The study of the polymorphic system of 2-chloro-4-nitrobenzoic acid†

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The solid-state behaviour of 2-chloro-4-nitrobenzoic (**2c4n**) acid has been reinvestigated. Infrared spectra, differential scanning calorimetry (DSC), and thermomicroscopy investigations as well as X-ray powder patterns for two modifications of the substance are described. Modification II is the thermodynamically stable crystal form from absolute zero to its transition point which occurs at approximately 97 °C according to the DSC analysis. Above that temperature, the material transforms to modification I which undergoes dramatic evolution of its lattice parameters with increasing temperature. Synchrotron powder diffraction measurements confirm the existence of two polymorphic forms. Both modifications have been structurally characterized by single-crystal X-ray diffraction. The acid molecules in both crystal structures are organized into $R_2^2(8)$ dimer units at the first graph set level in modification I and at the second level in modification II.

Introduction

The term polymorphism in the field of chemistry describes multicrystalline forms of any given material.[1] Polymorphs can differ in their chemical, physical and biological properties. This variety can be beneficial or detrimental to the pharmaceutical industry. Changes of the solubility and the dissolution rate accompanying changes in crystal form can affect the bioavailability of a drug compound. In this connection, the case of Ritonavir, Abbott Laboratories' drug for patients with AIDS, is a representative example of new polymorph appearing with serious financial and public relations consequences.[2] In 1998, a lower energy, more stable polymorph (form II) appeared, causing slowed dissolution of the marketed dosage form and compromising the oral bioavailability of the drug. This event forced the removal of the oral capsule formulation from the market and a year long development of a new formulation. There are many other cases where insufficient exploration of possible crystallization and interconversion conditions also caused serious delays in market launch and losses of revenue. Hence, the understanding and control of polymorphism and polymorphic behaviour is of considerable fundamental and practical importance.

In 1952 Ebert and Gottlieb were the first to show that infrared spectrometry could be a useful tool for the study of polymorphism in organic compounds.[3] One of the examples of its use was 2-chloro-4-nitrobenzoic acid (**2c4n**) (Fig. 1) which is known today as a potentially novel therapy for immunodeficiency diseases, including HIV infection.[4,5] This substance was obtained by them in two polymorphic forms: modification I was crystallized from benzene while modification II was produced *via* slow cooling from the melt. The two forms exhibited appreciable differences in their infrared spectra.

Subsequently the system was studied by Kuhnert-Brandstätter and Riedmann[6] who used hot-stage microscopy, DSC and solid FT-IR to identify and characterize two polymorphic forms. The compound crystallized concomitantly as a mixture of crystals of two polymorphs, modifications I and II. Modification II transformed to modification I between 60 and 90 °C. No structure determinations were carried out.

In a reinvestigation of the crystal chemistry of this system, our goals were to crystallize the two earlier reported polymorphs and to characterize them in considerable detail by a variety of methods including single-crystal analysis, thermal studies and spectroscopic (FT-IR) analysis. Temperature-dependent powder X-ray diffraction (PXRD) and differential scanning calorimetry (DSC) have been used to understand the nature and thermodynamics of the polymorphic transition. We also report here the determination of the crystal structures of both polymorphic forms.

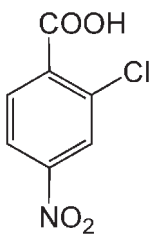

Fig. 1 Molecular structure of 2-chloro-4-nitrobenzoic acid (**2c4n**).

Experimental

Material

The studies of 2-chloro-4-nitrobenzoic acid were carried out using the commercial product provided by Aldrich. Samples of

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† Electronic supplementary information (ESI) available: X-Ray crystallographic information files (CIF) of modification I and II, SPXRD refinements of modification I at temperatures of 25, 83, 88, 104, 129 °C and DSC measurements for determination of the melting point of modification II. CCDC reference numbers 698967, 698968 and 700780–700784. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b812017j

the compound were purchased twice from the company with different lot numbers: S14087-034 and S42781-337.

