Electronic Supplementary Material (ESI) for CrystEngComm.

Structure and Stability of Two Polymorphs of Creatine and its Monohydrate

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1. Results of Automated Parallel Crystallisation Search

Experimental crystallisations were performed using a Chemspeed Accelerator SLT100 parallel synthesizer.[1] Details of the experimental crystallisations completed in the automated parallel crystallisation search, are given in Table S1 alongside corresponding results. In summary, the search included 134 crystallisations of CTN from 67 solvents in duplicate. From 134 crystallisations only 16 yielded sufficient material for XRPD analysis and of these the majority of samples had decomposed to form creatinine (10/16), 2 crystallisations from acetic acid yielded a new phase, possibly creatinine acetate, 3 crystallisations yielded the known monohydrate form and 1 crystallisation from formic acid gave a novel form, CTN formic acid solvate. The relatively high number of creatinine versus CTN forms obtained from crystallisations may be attributable to the high temperature at which solutions were heated to prior to inducing crystallisation (Table S1).

Table S 1. Automated crystallisation experiments; 3 ml solvent were added to an excess of CTN, solutions were heated to T_{prep} filtered prior to crystallisation induced by cooling to T_{cool}. Solutions were vortexed at 1000 rpm during crystallisation

Refcode	Solvent 1	Tprep (°C)	Tcool (°C)	Form
002750	pentane-1,5-diol	142	10	0
002751	pentane-1,5-diol	142	10	0
002752	1-methylnapthalene	142	10	0
002753	1-methylnapthalene	142	10	0
002754	2-phenylethanol	142	10	0
002755	2-phenylethanol	142	10	0
002756	n-dodecane	142	10	0
002757	n-dodecane	142	10	0
002758	nitrobenzene	142	10	0
002759	nitrobenzene	142	10	0
002760	1-methyl-2-pyrrolidinone	142	10	0
002761	1-methyl-2-pyrrolidinone	142	10	0
002762	octan-1-ol	142	10	0
002763	octan-1-ol	142	10	0
002764	Dimethyl sulfoxide	142	10	0
002765	Dimethyl sulfoxide	142	10	0
002766	aniline	142	10	MH

002767	aniline	142	10	Creatinine
002768	octan-2-ol	142	10	0
002769	octan-2-ol	142	10	0
002770	2-butoxyethanol	142	10	0
002771	2-butoxyethanol	142	10	0
002772	N,N-dimethylacetamide	142	10	0
002773	N,N-diemthylacetamide	142	10	0
002774	furfural	142	10	0
002775	furfural	142	10	0
002776	2-methoxyethylether	142	10	0
002777	2-methoxyethylether	142	10	0
002778	cyclohexanol	142	10	0
002779	cyclohexanol	142	10	0
002780	hexan-1-ol	142	10	Creatinine
002781	hexan-1-ol	142	10	0
002782	anisole	110	10	0
002783	anisole	110	10	0
002784	pentyl acetate	110	10	0
002785	pentyl acetate	110	10	0
002786	butyl ether	110	10	0
002787	butyl ether	110	10	0
002788	xylene	110	10	0
002789	xylene	110	10	0
002790	pentan-1-ol	110	10	0
002791	pentan-1-ol	110	10	0
002792	2-ethoxyethanol	110	10	0
002793	2-ethoxyethanol	110	10	0
002794	3-methylbutan-1-ol	110	10	0
002795	3-methylbutan-1-ol	110	10	0
002796	N,N-dimethylformamide	110	10	0
002797	N,N-dimethylformamide	110	10	0
002798	butylacetate	110	10	0
002799	butylacetate	110	10	MH + creatinine
002800	diethylcarbonate	110	10	0
002801	diethylcarbonate	110	10	0
002802	2-methoxyethanol	110	10	0
002803	2-methoxyethanol	110	10	I + III + creatinine
002804	tetrachloroethene	110	10	0
002805	tetrachloroethene	110	10	0
002806	acetic acid	110	10	Creatinine + new phase possibly creatinine acetate

