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Dehydration and rehydration of a hydrate diclofenac salt at room temperature

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Abstract

The salt diclofenac/*N*-(2-hydroxyethyl) pyrrolidine crystallizes from water as a dihydrate, while it precipitates from organic solvents anhydrously: the two salts have different crystal structures. Dehydration of the dihydrate salt was carried out in a desiccator over silica gel at room temperature: the process occurs with the retention of the crystal structure. Slight changes observed in the diffractograms suggest that, soon after dehydration, a phase transition starts, slowly due to the low temperature of the process. The reaction was followed determining the loss of weight as a function of time and by thermal analysis, since the dihydrate and the dehydrate forms have different thermograms, but similar diffractograms. The reaction was complete after 24 h. The analysis of the experimental data suggests a kinetic process related to a one-dimensional diffusion of the crystallization water molecules outwards the solid particles. At room temperature, the dehydrate material rapidly back-absorbs the two molecules of crystallization water from the atmosphere moisture. The interaction with water of the different forms of the salt was discussed as a function of their solid structures as well as of the complex equilibria present in aqueous solution: these can explain previous apparently anomalous results. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Diclofenac/*N*-(2-hydroxyethyl) pyrrolidine; Dihydrate and dehydrate forms; Dehydration and rehydration; Thermal analysis; Diffractometry; Behaviour in solution

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1. Introduction

Diclofenac, a potent non-steroidal anti-inflammatory drug, is an acid poorly soluble in water in its unionized form. It is widely used as a salt, mainly sodium, potassium and diethylamine salts (Martindale, 1996). Recently, a new chemical form with the organic base *N*-(2-hydroxyethyl) pyrrolidine (DHEP) was proposed as a suitable form for transdermal delivery of the active agent (Galzigna et al., 1989; Assandri et al., 1993). This salt demonstrated the highest solubility in water among a series of salts prepared with a large

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variety of metal hydroxides and organic bases (Fini et al., 1992; Fini and Fazio, 1994). The preparation of this salt from water results in the formation of a dihydrate form (Ledwige et al., 1996), which X-ray analysis demonstrated to possess a different crystalline structure from that of the form prepared from organic solvent (Castellari and Sabatino, 1994; Ledwige et al., 1996). As a consequence, the hydrate salt also displayed expected differences in the solution behaviour, concerning dissolution rate and solubility in water (Ledwige et al., 1996), and an extent of self-association of the diclofenate anions (Fini et al., 1991).

Since some pharmaceutical forms contain DHEP as the active agent, the use of water in its processing is obviously preferred for ecological, economical and technical reasons (Otsuka et al., 1990). Therefore, a research programme was started to understand the differences between the two forms and to suggest the optimum experimental conditions for the dehydration of the dihydrate form, and for the phase transition from the dehydrate towards the anhydrous form: in such a way, the whole preparation of the suitable form of DHEP could be performed in the absence of organic solvents.

In this paper, we present a study dealing with the dehydration of the dihydrate DHEP salt at room temperature in a desiccator over silica gel and the rehydration of the final product at controlled humidity. The processes were followed measuring the change of weight as a function of time and by thermal analysis, since the dihydrate, the dehydrate and the anhydrous forms have different thermograms.

2. Experimental

2.1. Materials

Acidic diclofenac (D) was a gift from IBSA (Lugano, Switzerland) and was used as received; *N*-(2-hydroxyethyl) pyrrolidine (HEP) was a commercial sample (Fluka, Buchs, Switzerland), distilled at reduced pressure, prior to use. DHEP samples of different industrial batches obtained from water and dehydrated were similarly obtained from IBSA.

2.2. Preparation of the DHEP salt

2.2.1. Dihydrate form (A)

Acidic diclofenac (0.01) was suspended in 10 ml water and an equivalent amount of HEP added under stirring. After complete dissolution of the suspended solid, a small excess of D was further added to control the pH of the solution. The mixture was heated to about 50°C and then filtered. The final solution was allowed to crystallize as $DHEP{\cdot}2H_2O$, after storage in a refrigerator at 4°C for a few days. The salt was recrystallized from water. The final material was kept in ambient conditions for 48 h to lose the excess crystallizing medium.

Alternatively, the dihydrate salt can be prepared by simply crystallizing the anhydrous salt from water, as already described, and keeping the solution at 4°C for 1 week.

