Journal of Thermal Analysis and Calorimetry, Vol. 73 (2003) 509–518

THERMAL DEGRADATION AND MELTING POINT DETERMINATION OF DICLOFENAC

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Abstract

The thermal behaviour of Diclofenac was investigated using Differential Scanning Calorimetry, Hot Stage Microscopy, and Thermogravimetric Analysis. A discrepancy was observed between the melting point values recorded under dynamic flow of either dry nitrogen (180°C) or air (160°C). By means of High Performance Liquid Chromatography/Mass Spectrometric analyses, it has been possible to ascribe this difference in melting points to the formation of three degradation products as a result of intramolecular cyclization and condensation reactions during the heating process in an oxidative atmosphere.

Keywords: diclofenac, drug degradation, HPLC-MS analysis, melting point, oxidative atmosphere, thermal decomposition

Introduction

Diclofenac, 2-[(2,6-dichlorophenyl)amino]benzeneacetic acid (DCNH, Fig. 1), is a non-steroidal anti-inflammatory drug, used in inflammatory and painful conditions of rheumatic and non-rheumatic origin. It is generally marketed as a salt derivative, in particular as a sodium salt (DCNNa).

As regards the physical properties of DCNH, most papers published so far are concerned with the preparation and the thermal characterization of a number of diclofenac salts [1–3]. Lately, the peculiar behaviour of DCNNa compacts in contact with water has been highlighted [4].

The polymorphism of DCNH has also been extensively investigated: two monoclinic [5] and one orthorhombic [6] crystal forms have been isolated and described. Both investigations focused only on the crystallographic aspects of DCNH polymorphs.

During solubility determinations of DCNNa dihydrate in supercritical CO_2 [7], the thermal behaviour of diclofenac acid (DCNH) was also studied and a melting

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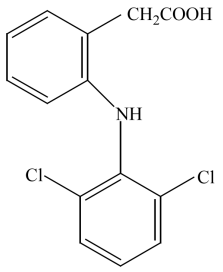

Fig. 1 Chemical structure of 2-[(2,6-dichlorophenyl)amino]benzeneacetic acid (DCNH)

point value of 180°C has been recorded. This value resulted in substantial disagreement with the melting point reported in literature (approximately 160°C) [8, 9] for DCNH. Unfortunately, no further information on the thermal behaviour of DCNH seems to be available to date.

Aiming to explain the discrepancy observed between the melting point values of DCNH, we investigated the thermal behaviour of DCNH using different instrumental techniques such as differential scanning calorimetry (DSC), thermogravimetry (TG) and hot stage microscopy (HSM). In addition, high performance liquid chromatography (HPLC), in combination with positive electrospray mass spectrometry (ES^+-MS) and tandem mass spectrometry (ES^+-MS/MS), was used in order to detect and identify possible degradation products formed during the thermal treatment of DCNH.

Experimental

Materials

Preparation of DCNH

DCNNa (Lisapharma, Como, Italy) water solution (10 g L^{-1}) was dropwise acidified with 1M HCl until a pH value of 3–4 was reached and a massive white precipitate had formed. The precipitate was then suction filtered, washed with distilled water, air dried and stored in a desiccator. Identification of DCNH was accomplished by means of HPLC and FTIR determinations.

Methods

Differential scanning calorimetry

Temperature and enthalpy measurements were performed by means of a Mettler DSC 821^e STAR^e system (Mettler Toledo, Switzerland). 4–5 mg samples were subjected to the following thermal programs:

- 30–190°C heating range, scan rate 10 K min^{-1}, in sealed and pierced crucible under dynamic flow of dry nitrogen (Method 1).
- 30–190°C heating range, scan rate 10 K min^{-1}, in open crucible under dynamic flow of air (Method 2).

• 30 –190°C heating range, scan rate 10 K min^{-1}, in sealed crucible (Method 3).

