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Preparation and solid state characterisation of chlorothiazide sodium intermolecular self assembly suprastructure

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Abstract

Chlorothiazide (CTZ), unlike other thiazide diuretics, can form salts. An injectable formulation containing the sodium salt is available; however neither the physicochemical characteristics of the salt nor its solid state form have been previously reported. This work reports on the crystal structure of chlorothiazide sodium. The structure was investigated by single crystal X-ray and nuclear magnetic spectroscopy (NMR) analyses and compared to chlorothiazide, while the solid state characteristics were assessed by thermal analysis, powder X-ray diffraction, infrared spectroscopy, dynamic moisture sorption and solubility analysis. The crystal structure of chlorothiazide sodium was determined to be triclinic; the crystal space group type was P-1. Chlorothiazide sodium presented a self-assembly polymeric-type suprastucture, where the unit cell comprised two chlorothiazide molecules bonded together with sodium cations through the water bridges. The coordinate centre comprised the following: $(CTZ)_3 \cdot (H_2O) \cdot Na(H_2O)_2Na \cdot (H_2O) \cdot (CTZ)_3$. The crystalline material was determined to be a monosodium dihydrate, stable in the range of 10-90% relative humidity (RH) at 25 ˚C. Additional processing of the salt resulted in a crystalline anhydrous form which was stable in the range 0-20% RH at 25 ˚C. The aqueous solubility of the chlorothiazide sodium dihydrate at 37 °C was found to be approximately 400-fold higher than that of chlorothiazide, which may present biopharmaceutical advantages for the salt compared to the non-salt form.

Key words

Chlorothiazide sodium, Calorimetry (DSC), Crystal structure, Hydrate, Moisture sorption, NMR spectroscopy, Solid state characteristics

1. Introduction

There is considerable interest in the impact of solid state physicochemical properties of drugs on their biopharmaceutical properties. Thus, for example, the fraction of a drug absorbed has been correlated with its solubility and with the inverse of the drug's melting point (Chu, Yalkowsky, 2009). Consequently, drug bioavailability may be improved by the preparation of lower melting point polymorphs, co-crystals, solvates of the drug (Stahly, 2007) or alternatively by preparing a more soluble salt form (Berge et al., 1977).

Chlorothiazide (CTZ), 6-chloro-4H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide (Beyer, 1982), with a molecular formula $C_7H_6ClN_3O_4S_2$, is a thiazide diuretic which is administered orally in the crystalline form as tablets or as an oral suspension (Brunton et al., 1996). Chlorothiazide is classified in class IV of the Biopharmaceutics Drug Classification System (BCS) (Amidon et al., 1995; Taub et al., 2002), indicative of its low solubility and low permeability. It is very slightly soluble in water, poorly soluble in acetone, ethanol and methanol but readily soluble in aqueous alkali solutions which is as a result of its acidic properties, allowing salt formation (European Pharmacopoeia, 2007). CTZ has a particularly high melting point (~350 °C with degradation), one of the highest for a molecular crystalline drug administered to man and it exhibits incomplete absorption following oral administration (Chu, Yalkowsky, 2009). The bioavailability of CTZ was found to decrease from 33% to 6% as the dose increased from 0.21 to 1.75 g (Corrigan, O'Driscoll, 1980). Considering the drug's poor physicochemical profile, it is considered advantageous to prepare a more soluble form, which may have a lower melting point.

The first report of X-ray crystallographic studies on CTZ was published by Dupont and Dideberg in 1970, while the crystal structure of CTZ was first solved and presented by Shankland and co-workers (1997). The structure of CTZ was subsequently confirmed by the joint use of powder neutron diffraction and powder X-ray diffraction (synchrotron) techniques (Leech et al., 2008). A good overview of CTZ physicochemical properties is contained in the European Pharmacopoeia (2010)., Furthermore, Oswald and co-workers recently described the formation of CTZ Form II, a new

polymorph created by applying high pressure to the crystals of Form I, the only known polymorph of CTZ recrystallised at ambient conditions (Oswald et.al. 2010). Unfortunately, CTZ Form II converts into the Form I at ambient pressure and any pharmaceutical advantages of CTZ Form II were not determined. The X-ray crystal structure of a range of solvated forms of chlorothiazide, such as chlorothiazide formic acid solvate (Johnston et al., 2007a), chlorothiazide N,N-dimethylacetamide disolvate (Johnston et al., 2007b), chlorothiazide DMSO solvate (Johnston et al., 2007c) and chlorothiazide-pyridine solvate (Johnston et al., 2008) have also been described but again, their physicochemical properties were not described.

In contrast, there appears to be no published reports on the crystal structure and the solid state characteristics of a sodium salt form of chlorothiazide in spite of its wide use as an injectable (parenteral) form of the drug as an intravenous solution. The soluble injection, to be reconstituted before use, is described as 6-chloro-2H-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide monosodium salt, with a molecular weight of 317.71 amu (empirical formula $C_7H_5ClN_3NaO_4S_2$), a sterile lyophilized white powder containing mannitol as an inactive ingredient (Hankins et al., 2001). This is available in the USA, marketed as Diuril Sodium®. The crystal and solid state profiles of this form of CTZ are unknown despite the apparent clinical and formulation benefits of the salt. Therefore the objective of this work was to prepare a crystalline sodium salt of chlorothiazide, determine its crystal structure and physicochemical properties and compare them to those of chlorothiazide with the aim of presenting comprehensive information on the solid state characteristics of the salt form.

2. Materials and methods

2.1. Materials

Materials used in experiments were chlorothiazide (CTZ) obtained from Sigma (Ireland), sodium hydroxide (NaOH) from Riedel de Haën (Germany) and potassium bromide (KBr, FT-IR grade) from Sigma (Ireland). The following solvents and other reagents were also used including deionised water obtained from Purite Prestige Analyst HP water purification system, acetone (analytical grade) and ethanol (H_2O<0.1%) from Corcoran Chemicals (Ireland), methanol HPLC grade from Sigma (Ireland), deuterated dimethyl sulphoxide (DMSO-D_6) from Apollo Scientific Limited (UK), hydrogen peroxide (H_2O_2, Ph. Eu., B.P. 30%) from Riedel de Haën (Germany), formic acid (HPLC grade) from Sigma (Ireland), nitric acid 69% (HNO_3, Aristar) from BDH (UK), hydrochloric acid 32% (HCl, extra pure) from Riedel de Haën (Germany) and Hydranal-Composite from Riedel de Haën (Germany).

2.2. Analytical methods

2.2.1. Sample preparation

Chlorothiazide sodium (CTZNa) was prepared by mixing equal molar amounts of 1M NaOH aqueous solution and chlorothiazide. The suspension obtained was evaporated under forced air flow at 70 ˚C until dry, re-suspended at 70 ˚C in water, filtered through a 0.45 µm membrane filter and the filtrate was recrystallised by solvent evaporation at 70 ˚C.

The crystals subsequently used for single crystal X-ray and other analyses were prepared from solutions. The solutions of CTZ and CTZNa were saturated for each solvent used. Saturation was obtained by introducing an excess of raw material into the appropriate solvent at ambient conditions by shaking for an hour on a WhirliMixer® (Fisons Scientific Equipment). 10 ml of the suspension obtained was filtered through a 0.22 µm membrane filter into a glass vial which was kept in a glove box (Clean Sphere CA 100, Safetech Limited) under constant nitrogen flow at room temperature (20-23 ˚C). The moisture

content in this controlled environment was less then 1% relative humidity. The dry nitrogen flow and temperature control were provided to maintain reproducible conditions of solvent evaporation. Compounds were crystallised by solvent evaporation. CTZ crystals were obtained by evaporation of acetone or ethanol and CTZNa crystals were obtained by evaporation of water or 1:1 acetone/water mixture or ethanol.

2.2.2. Single crystal X-ray diffraction (SC-XRD)

Single crystal XRD data was collected on a Rigaku Saturn 724 CCD diffractometer. Suitable crystals were selected and mounted using inert oil on a 0.30 mm quartz fibre tip and immediately placed on the goniometer head in a 150K nitrogen gas stream. Each data set was collected using Crystalclear-SM 1.4.0 software and 1680 diffraction images, of 0.5° per image, were recorded. Data integrations, reductions and corrections for absorption and polarization effects were all performed using Crystalclear-SM 1.4.0 software. Space group determinations, structure solutions and refinements were obtained using Crystalstructure ver. 3.8 and Bruker Shelxtl ver. 6.14 software. To evaluate the data, Mercury 2.2 software was used (Blessing, 1995).

