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Comparison of the physicochemical properties of the *N*-(2-hydroxyethyl) pyrrolidine, diethylamine and sodium salt forms of diclofenac

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Abstract

Non steroidal anti-inflammatory agents (NSAIDs) such as diclofenac have very low aqueous solubilities and consequently salt formation may be used to enhance solubility and dissolution rate. In this study, we examined the physicochemical properties of three diclofenac salts, diclofenac sodium (DNa), diclofenac *N*-(2-hydroxyethyl)pyrrolidine (DHEP) and diclofenac diethylamine (DDEA), and their different solid state forms to determine the influence of salt form on solubility, dissolution rate and membrane transport. The equilibrium solubility of DDEA at 25 °C was determined as 33 mM, lower than the solubilities of DHEP (273 mM) and DNa (66 mM) previously reported (Ledwidge and Corrigan, 1998). In addition to the dihydrate form of DHEP previously characterised, monohydrate forms of DHEP and DDEA were identified. Intrinsic dissolution rate studies were used to determine the solubility ratios of the hydrated and anhydrous forms. The monohydrate form of DHEP was found to be 1.8 times less soluble than the anhydrate, whereas DDEA anhydrate was approximately 1.7 times as soluble as the monohydrate form. On investigation of the pH-solubility profile (25 °C) of DDEA, appreciable supersaturation (76 mM) relative to the theoretical profile, was detected at the pH_{max}. This contrasts with values of >800 and 67 mM for DHEP and DNa, respectively. The transport of salt solutions through a porous membrane (Visking®) was investigated. A linear relationship between concentration (mM) and rate of transport (mmol/h) was established for DNa and DHEP solutions. The mass transfer coefficient determined for DHEP was lower than that for the other two salts. Nevertheless, the maximum transport rate obtained for DHEP is almost six times higher than that obtained for DDEA.

Keywords: Diclofenac salts; *N*-(2-hydroxyethyl)pyrrolidine; Diethylamine; Sodium; Solubility; Dissolution; Membrane transport

1. Introduction

Improvement of the physicochemical properties of drugs is a common approach to optimising their bioavailability. Salt formation is a commonly employed method for enhancing the solu-

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bility and dissolution rate of poorly water soluble weak electrolyte drugs. Diclofenac, 2-[(2,6-dichlorophenyl)amino]benzeneacetic acid is an acidic compound (pK_a 3.80 at 25 °C) with very low aqueous solubility (6×10^{-5} M at 25 °C) in the unionised form (Chiarini et al., 1984). The salts of diclofenac available in pharmaceutical products include diclofenac *N*-(2-hydroxyethyl)pyrrolidine (DHEP), diclofenac sodium (DNa) and diclofenac diethylamine (DDEA). DHEP demonstrated the highest solubility in water (46 mM at 25 °C) among a series of diclofenac salts (Fini et al., 1993a). Subsequently, a number of publications have explored the physicochemical characteristics of DHEP (Fini et al., 1993b, 1994a,b, 1995a; Holgado et al., 1995; Ledwidge et al., 1996; Ledwidge and Corrigan, 1998).

Conflicting values for the aqueous solubility of DDEA are reported in the literature: 11 mM at 25 °C (Fini et al., 1995b) and 35 mM at 20 °C (Kriwet and Müller-Goymann, 1993). Information on the physicochemical characteristics of DDEA is limited in comparison to that for DHEP. Only one form of DDEA has been reported in the limited published data on the salt. In contrast, four forms of DHEP have been reported: DHEP anhydrate (Fini et al., 1993b), DHEP dihydrate (Ledwidge et al., 1996), D_2HEP anhydrate (consisting of two molecules of diclofenac to each molecule of HEP), and D_2HEP monohydrate. The latter two forms were isolated in the course of a study on the pH-solubility profile of DHEP (Ledwidge and Corrigan, 1998).

In this study, we compare the physicochemical properties of three diclofenac salts and their different solid state forms: DNa, DHEP and DDEA. The influence of salt form on solubility, dissolution and membrane transport was investigated.

2. Materials and methods

2.1. Materials

DNa was obtained from Sigma Chemical Co. DDEA and DHEP were prepared from diclofenac acid (AMSA) and diethylamine (Aldrich) or *N*-(2-hydroxyethyl)pyrrolidine (Aldrich). The solvents used for salt preparation were acetone (BDH) and methanol (Riedel-de Haën).

2.2. Salt preparation

Salts were prepared by dissolving diclofenac acid in an appropriate solvent (e.g. water, methanol) and adding equimolar amount of base dissolved in the solvent (Ledwidge et al., 1996). The resulting product was recovered by filtration under vacuum (in cases where a precipitate resulted) or by removing excess solvent using a Büchi RE 111 Rotavapor apparatus linked to a Büchi B-169 vacuum system. Products were initially dried under ambient conditions for 48 h. Hydrate forms were prepared by dissolving the anhydrate form in deionised water at $\sim$40 °C and recrystallising the hydrate form by allowing slow evaporation of water at room temperature.

2.3. Powder X-ray diffraction (XRD)

X-ray diffraction (Siemens D500) measurements were carried out on powdered samples mounted in conventional cavity mounts; compressed discs were mounted in a holder with a 13 mm diameter aperture (Ledwidge et al., 1996).

