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Short communication

Different crystal morphologies arising from different preparation methods of a same polymorphic form may result in different properties of the final materials: The case of diclofenac sodium trihydrate

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ABSTRACT

Diclofenac sodium is a nonsteroidal anti-inflammatory drug widely used in painful and inflammatory diseases. It can exist in different hydrate phases.

Recently the physico-chemical and pharmaceutical properties of a trihydrate form, named DSH3 were reported by the same authors.

This short communication discusses how samples of a same polymorphic form can display dissimilar analytical signatures when obtained by different routes. Data from hot-stage microscopy, FT-IR spectroscopy, X-ray powder diffraction (XRDP) and thermal analysis were used to characterise the DSH3 samples prepared by different methods.

Through the case study of diclofenac sodium, this work highlights how the method used to prepare a specific crystal modification can generate samples with different morphologies and therefore different properties and physical stability.

1. Introduction

When seeking a thorough understanding of solid-state aspects of an active pharmaceutical substance, all solid forms of the same molecule that can be produced by standard pharmaceutical processes should be carefully considered: true polymorphs, solvates, hydrates, desolvated and amorphous forms [1,2].

When an active pharmaceutical ingredient showing polymorphism is manufactured in a solid dosage form, maximum care is given to the correct preparation of the desired polymorphic modification, because polymorphism can indeed affect solid state properties of utter importance such as packing properties (e.g. density, hygroscopicity), thermodynamic properties (melting temperature, solubility, etc.), spectroscopic properties, mechanical properties (e.g. hardness, compressability and compactability), kinetic properties such as dissolution rate and stability and, last but not least, bioavailability [1–3].

However differences in solid-state properties of powders may arise even in samples of the same polymorphic form: these differences are often related to crystal morphology, which in turn may be linked to the method employed for the preparation.

In particular, the case of a recently discovered trihydrate form of diclofenac sodium, named DSH3 [4], was investigated to elucidate this issue.

DSH3 was alternately prepared by two different methods and the samples obtained were screened through various spectroscopic and calorimetric techniques both to ensure that the desired polymorphic form DSH3 was actually obtained and to demonstrate that samples prepared by different methods displayed different analytical profiles.

2. Materials

Diclofenac sodium reference substance was supplied by Sigma–Aldrich (minimum 99.5% purity by the Ph. Eur. HPLC assay procedure) and was used without further purification.

Analytical grade organic solvents were purchased from Sigma–Aldrich (Milan, Italy).

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3. Methods

3.1. Preparation of the trihydrate form DSH3

DSH3 (water content about 16%) was prepared with the following methods previously described [4]:

- *Method 1*. By storing diclofenac sodium anhydrous form (DS) in an incubator (M80-RH Incubator, MPM Instruments, Italy) at 40 (±2) °C and 75 (±5)% RH (ICH accelerated storage conditions [5]) for 20 min.
- *Method 2*. By crystallisation. A solution of about 50 mg of anhydrous DS in 5 ml of isopropanol (or ethyl acetate) is stirred and evaporated in an incubator (M80-RH Incubator, MPM Instruments, Italy) for 1 h at 20 °C and 30% RH. It is interesting to note that an hydrate species is obtained even if crystallisation occurred in anhydrous solvents. It may be hypothesized that water is absorbed from the ambient moisture when crystals begin to form or that the extremely small amount of water present in the solvents is readily and completely captured by the hygroscopic diclofenac sodium crystals growing in solution.

Samples were homogenised in size by a gentle grinding process, carried out by a PerkinElmer vibrating micro-mill for 10 min.

The resulting samples were tested by hot-stage microscopy, differential scanning calorimetry (DSC), FT-IR and XRDP to confirm that DSH3 was obtained with both methods. Thermogravimetric analysis (TGA) was used to confirm the expected water content.

Identification of water as solvent of crystallisation was performed firstly by TG coupled with FT-IR and then by ^{1}H NMR spectroscopy.

The RH was checked by a digital thermo-hygrometer (Escort Junior Data Logger, Escort Data Logging Systems Ltd., Auckland, New Zealand).

HPLC analysis by the Ph. Eur. assay procedure [6] performed on samples obtained by both methods indicated that no degradation had taken place.