2.2.3. Powder X-ray diffraction (PXRD)

Powder XRD analysis was conducted using a Miniflex II Desktop X-ray diffractometer Rigaku with Ilaskris cooling unit. The tube output voltage used was 30 kV and tube output current was 15 mA. A Cu-tube with Ni-filter suppressing Kβ radiation was used. Measurements were taken from 5 to 40 on the 2 theta scale at a step size of 0.05˚ per second in each case (Tajber at al., 2009). Scans were performed at room temperature.

2.2.4. Differential scanning calorimetry (DSC)

DSC experiments were performed using a Mettler Toledo DSC 821^{e} with a refrigerated cooling system LabPlant RP-100. Nitrogen was used as the purge gas. Aluminium sample holders were sealed with a lid

and pierced to provide three vent holes. Sample volume was sufficient to provide proper contact between the powder and the bottom of the pan, and sample weight was ≥5 mg. DSC measurements were carried out at a heating/cooling rate of 10 ˚C/min (Tajber et al., 2005). The DSC system was controlled by Mettler Toledo STAR^e software (version 6.10) working on a Windows NT operating system. The unit was calibrated with indium and zinc standards.

2.2.5. Thermogravimetric analysis (TGA)

TGA was performed using a Mettler TG 50 module linked to a Mettler MT5 balance. Samples were placed into open aluminium pans (5-12 mg). A heating rate of 10 ˚C/min was implemented in all measurements (Tajber et al., 2005). Analysis was carried out in the furnace under nitrogen purge and monitored by Mettler Toledo STAR^e software (version 6.10) with a Windows NT operating system.

2.2.6. Karl Fischer titrimetry

0.5 g of sample was dissolved in pre-titrated 50 ml of methanol. As Karl-Fischer reaction is not stoichiometric in alkaline solutions (Scholz, 1987), the pH was adjusted with 50 µl of 32% hydrochloric acid. Metrohm 841 Titrando was used for titration and the unit was calibrated with 20 µl of water.

2.2.7. Solid state Fourier transform infrared spectroscopy (FTIR)

Infrared spectra were recorded on a Nicolet Magna IR 560 E.S.P. spectrophotometer equipped with MCT/A detector, working under Omnic software version 4.1. A spectral range of 650-4000 cm^{-1}, resolution 2 cm^{-1} and accumulation of 64 scans were used in order to obtain good quality spectra. A KBr disk method was used with a 0.5-1% sample loading. KBr disks were prepared by direct compression under 8 bar pressure for 1 minute (Healy et al., 2008). The sample preparation did not affect the spectra as confirmed with an attenuated total reflectance (ATR) spectrometer (data not shown).

2.2.8. Nuclear magnetic resonance (^{1}HNMR, ^{13}CNMR)

A Bruker Avance 400 NMR with 4-nucleus (^{1}H, ^{13}C, ^{31}P and ^{19}F) probe was used for NMR studies. Deuterated DMSO-D_6 was used to prepare the samples. Sample concentration was in the range 20-40 mg/ml. Spectrometer frequency was 400 MHz with an acquisition time of 2s. The number of scans was appropriate to gain good quality spectra. Standard Pulse Sequence supplied by Bruker was used for 1H, 13C and 2-dimensional experiments.

2.2.9. Elemental analysis

Elemental analysis was carried out using an Exeter Analytical CE440 CHN analyser. The molar amount of carbon as carbon dioxide, nitrogen, as nitrogen oxide and hydrogen as water, was determined by oxidation of the sample (around 10 mg) and the thermal conductivity analysis of obtained gases and water vapour.

2.2.10. Inductively coupled plasma–mass spectrometry (ICP-MS)

Known weights of the samples were placed in separate digestion vessels. 7 ml of 69% HNO_3 and 1 ml of 30% H_2O_2 were then added to each sample. The vessels were sealed and placed in a microwave digester where they were heated to 200 ^{o}C at 1000W and held for 20 minutes at these conditions. The samples were then made up to a final volume of 50 ml with deionised water. This digest was then analysed by ICP-MS Varian 820, to measure the levels of sodium and potassium. Certified calibration standards were used to calibrate the ICP-MS and the samples were diluted where necessary to bring them into the range of the calibration curve.

2.2.11. Gas chromatography – mass spectrometry (GC-MS)

A known weight of each sample was placed in a headspace vial to which 1 ml of deionised water was added. The vials were sealed. The vials were then heated and agitated at 80 ˚C for 10 minutes in a headspace auto-sampler where the volatile organics in the samples were driven into the headspace of the

vial (the air above the sample). 1 ml of the headspace gas was then extracted by syringe from the vial and injected onto the GC-MS Varian Saturn 22700 column. The ethanol eluted at 8.1 minutes and acetone at 8.7 minutes. The column used for the analysis was a WCOT fused Silica 60m x 0.32mm ID coating CP-SELECT 624 CB DF=1.8.

2.2.12. Density measurement by helium pycnometry

The true density was measured by AccuPyc 1330 Pycnometer Micromeritics™ using helium (99.995% purity) to determine the volume of the sample. A 1 cm^3 sample holder was used. The instrument was calibrated immediately before performing the analysis at room temperature. Each sample was analysed in duplicate and averaged results are presented in this paper. Samples of CTZ and anhydrous CTZNa were dried for at least 12 hours prior to analysis using a vacuum oven (Gallencamp, UK) operated at 30 ºC and -600 mbar pressure. Crystals of CTZNa dihydrate where dried in a glove box (Clean Sphere CA 100 by Safetech Limited) under constant nitrogen flow at room temperature (20-23 ˚C) for three hours. The moisture content in the environment was controlled and was less then 1% relative humidity.

2.2.13. Solubility

For CTZNa, powdered CTZNa dihydrate was added in excess (approximately three times the expected saturated concentration) directly to water at 37 ˚C, shaken at 100 cpm and analysed after 5 min, and every 10 min until 30 min. The concentrations obtained at the final three time points were the same and were averaged to give the saturated stoichiometric solubility (Anderson, Flora, 1996). All alkali solutions of CTZNa were analysed within one hour, as within this timeframe alkaline decomposition of the compound is insignificant (Charnicki et al., 1959).

For CTZ, excess of CTZ powder (approximately three times of the expected saturated concentration) was suspended in 10 ml of water in a 20 ml volumetric flask. The suspension of chlorothiazide was shaken at 70 cpm at 37 ˚C for 24 hours.

Suspensions were filtered through a 0.45 µm membrane filter. In the case of CTZNa, the excess of material recovered after 30 minutes was analysed using DSC and PXRD. Analysis confirmed the residue of material to be CTZNa dihydrate with no traces of pure CTZ precipitation (data not shown).

The pH of the aqueous saturated solutions was measured using a Thermo Orion 420+ pH-meter.

The quantity of CTZ and CTZNa dissolved in water was assayed by UV spectrophotometry at 292 nm (Shimadzu UV-1700 Pharmaspec). CTZ aliquots were diluted with 0.1M NaOH and water was used to dilute CTZNa samples to obtain the appropriate concentration range.

2.2.14. Dynamic vapour sorption (DVS)

Vapour sorption experiments were performed on a DVS Advantage-1 automated gravimetric vapour sorption analyzer (Surface Measurement Systems Ltd., London, UK). The DVS-1 measures the uptake and loss of water vapour gravimetrically with a mass resolution of ± 0.1 µg. The temperature was maintained constant at 25.0 ± 0.1 °C. A mass of around 10 mg of powder was loaded into a sample net basket and placed in the system. The samples were equilibrated at 0% of relative humidity (RH) until dry, reference mass was recorded. The samples were exposed to the following % of RH profile: 0 to 90% in 10% steps and the same for desorption. At each stage, the sample mass was equilibrated (dm/dt ≤ 0.002 mg/min for at least 10 min) before the change of relative humidity. An isotherm was calculated from the complete sorption and desorption profile. Amount of water was expressed as a percentage of the dehydrated chlorothiazide sodium (reference mass).

2.2.15. Data analysis

The following software was implemented to analyse the data: Omnic 4.1^{TM} - FTIR spectra analysis with baseline auto correction, Chem Sketch^{TM} freeware - to calculate theoretical element contributions for elemental analysis, TopSpin 2.1^{TM} - to evaluate ^{1}HNMR and ^{13}CNMR results. The crystallographic data was analysed in detail using Ortep-3 (Faruggia, 1997) and Platon (Spek, 2003) software.

