

THERMOANALYTICAL AND SPECTROSCOPIC CHARACTERIZATION OF SOLID STATE DIPYRIDAMOLE

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Abstract

A physico-chemical characterization of dipyridamole ($C_{24}H_{40}N_8O_4$), a widely used anti-aggregating agent, has been performed by using a combination of thermoanalytical (DSC) and spectroscopic (XRPD and FT-IR/PAS) techniques. A solid state transition, already reported in literature, has been ascribed to the breaking of an intramolecular H-bonds network. The rupture of a network of intermolecular H-bonds is thought to accompany the fusion. The solid state transition has been shown to be reversible provided the sample has not undergone melting.

Mechanical milling and thermal annealing have been shown to decrease melting temperature and enthalpy. The effect brought about by mechanical and thermal treatment on the solid state transition is different. In the milled samples the transition peak shifts towards lower temperatures and its enthalpy suggests that all intramolecular H-bonds have been transformed into intermolecular H-bonds.

Keywords: dipyridamole, polymorphism, solid state characterization, thermoanalysis

Introduction

It is well known that different crystal phases of the same molecule may have different solubility, dissolution rate and, hence, different bioavailability. In this sense a thorough characterisation of the possible polymorphs of a drug substance is not only highly recommended but has quite recently become mandatory.

Dipyridamole ($C_{24}H_{40}N_8O_4$, Fig. 1) is a widely used anti-aggregating agent mostly employed to prevent repeated heart attacks. Its crystal structure has been determined by single crystal X-ray diffraction [1] and two polymorphic forms (form I and II) have been put into evidence [2–4]: form II is thermodynamically stable at room temperature and transforms at $\approx 130^\circ\text{C}$ into form I which undergoes melting at $163\text{--}168^\circ\text{C}$ and is the only metastable form that can be obtained by cooling the melt at room temperature. A polymorph, though it is not yet clear which one (form I or II), is obtained by milling of dipyridamole.

What is apparent at first sight is the lack of detailed information on the thermal properties of dipyridamole such as the exact temperatures of transition and melting and the relevant enthalpies. Furthermore, no exhaustive description is available of the

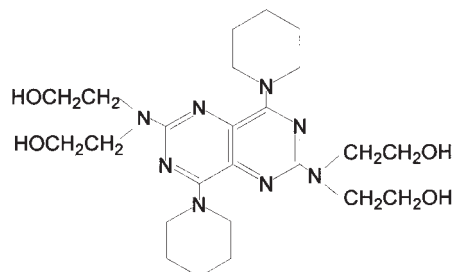


Fig. 1 Dipyridamole (molecular structure)

effects of thermal and/or mechanical (milling) treatments on dipyridamole physical properties. In the present work a thorough physico-chemical characterisation of polymorphism in dipyridamole has been attempted by using a combination of thermo-analytical (DSC) and spectroscopic (XRPD and FT-IR/PAS) techniques.

Experimental

A commercial product (Aldrich Chimica, Italy, purity 98%) was made of use. This was examined as it is (sample C), thermally annealed (sample A – i.e. heated up to a predetermined temperature where it has been kept for different times before to be cooled down to room temperature) or milled (sample BM – Pulverisette 7 planetary mill Fritsch, Germany, at 250 rpm with agate balls and jar. A ball: sample mass ratio of 10 has been adopted).

Simultaneous TG/DSC measurements have been carried out by a STA 625 apparatus (Polymer Lab., UK) connected to a Data Station (Rheometric Scientific, UK). Samples (4–5 mg) have been put in open aluminum pans and heated at 5 K min⁻¹ up to 190°C under nitrogen flux (25 mL min⁻¹).

FT-IR/PAS measurements were performed by a Nicolet 730 FT-IR spectrometer fitted with an MTEC 200 photoacoustic detector. The spectra (4000–400 cm⁻¹) were obtained by fast Fourier transformation (FFT) of 64 coadded interferograms with a resolution of 4 cm⁻¹. The spectrometer was purged with dry nitrogen flow of 15 L min⁻¹. The spectra were collected on all kind of samples (C, A or BM). No further sample manipulation was needed before recording the spectrum.

