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Solid-state characterization of non-stoichiometric hydrates of ester-type local anaesthetics
Part XI. Crystal polymorphism of local anaesthetic drugs

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Abstract

Three structurally closely related local anaesthetic drugs, hydroxyprocaine hydrochloride (4-butylamino-2-hydroxybenzoic acid 2-dimethylaminoethyl ester hydrochloride, HPCHC), tetracaine hydrochloride (4-butylamino-2-hydroxybenzoic acid 2-dimethylaminoethyl ester hydrochloride, TCHC) and hydroxytetracaine hydrochloride (4-butylamino-2-hydroxybenzoic acid 2-dimethylaminoethyl ester hydrochloride, SLCHC) are found to form hydrated crystals. Those were characterized by thermal analysis (hot stage microscopy, differential scanning calorimetry, thermogravimetry), vibrational spectroscopic methods (FTIR-, FT-Raman-spectroscopy), powder X-ray diffractometry, solid-state NMR and water sorption/desorption analysis. The formation and the stability of the hydrated solid phases are evaluated by sorption isotherms derived from different sorption/desorption analytic methods. The three substances investigated show conformational polymorphism with the anhydrated phases including a high temperature form mod. I, which is highly hygroscopic and isostructural with the hydrate. The hydrated form is present in commercial products at various contents. These hemihydrates crystallize from water, whereas the anhydrates crystallize from all other tested organic solvents. Different methods of water sorption/desorption analysis indicate the formation of non-stoichiometric hydrates. Different methods of drying lead to the same results. Solid-state NMR spectra were used to obtain both structural and molecular level mobility information.

Keywords: Local anaesthetics; Non-stoichiometric hydrate; Crystal polymorphism; Thermal analysis; Solid-state properties

1. Introduction

Local anaesthetic drugs (LA) are well known for the formation of polymorphs and solvates (Giron, 2001; Borka and Haleblian, 1990; Schmidt, 2005c,d). The hydrophilic group of the general formula lip–CO–hydr (lip = lipophilic end, mostly phenyl ring; CO = negatively charged linkage, commonly ester or amide; hydr = hydrophilic group, tertiary or secondary amine) is responsible for the receptor binding whereas the lipophilic and the linking group affect the duration of action (Goodman and Gilman, 1998).

Local anaesthetic compounds are commonly separated into two main groups, the ester-type LA with a short duration of anaesthesia and the amide type LA with a longer duration of action. A third smaller group unites compounds with different molecular structures and different pharmacological properties. Our systematic investigations on the solid-state properties of numerous local anaesthetic drugs showed that the ester-type LA tend to form polymorphs, whereas the amide type LA mainly form hydrates or solvates (Schmidt et al., 2004) due to the ability to form intermolecular hydrogen bonds with the N1 amine group.

The present paper compares the solid-state properties of the three local anaesthetics hydroxyprocaine hydrochloride (4-butylamino-2-hydroxybenzoic acid 2-dimethylaminoethyl ester hydrochloride, HPCHC), tetracaine hydrochloride (4-butylamino-2-hydroxybenzoic acid 2-dimethylaminoethyl ester hydrochloride, TCHC) and hydroxytetracaine hydrochloride (4-butylamino-2-hydroxybenzoic acid 2-dimethylaminoethyl ester hydrochloride, SLCHC), which are the only LA of the ester type (Fig. 1), that are able to form both, polymorphs and solvates (hydrates). TCHC is officially in the European (Ph. Eur.) and the United States Pharmacopoeia (USP) and is mainly used as ophthalmic solution to cause numbness or

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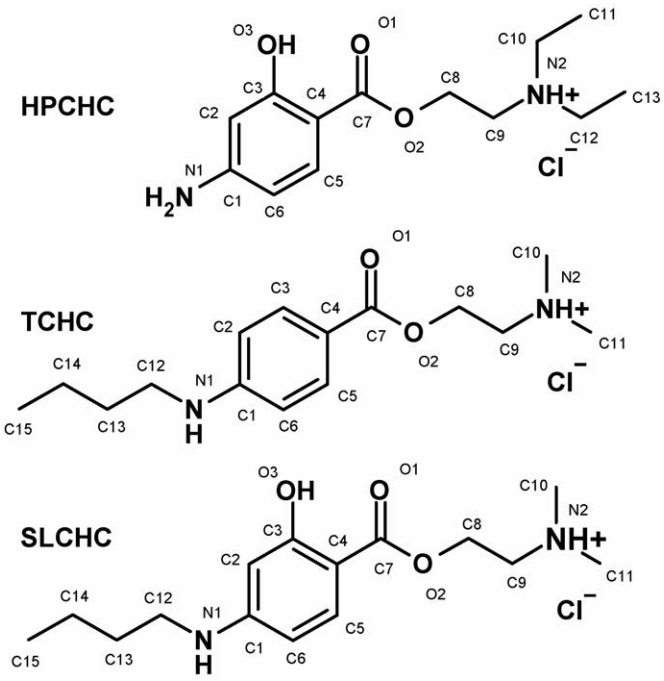

Fig. 1. Molecular structures of the three local anaesthetics HPCHC, TCHC and SLCHC with atomic numbers.

loss of feeling of the eye in therapeutic and diagnostic applications.

In previous analytical studies the polymorphs and hydrates of HPCHC (Brandstaetter and Grimm, 1956; Kuhnert-Brandstätter et al., 1983; Schmidt and Schwarz, 2005), of TCHC (Doser, 1943; Wickstrøm, 1953; Brandstaetter and Grimm, 1956; Burger and Ramberger, 1979b; He and Han, 1993; Giron et al., 1997; Schmidt and Schwarz, in preparation) and of SLCHC (Brandstaetter-Kuhnert and Grimm, 1957; Schmidt et al., 2006) were described, but none of these studies draw relations between the molecular/polycrystalline structure and the pseudo-/polymorphism of these compounds.

The crystal structures of the hydrated form and the anhydrate of SLCHC were solved by single crystal X-ray diffraction and were discussed earlier (Schmidt et al., 2006). The crystal polymorphism of the LA is caused by the conformational flexibility of the molecules (Schmidt, 2005a). The rotational flexibility of the side chains additionally is increased by the N2 alkyl chains on the hydrophilic side of the molecule (Schmidt et al., 2002). If there is a substituent of the phenyl ring in *ortho* position (SLCHC, HPCHC) of the benzoic acid function, an additional intramolecular hydrogen bond (O3–H···O1) reduces the conformational flexibility and also the number of polymorphs. A missing flexible butyl side chain in molecules like HPCHC compared to SLCHC reduces the number of possible conformations which is relevant for the formation of different (conformational) polymorphs like in many representatives of the LAE. Caused by the missing butyl side chain the HPCHC molecule forms less polymorphs but the formation of a hydrate is not affected by the reduction of the flexibility of the molecule.