3. Results and discussion

3.1. Crystal structure

CTZNa crystallised from water, 1:1 water/acetone mixture and ethanol resulted in the same crystal and molecular structure, as determined by single crystal XRD studies. The Ortep view of CTZNa is presented in Figure 1. The crystal structure of CTZNa determined in this work is triclinic and the crystal space group type is P-1. A summary of the key crystallographic information for CTZNa is given in Table 1.

In comparison to CTZNa, the crystal structure of CTZ is triclinic with the crystal space group type of P1 as presented by Shankland (1997) and Leech (2008). The CTZNa unit cell comprises two chlorothiazide molecules bonded together with sodium cations through water bridges. The coordinate centre consists of the following sequence: $(CTZ)_3 \cdot (H_2O) \cdot Na(H_2O)_2Na \cdot (H_2O) \cdot (CTZ)_3$ (Fig. 2). Additionally, some molecules of water (O7) were found on two opposite planes of the crystal cell; these are non-coordinated and most likely of an interstitial type, trapped in the void of the crystal cell. The polymeric-like chains including sodium cations are built from distorted Na1-O6 fragments forming a distorted octahedral unit stretching along the b-axis. Every asymmetric unit of the sodium linkage consists of two sodium cations each coordinating six oxygen atoms. Each of the sodium cations coordinates three molecules of chlorothiazide through a SO_2 group. Two CTZ molecules are bridged to each sodium(I) through an oxygen atom of the SO_2 group attached to the benzene ring. The third chlorothiazide molecule is bridged to the sodium cation through the oxygen of SO_2 belonging to the heterocyclic ring. The second SO_2 of this CTZ molecule attached to the benzene ring connects with a second layer of the polymeric structure (Fig. 2).

The distance between the two sodium cations sharing water molecules is 3.46Å (determined using the "measurement distance" tool in the Mercury software) and that between two sodium cations linked via the SO_2 group is significantly longer at 5.73Å. The parallelogram formed by the two sodium cations and two water molecules (Fig. 2) can be characterised by the bond lengths of 2.44Å and 2.34Å

between Na1 and O6 and angles: Na1-O6-Na1 of 92.7° and O6-Na1-O6 of 87.3°. One of the two distances between the sodium cation and oxygen of SO_2 bonded to the benzene ring is 2.47Å and the other is 2.36Å, which is the same as the distance formed between the same sodium cation and the heterocyclic SO_2 group.

Some of the bond distances are different for CTZNa compared to CTZ, reflecting the different neighbourhood around the chemical groups and their engagement in different types of bonds. A shortening of the S1-N1 (1.613(5)Å in CTZ to 1.584(5)Å in CTZNa) bond is observed, consistent with the disappearance of H-bonds formed in CTZ by the primary sulphonamide group. Shorter N2-C5 (1.392(6)Å in CTZ to 1.380(5)Å in CTZNa) and N2-C7 bonds (1.350(7)Å in CTZ to 1.327(5)Å in CTZNa) suggest significant changes in the heterocyclic ring associated with changes at N2, probably due to proton migration. On the other hand an increase of the S2-O3 distance 1.457(4)Å in CTZNa compared to CTZ 1.438(5)Å indicates involvement of the group in bonds forming the complex Na-O octahedral structure. The greatest lengthening of the bond is seen for N3-C7 (1.291(6)Å in CTZ to 1.345(6)Å in CTZNa) and supports the hypothesis of proton relocation around the heterocyclic ring. Other major differences in torsion angles between CTZ and CTZNa involving the sulphonamide parts of the molecule were also noticed.

Shankland and co-workers (1997) determined the hydrogen bond distances in the crystal lattice between the molecules of CTZ and stated that they were typical for organic molecules with those functional groups and were as follows: N1-H5...N3, N1-H6...O3, C4-H4...O4 and N1-H6...O1. A more extensive network of hydrogen bonds in the CTZ crystal than previously reported has been determined in the present work and is compared to H-bonds present in CTZNa in Table 2. Hydrogen bonding between the C-H groups as proton donors and the oxygen of the sulphonyl groups is not observed in the crystal lattice of, for example, hydrochlorothiazide (Dupont, L., Dideberg, O., 1972). The rich H-bond network found in CTZ is consistent with the high stability of the crystal lattice. Only a few hydrogen bonds can be observed in CTZNa (Table 2) as a consequence of some of the groups being now engaged in Na-O interactions.

A literature search was undertaken to compare the molecular structure of CTZNa with similar compounds. The work of Mizuno et al. (1969) describing sodium hexa- water coordinates of a xanthine sodium salt reported that the O-Na-O angles ranged from 84.5˚ to 97.0˚. The angles reported here for CTZNa are about the same size, however, the pattern of Na-O bonds is far more complex than that in xanthine sodium as oxygen atoms participating belong not only to water, but also to the sulphonyl groups. The crystal structure of sulfadimidine sodium (SDMNa) was reported by Patel (1995). SDMNa was recrystallised from an aqueous solution at ambient temperature. The sodium cation of SDMNa coordinates a molecule of SDM directly through the oxygen of the sulphonyl group of SDM in a similar way as in CTZNa. Na(I) is able to coordinate one molecule of water, but it does not bridge to the other sodium cation through two molecules of water, as observed for CTZNa. The crystal structure of SDMNa has an interstitial oxygen atom (most likely of water) similar to data of CTZNa.

The type of sodium complex presented here is commonly referred to as a polymeric self-assembly (Whitesides et al., 1991). To the best of our knowledge, no similar structure with the same architecture of the hydrated cation-ligand complex in which the ligand is an active pharmaceutical ingredient has been published. Work on the crystal structure of pharmaceutical sodium salts including cromolyn, cefazolin and fenoprofen sodium hydrates (Stephenson, Diseroad, 2000) and naproxen sodium (Kim et al., 1990) reveals that those highly ordered and polymeric intermolecular hydrate structures of sodium salts of pharmaceutical substances are rather uncommon.

Stephenson and Diseroad (2000) stated that water molecules in cromolyn sodium and cefazolin sodium have the character of channel hydrate and ion-coordinated type. Based on SC-XRD, in CTZNa two of the water molecules are coordinated by the sodium cation, but a third one may be of an interstitial type. Figure 2, which presents the crystal structure of CTZNa, indicates that the orientation of CTZ molecules facilitates the formation of similar channels capable of holding water molecules. Additional evidence of very weak bonding of interstitial water was obtained by Ortep analysis of the single crystal X-ray data. There is less certainty associated with the placement of this particular oxygen compared to the water

molecules which are bonded in the crystal structure. This means that even at low temperature conditions, as in single crystal X-ray analysis, this oxygen atom position tends to be unstable.

3.2. NMR studies

Recrystallised samples of CTZNa and CTZ were subjected to solution nuclear magnetic resonance (NMR) analysis. The structure of CTZ was reported previously by Jacobsen and Treppendahl (1979) and comparative studies were carried out for CTZNa to elucidate the differences between the two molecules.

^{1}HNMR analysis of CTZNa detected three CH groups (Table 3). The N1-H5 (ν7.42ppm) group was detected to be attached to the O1-S1-O2 group. Peak integration of the former confirmed that this group in fact is ionised to NH^{-} (with the integral of 0.96), consistent with results of the single crystal X-ray analysis, suggesting that only one proton is attached to N1. It is hypothesised that this deprotonated amine group will neutralise the positive charge coming from the two sodium cations in its close neighbourhood (N-Na^{+} distances are approximately 3.6Å and 3.7Å). A similar phenomenon was described by Garcia-Raso and co-workers (2000) in Hg(sulfamide)$_2$ complexes where a deprotonated -SO_2NH- group interacted with the Hg cation. Barbosa et al. (1998) reported values of pK_1 and pK_2 of chlorothiazide to be 6.7 and 9.5, respectively. The pK_1 refers to deprotonation of the secondary amine group in the heterocyclic ring, while the pK_2 relates to deprotonation of the primary amine group. The aqueous solution of CTZNa had a pH of 9.0±0.3 therefore it is likely that the primary amine group is deprotonated. No proton was detected by ^{1}HNMR in the heterocycle, which may perhaps indicate its delocalised nature.

^{13}CNMR (Table 4) analysis confirmed the presence of all seven carbons in the chlorothiazide molecule. CH COSY analysis confirmed that two protons (ν8.19 ppm and ν7.34 ppm) are attached to the carbons of the benzene ring (ν125 ppm and ν126 ppm respectively) and one proton (ν7.2 ppm) is attached to the carbon (ν155 ppm) of the heterocyclic ring of CTZ.