2.2.2. Dehydrate form (B)

Three grams of the dihydrate form were stored in a desiccator over silica gel for 48 h at room temperature; in these conditions, dehydration occurs with limited changes of the crystalline structure of the dihydrate form.

2.2.3. Anhydrous form (C)

Equivalent amounts (0.01 mol) of D and HEP were separately dissolved in 10 ml of ethyl acetate; the two solutions were mixed and shortly stirred, heated on a water bath, filtered and allowed to crystallize. The salt was recrystallized from the same solvent. The solid material in the form of nearly regular cubes was filtered and stored in a desiccator.

2.3. Dehydration and rehydration kinetics

2.3.1. Dehydration kinetics

Two grams of the hydrate sample (previously sieved: the fraction between 100–200 and 300–400 μm was used for the experiments) were kept in a desiccator over silica gel at room temperature and weighed at a prefixed time range for 24 h.

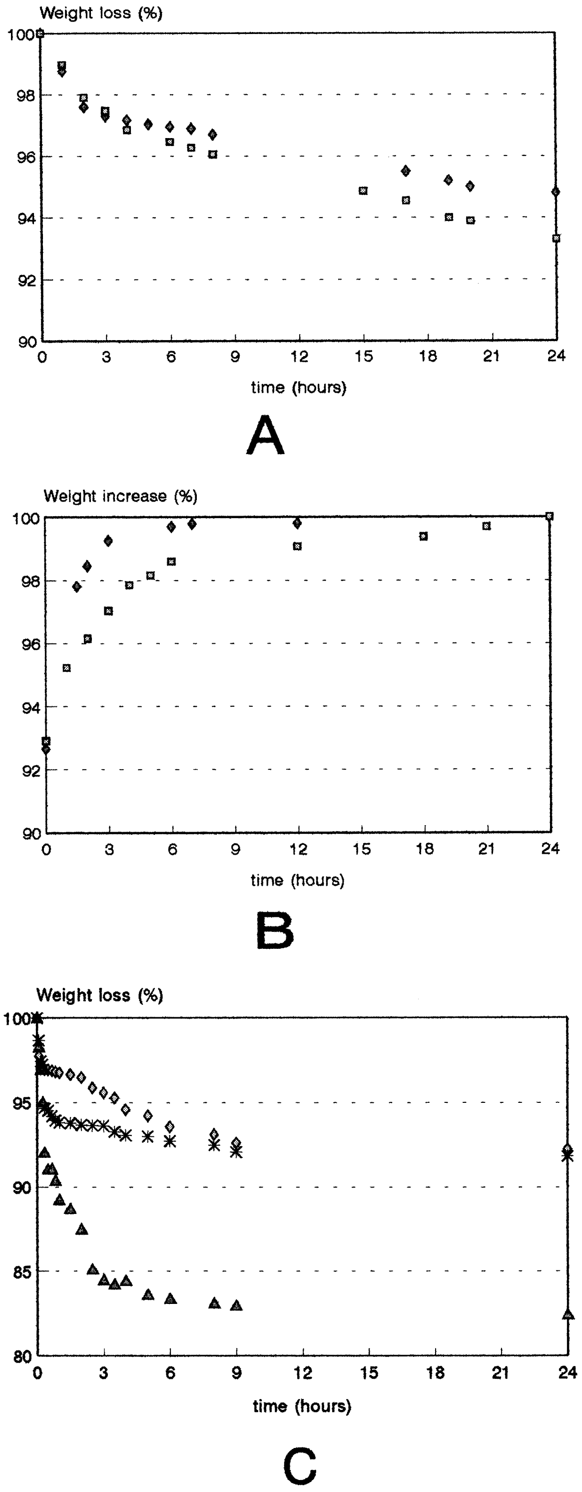

Fig. 1.

The weight loss percentage was plotted as a function of time, as shown in Fig. 1A. For comparison, three samples from different batches of the industrial production were also considered (without sieving, Fig. 1C).

2.3.2. Rehydration kinetics

The samples obtained by previous dehydration for 24 h in a desiccator over silica gel (Fig. 1A) were kept in a box with 75% relative humidity (saturated NaCl aqueous solution). Each sample was weighed at a prefixed time. The percentage increase in weight of water was plotted as a function of time (Fig. 1B).

