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Solid-state investigation of fluocinolone acetonide

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Abstract

Three crystalline modifications of fluocinolone acetonide, A, B and C, were characterized by means of FTIR, DSC, TG-FTIR, MICRO-FTIR and X-ray diffractometry. They were easily differentiated by their IR absorption bands in the 3600–3400 cm^{-1} range. The thermal behaviour was also elucidated using combined techniques; thermomicroscopy and thermogravimetry coupled with Fourier transform infrared spectroscopy were found to be very useful tools for a better understanding of thermal events. On heating, form A and C were fully converted into form B. Polymorph A was found to be enantiotropically related to B, while form C was monotropically related to B. The existence of three polymorphs was confirmed by means of their different X-ray diffraction patterns. Detailed methods of preparation of the three modifications are also described. © 1997 Elsevier Science B.V.

Keywords: Fluocinolone acetonide; Physico-chemical characterization; Polymorphism; Infrared spectroscopy; Thermal analysis; X-ray diffractometry

1. Introduction

Fluocinolone acetonide (6α,9α-difluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone, FA), is a corticosteroid topically used in the treatment of various skin disorders and of inflammatory eye, ear and nose diseases. It exhibits high anti-inflammatory activity and is usually employed in formulation as a cream, gel, lotion or ointment. The chemical structure is shown in Fig. 1.

The identification and characterization of polymorphic behaviour in a pharmaceutical substance is an essential aspect of drug development. It is well-known that the existence of a compound in more than one crystalline form or the existence of metastable crystalline forms may lead to difficulties in formulation of creams, ointments and suspensions. Any polymorphic change in the dosage form can influence the bioavailability and stability of a formulation [1].

The investigation of FA arose from the finding that samples of industrial-scale lots (all with HPLC purity >98%) showed poorly-defined thermal behaviour and different IR spectra. Mesley and Johnson reported FA to exist in more than one solid form. They distinguished two crystalline forms by IR spectroscopy: these two solid phases were denominated A and B [2,3]. The

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presence of discrete polymorphic forms was confirmed by means of X-ray powder diffraction patterns.

The thermal behaviour of FA was also characterized by means of TGA, DTA and DTG [4] and by thermomicroscopy with a Kofler apparatus [5], but nothing about its polymorphism was mentioned in the two forementioned papers.

Despite these experimental findings, FA is not included in the list of drugs reported as having polymorphism [6,7] and furthermore, in most recent European Pharmacopoeia no reference at all is made to this phenomenon.

The purpose of the present work was, therefore, to re-examine the preparation and characterization of the different crystalline forms of FA in more detail and to elucidate its thermal behaviour with particular regard to polymorphism.

2. Materials and methods

Fluocinolone acetonide was purchased from Sigma (minimum 98% purity) and was used without further purification.

The different polymorphic forms were prepared by crystallization from acetone, ethyl acetate, chloroform, absolute ethanol, 95% aqueous ethanol, methanol and acetone/hexane. All solvents were of analytical grade.

By crystallization from hot ethanol solution on a water-bath, Mesley was able to obtain form B while all other solvent treatments gave mixtures of the two forms [2,3].

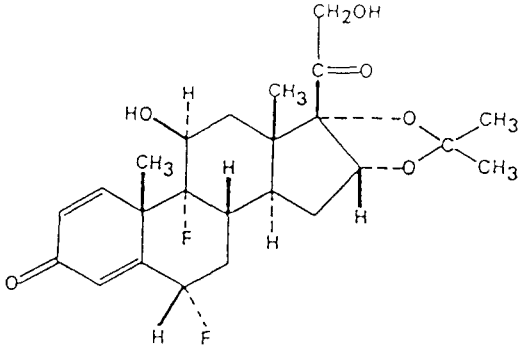


Fig. 1. The chemical structure of Fluocinolone acetonide.

Using the same crystallization procedure, we obtained form A (the polymorphic form which is found in commercial samples) by crystallization from cold acetone, cold chloroform and cold methanol, while form B was prepared by crystallization from cold absolute ethanol, both in the absence and in the presence of water. Form C was prepared by crystallization from ethyl acetate or 2:1 acetone/hexane mixtures and by freeze drying of the FA solution in absolute ethanol. All solutions were evaporated at room temperature and the resulting samples were stored in dryness for 3 h. Form B was also obtained by heating above 210°C each other form. UV and ^{1}H NMR analysis performed on materials recovered immediately after heating indicated that no significant degradation had taken place.

