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Solid-state studies on the hemihydrate and the anhydrous forms of flunisolide

M. Bartolomei *

Laboratorio di Chimica del Farmaco, Section of Physical–Organic Chemistry, Instituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

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Abstract

Flunisolide exists in at least two different anhydrous crystalline forms (I and II) and in a hemihydrate form with distinctly different physico-chemical properties. Modification II and the hemihydrate form are the commercial products. Form I was obtained by heating all other forms above 230°C. The different crystalline forms of flunisolide were investigated by FTIR spectroscopy, X-ray powder diffractometry, differential scanning calorimetry (DSC), thermogravimetric analysis and thermomicroscopy both coupled with FTIR spectroscopy (TG-FTIR and FTIR thermomicroscopy). The three forms were easily differentiated by their IR spectra, X-ray powder diffraction patterns and thermal behaviour. Their stability was investigated under different experimental conditions to verify the tendency to solid–solid transition and to study the existence range of the three forms. The relationship among the two anhydrous polymorphs and the hemihydrate form and their equilibrium solubilities in water at 20°C were also investigated.

Keywords: Flunisolide; Polymorphism; Physico-chemical characterisation; Infrared spectroscopy; Thermomicroscopy; Thermal analysis

1. Introduction

Understanding the role of the crystal structure in determining the properties of a pharmaceutical substance is a crucial aspect of drug development. In fact, the thorough knowledge of the crystal structure permits to forecast the physico-chemical properties of bulk powder, ensuring batch to batch reproducibility and a higher quality and greater bioavailability of dosage form [1].

Flunisolide (6α-fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone) is a glucocorticoid used as a nasal spray for the prophilaxis and treatment of allergic rhinitis and, as a metered aerosol, in the management of asthma. It is used in the clinical formulation either as hemihydrate or as anhydrous. In spite of the wide clinical use of flunisolide, there were no published reports on the physico-chemical or the solid-state properties of this drug.

* Fax: +39-6-49387100.
E-mail address: bartolom@iss.it (M. Bartolomei).

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It is well known that steroid hormones exhibited polymorphism [2,3], in particular it was found that more than 50% of deoxycorticosterone derivatives could exist in different polymorphic forms [4]. Furthermore, the investigation of flunisolide arose from the finding that sample of industrial scale lots of this drug showed batch to batch variations and no consistency in their thermal behaviour and IR spectra, suggesting that different crystalline forms coexisted in the commercial samples. Moreover, a recent study on an alternative micronisation technique using the aerosol solvent extraction system (ASES) reported that during this process the crystal properties of flunisolide changed. This was proved by means of X-ray powder diffractometry, but no further details on the mentioned crystalline change or on the characterisation of the starting materials and products have been reported [5].

The purpose of the present work was to investigate in detail the solid-state properties of the hemihydrate form and of the two anhydrous polymorphs with particular regard to the relationships among the different crystalline forms of flunisolide, their relative physical stabilities and equilibrium solubilities at room temperature.

2. Materials and methods

Micronised hemihydrate and anhydrous flunisolide were a generous gift of Sicor (99% purity by the USP HPLC assay procedure) and used without further purification.

2.1. Preparation of flunisolide crystalline forms

Flunisolide anhydrous form II and the hemihydrate form were obtained from Sicor and used as such.

Flunisolide anhydrous form I was prepared by heating flunisolide II above 230°C and then cooling the sample at room temperature. In the first step, heating was carried out directly on thermal analysis apparatus (DSC, TGA and hot stage of thermomicroscope), in the second step, a larger quantity of form I was obtained by heating anhydrous flunisolide in a suitable container in an oven. This method produced form I, that, after grinding to comminute larger crystals in order to obtain good infrared spectra and X-ray powder diffraction pattern, consisting of fine particles of homogeneous size.

Efforts were made to obtain the two crystalline anhydrous phases and the hemihydrate form by crystallisation from solvents, but they could not be isolated in a suitable grade of polymorphic purity or in a suitable amount to permit a complete physico-chemical characterisation of each form. Form II was obtained by evaporation of 2:1 acetone/hexane mixture solution at room temperature. The resulting sample was stored in an oven at 110°C for 3 h. Form I was prepared by crystallisation from absolute ethanol solution on water-bath at about 100°C and then storing the sample in dryness for 3 h, but form I was obtained more easily and successfully by heating any other form above 230°C. Hemihydrate form was prepared by suspension in water of form II. The resulting sample was stored in a desiccator for 3 h. This method easily led to production of the form II and hemihydrate form from the same solution. Therefore, it was often necessary to repeat this crystallisation more than once.

In the light of crystallisation experiment results, the samples used for the physico-chemical characterisation were commercial anhydrous form, commercial hemihydrate form and preground form I obtained on heating form II above 230°C and cooling it at room temperature.

To check the purity of the starting materials and of the products obtained by heating above 230°C, TLC and HPLC analysis (as described in USP) were used: analysis performed on materials recovered immediately after heating indicated that no significant degradation had taken place.

The repeatability of form I formation was verified by FTIR and DSC in each batch. The resulting samples were tested by means of TGA for water content.

2.2. Physico-chemical characterisation

Thermomicroscopy experiments were performed on an i-Series Perkin Elmer hot-stage

microscope coupled with a Perkin Elmer System 2000 FTIR spectrometer, employing a MCT (mercury–cadmium–telluride) detector. The apparatus was set to collect transmittance spectra. A small amount of sample was placed as a crystalline layer into the hot stage compartment. The thermomicroscopy experiments were performed at 5 and 10°C min^{-1} to enable a correct comparison with DSC experiments. The heating and cooling rate of 5°C min^{-1} was suitable for the microscopic observation of thermal phenomena.