Numerous shifts are observed in ^{1}HNMR and ^{13}CNMR spectra of CTZ compared to CTZNa consistent with the changes in chemical structure determined by single X-ray diffraction (Table 4 and 5). Upfield shifts were noted for H1 and H7 in contrast to a downfield shift for H4. A large downfield shift was observed by ^{13}CNMR for C5 directly bonded to N2 and the carbon (C3) to which the chlorine is attached (shifts of +12.0 and +11.1, respectively). The most affected signal, compared with CTZ, was that of the C5 of the benzene ring and, together with the upfield shift of H7 bonded to C7, this suggests a change in protonation of N2 and migration of H2 to N3. However, no signal from proton bonded to N3 was detected to directly confirm this hypothesis (Table 3).

3.3. Thermal analysis

CTZNa obtained after synthesis was a white crystalline powder. The DSC presented an endotherm with an onset at 83.7 ± 0.5 ˚C possibly related to the loss of hydrated water (Figure 3). An onset of the melting/decomposition point of CTZNa was 289 ± 1 ˚C. This is lower that the melting/decomposition point of CTZ, which was determined to be 363.51 ± 2.00 ˚C. The first endotherm corresponds to a 10.4 ± 0.5% mass loss observed by TGA which, when converted to a molar ratio, indicates that 1 mol of chlorothiazide sodium contains two moles of water (theoretical calculated water content is 10.18%) (Fig. 3b). This result was confirmed by Karl-Fischer titration where the water content was estimated to be 9.8 ± 0.3%. Therefore, it can be said that this material was a dihydrate.

24 hours of drying allowed the hydrated water to be removed and converted the dihydrate to a practically anhydrous state (Fig. 3a.c). The DSC/TGA analysis of anhydrous CTZNa gave different patterns to those of the dihydrate form. The DSC of anhydrous form presents an endotherm with an onset at 55 ˚C, related to just a 1.0 ± 0.2% mass loss. A similarly shaped TGA pattern to that of the hydrate was reported by Zhu et al. (1997) for water evaporation from nedocromil zinc hydrates.

3.4. Dynamic vapour sorption (DVS)

To investigate the hydration behaviour of chlorothiazide sodium under changing conditions of relative humidity (RH%) a dynamic vapour sorption (DVS) isothermal (25˚C) experiment was conducted (Fig. 4). To evaluate concurrent changes in the material PXRD (Fig. 5), DSC (Fig. 6) and TGA were also undertaken.

The starting material was dehydrated CTZNa, crystalline by PXRD (Fig. 5. g). Analysis after equilibration of the sample at 0% RH indicated a residual moisture content of about 2.6% mass loss by TGA This mass loss corresponded to a starting endotherm with onset at 30.4˚C, peaking at 54.8˚C on the DSC. Dehydrated CTZNa was stable during hydration (Fig. 4) in the range from 0 to 20% RH, where adsorbed moisture, most likely surface adsorbed, did not exceed 1% of the initial sample mass (m_0).

From 20% RH the sample started to gain water more rapidly in the range from 20-30% RH to reach a possible first mass equilibrium at 30-40% RH. The sample recovered from the DVS after equilibration at 30% RH had a residual water content of about 6.6% (by TGA), corresponding to an endotherm in the DSC (Fig. 6b) peaking at 77.2 ˚C. The m_0 increased by nearly 5% (by DVS), which corresponds to the theoretical percentage of water content calculated for a chlorothiazide sodium monohydrate. The PXRD analysis (Fig. 5. f) shows significant differences in the intensity of Bragg peaks (at 16.64, 19.63 and 26.52 2θ) in comparison to the pattern of the dehydrated form, with the disappearance of some peaks, e.g. at 17.72 and 26.52 2θ. However, the overall powder diffractogram of this sample resembles that of anhydrous CTZNa. Attempts were made to produce CTZNa monohydrate, but were unsuccessful, indicating that if the monohydrate exists, it is very unstable and can only be observed to form *in situ* by DVS. It may also be a transient structure occurring upon hydration of the dehydrated CTZNa.

A second period of rapid water uptake occurred in the range from 40 to 60% of RH and was followed by a second mass equilibration in the range of 60-80% RH. The sample, equilibrated at 60% RH, had a TGA mass loss of 11.8%, corresponding to an endothermic DSC (Fig. 6c) event with a peak

at 114.6 ˚C. The increase by 12% in mass, correlates with the theoretical 10% water content corresponding to CTZNa dihydrate. The PXRD analysis (Fig. 5. d) presents a different Bragg peak pattern in comparison to the dehydrate (Fig. 5.g) and it is consistent with the PXRD pattern of the recrystallised CTZNa used for single crystal X-ray analysis (Fig. 5. a).

Further hydration of the material did not result in the production of other forms characterised by a different PXRD pattern than that of the dihydrate (Fig. 5. c). The sample finally equilibrated at 90% RH gaining 19% of the starting sample weight. This is well above the 10% of water content calculated for the dihydrate and could be attributed to surface adsorbed moisture and/or a channel/interstitial type hydrate, where water molecules can be placed in the interstitial spaces between the CTZNa molecules. This type of structure was suggested by the single crystal XRD analysis and discussed in the sections related to crystal structure above, but was not subsequently confirmed by the thermal studies and Karl-Fischer titration. The discrepancy can be related to different environmental conditions (relative moisture, temperature) of the techniques used. However, it is certain that at high RH% at least the dihydrate, as indicated by PXRD, is formed. A variable level of hydration, due to the interstitial/channel space was previously reported for cromolyn sodium, which is believed to be able to accommodate up to nine water molecule in the lattice and for cefazolin sodium, which is characterised by an extensive network of tunnel space which can accommodate water (Stephenson and Diseroad, 2000). The observed variation in hydration levels for CTZNa may complicate its developability and manufacture, as more stringent environmental control may be needed.

An interesting behaviour of this sample was revealed by desorption/drying of the material in the DVS. The material dries slowly from 90% RH down to 10% RH, with a relatively constant drying rate between 60% RH and 10% RH. The sample recovered from the DVS instrument after desorption and equilibration at 10% RH showed a 12.9% mass loss by TGA corresponding to a DSC endotherm with an onset at 88˚C, peaking at 114.6 ˚C. Results obtained for the materials recovered during sorption at 60 and 90%RH in comparison to material recovered after desorption at 10%RH were the same regarding DSC pattern (Fig. 6d). The PXRD patterns of all four compared samples (Fig. 5. a-d) are the same and

correlate well with simulation of PXRD pattern based on X-ray single crystal analysis (data not shown). Below 10% RH the crystal structure collapses and the hydration water is released quickly, until at 0% RH the sample becomes again the dehydrate.

3.5. Other physicochemical characteristics

Both CTZNa forms, the dihydrate and anhydrous, were subjected to elemental analysis. With respect to CTZNa dihydrate, the theoretically calculated contents of carbon, hydrogen and nitrogen were 23.77%, 2.56% and 11.88%, respectively, consistent with those obtained experimentally: 23.49±0.06%, 2.62±0.01% and 11.57±0.00%. CTZNa dihydrate was subjected to additional drying to obtain the anhydrous salt. Theoretical contributions of carbon, hydrogen and nitrogen for the anhydrous form were 26.46%, 1.59% and 13.23%, respectively, which are also consistent with the experimental values of 25.82±0.04%, 1.65±0.01% and 12.59±0.01%, respectively. If the molar ratio of sodium to chlorothiazide in the salt is 1:1, then the theoretical sodium content in the salt is 6.9%. The results for sodium content obtained from ICP-MS analysis indicate that the sodium content in the sample is 7.6±0.9%, consistent with a stoichiometric ratio of chlorothiazide to sodium of 1:1.

The content of organic solvents in the recrystallised CTZNa was assayed by GC-MS. The residual content of acetone was 0.005% w/w for the sample recrystallised from 1:1 acetone/water mixture and 0.001% w/w of ethanol was present in the sample obtained from ethanol.

The true density calculated from the single crystal X-ray analysis (at 150K) of CTZNa dihydrate was 1.836 g/cm^3, while that measured by helium pycnometry (room temperature) was 1.728±0.004 g/cm^3 and for the anhydrous form it was 1.883±0.001 g/cm^3.The true density of CTZ raw material measured by helium pycnometry was 1.798±0.001 g/cm^3, while the density calculated from the single crystal X-ray analysis was 1.861 g/cm^3.

