

Characterization of Physical Forms of Donepezil Hydrochloride

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Abstract

Different crystal forms and amorphous form of donepezil hydrochloride were prepared and characterized, using various analytical techniques. Hydrate form I is stable only at lower relative humidity (RH), while at higher RH it sorbes additional water. Forms II, III and V were identified as polymorphs, whereas form III is thermodynamically stable. Form V was prepared by heating of hydrate IV at high temperature (about 150 °C). Three different forms of hydrate IV were obtained and characterized. Differential scanning calorimetry and X-ray powder diffraction are the most promising analytical methods for differentiation between crystal forms, both polymorphs and hydrates. Methods could be also used in determination of physical form of a drug, incorporated into solid dosage form. Thermal methods explain the most significant difference in dehydration of different forms IV: transformation to pure polymorphic forms or mixture thereof. On heating of hydrates I and IV-1, two novel crystal forms were prepared and characterized, namely crystal forms VII and VIII.

Keywords: donepezil hydrochloride, polymorphs, hydrates, characterization

1. Introduction

Majority of pharmaceutical solids, both active ingredients and excipients, exhibit a phenomenon of occurrence in different unsolvated and solvated crystal forms by the same solid substance, namely polymorphism and pseudopolymorphism.^{1–3} A novel, supramolecular definition of polymorphism defines it as »the existence of more than one type of network superstructure for the same molecular building blocks«, and pseudopolymorphism as the case where the solvent is one of molecular components in the network.⁴ Pseudopolymorphism refers to solvated forms, where solvent in crystal network of majority of pharmaceuticals is water. Hydrates are further classified according to the nature of release of water from the crystal lattice as stoichiometric (crystal lattice collapses upon dehydration) and non-stoichiometric hydrates (crystal lattice is essentially retained upon dehydration) and can be used in description of transformations of crystal forms during technological processes, e.g. crystallization, milling/grinding, granulation, drying, tableting, and rarely in film coating.⁵

Characterization of solid active ingredient in bulk and solid dosage form is very important in quality assurance of final product. The most frequent analytical methods are differential scanning calorimetry (DSC), thermo-

gravimetry (TGA), moisture sorption/desorption isothermal analysis, infrared (IR) and Raman spectroscopy, X-ray powder diffraction (XRPD), solubility, microscopy, solid-state nuclear magnetic resonance (ss-NMR), and should be used in a proper combination to determine the most important differences between various forms.⁶

Donepezil (D), (\pm)-2,3-dihydro-5,6-dimethoxy-2-((2-(phenylmethyl)-4-piperidiny)methyl)-1H-inden-1-one, is piperidine-based, long-acting reversible acetylcholinesterase (AChE) inhibitor that exhibit a relatively high degree of selectivity for neuronal AChE. It increases a level of acetylcholine (ACh) in the brain of patients with mild to moderately severe Alzheimer's disease (AD).^{7–9}

The most obvious neurochemical change observed in patients is rigorous deficiency of choline acetyltransferase, which is responsible for the synthesis of ACh.

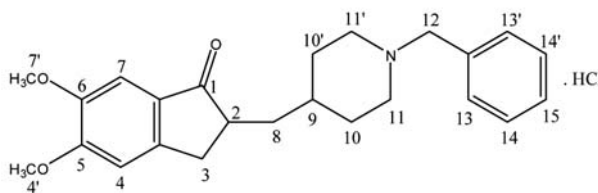


Figure 1. Chemical structure of donepezil hydrochloride.

Between several therapeutic approaches, inhibition of cholinesterase has been most investigated⁹. Symptomatic treatment of AD may at least maintain function in activities of daily living¹⁰.

In solid dosage forms, (D) is in the form of donepezil hydrochloride (DH)^{7–9} (Figure 1). Besides (DH), another halogenide salt, donepezil hydrobromide has been studied, where six physically distinct forms were characterized: four polymorphs, hydrate and amorphous form¹¹. Organic salts of (D) were also obtained by use of tosyllic, mesyllic, benzoic, salicylic, tartaric, citric acid and the like, and are described to be more chemically stable than (D) and (DH) in solution by exposure to light (1000 Lux, 1 month) and temperature (60 °C, 1 month), subsequently¹².

Different crystal forms of (DH) have been prepared and partially characterized. Five crystal forms (I, II, III, IV and V) and amorphous form were characterized by melting point, XRPD and IR spectroscopy. From the literature data on water content it can be concluded, that forms I and IV can be described as monohydrate and trihydrate, respectively. Crystal forms I–IV are preferential over amorphous form due to their chemical stability if exposed to temperature (–20–80 °C) up to 3 month and several RH (10.6–100%) at controlled room temperature¹³. Crystal form VI was prepared from donepezil oxalate¹⁴. Further, another four crystal forms (H1, H2, monohydrate, sesquihydrate) were prepared and characterized by XRPD¹⁵.

However, different crystal forms (A, B and C) were obtained and characterized also in (D)¹⁶.

In the present article, preparation and physical characterization of different crystal forms and amorphous form of (DH) is highlighted. In order to assure clearness of nomenclature, »polymorph« and »polymorphic form« refer only to anhydrous crystalline forms, while terms »hydrate« and »hydrate form« refer to hydrated crystalline forms.

2. Experimental

2.1. Materials

(D) and (DH) were obtained by Krka, d.d., Novo mesto (Slovenia). Polymorphs II, III and hydrates IV-1 and IV-2 were prepared by crystallization of (DH) from different solvents. Hydrates I and IV-3 were prepared by *in situ* formation of (DH) from (D) by addition of hydrochloric acid. Polymorph V was prepared by heating of hydrate forms IV (dehydration). Amorphous form was prepared by freeze-drying¹³. Crystal forms VII and VIII were prepared by heating of hydrates I and IV-1 on 90 °C.

Solvents used for crystallization were of analytical grade and used as received. Hydrochloric acid was obtained by Merck (Germany). Solid carbon dioxide was obtained by Messer (Slovenia).

2.2. Methods

Thermal properties of physical forms were characterized, using Mettler Toledo DSC822e differential scanning calorimeter and Perkin Elmer TGA-7 thermogravimetric analyzer. DSC curves were recorded under nitrogen atmosphere at heating rate 10 °C/min in temperature range 30–250 °C.

Particle size distribution was measured on Malvern Mastersizer S/Femtosystems. Samples were dispersed in sunflower oil.

Hot stage microscopy was performed on Mettler Toledo termosystem FP900 with FP82HT hot-stage cell.

Mid-IR spectroscopy was obtained on Perkin Elmer FT-IR Spectrometer 1720X in range 400–4000 cm⁻¹ (resolution 4 cm⁻¹) as KBr pellets.

Near-IR spectroscopy was performed on Perkin Elmer FT-IR Spectrometer GX in range 10000–4000 cm⁻¹ (resolution 8 cm⁻¹).

Raman spectroscopy was performed on Perkin Elmer FT-IR Spectrometer GX in range 4000–100 cm⁻¹ (resolution 4 cm⁻¹).

X-ray powder diffraction (XRPD) analysis was performed on powder diffractometer Philips X'pert Pro under controlled conditions (CuK α radiation, $\lambda = 1.54187 \text{ \AA}$, 2 θ range 2.9280–31.0615°, step 0.0167° 2 θ).

Inverse gas chromatography (IGC) was tested in arranged gas chromatograph Agilent Technologies 6890N by the following adsorptives: methane, n-hexane, n-heptane, n-octane, n-nonane, n-decane, tetrahydrofuran (THF), acetone, ethylacetate and chloroform.

True density was determined by Accupyc 1330 helium pycnometer.

Dynamic vapour sorption (DVS) was studied on automatic water sorption analyzer DVS-1 (Surface Measurement Systems Ltd.) under following conditions: controlled room temperature (25 °C), nitrogen flow: 200 mL/min, 2 complete cycles 0–95% RH and backwards in 11 steps, minimal time in 1 step (dm/dt < 0.002%): 10 minutes, maximal time in 1 step: 360 minutes.

Scanning electron microscopy (SEM) was performed on microscope Olympus BX50, connected to CCD camera Sony XC-75CE.

¹³C-solid state nuclear magnetic resonance (¹³C ss-NMR) spectra were obtained by Varian Unity Inova 600 MHz NMR spectrometer, using RAMP/CW technique at 10 kHz.