2.4. Thermal analysis

2.4.1. Differential scanning calorimetry

Thermal analysis using a differential scanning calorimetry (DSC) method was carried out employing an automatic thermal analyzer system (Mettler FP80 HT Central Processor and FP85 TA Cell). The data processing system (Mettler FP89 HT) was connected to the thermal analyzer. Sealed and holed aluminium pans were used for the experience for all the samples. Temperature calibrations were made using indium as standard. An empty pan, sealed in the same way as the sample, was used as reference. The thermograms were run at a scanning speed of 10°C/min, from 30 to 320°C.

Thermograms of the three different forms of the DHEP salt were recorded in order to have evidence both of their identity and of the extent of transformations occurring under the different conditions (Fig. 2).

Fig. 3 shows the changes of the thermogram of a dihydrate sample during the dehydration/rehydration cycle.

Fig. 1. Dehydration/rehydration cycle for dihydrate DHEP salt. (A) Dehydration in a desiccator over silica gel at room temperature. (B) Dehydration in a desiccator over silica gel at room temperature of DHEP samples of different industrial batches. (C) Rehydration of the DHEP sample (previously dehydrated, see (A)) kept at environmental humidity.

2.4.2. Hot stage microscopy

The DSC peaks associated with the melting of the different forms were identified by direct microscopy examination at the hot stage.

2.5. Diffractometric analysis (X-ray diffraction)

To perform X-ray diffratometric analysis, we used a Philips PW 3719 diffractometer controlled by a computer: radiation, Cu Kα; 40 kV and 30 mA; scanning interval, 3–35°; scanning speed, 1°C/min; graphite monochromator on the diffracted beam.

2.6. Solubility measurements

Two grams of each form of the DHEP salt was added to 50 ml water at room temperature under stirring. The anhydrous form C (from organic solvent) quickly dissolves, while the other two forms leave undissolved material. The three samples were kept at 25°C to achieve equilibrium. The sample containing the anhydrous form started to precipitate the excess solute very slowly after 1 week. The concentration of the dissolved salt was assayed spectrophotometrically.

2.7. Self-association

Solutions at increasing concentration of DHEP (10–46 mM) were added to a few crystals of Orange OT, an azo-dye largely used to detect micelle-like association. After 1 week at room temperature, the solubilized dye was detected spectrophotometrically at $\lambda = 490$ nm. Absorbance starts to increase above 35 mM.

3. Results and discussion

The salt DHEP exists in two different forms that can be obtained separately by carrying out the preparation of the salt in organic solvents (for the anhydrous form) or in water (for the dihydrate form). A third form can be prepared from the dihydrate form by dehydration in controlled conditions. The three forms can be described as follows: the dihydrate form contains two water molecules per formula (form A); the dehydrate form has lost the crystallization molecules, but retained the crystalline structure (form B); and the anhydrous form has a crystal structure different from that of the dehydrate form (form C).

$$\mathrm{DHEP{\cdot}2H_2O(s)\ (A) \Leftrightarrow DHEP^*(s)\ (B) + 2H_2O(g)}\ \text{dehydration/rehydration} \quad (1)$$

$$\mathrm{DHEP^*(s)\ (B) \Leftrightarrow DHEP(s)\ (C)}\ \text{phase transition} \quad (2)$$

Therefore, form A is the hydrate form of B; B and C are polymorphs of the same form lacking crystallization water (Byrn, 1982; Ford and Timmins, 1989).

Since water molecules in the dihydrate form A are loosely bound (Ledwige et al., 1996), form B can be easily obtained by removing the crystallization water molecules at low temperature: the process is reversible (see later).

Dehydration was performed maintaining the dihydrate samples in a desiccator over silica gel at room temperature (20°C) (Holgado et al., 1995) and recording the weight at preset times. Fig. 1A,C shows the profile of the weight loss as a function of time for two sets of samples. The first consisted of DHEP·2H$_2$O prepared in this laboratory and sieved (two different dimensional ranges for the powder to be analyzed were chosen: 100-200 and 200–300 μm). An almost regular dehydration profile can be observed for both samples; powder particles having a larger size range release crystallization water at a slower rate and need much time to lose two water molecules (corresponding to a decrease of about 8% of the original weight).