Room temperature and variable-temperature infrared spectra were recorded from 4000 to 580 cm^{-1}; 16 scans were collected for each sample at a resolution of 4 cm^{-1}.

X-ray powder 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 one step per s.

DSC curves were recorded using a Perkin Elmer DSC7 instrument. To obtain a better understanding of the thermal events for the two crystalline anhydrous phases and the hemihydrate, a series of increasing heating rates and different pan types were used. Approximately 1.5 mg of sample was accurately weighed into a DSC pan. The DSC profiles were recorded at 5, 10 and 30°C min^{-1}, under nitrogen flux, from 40 to 270°C. The DSC experiments were run using pans that were open, closed or closed with a cover hole. As it was impossible to obtain a better resolution of thermal events from these different experimental conditions, open pans and a heating rate of 10°C min^{-1} were routinely used for a meaningful comparison with the thermomicroscopy and TGA data. A heating rate of 30°C min^{-1} was helpful for a correct comparison with TG-FTIR data.

Programmed heat–cool cyclic DSC studies were also performed at 10°C min^{-1}.

Each experiment was repeated at least three times.

The DSC temperature scale was calibrated using extrapolated onset temperatures of the fusion endotherms of indium and zinc pure standards, heated at same rates used for the samples.

The TG curves were recorded with a Perkin Elmer TGA7 instrument coupled with a System 2000 FTIR spectrometer that allowed simultaneous analysis of gaseous products, at two heating rates — 10 and 30°C min^{-1}. The first represented a reasonable high heating rate suitable to permit a correct comparison with DSC experiment; the second the minimum rate adequate for obtaining a good infrared spectra of the gas phase. For TGA determinations, approximately 10 mg of sample was 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 temperatures 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 being 4 cm^{-1}.

2.3. Stability studies

Samples of flunisolide form I and II were stored at room temperature for 1 year in open air (about 55% RH) and in a desiccator to test the physical stability and the different ability of the two forms to uptake water from the environment.

The two forms were also manually ground and milled in an agate mortar with a pestle, separately for 1 and 5 min and tested for polymorphic transition. Aliquots of the two forms were stored in a crystalline layer on a KBr disc or on a glass slide in open air (about 55% RH) and in a desiccator for 30 days to test the physical stability of the two different forms.

The hemihydrate form was stored in a vacuum desiccator for 5 h.

The hemihydrate form was also manually ground and milled in an agate mortar with a pestle separately for 1 and 5 min. The resulting samples were tested for solid–solid transition by FTIR and water content by TGA. Aliquots of the ground materials were stored in a vacuum desiccator for 2 h. The samples were investigated by TG-FTIR to test the water content variations.

2.4. Equilibrium solubility studies

The solubility values of the different crystalline forms of flunisolide were determined in water at 20°C. Saturated solutions were prepared by introducing excess amounts of each form (about 1.5 mg) into 5 ml of water in volumetric flasks. The samples were placed in a thermostated water bath maintained at 20°C for 60 min and subjected to magnetic stirrer. The solutions were withdrawn with a syringe, filtered through a 0.22-μm membrane filter (Millex-GS Millipore) and the concentration of the drug was measured spectrophotometrically at 246 nm (λ_{max} of absorption of flunisolide determined in water). The equilibrium solubility value was confirmed by storing saturated solutions for 24 h without agitation at 20°C. The samples were then filtered and their concentration in water were determined by measuring the intensity of the UV absorption band at 246 nm by a Perkin Elmer lambda 16 spectrophotometer.

Each experiment was repeated at least three times.

3. Results and discussion

3.1. FTIR-microscopy and X-ray powder diffractometry studies

Room temperature infrared microscopy was used to record the spectra of the different forms of flunisolide directly on to KBr window in the hot stage compartment. Fig. 1 shows the spectra of polymorphs I and II and of the hemihydrate form (H). They exhibited significant differences in the observed vibrational transitions. Particularly noteworthy was the shift of O–H stretching in the 3600–3300 cm^{-1} region. In the 2000–650 cm^{-1} range the fundamental frequencies positions were nearly the same, the differences consisting of the relative intensity ratio of the bands and in the presence of specific peaks for each forms, suitable for identification in mixture. The attributions for the major bands of the three forms are summarised in Table 1.

The shifting of the stretching vibrations in the three crystal modifications reflected slightly different geometries in crystal packing. This was clearly

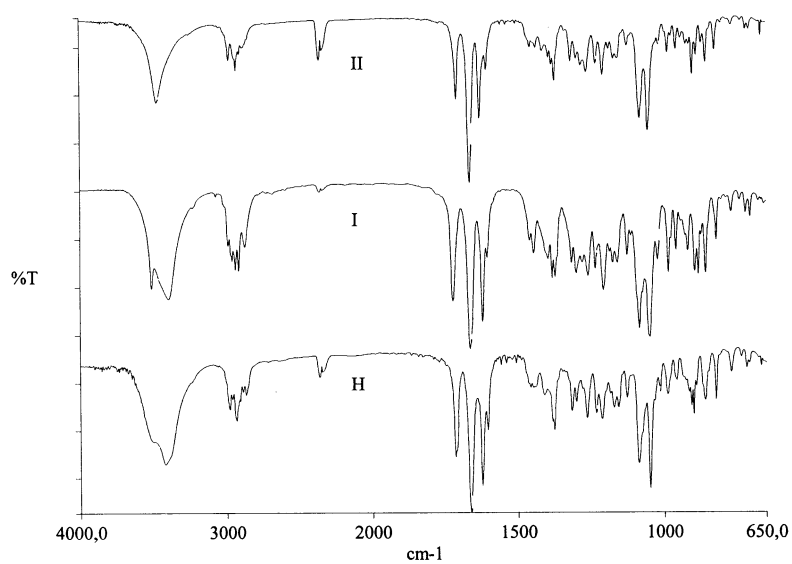


Fig. 1. FTIR spectra in the 4000–650 cm^{-1} range of the polymorphs I and II and the hemihydrate form (H) of flunisolide at room temperature.