In the present study, we characterize the formation, the physical properties and similarities of the hydrated forms of HPCHC, TCHC and SLCHC by thermal analytical methods, vibrational spectroscopy, powder X-ray diffraction and different water–vapour sorption analytic methods evaluating the influence of structural features and the resulting stabilities. Solid-state ^{1}H NMR shows the number of molecules in the crystallographic asymmetric unit and the strength of hydrogen bondings.

2. Materials and methods

2.1. Materials

HPCHC: two samples of HPCHC ("Farbwerke Hoechst S" and "Oxyprocain") were available for this study.

TCHC: nine samples of TCHC ("Hoechst Op. B 029", "Pantocain Hoechst", "Pantocain 173A003", "Hoechst Pantocain 100A003", "Bayer Pantocain", "Tetracainhydrochlorid Chemosan", "Tetracainum Hydrochloricum Gatt 1992/0298 3398", "Tetracainum Hydrochloricum Apoka 1784 1/1294/83", "Pantocain Bayer IFB/K-0542") were used for this study.

SLCHC: two samples "Salicain HCl Farbwerke Höchst op-16" and "Salicain HCl Rheinpreussen" were tested in this study.

All solvents used in this study were of p.a. ("pro analysis") quality and applied without further purification/desiccation.

2.2. Methods

2.2.1. Hot stage microscopy

The thermal behaviour of the solid-state forms was observed with a Reichert Thermovar® polarized microscope, magnification 100× (Reichert, Vienna, A) equipped with a Kofler hot stage (Reichert, Vienna, A).

2.2.2. Differential scanning calorimetry (DSC)

DSC-thermograms were recorded with a DSC 7 system (Perkin-Elmer, Norwalk, CT, USA) using the Pyris 2.0 software. Samples of approximately 2 mg (weights controlled to ±0.0005 mg using a UM3 ultramicrobalance, Mettler, Greifensee, CH) were weighed into aluminium pans (25 μl) with perforated covers. Dry nitrogen was used as purge gas (purge: 20 ml min^{-1}), and the system was calibrated with caffeine (236.4 °C) and indium 99.999% (156.6 °C, 28.45 J/g). Heating rates of 5 K min^{-1} were routinely used.

2.2.3. Thermogravimetric analysis (TGA)

Thermogravimetric analysis was done with a TGA7 system (Perkin-Elmer, Norwalk, CT, USA) using the Pyris 2.0 software. Samples of approximately 5 mg (weights controlled to ±0.0005 mg using a UM3 ultramicrobalance, Mettler, Greifensee, CH) were weighed into aluminium pans (15 μl). Two-position calibration of the temperature was done with ferromagnetic Ni (ferromagnetic transition 163 °C) with respect to the standard of Perkin-Elmer. Heating rates of 5 K min^{-1} were routinely used (except isothermic runs). Dry nitrogen was used as purge gas (sample purge: 20 ml min^{-1}, balance purge: 40 ml min^{-1}).

2.2.4. Infrared spectroscopy

Fourier transformed infrared (FTIR) spectra were acquired on a Bruker IFS 25 spectrometer (Bruker Analytische Messtechnik GmbH, Karlsruhe, D). Spectra over a range of 4000–400 cm^{-1} with a resolution of 2 cm^{-1} (50 scans) were recorded using KBr pellets (approximately 2 mg sample compound per 300 mg KBr). For temperature-controlled FTIR spectra the samples were prepared on ZnSe disks using a heating device (Bruker) and the Bruker IR microscope I (Bruker Analytische Messtechnik GmbH, Karlsruhe, D), with 15×-Cassegrain-objectives (spectral range 4000–600 cm^{-1}, resolution 4 cm^{-1}, 100 interferograms per spectrum).

2.2.5. Raman spectroscopy

Raman spectra were recorded with a Bruker RFS 100 Raman spectrometer (Bruker Analytische Messtechnik GmbH, Karlsruhe, D), equipped with a Nd:YAG laser (1064 nm) as excitation source and a liquid-nitrogen-cooled, high-sensitivity Ge-detector. The spectra were recorded in aluminium sample holders at a laser power of 300 mW (64 scans per spectrum).

2.2.6. Powder X-ray diffraction (PXRD)

The X-ray diffraction patterns were obtained using a Siemens D-5000 diffractometer (Siemens AG, Karlsruhe, D) equipped with a theta/theta goniometer, a Goebel mirror (Bruker AXS, Karlsruhe, D), a 0.15° soller slit collimator and a scintillation counter. The patterns were recorded by Cu Kα-radiation at a tube voltage of 40 kV and a tube current of 35 mA, applying a scan rate of 0.005° $2\theta\,s^{-1}$ in the angular range of 2–40° in 2θ. Temperature- and moisture-controlled experiments were done with a low-temperature camera TTK (Anton Paar KG, Graz, A) and a SYCOS-H humidity control system (Asynco, Karlsruhe, D).

2.2.7. Water sorption/desorption analysis

Interval method. The interval moisture sorption isotherms were acquired using a SPS-11 moisture sorption analyzer (MD Messtechnik, Ulm, D). The measurement cycles were started at 0% relative humidity (RH) and increased in 10% steps up to 90% RH and back to 0% RH with sample masses of about 100 mg. The equilibrium condition for each step was set to a weight constancy of ±0.007% over 40 min. The temperature was 25 ± 0.1 °C. *Integral method*. Integral moisture sorption was done in humidistats with the samples stored over saturated salt solutions to adjust different relative humidities (sample masses of about 300 mg). *Thermogravimetric isotherms*. Isothermal gravimetric desorption was carried out with the Perkin-Elmer TGA7 (see above) with sample masses of 5 mg.

2.2.8. Lyophilization

Freeze-drying was performed with a Lyolab B, LSL (Secfroid Lyophilisator Inula, Wien, A) equipped with vacuum pump 2400 A (Alcatel Cit, Annecy Cedex, F). The frozen aqueous solutions (10%, w/w) were dried at −60 °C and 0.05 mbar.

2.2.9. Solid-state nuclear magnetic resonance spectroscopy (NMR)

^{1}H spectra were run using an InfinityPlus 500 spectrometer and 2.5 mm rotors to allow high spinning speeds.

3. Results and discussion

The crystal forms are named according to the Kofler notation using roman numerals in the order of the melting points (i.e. the form with the highest melting point is called mod. I). The modification which is thermodynamically stable at 20 °C is marked with a superscript zero.

