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Physico-chemical characterisation and intrinsic dissolution studies of a new hydrate form of diclofenac sodium: comparison with anhydrous form

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Abstract

Diclofenac sodium is a non-steroidal anti-inflammatory drug widely used in painful and inflammatory diseases. In standard conditions, by exposure to relative humidity even below 60% at 25 °C, the anhydrous form DS gives rise to a hydrate species DSH, a tetrahydrate form different from that obtained by crystallisation from water and previously described. The method of preparation and the physico-chemical properties of the hydrate form were investigated. Data from FTIR spectroscopy, X-ray powder diffraction and thermal analysis were used for the identification and the characterisation of DSH. DS and DSH were easily differentiated by their IR spectra, X-ray patterns and thermal behaviour. DSH stability was followed at room temperature over a period of 1 year and under different conditions of temperature to verify the tendency to solid–solid transition and to study its existence range. Solubility and intrinsic dissolution studies were performed to compare the physico-chemical properties of DS and DSH. Differences in solubility and intrinsic dissolution rates were pointed out: these studies showed that DS dissolved faster than DSH. Storage under uncontrolled environmental conditions or contact with water vapour during manufacturing process could thus influence the performance of the final dosage form.

Keywords: Diclofenac sodium; Solid-state; Hydrate form; Infrared spectroscopy (FTIR); Physical stability; Thermal analysis; Intrinsic dissolution

1. Introduction

Pharmaceutical solids can be in contact with water during processing steps such as crystallisation, lyophlisation, wet granulation, aqueous film coating or spray drying and can be exposed to water during storage in an atmosphere containing water vapour [1,2].

Water can be absorbed onto the solid surface and/or into the bulk solid structure. The formation of a hydrate form can strongly affect physico-chemical properties such as stability, solubility, dissolution rate and hence bioavailability. Concerning the solubility in water, the anhydrous form of a substance is generally more soluble in water than the corresponding hydrate [3].

Assessment of the solid state characteristics of drugs is a regulatory prerequisite for the correct formulation of solid dosage forms: controlling polymorphism in pharmaceutical solids must include previsions for hydrate formation [4,5].

Diclofenac sodium, sodium salt of [2-(2,6-dichlorophenyl amino) phenyl] acetic acid, is a well-known drug available in various pharmaceutical dosage forms. It is a potent non-steroidal anti-inflammatory drug with pronounced analgesic and antipyretic properties. It is widely used in the long-term treatment of degenerative joint diseases. Nevertheless, it produces a relatively high incidence of gastrointestinal side effects due to the physico-chemical action on the gastric mucous and the inflammatory action on both small bowel and the colon [6]. It has weak acidic properties (pk_a about 4) and its solubility depends on the pH of the medium. It is slightly soluble in water, very slightly soluble in phosphate buffer at pH 6.8 and practically insoluble in hydrochloric acid at pH 1.1 [7].

Extensive literature on chemical and spectroscopic characteristics of the anhydrous form DS is available and its crystal structure has been also described [7,8]. Thermal behaviour, decomposition and melting characteristics of DS have been previously investigated by differential thermal analysis (DTA), differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) [9,10].

Solid-state properties of diclofenac sodium, with particular reference to its pharmaceutical and technological characteristics

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both as active substance and in the finished product, have been subject to some investigations. A tetrahydrate form of diclofenac sodium, re-crystallised from water or obtained by suspending it in boiling water has been previously described [11,13]. A tetrahydrate form obtained by crystallisation and a pentahydrate form precipitated from a matrix of chitosans were characterised by single crystal X-ray diffractometry [14,15].

As a part of a recent study on the dissolution profiles of diclofenac sodium multisource prolonged release tablets, it was found that the release characteristics varied considerably among different manufacturers and even identical formulations showed rather dissimilar release profiles in all the studied media. This suggested likely implications for the bioavailability of the active ingredient [16].

Furthermore, our interest in diclofenac sodium arose from the finding that samples of industrial scale lots of this drug showed batch to batch variations and poor consistency in their thermal behaviour and IR spectra, suggesting that different crystalline forms coexisted in the commercial samples.

The purpose of the present work was to investigate the capability of the anhydrous form DS to uptake water from the environment giving rise to hydrate formation and to evaluate how the different physico-chemical properties can affect the shelf life, the process behaviour and in the end, bioavailability.

The present work summarises the available data on solid state properties of diclofenac sodium with particular regard to the new tetrahydrate form DSH obtained by exposure to water vapour even below 60% RH at 25 °C, including its preparation and its characterisation by comparison with both DS and the tetrahydrate form reported in literature. Solubility and intrinsic dissolution studies were performed to compare the physico-chemical properties of DS and the new form DSH.

2. Materials

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

Analytical grade potassium dihydrogen phosphate, sodium chloride, sodium hydroxide, hydrochloric acid and methanol were purchased from Sigma–Aldrich.

Deionised water obtained from an Ultra Pure Water System Type Integra (SG, Barsbüttel, Germany) was used for the preparation of dissolution media.

3. Methods

3.1. Preparation of the hydrate form DSH

Tetrahydrate form DSH (20% water content) was obtained by storing DS in a water saturated chamber without a drainage system (100% RH) at room temperature for 24 h.