The second set consisted of samples from different batches of the industrial preparation; two of these samples behave as expected (taking into account that in this case there has been no previous sieving). The anomalously high loss of water observed for the third sample can suggest that it also contained, together with the crystallization water molecules, moisture or traces of the crystallization medium (Fini et al., 1998). In fact, the drying conditions during the filtration of the precipitate must be mild, in order not to start dehydration. Since this circumstance can frequently occur, the nature of the final product can be simply checked by a thermal analysis (Criado et al., 1978).

Kinetic data were examined in terms of α, the fraction of the dehydrated salt at time t. The following equation can be proposed to fit the experimental data:

$$g(\alpha) = \mathrm{d}\alpha / f(\alpha) = kt$$

where $g(\alpha)$ should be linearly related to time, when the appropriate form for $f(\alpha)$ was chosen as the operative equation. The equations for the most common mechanisms operating in the dehydration process in the solid state have been taken from the

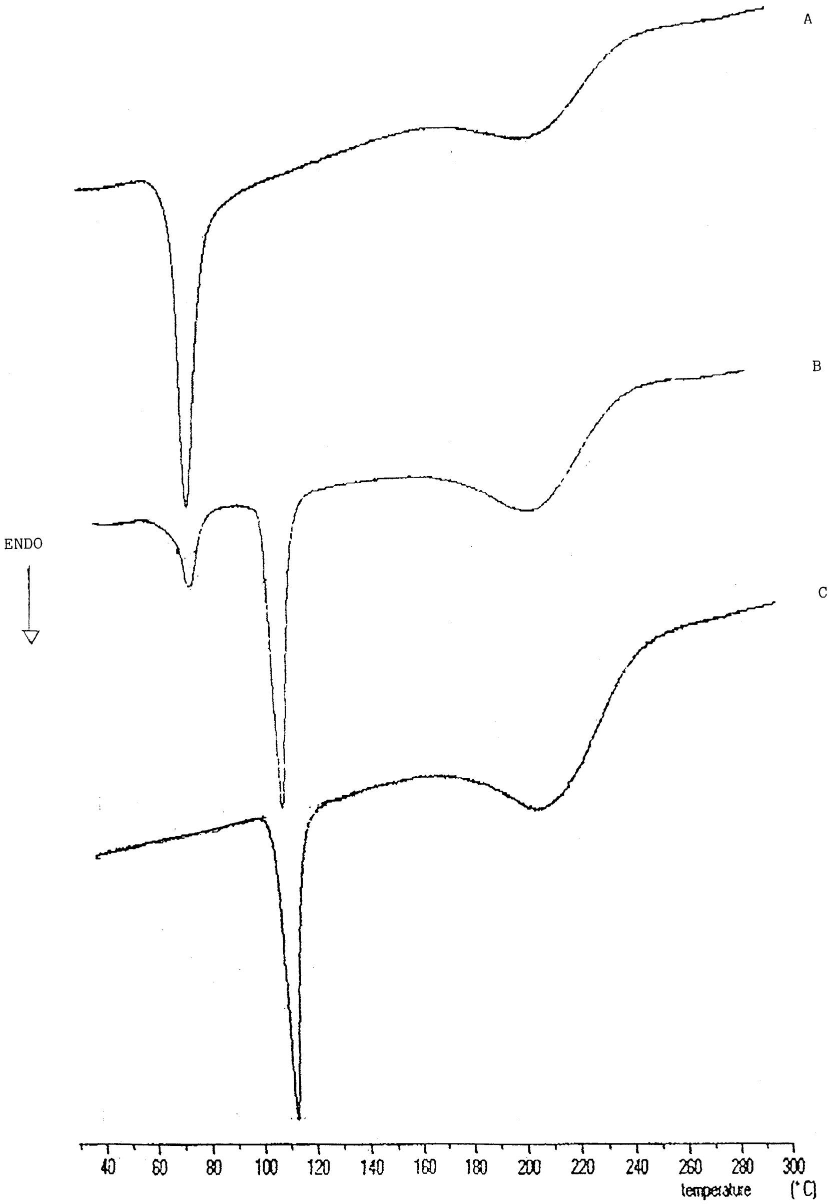

Fig. 2. Thermograms of the three forms of the DHEP salt. (A) Dihydrate (form A); (B) dehydrated over silica gel (form B); (C) anhydrous (from ethyl acetate) (form C).