• 30–100°C, scan rate 10 K min^{-1} and 100–190°C, scan rate 1 K min^{-1}, heating ranges in open crucible under dynamic air flow (Method 4).

Purging gases (dry nitrogen or air) were allowed to flow at 100 mL min^{-1}.

Instrument calibration was performed with standard indium and zinc samples (purity>99.99%) of known melting temperature and enthalpy.

Thermogravimetric analysis

Thermogravimetric analyses (TG 50, Mettler Toledo, Switzerland) were carried out on samples placed in alumina pans with a pierced cover. All samples were subjected to the same thermal program (30–190°C heating range, scan rate 10 K min^{-1}). The experiments were carried out under dynamic flow of either dry nitrogen or air (200 mL min^{-1}).

Hot stage microscopy

A hot stage apparatus (HSF 91, Linkam Scientific Instruments, Tadworth, U.K.) equipped with a polarising microscope (Labophot II Nikon, Tokyo, Japan) and a color video camera (XC-003P Sony, Tokyo, Japan), supported by Image-Pro Plus 4.0 software (Media Cybernetics, MD) allowed the recording of images during temperature scans. Samples, placed between two glass cover slides, were heated from ambient temperature up to 190°C, scan rate 10 K min^{-1}.

HPLC/MS apparatus and methods

HPLC analyses were carried out by using an HP 1100 binary pump, equipped with an autosampler G1329A and an UV detector G1315A (Agilent Technologies, Palo Alto, CA, USA). LC/MS and LC/MS/MS analyses were performed on a triple quadrupole instrument QUATTRO-LCZ (Waters-Micromass, Manchester, U.K.) operating in positive electrospray (ES^+) mode. The LC effluent coming from the HP1100 pump was split 1/10 prior to the introduction into the mass spectrometer ion source. LC/MS (full-scan) data were acquired over the *m/z* range 150–800. MS/MS experiments were performed by using argon at a pressure of $\approx 1 \cdot 10^{-3}$ Torr and collision energies ranging from 20 to 24 eV. The source parameters were: 110°C source temperature and 180°C desolvation temperature. The instrument calibration (from mass 12 to mass 3925) was performed using an isopropanol/water 1:1 solution of NaI (2 μg/μL) and CsI (0.05 μg/μL).

Samples of molten DCNH were prepared using a hot stage Köfler apparatus (C. Reichert Optische Werke A.G., Wien, A) with a large heating surface (~45 cm^2) and accurate temperature control. The drug was placed above a glass cover slide and heated up to complete fusion (approximately 160°C), removed from the hot stage and eventually allowed to cool down to ambient temperature spontaneously.

Untreated DCNH, used for preparing the standard reference solution, and the previously molten sample were separately dissolved in acetonitrile/water (1:1, vol/vol) mixture, thus affording working solutions of proper concentration (~0.7 mg/mL). The HPLC analyses were carried out on a Hypersil MOS C8 column (100×4.6 mm i.d., 5 μ) in a gra-

dient mode with a mobile phase A composed of water acidified with glacial acetic acid (pH 3.3) and a mobile phase B composed of acetonitrile. The samples were eluted according to the following gradient: isocratic elution of A/B (1/1) for 12 min, linear gradient from A/B (1/1) to A/B (1/9) in 53 min, linear gradient from A/B (1/9) to A/B (1/1) in 2 min, isocratic elution of A/B (1/1) for 8 min. The sample injection volume and the flow rate were 10 μL and 1 mL min^{-1}, respectively; detection was performed at 280 nm.

Results and discussion

Thermal behaviour of DCNH

The DSC trace of DCNH (Method 1) shows a sharp endothermic peak (ΔH= –38.4 kJ mol^{-1}) at around 180.5°C (s.d. 0.8) (Fig. 2), corresponding to fusion.