				new phase possibly creatinine
002807	acetic acid	110	10	acetate
002808	pentan-2-ol	110	10	0
002809	pentan-2-ol	110	10	0
002810	4-methyl-2-pentanone	110	10	0
002811	4-methyl-2-pentanone	110	10	MH
002812	butan-1-ol	110	10	0
002813	butan-1-ol	110	10	0
002814	isobutylacetate	78	10	0
002815	isobutylacetate	78	10	MH
002816	pyridine	78	10	0
002817	pyridine	78	10	0
002818	toluene	78	10	0
002819	toluene	78	10	0
002820	2-methylpropan-1-ol	78	10	0
002821	2-methylpropan-1-ol	78	10	0
002822	1,4-dioxane	78	10	0
002823	1,4-dioxane	78	10	0
002824	nitromethane	78	10	0
002825	nitromethane	78	10	0
002826	iso-octane	78	10	0
002827	iso-octane	78	10	0
002828	water	78	10	Creatinine
002829	water	78	10	0
002830	n-heptane	78	10	0
002831	n-heptane	78	10	0
002832	butan-2-ol	78	10	0
002833	butan-2-ol	78	10	0
002834	propan-1-ol	78	10	0
002835	propan-1-ol	78	10	0
002836	N,N,N-triethylamine	78	10	0
002837	N,N,N-triethylamine	78	10	0
002838	trichloroethylene	78	10	Creatinine
002839	trichloroethylene	78	10	0
002840	1,2-dimethoxyethane	78	10	0
002841	1,2-dimethoxyethane	78	10	0
002842	dichloroethane	78	10	0
002843	dichloroethane	78	10	0
002844	propan-2-ol	78	10	0
002845	propan-2-ol	78	10	MH + creatinine
002846	acetonitrile	45	10	0

002847	acetonitrile	45	10	0
002848	cyclohexane	45	10	Creatinine
002849	cyclohexane	45	10	0
002850	benzene	45	10	0
002851	benzene	45	10	0
002852	methylethylketone	45	10	0
002853	methylethylketone	45	10	0
002854	1-chlorobutane	45	10	0
002855	1-chlorobutane	45	10	0
002856	ethanol	45	10	Creatinine + MH
002857	ethanol	45	10	Creatinine + MH
002858	ethylacetate	45	10	0
002859	ethylacetate	45	10	0
002860	carbon tetrachloride	45	10	0
002861	carbon tetrachloride	45	10	0
002862	hexane	45	10	0
002863	hexane	45	10	0
002864	Tetrahydrofuran	45	10	0
002865	Tetrahydrofuran	45	10	0
002866	chloroform	45	10	0
002867	chloroform	45	10	0
002868	methyl acetate	45	10	0
002869	methyl acetate	45	10	0
002870	tert-butyl methyl ether	45	10	0
002871	tert-butyl methyl ether	45	10	0
002872	cyclopentane	45	10	0
002873	cyclopentane	45	10	0
002874	methanol	45	10	0
002875	methanol	45	10	0
002876	acetone	45	10	0
002877	acetone	45	10	0
002878	formamide	65	10	0
002879	formamide	65	10	0
002880	formic acid	65	10	S
002881	formic acid	65	10	0
002882	2,2,2-trifluoroethanol	65	10	0
002883	2,2,2-trifluoroethanol	65	10	0

0 = insufficient sample obtained for analysis; I = form I, II = form II, MH = monohydrate; creatinine = decomposition product.

2. Details of Slurry Experiments

Slurry experiment were conducted for 51 solvents using a Chemspeed Accelerator SLT100 parallel synthesizer.[1] The majority of the crystallisations were performed by slow cooling from a temperature close to the boiling point of the solvent, down to room temperature. Suspended samples were reclaimed by filtration and transferred to a multiposition sample holder for identification using multisample foil transmission XRPD.

Details of CTN Slurry experiments and results are provided in Table S2. In summary the following forms were observed: 15 pure form I, 8 Pure form II, 0 pure form III but form III was observed as a mixture with forms I, II or monohydrate in 3 instances, 2 pure monohydrate, 1 formic acid solvate and 21 mixtures comprising Forms I, II and MH. In addition creatinine acetate was also obtained from slurrying with acetic acid.

Table S 2. Slurry experiments; an excess of CTN was mixed with 3ml of solvent and vortexed at 1000 rpm for 1 hour at the temperature Tprep.

Solvent	Tprep (°C)	Form
pentane-1,5-diol	142	I
1-methylnapthalene	142	I
2-phenylethanol	142	I
n-dodecane	142	I
nitrobenzene	142	I
1-methyl-2-pyrrolidinone	142	I
octan-1-ol	142	I
Dimethyl sulfoxide	142	I + III
aniline	142	I
octan-2-ol	142	I
2-butoxyethanol	142	I
N,N-dimethylacetamide	142	I + III
furfural	142	II
2-methoxyethylether	142	I
cyclohexanol	142	I
hexan-1-ol	142	I
anisole	110	I + II
pentyl acetate	110	I + II
butyl ether	110	I + II
xylene	110	I + II
pentan-1-ol	110	I + II