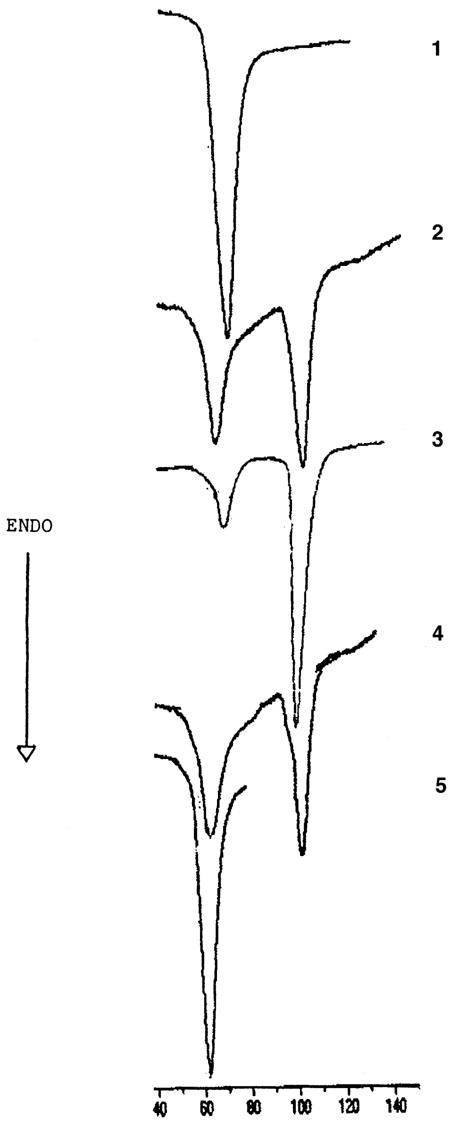

Fig. 3. Thermograms for different stages of dehydration (1, 2, 3) and rehydration (4, 5, 6). (1) Dihydrate (form A); (2) over silica gel, after 16 h, partial dehydration; (3) over silica gel after 24 h, complete dehydration (form B); (4) at environmental humidity, after 2 h, partial rehydration; (5) dihydrate, complete rehydration (form A).

literature (Bettinetti et al., 1994). The integrated form of the function $f(\alpha)$, which best fits the experimental data (on the basis of the correlation coefficient), was found to be:

$$f(\alpha) = \alpha^2$$

The result of the fit suggests a one-dimensional diffusion of the water molecules out of the solid particles, a mechanism which well agrees with the results reported for the dehydration of the salt in different experimental conditions (Ledwige and Corrigan, 1997). This can result from the proposed crystal structure of the hydrated form, which contains tunnels, filled by the water molecules (Ledwige et al., 1996), and which also represents easy escaping pathways.

The different profiles of thermograms of the three forms provide a useful tool to follow the changes occurring inside the sample during the dehydration process. Fig. 2 shows the thermograms for the three forms. After having tested each sample for the change of weight at preset times, a small aliquot was also analyzed by DSC. Forms A and C display a thermogram with one endotherm, at 66 and 104°C, respectively, that hot stage microscopy examination associated with the melting of the pure forms. The thermogram of form B has a small endotherm centred at 77°C, associated with the phase transition (B → C), and a second sharper endotherm at 104°C due to the melting of form C, thus formed. All the forms also show a large endotherm at about 200°C, possibly due to the decomposition of the salt.

Fig. 3 shows the thermograms for the dehydration and rehydration cycles. Up to 2 h, the thermogram of the sample under dehydration has the same shape of that of form A. After 16 h, an intermediate situation is present, which is a partial dehydration. In fact, the first endotherm at 67°C can be associated with the melting of the original portion, not yet dehydrated at the moment of analysis; possibly, it results also from the contribution of the thermal events associated with the phase transition of the B aliquot, formed after dehydration; this process in turn generates form C (see later). The second endotherm can be attributed to the melting of form C. After 24 h, the dehydration was complete: the thermogram of the sample at this moment is similar to that reported in Fig. 2 for the dehydrate form B.

The final product thus obtained can again absorb water, in moisture-rich environments (Fig. 1B). This fact was documented by the change of the thermogram of the material, dehydrated in the desiccator, after 2 h exposure to a 76% moisture atmosphere: the thermal profile for this partial rehydration recalls that of the partial dehydration. After 2 days of the exposure to this atmosphere, the material recovered the complete original hydration (Fig. 3), as suggested by thermograms similar to that in Fig. 2 for the dihydrate salt. No change was observed during a prolonged treatment of 48 h. These results document the reversibility of the dehydration and rehydration processes at low temperature.