FTIR spectra of the three forms were obtained using a mull in liquid paraffin (nujol) and a dispersion in alkali halide (KBr) disc. The IR spectrum of FA Sigma in chloroform solution was also obtained. Spectra were recorded at room temperature from 4000 to 650 cm^{-1} on a Perkin Elmer System 2000 spectrometer. For each sample 32 scans were collected at a resolution of 4 cm^{-1}, employing a deuterated triglycine sulphate detector.

X-ray diffraction patterns were obtained with a Philips P.W. 1710 diffractometer in the 2ϑ range between 3 and 60° using Cu Kα radiation-Ni filtered (40 kV; 40 mA). The step scan mode was performed with a step width of 0.02° at a rate of 1 step s^{-1}.

DSC thermograms were recorded using a Perkin Elmer DSC7 instrument. Approximately 1.5 mg of sample were accurately weighted into a DSC pan, the pan was hermetically sealed, and a pinhole punched into the pan lid. The use of the pinhole allows for pressure release, but still ensures that the thermal reactions proceed under controlled conditions. The DSC profiles were recorded at 10 and 30°C min^{-1}, under nitrogen flux, from 40 to 310°C. The DSC temperature scale was calibrated using the extrapolated onset temperatures of the fusion endotherms of Indium and Zinc pure standards, heated at a rate of 10 and 30°C min^{-1}.

The thermogravimetric curves were recorded with a Perkin Elmer TGA7 instrument coupled with a System 2000 FTIR spectrometer, at two heating rates: 10 and 30°C min^{-1}. The first represented the minimum rate adequate for obtaining good infrared spectra of the gas phase; the second a reasonable high heating rate suitable to permit a correct comparison with DSC experiment. For TGA determinations, approximately 10 mg of sample were used. Each experiment was repeated at least three times. A temperature calibration of the thermogravimetric apparatus was performed using two standards, alumel and nickel whose magnetic transition temperature are 163 and 354°C, respectively.

The IR spectra of the gas phase were obtained by Fourier transform of 32 and 64 interferograms; the spectral resolution was 4 cm^{-1}.

Variable temperature experiments were performed with a hot stage microscopy coupled with a Perkin Elmer FT-IR 16PC apparatus (MICRO-FTIR).

A small amount of sample was placed in the hot stage compartment on a KBr window and the area of interest was selected with a variable aperture mask using a window of 400 μm^2 in order to avoid the effect of diffraction of the IR radiation. Each spectrum was recorded with a 6 cm^{-1} resolution; the heating rate was fixed at 3°C min^{-1}.

3. Results and discussion

3.1. FTIR and X-ray diffractometry studies

The influence of the sample preparation technique on the FTIR spectra was first investigated: for pure crystalline forms, the spectra obtained using either a mull in liquid paraffin or a dispersion in KBr disc were not markedly different from each other, particularly with respect to the positions, sharpness and intensity ratio of the bands. The crystalline structure seemed to be neither altered nor destroyed by pelleting. Instead, for samples in which different crystalline forms coexisted the nujol mull technique was found to give rise to more resolved spectra.

Moreover dispersion in potassium bromide affected the kinetics of interconversion.

Thus great caution should be exercised in the use of KBr pellet for the identification of the FA polymorphs in mixture even if this is the technique of choice to compare the three polymorph spectra in the C–H stretching range.

Fig. 2 shows the KBr spectra for A, B and C forms, respectively. They exhibited significant differences in the observed vibrational transitions.

Particularly noteworthy was the shift of O–H stretching in the 3600–3400 cm^{-1} region.

Form A showed a quite sharp band at 3400 cm^{-1} corresponding to the absorption of a hydrogen-bonded hydroxyl group in polymeric structures [3].

Form B exhibited a broad band in the 3500–3200 cm^{-1} region, due to hydroxyl groups hydrogen-bonded in a dimeric structure [3].