XRD data were obtained by a Philips PW 1710 powder diffractometer equipped with a Philips PW 1050 vertical goniometer and a graphite monochromator for the CuK_α radiation (K_{α_1} = 1.5406 Å; K_{α_2} = 1.5443 Å). Measurements were performed in the temperature range from room temperature (*r. t.* in the following) to 150°C using a home-made polythermal attachment. The nominal temperatures were controlled by acquiring diffraction patterns of AgI around its $\gamma \rightarrow \alpha$ transition (147°C). The angular range 5° < 2 θ < 40° was covered in the step scan mode (step width: 0.03°; counting time of 1 s; 40 KV 20 mA). Silicon was used as an internal standard for zero error correction ($\approx 0.17^\circ$).

Results

DSC evidence

Figure 2 reports the DSC/TG trace of the C sample. A DSC broad hump (spread over 80–150°C temperature range) is due to a solid state transformation. A further sharp DSC peak represents the fusion of dipyridamole. No mass variation is recorded in the whole temperature range so indicating that dipyridamole is thermally stable over melting. The enthalpies of transition and melting of sample C (Table 1) are the mean of 10 independent measurements. Solid state transition still occurs in samples A (Table 1) but the relevant enthalpies depend, as the melting ones, on the thermal treatment. Namely 90°C annealing does not appreciably affect either of them while annealing at 150°C results in a nearly equal decrease of both transition and melting enthalpies. The transition peak does no longer show up in samples previously melted:

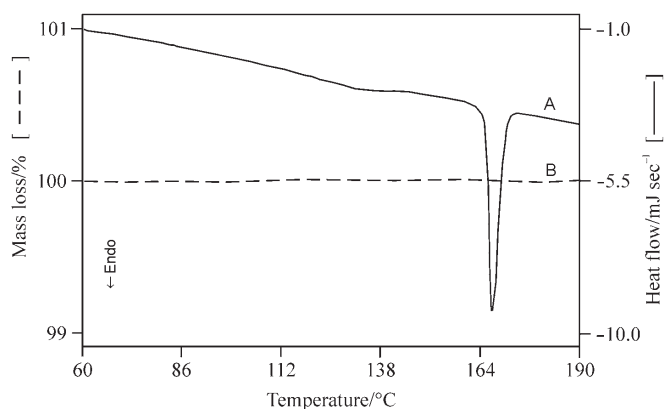


Fig. 2 DSC/TG scan of a sample C. Curve A=DSC trace; B=TG trace

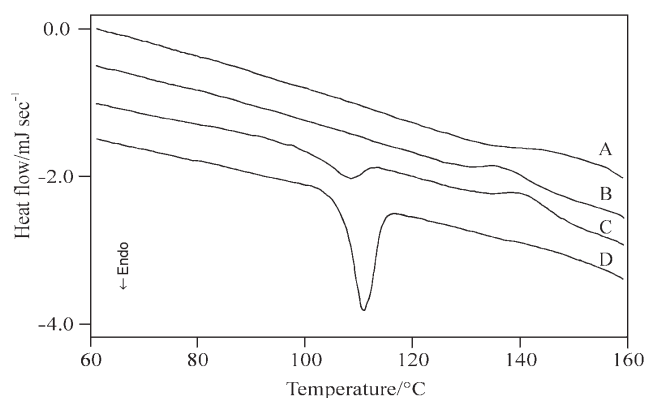


Fig. 3 DSC curves of samples milled for different times: curve A=10 min; curve B=40 min; curve C=90 min; curve D=300 min

they show, during cooling, a crystallisation peak and, on a second heating, a melting peak of very similar enthalpy but lower than first melting enthalpy.

Table 1 Transition ($\Delta_t H$) and melting enthalpies ($\Delta_{\text{fus}} H$) of dipyridamole samples. The melting temperatures (T_{fus}) are also reported. C=commercial sample; A=annealed sample; BM=ball milled sample