Single-crystal X-ray diffraction

Single-crystal crystallographic data were collected at room temperature on a Bruker Smart 1000 K diffractometer using Mo-Kα radiation (λ = 0.71073 Å) with a graphite monochromator. All the heavy atoms were located in the direct methods solution. The hydrogen atoms of the benzene ring were calculated and the hydrogen atom of the carboxyl group was located from the Fourier difference map. The data were reduced by SAINT[7] while absorption corrections were applied using SADABS.[8] The structure was solved using SHELXS,[9] and then refined with SHELXL[10] in SHELXTL.[11]

Laboratory powder X-ray diffraction (PXRD)

X-Ray powder data were collected on a Huber Imaging Plate camera. Cu-Kα_1 radiation (λ = 1.5406 Å) was produced by a Rigaku rotating anode generator, operated at 40 kV and 90 mA.

Synchrotron powder X-ray diffraction (SPXRD)

High-resolution synchrotron X-ray powder diffraction patterns were collected at the X16C beamline at the National Synchroton Light Source at Brookhaven National Laboratory. X-rays of wavelength 0.6986 Å were selected with a Si(111) channel cut monochromator. After the sample was mounted, the diffracted beam was analyzed with a Ge(111) crystal and detected by a NaI scintillation counter. Wavelength and diffractometer zero were calibrated using a sample of NIST Standard Reference Material 1976 (a sintered plate of Al_2O_3). The sample, sealed in a glass capillary, was loaded in a locally made heating stage. We observed a significant temperature difference between sample and thermocouple, and therefore the melting point of a sample of Ibuprofen was used to calibrate the temperature over the same range. The correction applied was $T_{sample} = 22 + 1.12\,(T_{thermocouple} = 22\,^{\circ}C$; the values are in Celsius). All fits to the data were performed using the program TOPAS Academic.[12]

FT-IR

FT-IR measurements were performed on KBr disks using a Nicolet Impact 410 spectrometer.

Differential scanning calorimetry (DSC)

DSC investigations were performed on a Mettler 820. All the measurements, except a determination of the melting point of modification II, were run with heating and cooling rate of 2 °C min^{-1} in sealed Al pans. To detect the melting point of modification II we used a faster heating rate of 10 °C min^{-1} in order to avoid the phase transition.

Hot stage microscopy (HSM)

HSM examinations were carried out on a Wagner and Munz Kofler Hot Stage equipped with digital video recording facilities.

Results and discussion

Preparation of crystals

The powder of 2-chloro-4-nitrobenzoic acid of pale yellow color as first obtained from Aldrich (lot number: S14087-034), was characterized by FT-IR spectroscopy as modification I (Fig. 2(a)).[3,6] The substance was recrystallized from a variety of solvents at room temperature. All crystallization conditions except water as a solvent resulted in the formation of modification II (Fig. 2(b)). Aqueous solutions resulted in the crystallization of the two polymorphic forms concomitantly.[13] Fig. 2(c) shows thin needles of modification II and smaller crystals which belong to modification I organized in a cluster.

For additional crystallizations the material was purchased again from Aldrich but this time with a different lot number (S42781-337). At first sight the commercial compound appeared the same pale yellow powder; however when examined by PXRD it turned out to be a mixture of the two modifications, in which modification II is the dominant one. Experimental diffractograms of the two commercial powders and calculated patterns from single crystal structures are shown in Fig. 3.

Thermal analysis

The DSC of modification II of **2c4n** exhibits two endothermic peaks (Fig. 4). The first peak at 97.1 °C corresponds to the phase transition from modification II to modification I which is not observed in the hot-stage microscopy. The second large peak on the DSC curve is due to melting of modification I at 141 °C.

As reported in the paper of Ebert and Gottlieb,[3] cooling of the melt results in the crystallization of modification II, which melts at 128 °C. Further, in order to verify the melting point temperature of modification II, a sample of the second polymorph was run on DSC with a faster heating rate of 10 °C min^{-1} to avoid the phase transition. According to the heat-of-transition and heat-of-fusion rules of Burger–Ramberger,[14] the heat of transition is positive for enantiotropic transitions from the low-temperature to the high-temperature form, and negative for monotropic transitions. In other words, if the phase transition is endothermic, as in our case, these two polymorphic forms are enantiotropically related with a very high probability.

The melting point according to the Aldrich catalog is 136–140 °C and is indeed that of modification I. But as noted above, a melting point determination of modification II will result in the same melting point as modification I due to the phase transition. This is another demonstration that one technique may often not be sufficient to identify or characterize a polymorphic system.