Stability studies results showed that DSH was also obtained by storing DS in chamber at 59% RH for 60 days and at 98% RH for 4 days.

Diclofenac sodium was also re-crystallised from water as previously described [12,13]. In each experiment about 100 mg were dissolved in 10 ml of water. Re-crystallisation was firstly obtained by evaporation of solvent at room temperature, then it was forced either by evaporating the solvent on a water-bath or by crystal precipitation on an ice-bath. As assessed by X-ray powder diffraction (XRPD), re-crystallisation performed by evaporation of water at room temperature gave rise to the tetrahydrate form DSH1 previously described by other authors [11–13].

The resulting samples were stored in open air and tested by DSC, FTIR and XRPD to assess the crystalline form and TGA for water loss.

The water stoichiometries of diclofenac sodium hydrates were deduced from TGA experiments.

HPLC analyses performed by the Ph. Eur. assay procedure on the hydrate forms obtained either by forced hydration or by crystallisation indicated that no degradation had taken place.

3.2. DSH characterisation by comparison with DS and DSH1

FTIR spectra of DS, DSH and DSH1 were obtained directly on untreated powder by means of an ATR sampling system (Golden Gate-Specac, England) coupled with a Perkin Elmer FTIR System 2000 spectrometer (Perkin Elmer, USA). Spectra were recorded at room temperature from 4000 to 370 cm^{-1} on a Perkin Elmer System 2000 spectrometer. For each sample 16 scans were collected at a resolution of 4 cm^{-1}.

XRPD patterns were obtained by a P.W. 1710 diffractometer (Philips, The Netherlands) 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}.

Samples were mildly pre-ground with a pestle in a agate mortar to make them homogeneous, to control crystals size and to minimise preferred orientation effects.

DSC curves were recorded using a Perkin Elmer DSC 7 instrument. Sample weight ranged from 1.5 to 5 mg. The DSC profiles were recorded at 10 °C min^{-1}, under nitrogen flux, from 25 °C to about 150 °C. The experiments were conducted using closed pans with a hole made by the Perkin Elmer's Accupik system.

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

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

Each experiment was repeated at least three times.

Thermogravimetric curves were recorded with a Perkin Elmer Pyris1 TGA at the heating rate of 10 °C min^{-1}. Approximately 10 mg of substance were weighed. The experiments were conducted using closed pans with a cover hole made by the Perkin Elmer's Accupik system.

A temperature calibration of the thermogravimetric apparatus was performed measuring the magnetic transition temperature of two standards, alumel and nickel (supplied by Perkin Elmer).

Each TGA experiment was repeated at least three times.

3.3. Stability studies: water uptake at various relative humidity percentages

Samples of DS were stored at controlled temperature and humidity conditions to check its ability to uptake water from the environment.

Samples of DS were stored in a desiccator at 25 °C at both 59 and 98% RH for 60 and 4 days respectively, using saturated sulphuric acid solutions with known relative humidity [17]. RH was checked by a digital thermohygrometer.

The water content of the equilibrated samples was determined by TGA and the solid phase was characterised by XRPD, FTIR and DSC.

Samples of DSH were stored at different temperature conditions (30 °C for 60 min, 50 °C for 10 min and 60 °C for 10 min) to test the stability of the DSH form. DSH was also stored at room temperature for 1 year.

DSC, TG, FTIR, XRPD analyses were carried out on each sample.

3.4. Physico-mechanical property studies: compression effect

Discs of the two crystalline forms were prepared by accurately weighing 200 mg (as anhydrous base) of the samples which were placed in an evacuable stainless-steel die and pressed in a Perkin Elmer electric press to obtain a 13 mm diameter sample disc. Three levels of compression were applied: 3 t for 30, 45 and 60 min, 5 t for 30, 45 and 60 min and 6 t for 30, 45 and 60 min.

Samples were taken from the core of each disc and screened for solid-state transition using DSC, TGA and FTIR.

Compressed discs obtained by a compression force of 5 t for 1 min were used for intrinsic dissolution studies.

3.5. Equilibrium solubility studies

The solubility of both DS and DSH was investigated in 2-propanol, allowing to use a small amount of substance.

Saturated solutions were prepared by introducing excess amounts of DS and DSH (100 mg) into 5 ml of 2-propanol in screw cap vials. The samples were placed on a thermostatic water-bath maintained at 20 ± 0.5 °C for 60 min and subjected to magnetic stirring. Aliquots of the solutions were withdrawn with a syringe, filtered through a 0.45 μm membrane filter (Gelman GHP Acrodisc) and appropriately diluted with methanol (1:250; 40 μl in 10 ml of solvent). The concentration of the drug was spectrophotometrically determined at 282 nm (λ_{MAX} of absorption of diclofenac sodium in methanol) by a Hewlett-Packard model 8452A diode array spectrophotometer (Agilent Technologies Italia Spa., Roma, Italy). The state of true equilibrium was obtained when the concentrations of the samples reached constant values.