2.4. Thermal analysis

Differential scanning calorimetry (DSC) (Mettler TA3000 Thermal Analysis System or Mettler Toledo DSC 821^e) and thermogravimetric analysis (TG) (Mettler TG 50 linked to a Mettler MT5 balance) were performed on powdered samples (5–10 mg) under nitrogen purge at a heating rate of 10 °C/min (unless specified otherwise). Integral enthalpies of solution (ΔH_{soln}) were determined using the Setaram MicroDSC III calorimeter operated in isothermal mode at 25 °C. Known amounts ($\sim$450 mg) of solvent were placed in the lower compartments of the batch mixing vessels (sample and reference vessels). A known weight of powdered sample ($\sim$1 mg) was placed in the upper compartment of the sample batch mixing vessel. The choice of solvent was based on the requirement for 'rapid' dissolution of solid for accurate determination of ΔH_{soln} values (Giron,

1995). The solvents used were water (DHEP) and water:methanol 50:50 v/v (DDEA). All readings were determined in triplicate.

Thermomicroscopy was carried out on a Reichert hot stage mounted on a light microscope with and without crossed polarising filters. Samples were mounted in air or in silicone oil (to detect desolvation).

2.5. Karl Fischer titration

Karl Fischer titrations (KFT), for the determination of water content, were carried out on powdered samples using Metrohm 701 KF Titrino linked to a Metrohm 703 Ti stand. All analyses were performed in triplicate.

2.6. Elemental analysis

Elemental analysis (Exetor Analytical CE440) for C, H and N was carried out on powdered samples ($\sim$ 2 mg).

2.7. Spectroscopy

Fourier transform infrared (FT-IR) analysis was carried out using either a Nicolet 205 FT-IR spectrometer or a Perkin Elmer Paragon 1000 FT-IR spectrometer using the drug salts in KBr. The signal at 1684/cm in the spectrum for diclofenac acid was attributed to C=O stretching of the carboxylic acid group (Silverstein and Webster, 1998; Palomo et al., 1999). Salt formation was confirmed by: (a) the absence of the carboxylic acid peak at 1684/cm; (b) the presence of bands characteristic of carboxylic acid salts, at 1650–1550 and 1440–1335/cm (Socrates, 1994); and (c) absorption at 3350–3150/cm, attributable to NH_3^+ stretching of solid amine salts (Socrates, 1994). Water of crystallisation in hydrates can be detected by the presence of medium intensity O – H stretching vibrations between 3600 and 3100/cm (Socrates, 1994).

The concentration of diclofenac in aqueous salt solutions was determined by UV assay. Absorbances were measured at λ, 276 nm (Adeyeye and Li, 1990) using a Hewlett Packard 8452A diode array spectrophotometer.

2.8. Solubility and dissolution rate

For dynamic solubility studies excess solid (2–3 times the equilibrium solubility) was placed with $\sim$ 30 ml deionised water in a water-jacketed vessel linked to a temperature controlled water bath held at 25 °C. Solutions were agitated constantly by overhead stirrers at 500 r.p.m. The solutions were analysed at hourly intervals up to 8, at 24 and at 48 h. Aliquots of 2 ml were withdrawn, filtered through a 0.45 μm filter (Gelman Sciences), diluted appropriately and assayed for drug content. Solubility determinations were carried out in duplicate.

Equilibrium solubilities were determined using a modification of the sealed ampoule method of Mooney et al. (1981). Excess solid (approximately 2–3 times the estimated solubility) was placed in 5 ml deionised water in a 10 ml glass ampoule, which was then heat sealed. Ampoules were placed in a shaker water bath (Precision Scientific) at 25 °C and agitated at 150 c.p.m. At 24, 48 and 72 h, samples were withdrawn from the ampoules, filtered, diluted appropriately and assayed for drug content. Determinations were carried out in triplicate and the pH of the filtered solution was determined.

The pH-solubility studies of the salts were carried out using a modification of the phase solubility technique of Dittert et al. (1964). A saturated solution of diclofenac acid or diclofenac salt was prepared by dissolving an excess in deionised water. The pH was adjusted by the dropwise addition of the appropriate base or acid (HCl). Following equilibration after each addition, the pH of the bulk solution was recorded (Orion Model 520A pH meter) and a sample taken, filtered through a 0.45 μm filter (Gelman Sciences), diluted appropriately and assayed for drug.

Intrinsic dissolution rate (IDR) studies, under sink conditions, were performed using the USP 24 paddle method (Sotax AT7) in 900 ml water at 25 °C and 50 r.p.m. on paraffin mounted compressed discs (13 mm). XRD confirmed that the crystal form of the original powder was retained following the compression procedure. The initial portion of each dissolution profile (0–15 min) was used to derive the intrinsic dissolution rate.

2.9. Membrane transport studies

A modification of the diffusion cell technique of Goldberg and Higuchi (1968) was used to investigate transport through Visking®, a porous membrane of regenerated cellulose tubing (Scientific Instrument Centre). The Visking® membrane (2.5 cm diameter), which had been soaked overnight and rinsed several times in deionised water, was placed between these ground-glass ends and the flasks were clamped together. A solution of drug in deionised water at 25 °C was placed in the donor flask. Each flask contained a 3.5 cm Teflon-coated magnetic stirring bar. The apparatus was immersed in a water bath at 25 °C, under which were placed single speed synchronous motors at 500 r.p.m. (Griffin). Samples of receptor solution (5 ml) were withdrawn at 30 min intervals for UV analysis and replaced with fresh medium (25 °C).

2.10. Surface active characteristics

Surface tension measurements of drug solutions in deionised water were carried out at 25 °C on a Lauda Tensiometer TD1 using the ring accessory. For each system examined, surface tension values (mN/m) were plotted against concentration (log scale). CAC (critical association concentration, Kriwet and Müller-Goymann, 1993) values were determined from the change in the concentration dependence of surface tension.