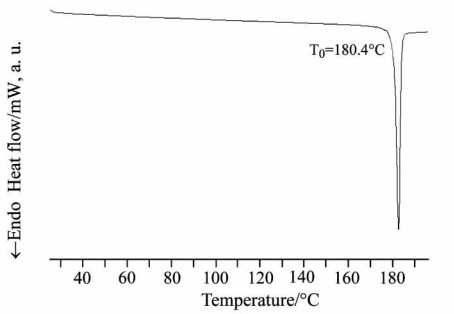

Fig. 2 Thermal behaviour of DCNH, submitted to heating program of Method 1; the onset temperature is reported

Polarised light HSM analysis revealed changes in morphology (edges rounding), movements and colour alterations of DCNH crystals starting at around 149°C; complete fusion was observed at approximately 160°C, i.e. close to the scheduled value reported in literature [8, 9].

This discrepancy was reasonably attributed to the fact that DCNH, when heated in presence of air, as in the case of HSM experiments, undergoes a decomposition process well before the melting temperature. The resulting degradation products can, therefore, be responsible for the mp lowering of the drug.

In order to support this hypothesis, samples of DCNH were subjected to thermal programmes in a controlled atmosphere.

In Fig. 3, the DSC profiles of DCNH samples, scanned according to Methods 2 and 3 (curves a and b, respectively), are reported. In both cases, an exothermic event can be observed at around 160°C, followed by an endothermic effect at about 173°C. Moreover, in the case of curve a, the endothermic effect is immediately followed by exothermic decomposition, due to the dynamic flow of air which supports the degradation process. When the experiment is carried out at a scan rate of 1 K min^{-1} (Method 4), DCNH undergoes a much more remarkable thermal stress, due to the

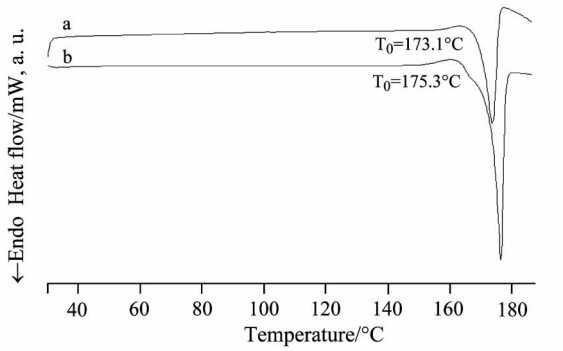

Fig. 3 Thermal behaviour of DCNH, submitted to heating programs of Methods 2 and 3: curve a and curve b, respectively; the onset temperatures are reported

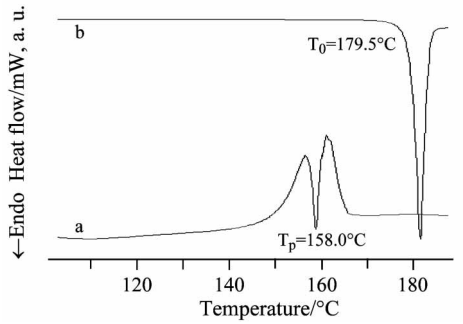

Fig. 4 Thermal behaviour of DCNH, submitted to heating program of Method 4 (curve a); the peak temperature of the endothermal effect is reported. For ease of comparison, the thermal behaviour of DCNH, submitted to Method 1, with the relevant onset melting temperature is also shown (curve b)

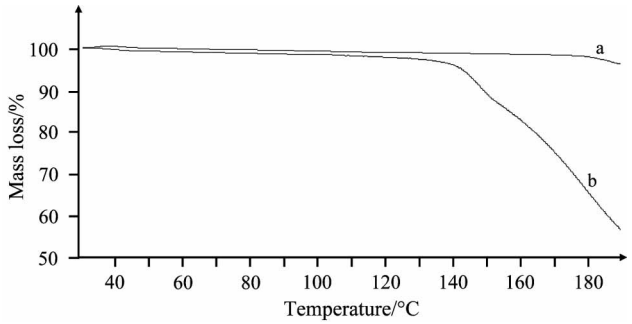

Fig. 5 TG analyses of DCNH performed under dynamic flow of dry nitrogen (curve a) or air (curve b)