Saturated solutions of each form were prepared in an appropriate volume of 2-propanol so that a sediment was left at 20 °C after 24 h without stirring. The samples were centrifuged and filtered through a 0.45 μm membrane filter, opportunely diluted with methanol and then quantitatively determined by UV absorption at 282 nm. The solids remaining after the solubility studies were analysed by DSC and FTIR. All reported data represent the mean values of at least three separate experiments that always showed a good reproducibility.

3.6. Intrinsic dissolution studies on DS and DSH

Selection of the dissolution testing conditions was based on EMEA guidelines [18,19].

For all dissolution tests the USP 28 paddle method, employing 900 ml of dissolution medium at a temperature of 37 ± 0.5 °C and rotational speeds of 50 and 100 rpm were used.

Each dissolution experiment was performed at least in triplicate.

The dissolution system was fitted with a DISTEK PREMIERE 5100 dissolutor (Distek Inc., NJ, USA), an HP 89092A 7-channel peristaltic pump (Agilent Technologies Italia Spa., Roma, Italy), PC directed control through the Idis EE software (Icalis Data System Ltd., UK). Released percentages of the active ingredient were automatically measured every 5 min at 276 nm using an HP 8452A diode array detector (Agilent Technologies Italia Spa.) equipped with a linear 7-cell transporter. The flow cell path length was 1 mm. Filtration of aqueous samples was performed on-line on Whatman GF/C (1.2 μm) filters (Whatman, Kent, England). Check for adsorption to the filters revealed no significant loss of drug.

3.6.1. Composition of dissolution media

The composition of dissolution media was chosen in such a way to cover the physiological pH range.

Medium A. Simulated intestinal fluid (SIF) without pancreatin (pH 6.8) according to USP 28 [20].
Medium B. Phosphate buffer solution (pH 8.0; 0.02 M) (Ph. Eur.) [21].
Water. Deionised water.
Medium C. Phosphate buffer solution, pH 4.5 (Ph. Eur.) [21].
Medium D. Simulated gastric fluid (SGF) without pepsin (pH 1.2) according to USP 28 [20].

3.6.2. Calibration curves

Calibration curves for diclofenac sodium reference substance were obtained by measuring the absorption in dissolution Media A and B at the maximum absorption wavelength. Due to the low solubility of diclofenac sodium in Media C and D, data from the calibration curve obtained in Medium A were used. Standards were prepared in the 0.006–0.264 mg ml^{-1} concentration range. The linearity of the calibration curves was confirmed over the concentration range between 2.5 and 120% dissolution of the drug.

3.6.3. Intrinsic dissolution rate determination

Intrinsic dissolution rate (IDR) studies were performed by the stationary disc method using the USP 28 paddle apparatus.

Discs were prepared compressing 200 mg of powder (as anhydrous base) in a Perkin Elmer hydraulic press, for 1 min under 5 t compression, using a 13 mm punch and die set. Analysis of the compressed discs by FTIR confirmed that the crystal form of the original powder was retained following the compression procedure. Attempts were made to use directly the die with the disc flat surface exposed to the medium but alteration of pellet surface was observed in a few minutes. Therefore paraffin wax was used to mount the discs in plexiglas disc holders, leaving one face exposed (1.327 cm^2 surface area). All dissolution runs were carried out in triplicate, under sink conditions. The linear portion of each dissolution profile, i.e. before depletion of the disc and alteration of its surface area, was used to derive the intrinsic dissolution rate.

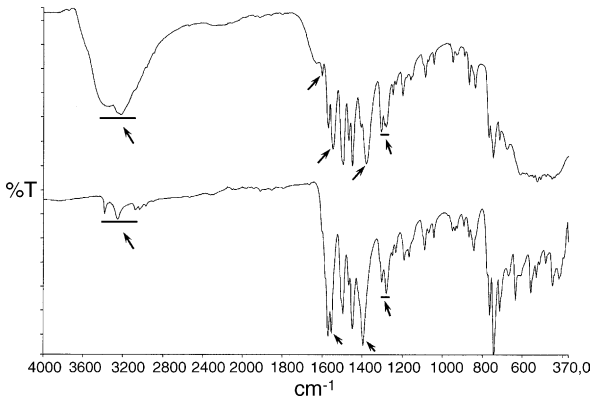


Fig. 1. FTIR spectra in the 4000–370 cm^{-1} range of anhydrous form DS and hydrate form DSH; untreated powder by means of an ATR sampling system.

4. Results and discussion

4.1. Preparation of DSH

Efforts were made to study the tendency of DS to uptake water, reproducing the hydration by water vapour exposure under not controlled conditions. A different hydrate form DSH, never described in literature, was obtained by storing the anhydrous form in a desiccator at a RH below 60%. Storage of the anydrous form at RH of 59%, 98% at room temperature and 100% without drainage system at different temperatures (20 and 30 °C) gave rise always to the same hydrate form DSH.

The DSH formation in a saturated chamber at 100% RH without drainage system at room temperature was routinely used as preparation method because it was an easier and time-saving method.

The repeatability of form DSH formation was verified by FTIR, XRPD, TGA and DSC on each batch.