3.1. Preparation of different crystal forms

Mod. II, the anhydrate which is thermodynamically stable at room temperature in the three compounds, is present in commercial products and crystallizes from all the organic solvents tested in this study, such as ethanol, ethanol 70%, methanol, 1-propanol, 2-propanol, 1-butanol, 1-pentanol, acetone, acetonitrile, methylene chloride, trichlormethylene, diethyl ether, ethyl acetate, *n*-hexane, cyclohexane, 1,4-dioxane, nitromethane, ethyl methyl ketone, dimethyl formamide, toluene, precipitated at room temperature and dried at ambient conditions.

Mod. I crystallizes from the melt at temperatures above the experimental transition temperature and, according to Ostwald's 'Rule of Stages' (Ostwald, 1897), also nucleates at lower temperatures by fast cooling of the melt.

The hydrated forms derive by crystallization from aqueous solutions. Freeze-drying (10% sample compound in water at −60 °C, 0.05 mbar) lead to fluffy powders of the hydrates except SLCHC, which crystallizes in a third anhydrate polymorphic crystal form (mod. III) under freeze-drying conditions.

3.2. Characterization of the hydrates

3.2.1. Thermal analysis

3.2.1.1. HSM. HPCHC. The commercial product consists of polyedric (100–200 μm), highly birefringent fine needles of the hydrate. On heating up to 60 °C using a heating rate of 5 K min^{-1} the crystals start to jump and crack intensely caused by dehydration. The loss of water does not cause significant change of polarized colours whereas the former plane crystal shapes show a fine lamellation after the thermal dehydration. At 100 °C the compound starts to condense and sublimates shaped as needles and stems precipitate on the slide increasing until the temperature reaches the melting point. The crystals inhomogenously melt at about 154 °C and mod. I simultaneously crystallizes from the melting drops and melts at about 160 °C. Together with the melting of the last crystals of mod. I at 162 °C, the compound starts to decomposite showing brown-coloured melting drops. In a preparation with highly viscous silicone oil the crystals are observed to loose solvent (water) above 40 °C continuing to 110 °C. By means of hot stage microscopy combined with thermogravimetry the third modification (mod. III) being described in previous studies (Kuhnert-Brandstätter et al., 1983) could now be identified as a hydrated form.

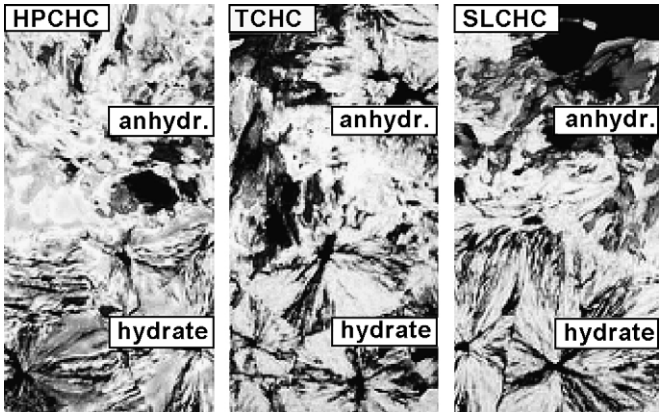

Fig. 2. Micrographs of HPCHC, TCHC and SLCHC crystal films at room temperature. From a drop of water placed on the crystal film of the anhydrate (shaped as small plates, above) the hydrated form crystallizes as raw spherulithes (bottom). The border between the two crystal forms proceeds in the middle of each picture.

TCHC. Most of the commercial products investigated in this study consist of polyedric (100–200 μm), highly birefringent stems of the stable anhydrate impured with the hydrated form characterized by a strong texture. On heating, using a heating rate of 5 K min^{-1}, the compound starts to condense and sublimates shaped as needles and stems precipitate on the slide increasing until the temperature reaches the melting point. The crystals inhomogenously melt at about 139.5 °C and mod. I simultaneously crystallizes from the melting drops and melts at about 149 °C. One of the commercial products consists of the pure stable anhydrate, which shows homogenous melting at 140 °C without recrystallization of mod. I. A drop of water placed beside the crystal film of the anhydrate on the slide results in a crystal film of a stable hydrated form shaped as raw spherulithes (Fig. 2). Samples recrystallized from water consist of highly birefringent fine needles (up to 1 mm in length) of the hydrate. On heating up to 40 °C using a heating rate of 5 K min^{-1} the crystals start to jump and crack intensely caused by dehydration. The loss of water does not cause significant change of polarized colours whereas the former plane crystal shapes show a fine lamellation after the thermal dehydration. The crystals melt at 149 °C (mod. I). On a preparation with highly viscous silicone oil the crystals are observed to loose solvent (water) above 50 °C with a maximum at 80 °C. In a special preparation of two slides glued together by a strong adhesive enclosing some crystals of the hydrate a solid transformation of the hydrate could be observed at about 84 °C by change of the colours of the polarized light starting from the center of each crystal (proved by DSC, transition endotherm at 80 °C, heat of transition ∼5 kJ mol^{-1}).

SLCHC. The commercial products consist of small (100–300 μm), highly birefringent stems of the stable form mod. II and hydrated mod. I. Upon heating, the crystals of mod. II show no change until they melt at about 152 °C, whereas the hydrated crystals start jumping and cracking at about 80 °C. One of the two tested commercial samples solely consists of crystals that jump and crack at about 80 °C. Keeping the temperature after the melting process at about 153 °C mod. I crystallizes from the melt shaped as highly birefringent polygonal plates.

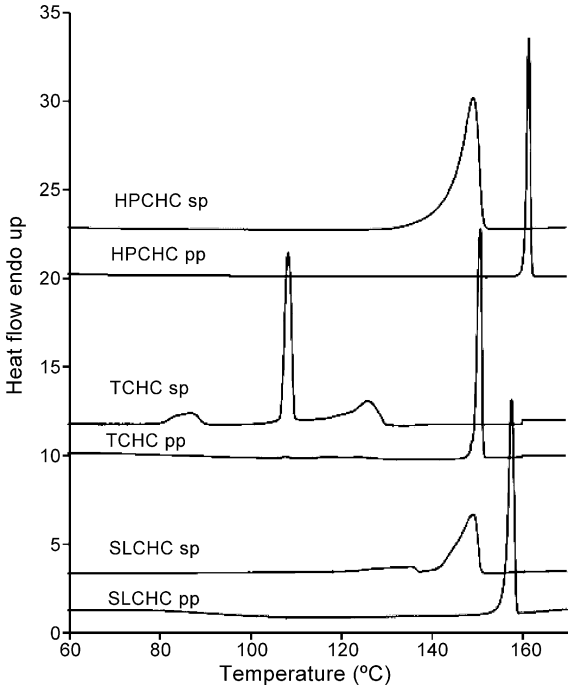

Fig. 3. DSC curves of the hydrated crystal forms prepared in sample pans with perforated covers (pp) and sealed covers (sp). From the perforated pans the water can leak and the melting peak of mod. I is registered. In the sealed pans the melting endotherm of the hydrated form is recorded. Heating rates: 5 K min^{-1}.