Table 1
Frequencies (cm^{-1}) and attributions of fundamental vibrations for the flunisolide crystalline modifications: hemihydrate form, form I and II

Attributions	Hemihydrate	Form I	Form II
ν O–H	3423	3513 3398	3476
ν C_{20}=O	1716	1724	1712
ν C_3=O	1663	1666	1666
ν C_4=C_5	1623	1622	1632
ν C_1=C_2	1604	1606	1608

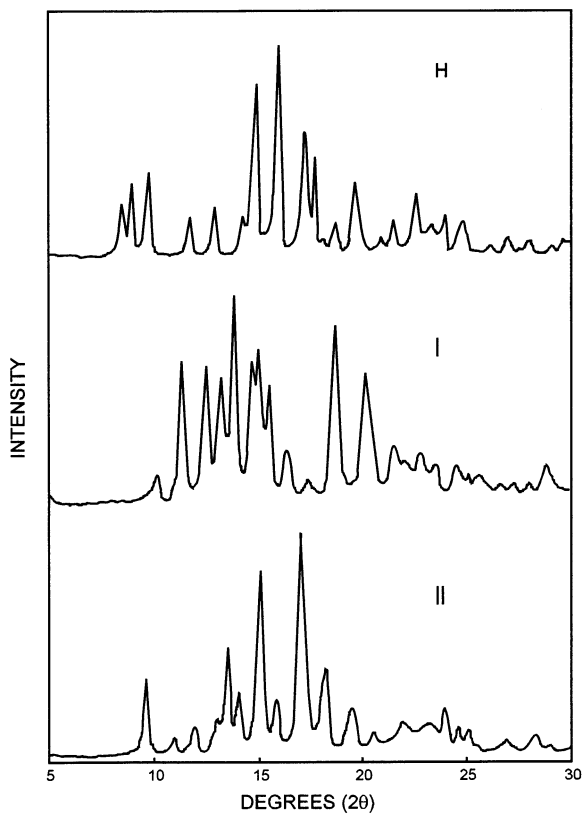


Fig. 2. X-ray powder diffraction patterns for flunisolide hemihydrate form (H) and polymorphs I and II.

confirmed by X-ray powder diffraction patterns of the three forms (Fig. 2): their profiles were sufficiently distinct to characterise each crystalline form. Moreover, 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 flunisolide molecules in the crystal lattice of each form. The characteristic angles of diffraction together with the four main peak intensities for the three crystal forms are listed in Table 2.

The X-ray powder diffraction pattern reported in [5] for flunisolide 'jet milled product' actually was consistent with the hemihydrate form pattern, probably in mixture with form II. Unfortunately the aforementioned paper did not report the X-ray patterns for the starting materials or the diffraction data for the products obtained by the different micronisation techniques, hence it not possible to make a more complete comparison with our results.

3.2. DSC, TG-FTIR and FTIR thermomicroscopy studies

The DSC profiles of the polymorphs I and II, respectively (open pan, heating rate of 10°C min^{-1}) are shown in Fig. 3.

Form II presented two characteristic endothermic peaks: a first broad small peak, often followed by a small exothermic peak and a second sharp endothermic peak due to the melting of the compound at about 255°C. At the beginning, the first endotherm was attributed to a solid–solid transition into form I, that occurred in the 210–235°C temperature range. Such transition has been verified in our experiments through room

Table 2
X-ray powder diffraction data for the two polymorphs (I and II) and the hemihydrate form (H) of flunisolide

Form	2θ (°)	I/I_0 (%)
I	13.90	100
	18.68	86.6
	15.04	69.4
	11.37	67.7
II	17.01	100
	15.07	82.6
	13.51	49.5
	18.21	39.8
H	16.05	100
	15.01	79.57
	17.25	57.61
	17.66	46.90