A modification of the technique employed by Brito and Vaz (1986) was used to study the solubilisation behaviour of the salts examined. The water insoluble fluorescent compound *N*-phenyl-1-naphthylamine (NPN) was added to the solutions ($\sim$1 mg/10 ml), which were agitated at 25 °C for 24 h in a temperature controlled shaker waterbath (Precision Scientific). Aliquots of 5 ml were withdrawn from the receptor flask and filtered (0.45 μm filter, Gelman Sciences) to remove excess solid NPN. The solutions were subsequently analysed for fluorescence of NPN using a Perkin-Elmer LS50B Luminescence Spectrometer (excitation λ, 356 nm; emission λ, 410 nm; minimum slit width). For each salt, relative fluorescence intensity was plotted against concentration and this plot used to determine the CMC of the salt.

3. Results and discussion

3.1. DHEP monohydrate

Dissolving DHEP samples from IBSA in water at $\sim$40 °C and re-crystallisation by slow evaporation at room temperature resulted in a crystalline form differing from the known dihydrate (DHEP-DH) or anhydrous (DHEP-A) forms. This form (DHEP-MH) was subsequently identified as DHEP monohydrate. The powder X-ray diffraction pattern and DSC profile of DHEP-MH are compared with those of the dihydrate and anhydrous forms in Fig. 1 and Fig. 2, respectively. The DSC scan for DHEP-MH at 2 °C/min revealed melting of the crystals with elimination of solvent of crystallisation, followed by recrystallisation of the anhydrate form from the melt. This thermal behaviour was characteristic of a Type 2 pseudopolymorph according to the classification system of Giron (1995). This new form was found, by Karl Fischer titration, to contain $4.48 \pm 0.24\%$ w/w water ($n = 3$), corresponding to a water:drug ratio of 1:0.93. The theoretical water content of the monohydrate form of DHEP is 4.20% w/w. The elemental analysis results, (C 56.04%; H 6.12%; N 6.36%) for the product recrystallised from water are consistent with the formation of a monohydrate form of DHEP.

Comparison of the FT-IR spectra for DHEP-MH and for DHEP-DH with the spectrum for DHEP-A indicated broader absorption bands for the new product and the dihydrate form between 3600 and 3100/cm. This was consistent with the presence of water of crystallisation which generates medium intensity stretching vibrations within this frequency range (Socrates, 1994). The band of absorption in this range was slightly broader for the dihydrate form than for the monohydrate, which was consistent with the greater number of water molecules in the dihydrate form.

The inclusion of a water molecule into the crystal lattice of DHEP would be expected to influence the intermolecular interactions (affecting the internal energy and enthalpy) and the crystalline disorder (entropy) and will therefore influence the free energy, thermodynamic activity, solubility, dissolution rate, stability and

bioavailability (Khankari and Grant, 1995). The hydrated form of a drug is generally less soluble in water than the corresponding anhydrate due to the loss of free energy that occurs during the hydration process (Khankari and Grant, 1995).

Assuming the values for the diffusion layer thickness in aqueous solution for the anhydrate and monohydrate are equivalent, that the diffusion coefficients are equal and that sink conditions apply, differences in intrinsic dissolution rate (IDR) will reflect differences in saturation solubility of the drug, Eq. (1) (Khankari and Grant, 1995):

$$\frac{\mathrm{IDR}_{\mathrm{anhydrate}}}{\mathrm{IDR}_{\mathrm{hydrate}}} = \frac{C_{\mathrm{S\ anhydrate}}}{C_{\mathrm{S\ hydrate}}}, \quad (1)$$

where C_S denotes the saturation solubility.

The dissolution rate of DHEP-MH was determined in water at 25 °C and compared with the dissolution rates obtained by Ledwidge et al. (1996) for DHEP-A and DHEP-DH (Fig. 3). The IDR value obtained for DHEP-MH at 25 °C was 4.19 ± 0.15 mg/min per cm^2. This value lies between the intrinsic dissolution rates of the anhydrate and dihydrate forms of DHEP, 7.31 and 1.49 mg/min per cm^2, respectively (Ledwidge et al., 1996). From these intrinsic dissolution rate values, the monohydrate was estimated to be approximately 1.8 times less soluble than the anhydrate and 2.8 times more soluble than the dihydrate.

Each crystalline form of a compound will have a characteristic enthalpy of solution (Giron, 1995). The enthalpy of solution (ΔH_{soln}) value gives information about the solution process, the relative effects of temperature on solubility and the heat of hydration (ΔH_{h}) between related solid forms. The ΔH_{h} value for a particular drug may be calculated from the ΔH_{soln} values for the hydrate and anhydrate forms, Eq. (2) (Giron, 1995):

$$\Delta H_{\mathrm{h}} = \Delta H_{\mathrm{soln(anhydrate)}} - \Delta H_{\mathrm{soln(hydrate)}}. \quad (2)$$

The integral enthalpy of solution (ΔH_{soln}) for the monohydrate form of DHEP was determined to be 26.9 ± 0.6 kJ/mol. Ledwidge et al. (1996) reported values of 22.6 ± 1.0 and 34.0 ± 0.6 kJ/mol for the enthalpies of solution of DHEP-A and DHEP-DH, respectively. The enthalpy change for the hydration of DHEP-A to the monohydrate form (ΔH_{h}), calculated from Eq. (2), is -4.3 kJ/mol. At constant temperature and pressure the free energy of hydration, ΔG_{h}, for