3. Results and Discussion

DSC curves of polymorphs II, III and V show no difference in basic line until sharp melting, which confirms high crystallinity of samples with low degree of admixed amorphous form (Figure 2). Pure amorphous form is softened at about 120 °C, which was confirmed also by hot stage microscopy. Distinction between individual physical

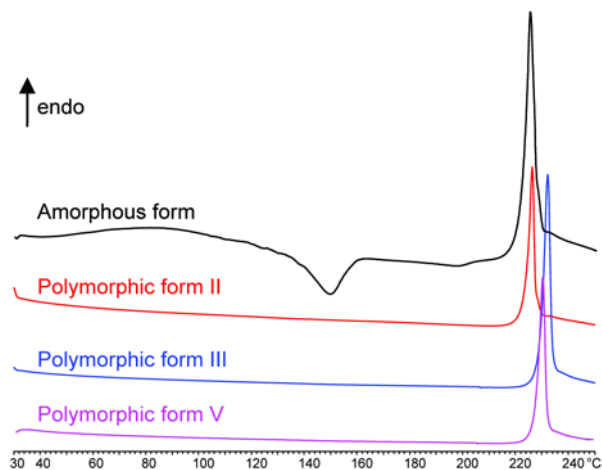


Figure 2. DSC curves of amorphous and polymorphic forms II, III and V.

Table 1: Melting points and enthalpies of transition of amorphous and polymorphic forms II, III and V.

Parameter	Amorphous form	Form II	Form III	Form V
T_{melting} (°C)	221.80*	223.49	229.09	227.62
$\Delta H_{\text{melting}}$ (J/g)	81.61	105.09	125.78	113.85

* melting of crystallized amorphous form

forms is based on melting points for crystal samples and enthalpies (Tables 1 and 2). Amorphous form shows two crystallization exothermic effects, where the first one is much stronger than the second. Broad melting endotherm with a shoulder is observed after both exothermic effects, showing the presence of two crystalline forms. From the comparison of thermal behaviour of crystalline and amorphous form, it can be concluded that forms II and III are present in the crystallized amorphous form.

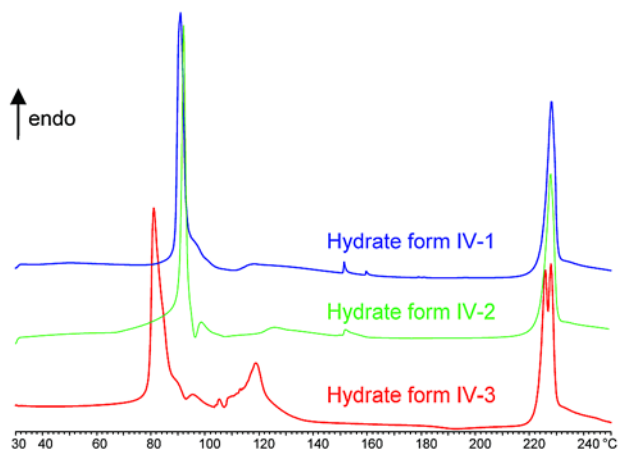


Figure 4. DSC curves of hydrate forms IV-1, IV-2 and IV-3.

Table 2: Dehydration of hydrate forms I, IV-1, IV-2 and IV-3.

Parameter	Hydrate I	Hydrate IV-1	Hydrate IV-2	Hydrate IV-3
$T_{\text{dehydration}}$ (°C)	79.33	88.83	90.44	79.25
$\Delta H_{\text{dehydration}}$ (J/g)	150.55	156.64	162.47	119.63

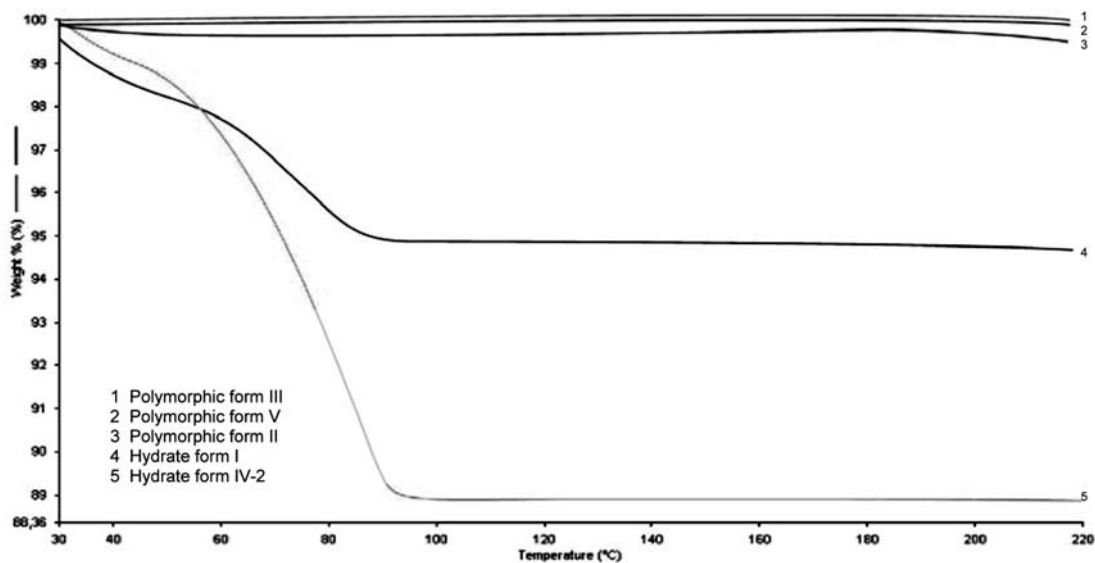


Figure 3. TGA of crystal forms I, II, III, IV-2 and V.

Table 3: Loss of weight in TGA and water content by Karl-Fischer analysis.

Parameter	Hydrate I	Form II	Form III	Hydrate IV-2	Form V	Amorphous form
Δm_1 (%)	1.8 (until 50 °C)	0.3 (until 55 °C)	0.0 (until 200 °C)	1.1 (until 44 °C)	0.2 (until 200 °C)	5.4 (until 100 °C)
Δm_2 (%)	3.4 (50–115 °C)	–	–	9.9 (44–120 °C)	–	–
Water content by KFA (%)	5.34	0.13	0.05	11.24	0.21	5.67
Theoretical water content (%)	4.15	0	0	11.49	0	0

Form III has the highest melting point (229.09 °C) and melting enthalpy (125.78 J/g), which confirms that this form is thermodynamically stable. The other two polymorphic forms are metastable and tend to transform to form III with different kinetics and is influenced by temperature and relative humidity. Lower melting point of these forms show also lower melting enthalpies: form II with melting point 223.49 °C has the lowest melting enthalpy (105.09 J/g) and form V with melting point 227.62 °C and melting enthalpy 113.85 J/g. The absence of water in forms II, III and V was confirmed by TGA, where less than 0.3% loss of weight was observed (Figure 3, Table 3). Loss of weight can be attributed to adsorbed, non-crystalline bound water.

Form I is defined as a hydrate crystal form by stoichiometric ratio of molecules of (DH) to water 1:1 and theoretical water content 4.15%. Further, hydrate form IV is defined by stoichiometric ratio of molecules of (DH) to water 1:3 and water content 11.49%¹³. DSC curves of hydrate forms IV-1, IV-2 and IV-3 (Figure 4) show dehydration until 120 °C, which is confirmed by TGA (Figure 3). Very complex thermal behaviour was obtained by all three forms of hydrate form IV.

Water content at room temperature in form I is 1.3 moles of water on 1 mole of (DH). Water can be sorbed in different ways, while TGA confirms loss of weight in two steps: in lower temperature surface adsorbed water (until 50 °C), and crystal bound water of hydrate mainly at higher temperature (50–115 °C). However, form I can be also defined by total amount of water, determined by Karl-Fischer titrimetric method. It is proposed that form I can

be defined as monohydrate, although it contains additional water (additional hydration), or it can also contain less water than theoretical monohydrate, which can be attributed to partial dehydration of monohydrate, or damaged crystal structure, or to the presence of small amount of hygroscopic amorphous form.

Form IV-2 contains 11–11.24% of water, depending on the method of measurement used, which is in good accordance to theoretical trihydrate (11.49% of water). It is assumed that it does not contain significant amount of surface bound water. Narrow dehydration peak could be attributed to isolated lattice sites of water in crystal form. First loss of weight in TGA (about 1%) is loss of water from sites near the surface of crystals, while the rest of water evaporates up to 100 °C. It does not represent a rapid removal of water from the inner structure which is confirmed by DSC curve with relatively extended dehydration peak. Endothermic peak after dehydration at about 90 °C is connected to transition to polymorph II. Broad endothermic peak after transition to form II is most obvious in dehydrated form IV-3.

Dehydration of form IV-3 is easier and starts at significantly lower temperature (79.25 °C) than at the other forms IV (88.83 °C for form IV-1 and 90.44 °C for form IV-2). Different forms IV are presumed also from experiments, performed by drying in heating process. Forms IV are finally completely converted to pure form V or mixture of form V with additional small amount of form II. This phenomenon is surprising since polymorphic forms II and V are metastable and spontaneously transit to stable form III. However, stability studies confirmed good stability of

Table 4: Particle size distribution of amorphous and crystal forms.