The thermodynamic behavior of this system may be summarized by preparing the qualitative energy *vs* temperature diagram for an enantiotropic dimorphic system[15] (Fig. 5). The energy/temperature diagram is constructed from the isobars of the Gibbs free energy G and of the enthalpy H of the two modifications, I and II, as well as of the melt (*liq*). At a given temperature, the thermodynamically stable modification has the lowest Gibbs free energy. Fig. 5 clearly demonstrates that

Fig. 2 Photographs of 2-chloro-4-nitrobenzoic acid polymorphs: (a) modification I, (b) modification II and (c) modifications I and II crystallized concomitantly.

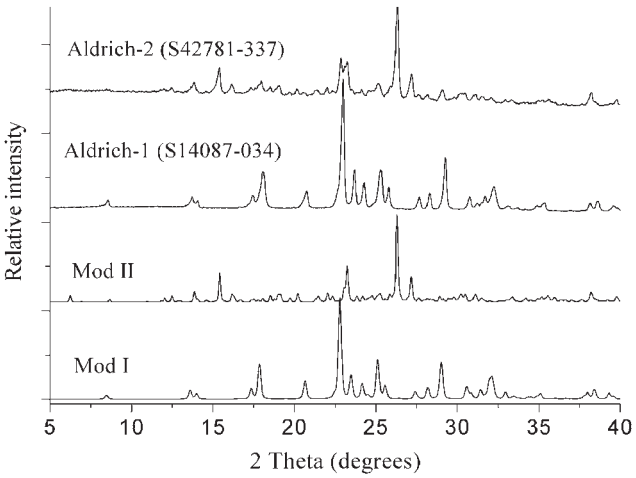

Fig. 3 Experimental diffractograms of Aldrich-1 (S14087-034) and Aldrich-2 (S42781-337) and the calculated powder diffraction patterns of modifications I and II. Data collected with Cu-Kα radiation on the laboratory source.

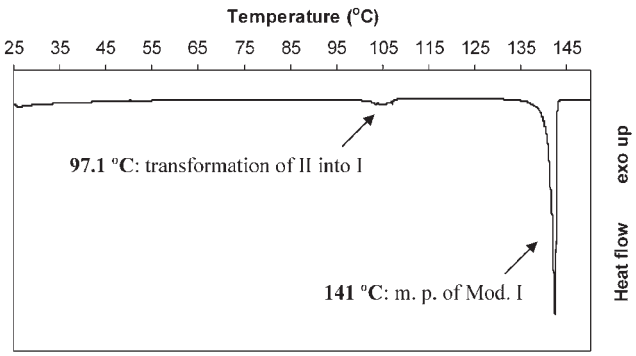

Fig. 4 DSC curve of modification II during heating and phase transition to modification I. The heating rate is 2 °C min^{-1}.

the relative stability at low temperature is II > I. Since the two forms are related enantiotropically, the *G* isobars of two crystal forms cross at the transition point (T_p) (at 97.1 °C), and the transition is reversible. ΔH_t represents the enthalpy of transition for the transformation II/I and ΔH_f the enthalpies of fusion for modification I and II, respectively. Since this is a concomitant polymorphic system, the enthalpy difference

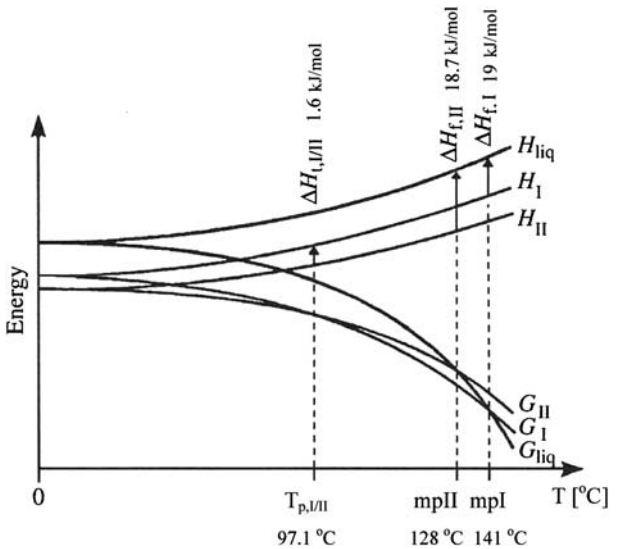

Fig. 5 Suggested schematic energy/temperature diagram for the **2c4n** enantiotropic dimorphic system.

between two polymorphs is expected to be small[13] as was found experimentally by the DSC.