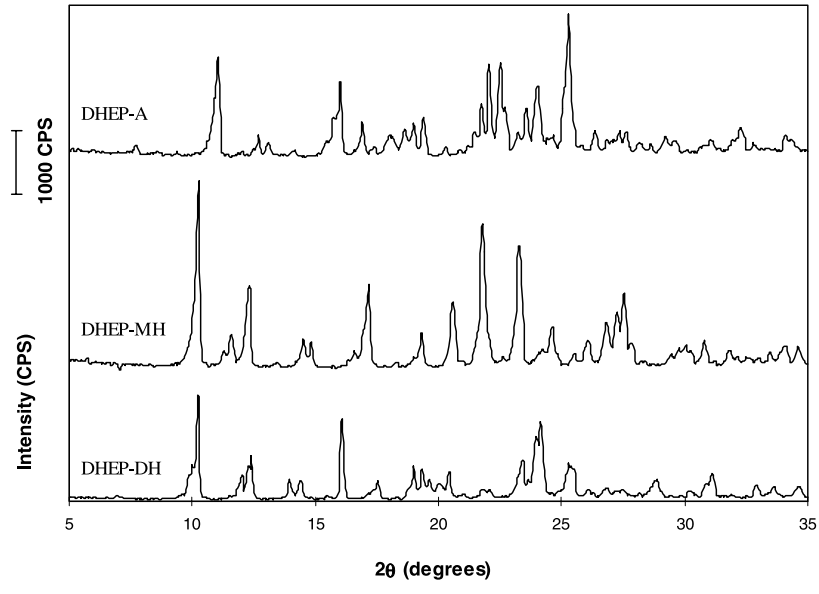

Fig. 1. XRD traces for DHEP-A, DHEP-MH and DHEP-DH.

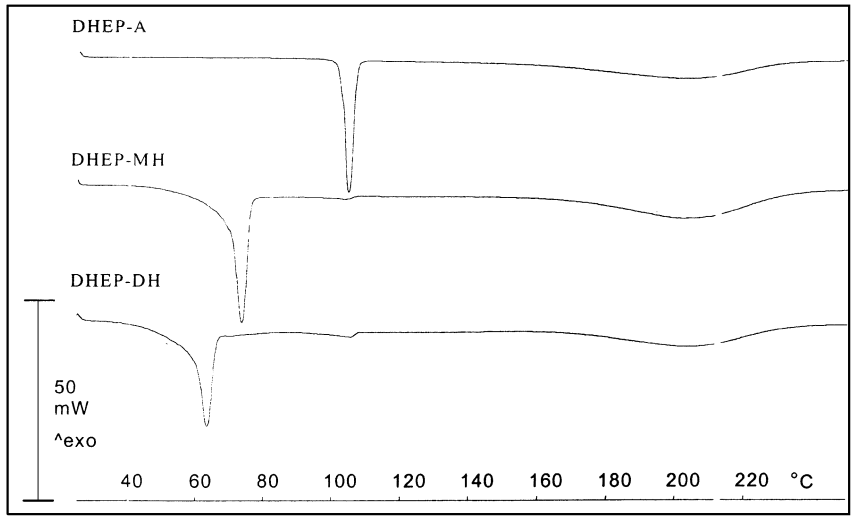

Fig. 2. DSC scans (10 °C/min) for DHEP-A, DHEP-MH and DHEP-DH.

DDEA may be calculated from the ratios of the solubilities of the hydrous and anhydrous forms according to Eq. (3) (Shefter and Higuchi, 1963):

$$\Delta G_h = RT \ln\left(\frac{C_{S\,hydrate}}{C_{S\,anhydrate}}\right). \quad (3)$$

Substituting the ratio of the intrinsic dissolution rates instead of that of the solubilities (Eq. (1)) yields the following equation:

$$\Delta G_h = RT \ln\left(\frac{IDR_{hydrate}}{IDR_{anhydrate}}\right). \quad (4)$$

The free energy lost on dissolution and further interaction with water is less for the hydrate than for the anhydrate. This occurs because the hydrate has already interacted intimately with the water (Khankari and Grant, 1995) and means that, with respect to an aqueous environment, the hydration process is accompanied by a free energy decrease. The ΔG_h value accompanying the conversion of DHEP-A to its monohydrate at 25 °C, calculated from Eq. (4), is -1.38 kJ/mol. The corresponding entropy change (ΔS_h) can be calculated from Eq. (5) (Shefter and Higuchi, 1963):

$$\Delta S_h = \left(\frac{\Delta H_h - \Delta G_h}{T}\right). \quad (5)$$

The ΔS_h value calculated was -9.80 J/mol per K. Dunitz (1994) states that the entropic cost of transferring a water molecule from liquid water into the crystalline structure of biomolecules can range from 0 to ~ 29 J/mol per K per molecule of water. The upper limit will only apply to molecules of water that are firmly bound within the crystalline structure. The relatively low value calculated for the conversion of DHEP to its monohydrate form suggests that the water of crystallisation present in the molecule is loosely bound.

3.2. Identification and characterisation of DDEA monohydrate

A new form of diclofenac diethylamine was isolated by recrystallisation following slow evaporation at room temperature of a saturated solution of DDEA in water. This new form of the salt, was characterised by powder X-ray diffraction, thermal analysis, Karl Fischer titration and elemental analysis and identified as DDEA monohydrate (DDEA-MH). The XRD trace of the new form differed significantly from that of the anhydrous phase (Fig. 4). The positions of the main diffraction peaks in the 2θ range 5–20° for DDEA-A and DDEA-MH were $2\theta =$

8.20, 12.80, 15.25 and 17.95° and $2\theta =$ 6.50, 10.85, 12.85, 13.70, 15.90 and 18.60°, respectively.