Mod. I melts at 157 °C, according to the melting point reported in the literature. In contrast to the closely related TCHC, SLCHC is thermally unstable and starts to decompose (changing from a white to a brown-coloured powder) at temperatures above 140 °C (probably due to the phenolic hydroxylic group which is absent in TCHC).

3.2.1.2. DSC. Fig. 3 shows the DSC curves of the hydrates in sealed and perforated pans, which causes impressive differences. In perforated pans the water can leak from the crystals on heating and the melting endotherms of the anhydrates (the high temperature polymorphs) is registered. In the sealed pans the water cannot escape and under the vapour pressure of the leaking water molecules the melting of the hydrates can be observed. TCHC shows the lowest melting temperature of both, the hydrate and the anhydrate and moreover the hemihydrated phase can be stabilized in two polymorphic forms (Schmidt and Schwarz, in preparation). The enthalpies of fusion of the anhydrates correspond between the three compounds, whereas the hydrate of TCHC shows a significant lower value as the two hydroxysubstituted compounds, which may be caused by the fact that the melting of TCHC hydrate is overlapped by a distinct liquidus curve, indicating that parts of the compound is dissolving in the released water, which is not the case with HPCHC and SLCHC. The peaks of dehydration are very broad and smooth, typical of sorbents without phase transitions. The thermophysical data are summarized in Table 1.

On storing the compounds in an oven at higher temperatures or in a desiccator at 0% RH the nucleation of mod. I can be suppressed. In Fig. 4 two different methods of drying are shown by

Table 1
Physicochemical data of the three hydrates

Compound	HPCHC	TCHC	SLCHC
Hydrate contained in commercial samples (%)	100	0–70	60–100
Crystallized from	Water	Water	Water
Water content (at ambient conditions, TGA)	2.5% = 0.42 mol[a]	2.8% = 0.48 mol[a]	3.5% = 0.63 mol[a]
Highest content of hydrated form allowed by Ph. Eur (%)	80	70	60
Number of possible hydrates	1	4	1
Dehydration temperature (TGA) (°C)	30–130	30–80	30–80
Water completely removed above (TGA) (°C)	50	40	60
Dehydration temperature (HSM)[b] (°C)	110	95	90
Isothermal desorption (60 °C)[c] (h)	4	2	1
Mod. I thermodynamically stable above (°C)	120	110	100
Melting point (°C)	144.8 ± 0.5[d]	106.8 ± 0.2[d]	142.6 ± 0.7[d]
Enthalpy of fusion (kJ mol^{-1})	36.2 ± 0.9	21.8 ± 0.2	34.2 ± 0.6
Max. water sorption at 90% RH[e] (%)	1.8	11.5	3.7
Kinetic stability at 25 °C, 30% RH	Years	Years	Years

[a] Depending on ambient RH.
[b] In viscous silicone oil.
[c] Sample weight ~5 mg.
[d] DSC onset.
[e] Mass change relating to the sample mass at 0% RH.

the example of TCHC. Drying in a desiccator at 0% RH represents a faster drying process as thermal drying at 80 °C. HPCHC and SLCHC need more time of drying and higher temperatures. But, at temperatures above 80 °C the hydroxysubstituted compounds are chemically unstable and start to decompose. Thus, the "gentle" method of drying by storing at 0% RH is recommended for these active compounds.

3.2.1.3. TGA. The mass losses of the three hydrates during heating are shown in Fig. 5. The water contents (Table 1) strongly depend on the ambient relative humidity and range from 2.5% to 3.5% (0.4–0.6 mol) which roughly estimated may be referred to as hemihydrates. At heating above 170 °C (not shown) the decrease in weight of the two hydroxyl-substituted compounds starts again with the thermal decomposition, whereas TCHC shows a significant higher thermal stability (decomposition starts >220 °C). The derivative curves show the mass loss with respect to time. HPCHC and SLCHC loose the most water right after the start of the heating cycle, whereas TCHC shows the highest desorption rate at 36 °C (~3 min). The two structurally related compounds TCHC and SLCHC finish dehydration at about 80 °C. HPCHC shows a nearly constant weight loss between 30 and 130 °C, which suggests a very loosely arrangement of water molecules within the crystal lattice, as in all of the three compounds.

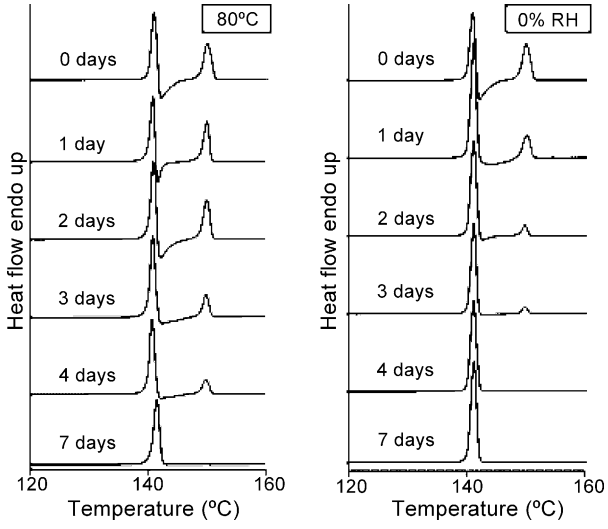

Fig. 4. Storage of TCHC at 80 °C (left) and 0% RH (right). Drying at low humidity is gentle and faster than the thermal method.

3.2.2. Spectroscopy

3.2.2.1. FTIR and Raman. The three hydrates show completely different FTIR (Fig. 6) and Raman spectra (Fig. 7). The spectra of the hydrated form and the isostructural mod. I are almost identical as shown with TCHC. The only difference between the IR spectra of the hydrated (H) and the anhydrated (I) form is the additional absorption band of the hydrogen bonds of the water molecule (TCHC H 3476 cm^{-1}). The wavenumbers of the water hydrogen bonds are of the order HPCHC > TCHC > SLCHC, which corresponds well with the NMR and SCXRD results and shows that the stronger hydrogen bonding absorbs at the higher wavenumber (Table 2).