Crystallisation experiments suggested that the solvent-mediated hydration could give rise to a hydrate form different from that obtained by water vapour exposure. In fact, crystallisation experiments clearly indicated that the simple crystallisation from water at room temperature gave rise to the different tetrahydrate form DSH1 previously described in literature [11–13]. The temperature at which the solvent was removed seemed to affect the form of the resulting solid. In fact, crystallisation from water at temperature over 25 °C often gave rise to DS.

4.2. DSH physico-chemical characterisation

FTIR spectroscopy was a useful tool to distinguish DS from DSH. The two forms exhibited significant differences in the observed vibrational transitions.

The crystalline structures seemed to be neither altered nor destroyed by pelletting. For each form the FTIR spectra directly obtained on the powder by ATR sampling system and on a dispersion in KBr pellet were not markedly different from each other with respect to the position, sharpness and intensity ratio of the bands. The two forms were easily differentiated by their IR absorption bands in the 4000–2000 cm^{-1} range, but were distinguishable over the whole 4000–370 cm^{-1} range of frequencies. In the 4000–2000 cm^{-1} range two bands at about 3387 (ν_{NH}) and 3255 cm^{-1} (ν_{NH-O}) were seen in the spectrum of DS, while a broad band with a higher intensity at about 3220 cm^{-1} and a shoulder at about 3365 cm^{-1} were observed for DSH. These spectra distinctly differed from each other in the location and intensity as well as in fine structure of some major absorption bands. The spectrum of DS and the frequencies recognised as diagnostic bands were in agreement with those previously reported by Bucci et al. [8]. The fingerprint region of the spectrum differentiated each form and could be used for the characterisation and identification of the different crystalline modifications.

Fig. 1 shows DS and DSH spectra in the 4000–370 cm^{-1} range. The specific bands for each form, also suitable for identification in mixture, are marked in the figure with arrows.

FTIR was not a suitable method to distinguish DSH from DSH1 because they have identical vibrational frequencies. Fingerprint regions of the two hydrate forms showed just a few subtle differences in the 1600–1300 cm^{-1} region with respect to the relative intensity of the bands.

The XRPD patterns of DS and DSH were sufficiently distinct to characterise each crystalline form: they showed differences both in the positions of the peaks and in the intensity ratios that could not be attributed to a preferred orientation of crystal growth. Differences in X-ray diffraction patterns indicated different arrangements of diclofenac sodium molecules in the crystal lattice of the hydrate forms. The X-ray powder diffraction pattern for DS was consistent with that previously reported [11]. DSH exhibited numerous different peak positions distinguishing it from both DS (Fig. 2) and DSH1 (Fig. 3).

The thermal behaviour of DS has already been extensively reported [9,10].

To obtain a better understanding of the thermal events for the hydrate forms and anhydrous form, a series of increasing heating rates and different pan types were used. The DSC profiles were recorded at 5 and 10 °C min^{-1}. The DSC experiments were run using pans that were open or closed with a cover hole. As it was impossible to obtain a better resolution of thermal events from these experimental conditions, pans with cover hole and a

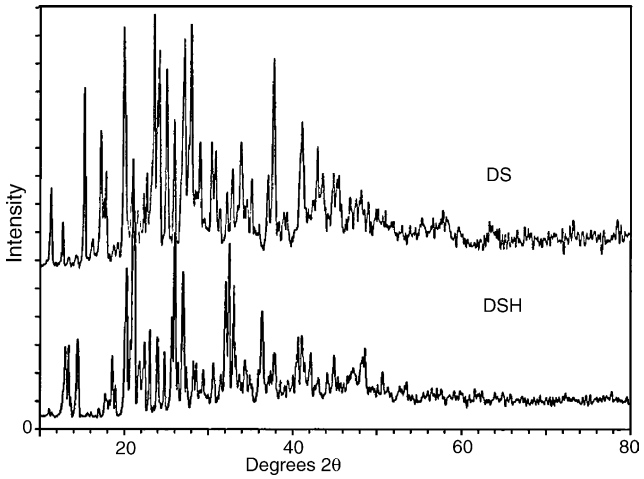


Fig. 2. XRDP patterns of anhydrous form DS and hydrate form DSH of diclofenac sodium.

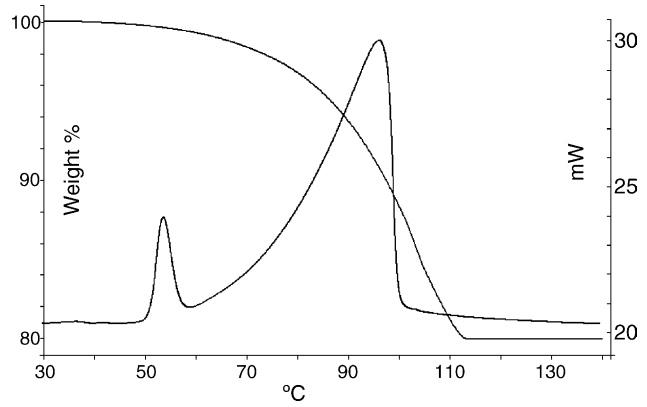


Fig. 4. DSC and TG curves of hydrate form DSH of diclofenac sodium; open pan with a hole by Perkin Elmer Accupik System; 10 °C min^{-1}, scan rate; heat flow, endothermic scale.

heating rate of 10 °C min^{-1} were routinely used for a meaningful comparison with TGA.