The DSC and TG scans for DDEA-A are shown in Fig. 5. The DSC scan revealed two overlapping endotherms with onset temperatures of 125 and $\sim$135 °C, lower than the reported melting point of 157–159 °C (Fini et al., 1995b). The broad endotherm from $\sim$160 °C could be attributed to the volatility of the molten drug and/or decomposition. The TG scan showed an increase in the rate of weight loss at $\sim$130 °C, corresponding to the second of the two overlapping endotherms in the DSC scan. The sharp increase in the rate of weight loss above $\sim$ 200 °C corresponded to the broad peak in the DSC scan, attributed to the volatile nature of the melt and/or the occurrence of decomposition. The sample was examined by thermomicroscopy. Prior to heating, the sample was present as prismatic crystals displaying birefringence. Melting of the sample was observed from 115 °C, followed by recrystallisation at 130 °C into acicular crystals displaying birefringence. Subsequently, melting was observed from 145 °C. The recrystallisation from the melt of a different crystal form and subsequent re-melting explained the presence of two endothermic peaks in the open pan DSC scan (Fig. 5).

The DSC and TG scans for DDEA-MH are shown in Fig. 6 and reveal an endothermic event at 54 °C not present in the scan of the anhydrous form. In order to determine if this lower temperature thermal event was reversible, a sample was held at 100 °C for 30 min and allowed to cool to room temperature. An open pan DSC was carried out immediately and again after the sample was left at room temperature overnight. The endotherm of onset temperature $\sim$54 °C was absent from the resultant scans, which were characteristic of DDEA-A. XRD analysis of the sample after cooling to room temperature confirmed that the sample had converted to DDEA-A. As with the TG scan for DDEA-A, an inflexion in the trace for DDEA-MH occurred at $\sim$150 °C, corresponding to an increase in the rate of weight loss. In contrast to DDEA-A, a weight loss of $4.32 \pm 0.17\%$ w/w occurred for the new form ($n =$ 3) over the temperature range corresponding to the first endotherm in the DSC scan. When a sample was examined by thermomicroscopy, the evolution of vapour on heating above $\sim$50 °C,

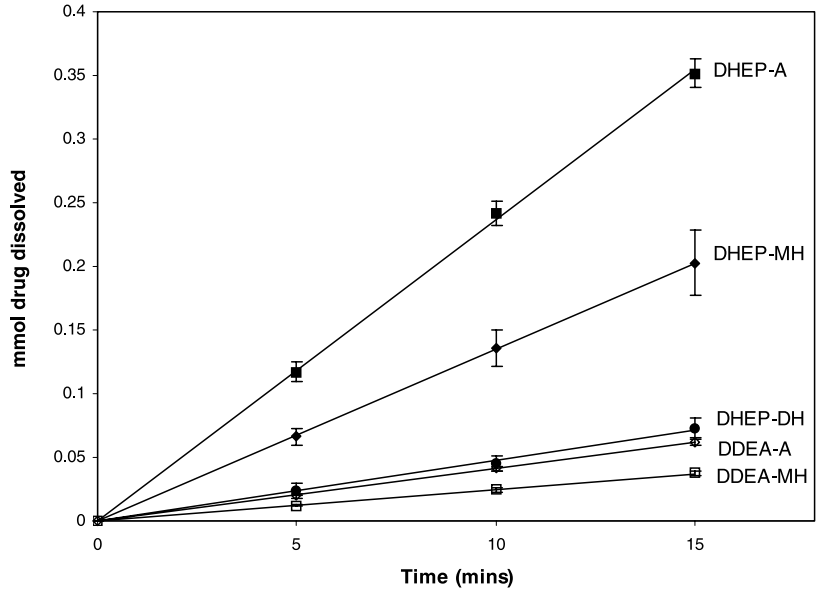

Fig. 3. Intrinsic dissolution profiles for DHEP-A, DHEP-MH, DHEP-DH, DDEA-A and DDEA-MH.

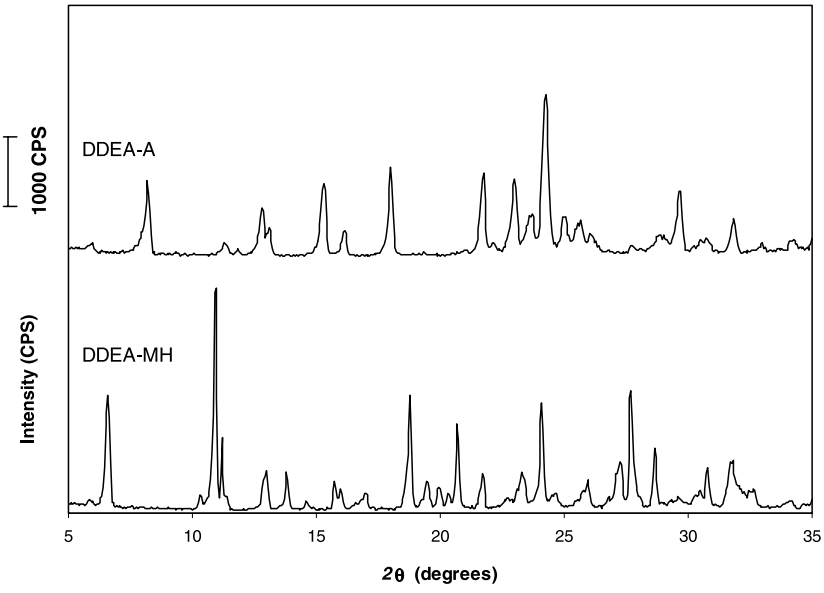

Fig. 4. XRD traces for DDEA-A and DDEA-MH.