long time of exposure to high temperatures in the presence of air. Consequently, a strong broad exothermic effect, starting at 110°C approximately, is clearly evident. The sharp endothermic peak at around 158°C is reasonably due to the presence of both unreacted DCNH and degradation products, and therefore this temperature rep-

resents the melting point of DCNH when assessed in an oxidative atmosphere (Fig. 4, curve a). By the way, if all DCNH would decompose during the thermal treatment (according to Method 4), no DCNH would be either detected in analogous molten samples prepared for HPLC analyses (see the relevant section).

All these phenomena occurred, in agreement with HSM analyses, at temperatures well below the melting point recorded when DSC scans were carried out under a dynamic flow of dry nitrogen (Fig. 4, curve b).

TG analyses, performed under dynamic flow of air, confirmed the presence of degradation reactions and formation of volatile end-products. In fact, the recorded mass losses of samples heated under dynamic flow of dry nitrogen or air at around 158°C were about 4.4 and 18.5%, respectively (Fig. 5).

HPLC/MS analysis

Chromatograms of the standard reference solution and of DCNH samples recovered after melting are reported in Figs 6 and 7, respectively. In the case of pure DCNH, only one peak, retention time (R_t) 4.12 min, is obviously observed. The HPLC chromatogram of

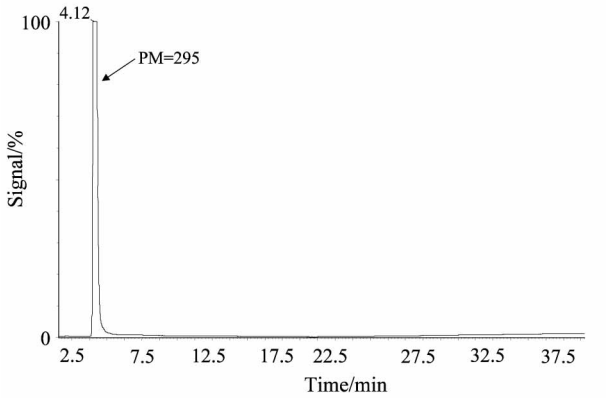

Fig. 6 HPLC profile of standard solution of DCNH

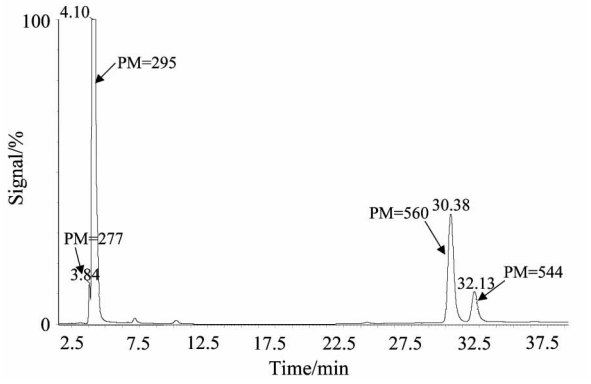

Fig. 7 HPLC profile of DCNH recovered after melting

the previously molten sample is characterised by the presence of three other major peaks (R_t values 3.84, 30.38, and 32.13 min, respectively), in addition to that corresponding to undegraded DCNH. Figure 7 reports also the relevant molecular mass of each degradation product measured by mass spectrometry, as well as that of undegraded DCNH.

The structures of the main degradation products were ascertained by means of ES^+-MS/MS investigations. In Table 1 the retention times and molecular masses of DCNH and of the three degradation products are collected, while in Fig. 8 the related chemical structures are reported.