IR spectroscopy

As suggested by Ebert and Gottlieb[1] modification I and modification II could be easily distinguished using FT-IR. Expanded solid FT-IR spectra are shown in Fig. 6. The significant differences were found in the area of 1200–1600 cm^{-1} in which vibration of the aromatic C=C bond occurs. The peaks that characterize the two polymorphic forms are summarized in Table 1.

Laboratory X-ray powder diffraction

The XRPD of modification II was examined as a function of temperature in a sealed capillary (Fig. 7). The diffraction was measured every 10–15 °C in the temperature range 25–60 °C, with no evidence for transformation to modification I. At ~75 °C it was possible to recognize the initiation of a phase transition. At 90 °C the transformation from modification II is completed; however the diffraction pattern shows significant differences from the room-temperature pattern of modification I. Subsequent cooling of the sample to room temperature gives

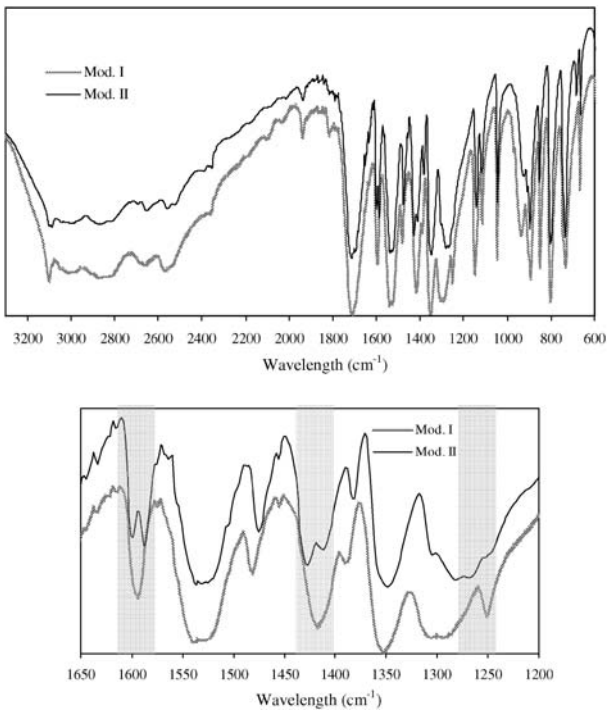

Fig. 6 FTIR spectra for KBr tablets of modification I and II, for full (upper) and limited, characteristic range (1200–1650 cm^{-1}). The grey-shaded regions are convenient for distinguishing the two forms (see text and Table 1).

the powder pattern of modification I consistent with that calculated from the single-crystal structure.

Due to the dramatic motion of the peaks through the range 70 to 100 °C, the thermal behavior of the powder diffraction pattern of the two modifications was examined on the synchrotron to determine if there is an additional phase between modification I and II. A powder sample of modification II was sealed inside a thin-walled glass capillary of 1.5 mm nominal diameter. The sample was heated in a furnace and the temperature monitored by a thermocouple placed near the sample. Diffraction data over the angular range of 6–7.5° 2θ were taken at several temperatures as shown in Fig. 8; peaks at 6.95, 7.30 and 7.35° 2θ indicate that the sample is pure modification II. Significant changes in the diffraction pattern were first noted when the sample reached 83 °C, and so the temperature was held at that value. After the transformation was complete, a full dataset covering the range of 1–30° 2θ was taken. Rietveld refinement of the structure of modification I to the