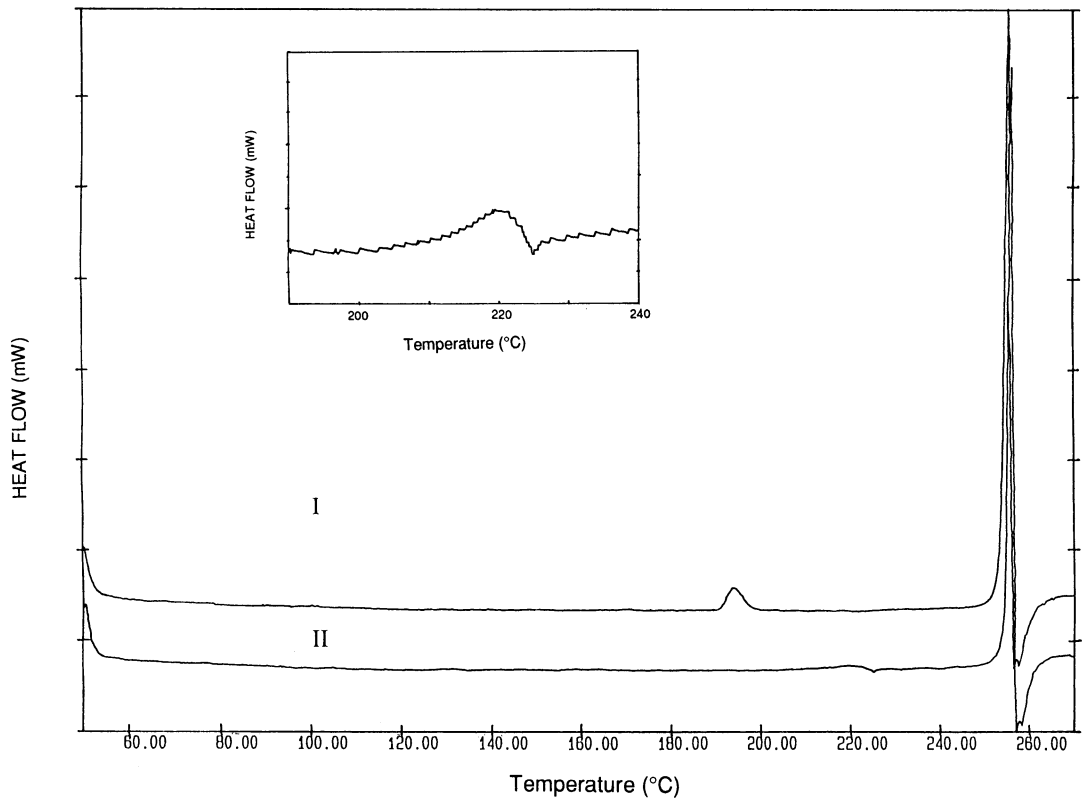


Fig. 3. DSC curves of flunisolide form I and form II; open pan; scan rate, 10°C min^{-1}; heat flow, endothermic scale. Inset showed the expanded transition temperature range of form II.

temperature FTIR spectra and X-ray powder diffraction pattern of the solid phases before and after the transition temperature. The solid–solid transition above 230°C was difficult to detect by DSC analysis using low heating rate (up to 10°C min^{-1}, open pan) due to the low transition enthalpy value (~ 2 J g^{-1}); this phenomenon could be made more evident using a heating rate of 30°C min^{-1}.

This transition was clarified by means of thermomicroscopy coupled with FTIR. On heating up to 235°C the opaque powder of form II changed into yellow needle crystals. Variable temperature IR spectra confirmed that a solid–solid transition took place on heating form II (Fig. 4). All spectra recorded until 230°C were identical to the spectrum at room temperature because no phase modifications occurred in this temperature range. Between 230 and 235°C the IR spectrum exhibited a shifting of the characteristic frequencies of form II, nevertheless, IR spectra from 235°C up to the melting temperature did not match the frequencies of form I; only after cooling at room temperature the characteristic frequencies of form I appeared. This behaviour was similar to the one previously reported for fluocinolone acetonide [6].

Form I (Fig. 3), obtained by heating the form II up to the transition temperature (235°C) and cooling it at room temperature, showed a first endotherm at about 190°C, sharper and bigger than the one of the form II (the transition enthalpy value was about 8–9 J g^{-1}) and a second sharp endotherm at about 255°C, due to the melting followed by decomposition of the compound and it was practically superimposed to the one of form II. This behaviour was consistent with heating–cooling cyclic DSC studies; a re-run on form II after heating up to 235°C and cooling

it at room temperature showed the same profile. The events recorded by DSC were clarified by FTIR thermomicroscopic experiments. By heating on the hot stage of thermomicroscope, form I showed at about 160°C crystal shrinkage. IR spectrum changed in the 160–220°C temperature range (Fig. 5), assuming the particular feature of the same intermediate product obtained on heating form II. On cooling the IR spectrum became identical to the one of form I.

From the above reported experimental evidence, it emerged that heating produced, for both forms I and II, an unstable intermediate state that only after cooling, spontaneously gave rise to

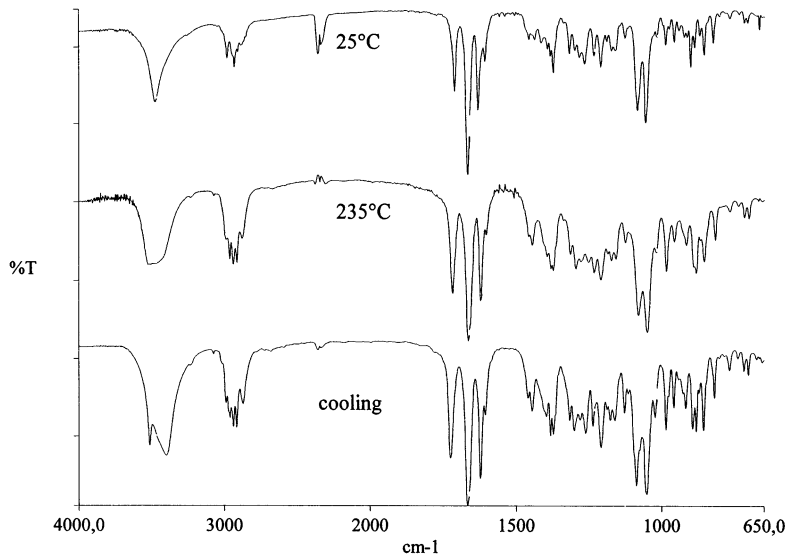


Fig. 4. Variable-temperature FTIR spectra of flunisolide form II in the 4000–650 cm^{-1} range, heating rate, 10°C min^{-1}.