FTIR spectrum of CTZNa systems, hydrated and anhydrous, reveals significant differences in FTIR pattern in the range of 2800-3600 cm^{-1}, where hydrogen bonds are expected (Fig. 11 c,d). Strong, multiple bands of O-H stretching modes can be seen between 3400 and 3600 cm^{-1} confirming the

presence of crystalline water in CTZNa (Fig. 11c). FTIR pattern of CTZ recrystallised from acetone was consistent with that obtained for the raw material (Fig. 7 a, b).

The solubility of the dihydrate form of CTZNa, the stable form in aqueous solution, was determined to be 79.4±9.5 mg/ml (pH = 9.5±0.2), while the aqueous solubility of CTZ at 37 °C was determined to be 0.200 ± 0.004 mg/ml (pH = 4.8±0.1) which is in agreement with the value of 0.2 mg/ml reported previously by Charnicki and co-workers (1959). Therefore the solubility difference between CTZ and CTZNa is of the order of 400-fold.

4. Conclusions:

We report in this paper, for the first time, the crystal structure of a dihydrate chlorothiazide sodium salt as an intramolecular self-assembly: $(CTZ)_3{\cdot}(H_2O){\cdot}Na(H_2O)_2Na{\cdot}(H_2O){\cdot}(CTZ)_3$, which has a triclinic crystal structure and a space group P-1, regardless of whether water, a 1:1 water/acetone mixture or ethanol was employed for recrystallisation. Each of the sodium cations coordinates six oxygen atoms. Sodium cations are bridged through two molecules of water. Each of the sodium cations coordinates an additional not shared molecule of water and three chlorothiazide molecules through the oxygens of the sulphonyl groups.

It has been established by NMR and SC-XRD that the presence of the sodium cation induces a negative charge in the SO_2NH_2 group i.e. SO_2NH^- is formed. The sodium cation displaces the H of the secondary amine group (NH) of the heterocyclic ring. SC-XRD confirmed the presence of NH group in the heterocyclic ring at the nitrogen atom next to the SO_2 moiety. In CTZNa, the proton is likely to be attached to N2 rather than N3 as in CTZ. A crystalline anhydrous form of CTZNa was also identified and its PXRD pattern presented.

DVS analysis confirmed the existence of CTZNa dihydrate over a broad range of relative humidities, 10-90% RH, at 25˚C. For material subjected to relative humidities between 30 and 40%, slight changes in the PXRD pattern, with differences in the intensity of some Bragg peaks (at 16.64, 19.63 and 26.52 2θ) and the disappearance of others (at 17.72 and 26.52 2θ), suggests the formation of another hydrated form, possibly a monohydrate. The presence of the anhydrous form was evident close to 0% RH.

CTZ has one of the highest melting-decomposition points of all APIs, at around of 363 ˚C, which is consistent with the extensive hydrogen bond network. The melting/degradation point of the dehydrated CTZNa was at around of 289 ˚C, ~70 ˚C lower than that of CTZ. The solubility of CTZNa dihydrate was determined to be 79.4±9.5 mg/ml, around 400-fold higher than of CTZ. Therefore it can be concluded that favourable pharmaceutical properties of CTZNa result from a significantly reduced melting point and improved solubility by salt formation.

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CCDC 757605 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

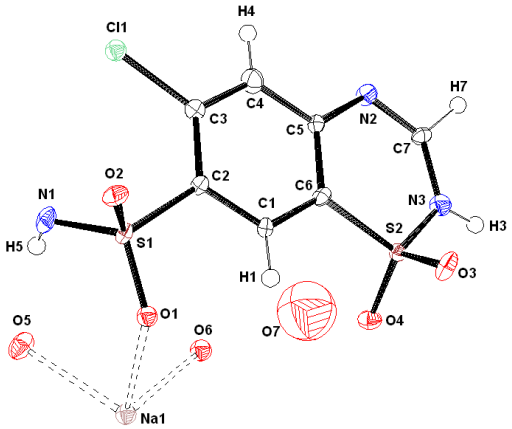
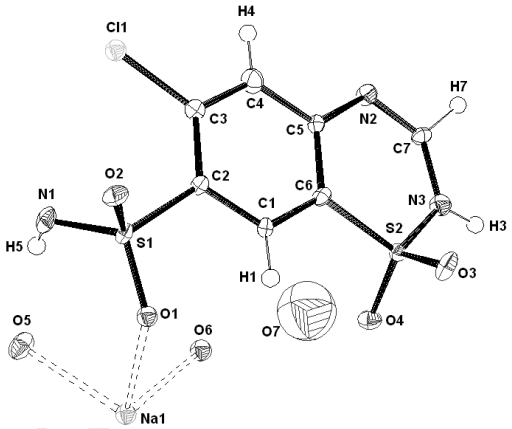


Fig.1. Ortep diagram of chlorothiazide sodium (with ellipsoids at 50% probability level)



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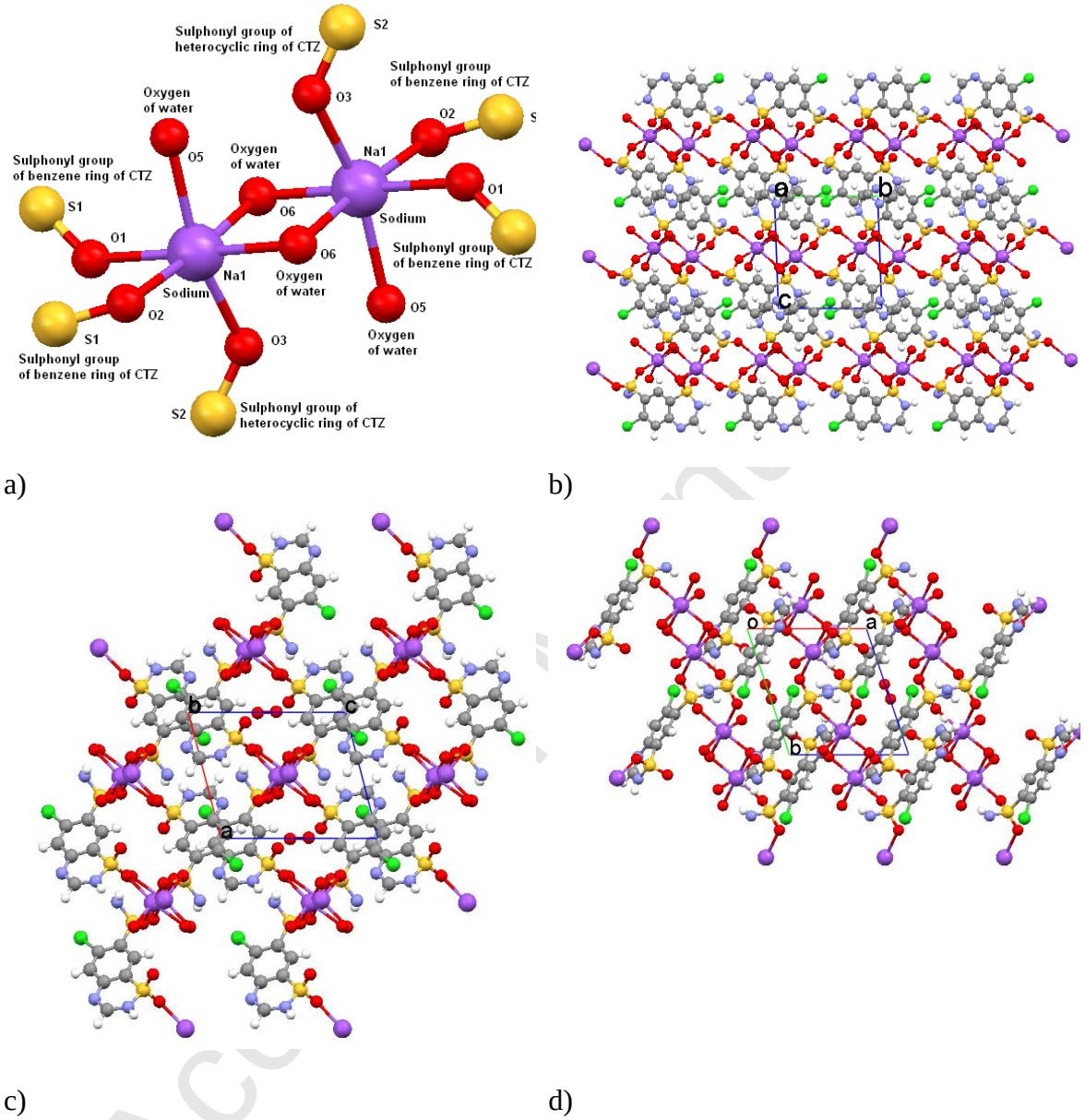
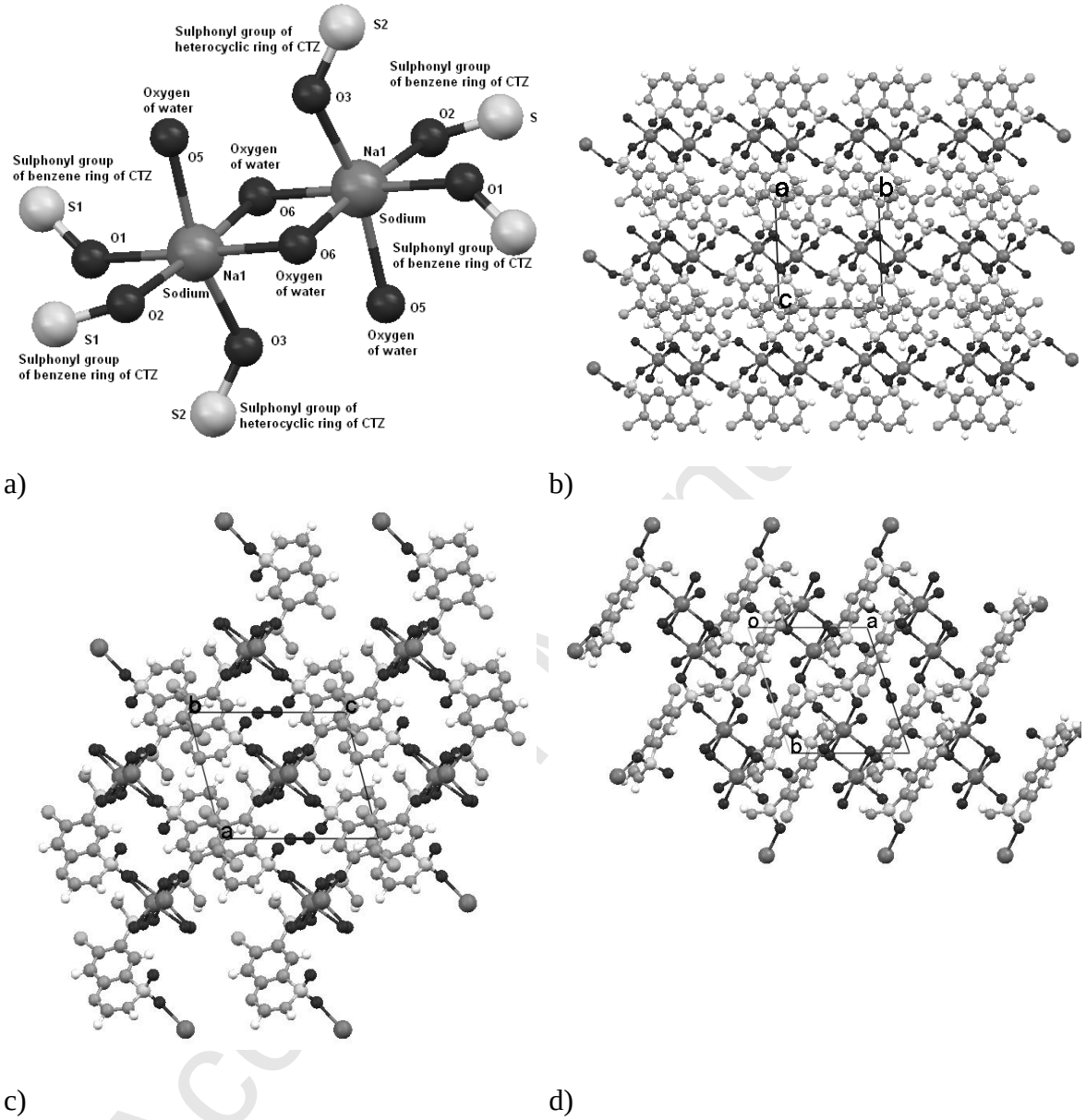