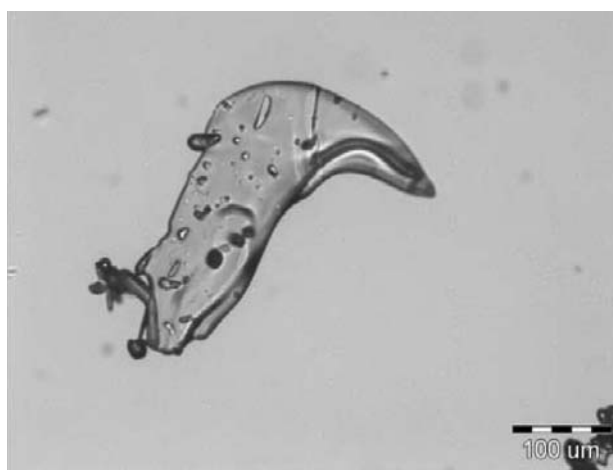
Range (µm)	Particle size distribution (volume %)				
	Amorphous form	Hydrate I	Form II	Form III	Hydrate IV-1
0.02–2.00	0.0	0.6	0.6	0.9	0.9
2.00–5.00	0.0	2.9	1.7	3.2	1.3
5.00–10.00	0.2	6.6	4.7	11.0	1.3
10.00–20.00	2.1	13.8	5.6	22.1	6.5
20.00–50.00	7.4	29.0	22.4	18.7	17.8
50.00–100.00	14.2	21.2	31.0	7.5	25.8
100.00–150.00	13.8	9.1	12.0	9.3	22.6
150.00–500.00	52.9	15.3	20.9	27.1	23.7
500.00–800.00	9.3	1.5	1.2	0.2	0.0
Average size (µm)	241	88	108	95	103

all crystal forms, even if exposed directly to extreme conditions (e.g. 40 °C/75% RH) for period of 10 days; stress with increased both temperature and RH show transformation of metastable forms to thermodynamically stable

polymorph III. Double melting peak of dehydrated form IV-3 could be attributed either to non-homogeneous particle size distribution and/or high proportion of bigger particles (100.00–500.00 µm) (Table 4), or to the presence of



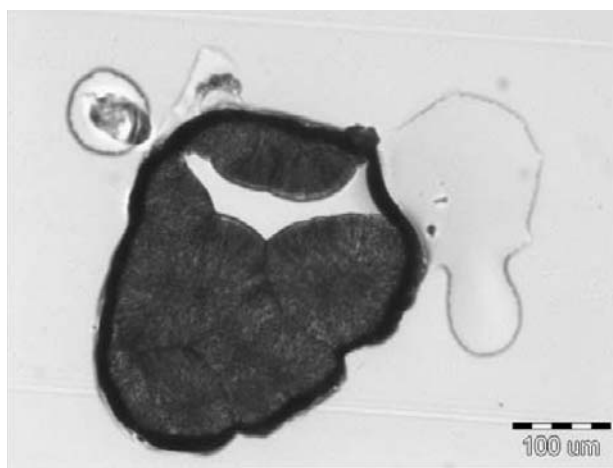
T = 30 °C – amorphous form



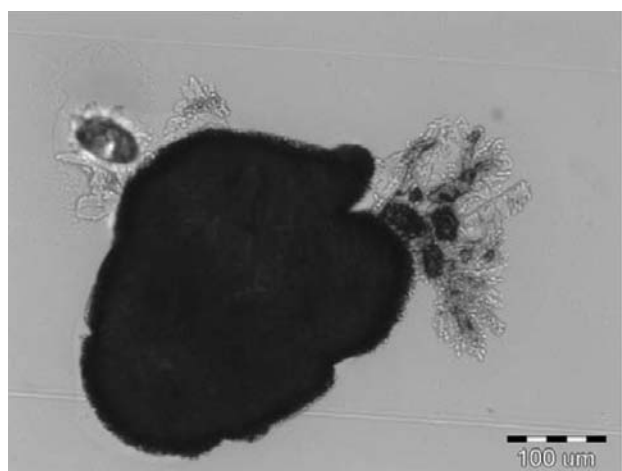
T = 120 °C – beginning of softening



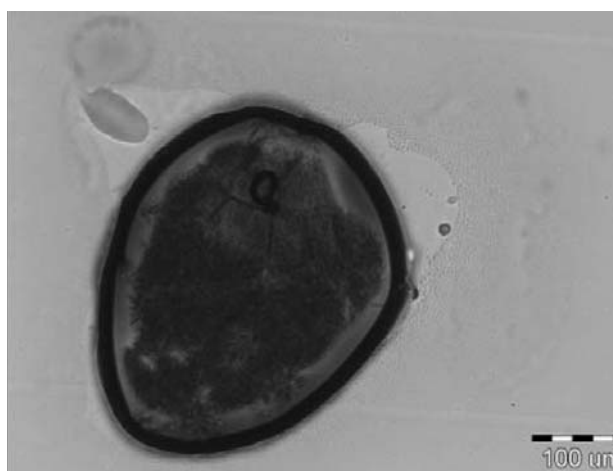
T = 125 °C – glass transition



T = 155–180 °C – recrystallization



T = 200 °C – crystal form



T = 211–220 °C – melting of crystal form

Figure 5. Hot stage microscopy of amorphous form.

mixture of polymorphic forms II (223.49 °C) and V (227.62 °C).

Form V was prepared by heating of hydrate forms IV-1 and IV-2 at about 150 °C in a drying chamber. The process is, however, not repeatable. By using the same, known parameters, prevailing form V was prepared with admixed form II. It was observed that form II that also appeared by heating of hydrate form IV-3. However, polymorph II obtained by dehydration of hydrate IV-3, remains stable even at heating to 170 °C. It is obvious that transformation of hydrate IV upon heating is through form II to form V or directly to form V, depending on parameters of samples preparation and difference between forms IV-1, IV-2 and IV-3. Nevertheless, form V cannot be prepared directly from form II, prepared by crystallization, upon heating, but only from hydrate forms IV. The process is influenced by factors which are not explained

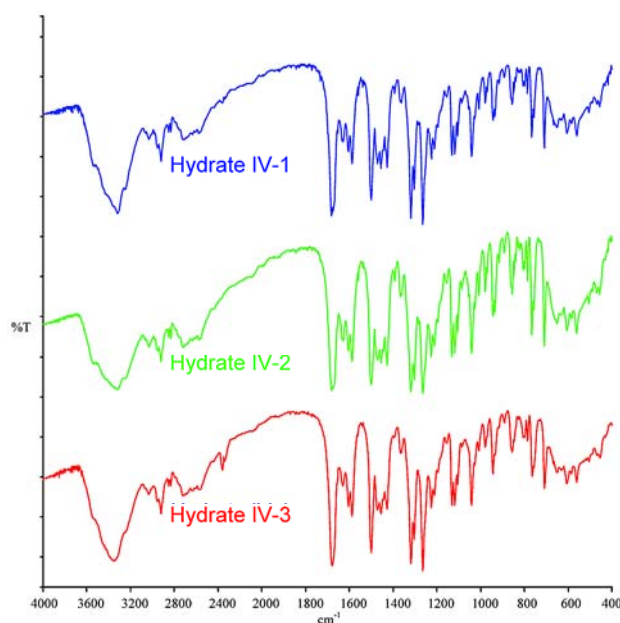


Figure 6. Mid-IR spectra of hydrate forms IV-1, IV-2 and IV-3.

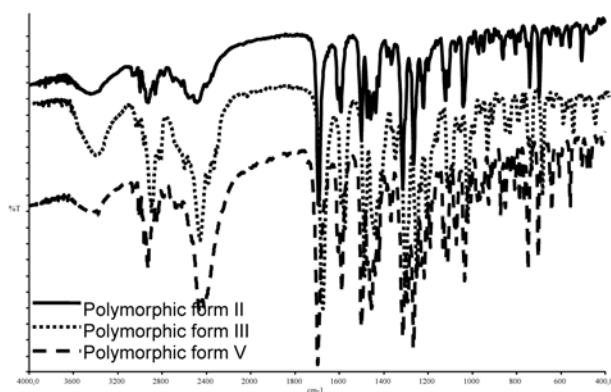


Figure 7. Mid-IR spectra of polymorphic forms II, III and V.

yet, with possible presence of nuclei that relieve appearance of prevailing form V, because samples were prepared in the same laboratory. If all crucial parameters are settled, every process is repeatable, as described in the literature^{17–20}.