suggested by the weight loss displayed in the TG trace, was confirmed by observation of a sample mounted in silicone oil. Karl Fischer titration results indicated a water content of $4.63 \pm 0.16\%$ w/w. The differences between the XRD traces for DDEA-A and DDEA-MH verified that the water present in DDEA-MH consisted of water of crystallisation. The value of 4.63% w/w for the water content of DDEA-MH represents a water:drug ratio of 1:1, confirming the new form as a monohydrate. Elemental analysis compared well with those values expected for a monohydrate form of diclofenac diethylamine. A broadening of the absorption bands in the FTIR spectrum between 3600 and 3100/cm was observed for DDEA-MH relative to anhydrous DDEA. This was consistent with the presence of water of crystallisation which generates medium intensity stretching vibrations within this frequency range (Socrates, 1994).

In conclusion, the new form of DDEA isolated in this study was identified as a monohydrate form. On heating, this monohydrate converts to the anhydrate form, which then generates the endotherm characteristic of DDEA-A (onset, $\sim$ 122 °C). This behaviour is characteristic of a Type 1 pseudopolymorph according to the classification of Giron (1995).

A dynamic solubility study was carried out on DDEA-A at 25 °C, using the paddle solubility method, which indicated a decrease from an initial solubility value of $\sim$40–45 mM to an equilibrium apparent solubility value of 33.3 mM (12.3 mg/ml), consistent with precipitation from solution of a less soluble phase. The solid phase recovered by filtration of the saturated solution used in the solubility study (after 48 h) was examined by XRD and was found to consist of DDEA-MH. This value of 33.3 mM was notably higher than the value of 4.1 mg/ml (11.1 mM) previously reported for the solubility of DDEA in water at 25 °C (Fini et al., 1995b). The lower solubility value reported by Fini et al. (1995b) was consistent with the higher melting point of the salt form identified in their study. A salt form with a melting point of 157–9 °C, the value quoted by Fini et al., was not isolated in the current work. The value obtained for the solubility of DDEA (33.3 mM) was closer in value to the reported solubility of DDEA at 20 °C (35 mM, Kriwet and Müller-Goymann, 1993).

An equilibrium solubility study of DDEA-A and DDEA-MH was carried out at 25 °C, using the ampoule solubility method. The solubility of

DDEA-A was determined as 34.96 ± 0.65 mM, which was consistent with the result obtained from the dynamic solubility study. As observed in the dynamic solubility study, the solid phase in equilibrium with the solution was shown by XRD to consist of DDEA-MH, indicating that the less soluble monohydrate precipitated from solution. The result from an equilibrium solubility study carried out using DDEA-MH as the starting material was consistent with the value obtained for the equilibrium solubility of the anhydrate form (32.77 ± 1.15 mM after 24 h).

An estimation of the solubility ratio between the two forms, DDEA-A and DDEA-MH, was determined using an intrinsic dissolution rate (IDR) study. The IDR values in water at 25 °C, as derived from the initial linear portion (0–15 min) of the dissolution plot (Fig. 3) were 1.15 ± 0.02 and 0.69 ± 0.01 mg/min per cm^2 for the anhydrate and monohydrate forms of DDEA, respectively. This indicates that the anhydrate is approximately 1.7 times more soluble than the monohydrate.

The integral enthalpies of solution (ΔH_{soln}) for the anhydrate and monohydrate forms of DDEA were determined as 26.3 ± 0.2 and 28.8 ± 0.6 kJ/mol, respectively. The ΔH_h value for DDEA monohydrate, calculated from Eq. (2), was -2.57 kJ/mol. The ΔG_h value at 25 °C, calculated using the intrinsic dissolution rates for the two forms (Eq. (3)), was -1.27 kJ/mol. Using the ΔH_h and ΔG_h values, the entropy of hydration value (ΔS_h) was calculated as -4.37 J/mol per K. The relatively low value calculated for the conversion of DDEA to its monohydrate form suggests that the water of crystallisation present in the molecule is loosely bound (Dunitz, 1994).

3.3. pH-solubility profile of DDEA

The pH-solubility profile of DDEA is shown in Fig. 7. Using the anhydrous form as the starting material, the solubility of the salt in water at 25 °C was found to be 34 mM at pH 8 (indicated by the arrow in Fig. 7). This value was consistent with the solubility determined in the dynamic solubility study (33.3 mM). Lowering the pH of a saturated solution of the salt with HCl resulted in a drop in the solubility and precipitation of the free acid. Addition of DEA to a saturated solution of DDEA did not cause a change in the solubility. The solubility above pH 8 remained constant at ~34 mM. The addition of diethylamine to the solution of diclofenac acid caused a sharp increase in the solubility of diclofenac acid to a maximum of 76 mM at pH 8.15. As with