DSC profiles of DS recorded at a heating rate of 10 °C min^{-1} by Perkin Elmer DSC 7 showed a broad fusion with decomposition between 170 and 180 °C.

DSH showed a more complex profile with two endotherms in the 40–110 °C range before the fusion with decomposition that is practically superimposed to the one of DS. The first small and sharp endotherm showed an onset temperature of 49.8 ± 0.3 °C (peak temperature: 51.8 ± 0.2 °C) and an enthalpy of fusion of 18.6 ± 1.2 J g^{-1}; the second bigger and broad endotherm showed an onset temperature of 71.3 ± 0.8 °C (peak temperature: 95.6 ± 2.3 °C) and an enthalpy of fusion of 385.9 ± 19.3 J g^{-1}(Fig. 4).

The first endotherm was due to a first water loss (less bound water), the second endotherm represented a composite heat effect due to the fusion of the solvate crystals and the evaporation of the main portion of bound water. This behaviour corresponding to a water loss of about 20.0% (±0.25) from 35 to 110 °C in the TGA curve is in agreement with that of a tetrahydrate. The water loss started slowly from 35 °C and showed a maximum between 90 and 100 °C.

The two tetrahydrate forms DSH and DSH1 resulted to have similar water content: in fact DSH1 showed a water loss of about 18.3% in the same temperature range, according to that previously reported [13].

DSH and DSH1 showed also very similar DSC profiles and onset temperatures quite close to each other. Heating–cooling cyclic DSC was a useful tool in distinguishing the two hydrate forms, showing that different solid–solid transitions took place for the two hydrates at a temperature below 50 °C, hidden by the first water loss endotherms (data not shown).

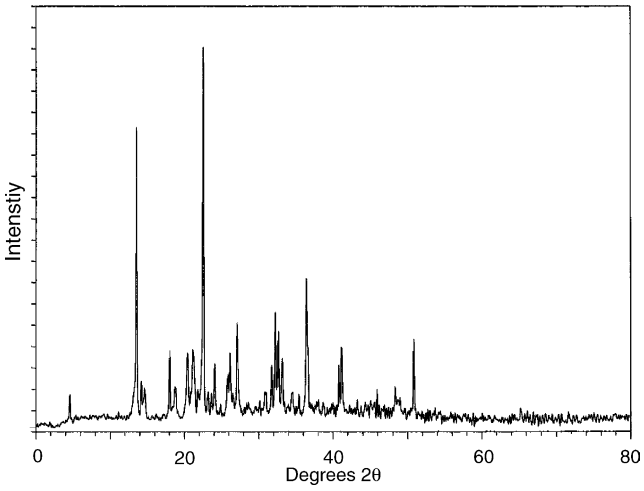


Fig. 3. XRPD pattern of hydrate form DSH1 of diclofenac sodium.

4.3. Physico-mechanical property and stability studies

Although systematic studies on grinding effect were not performed, both forms seemed unaffected by mild grinding and pelletting.

The influence of compression on solid-state properties was investigated: compression force of 6 t for 60 min did not alter the crystalline structure. In fact no differences in the IR spectra, TGA and DSC profiles could be detected after compression.

The compression force employed in the tabletting process greatly influences the apparent density, porosity, hardness, disintegration time and average primary particle size of compressed tablets. Moreover different water content of powder before compression can influence the tablet strength indirectly by affecting the volume reduction of the mass during compression.

DS and DSH showed different compression properties. In fact the apparent densities measured were respectively 36.96 mg ml^{-1} for DS and 60.06 mg ml^{-1} for DSH [12].

Stability studies were also performed on DS and DSH.

DS showed a tendency to uptake water from the environment and to give rise to a solid–solid transition to a hydrate form with characteristic physico-chemical properties. Hydrate form DSH was stable at room temperature for at least 1 year. Upon heating for 10 min at 50 °C DSH was completely transformed into DS as checked by FTIR, DSC and TGA analysis.

4.4. Technological properties and equilibrium solubility studies

Particle size distribution was initially not investigated. However, in order to obtain good infrared spectra and X-ray powder diffraction patterns, the two forms were pre-ground with a pestle in an agate mortar and large crystals comminuted. In order to complement solubility studies results, microscopic observation was performed by scanning electron microscopy (SEM Philips XL30): crystals of DSH and DS forms were not very similar in their morphology, while the main portion of the crystals showed the same particle size.

DS and DSH powders showed very different technological properties as wettability, cohesivity and flowability.

The DSH powder showed better flowability properties than DS one mainly due to the lower cohesivity of the hydrate particles. These characteristics were confirmed by the easier tablets ejection from the die after compression.

DS and DSH powder cohesion and aggregation produced the formation of a hard cake in water suggesting they had low wettability property. Caking is the state in which the powder cannot be moved by vigorously shaking or tapping the container [22]. DS tablets showed higher wettability than DSH ones according to their different friability suggesting a direct proportionality between these two parameters. Friability was investigated by the "Friability of uncoated tablets test" according to the European Pharmacopoeia, 5th ed. on a TAR Tablet Friability Tester (Erweka Italia, Milano, Italy).