This represents an interesting result. In fact, since water molecules are loosely bound, a severe drying process of the material crystallized from water could also produce a loss of crystallization water. On the contrary, too mild a process could cause retention of some aqueous medium (see, for example, the thermogram of the sample obtained from an industrial preparation; Fig. 1C): in this case, the weight loss exceeds the 8% value, related to the loss of only two crystallization water molecules. The result obtained with the rehydration process suggests that the drying process can be as strong as necessary, provided it is at low temperature; the exposure of the final material at an appropriate humidity provides the complete hydration of the dihydrate form, if necessary. Similarly, to prevent the transition from the anhydrous toward the dihydrate form, storage of the anhydrous form needs dry conditions. Fig. 4 shows the thermogram of a DHEP sample obtained from ethyl acetate and kept for 4 days in the presence of 76% relative humidity and suggests that, under suitable conditions, the anhydrous form C can be transformed into the dihydrate A (Ledwige and Corrigan, 1997); in other words, processes (1) and (2) are reversible.

Fig. 5 shows the diffractograms for the dihydrate (A) and anhydrous (C) forms of the DHEP salt. Forms A and C display different diffractograms (Fig. 5A and D, respectively) since they have different crystal structure. Differences could be clearly observed at $2\Theta = 20°$, where a peak present in A is absent in C. The diffractogram in Fig. 5B corresponds to the end of the dehydration process and the profile in Fig. 5C is related to the complete recovery of the crystallization water. During the dehydration/rehydration cycle (A →

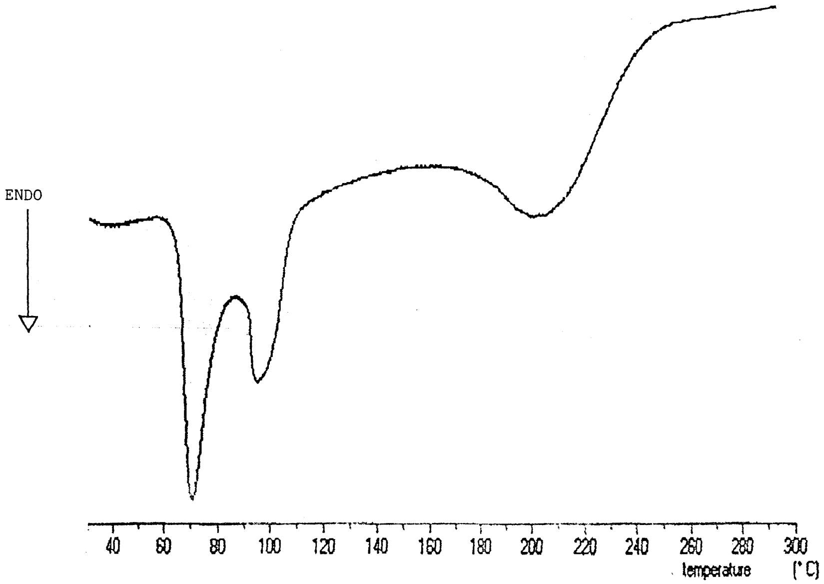

Fig. 4. Partial rehydration of anhydrous DHEP (from ethyl acetate) after 4 days in a box at 76% RU (saturated NaCl aqueous solution).

