conditions by X-ray and DSC techniques

Technique	Storage conditions	Percent crystallinity			
		Initial	15 days	1 month	3 months
X-ray	–	32	–	–	–
	35 °C	–	–	29	26
	55 °C	–	–	31	24
	25 °C + 80% R.H.	–	42	71	74
	25 °C + 90% R.H.	–	77	–	84
	35 °C + 80% R.H.	–	31	39	82
DSC	–	[a]	–	–	–
	35 °C	–	–	[a]	[a]
	55 °C	–	–	[a]	[a]
	25 °C + 80% R.H.	–	25 [b]	36 [b]	38 [b]
	25 °C + 90% R.H.	–	40 [b]	–	30 [b]
	35 °C + 80% R.H.	–	23 [b]	29 [b]	26 [b]

[a] Not detectable.
[b] Broader peak at 183–184 °C.

portant role in morphological modification: after 3 months the quantities of crystalline substance detected are practically independent of the storage conditions and the diffractographic pattern is compatible with that of a 1 : 2 mol/mol complex, which can be readily obtained via other preparation techniques such as the coprecipitation or kneading methods (Torricelli et al., 1990) (Fig. 3). Some authors (Kawano and Nakai, 1985) mentioned that cyclodextrin molecules in the roll mixed system became mobile when water vapor was absorbed and observed amorphous drug crystallizing spontaneously, forming the inclusion compound.

Consequently, it is possible to suggest for FCE24304 that the active substance partially interacts with β-cd to form a 1 : 2 mol/mol crystalline complex and partially remains dispersed in its unaltered amorphous or in a microcrystalline form.

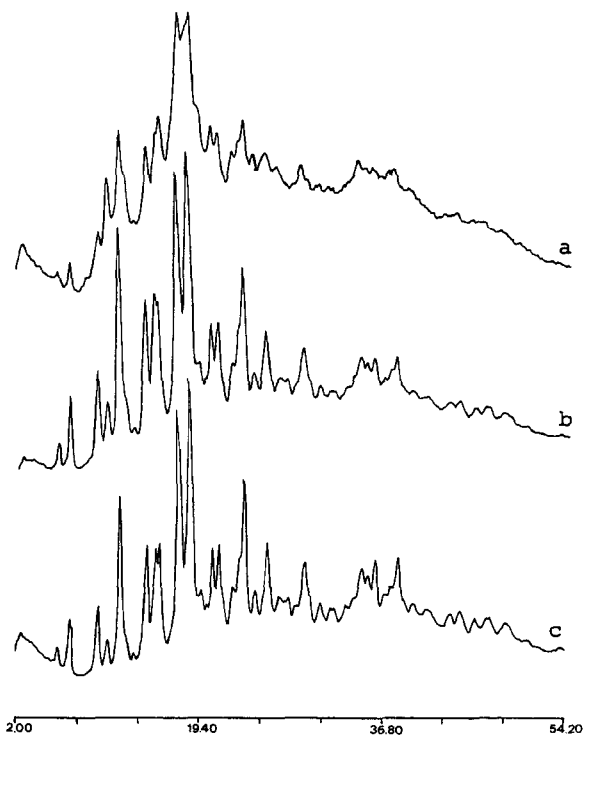

Fig. 6. X-ray diffractograms of FCE24304/β-cd coground system at 35 °C + 80% R.H.: (a) 15 days storage; (b) 1 month storage; (c) 3 months storage.

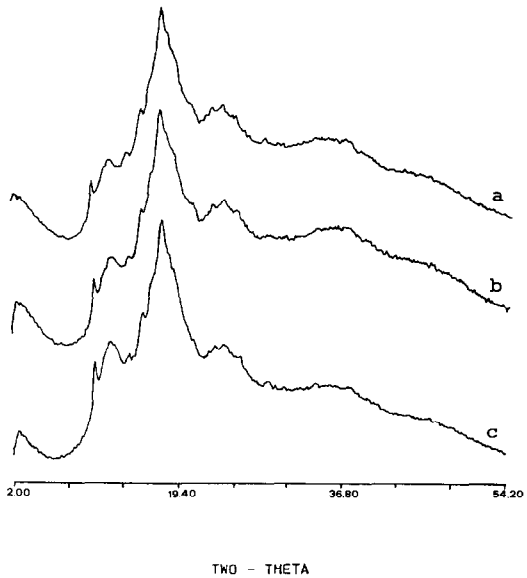

Fig. 7. X-ray diffractograms of FCE24304/β-cd coground system: (a) 3 months at 55 °C storage; (b) 3 months at 35 °C storage; (c) initial.

The rate of crystalline complex formation is influenced by temperature and relative humidity; in fact, as shown in Figs. 4–6, under conditions of constant temperature, the degree of amorphization decreases faster when the environmental moisture content is higher, whereas at constant humidity, increasing the temperature lessens the initial crystallization. No physical changes were observed at 35 and 55 °C (see also Fig. 7).

Dissolution rate data of samples stored for 3 months are listed in Table 3 in comparison to the initial values. The unaltered physico-chemical properties of the ground system stored at 35 °C are reflected in the unchanged drug dissolution profile while the aging at 55 °C negatively influences the release because of the thermoinduced degradation of the drug.

On the other hand, samples exposed to moisture show very fast dissolution behavior; the morphological change detected by X-ray diffractometry plays a fundamental role: the loss of amorphization which generally lessens the release rate in this case is accompanied by partial formation of the complex. This is the governing factor in the overall dissolution process for its very interesting

TABLE 3

Dissolution rate behavior under sink conditions of samples stored in different temperature and humidity conditions

Time (min)	Percent in solution					
	Initial	35°C	55°C	25°C +80% R.H.	25°C +90% R.H.	35°C +80% R.H.
1	46	44	23	63	60	61
3	68	70	46	76	76	76
5	76	78	58	81	79	80
10	82	86	69	83	83	85
15	85	88	74	85	84	87
30	88	91	78	87	87	88
60	90	94	80	89	89	90

characteristics of dispersibility and dissolution rate.

Conclusions

On the basis of the reported data, it is concluded that there are no differences between the chemical stability of the FCE24304/β-cd mechano-chemical activated system and the parent drug.

The system is stable under storage conditions up to 35°C but other preparation techniques, which generate a solid complex, give better results both for stability and dissolution properties. Such systems will be discussed in a following paper.

From the physico-pharmaceutical point of view, it is evident that the crystallization of the coground system to a complex in the presence of a moisturized atmosphere could be an interesting approach for both increasing stability and dissolution rate performances of FCE24304/β-cd systems.

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