Table 1 Retention times and molecular masses of DCNH and of the three major degradation products

Compounds	Retention time/ min	Molecular mass
1-(2,6-Dichloro-phenyl)-1,3-dihydro-indol-2-one (**1**)	3.84	277
2-[2,6-dichlorophenyl)amino]benzeneacetic acid	4.10	296
[2-(2,6-Dichloro-phenylamino)-phenyl]acetic acid [2-(2,6-dichloro-phenylamino)-phenyl]-hydroxy-methyl ester (**2**)	30.38	560
[2-(2,6-Dichloro-phenylamino)-phenyl]-acetic acid 2-(2,6-dichloro-phenylamino)-benzyl ester (**3**)	32.13	544

MS/MS spectra of the decomposition products are shown in Figs 9–11. Compound (**1**) derives from the loss of one water molecule from DCNH and subsequent intramolecular cyclization between the amino and carboxyl groups [3]. As regards com-

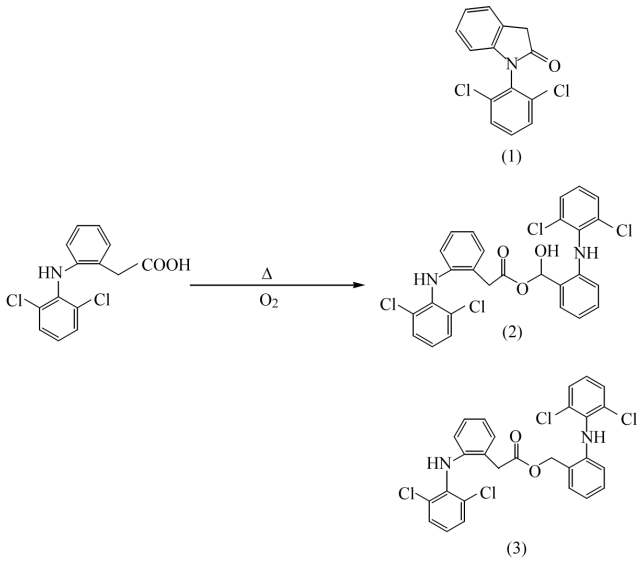

Fig. 8 Schematic view of the three major degradation products, obtained by thermal treatment of DCNH

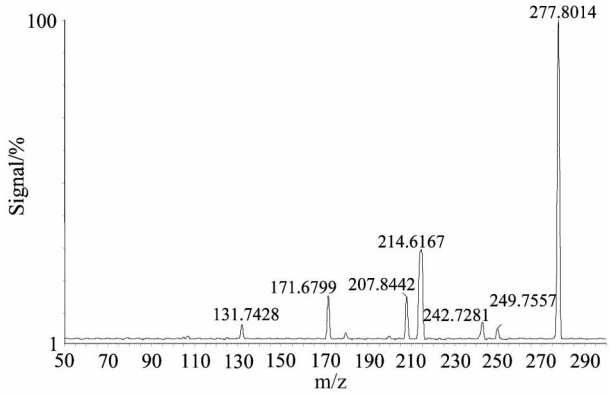

Fig. 9 Positive electrospray MS/MS spectrum of $[M+H]^+$ ion of compound **1**

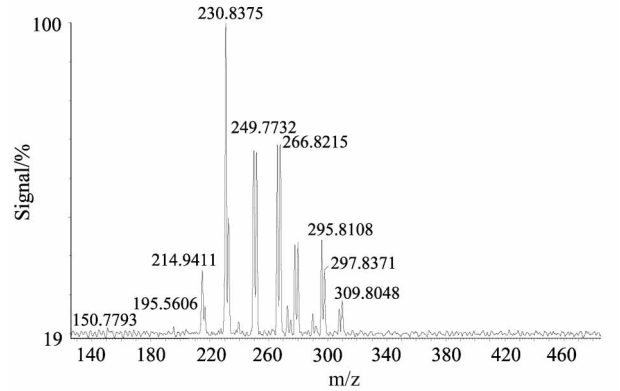

Fig. 10 Positive electrospray MS/MS spectrum of the most abundant signal of the isotopic pattern of the $[M+H]^+$ ion of compound **2** (*m/z* 563)