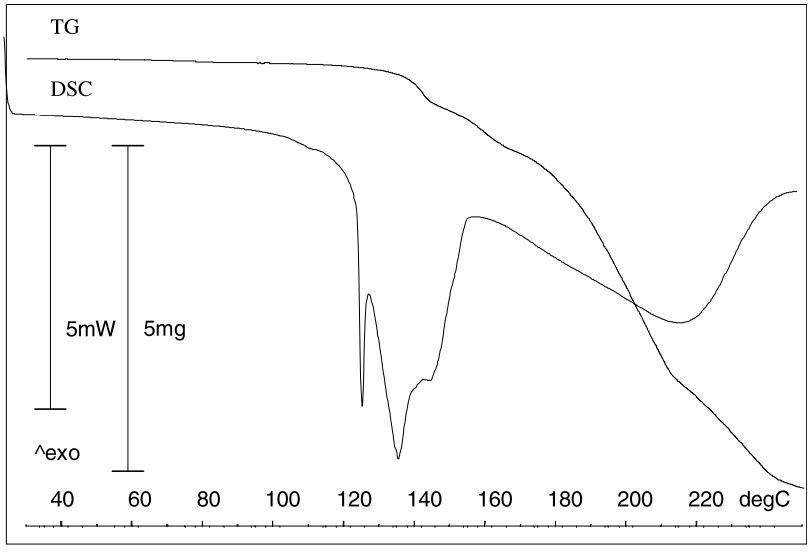

Fig. 5. DSC and TG scans for DDEA-A.

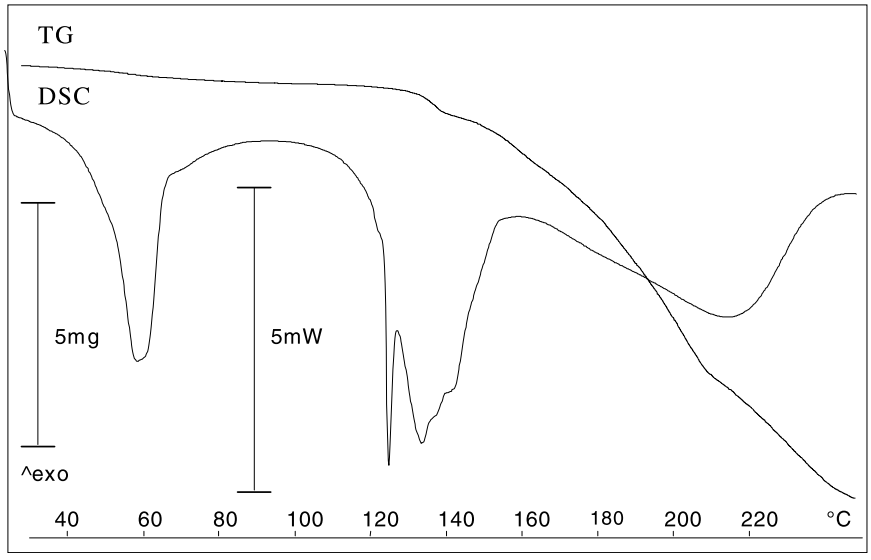

Fig. 6. DSC and TG scans for DDEA-MH.

DHEP (Ledwidge and Corrigan, 1998), a supersaturation phenomena was observed at the pH of maximum solubility. The supersaturated solution had a concentration considerably higher than the aqueous solubility of DDEA (33.3 mM). The *apparent supersaturation* is defined as the solubility achieved at the pH_{max} relative to a saturated solution. According to this definition the level of supersaturation observed in the DDEA system was 2.3. The apparent supersaturation values previously determined for DHEP and DNa were >3 and ~ 1.03, respectively (Ledwidge and Corrigan, 1998). Further addition of diethylamine caused a lowering of the solubility of the solution to ~ 34 mM at pH ~ 10. The solubility remained at ~ 34 mM on further addition of DEA, at pH values >10. This behaviour was consistent with the solubility value of 34 mM obtained when DDEA was used as the starting material.

The solubility data for pH values $< pH_{max}$ was fitted to the equation which describes the pH-solubility profile for weak organic acids below the pH_{max} (Chowhan, 1978). Using 0.037 mM as the estimate of intrinsic solubility (Ledwidge and Corrigan, 1998), a value of 4.87 was obtained for the pK'_a at 25 °C.

3.4. Surface active properties

The surface activity and self-association properties of DDEA in solution were investigated using techniques identical to those used in the study of DHEP and DNa properties (Ledwidge and Corrigan, 1998). The results obtained were compared with those reported for the other two salts.

In the case of DHEP, a change in the concentration dependence of surface activity was observed at 30 mM, the critical association concentration (CAC) for the salt (Ledwidge and Corrigan, 1998). Solutions of DDEA with concentrations greater than this value were not investigated. This was because of the limited solubility of the salt (33 mM). Consequently, the plot of concentration versus surface tension for DDEA did not reveal a change in the concentration dependence of surface tension, i.e. no CAC was detected.

Using the water insoluble fluorescent compound, *N*-phenyl-1-naphthylamine (NPN), fluorescence was detected for DHEP solutions with concentrations greater than 30 mM, a value consistent with results obtained previously (Ledwidge and Corrigan, 1998). No fluorescence was observed for DDEA solutions in the concentra-

tion range examined (10–30 mM). This may be attributed to the solubility of DDEA (33 mM) limiting the concentrations examined to less than that necessary for micelle formation and solubilisation of NPN.

Kriwet and Müller-Goymann (1993) investigated the micellar behaviour of DDEA-water systems and the transition from a micellar solution to a liquid crystalline phase via vesicle formation. A CAC value of 20 mM (20 °C) was determined for the salt from the plot of surface tension versus concentration. However, whereas the solubility at 20 °C was determined to be 35 mM, the surface tension measurements included solutions of DDEA with concentrations above 100 mM, i.e. supersaturated solutions. Kriwet and Müller-Goymann (1993) reported that, whereas viscosity measurements at 45 °C indicated the presence of spherical micelles, a concentration-dependent increase in viscosity was observed at room temperature, suggesting the formation of vesicles and lyotropic liquid crystals. Their results from transmission electron microscopy indicated that vesicles occurred only at temperatures below 32 °C, the transition temperature from liquid crystals to an isotropic micellar solution. Further investigations by transmission electron microscopy showed that the region of existence of a micellar solution of DDEA at room temperature was small. A vesicle dispersion was shown to exist at a concentration as low as 2%.