The band shifts of the polymorphic anhydrates (not shown) average at 3–6 cm^{-1}, comparable to other conformational polymorphic LA. The most striking differences can be realized in the range of the C–H stretching vibrations of the alkyl chain (2980–2960 cm^{-1}, Raman), the valence vibrations of the carbonyl and amine group (1700–1600 cm^{-1}, FTIR), the molecule

Table 2
Significant infrared spectroscopic data of the three hydrates

Assignment	HPCHC	TCHC	SLCHC
νH_2O	3491	3476	3452
νN–H mod. II	3403	3380	3380
νN–H mod. I	3384	3367	3266

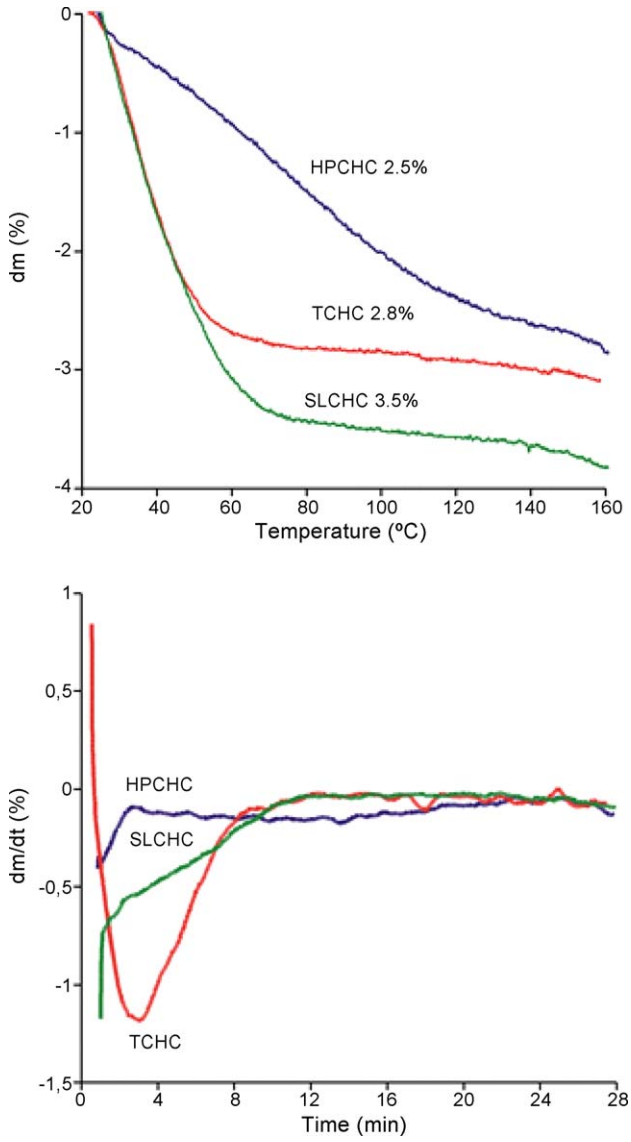

Fig. 5. Mass loss (top) and the rate of mass loss (bottom) of the hydrated crystal forms. The difference between starting and final mass was considered to be the water content of the sample. Heating rates: 5 K min^{-1}.

vibrations (1500–1000 cm^{-1}, FTIR) and the lattice vibrations (200–50 cm^{-1}, Raman).

The N–H stretching vibrations (FTIR) of the stable anhydrates (mod. II) lie at higher wavenumbers than that of the metastable mod. I. This is contradictory to Burger's 'infrared rule' (Burger and Ramberger, 1979a) and suggests that intermolecular hydrogen bonding to the N1 amino-function plays not the significant role in the molecular arrangements of the anhydrated crystal forms of the three compounds.

3.2.2.2. NMR. The chemical shifts of the peaks in the ^{1}H NMR spectra are caused by the protons that are involved in the H-bonding. A high chemical shift corresponds to strong H-bonds (Fig. 8). The strength of H-bondings in the hydrated crystals of the compounds tested in this study is of the order SLCHC > TCHC > HPCHC. Moreover, the solid-state ^{1}H NMR spectra give immediate information about the number of

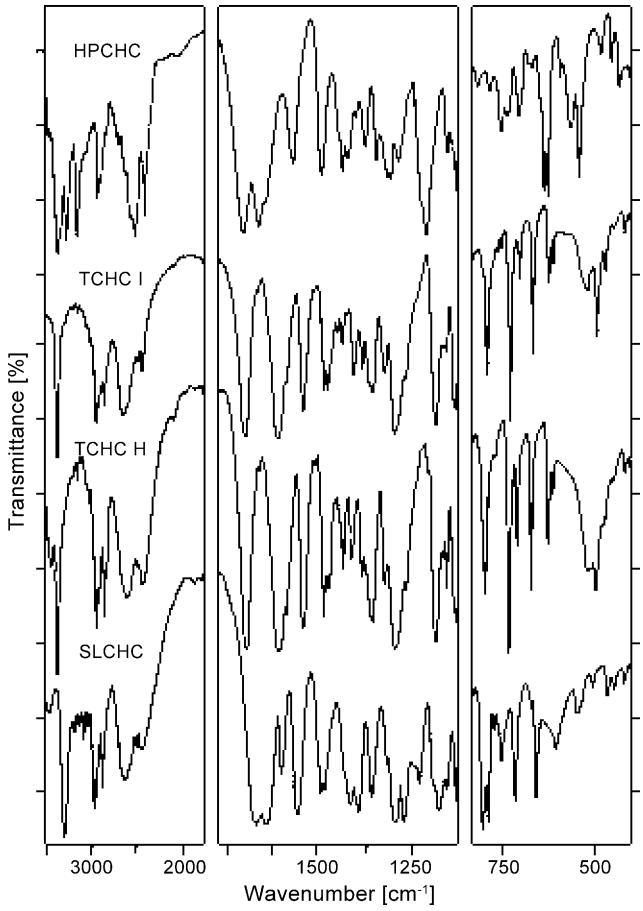

Fig. 6. Most important sections of the FTIR spectra of the three hydrates (H) and TCHC mod. I (I), recorded at ambient conditions (KBr method).

molecules in the asymmetric unit. TCHC and SLCHC show each one conformer with the anhydrates, but two conformers with the hydrated forms. Recording of HPCHC anhydrate failed by conversion to the hydrate under ambient conditions, but the spectrum of the hydrated form indicates one conformer in the asymmetric unit different to the others. Compared to PXRD the solid-state NMR spectra show distinct differences between the hydrates and the isostructural mod. I. A full report on the NMR results will be published at a later date with expanded authorship.