Sample	$\Delta_t H/J \text{ g}^{-1}$	$\Delta_{\text{fus}} H/J \text{ g}^{-1}$	$T_{\text{fus}}/^\circ\text{C}$
C	17.66±0.98	55.55±1.45	165.7±0.1
A 90°C/5 h	17.64	51.32	165.78
A 90°C/12 h	19.23	57.75	165.70
A 90°C/16 h	17.72	52.39	165.15
A 90°C/20 h	20.00	53.15	165.34
A 150°C/1 h	17.40	56.49	165.46
A 150°C/5 h	13.96	55.66	165.19
A 150°C/7 h	14.13	54.77	165.12
A 150°C/12 h	12.68	51.91	164.32
A 150°C/20 h	11.03	50.49	163.51
BM 10 min	9.94	52.94	162.3
BM 20 min	7.80	53.47	162.9
BM 30 min	5.15	52.73	162.4
BM 40 min	–	53.39	162.4
BM 60 min	–	52.07	162.6
BM 90 min	–	52.32	162.5
BM 120 min	–	52.11	162.4
BM 180 min	–	52.11	162.4
BM 210 min	–	51.09	162.1
BM 300 min	–	49.68	162.3
BM 420 min	–	48.35	162.4

Figure 3 reports the DSC curves of some milled samples. By increasing milling time a progressive decrease of the area of the transition peak is observed (Table 1) for milling up to 30 min. By further milling the transition peak vanishes and a small exothermic peak appears instead between 120 and 150°C (curves B and C) that disappears for milling times >180 min (curve D). Moreover, an endothermic peak shows between 90 and 120°C for milling times >90 min (curves C and D) that increases until it reaches (milling time ≈300 min) nearly the same area of the transition peak

(curve D). Temperatures and melting enthalpies are nearly equal to those of the samples annealed at 150°C for long times. It seems as if the energy added either mechanically (by milling) or thermally (by annealing) would yield the same solid phase. However the exothermic/endothermic peak connected to mechanical milling disappear by a mild annealing (1–3 h at 120°C); afterwards the curves resemble those of the melted and recrystallised samples.

FT-IR/PAS evidence

Figure 4 reports PAS spectrum of sample C. It has to be noted that the spectra of A samples are identical to this one.

With respect to the spectrum of Fig. 4 the following spectral variations arise in samples that underwent melting and recrystallisation:

- the broad band (with maxima at 3382 and 3300 cm^{-1}) due to stretching of OH groups is much narrower and does no longer show the two maxima,
- a peak at $\approx 3040 \text{ cm}^{-1}$ appears,
- the intensity of the peak at 1281 decreases with respect to that of the peak at 1216 cm^{-1} (both peaks are due to OH in plane deformation),
- the intensity of the peak at 1052 decreases with respect to that of the peak at 1015 cm^{-1} (both peaks are due to C–O stretching).