Many efforts were made to study equilibrium solubility of DS and DSH. Many solvents were screened to obtain a reasonable equilibrium solubility value: water, methanol, ethanol, 2-propanol, acetone, chloroform, dioxane, DMSO, ethyl acetate, cyclohexane, 1-butanol and 1-hexanol.

We chose 2-propanol because it was the most convenient solvent to discriminate between the two forms from a chemical point of view, as a further way of characterisation of the new form.

The results of equilibrium solubility studies are reported in Table 1. It can be seen that DS attained a higher concentration than DSH, being the amount dissolved from DS about 37% higher than that from DSH. The solubility ratio at 20 °C between DS and DSH subjected to magnetic stirring for 1 h in 2-propanol was found to be 1.37.

FTIR and DSC studies were performed on the solid remaining after solubility studies had been completed: in all cases the solid phase isolated at the end of each experiment was constituted of DS.

An attempt to study solubility by storing saturated solutions of the two forms without stirring for 24 h was performed. The results obtained were not significant probably due to degradation phenomena occurring in the solvent during the 24 h solubility test.

Table 1
Equilibrium solubilities of DS and DSH measured at $T = 20\,^{\circ}C$

Form	Equilibrium solubility after 1 h in mg ml^{-1}
DS	2.61 ± 0.04
DSH	1.90 ± 0.02

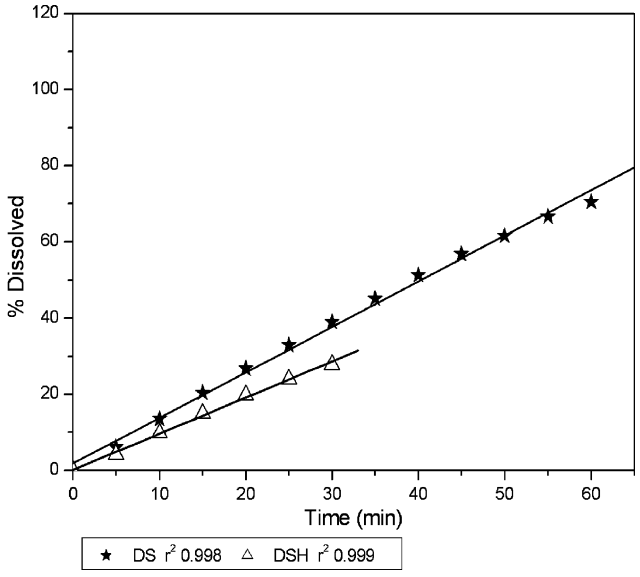


Fig. 5. Linear portion of mean dissolution profiles of anhydrous DS and hydrate DSH in SIF pH 6.8.

4.5. Intrinsic dissolution studies

Intrinsic dissolution studies were performed to further characterise DS and DSH. Figs. 5–7 show the mean release profiles for the two forms in the selected dissolution media.

Influence of the rotational speed on the dissolution behaviour was studied. Dissolution profiles in Medium A obtained at 50 rpm were compared with those obtained at 100 rpm. Great differences in the release were observed, the lower rotational speed providing better discrimination between the different forms. In fact the difference in the extent obtained at 100 rpm was only

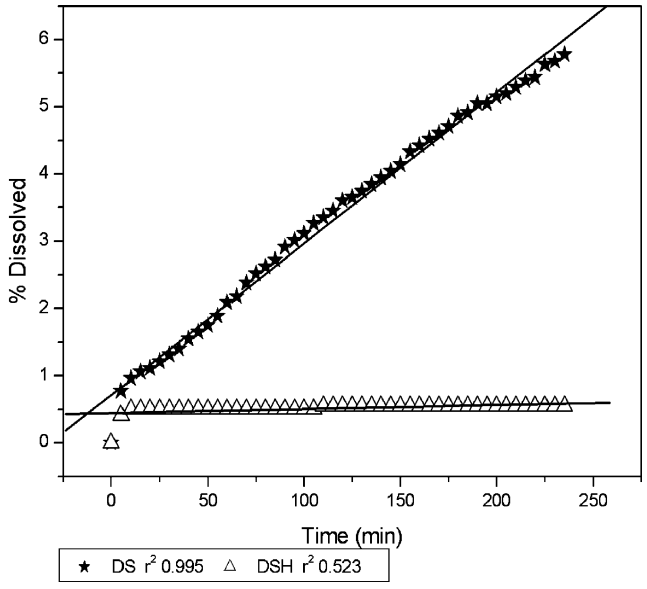


Fig. 6. Linear portion of mean dissolution profiles of anhydrous DS and hydrate DSH in phosphate buffer solution pH 4.5.