3.2. Characterisation of DSH3 prepared by methods 1 and 2

Thermo-microscopy experiments were performed on the i-Series PerkinElmer hot-stage microscope coupled with a PerkinElmer System 2000 FT-IR spectrometer, employing a MCT (mercury-cadmium-telluride) detector. A small amount of sample was placed as a crystalline layer into the hot stage compartment. The thermo-microscopy experiments were performed in the 25–100 °C range at 1, 5 and 10 °C min^{-1} to enable a correct comparison with DSC and TGA experiments. The heating and cooling rate of 5 °C min^{-1} was suitable for the microscopic observation of thermal phenomena.

Variable temperature FT-IR experiments were performed by means of a PerkinElmer FT-IR spectrometer equipped with an ATR (attenuated total reflection) sampling system with the Heated Golden Gate Controller (Specac, UK) and recorded from 4000 to 370 cm^{-1} at a resolution of 4 cm^{-1}.

X-ray powder diffraction (XRPD) patterns were obtained with a Philips P.W. 1729 diffractometer equipped with a personal computer for data acquisition and analysis (software Philips APD) in the 2θ range between 5° and 35° using Cu Kα radiation-Ni filtered (40 kV; 30 mA). The step scan mode was performed with a step width of 0.01° at a rate of 1 step s^{-1}.

DSC curves were recorded using a PerkinElmer DSC7 instrument and a Pyris 1 DSC. Approximately 1 mg of powder was weighed into a DSC pan. The DSC profiles were recorded at 10 °C min^{-1}, under nitrogen flux, from 25 °C (DSC7) or 5 °C (Pyris 1 DSC) to about 150 °C. The experiments were conducted using closed pans with a cover hole made by the PerkinElmer's Accupik system. The DSC temperature scale was calibrated using onset temperatures extrapolated from the fusion endotherms of indium and lead pure standards, heated at the same rates used for the samples. Each DSC experiment was repeated at least three times. Programmed heat-cool cyclic DSC studies were also performed at 10 °C min^{-1}.

Thermogravimetric curves were recorded with a PerkinElmer Pyris 1 TGA at the heating rate of 1 and 10 °C min^{-1}. Approximately 5 mg of substance were weighed. The cooling accessory C6 chiller (PerkinElmer) allowed starting from 15 °C. A temperature calibration of the thermogravimetric apparatus was performed measuring the magnetic transition temperature of two standards, alumel and nickel. Each TGA experiment was repeated at least three times.

4. Results and discussion

Different samples of the same hydrate form may exhibit different properties and thermal behaviour if their crystal size and/or morphology are different. For example dehydration will be faster for samples with smaller particles. Thus, to focus the present study on the sole morphology, the size of the two investigated samples of DSH3 were homogenised by gentle grinding carried on for 10 min in a vibrating micro-mill (PerkinElmer). No specific measures of the crystal size distribution were performed due to the low amount of material available. Furthermore such measures resulted quite prone to sampling errors, especially because particles tended to agglomerate and in such a case dehydration kinetic is often related to agglomerate material and not to primary particles size.

Methods 1 and 2 produce almost identical samples, that nonetheless show interesting differences in their analytical profiles. Although the two samples belong to the same pseudo-polymorphic structure DSH3, as showed by their XRPD patterns, FT-IR spectra and water content (see Figs. 1, 2 and 4), they display different crystal habits: optical microscopy experiments showed that method 1 produces a polycrystalline DSH3 consisting of agglomerates of dark particles. Method 2 instead, produces crystals with a flake-like shape according the classification reported in [2]: thin, flat, platy crystals that have similar breadth and width.

Also X-ray diffraction powder patterns showed significant differences in peak intensities and peak intensity ratios. These differences are probably due to preferred orientation effects. Method 2 samples in fact, having flake-like morphology are prevented from casual orientation of crystallites during packing in the sample compartment of the diffractometer. Such effects are maximised when grinding is prolonged (manual grinding by agate mortar and pestle), probably because the crystallites are forced to assume a preferred orientation (see Fig. 1).

IR spectra of the samples obtained by methods 1 and 2 are identical at room temperature. Yet they diverge considerably upon heating: in variable temperature ATR FT-IR experiments, samples obtained by method 1 (Fig. 2A) retain their IR spectrum up to 50 °C, then they give rise to an intermediate structure stable up to 70 °C, and finally revert to the original spectrum upon cooling to ambient temperature. On the contrary samples obtained by method 2 (Fig. 2B) lose their characteristic IR spectrum upon heating: at about 55 °C they give rise to the DS spectrum, the process being irreversible. TGA results proved the sample is not completely desolvated at this temperature.