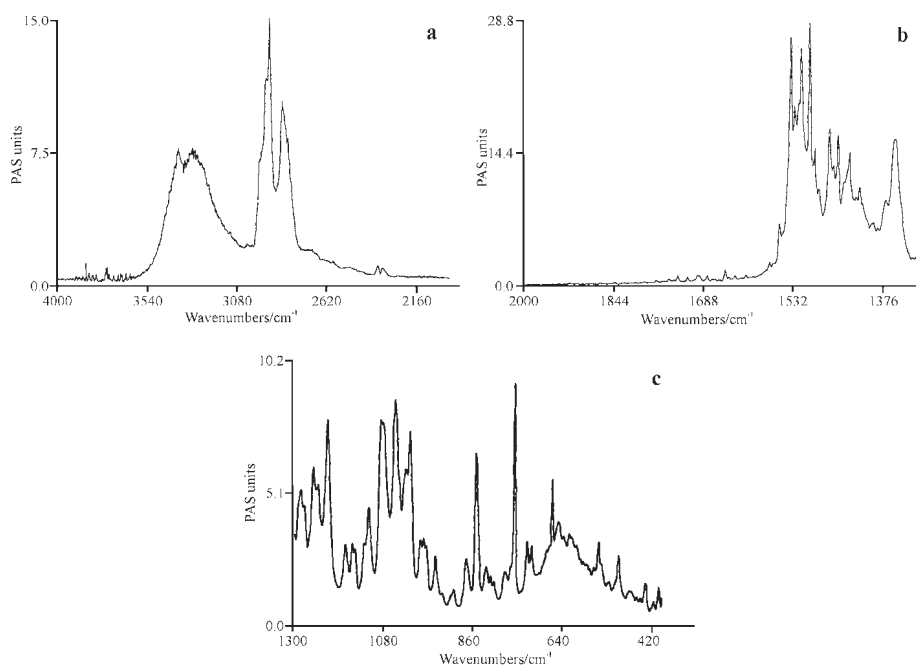


Fig. 4 PAS spectrum of sample C

It is here worth noting that the fingerprint region of FT-IR spectrum of sample M is appreciably different from that of form reported in [3]. It is known from literature [1] that both intermolecular and intramolecular H-bonds exist in dipyrindamole. Namely the bonds C–O–H···N are preferably (or exclusively) intramolecular while those C–O–H···O–C can be both intra- and intermolecular. The disappearance of the two maxima of OH stretching band (point a) suggests that during fusion the H-bonds are evened.

The remark d) agrees with the above observation. The oxygen has greater electronegativity so that the bonds C–O–H···O–C should be stronger than the C–O–H···N ones. The lower frequency peak of C–O stretching should correspond to the stronger bond. Therefore, it is the relative amount of C–O–H···N bonds that decreases due to melting. Such an interpretation also accounts for remark c.

Table 2 Intensities of the PAS peaks of ball milled samples. The numbers express the time of milling in min

Sample	cm ⁻¹									
	2925	2850	$\Delta v_{1/2}$	1281	1251	1081	1052	1015	860	760
BM 60	54.0	46.0	398.4	28.5	31.9	33.7	30.5	35.8	46.2	53.8
BM 90	54.0	46.0	375.8	27.8	31.8	32.3	30.4	37.3	42.9	57.1
BM 210	58.0	42.0	285.2	24.2	35.0	29.0	28.2	42.8	39.1	60.9
BM 420	61.4	38.6	244.5	22.9	34.4	28.2	26.2	45.7	36.3	63.7
BM 300 +1 h 120°C	55.4	44.6	380.3	26.5	33.8	34.3	29.9	35.8	44.9	55.1

A prolonged (≈ 7 h) milling also results into noticeable changes of spectral intensities that are summarized in Table 2 (reference is made to spectrum of Fig. 4). Namely:

- the broad band due to stretching of OH groups considerably narrows and, as in the case of melted and recrystallised samples, no longer shows two maxima,
- the intensity of the peak at ≈ 3040 cm⁻¹ increases,
- the intensity of the CH₂ asymmetric stretching (2925 cm⁻¹) increases with respect to that of the symmetric CH₂ stretching (2850 cm⁻¹),
- the intensity of the peak at 1281 decreases while that of the peak at 1251 cm⁻¹ increases,
- the intensity of the peak at 1015 increases while those of the peaks at 1081 and 1052 cm⁻¹ decrease,
- the intensity of the peak at 760 increases while that of the peak at 860 cm⁻¹ decreases.

The remarks under a), d) and e) are analogous to those made when describing the behaviour of the melted and recrystallized samples. They have been interpreted on

the basis of a H-bonds network rearrangement. The further narrowing of the band of OH stretching suggests that milling evens the H-bonds more than melting does. The remarks under b), c) and f) are related to stretching (b and c) and rocking (f) of methylene groups and can be explained by allowing for the presence, in the milled samples, of CH₂ groups in strained cyclic systems.

Finally, it has to be noted that some of the mentioned spectral changes occurring by milling, disappear by mild annealing. Indeed the evidence of CH₂ groups in strained cyclic systems disappears after 1 h annealing at 120°C (last row of Table 2).

XRD evidence

Figure 5 reports the XRD patterns of a sample C at *r.t.* (5A), heated up to 100°C (5B), 150°C (5C) and after it has been cooled down to *r.t.* (5D). Apart from some minor differences in the $2\theta=15-20^\circ$ range, spectrum 5B is similar to spectrum 5A. Sensible differences arise in spectrum 5C and this piece of information agrees with DSC evidence: the transition has taken place leading to a different crystal phase. The spectrum of the same sample after cooling down to *r.t.* (5D) is again very similar to 5A that indicates, in agreement with DSC evidence, the phase transition is a reversible process provided the sample did not undergo melting. It is to note that a XRD spectrum recorded at *r.t.* on a sample kept at 150°C for 7 h is practically indistinguishable with respect to 5D (where the annealing at 150°C was only 10 min). Thus a prolonged annealing at 150°C, which has been seen to trigger changes in both transition and melting enthalpies (determined by DSC), fails to produce significant structural variations.