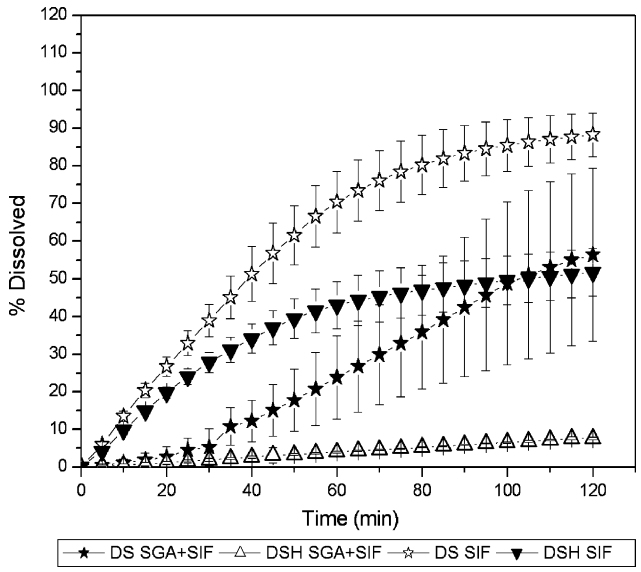


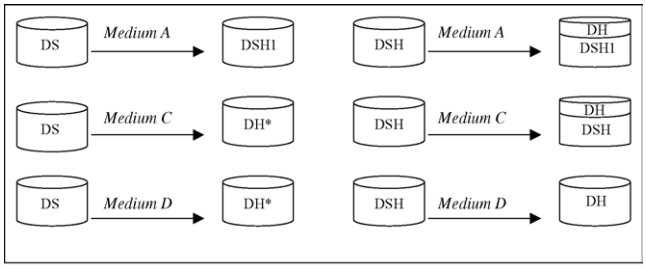
Fig. 7. Mean dissolution profiles of anhydrous DS and hydrate DSH in Medium A and in Medium A after a 2 h period in Medium D.

5% versus 35% obtained at 50 rpm. Moreover lower variability of the data was obtained at the lower rotational speed. All experiments were therefore run at 50 rpm.

In vitro release profiles of DS and DSH in Medium A showed remarkable differences in slope (Fig. 5) and extent (Fig. 7).

In fact anhydrous pellets released 90% after 2 h whereas hydrate discs released only 50% in the same time despite they reached the plateau.

FTIR analysis of the residual powder after dissolution showed a spectrum consistent with the DSH1 form from anhydrous pellet, while a two-layer pellet arose from the hydrate one, the upper layer being the non-ionised diclofenac acidum form (DH) with a DSH1 layer underneath (Scheme 1). The insoluble DH layer prevented full release from pellets, thus explaining the lower release extent. Two competitive phenomena seem to exist in solution: superficial layer neutralisation and saline layer dissolution. The former seems to be prevalent at 50 rpm, the latter one at 100 rpm. In fact increasing paddles rotational speed decreased the differences between the two forms, probably due to the mechanical removal of the DH superficial coat. In fact, during dissolution test, some insoluble particles rotating around the paddles were noticed. Analyses on these floating particles were performed by FTIR and the DH form was identified. The DH layer removal



* swollen disc

Scheme 1. Residual composition of tablets after dissolution tests, analysed by means of FTIR and DSC.

Table 2
Intrinsic dissolution rate at 37 °C, 50 rpm for the compressed anhydrous DS and hydrate DSH

Medium	Form	IDR (mg/min/cm^2)	S.D.	R.S.D. (%)
A	DS	1.98	±0.09	4.39
	DSH	1.43	±0.08	5.75
C	DS	0.04	±0.03	64
	DSH	0.01	–	–

caused the exposure of the underneath saline layer to the medium thus letting dissolution overcome neutralisation.

The calculated IDRs of DS and DSH in Medium A was significantly ($p < 0.05$) different and their values obtained at 50 rpm and 37 °C are shown in Table 2.

Dissolution curves in Medium B reached the plateau very fast (data not shown) because of the higher solubility of diclofenac sodium at pH 8.0. The releases observed for DS and DSH were very similar each other but not completely coincident. The curves seemed to converge but the test was stopped before 100% release was reached to save enough residues for FTIR analysis.

FTIR spectra of residues were not consistent with any of the studied forms.

Sigmoidal shapes instead of linear profiles were observed, therefore IDRs could not be calculated. This phenomenon will be subjected to further investigation.

Both forms showed fast dissolution rate in water, without resulting in noteworthy differences during the entire dissolution test, either in the extent or in the profile (data not shown). The curve superimposition seemed to depend more on the active ingredient higher solubility in water than in Medium A [23], than on an interface conversion. In this latter case a lag phase was expected instead of the similar release obtained. Moreover the complete coincidence of the profiles final portion, predicted in the above-mentioned case, was not attained.

In the central portion of the curve small variations could be observed: they could be due to some differences in technological properties as wettability and flowability.

The calculated IDR in water was 1.91 demonstrating that diclofenac sodium undergoes hydrolysis increasing the medium pH value, thus enhancing its solubility and increasing its dissolution rate. Consequently, water cannot be considered the ideal medium to give discrimination between the two forms.

The amount dissolved in Medium C was very small during the whole test period according to the very low solubility of diclofenac sodium in acidic media (Fig. 6).

The calculated IDRs of DS and DSH in Medium C was significantly ($p < 0.05$) different and their values obtained at 50 rpm and 37 °C are shown in Table 2.