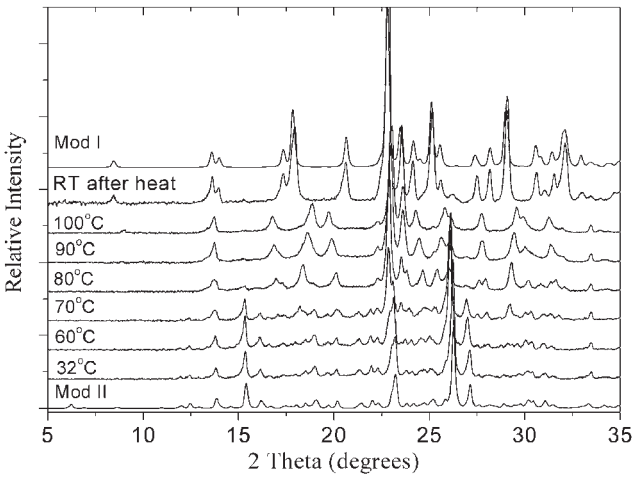

Fig. 7 Variable-temperature laboratory XRPD. Only modification II is present between 32 and 60 °C. The transition to modification I begins at ~80 °C and is completed at ~90 °C. The uppermost and lowermost diffractograms belong to the powder diffractions of the two modifications calculated from the single-crystal structure determinations.

data taken at 83 °C was found to give a satisfactory fit, as shown in Fig. 9. The sample was subsequently cooled back to room temperature, and a dataset covering the range of 6–12° 2θ was taken. These room temperature data were also found to be consistent with the structure of modification I.

The sample, having been transformed from modification II to modification I by heating, was again studied with *in situ* measurements performed during the heating process. Powder diffraction patterns were collected over 2θ values of 2–12° during two heating cycles at temperatures from 25 to 154 °C, as shown in Fig. 10. The lattice parameters at each temperature were determined with a Pawley fit using the lattice of modification I, and are shown in Fig. 11. Between room temperature and the melting point, the *b* axis grows and the perpendicular *a* axis shrinks significantly, but the volume changes by a much smaller fraction. Full SPXRD patterns (extending from 1–30° 2θ) of modification I were measured at temperatures of 25, 83, 88, 104 and 129 °C; all refinements are consistent with the structure of modification I. Having melted the sample, the heater was turned off and the sample quenched to room temperature over an interval of approximately 30 s. Subsequent measurement of the cooled sample showed that it consisted of modification II, although the granularity of the diffraction pattern made a refinement impossible.

Table 1 Solid FT-IR assignments and comparison of wavenumber peaks (cm^{-1}) of the polymorphs of 2-chloro-4-nitrobenzoic acid

Assignment	Modification I	Modification II
O–H stretching vibrations	3100–2500	3100–2500
C–H stretching vibrations	3103	3095, 3103
C=O stretching vibrations	1716	1718
Asymmetric and symmetric vibrations of NO_2	1541, 1356	1529, 1352
Aromatic C=C stretching vibrations	1595, 1419	1601, 1589, 1429, 1414
C–H in-plane bending vibrations	1149, 1115	1142, 1118
C–Cl stretching	1047	1045
O–H deformation vibrations	939, 893	928, 897
C–H out-of-plane bending vibrations	804, 735, 667	802, 737, 683, 663

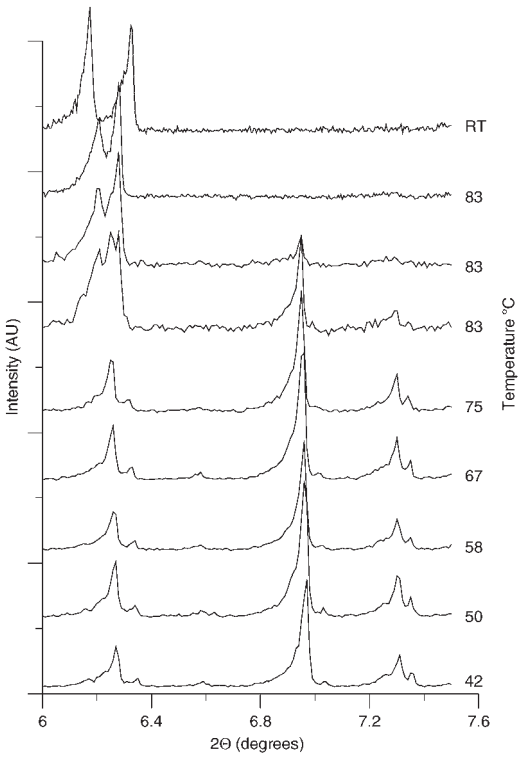

Fig. 8 Synchrotron powder diffraction patterns taken during the heating of modification II. The transformation at 83 °C can be seen in the three patterns at that temperature which are roughly 4 min apart in time. The top pattern was taken at room temperature after the transformation was complete.