Figure 6 reports XRD patterns of samples milled for different times. In some instances (spectra 6B and 6D) the sample, after being milled, has been annealed for 10 min before the spectrum has been taken. The spectrum of the sample milled 60 min (6A) is similar to that of sample C (Fig. 5A) though the peaks are less intense due to

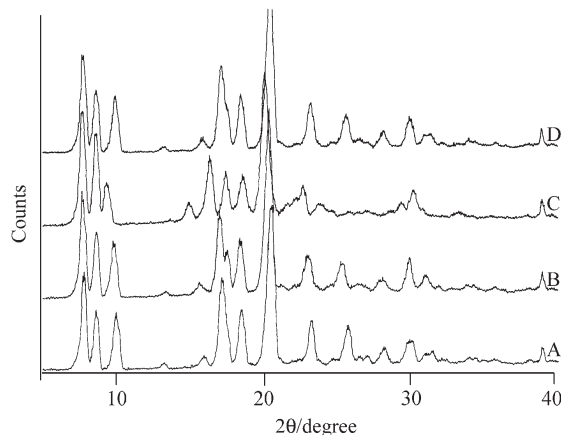


Fig. 5 XRD spectra of sample C. They have been collected at: A – room temperature; B – 100°C (10 min); C – 150°C (10 min); D – sample of spectrum (C) after cooling at room temperature

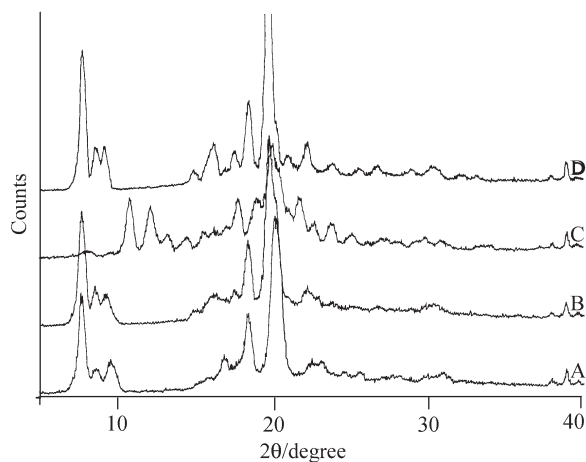


Fig. 6 XRD spectra of milled samples. They have been collected on samples: A – milled 60 min (25°C); B – milled 60 min then annealed at 110°C; C – milled 300 min (25°C); D – milled 300 min then annealed at 120 °C

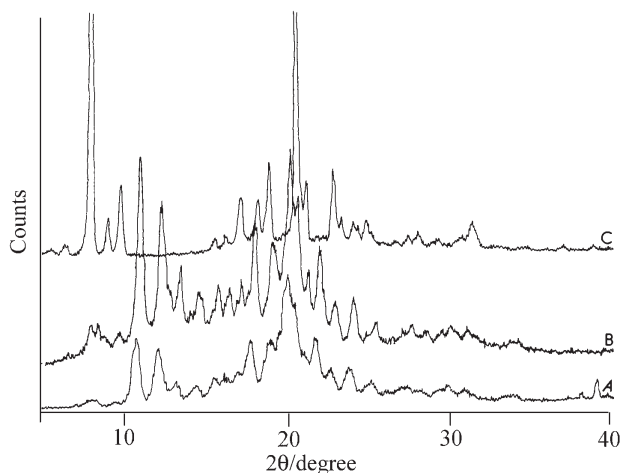


Fig. 7 XRD spectra collected on: C – melted sample; B – sample melted and afterwards milled 300 min; A – sample milled 300 min

the smaller grain size. In the case of short-milled samples FT-IR/PAS experiments did not show noticeable changes whereas DSC showed quite significant differences in both transition and melting enthalpies. The spectrum of the sample ball milled at for 60 min and then annealed at 110°C (6B) is similar (if the general diminished peak intensities are discounted) to that recorded on a commercial sample annealed at 150°C (5C). This emphasizes that solid state transition can occur also at $T < 150^\circ\text{C}$ if

thermal energy (provided by annealing) is compensated for mechanical energy (by milling). Spectrum 6C shows the structural consequences of long-time milling (300 min) while spectrum 6D brings into evidence that even a mild annealing (120°C) cancels out the changes seen in spectrum 6C. This has been previously reported under DSC section: by annealing a milled sample, a DSC curve is obtained similar to that of a melted and recrystallised sample. This has been interpreted as being due to the disruption of the strained cyclic systems that form (FT-IR/PAS experiments) by prolonged (≈ 7 h) milling.