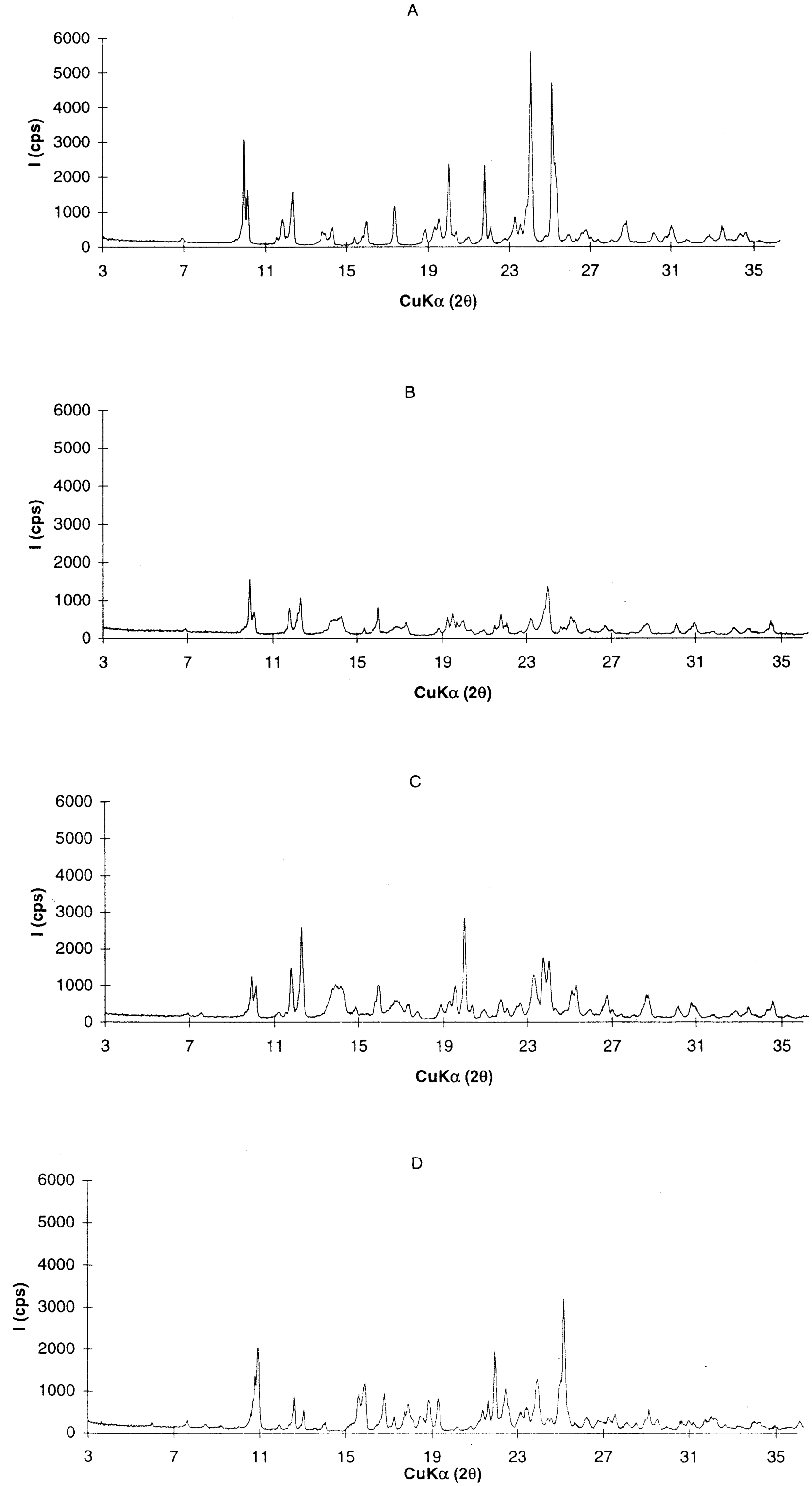

Fig. 5. Diffractograms of the different forms of DHEP salt: (A) dihydrate (form A); (B) dehydrated over silica gel for 24 h (form B); (C) rehydrated at high RU (form A); (D) anhydrous (from ethyl acetate) (form C).

B → A), slight modifications present in the diffractogram of Fig. 5B suggest that the phase transition (B → C) starts after (or during) dehydration. The dehydrated form B is not so stable as initially supposed and must be stored at low temperature in dry atmosphere, to slow down the phase transition.

At short times, such as those of the present experimental conditions, the starting crystal structure of A is maintained practically unchanged during the dehydration (form B): therefore, rehydration occurs rapidly compared with what was observed in the case of the form C, kept in a moist atmosphere (Fig. 4), where the hydration process must be accompanied by the phase transition.

An aqueous medium is even more effective in the hydration not only of form B, but also of form C.

Since the nature of the solid state of the dissolving particle affects solubility in water, differences to this respect should be expected among the three forms. However, after dissolution, the nature of the solute is the same whatever the nature of the starting A, B or C forms, these being different forms of the same chemical compound. Moreover, the only solid form which can exist in equilibrium with the solute in aqueous solution is the dihydrate (A). Therefore, in each case, after the equilibrium conditions were achieved, the only system present in the aqueous solution is:

$$\text{Dihydrate(s)} \Leftrightarrow \text{solute}$$

and the solubility value measured in these conditions is that of the A form (46 mM). This result explains why only the dihydrate form can be obtained crystallizing either the B or the C form from water, and that the dihydrate form precipitates from even partially aqueous organic solvents (e.g. 95% ethanol).