The sharp band at 3600 cm^{-1} of polymorph C may arise from an unbounded O–H group [3,8]; the high frequency has been attributed to steric hindrance within the crystal.

According to the thermodynamic infrared rule, form C which absorbs at higher frequencies may be assumed to have the larger entropy and to be also less stable a 0°K [9].

All other regions of the spectra showed a few subtle differences. Similar spectral differences were obtained for samples prepared as nujol mull. The nujol mull spectra of form A and B were in agreement with those previously reported by Mesley [3].

The assignments for the major IR bands of the three polymorphs are summarized in Table 1.

The shifting of the stretching vibrations in the three forms reflects slightly different geometries in the crystal packing. This was clearly confirmed by X-ray diffraction patterns of the three polymorphs: their profiles were sufficiently distinct to characterize each crystalline form (Fig. 3). In fact the differences either in the positions or in the intensity ratio of the peaks may not be attributed to preferred orientation crystal growth, but suggested different arrangements of FA molecules in the crystal lattice of each form. Unfortunately Mesley did not report X-ray diffraction patterns of form A and B, hence it is not possible to make

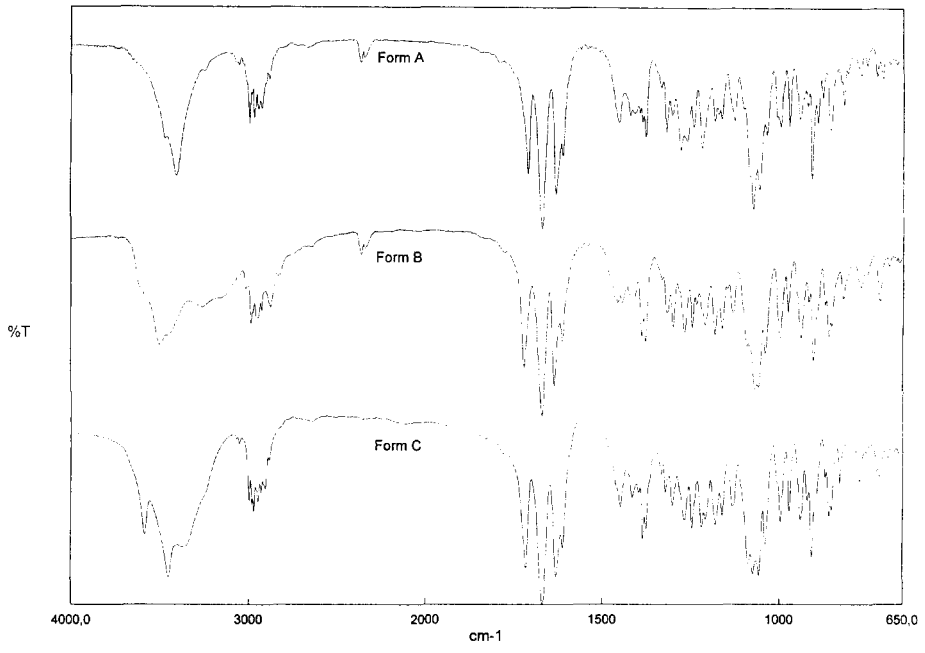


Fig. 2. Fluocinolone Acetonide FTIR spectra of polymorphs A, B and C (0.5% in potassium bromide disc).

comparison with our results. Single crystal X-ray measurements were not performed, due to the intrinsic difficulty of growing pure polymorph crystals of sufficient dimensions to allow such an analysis.

3.2. DSC, TGA-FTIR and MICRO-FTIR studies

The DSC profiles of form A, B and C, (heating rate of 30°C min^{-1}) are shown in Fig. 4.

Form A presented two characteristic endothermic peaks: the first broad small peak was attributed to a solid-solid transition into form B (such transition has been verified in our experiments through FTIR, MICRO-FTIR and X-ray diffractometry pattern of the solid phases before and after the transition temperature), that occurred in the 180–220°C range. The transition energy was so small (3–4 J g^{-1}) and the process rate so slow, that DSC could not give any clear indication of the transition temperature in mixture. The second sharp endothermic peak was due to the melting of form B at about 280°C.