3.2.3. X-ray diffraction

3.2.3.1. PXRD. The X-ray powder patterns of the crystal forms are illustrated in Fig. 9. The diffractograms of the two hydroxy-substituted compounds show related polycrystalline structures, although the powder diffractograms are severely affected by preferred orientation. The peaks of SLCHC are shifted to smaller angles 2θ indicating greater interplanar distances of the crystalline structure than HPCHC. TCHC shows a very different pattern with the greatest interplanar distances. Diffraction patterns before and after dehydration show nearly identical peaks and intensities. The only difference may be seen in the first peaks of the patterns (Table 3) which are slightly shifted to higher angles 2θ (smaller interplanar distances) after dehydration, even with the mono- and dihydrate of TCHC, although the patterns are recorded at raised temperature. All the other reflexes are

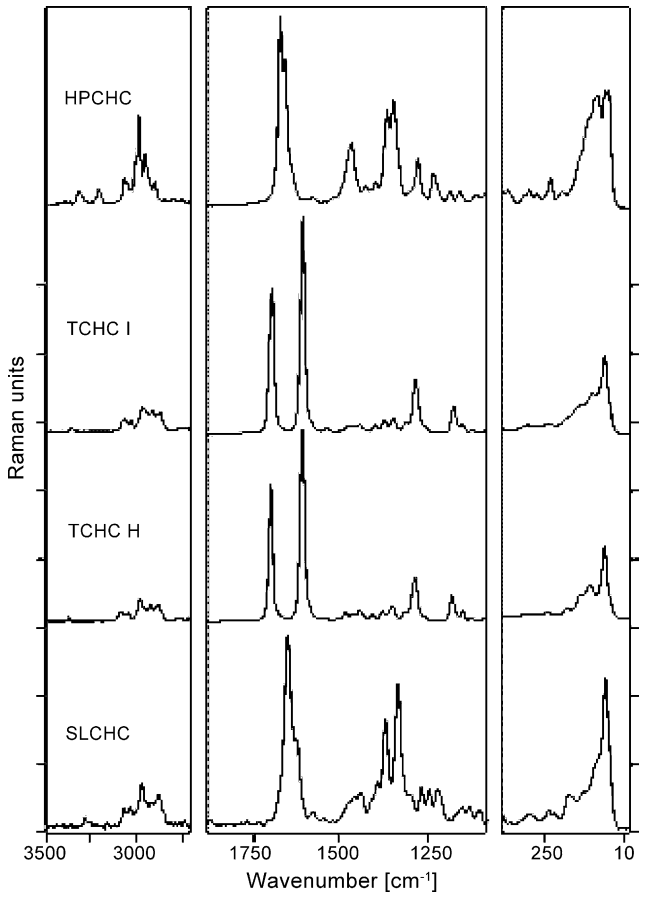

Fig. 7. Most important sections of the FT-Raman spectra of the three hydrates (H) and TCHC mod. I (I), recorded at ambient conditions.

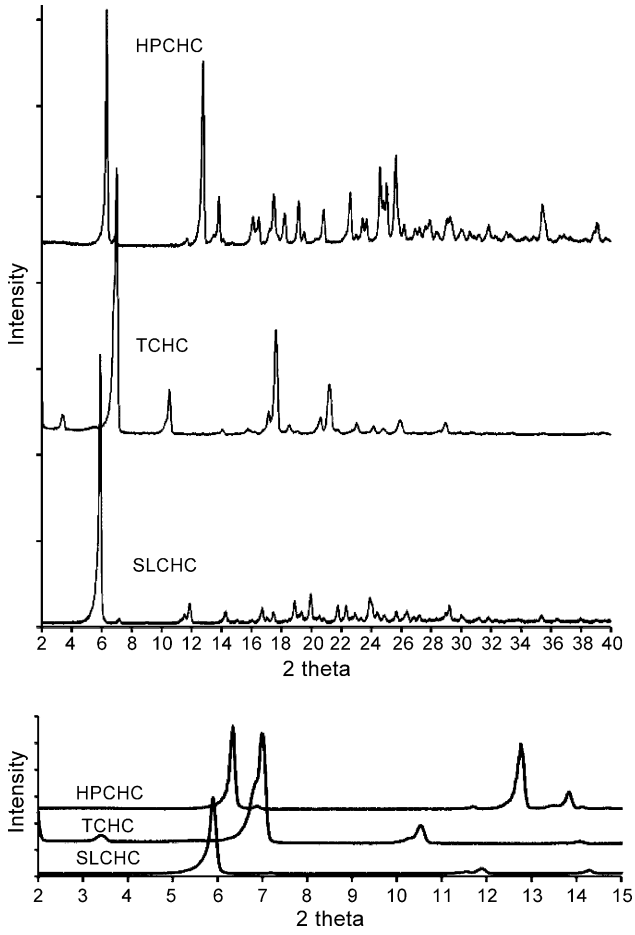

Fig. 9. Powder X-ray diffraction patterns of the three hydrated crystal forms, recorded at ambient conditions. The patterns at the bottom show the higher angle diffraction in detail.

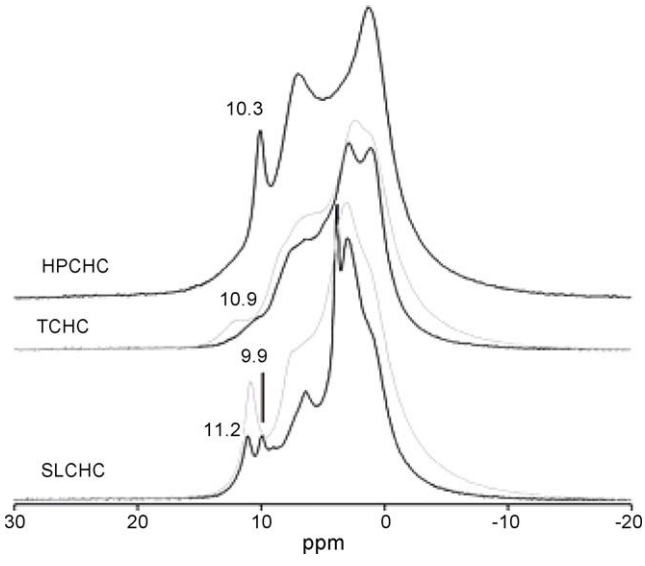

Fig. 8. ^{1}H NMR spectra of the three hydrates at ambient conditions. The data represent the chemical shift of hydrogen bondings of the hydrates. Bright grey lines show ^{1}H NMR spectra of the anhydrates of TCHC and SLCHC each with one molecule in the asymmetric unit.

unchanged. PXRD indicates a structural relationship between HPCHC and SLCHC.