In amorphous form, glass transition temperature could only be determined by hot stage microscopy (Figure 5).

Glass transition of amorphous form begins at 120 °C. Wide exothermic peak at about 150 °C represents crystallization. Melting of crystal form is at about 220 °C.

Intensive absorption in mid-IR spectra at 1690 cm^{-1} is connected to vibrations of C=O group in the structure of (DH). Frequency of absorption of C=O group in hydrate form IV-1 (1683 cm^{-1}) is less intensive than in polymorphic forms II, III and V (1690, 1697 and 1693 cm^{-1} , respectively), probably because of forming of hydrogen bonds to water molecules (Figures 6 and 7).

Crystal forms are distinguished in vibration area of terc-amino group in range 2200–2700 cm^{-1} . Strongest absorption of sp^3 hybridized carbon atoms of C–H bonds is at 2922 cm^{-1} in all polymorphic forms, with exception of form II (2928 cm^{-1}). Double absorption band at 1590 and 1600 cm^{-1} is of vibrations of C=C aromatic bonds.

The most significant differences between hydrates and polymorphs are in the functional groups area (1200–4000 cm^{-1}) and are effected by interactions of water molecules through hydrogen bonding²¹. Additional absorption band at 1650 cm^{-1} that is present only in hydrate forms, is contributed by water molecules that are annotated to carbonyl group through hydrogen bonding. In hydrates IV, absorption bands at 3590 cm^{-1} are indicated, but are overlaid because of presence of multiple OH groups of water molecules in crystal structure of trihydrate. They are forming hydrogen bonds, because of which they can be seen as wide absorption bands in region 3100–3700 cm^{-1} with most intensive absorption bands at 3320, 3350 and 3420 cm^{-1} . Absorption bands in this region indicates possible presence of free OH group (i.e. tautomerism of (DH)).

Partial dehydration of hydrate form IV-1, obtained by heating, results in formation of form II in a mixture with hydrate form. Presence of crystal bound water was

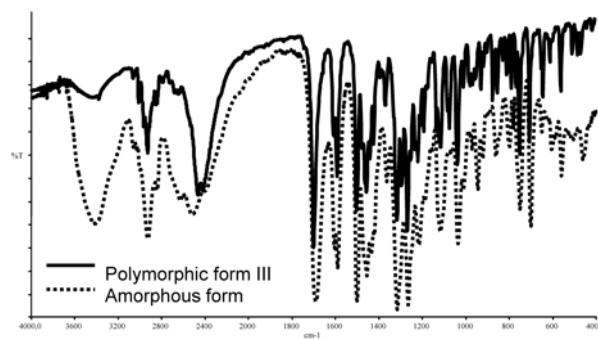


Figure 8. Mid-IR spectra of polymorphic form III and amorphous form.

confirmed by a band at 1650 cm^{-1} , which is not present in form II. Karl-Fischer titration method confirms presence of 0.8% of water in partial dehydrated form IV-1. Partial dehydration of form IV-1 to form II and hydrate form, again confirms transformation of forms IV through form II to form V. Kinetics of transformation differs in hydrate forms IV-1, IV-2 and IV-3, which was observed also in thermal analysis. Differences in dehydration result in appearance of different forms and mixture thereof: dehydration of form IV-1 results in form V or mixture of forms II and V, while hydrate IV-3 dehydrates to pure form II, which remain in this form even at heating to $170\text{ }^{\circ}\text{C}$.

Amorphous form presents weaker and widened absorption bands, characteristic for non-crystalline forms and are significantly different from crystalline forms (Figure 8 shows comparison to thermodynamically stable form III).

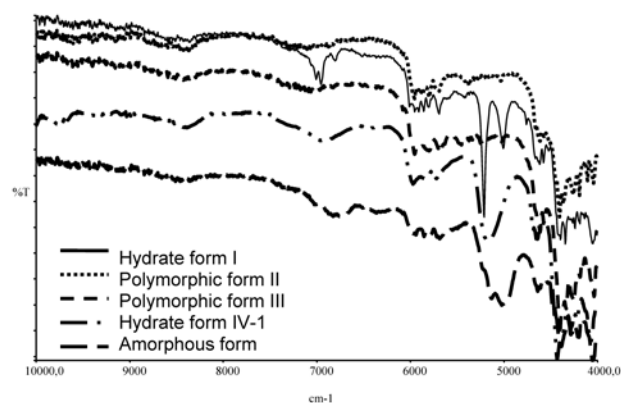


Figure 9. Near-IR spectra of crystal forms I, II, III, IV-1 and amorphous form.

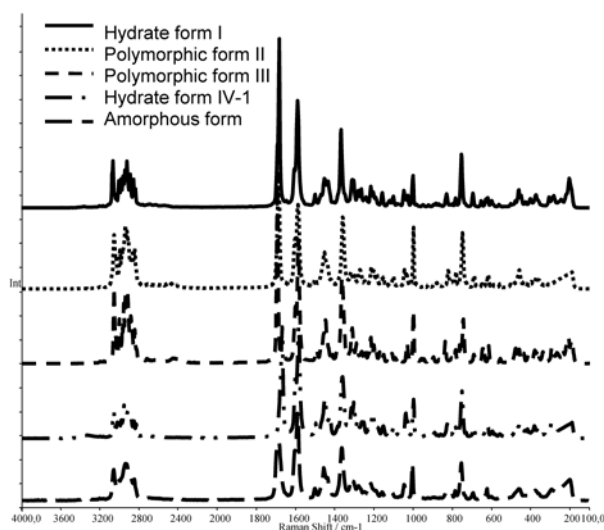


Figure 10. Raman spectra of crystal forms I, II, III, IV-1 and amorphous form.

Absorption of hydrate forms and amorphous form in near-IR spectra at 5000 and 7000 cm^{-1} are significantly different from polymorphic forms II and III (Figure 9). The bands are typical for pure water (5150 and 6900 cm^{-1})²¹. Connecting of water through hydrogen bonding on other molecules shifts values towards higher frequencies. Near-IR spectra of form I with splitted signal of OH group of water at 5000 cm^{-1} is attributed to its bonding in the crystal lattice.

Polar groups (CO, OH) of mid-IR absorption spectra are less visible in Raman emission spectra. However, non-polar simetric and almost simetric bonds like C–C and C=C are more intensive in the latter and influence of

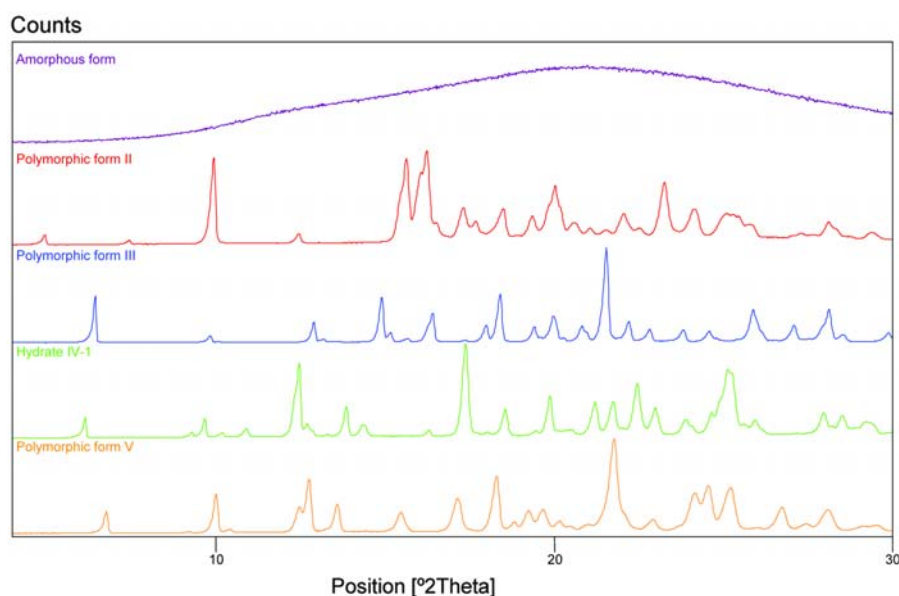


Figure 11. X-ray powder diffactograms of crystal forms II, III, IV-1, V and amorphous form.