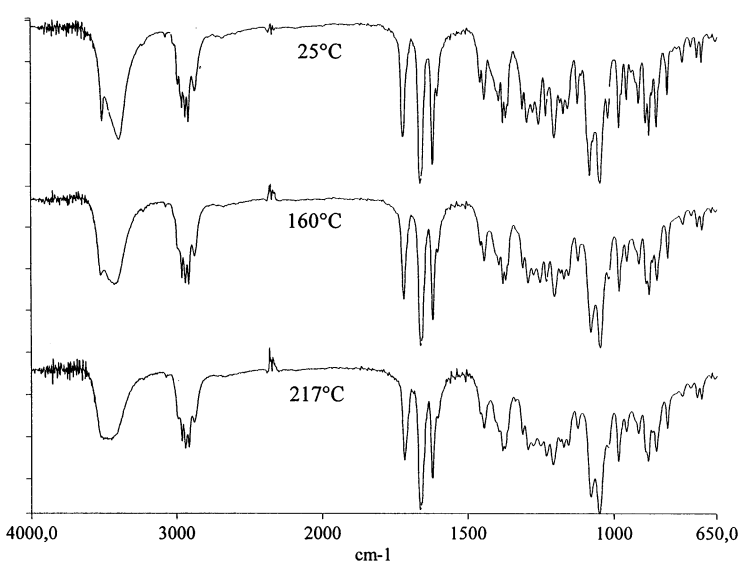


Fig. 5. Variable-temperature FTIR spectra of flunisolide form I in the 4000–650 cm^{-1} range, heating rate, 10°C min^{-1}.

form I. It was impossible to isolate this intermediate form as it converted into form I below 180°C. The intermediate form existed only at high temperature, from the solid–solid transition temperature up to the melting temperature. It was impossible to stabilise the intermediate form or to transform it into phase I by isothermal holding at 235°C.

Heating–cooling cyclic DSC studies were also performed to clarify further the thermal behaviour of the two anhydrous forms. Heating–cooling experiment results confirmed that the transition II → I was always mediated by the formation of an intermediate product that only by cooling gave rise to form I.

To exclude the possibility of solvent being present, the forms I and II were investigated by TGA; no weight loss was detected in the 40–240°C temperature range.

DSC studies on commercial samples showed large variations in thermal behaviour either in shape or in the temperature range of solid–solid transition or in the melting range. In fact in some commercial lots of form II, the solid–solid transition of form II into the intermediate product occurred at a lower temperature, at about 200°C with an endothermic peak larger and broader. Furthermore, the melting peak could fall in a lower temperature range (245–250°C). In spite of these differences in thermal behaviour, these samples exhibited identical X-ray patterns and IR spectra. A possible explanation for this behaviour might be that the sample had insufficient polymorphic purity. Some nuclei of the form I, whose presence was not detected by the X-ray diffractometry and IR spectroscopy, could favour the transition of form II during heating. This was confirmed by the fact that these samples showed a less clear transition by thermomicroscopic examination; in fact form II evolved into the intermediate form through a solid–solid transition confirmed by dynamic FTIR, without needle crystal formation. Fig. 6 showed that this behaviour could make difficult to distinguish DSC pure polymorph I from form II in mixture with a little amount of contaminant polymorph I. The shifting of the fusion to a higher temperature for sample of pure polymorph II could be attributed to the poor transfer of heat caused by larger size of needle crystals.

Hemihydrate form showed a more complex thermal profile. DSC curve (10°C min^{-1}, open pan) showed a first big broad endothermic peak in the 60–160°C temperature range that represented a composite heat effect due to fusion of the solvate crystals and evaporation of water. This endothermic peak, corresponding in the TGA curve to solvent loss, usually exhibited a shoulder between 60 and 130°C and a maximum above 140°C. This phenomenon in the DSC curve was followed by a sharp big exothermic peak at about 170°C due to the rapid re-crystallisation of an anhydrous form that consisted primarily of flunisolide form II in mixture with a little amount of form I (checked by means of FTIR thermomicroscopy).

A broad small endothermic peak closely followed the exotherm in a temperature range from 180 to 200°C and represented the solid–solid transition (~ 6 J g^{-1}) into the unstable intermediate form, that occurred at lower temperature according to the presence of form II in mixture with I. The last endotherm at about 250°C represented the fusion of the intermediate compound.

The thermal behaviour of hemihydrate form was confirmed by thermomicroscopy coupled with FTIR. On heating from 80 to 140°C increasing crystal shrinkage appeared; at about 145°C crystals melted and began to re-crystallise at about 155°C. IR spectra of the crystals obtained after complete re-crystallisation at 170°C showed that the desolvation of hemihydrate form gave rise to a mixture of the two anhydrous forms, with form II being predominant. At about 210°C microscopic observation showed a feature change, confirmed by the appearance of the characteristic infrared frequencies of intermediate form.

Fig. 7 showed TGA and DSC profiles of hemihydrate form recorded at 30°C min^{-1} in open pan. This heating rate shifted thermal events to higher temperature, but it represented the minimum rate adequate for obtaining good infrared spectra of the effluent gas phase. The desolvation of hemihydrate form occurred from 80 to 190°C with a weight loss in the 2–2.2% range in the different samples, which is in agreement with that