3.2.3.2. Single crystal X-ray diffraction (SCXRD). X-ray diffraction of single crystals of SLCHC (Schmidt et al., 2006) and TCHC (Ellern, unpublished results) proved the number of molecules in the asymmetric units found by solid-state NMR (TCHC anhydrate 1 conformer, SLCHC anhydrate 1 conformer and SLCHC hydrate 2 conformers). The intermolecular hydrogen bond lengths observed in hydrated SLCHC are N–H···O 2.641 Å (conformer 1) and N–H···O 2.632 Å (conformer 2),

Table 3
Significant powder X-ray diffraction data of the three hydrates

	HPCHC		TCHC		SLCHC	
	2θ (°)	d (Å)	2θ (°)	d (Å)	2θ (°)	d (Å)
Hydrate	6.328	13.957	3.355 6.769	26.311 13.047	5.897	14.975
Mod. I	6.350[a]	13.907	3.399[b] 6.888	25.971 12.823	5.985[c]	14.754

[a] At 140 °C.
[b] At 112 °C.
[c] At 130 °C.

which represents a stronger intermolecular hydrogen bonding than in the anhydrated form (N–H···Cl 2.661 Å) and proves the ^{1}H NMR results. Compared to other drug compounds, e.g. oxybuprocaine hydrochloride (Schmidt, unpublished results), which has H-bondings of 2.226 Å, the H-bondings of the three hydrate forming LA of this study are relatively weak, which prove the conclusion form the contradiction to the 'infrared rule'. The hydrates structurally can be classified as channel hydrates (Morris, 1999), which supports the observations (TGA, TGA isotherms, water sorption/desorption isotherms) of loosely arranged water in the crystals.

3.2.4. Water sorption/desorption analysis

The compounds of this study were examined by three water sorption/desorption analytic methods: 1, interval method; 2, integral method; 3, thermogravimetric isotherms.

3.2.4.1. Interval method. Even less hygroscopic anhydrated polymorphic forms can be differentiated by sensitive interval water sorption/desorption analysis, which can be a very useful method to differentiate and identify the forms or to rank the polymorphic forms (Schmidt, 2005b) by their relative thermodynamic stability (with regard to surface area and crystal transformations). The thermodynamically stable form is used to adsorb the smallest amount of water, whereas the metastable and unstable forms show a far higher extent of adsorption. In some cases a transformation from one crystal form into another can be observed using this method (Schmidt et al., 2004). The interval water sorption/desorption isotherms of the three hydrates are depicted in Fig. 10. All of the three isotherms show a very small hysteresis, which indicates that the water molecules easily can leave the polycrystalline material. The water sorption of all three hydrates is very similar in the range of 0–40% RH. The amount of adsorbed water at ambient conditions (25 °C, 40% RH) is of the order SLCHC > TCHC > HPCHC, which correlates with the order of strength of the H-bondings found by ^{1}H NMR. Whereas the hydrates of the hydroxysubstituted compounds HPCHC and SLCHC sorb only a little amount of water at RH > 40%, the hydrate of TCHC converts to a monohydrate at 50% RH and to a dihydrate at 75% RH. The large steps in the adsorption of the water suggest transformations to other crystal systems, which could be disproved by moisture-controlled PXRD. All the hydrated forms found in this study are non-stoichiometric.

3.2.4.2. Integral method. The integral sorption/desorption isotherms of TCHC are shown in Fig. 11. The isotherm at 43% RH does not change during the measurement cycle of 240 h. The isotherms at lower RH show desorption and the isotherms at higher RH show sorption of water. The water of TCHC can completely be removed at a relative humidity below 23% RH. The isotherms of HPCHC and SLCHC show the same process with different values of adsorption (see TGA). Both, the integral and the interval method show that the water in all of the three compounds completely can be removed in dry air (0% RH).

3.2.4.3. Thermogravimetric isotherms. Isothermal thermogravimetry was performed at 30, 40, 50 and 60 °C. Fig. 12 shows

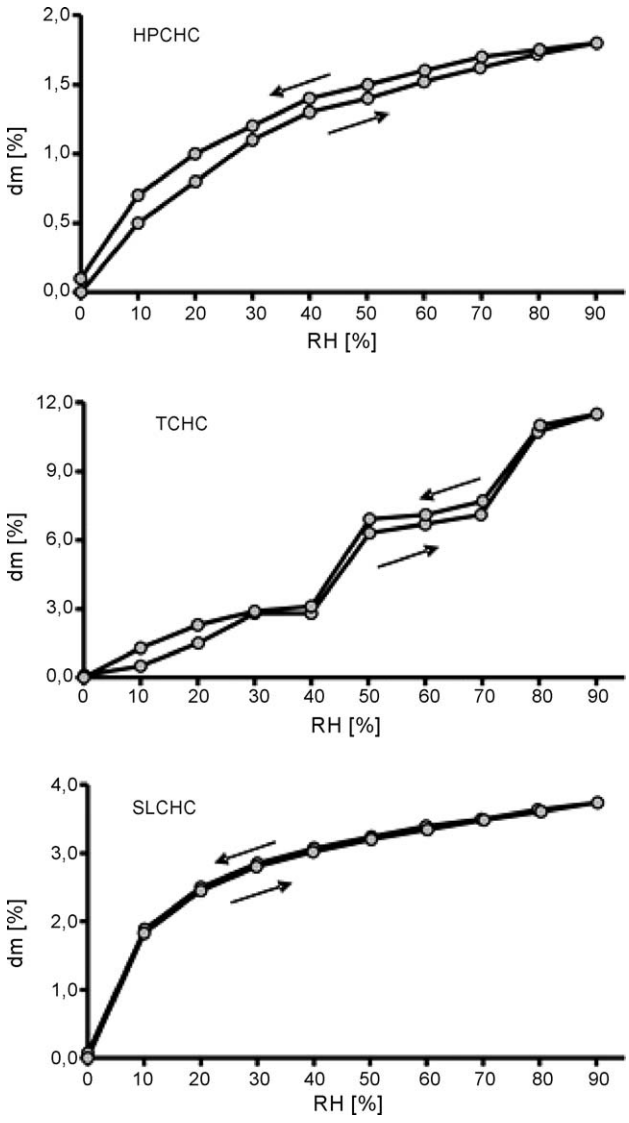

Fig. 10. Moisture sorption isotherms of the three compounds at 25 °C. The originally hydrated forms were dehydrated at 0% RH. After equilibration the measurement cycle started at 0%. The mass change is corrected for the minimum mass value at 0% RH.

that the water cannot be completely removed at 30 and 40 °C, but above 50 °C in about 2 h (sample mass 5 mg). The thermogravimetric isotherms of HPCHC and SLCHC are similar, but the water of SLCHC can completely be removed above 60 °C and that of HPCHC above 70 °C. So the order of thermal dehydration is TCHC < SLCHC < HPCHC. Storage of the compounds in an oven at analogous temperatures and subsequent determination of the water content (by TGA) exactly leads to the same results.