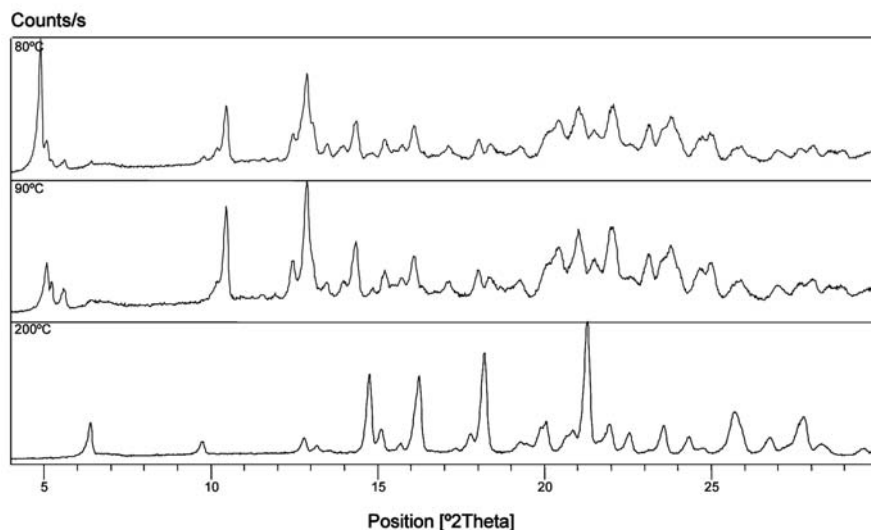


Figure 12. X-ray powder diffactograms of heated hydrate form I.

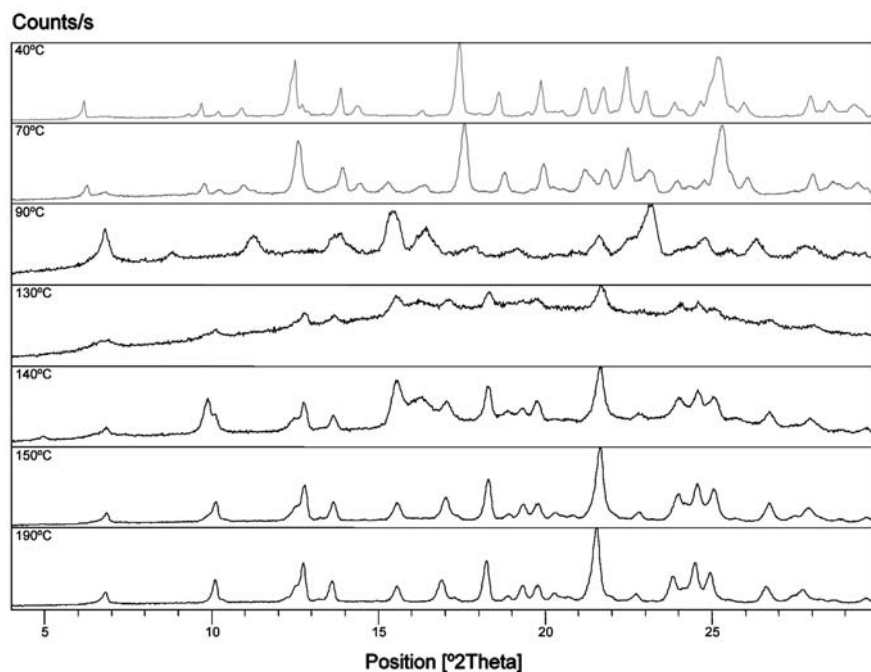


Figure 13. X-ray powder diffactograms of heated hydrate form IV-1.

water is minimized. Absorption peaks due to hydrate bound water are not significant and difference between polymorphs and hydrates is not obvious (Figure 10). Due to faster depreciation of Raman influence, neighbouring molecules have only a minor influence on vibrating molecules, which result in sharper peaks and less overlaying.

XRPD analysis is shown to be suitable tool for detection of mixture of crystal forms and presence of them in amorphous form, e.g. during shelf-life in both bulk drug (Figure 11) and solid dosage form, containing (DH).

XRPD after exposing to different temperatures were obtained for hydrates I and IV-1 (Figures 12 and 13).

Heating of hydrate I results in appearing of a novel crystal form at 80 °C, that is named »crystal form VII«. At 90 °C–120 °C, it is present in pure form.

Table 5: XRPD data of crystal form VII.

No.	Pos. [°2Th.]	d-spacing [Å]
1	5.1	17.4
2	5.2	16.9
3	5.6	15.8
4	10.5	8.5
5	11.5	7.7
6	12.5	7.1
7	12.9	6.9
8	14.4	6.2
9	15.2	5.8
10	16.1	5.5
11	17.1	5.2
12	18.0	4.9
13	18.4	4.8
14	19.3	4.6
15	20.0	4.44
16	20.4	4.35
17	21.0	4.22
18	22.0	4.04
19	23.1	3.85
20	23.8	3.74
21	24.7	3.61
22	25.0	3.56
23	25.9	3.44
24	27.0	3.30
25	27.7	3.22
26	28.1	3.18

Table 6: XRPD data of crystal form VIII.

No.	Pos. [°2Th.]	d-spacing [Å]
1	6.8	13.0
2	8.8	10.0
3	11.2	7.9
4	13.1	6.7
5	13.7	6.4
6	15.3	5.8
7	15.6	5.7
8	16.4	5.4
9	17.8	5.0
10	19.1	4.6
11	21.6	4.11
12	22.5	3.96
13	23.2	3.84
14	24.1	3.69
15	24.9	3.58
16	25.5	3.49
17	26.4	3.38
18	27.7	3.22
19	28.2	3.17
20	29.0	3.08
21	29.5	3.03

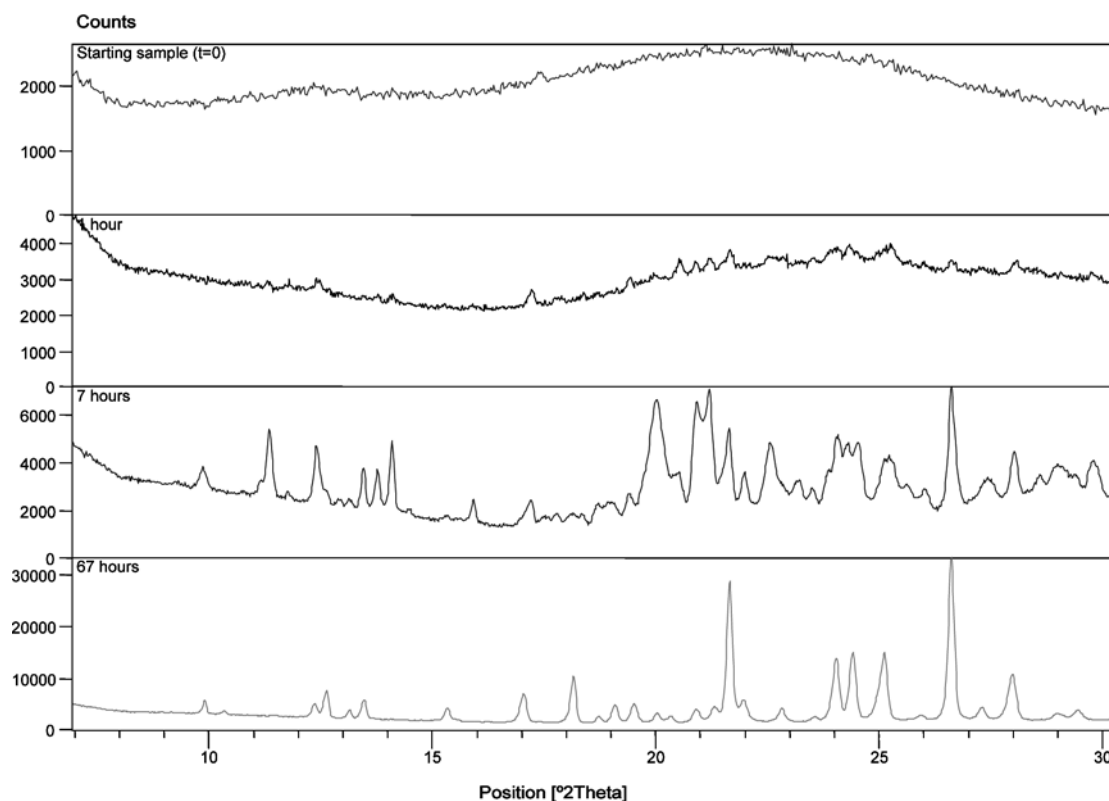


Figure 14. X-ray powder diffractograms of amorphous form, exposed to 40 °C/60%RH, over the time.

Further heating up to 200 °C results in appearing of pure polymorphic form III. Crystal form VII is characterized by XRPD data (Table 5).

Heating of hydrate IV-1 results in appearing of a novel crystal form at 70 °C, namely »crystal form VIII« that is present in pure form at 90 °C. At 130 °C, the sample shows low crystallinity with appearing of polymorph V. At 140 °C, better crystallinity results in appearing of

polymorphic form V with admixed polymorphic form II; the latter disappears at 190 °C, where only polymorph V is presented. Crystal form VIII is characterized by XRPD data (Table 6).