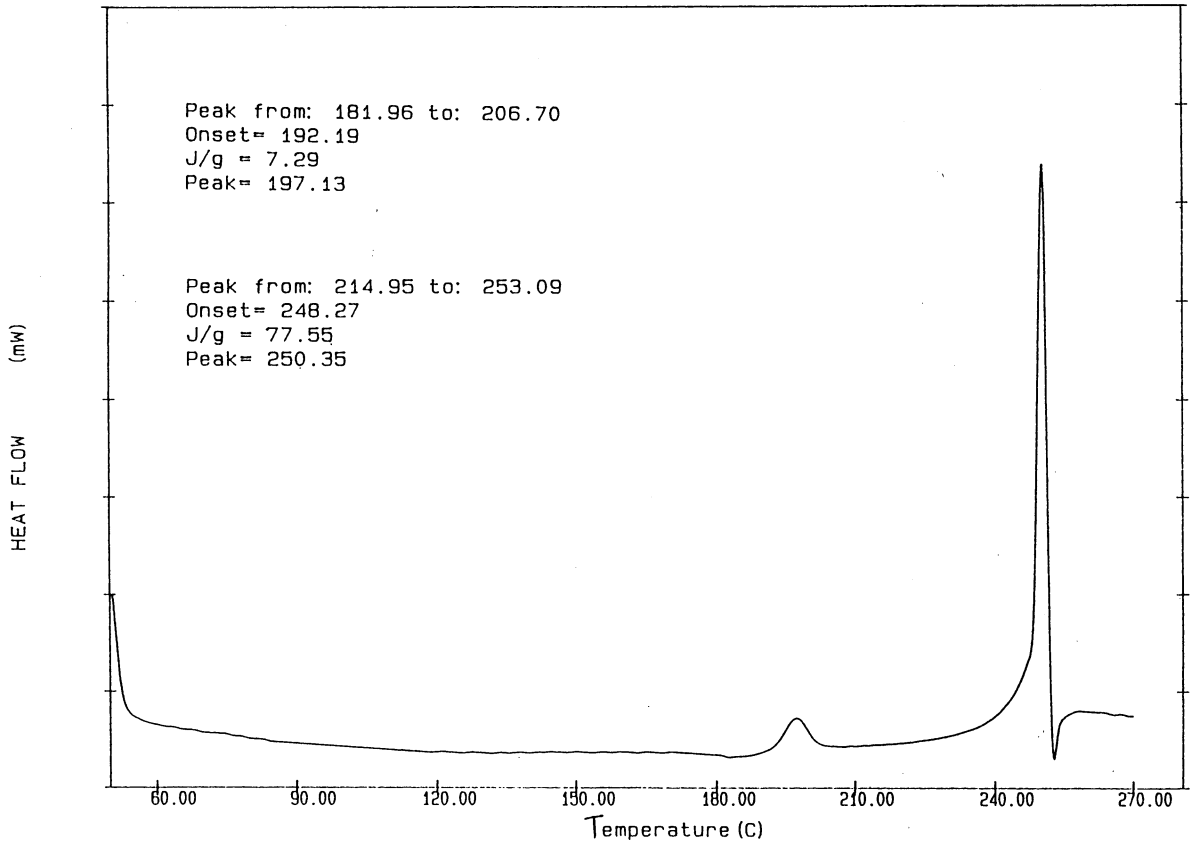


Fig. 6. DSC curve of a commercial lot of micronised flunisolide anhydrous form, consisting of form II with a contaminant amount of form I (open pan; heating rate, 10°C min^{-1}; heat flow, endothermic scale).

of a hemihydrate. The IR spectra of corresponding effluent gas phase revealed the presence of the characteristic frequencies of water together with a little amount of hexane (Fig. 8). On heating, hexane was eliminated with water. Grinding could decrease the hexane content without altering the crystalline structure of the hemihydrate form. This behaviour suggested that the interactions between hexane and the crystalline lattice were very slight; hexane was probably physically trapped in closed cavities provided by the crystalline structure without affecting its overall structure. Moreover some batches of commercial flunisolide hemihydrate form showed a hexane loss of about 0.3% at 200°C. Accordingly, it has been previously reported for fluocinolone acetonide [6], solvent dislocation was probably caused by II → unstable form solid–solid transition that occurred at the same temperature. The formation of adducts with organic solvents of the clathrate type, has been previously reported for other steroids like prednisolone form B, desametasone acetate form C and hydrocortisone form B [7,8].

3.3. Physical stability and equilibrium solubility studies

The stability of the two anhydrous polymorphic forms and the hemihydrate form under different experimental conditions was investigated to forecast the tendency of crystalline structural changes.

From stability studies it emerged that, form I and II were stable for at least 1 year at room temperature either in open air or in a desiccator; in fact under these conditions they did not show

polymorphic transitions or water content variations.

Stability of form I and II of flunisolide depended significantly on the grinding conditions. The crystalline structure seemed not to be altered by manually grinding for 1 min. Under this experimental condition, both the polymorphic forms, stored in a crystalline layer on a KBr window or on a glass slide in open air or in a desiccator, did not show a tendency to uptake water, as checked by thermogravimetric experiments. In spite of this, form I previously ground for 5 min and stored in open air in a crystalline layer on KBr disc or on a glass slide converted into hemihydrate form in 24 h. Form II needed more than 30 days to be partially converted to the hemihydrate form under the same experimental conditions. Form I and II after grinding for 5 min could be stabilised storing them in a desiccator.

The storage of the hemihydrate form in a vacuum desiccator for 5 h, generally did not significantly decrease the solvent content. Some commercial lots of hemihydrate form could show under this condition a solvent loss up to 0.5% between 50 and 110°C. This amount was variable in the different samples and could be attributed to absorbed water on the surface of crystals.

Hemihydrate form seemed not to be altered by manually grinding for 1 min; in fact, under these conditions it did not show polymorphic transition or water content variation, as checked by FTIR and TG-FTIR.

The hemihydrate form before and after grinding for 5 min exhibited the same IR spectrum and X-ray diffraction powder pattern. In spite of this, TGA profile of hemihydrate form after grinding for 5 min showed a feature change. In TGA profile the solvent dislocation could be separated in two different steps; the first from 50 to 100°C