The different stability to thermal stress showed by variable temperature IR experiments, is confirmed by hot-stage microscopy. Upon heating in the hot stage, the DSH3 flake-like crystals obtained by method 2 changed to a polycrystalline aggregate that retains their outward shape: these crystals, originally clear and

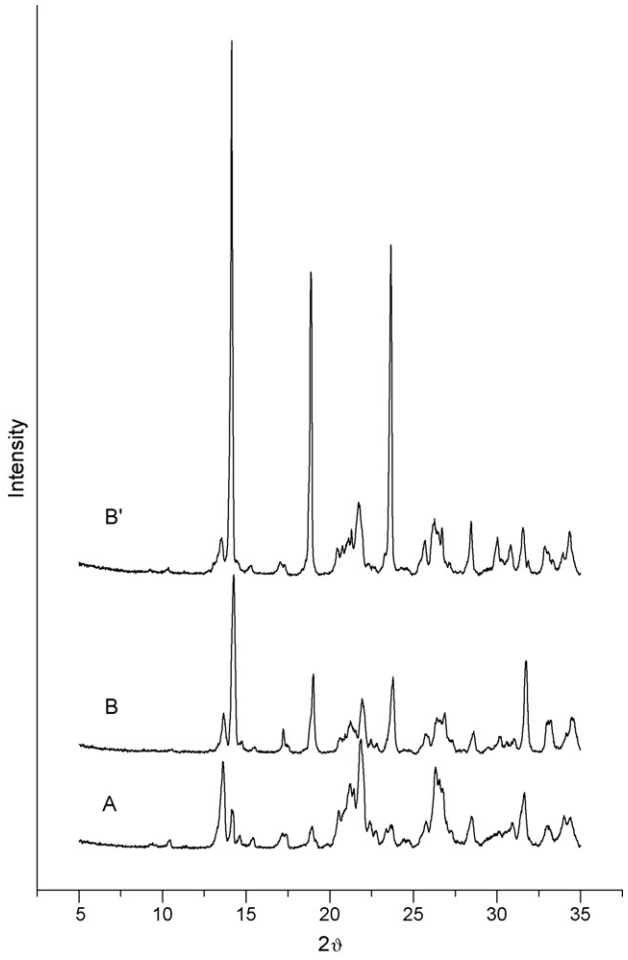

Fig. 1. XRDP patterns of DSH3 obtained by method 1 (A) and by method 2 before (B) and after prolonged grinding (B′).

transparent, became opaque at about 40 °C and brown in colour within the 40–70 °C temperature range. Darkening was isotropic, i.e. progressive and simultaneous in every part of the crystal. Crystals of DSH3 obtained by method 1, instead, lost water without visible effects during thermo-microscopy experiments. The observed differences in behaviour of DSH3 produced by methods 1 and 2 upon heating can be explained as a result of diverse crystal habit and vent hole size.

DSH3 produced by method 1 shows small crystallites that after dehydration via heating can spontaneously re-hydrate to DSH3 taking advantage of the self-seeding effect due to the presence of residual trihydrate particles at 70 °C. Flake-like crystals obtained by method 2 instead, lose water at 55 °C changing irreversibly into DS.

Also the DSC curves of DSH3 produced by the two methods, although very similar, are not identical. DSH3 produced by method 2 shows a first endotherm sharper and higher than the one showed by DSH3 produced by method 1 (Fig. 3). The onset temperatures are almost identical.

Also TGA experiments show some slight differences between the samples prepared with the two methods. When heated at 1 °C min^{-1}, they show thermogravimetric profiles that do not completely overlay (Fig. 4). Samples obtained by method 1 display an earlier leakage of water from the crystals at low temperature that forego the loss of the main portion of water from the lattice. Samples prepared by method 2 instead, lose water continuously in the 30–100 °C range.

The loss of water for both samples in this experimental condition is complete at about 63–65 °C.