Fig. 2. Crystal structure of chlorothiazide sodium dihydrate: a) coordinates of the sodium cations, b) crystal cell structure packing along **a** axis, c) crystal cell structure packing along **b** axis, d) crystal cell structure packing along **c** axis. Atom colours: carbon: grey, nitrogen: blue hydrogen: white, oxygen: red, sulphur: yellow, chlorine: green, sodium: violet.



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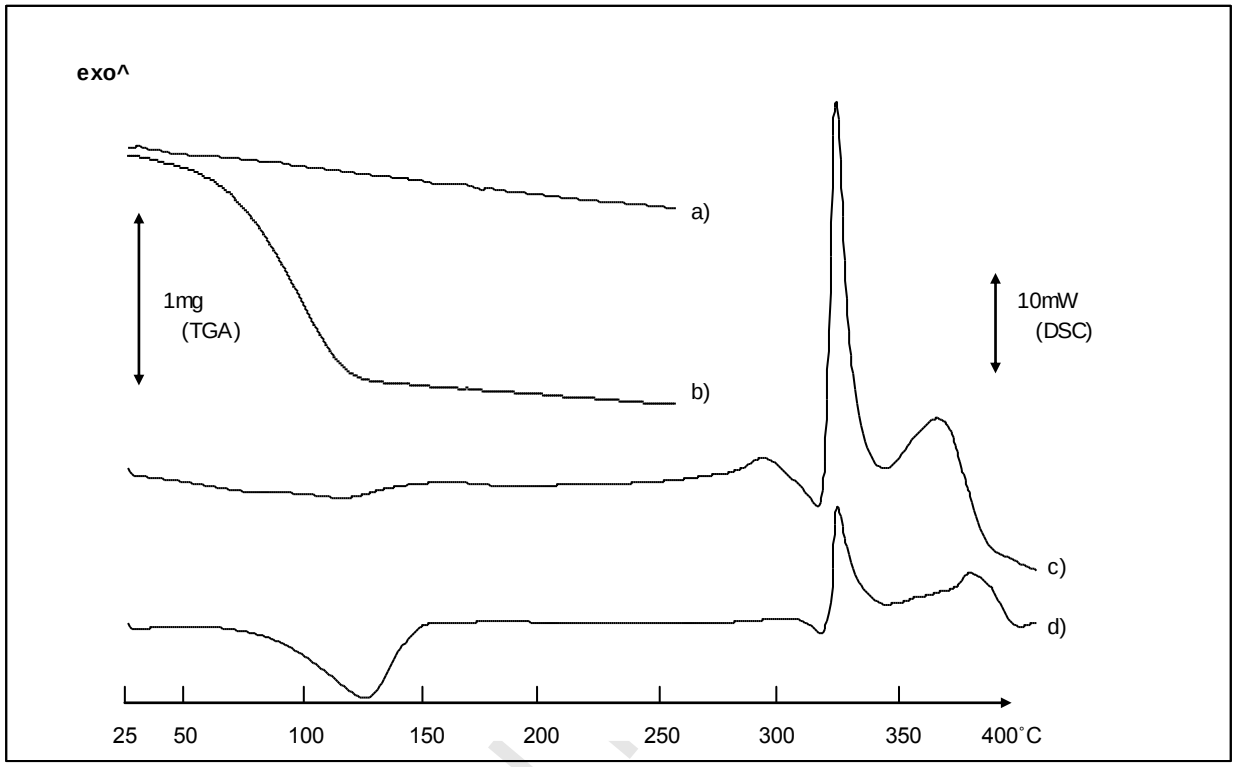

Fig. 3. Thermal analysis of anhydrous CTZNa: a) TGA, c) DSC and CTZNa dihydrate: b) TGA,

d) DSC.