Amorphous form was exposed to both increased temperature (40 °C) and controlled RH (60%). Crystallization and formation of crystal forms was observed over 67 hours. After 1 hour, crystallization to form V is

Table 7: Parameters of surface characteristics of crystal forms and amorphous form.

	$\gamma_s^b \pm SD$ (mJ/m ²)	$\Delta G^{sp} \pm SD$ (kJ/mol)			
		THF	acetone	ethylacetate	Chloroform
Hydrate I	46.20 ± 0.51	4.07 ± 0.31	6.27 ± 0.32	5.58 ± 0.33	5.40 ± 0.17
Form II	45.08 ± 0.41	2.80 ± 0.35	5.79 ± 0.24	4.78 ± 0.13	4.30 ± 0.03
Form III	41.07 ± 1.20	2.67 ± 0.22	6.13 ± 0.18	5.15 ± 0.20	4.37 ± 0.12
Hydrate IV-1	43.80 ± 2.85	2.20 ± 0.64	6.02 ± 0.84	4.75 ± 0.36	4.96 ± 0.09
Amorphous form	27.23 ± 1.66	3.81 ± 0.33	4.93 ± 0.64	3.99 ± 0.31	1.99 ± 0.01

Table 8: Relation of specific components of free enthalpies of adsorptives for crystal forms and amorphous form.

Ratio	Hydrate I	Form II	Form III	Hydrate IV-1	Amorphous form
$\Delta G_{\text{chloroform}}^{sp} / \Delta G_{\text{THF}}^{sp}$	1.33	1.54	1.64	2.25	0.52
$\Delta G_{\text{chloroform}}^{sp} / \Delta G_{\text{ethylacetate}}^{sp}$	0.97	0.90	0.85	1.04	0.50
$\Delta G_{\text{chloroform}}^{sp} / \Delta G_{\text{acetone}}^{sp}$	0.86	0.74	0.71	0.82	0.40
$\Delta G_{\text{ethylacetate}}^{sp} / \Delta G_{\text{acetone}}^{sp}$	0.89	0.83	0.84	0.79	0.81
$\Delta G_{\text{THF}}^{sp} / \Delta G_{\text{ethylacetate}}^{sp}$	0.73	0.59	0.52	0.46	0.96
$\Delta G_{\text{THF}}^{sp} / \Delta G_{\text{acetone}}^{sp}$	0.65	0.48	0.44	0.37	0.77

Table 9: True densities of crystal forms and amorphous form.

Form	Hydrate I	Form II	Form III	Hydrate IV-1	Amorphous form
$\rho_T \pm SD$ (g/cm ³)	1.263 \pm 0.000	1.337 \pm 0.014	1.442 \pm 0.004	1.366 \pm 0.019	1.585 \pm 0.000

completed with additional new crystal form with the following 2θ values: 11.3, 13.8, 14.1, 15.9, 22.6, 23.2, 28.6 and 29.8°. After 67 hours, the new crystal form is completely converted to crystal form V (Figure 14).

IGC could be used for differentiation on the basis of specific surface characteristics of crystal and amorphous form. Both acetone and ethylacetate are capable of interactions of Lewis acid and base. Due to stronger electron acceptor action of acetone, ΔG^{sp} values of it are higher than of ethylacetate (Table 7, results are average of 3 measurements). Reproducibility of measurements does not offer a strong confidence, which is confirmed by relatively high standard deviation (SD).

Relation $\Delta G_{\text{chloroform}}^{sp}/\Delta G_{\text{THF}}^{sp}$ is appropriate for differentiation between individual forms with exception of polymorphic forms II and III (Table 8). Chemical groups on surface of crystal forms are capable of formation of stronger interactions to Lewis acids than bases. Hydrate IV-1 have the weakest interactions with basic adsorptives, and strongest interactions to Lewis acids. Hydrate I was

relatively strongest interactions to Lewis bases, which could be important in bounding of water at lower RH values (i.e. OH groups of water act as Lewis acids)²². $\Delta G_{\text{chloroform}}^{sp}/\Delta G_{\text{THF}}^{sp}$ of amorphous form is significantly lower than in the case of crystal forms. Weaker electron donor surface characteristics of amorphous form are confirmed also by $\Delta G_{\text{chloroform}}^{sp}/\Delta G_{\text{ethylacetate}}^{sp}$ and $\Delta G_{\text{chloroform}}^{sp}/\Delta G_{\text{acetone}}^{sp}$. However, interactions of amorphous surface to electron donor functional groups of adsorptives are stronger when comparing to crystal forms. Weaker electron donor or stronger electron acceptor characteristics of amorphous form surface in comparison to crystal forms are confirmed by $\Delta G_{\text{THF}}^{sp}/\Delta G_{\text{acetone}}^{sp}$ and $\Delta G_{\text{ethylacetate}}^{sp}/\Delta G_{\text{acetone}}^{sp}$, too.

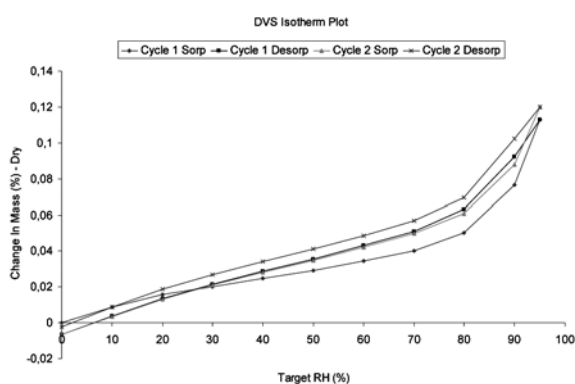
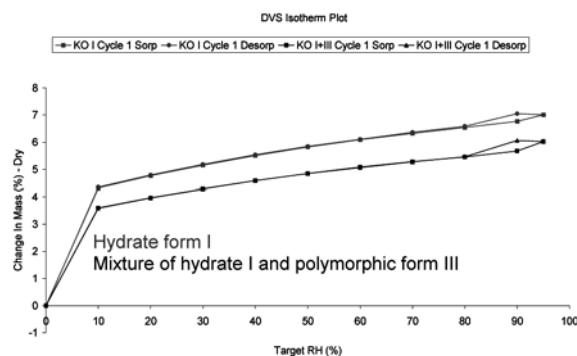
True density (ρ_T) is not the most significant parameter to distinct between individual forms (Table 9). The lowest value of true density was obtained for hydrate form I. True density of polymorphic form III has the highest value (1.442 \pm 0.004 g/cm³) which is attributed to small proportion of free spaces between molecules in thermodynamically stable form. High value of true density of amorphous form is explainable by presence of bigger particles (Table 4).

No significant difference in solubility and dissolution rate was observed between different forms, which is attributed to good solubility of (DH) according to BCS,²³ which is also in good agreement with difference between both polymorphic forms and polymorphic and hydrate forms; the ratio of solubility between them is typically less than 2.²⁴

DVS distinguishes forms I and III and is suitable method form determination of presence of them in mixtures (Figures 15 a and 15 b).

Thermodynamically stable form III adsorbes only a minor amount of water, i.e. only 0.13% up to 95% RH. In first cycle, replacement of residual methanol, which is solvent of crystallization is observed. Open hysteresis loop explains presence of pores, that retain water after drying.

Comparing different hydrate forms IV, a significant difference is observed in sorption of water at different RH values (Figures 16 a, 16 b and 16 c). Form IV-1 represents the most accurate course of sorption and desorption isotherms, that at already 20% RH sorbes amount of water that corresponds to 3 moles per 1 mole of (DH), which is in good accordance with theoretical trihydrate. Further, only small amount of water is adsorbed to the surface. First sorption cycle of form IV-2, brings adsorption of 2 moles of water per 1 mole of (DH) up to 90% RH. Up to 95% RH, additional 1 mole of water is adsorbed for formation of trihydrate, while amount of water over theoretical trihydrate is bound to the surface. In the second cycle,

**Figure 15 a.** Sorption-desorption isotherms of polymorphic form III.**Figure 15 b.** Comparison of sorption-desorption isotherms of hydrate I and mixture of hydrate I and polymorphic form III.

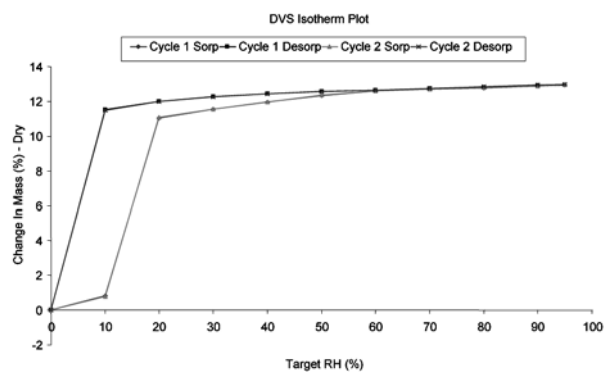


Figure 16 a. Sorption-desorption isotherm of hydrate form IV-1.