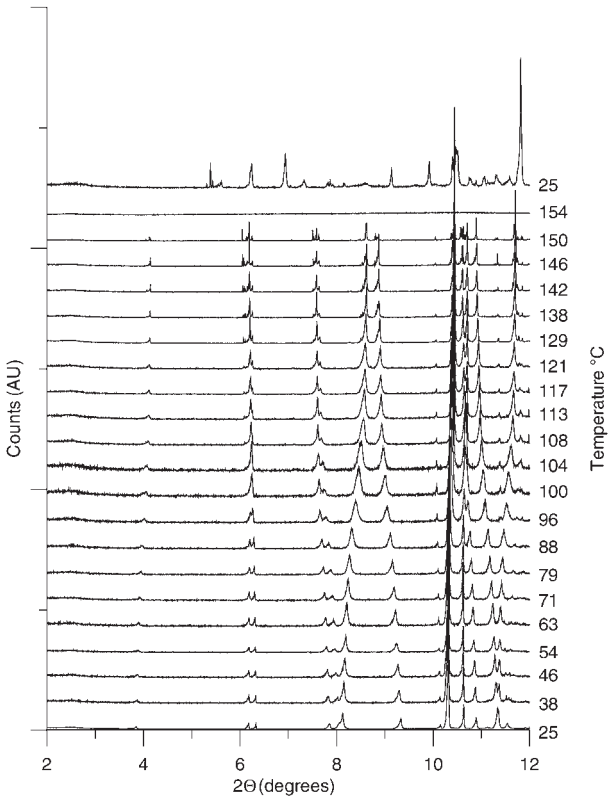

Fig. 10 Synchrotron powder diffraction patterns taken during the heating of modification I. The continuous evolution of the lattice parameters can be seen, although no changes suggestive of a phase transformation are observed.

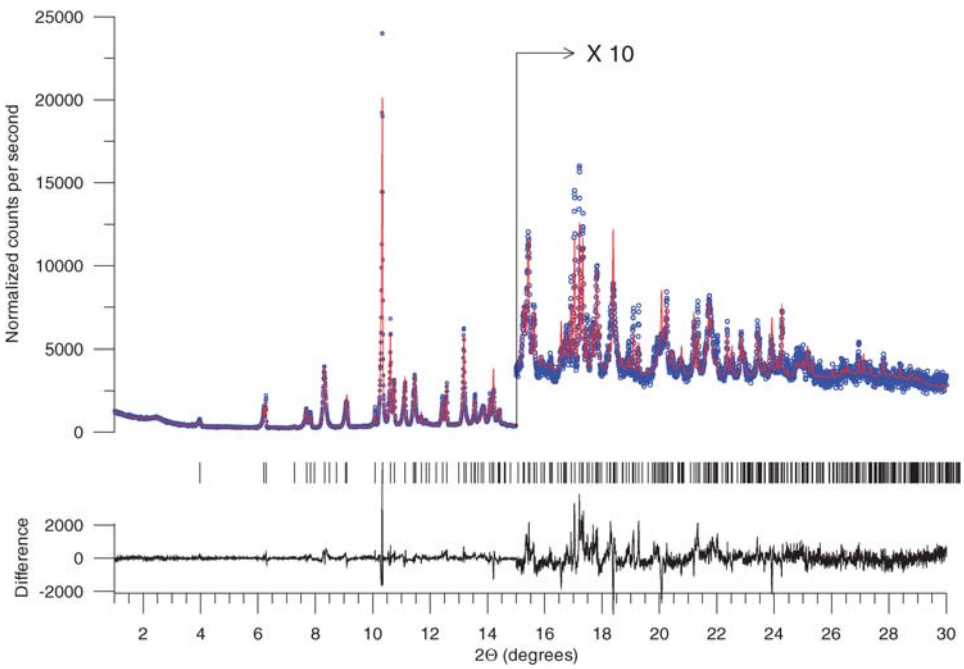

Fig. 9 Rietveld fit of the structure of Modification I to the synchrotron PXRD data taken at 83 °C, immediately after transformation from Modification II. Open circles are the data, the solid line is the calculated pattern. The curve below shows the difference between the two and the tick marks indicate allowed peak positions.