Differences among the forms with respect to their behaviour in aqueous solutions can be better evidenced before the achievement of the equilibrium: in this case, the differences in the crystal structure, and the relative rates of some processes occurring in the solution play important roles.

The solubility of form C, estimated by indirect methods (intrinsic dissolution rate), is fivefold higher than that of the A form (Ledwige et al., 1996). This can explain the difficulty in obtaining a heterogeneous system starting from the anhydrous form to determine its solubility, under equilibrium conditions (Fini et al., 1991). In fact, the process needs the addition of an amount of solid material exceeding the estimated value of solubility (> 230 mM). When aqueous solutions were prepared with the anhydrous form, they were limpid and homogeneous, but above 100 mM they became increasingly dense and viscous. In these conditions, nucleation phenomena of the seeds for mass crystallization are slowed down, as well as the diffusion to grow the possible microscopic crystals at saturation: the formation of whatever solid (hydrate or anhydrous) is delayed.

In concentrated aqueous solutions of DHEP, above 35 mM, the formation of self-association structures $[D]_n^{n-}$ of $n\text{D}^-$ diclofenac anions was suggested (Fini et al., 1991) by the behaviour of the solution towards the solubilization of hydrophobic substances (azo-dye, lecithins); an active role of the organic cations in this process cannot be excluded.

The aggregates somehow recall those of association colloids:

$$n\text{D}^- \Leftrightarrow [\text{D}]_n^{n-}$$

The driving force for the aggregation of this type is attributed to a hydrophobic effect that brings the hydrophobic portions of the diclofenac anions far from the contact with water. It is possible that the stacking of diclofenac anions in solution resembles the organization of the anhydrous form crystals (C form). In fact, the examination of the crystal structure of this form (Ledwige et al., 1996) reveals the presence of a completely hydrophobic zone inside the crystal cell, where the phenyl moieties of the anions and the aliphatic cycle of the cations are contained, while the charged groups face outside this site. On dissolving, this type of packing could be maintained, even due to the difficulty of the water molecule reaching the inner portion of this structure: in other words, aggregates could be formed directly during dissolution in the case of the anhydrous form. A similar situation was reported in the case of choleic acids formed by sodium deoxycholate: because of the similar organization both of the solid state and of micellar aggregates of the bile salt in solution, no discontinuity of assembly could be observed during dissolution (Giglio, 1981).

The structure of the dihydrate and dehydrate forms of the salt DHEP (forms A and B) are different, where single formulae of the salt are separated from each other by the crystallization water molecules and therefore must enter the solution as monomers. In this case, aggregation should occur as a subsequent step above 35 mM.

The association phenomena can also explain the delay of the precipitation. Since the dihydrate form finds its solubility equilibrium at 46 mM, this concentration value does not also allow, in the saturated solution, either a high number of aggregates or a high aggregation number (n):

$$\text{DHEP (A or B form)(s)} \Leftrightarrow \text{D}^- + \text{HEP}^+\text{(soln.)}$$

$$n\text{D}^- \Leftrightarrow [\text{D}]_n^{n-}$$

In these conditions, the achievement of the solubility equilibrium is easy and relatively rapid and excess crystals remain undissolved (equilibrium shifted to the left).

The stability of highly concentrated aqueous solutions of anhydrous DHEP and their resistance to form a precipitate can be explained also from another point of view. In fact, 35 mM, the concentration above which self-aggregates start to be formed, represents the highest concentration of monomers: this value remains practically constant in the presence of aggregates (Florence and Attwood, 1987). As a consequence, when the solute is present mainly as aggregates, this value is lower than that necessary to achieve the saturation of the solution with respect to the dihydrate form (46 mM).

Finally, due to the presence of the solute in solutions in the form of self-aggregates, terms such as solubility in water of anhydrous DHEP or supersaturation of its aqueous solutions, as previously reported (Fini et al., 1991), need to be interpreted at the light of the complex equilibria involving the different solid forms and the state of the solute at increasing concentration.

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