This evidence together with the continuous dehydration profile showed by DSC experiments in the 40–110 °C and the wide OH stretch visible in IR spectra, indicate that DSH3 is a channel type hydrate, even if the hot-stage data seem not to corroborate this hypothesis (the expected anisotropic darkening upon water loss was not observed). However such a structure is rather common among the alkaline diclofenac salts [7]. Moreover the early dehydration onset visible in DSC profiles, opposed to the high T peak, seems to suggest that an "inverse cooperativity" dehydration mechanism is involved (see [8]).

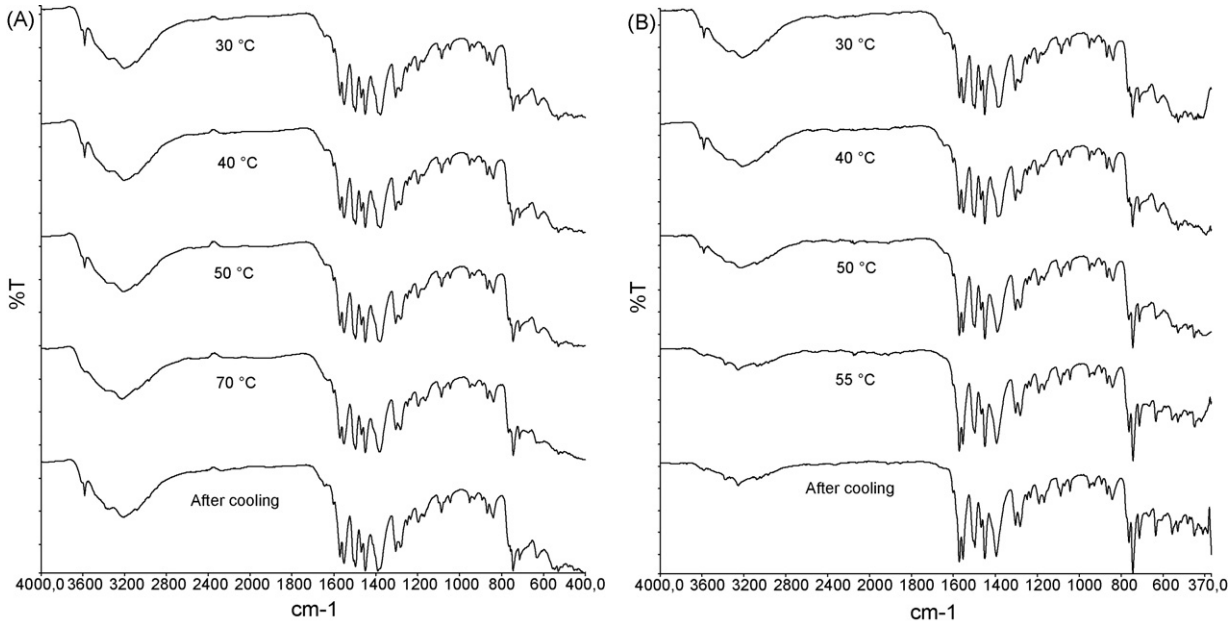

Fig. 2. Variable temperature FT-IR spectra by ATR of DSH3 prepared by method 1 (A) and 2 (B).