Table 1
IR assignments for the fluocinolone acetonide forms A, B and C[a]

	A	B	C
ν O–H	3400	3495	3583 3448
ν C_{20}=O	1708	1719	1714
ν C_3=O	1669	1670	1668
ν C_4=C_5	1629	1634	1629
ν C_1=C_2	1606	1611	1610

[a]Wavenumber given in cm^{-1}.

Form B showed only one endothermic peak at about 280°C corresponding to the fusion followed by decomposition of the compound.

Form C exhibited a small exothermic peak from 150 to 180°C (1–2 J g^{-1}), which may be ascribed to a solid-solid phase transition from form C to form B; such phenomenon was verified through FTIR, X-ray diffraction and MICRO-FTIR analysis of the solid phase. The exothermicity of the process is a characteristic associated with 'disorder-order' type transition. Irreversible solid-solid transition indicated that form C is monotropically related to form B and confirms

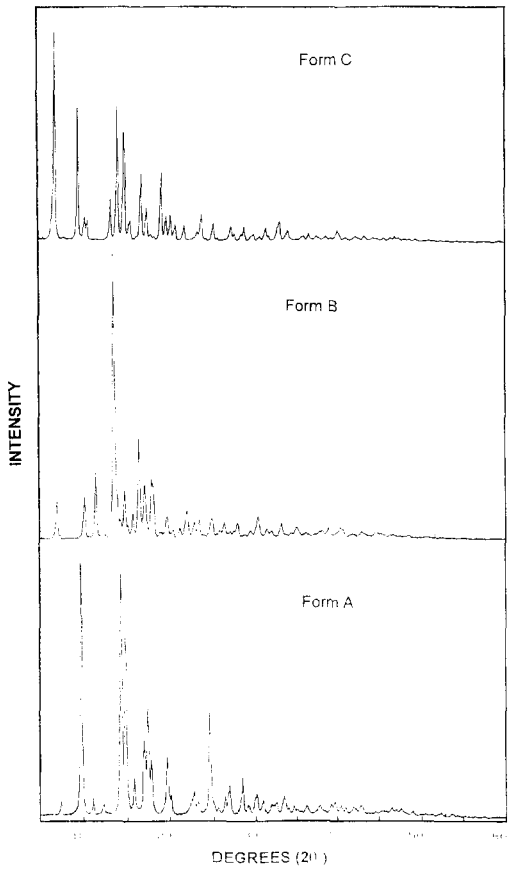


Fig. 3. Fluocinolone acetonide XRPD patterns for forms A, B and C.

that form B is the high temperature stable phase of FA.

A rerun on form A and C after heating at 210°C showed only one peak corresponding to the final melting process of form B.

In order to avoid any misinterpretation of the DSC profiles, we recorded the TGA curves of the three polymorphs. Coupling of TGA and FTIR permitted the simultaneous analysis of the gaseous products, giving a better interpretation of the thermal events.

The TGA curve of form B showed only two stages of weight loss: the first from 35 to 120°C (about 2%) due to the absorbed water. This weight loss decreased to about 0.5% if the sample was stored in dryness under vacuum for 3 h. The second weight loss, at about 270°C was due to the fusion and rapid decomposition of FA.

Polymorphs A and C had more complex TGA curves. Form A showed three distinct stages of weight loss: the first from 30 to 100°C due to the absorbed water on the surface of crystals which was easily eliminated by storing the sample in dryness; the second of about 0.5% from 120 to 220°C due to the removal of crystallization solvent from the lattice. This phenomenon was confirmed by the IR spectrum of the gas phase originating during the second weight loss, showing frequencies characteristic of solvent of crystal-