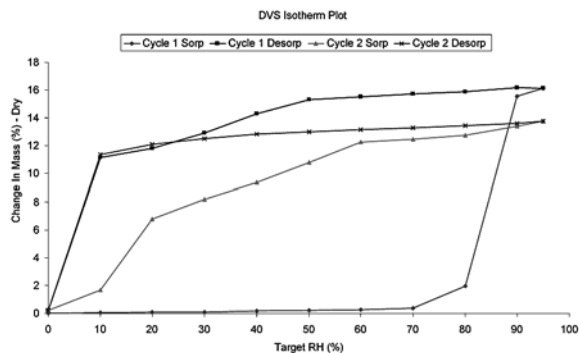


Figure 17. Sorption-desorption isotherm of polymorphic form II.

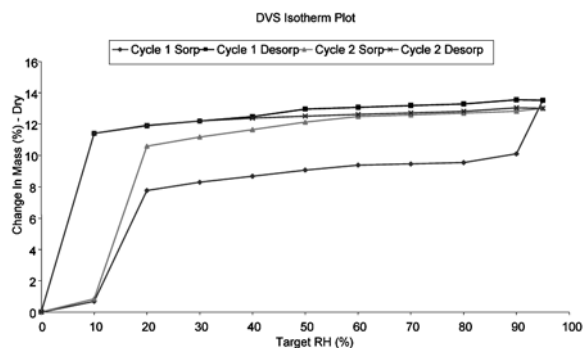


Figure 16 b. Sorption-desorption isotherm of hydrate form IV-2.

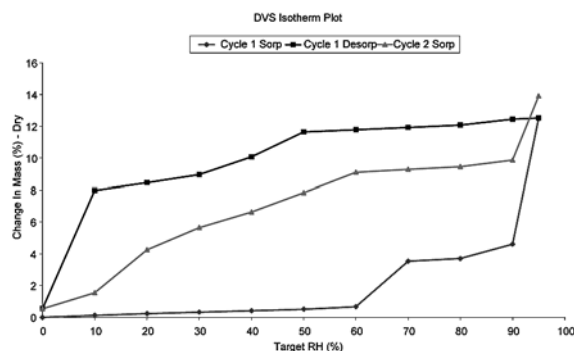


Figure 18. Sorption-desorption isotherm of polymorphic form V.

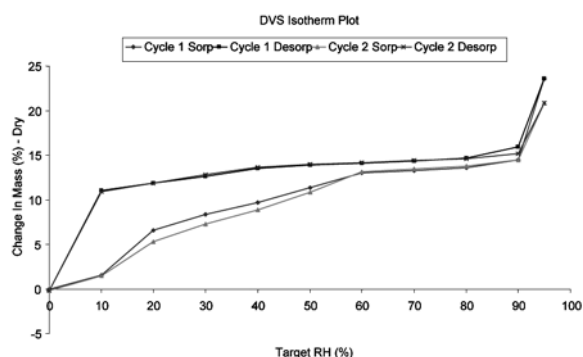


Figure 16 c. Sorption-desorption isotherm of hydrate form IV-3.

adsorption course of form IV-2 is similar as for form IV-1. It can be concluded that a physical change happened during the first cycle to the form similar to hydrate IV-1. Form IV-3 adsorbs 1.5 mole of water per 1 mole of (DH) up to 20% RH, while it further linearly adsorbs 1.5 moles of water per 1 mole of (DH). Sorption over 90% RH is connected to deliquescence. For all forms IV, it is characteristic that in desorption cycle below 10% RH, 3 moles of water per 1 mole of (DH) are released. Difference in sorption of water between hydrate forms IV is ascribable by different porosity of samples, i.e. different surface charac-

teristics. Similarity of forms IV-1 and IV-2 are ascribed to the same method of preparation. Difference between forms IV has been noticed also in thermal behaviour where dehydration occurred on heating of different samples (Figure 4).

Polymorph II uptakes 4.5 moles of water per 1 mole of (DH) up to 90% RH. In the first cycle, the water is retained until 50% RH, while further dehydration drop down to the value, which is characteristic for trihydrate (Figure 17). Transformation to “meso” form is seemed because of a large uptake of water, which can induce phase change. In that case, desorption is denoted by minor drop of water content, which depends on stability of formed hydrate, until final release of water at low RH.

Polymorphic form V uptakes <0.5 moles water per 1 mole of (DH) up to 60% RH in first sorption cycle. Above 60% RH, it is transformed through monohydrate to trihydrate with additional surface bound water. Transition in the second cycle is not finished yet which is confirmed by not fulfilled desorption in first cycle (Figure 18).

Surface characteristics of crystal and amorphous forms were observed also by SEM (Figure 19). Differences between crystal forms can be described as different crystallinity; the term is ascribed to crystal habit. Zero-crystallinity of amorphous form is determined only by XRPD and not by specific SEM micrograph.

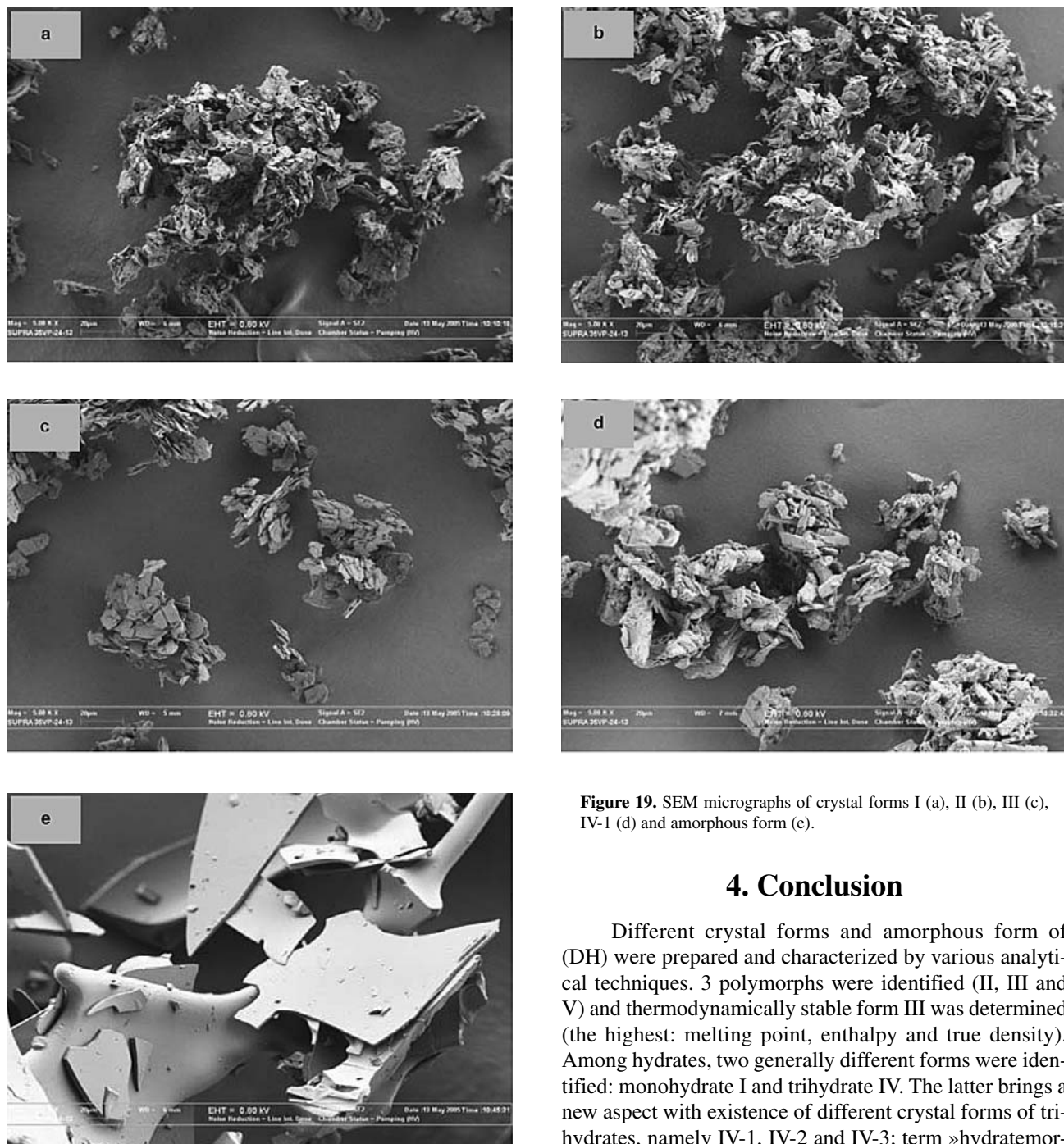


Figure 19. SEM micrographs of crystal forms I (a), II (b), III (c), IV-1 (d) and amorphous form (e).