Hydrate discs showed releases lower than 1% while the anhydrous discs reached about 6%.

The DS discs swelled looking like sponges, increasing their volume whereas DSH pellets maintained their smoothing and thickness.

After the test completion the superficial layer was removed from the residual discs to be analysed by FTIR and was found to be constituted of DH. In the anhydrous discs the underneath

layer too was constituted of DH, whereas DSH pellets remained generally unchanged (Scheme 1).

The competitive phenomena previously mentioned for Medium A, can be envisaged.

This behaviour could be attributed to the lower wettability of the hydrate powder. In fact wettability seemed to be the driving parameter in this case.

Therefore at pH 4.5 and 6.8 the neutralisation of the outer layer prevented solvent uptake so that the inner layer remained as a salt. On the contrary the greater wettability of the anhydrous discs justify their higher solubility in all the studied media, also in the less favourable ones.

In Medium C neutralisation was predominant (diclofenac sodium pk_a = 4) whereas solubilisation prevailed in Medium A being faster than neutralisation. High wettability enables fast diffusion of the solvent front inside the anhydrous discs attaining an almost complete neutralisation of the powder.

After DH layer mechanical removal, the pellets were transferred into deionised water and a dissolution test was performed again.

Hydrates showed releases of about 88% whereas anhydrous forms reached only 23%. This experiment confirms that the discs were almost intact and the active ingredient was not precipitated in the Medium C.

According to USP 28 ⟨724⟩ Method A a two-step dissolution test was performed. After a few minutes in Medium D, DS pellets swelled up showing longitudinal fissured planes looking like superimposed soaked sheets while DSH discs maintained their appearance unchanged during the entire two-hour period.

Solvent seemed to penetrate faster into the DS discs suggesting a lower contact angle at the liquid–solid interface with respect to DSH.

After the dissolution step in Medium D, both outer and inner pellet layers resulted by FTIR analysis constituted of DH insoluble form (Scheme 1). The DS discs also resulted to be neutral; anyway the swollen DSH pellets exposed to solvent different portions both in shape and in surface area: this could account for the higher dissolution values obtained for SD. Therefore, data obtained by the second dissolution step (Medium A) could not be regarded as an IDR study.

In Medium A DS discs reached the plateau after 4–10 h whereas DSH discs could not reach equilibrium even after 21 h (Fig. 7).

5. Conclusions

This study enabled to enrich the knowledge about diclofenac sodium with some unexplored aspects of its solid state and physico-chemical properties.

Anhydrous form DS (the commercial form), showed a tendency to uptake water from the environment and to spontaneously give rise even under standard conditions (25 °C and RH below 60%) to a solid–solid transition to a hydrate form DSH with characteristic physico-chemical, biopharmaceutical and technological properties.

DSH is a tetrahydrate form never described in literature and different from DSH1, the tetrahydrate form obtained by crystallisation from water and previously described.

DSH was characterised by means of FTIR, XRPD, DSC and TGA in comparison with DS and DSH1.

Infrared spectroscopy provided a useful mean for the identification of both DS and DSH. Heating–cooling cyclic DSC was a useful tool in distinguishing the two hydrate forms. XRPD permitted to assess the existence of the different crystalline forms.

Stability of DS and DSH was investigated under different experimental conditions to verify the tendency to solid–solid transition, to uptake water from the environment and to study the existence range of the different forms.

DSH was shown to have good physical stability: in fact it was able to exist for at least 1 year at room temperature; no change in its crystalline form was observed by mild grinding and compression up to 6 t for 60 min Inter-conversion between the hydrate form DSH and the anhydrous form DS was detected after storing DSH at 50 °C for 10 min.

Solubility and intrinsic dissolution studies were performed to compare the physico-chemical properties of DS and DSH. Differences in their solubility and intrinsic dissolution rates were pointed out, DS showing a higher solubility and a faster dissolution than DSH even at the physiological pH 6.8, involving difference in bioavailability when dissolution is the limiting step.

Intrinsic dissolution studies confirmed firstly the pH dependence of solubility of diclofenac sodium, secondly the formation of acidum diclofenac (DH) under acidic conditions which causes the salt to become inactivated according to [11]. DS and DSH showed to have a different chemical behaviour during dissolution, probably due to different wettability of the powders.

The results obtained by intrinsic dissolution studies suggested that other hydrate modifications different from both DSH and DSH1 could exist.

All the experimental evidences confirmed that the commercial product DS is not stable at room temperature under standard conditions.

As a general conclusion, it could be postulated that standard conditions proposed by ICH guidelines for long term stability studies [24] (25 ± 2 °C/60 ± 5% RH or 30 ± 2 °C/65 ± 5% RH) could be not sufficient to avoid the hydration of DS form and the conversion into the lower soluble DSH form. In fact, storage under standard conditions or exposure to a RH of about 60% even if without a direct contact with water during manufacturing process could modify the solid state of diclofenac sodium and influence the performance of the final dosage form. The occurrence of this event can be effected by the excipients capability to enhance or prevent water absorption.

Manufacturer of diclofenac sodium should take care of controlling solid state properties and assessing crystal form during stability studies to assure batch to batch reproducibility of the active substance.

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