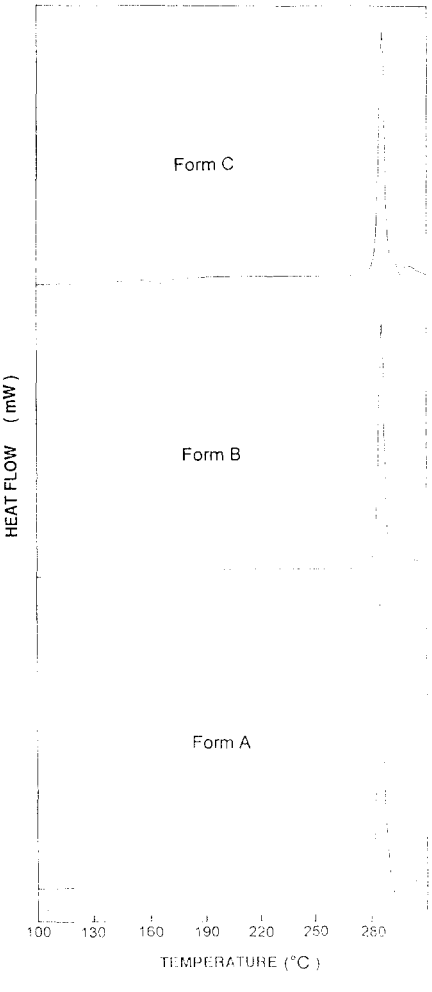


Fig. 4. Fluocinolone acetonide DSC data for polymorphs A, B and C (obtained by freeze drying); scan rate, 10°C/min; heat flow, endothermic scale.

lization. The last weight loss, superimposed with that of form B, took place at about 270°C, corresponding to FA decomposition process.

The TGA curve of form C obtained by freeze drying showed only one weight loss at about 270°C.

Form C obtained by crystallization, exhibited a variable (from 0.5 to 3%) weight loss that took place at about 150°C. The IR spectrum of the gas phase revealed the presence of solvent of crystallization.

These experiments clearly showed that the three polymorphs exhibited a different capacity to retain solvent. Form B is more hygroscopic, while forms A and C were found to form non stoichiometric adducts, probably of the clathrate type, with solvents of crystallization [10]. The existence of these inclusion compounds was confirmed by the fact that form A and C, with and without solvent, showed the same IR spectra and the same X-ray diffraction patterns. Furthermore the amount of retained solvent was variable and non stoichiometric, thus ruling out the presence of solvates.

As a general conclusion, the first thermal event in the DSC curve of polymorphs A and C arose from two overlapped processes: a solid-solid transition, that was associated with the internal packing rearrangement of the crystalline structure and a consequent removal of the solvent intrapped in the crystalline cages. When form C was prepared by crystallization, the endothermic peak due to the solvent removal completely obscured the concomitant exothermic transition into form B which was no more detectable.

Hot stage microscopy coupled with a FTIR spectrometer was used to further characterize the polymorphic system.

Video taping of hot stage microscopic observations was helpful for clearly distinguish the different crystal habits of three polymorphs. Form A showed prismatic crystals, while form B and C exhibited big and smaller, needle shaped crystals, respectively.

By heating, forms A and C showed at about 150 and 220°C, respectively, crystals shrinkage due to the rearrangement to form B, in accord to what has been previously reported by Kuhnert-Brandstatter [5].

Variable temperature IR spectra confirmed that a solid-solid transition took place on heating form A and C (Fig. 5). The IR spectrum at 230°C exhibited a shifting of the frequencies characteristic of form A transforming into B, but did not match the frequencies of form B. Form C showed the same behaviour, but the shifting of the frequencies showed up at lower temperature (about 150°C).

From the above reported experimental evidence, it emerges that thermal activation was probably not enough to pass the energy barrier: heating produced for both forms A and C an instable activated state that only after cooling, spontaneously gave rise to form B. This was confirmed by the fact that form B on heating showed at 70°C the same intermediate product: the shifting of frequencies of form B began at about 50°C.

4. Conclusions

FA exists in at least three crystalline forms with unique physical and spectroscopic properties. These forms appear to have characteristic FTIR spectra and X-ray diffraction patterns. FTIR was found to be useful in identifying each solid phase and in determining the polymorphic purity.

The DSC data obtained with a high scanning rate illustrated differences in the thermal behaviour of the three forms.

The thermal behaviour and relationships between different forms of FA were also elucidated using combined techniques: TG-FTIR and MICRO-FTIR.

Polymorph A, the form used in the clinical formulation, is the stable form at room temperature.

Although systematic stability studies have not been carried out, we observed that forms B and C show tendency to be transformed by ageing in the most stable modification; the presence of seed crystals enhances the interconversion rate.