4. Conclusion

Different crystal forms and amorphous form of (DH) were prepared and characterized by various analytical techniques. 3 polymorphs were identified (II, III and V) and thermodynamically stable form III was determined (the highest: melting point, enthalpy and true density). Among hydrates, two generally different forms were identified: monohydrate I and trihydrate IV. The latter brings a new aspect with existence of different crystal forms of trihydrates, namely IV-1, IV-2 and IV-3; term »hydratemorphism« of (DH) trihydrates is proposed. Difference between trihydrates is attributed to different methods of preparation and results mainly by changes in surface characteristics, e.g. orientation of functional groups of (DH) in crystal lattice. They were determined by thermoanalytical methods, mid-IR spectroscopy and DVS, but less applied with IGC that is otherwise appropriate analytical tool for determination of difference in surface characteristics. Obvious difference among them was observed also in both partial and total dehydration with appearance of forms II and V and mixtures thereof. Appearance of polymorphs II and V depends on heating rate: by heating of

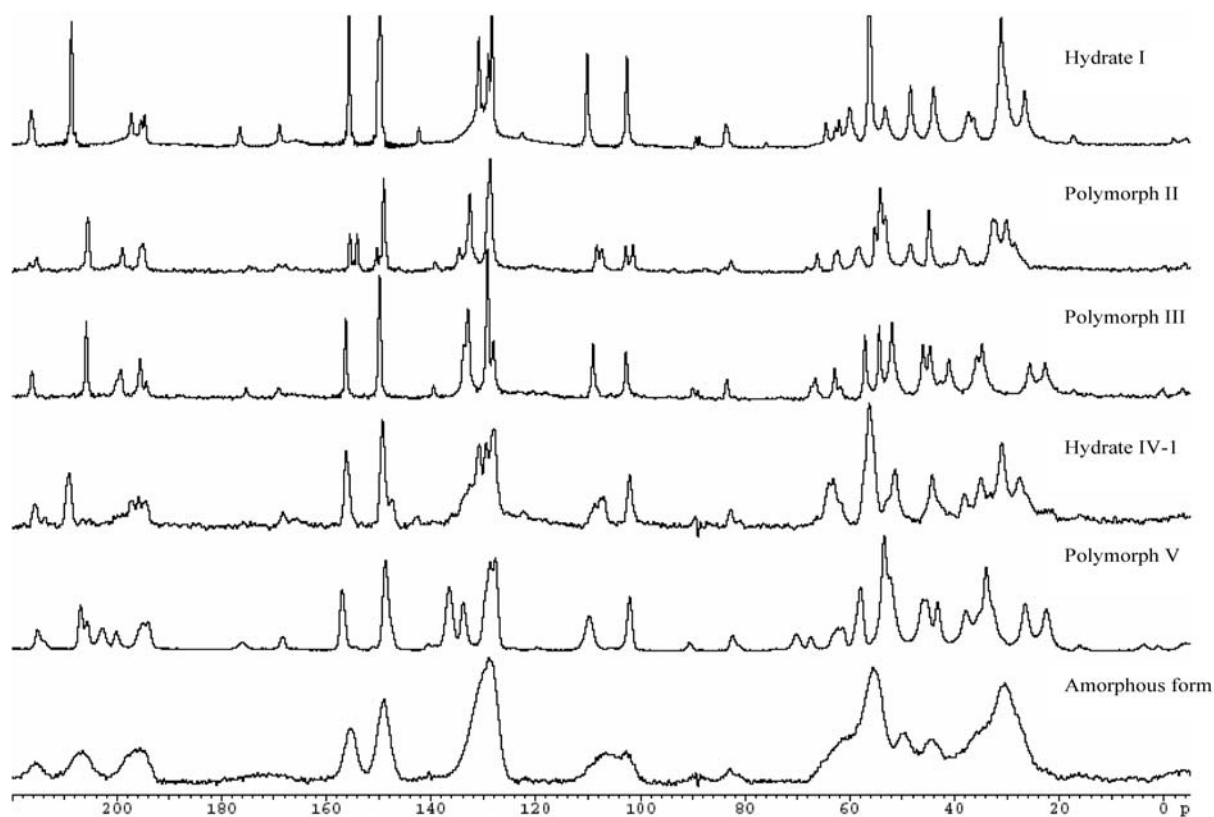
^{13}C -solid state NMR is the only analytical method where it can be possible to determine a structure of individual physical forms on the basis of chemical shifts for all C atoms in the structure (Table 10, Figure 20). The method is very useful also in determination of mixture of different crystal forms, where specific chemical shifts are connected to individual crystal forms. Amorphous form with broad peaks is different from all analyzed crystal forms with sharp peaks for certain C atoms.

Table 10: ^{13}C solid state NMR assignments for amorphous and crystal forms.

Amorphous form	Hydrate I	Form II	Form III	Hydrate IV-1	Form V	Assignment
21–68	26.5–60.0	26.0–59.0	22.0–57.0	27–56	22–58	C10 C10' C9 C3 C8 C2 C11 C11' C12 (OMe) ₂
100–112	101.1 102.5	101.3 102.7 107.3 108.3	102.7 109.0	102.0 107.0	102.0–109.8	C4 C7
124–135	128–130	128–135	128–132	128–132	127.6–136.5	C4' C12' C13 C13' C14 C14' C15
100–112	101.1 102.5	101.3 102.7 107.3 108.3	102.7 109.0	102.0 107.0	102.0–109.8	C4 C7
124–135	128–130	128–135	128–132	128–132	127.6–136.5	C4' C12' C13 C13' C14 C14' C15
148	149.6	149.0	149.7	149.2	148.7	C5 C6 C7'
155	155.6	154.0 154.6	156.2	156.2	157	
207	208.6	205.5	205.8	209.1	206.8	C1

hydrates by DSC at heating rate 10 °C/min, preferential form II is formed and further transformation to form V is not detected, while at heating to 150 °C, form V is preferentially formed with possible admixture of minor quantities of form II; the latter depends on starting form IV. Heating

of forms IV-1 and IV-2 results in formation of form V through form II, while hydrate IV-3 dehydrates to form II which remain in this form even at heating on 170 °C. It has to be focused out that hydrate form IV-1 is most similar to the known trihydrate form IV¹³.

**Figure 20.** ^{13}C solid state NMR spectra of crystal and amorphous forms of (DH).

Two novel crystal forms were prepared by heating of hydrates I and IV-1 and tracked by XRPD. According to the first settled nomenclature for crystal forms of (DH)¹³, the novel crystal forms were named »crystal form VII« and »crystal form VIII«. They are stable at certain temperature range. Upon heating, crystal forms VII and VIII transform to polymorphic forms III and V, subsequently.

Form I contains 1 mole of water per 1 mole of (DH) already at 10% RH, while later adsorption of water is mainly to the surface of the crystal lattice.

Near-IR resolves hydrates from polymorphs, while thermal analysis brings difference between crystal forms although minor difference is observed in melting points and enthalpies of both polymorphs and hydrates. A significant difference is observed by XRPD among all crystal forms, while amorphous form brings expected diffractogram with broad peak.

The confirmation of the structure difference between polymorphs cannot be determined by universal analytical technique – different methods should be used to complete characterization of (DH), although ¹³C ss-NMR brings the most obvious differences between individual physical forms on the basis of chemical shifts for all C atoms of the structure.

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Povzetek

Pripravili in karakterizirali smo različne kristalne oblike in amorfnu obliko donepezil hidroklorida. Hidrat I je stabilen le pri nizki relativni vlažnosti in pri višji relativni vlažnosti absorbira vodo. Oblike II, III in V so polimorfi, termodinamsko je stabilna oblika III. Oblika V nastane s segrevanjem hidrata IV pri temperaturi okrog 150 °C. Pripravili in karakterizirali smo tri oblike hidrata IV. Kot najprimernejši metodi za določitev kristalnih oblik, tako polimorfov kot hidratov, sta se izkazali diferenčna dinamična kalorimetrija (DSC) in rentgenska praškovna analiza. Metodi sta uporabni tudi za določitev oblike donepezil hidroklorida v trdni farmacevtski obliki. S termično analizo smo ugotovili razlike v dehidraciji različnih oblik hidrata IV, to je prehod v čisto polimorfno obliko ali v zmes polimorfov. Segrevanje hidratov I in IV-1 daje novi kristalni obliki VII in VIII.