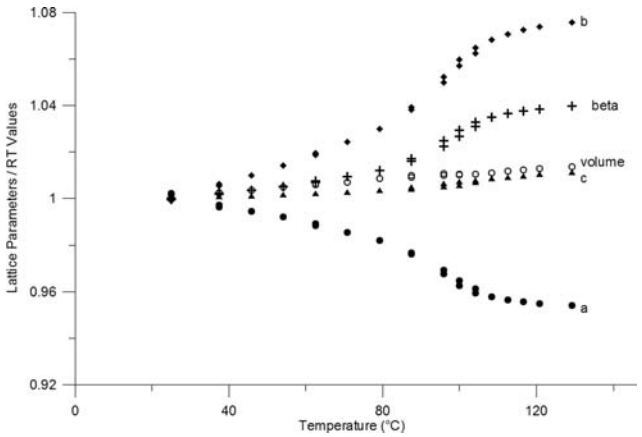

Fig. 11 Lattice parameters and cell volume of Modification I normalized to the values at room temperature. Note the change in slope in the vicinity of 90 °C. Filled circles are the *a* lattice parameter, diamonds are the *b* lattice parameter, triangles are the *c* lattice parameter, plus signs are the angle β, and the open circles are the unit cell volume.

Single-crystal analysis

The crystal structures of the two modifications were determined from crystals grown concomitantly in aqueous solution. Crystallographic data of the two polymorphs are given in Table 2. Both polymorphs crystallize in space group $P2_1/c$ but with different number of molecules in the asymmetric unit. The asymmetric unit of modification I is comprised of a single **2c4n** molecule. However in modification II $Z' = 4$. The structure and atomic numbering for modification I of **2c4n** are given in Fig. 12. The most significant difference in torsion angles between four molecules of the second polymorph (Table 3) is in the rotation of the nitro group with respect to the aromatic ring in molecules C and D. In both molecules the $-NO_2$ group is significantly out of the plane of the benzene ring. The dihedral angles between the planes are 19.82 and 11.70°, respectively. On the other hand, the nitro group in molecule B makes a small angle of 1.24° with the benzene ring plane. In the case of molecules C and D, the lack of coplanarity of $-NO_2$ substituent with the ring is essentially due to a twist about the C4–N1 bond (Table 3).

Table 2 Comparison of crystallographic data obtained for both polymorphic forms

	Modification I	Modification II
T/°C	25	25
Color	Pale yellow	Pale yellow
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/c$	$P2_1/c$
a/Å	10.833(3)	14.209(10)
b/Å	5.857(14)	29.430(2)
c/Å	13.497(3)	7.735(5)
β/°	105.734(4)	93.619(12)
V/Å^3	824.3(3)	3228(4)
Z (Z')	4 (1)	16 (4)
D_c/Mg m^{-3}	1.624	1.659
F(000)	408	1632
Reflections collected	5010	20198
Reflections used	1922	7496
R_1; R_w	0.046; 0.132	0.047; 0.100

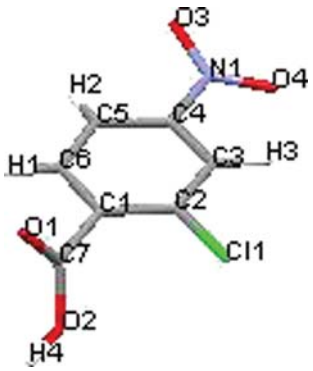

Fig. 12 Structure of **2c4n** in modification I together with the labeling scheme.

The calculated density of modification II is 1.659 Mg m^{-3}, while that of modification I is 1.624 Mg m^{-3}. According to the density rule of Burger and Ramberger[16]—*if one modification of a molecular crystal has a lower density than the other, it may be assumed to be less stable at absolute zero*. This is in agreement with the observation that modification II is the stable form at lower (room) temperature.

Packing diagrams and hydrogen bonds for both modifications are given in Fig. 13. In modification I the benzoic acid dimer creates a motif with graph set[17] $R_2^2(8)$. The two hydrogen bonds in the motif are equivalent by crystallographic inversion symmetry, so the motif appears at the first level.[18] In modification II the two hydrogen bonds are not crystallographically equivalent. The first-level graph set of the second polymorph that involves the two kinds of hydrogen bonds is therefore *D*. The $R_2^2(8)$ pattern appears at the binary level (Fig. 13).