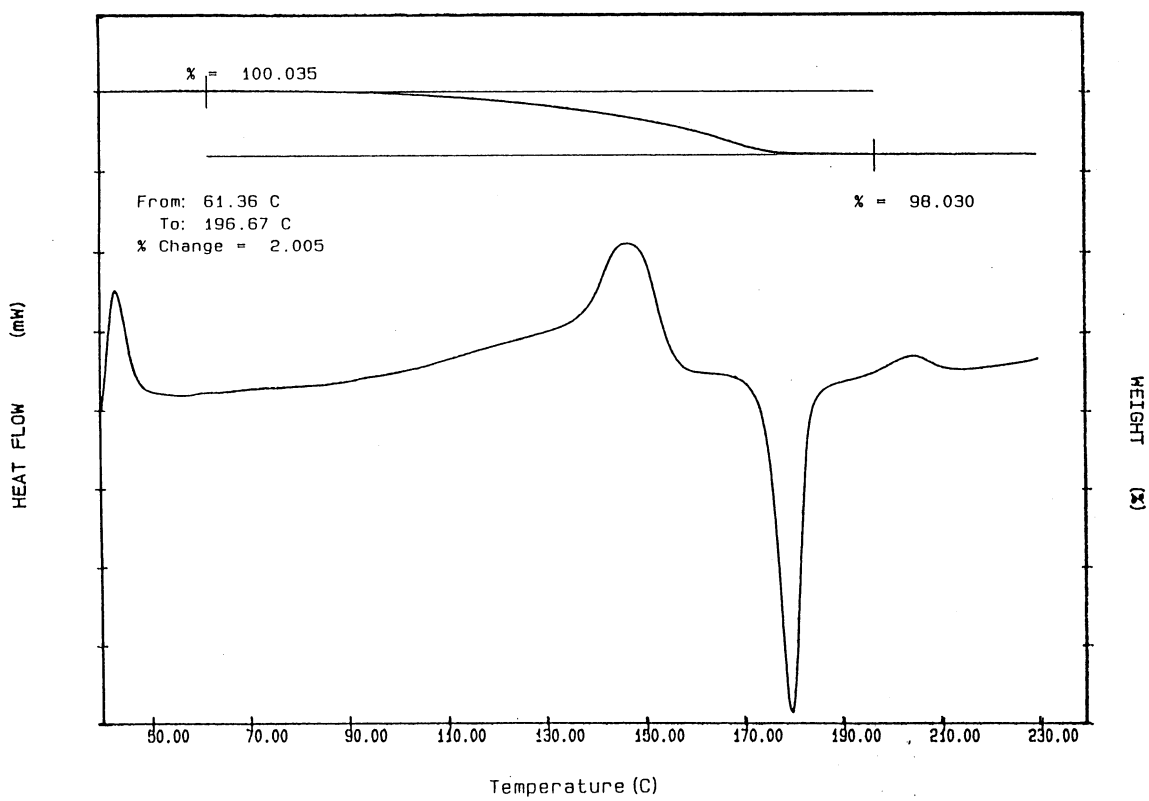


Fig. 7. DSC (open pan) and TG curves of flunisolide hemihydrate form; 30°C min^{-1}, scan rate; heat flow, endothermic scale.

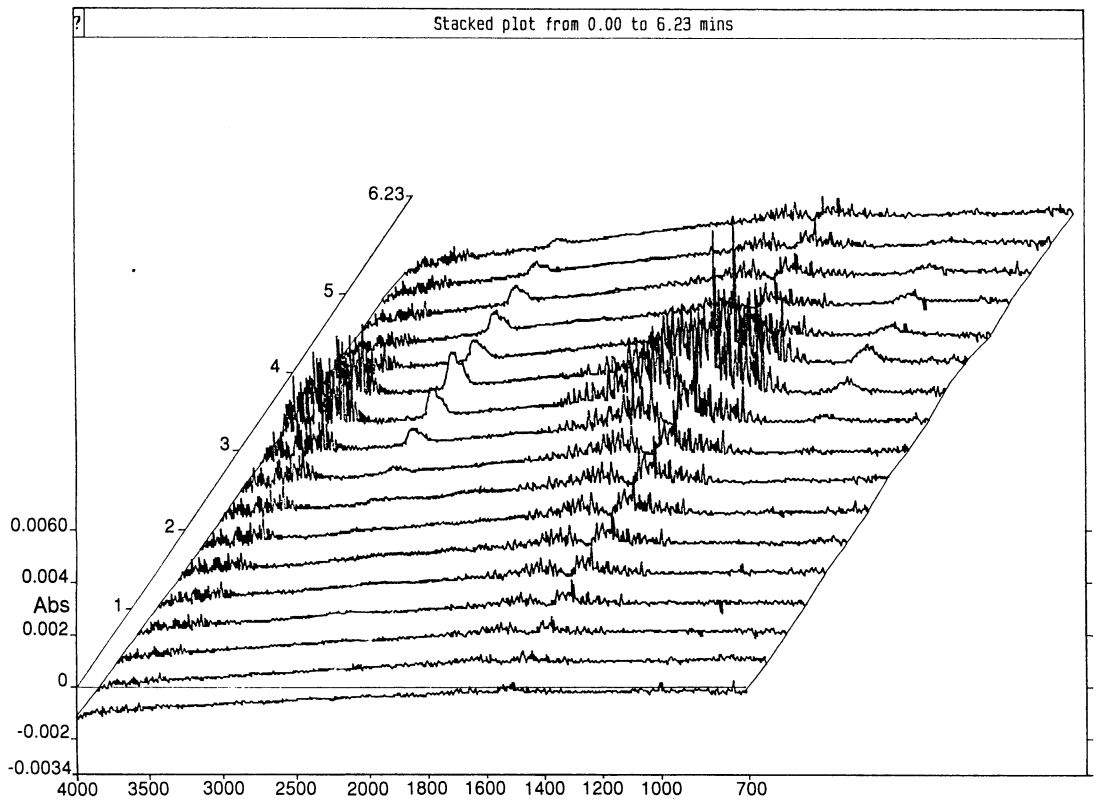


Fig. 8. Stacked plot of the IR spectra of the gas phase originating during the flunisolide hemihydrate form desolvation process, heating rate, 30°C min^{-1}, under nitrogen flux.

of about 0.3–0.4% and a second from 105 to 170°C of about 1.7%. The first weight loss decreased storing the pre-ground sample in a vacuum desiccator for 2 h.