3.5. Membrane transport

Transfer rates (mmol/h) were obtained from linear plots of total amount of drug transported (mmol) against time (h). Linear relationships between concentration (mM) and rate of mass transport (mmol/h) were established for DNa and DHEP solutions (Fig. 8). The maximum rate of transport observed for DDEA solutions is lower than that observed for DNa solutions, a trend consistent with a lower saturation solubility value for DDEA. Despite a lower mass transfer coefficient for DHEP, the maximum transport rate obtained for DHEP solutions, 0.1248 mmol/h, was almost six times higher than that obtained for DDEA solutions, 0.0209 mmol/h.

A trend towards decreasing mass transfer coefficient (P_e) with increasing counterion size was suggested. Self-association of a drug may hinder diffusion. CAC values have been determined for DNa and DHEP (Ledwidge and Corrigan, 1998). The solubility of DDEA limited the concentrations examined to less than those necessary to achieve self-association. DHEP was shown to sol-

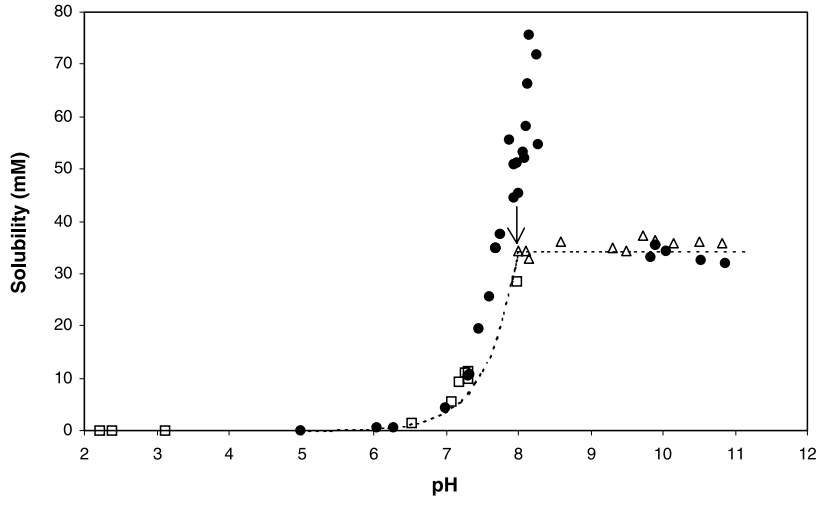

Fig. 7. pH-solubility profile for DDEA: addition of DEA to diclofenac acid (●), addition of HCl to DDEA (□) and addition of DEA to DDEA (Δ).

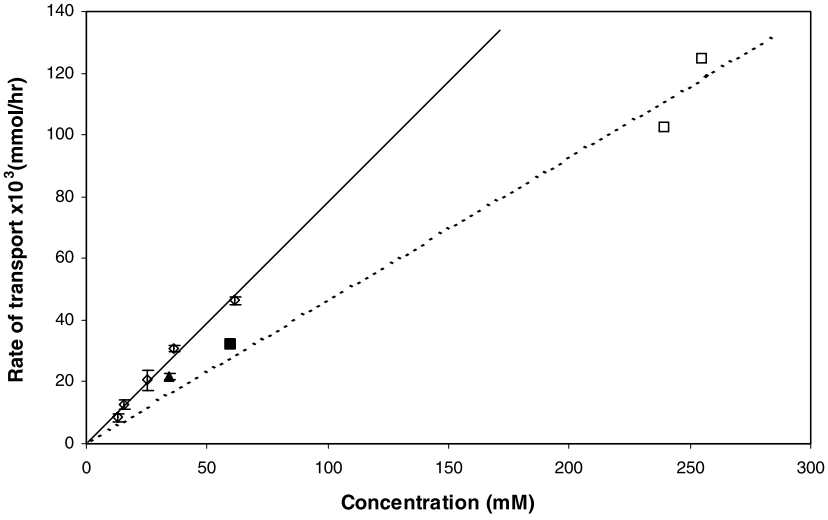

Fig. 8. Membrane transport of diclofenac salts: DNa solutions (◇), saturated DDEA solution (▲), unsaturated DHEP solution (■), saturated DHEP solutions (□), trendline for DNa solutions (—) and trendline for DHEP solutions (---).

ubilise NPN, whereas no solubilisation was detected for DNa (Ledwidge and Corrigan, 1998) or DDEA solutions. The lowest P_e value for transport across Visking® was observed for DHEP, the salt showing the greatest potential to self-associate and form micelles.

4. Conclusions

Considerable differences in the biopharmaceutically relevant physicochemical properties, solubility, dissolution rate and membrane transport, between the three salts of diclofenac were observed.

DDEA showed appreciable supersaturation at the pH_{max}. The apparent supersaturation level observed (2.3) was intermediate between the values obtained by Ledwidge and Corrigan (1998) for DHEP (>3) and DNa (~ 1.03).

DHEP precipitates from water as the dihydrate form at 25°C, whereas DDEA converts to its monohydrate under identical conditions. IDR results suggest that DDEA-A is almost twice as soluble as DDEA-MH.

The mass transfer coefficient, through Visking, determined for DHEP was lower than that for DNa. Nevertheless, the maximum transport rate obtained for DHEP was almost six times higher than that obtained for DDEA.

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