Summary

We have reinvestigated the crystal growth, thermal and spectroscopic properties of the polymorphs of 2-chloro-4-nitrobenzoic acid. In addition, their crystal structures have been determined by single-crystal X-ray methods. modification I appeared concomitantly with modification II in water and also as a pure form in the first commercial sample. Modification II readily crystallizes from various solvents at room temperature. This is also the stable form according to the Burger–Ramberger rules.[14,16]

In this investigation we also studied the thermal behavior of the **2c4n** forms by a number of methods. The information obtained was not always compatible among all techniques. Hot stage microscopy is an important tool for understanding the thermal properties of a system, but alone it is not always a reliable method for polymorph identification. For this reason it is important to use other complementary thermal methods such as DSC and variable-temperature PXRD analysis as discussed here. These two techniques, especially variable-temperature PXRD performed on a synchrotron gave us a detailed picture regarding the system's thermal behaviour. It was demonstrated that modification I has a very strong thermal expansion anomaly around 90 °C upon heating. The modification I PXRD has a different appearance at low and high temperature, but the lattice parameters evolve

Table 3 Torsion and dihedral angles (°) of modifications I and II

		Mod. II			
	Mod. I	Molecule A	Molecule B	Molecule C	Molecule D
–NO_2–benzene ring	19.29	4.51	1.24	19.82	11.70
O3–N1–C4–C5	19.90	3.10	−3.12	−19.37	11.49
O4–N1–C4–C3	18.30	5.28	0.78	−18.64	11.23
O3–N1–C4–C3	−159.48	176.55	178.64	159.57	−167.66
O4–N1–C4–C5	162.31	175.06	179.01	162.42	−169.61
–CO_2H–benzene ring	70.21	34.45	35.40	36.94	30.20
O2–C7–C1–C6	107.43	33.34	−144.94	−36.40	149.50
O1–C7–C1–C2	111.57	32.50	−144.59	−35.87	149.61
O2–C7–C1–C2	−72.03	−150.14	35.54	146.84	−31.32
O1–C7–C1–C6	−68.98	−144.01	34.93	140.90	−29.57

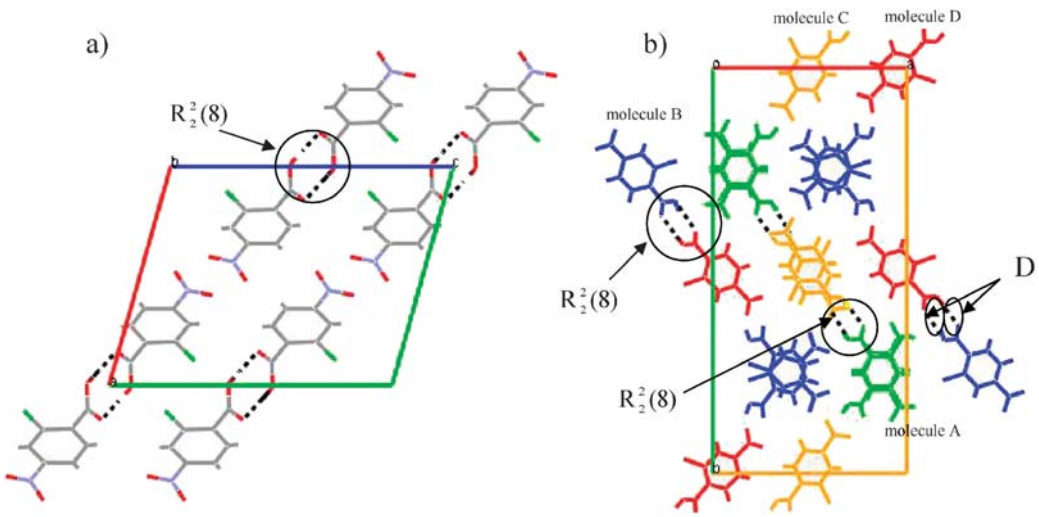

Fig. 13 Hydrogen bonds in modification I (a) and II (b) including graph sets. $R_2^2(8)$ appears at the first level in modification I and at the second level in modification II. The two *D* motifs at the first level in modification II are crystallographically independent (see text). The colors indicate different molecules in the crystallographic asymmetric unit.

continuously with temperature, and the space group with the molecular packing remains the same. The conclusion is that this is a continuous evolution and not an additional phase of the **2c4n** compound.

Acknowledgements

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