These results could be in agreement with the fact that on grinding the physically trapped solvent, moved on the crystal surface and could be removed on drying. The IR spectra of the gas phase originating on heating in TG analysis of dried samples showed the almost disappearing of characteristic frequencies of hexane.

Equilibrium solubilities of I, II and the hemihydrate forms were determined by investigating the saturation conditions of the different crystalline forms at 20°C in water. At 20°C all the three crystalline forms reached saturation within 60 min. The equilibrium solubility value at 20°C was confirmed by comparison of the results obtained at 20°C by stirring for 1 h and storing saturated solutions of the two forms for 24 h. The results of equilibrium solubility studies at 20°C by stirring for 1 h are reported in Table 3.

On comparing the saturated states of these samples, it can be seen that form I attained the highest concentration; the amount dissolved from form I was about 30% higher than that form II. Less relevant differences in solubility were found

Table 3
Equilibrium solubilities of form I, II and hemihydrate form in water at $T = 20$°C

Form	Equilibrium solubility[a] (μg ml^{-1})
I	45.3 ± 0.19
Hemihydrate	37.9 ± 0.17
II	34.7 ± 0.26

[a] Subjected to a constant stirring rate for 1 h.

between II and the hemihydrate. In fact the hemihydrate form was more soluble than form II of about 9%. The free energy difference ($\Delta G_{I,II}$) between the two polymorphs at a particular temperature (20°C) is an indication of their relative stability and can be obtained by means of solubility measurements according to the formula:

$$\Delta G_{I,II} = RT \ln \frac{\text{solubility of form I}}{\text{solubility of form II}}$$

where T is the temperature (K) at which the solubilities were determined and R is the gas constant [9–11]. The solubility ratio between form I and II at 20°C subjected to magnetic stirrer for 1 h in water was found to be 1.3 and the free energy difference between the two polymorphs was 0.65 kJ mol^{-1}. Analogously the free energy change ($\Delta G_{I,II}$) for the hydration of the anhydrous form II resulted as 0.21 kJ mol^{-1}.

4. Conclusions

Flunisolide existed in multiple crystalline forms with unique physical and spectroscopic properties.

The commercial anhydrous and hemihydrate flunisolide samples had different IR spectra, X-ray powder diffraction patterns and very different thermal behaviour.

From thermal studies on flunisolide emerged the existence of at least another stable anhydrous polymorph, denominated form I, in which the two commercial forms were converted by heating above 230°C and cooling them at room temperature. The presence of a discrete crystalline form was confirmed by X-ray powder diffractometry.

Infrared spectroscopy provided a very useful mean of identifying the hemihydrate and the two anhydrous forms. DSC analysis was not recommended as a primary tool for identifying the two anhydrous polymorphic forms, but the knowledge of their thermal behaviour was found to be helpful in evaluating the polymorphic purity of the samples. In fact, it was found that the presence of a small amount of polymorph I in samples which consisted primarily of form II, could have a profound effect on the resulting thermal profiles.

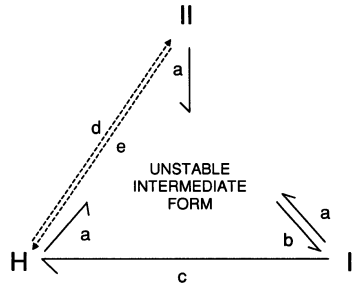
Thermomicroscopy coupled with FTIR has proved to be advantageous to further characterise the polymorphic system; it emerged that heating produced for both form I and II an unstable intermediate state that only after cooling, spontaneously gave rise to form I. It was impossible to isolate and characterise this unstable form; the transition between the two polymorphic forms were always mediated by the formation of this intermediate product that only by cooling at room temperature gave rise to form I.

The relationship between the two anhydrous polymorphs and the hemihydrate form was also investigated. The desolvation of hemihydrate form gave rise to a mixture of the two anhydrous forms, with form II being predominant.

The hemihydrate form was found to retain a little, non-stoichiometric amount of hexane, probably trapped in crystalline cages, in accordance with that previously reported for other semisynthetic glucocorticoids.

Furthermore, the position of the desolvation peak in DSC curve varied considerably from one commercial sample to the next. There was no significant change in water content, but this behaviour permitted to suppose that different types of binding sites for water in the crystal lattice existed; this was confirmed by the fact that some samples showed two distinct stages of water loss in TG-FTIR experiments.

Form I, showed a higher solubility than the other anhydrous form and than the hemihydrate form with decreasing equilibrium solubility value in the order I > hemihydrate > form II at 20°C. Taking into account the less relevant differences in solubility found between the form II and the hemihydrate, these results were in agreement with the stability studies results. Although both the anhydrous forms were able to exist for at least 1 year at room temperature, the grinding experiments confirmed that form I was the least stable form at room temperature. In fact, form I converted to hemihydrate form after grinding for 5 min and storing it as a crystalline layer in open air for 24 h. The commercial products, form II and the hemihydrate form were the most thermo-



a = heating up to transition temperature.
b = cooling at room temperature.
c = grinding for 5 min and storing the sample as a crystalline layer on open air for 24 h.
d = heating at about 170°C → mixture of the two anhydrous forms with form II predominant.
e = grinding for 5 min and storing the sample as a crystalline layer on open air for at least 30 days → form II partially converted to the hemihydrate form.

Solid-solid transition

Incomplete solid-solid transition

Scheme 1.

dynamically stable at room temperature. Furthermore from the stability studies emerged a little tendency of form II to uptake water from the environment and to change its structure into hemihydrate form after grinding 5 min and storing it as a crystalline layer in open air for 60 days. The assumed phase relationships among the flunisolide anhydrous phases and the hemihydrate form are summarised in Scheme 1.

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