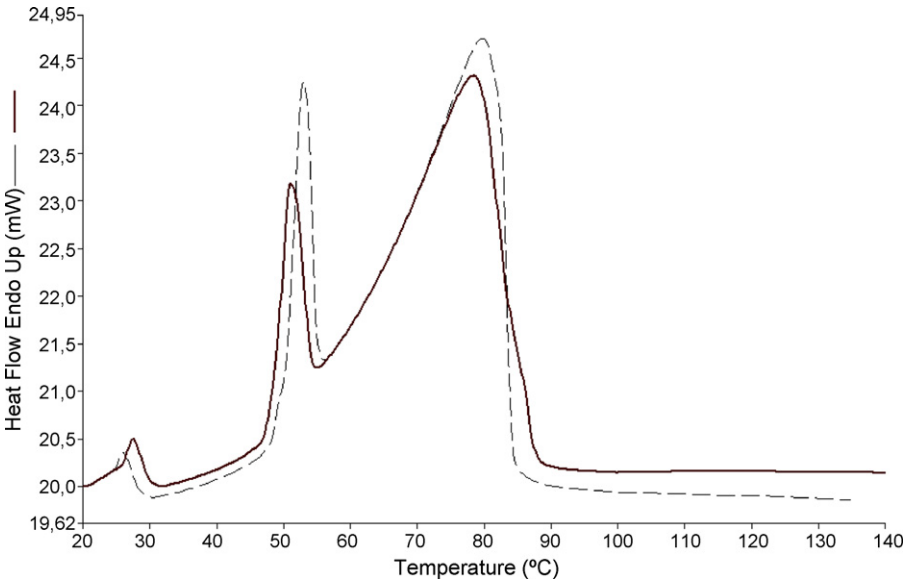

Fig. 3. DSC profiles of DSH3 obtained by method 1 (dashed line) and by method 2 (solid line), 10 °C min^{-1}, scan rate; heat flow, endothermic scale.

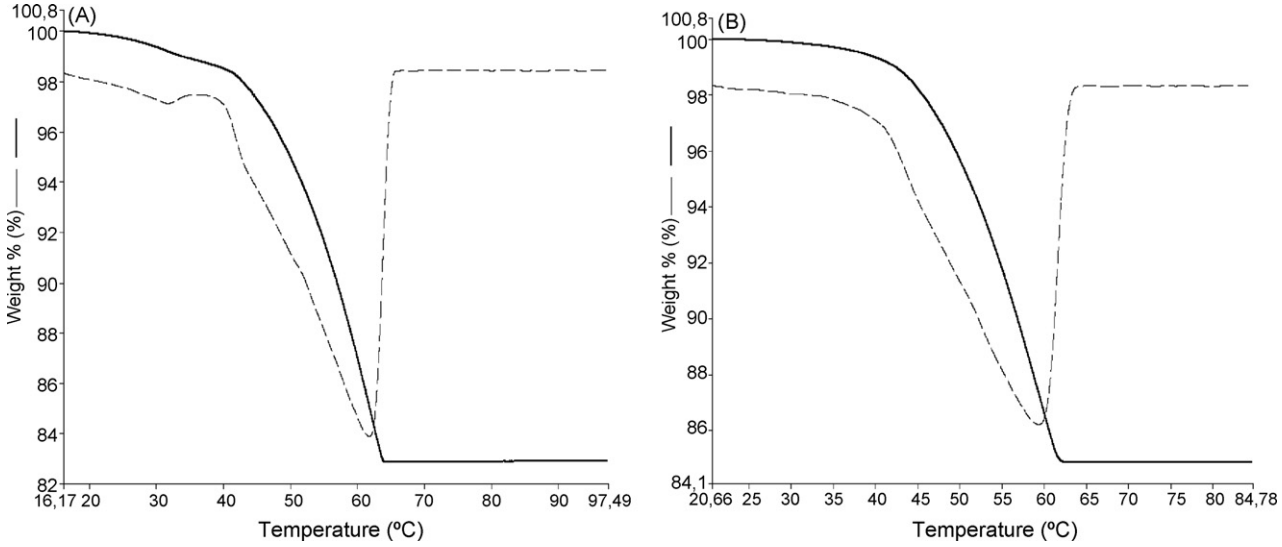

Fig. 4. TG profiles (1 °C min^{-1}) with derivative (dashed lines) of DSH3 prepared by method 1 (A) and 2 (B).

5. Conclusions

Diclofenac sodium can exist in different hydrate phases [9–17].

During the investigation of the physico-chemical and pharmaceutical properties of the trihydrate form DSH3 it emerged that the two different methods used to prepare this form gave rise to materials with different characteristics, in particular with diverse stability to thermal stress.

Hot-stage microscopy and variable temperature ATR experiments were very useful tools to explain the different thermal behaviour of the DSH3 samples.

Various differences between the analytical profiles of the two samples were reported. Differences in peak intensity and intensity ratio in XRPD patterns, different thermal behaviour at variable temperature ATR FT-IR and hot-stage-microscopy and a few minor differences in DSC and TGA profiles. All these discrepancies were attributed to macroscopic characteristics of the powders and in turn they resulted to be related to the two methods of preparation employed.

Thus it was shown how the method chosen for the preparation of a specific polymorphic form may occasionally produce samples of different morphologies that in turn may critically affect characteristics and physical stability of the final material. In conclusion this work intends to stress the importance of proper selection of processing parameters to guarantee the correct manufacturing of active pharmaceutical substances.

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