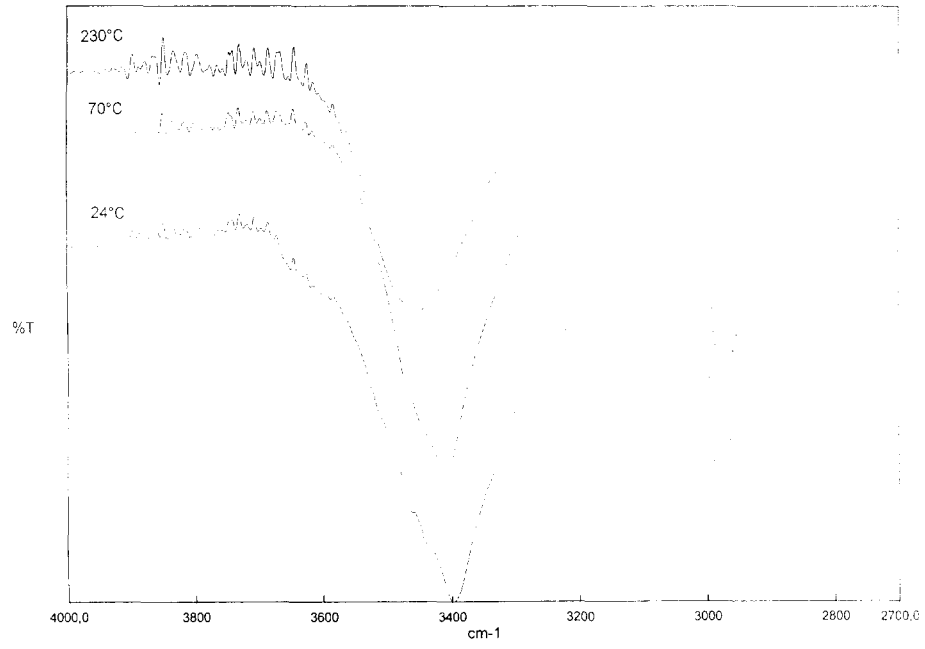


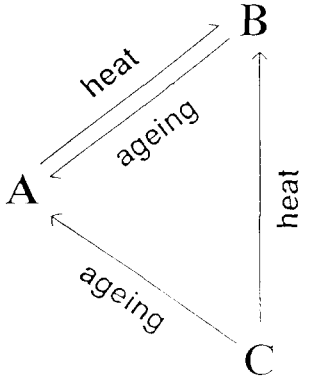
Fig. 5. Fluocinolone acetonide form A variable temperature FTIR spectra in the 4000–2700 cm^{-1} range; heating rate, 3°C min^{-1}.

Form B (the high temperature stable form), can exist for a longer time at room temperature, but it tends to be transformed partially or fully by seeding. This is in agreement with the endothermicity of the phase transition A → B.

Form C shows the highest degree of disorder, probably through the formation of defects and of amorphous regions. The exothermicity of the solid-solid transition to form B is consistent with a disorder-order type transition.

The phase relationships between the FA polymorphs are summarised in Scheme 1.

Further studies are planned to investigate the influence exerted by the different crystalline forms of FA on the stability and biopharmaceutical properties of the corresponding formulations.



Scheme 1.

Acknowledgements

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References

[1] J.K. Haleblian and W. Mc Crone, J. Pharm. Sci., 58(8) (1969) 911–929.

[2] R.J. Mesley and C.A. Johnson, J. Pharm. Pharmacol., 17 (1965) 329–340.
[3] R.J. Mesley, Spectrochim. Acta, 22 (1966) 889–917.
[4] F.I. Khattab, Thermochim. Acta, 74 (1984) 371–376.
[5] M. Kuhnert-Brandstatter, P. Gasser, P.D. Lark, R. Linder and G. Kramer, Microchem. J., 17 (1972) 719–738.
[6] L. Borka, Pharm. Acta Helv., 66(1) (1991) 16–22.
[7] L. Borka, Pharmaeuropa, 7(4) (1995) 586–593.
[8] K. Nakanishi, Infrared Absorption Spectroscopy, Holden-Day, San Francisco and Nankodo, Tokyo, 1962.
[9] A. Burger and R. Ramberger, Mikrochim. Acta II (1979) 259–271.
[10] R.J. Mesley, Chem. Ind., (1965) 1954–1955.