3.3. Stability of the hydrates

Table 1 summarizes the most important thermoanalytic data of the three hydrates. The hydrates are stable enough to coexist with the anhydrated stable crystal form in commercial samples, but the water contents strongly depend on the ambient relative humidity. Desorption by thermal or humidity methods results in the isostructural mod. I, which at the same time is the thermody-

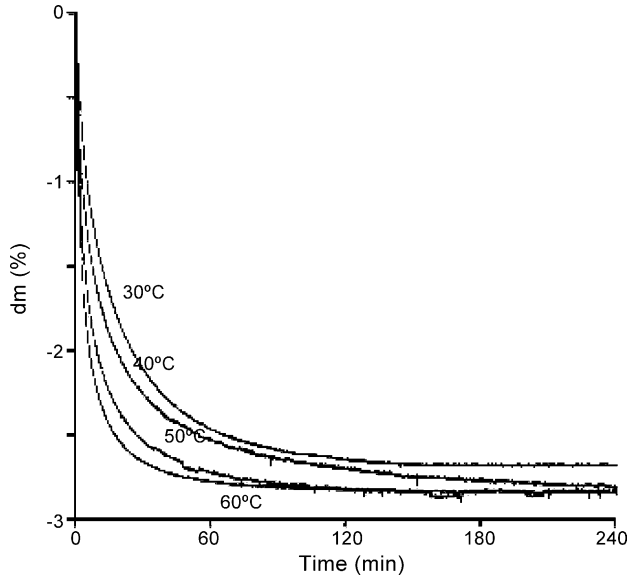

Fig. 11. Thermogravimetric desorption isotherms of TCHC at 30, 40, 50 and 60 °C. The included water can easily be removed above 40 °C, whereas drying at 50 °C takes half the time.

namically unstable crystal form of the anhydrate (thermodynamically stable above 100–120 °C). Mod. I, recrystallized from the melt at raised temperature, is a very hygroscopic crystal form and transforms to the hydrate (sorbs water) on decreasing the temperature under ambient humidity conditions. The hydrates are non-stoichiometric, which means that they are not stable to changing temperature and/or relative humidity. The water molecules seem to be loosely arranged in the crystal lattices and may enter or leave the crystals depending on the ambient humidity and temperature conditions. The broad peaks of the TGA derivative curves and the very low hysteresis of the water sorption/desorption isotherms prove the assumption that the hydrates are of low stability. But with the water desorption isotherms of HPCHC anhydrate (Schmidt and Schwarz, 2005) it could be shown, that the stable crystal phase of the anhydrate converts to the crystal system of the hydrated phase on raising the relative humidity (50% RH) without re-conversion on decreasing the humidity conditions.

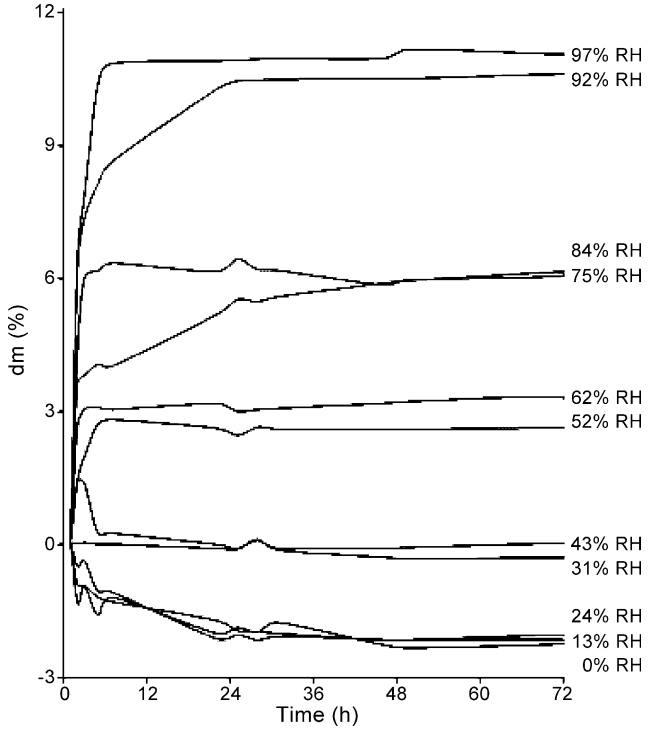

Fig. 12. Integral desorption isotherms of TCHC at 25 °C. The water content nearly stays constant at 31–43% RH (room conditions). At lower humidities water is desorbed, at higher humidities water is absorbed by the crystals.

4. Conclusions

The three on molecular level closely related local anaesthetics HPCHC, TCHC and SLCHC are the only compounds of the series of investigated ester-type local anaesthetics that are able to form non-stoichiometric hydrates. The compounds show very closely related thermal analytic and sorption effects, such as the melting behaviour, thermal, interval and integral sorption/desorption and the isomorphism of the hydrate with the unstable high temperature form of the anhydrate. In contrast, the compounds differ strongly in their X-ray diffractional and spectroscopic properties. TCHC and SLCHC which on molecular level only differ in the C3 hydroxy group show very closely related solid-state properties, although the C3 hydroxy group of SLCHC obviously inhibits the formation of more than one hydrated form (TCHC is able to form four different hydrates). The presence of the C3 hydroxyl group in HPCHC and SLCHC significantly reduces the thermal stability and put a risk on formulation processes at higher temperatures.

The crystal structure of the stable anhydrate and the hydrate of SLCHC has been solved, so it may be concluded, regarding the NMR results, that the asymmetric unit of TCHC anhydrate consists of one conformer (proved by single crystal X-ray diffraction) whereas the hemihydrate consists of two. The solid-state properties of HPCHC strongly differ from the others probably caused by the missing flexible N1 butyl side chain, which plays an important role in the formation of polymorphs.

The contents of hydrated form in the commercial products vary from 0% to 100%, which is not a problem to formulation processes, since HPCHC, TCHC and SLCHC are drug compounds with a high solubility in water (even the hydrates). However, the hygienic stability of the compounds is affected and strongly depends on the storage conditions, at which a content of hydrated form higher than 60–80% exceeds the content of water allowed by the pharmacopeias (2% content of water, recommended is 1%).

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