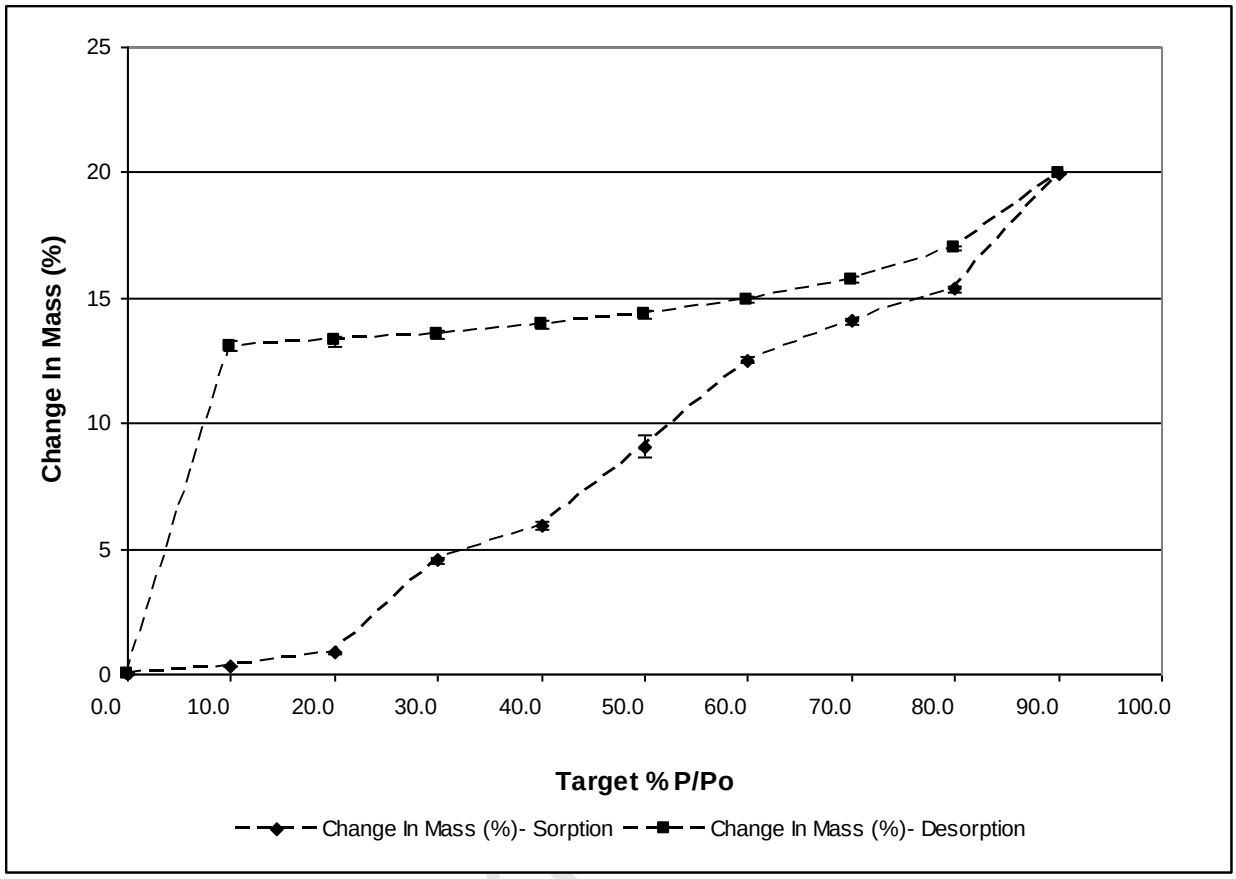

Fig. 4. Isothermal (25˚C) plots of sorption and desorption of water for CTZNa.

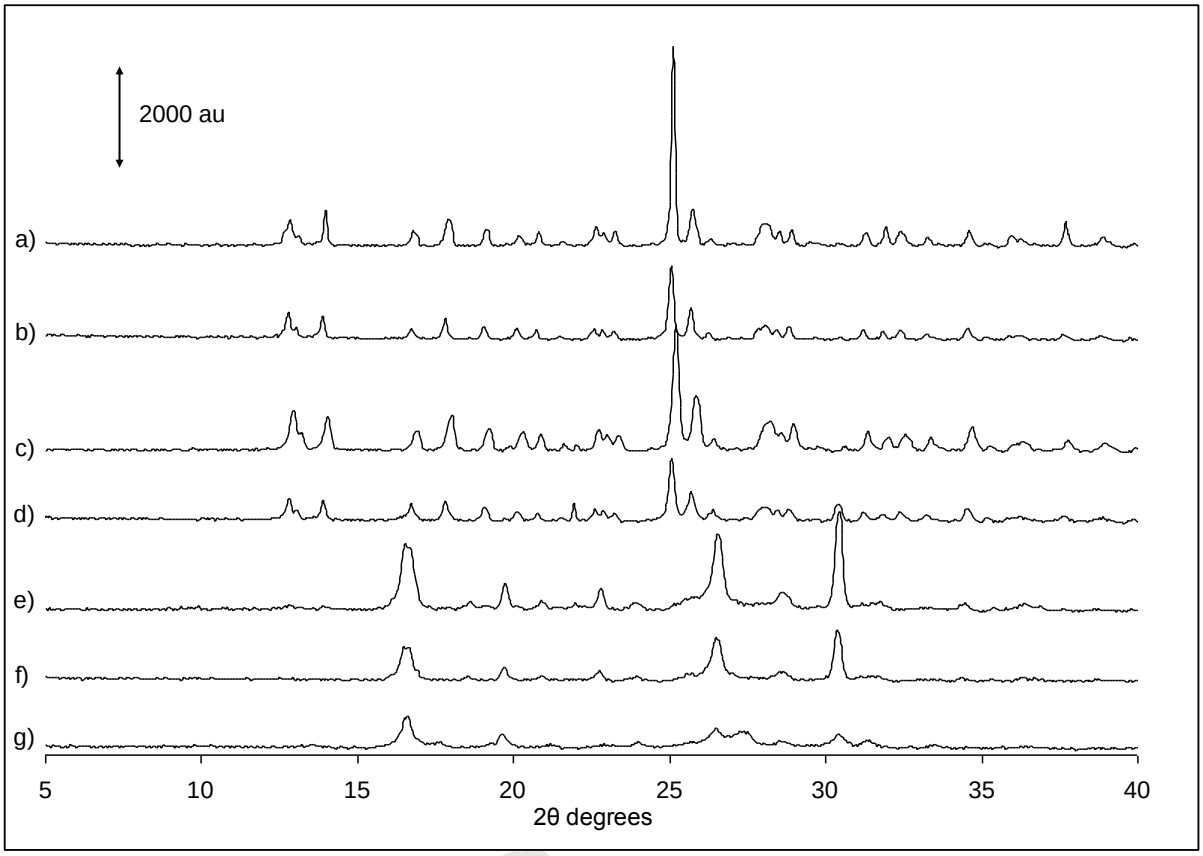

Fig. 5. Powder XRD patterns of: a) CTZNa dihydrate single crystal X-ray material, b) CTZNa dihydrate recovered from DVS after desorption at 10% RH, c) CTZNa dihydrate recovered from DVS after sorption at 90% RH, d) CTZNa dihydrate recovered from DVS after sorption at 60% RH e) CTZNa recovered from DVS after sorption at 40% RH, f) CTZNa recovered from DVS after sorption at 30% RH, g) CTZNa dehydrate recovered from DVS after equilibration of reference mass at 0% RH.