Figure 7 reports the spectrum (7C) of a melted sample M that (DSC and FT-IR/PAS evidence), it is a different polymorphic form of dipyridamole. The same figure reports the spectrum of a melted sample that has been milled for 300 min (7B) and that of the sample milled 300 min (7A). From the comparison between these two spectra it can be seen that melting brings about structural changes that can be substituted for by other ones caused by prolonged milling.

Discussion

XRD measurements brought into evidence four different 'structural patterns'. Namely that of the commercial sample (C), of the milled sample (BM), of the solid state transitioned sample (TS) and of the melted and recrystallised sample (M). Three of them (C, BM and M) can be obtained at room temperature while TS is only visible at 150°C and it is likely to represent form I of literature indeed Kuhnert-Brandstätter and Wurian [3] found 130°C as the transition temperature from form II to form I. The patterns of sample M can transform, by high energy milling, into those of BM (Fig. 7). These ones, in turn, change as a function of time (Fig. 6). Both these patterns (M and BM) correspond to a metastable phase at *r. t.* Form II of literature is sample C. What does not fit with our findings, is the claim that form I recrystallizes from the liquid phase.

By FT-IR/PAS measurements, three distinct 'molecular patterns' were singled out: the commercial sample (C), the milled sample (BM) and the melted and recrystallised sample (M). The fact that no TS 'molecular pattern' has been obtained is coherent with the reversibility of the transition (all FT-IR/PAS measurements were performed at room temperature). The original indication of the FT-IR/PAS measurements is that both melting and milling are leading to permanent effects in the molecular geometry. It has to be noted that the term permanent has to be taken in a kinetic rather than in a thermodynamic sense since both M and BM represent metastable phases at *r. t.*

It is very likely that dipyridamole solid state transition implies the breaking of intramolecular H-bonds while the intermolecular ones are ruptured by melting. The intramolecular H-bonds can be formed again provided the intermolecular ones have not been destroyed by fusion. Indeed the transition has been seen to be reversible if no melting has taken place. Due to the annealing at 150°C (i.e. next to melting temperature) the intramolecular H-bonds and, though to a limited extent, also some of the intermolecular ones can be broken: this is raising a certain thermal irreversibility mir-

rored by the decreasing both of transition and melting enthalpies. The fact that such a decrease is the same for both enthalpies suggests the existence of a relation between the number of inter- and intramolecular H-bonds which do not form again during cooling. The outlined phenomenology is confirmed from the thermal behaviour of samples M that, during a second heating, do not have the transition peak and show a melting enthalpy 85% of that during first heating. What it is likely to have occurred is that a share of the C–O–H···N intramolecular H-bonds transforms to intermolecular bonds that are however weaker than the C–O–H···O–C ones. This change of H-bonds network could also account for the sensible decrease ($\sim 4^\circ\text{C}$) of the fusion temperature during second heating.

Finally, both milling and annealing act as to decrease melting temperature and enthalpy but show different effects on the transition peak. Indeed a prolonged annealing at 150°C produces a decrease of transition enthalpy while a prolonged milling seems to shift the peak (with similar enthalpy) at lower temperature and in a narrower temperature range. It is argued this peak stems from the breaking of the strained cyclic systems whose presence is seen by FT-IR/PAS spectroscopy. The enthalpy of this peak, which is nearly the same of the vanished transition peak, suggests that all intramolecular H-bonds have been transformed into intermolecular H-bonds of the cyclic systems.

The irreversibility of the solid state transition arises from the intervention of the liquid phase. Indeed its enhanced conformational freedom yields, by recrystallisation, a metastable phase at *r. t.* The same kind of conformational changes can be obtained either by prolonged annealing at 150°C or by high energy milling. Indeed the grain size reduction induced by milling allows to produce the same conformational changes with a lower energy expense than in large particle aggregates.

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