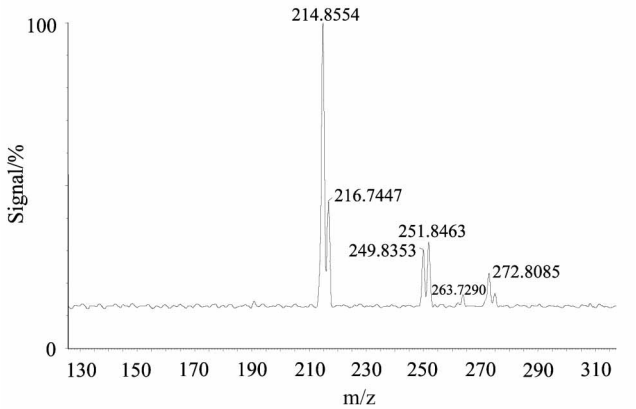

Fig. 11 Positive electrospray MS/MS spectrum of the most abundant signal of the isotopic pattern of the $[M+H]^+$ ion of compound **3** (*m/z* 547)

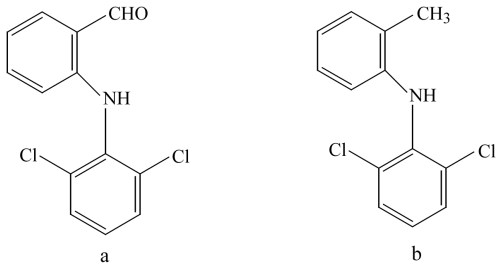

Fig. 12 Chemical structures of a – (2,6-dichloro-phenylamino)-benzaldehyde and b – (2,6-dichloro-phenyl)-*o*-tolyl-amine

pounds (**2**) and (**3**), their formation can be explained as a result of a condensation process between reacted and non-reacted molecules of DCNH, probably through a radical mechanism. As can be observed in the MS/MS spectrum of the most abundant signal of the isotopic pattern of the 4-chlorine-containing $[M+H]^+$ ion of compound (**2**) (*m/z* 563) (Fig. 10), the peak at *m/z* 266 can be attributed to the (2,6-dichloro-phenylamino)-benzaldehyde fragment (Fig. 12a), while the peak at *m/z* 296 corresponds to undecomposed DCNH. The peaks at *m/z* 231 and 215 can be attributed to the loss of chlorine atoms from the fragments at *m/z* 266 and 250, respectively.

As far as identification of compound (**3**) is concerned, a fragment corresponding to (2,6-dichloro-phenyl)-*o*-tolyl-amine (at *m/z* 252) (Fig. 12b) is observed in the mass spectrum of the most abundant signal of the isotopic pattern of $[M+H]^+$ ion of compound (**3**) (*m/z* 547) (Fig. 11).

Conclusions

The melting point is commonly used as a preliminary identification parameter for crystalline organic compounds. Köfler apparatus and capillary method are used to this purpose. Microcalorimetry (DCS and DTA) represents a much more sofisticated although routine method able to afford also a quantitative evaluation of the heat exchanged during the solid/liquid phase transition. Moreover, controlled conditions (e.g. atmosphere, pressure, …) can be easily adopted.

A melting point around 160°C is reported for DCNH according to literature [8–9], while DSC measurements in a controlled inert atmosphere showed that the fusion endotherm occurs approximately at 180°C.

Both thermal data and HPLC/MS experiments demonstrated that, on heating, DCNH undergoes a decomposition process when a potentially oxidative atmosphere is present and, therefore, the low value of fusion temperature for DCNH (160°C) is due to the formation of decomposition products at high temperatures. The structures of these degradation products have been elucidated by MS/MS analyses.

In conclusion, only the melting point determination under a dynamic flow of dry nitrogen can provide a reliable value for the DCNH fusion temperature.

The financial support of Italian MIUR (Ministero dell'Istruzione Università e Ricerca) and of Italian CNR (Consiglio Nazionale delle Ricerche) is gratefully acknowledged.

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