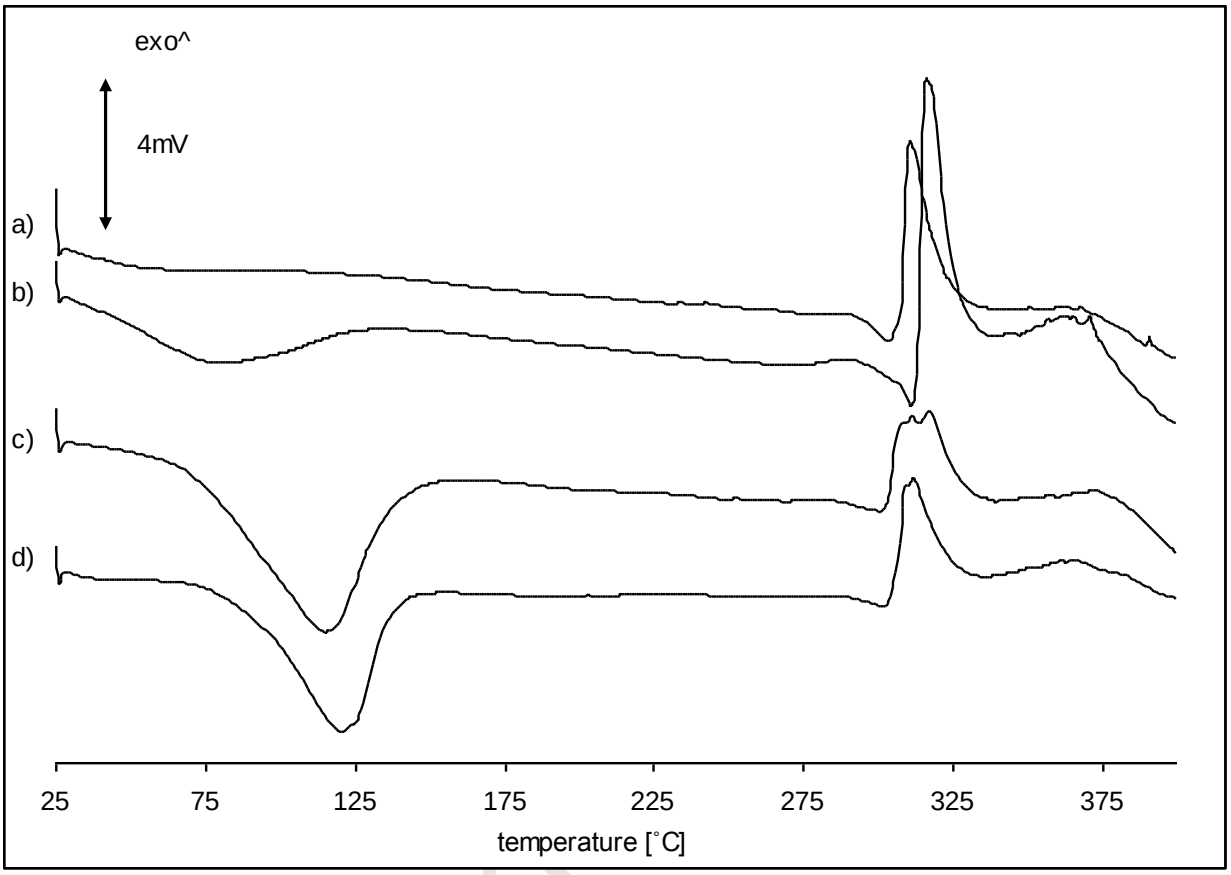

Fig. 6. DSC sans of chlorothiazide sodium samples recovered from DVS instrument after equilibration at: a) 0%RH, b) 30%RH, c) 60% RH and d) 90%RH

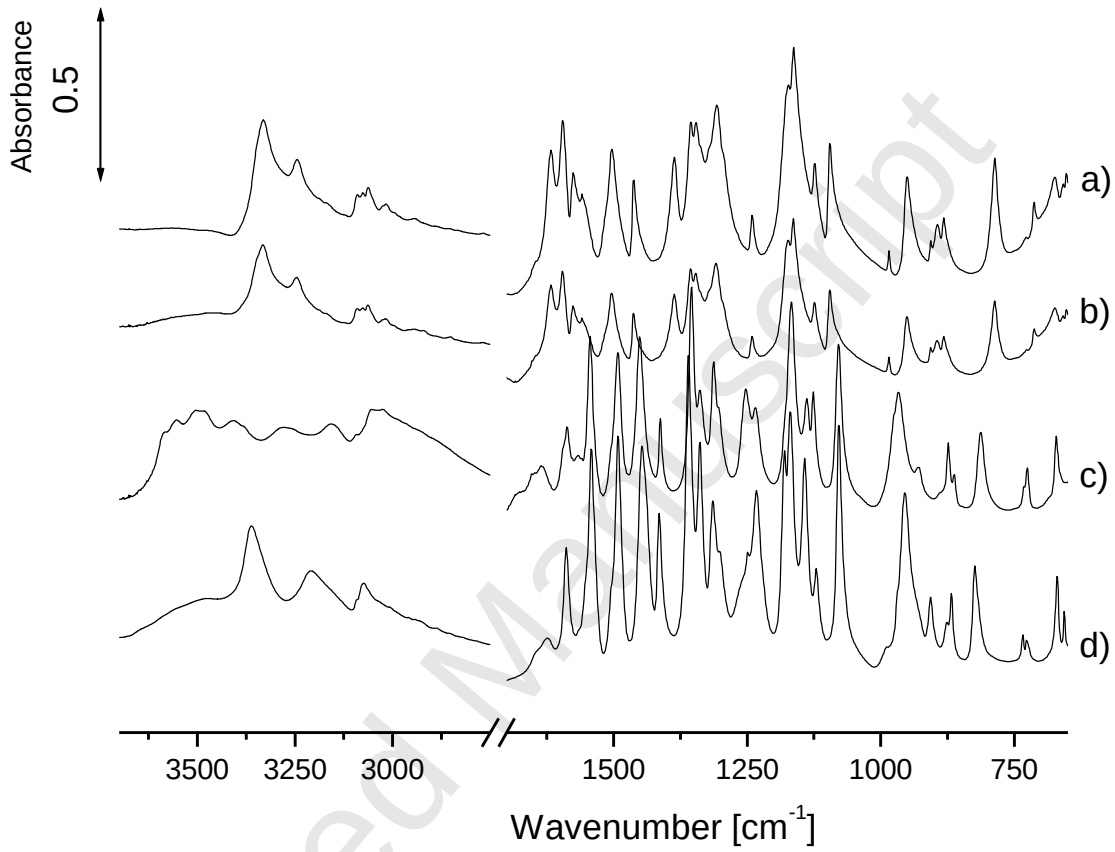

Fig. 7. FTIR spectra of: a) CTZ starting material, b) CTZ recrystallised from acetone, c) recrystallised CTZNa dihydrate and d) CTZNa anhydrous form.

Table 1. Crystal data and structure refinements for chlorothiazide sodium.

Analysis parameters	**Chlorothiazide sodium dihydrate**	
Identification code	Shelxl	
Empirical formula	C14 H10 Cl2 N6 Na2 O14 S4	
Formula weight	731.40	
Temperature	150K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	α = 82.80(3)°	a = 8.3567(17) Å
	β = 73.93(3)°	b = 9.0728(18) Å
	γ = 70.31(3)°	c = 9.6486(19) Å
Volume	661.4(2) Å3	
Z	1	
Density (calculated)	1.836 Mg/m3	
Absorption coefficient	0.674 mm-1	
Crystal size	0.2 x 0.2 x 0.2 mm3	
Reflections collected	4591	
Completeness to theta = 25.00°	96.1 %	
Goodness-of-fit on F2	1.205	
Final R indices [I>2sigma(I)]	R1 = 0.0654, wR2 = 0.1948	
R indices (all data)	R1 = 0.0705, wR2 = 0.2157	

Table 2. Bond lengths [Å] and dihedral angles [°] for hydrogen bonding interactions in chlorothiazide and chlorothiazide sodium.

Bond type (D-H...A)	**D-H [Å]**	**H..A [Å]**	**D...A [Å]**	**D-H...A [°]**
Chlorothiazide				
N2-H2...O3[(a)]	0.86	2.57	2.887(6)	103
N2-H2...N1[(b)]	0.86	2.61	3.327(7)	142
N1-H5...N3[(c)]	1.13(6)	2.12(6)	3.132(7)	148(4)
N1-H6...O1[(d)]	0.91(6)	2.59(7)	2.917(6)	102(5)
N1-H6...O3[(e)]	0.91(6)	2.14(7)	3.015(6)	161(6)
C1-H1...O2	0.95	2.41	2.831(6)	106
C4-H4...O4[(f)]	0.95	2.26	3.152(7)	157
C7-H7...O [(g)]	0.95	2.43	3.369(6)	170
Chlorothiazide sodium				
N1-H5...O5	0.65(7)	2.27(7)	2.913(6)	169(7)
N3-H3...N [(a)]	0.86	2.10	2.949(6)	169
C1-H1...O1	0.93	2.34	2.756(5)	107

Symmetry codes: (a) 1+x, -1+y, z.; (b) x, 1+y, -1+z; (c) -1+x, y, 1+z; (d) x, y, 1+z; (e) 1+x, y, z; (f) -1+x, 1+y, z; (g) 1+x, 1+y, -1+z.

Table 3. Results of ^{1}HNMR analysis of chlorothiazide sodium and chlorothiazide

Peak	**CTZNa**	**CTZ**	**Peak shift**
	ν(F1) [ppm]	ν(F1) [ppm]	Δδ H
C(4)H(4)	8.19	7.54	+0.65
C(1)H(1)	7.34	8.29	-0.95
C(7)H(7)	7.22	8.12	-0.90
N(2)H(2)	-	12.62	-
N(1)H_2(5,6)	-	7.89	-
N(1)H(5)	7.43	-	-

Table 4. Results of ^{13}CNMR analysis of chlorothiazide sodium and chlorothiazide.

Peak	**CTZNa**	**CTZ**	**Peak shift**
	ν(F1) [ppm]	ν(F1) [ppm]	Δδ C
C(4)H(4)	124.8	120.3	+4.5
C(1)H(1)	126.5	125.0	+1.5
C(7)H(7)	155.5	148.6	+6.9
C(5)	150.9	138.9	+12.0
C(6)	134.5	137.8	-3.4
C(2)	122.6	134.4	-11.9
C(3)	131.3	120.2	+11.1