2-ethoxyethanol	110	I + II
3-methylbutan-1-ol	110	I
N,N-dimethylformamide	110	I + II
butylacetate	110	II
diethylcarbonate	110	I, II
2-methoxyethanol	110	I
tetrachloroethene	110	I + II
acetic acid	110	New phase possibly CA
pentan-2-ol	110	II
4-methyl-2-pentanone	110	II
isobutylacetate	78	II + MH
pyridine	78	I + II
toluene	78	II + MH
2-methylpropan-1-ol	78	I + II
1,4-dioxane	78	I + II
nitromethane	78	I + II
iso-octane	78	I + II
water	78	MH
trichloroethylene	78	II
propan-2-ol	78	I + II
acetonitrile	45	I + III + MH
cyclohexane	45	II
benzene	45	I + II + MH
methylethylketone	45	II
1-chlorobutane	45	I + II
ethanol	45	I + II
ethylacetate	45	II
carbon tetrachloride	45	I + II + MH

I = Form I, II = form II, III = Form III, MH = monohydrate; CA = creatinine acetate.

3. Infrared (IR) spectroscopy

The FT-IR spectrum for each CTN form is clearly different (see Figure S 1) The frequencies of some characteristic IR bands for each CTN form are listed in Table S 3. All the different CTN form show a broad absorption band due to N-H···O bond stretching in the 3600-2600 cm^{-1} range. In this range, CTN monohydrate show a broader band compared to the others and an extra peak at 3398 cm^{-1} due

to the stretching vibrations of the water molecule. In the 1800-950 cm^{-1} range, they also show very similar IR absorption bands characteristic of the guanidinium ion and the carboxylate stretching vibrations. Between the four forms, the monohydrate show an extra absorption band is the δ OH water at 1661 cm^{-1}. The fact that IR spectra are very similar underlines the zwiterionic character of CTN anhydrous forms just like the monohydrate and also the similar environment and conformation of CTN molecules in these forms.

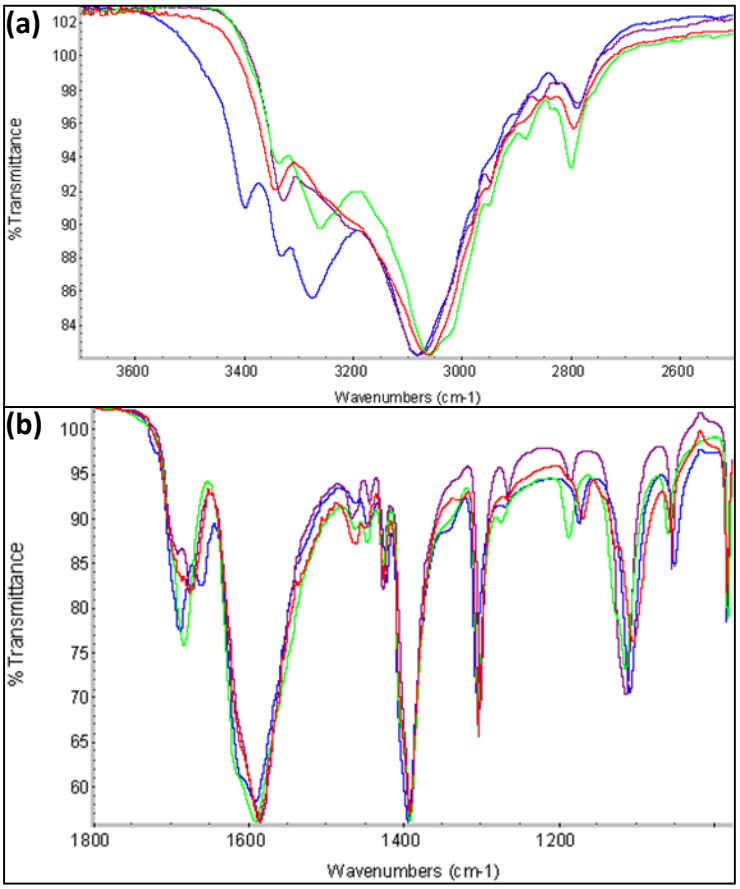

Figure S 1. FT-IR Spectra of monohydrate (blue), form I (purple), form II (green) and form III (red) of CTN.

Table S 3. Wavenumber in cm $^{-1}$ for IR absorption bands in CTN forms

Compound	CTN monohydrate	CTN form I	CTN form II	CTN form III
Broad band 3450 – 2900 cm^{-1} For ν_s(N-H…O) and (O-H…O)	3081 br	3079 br	3056 br	3060 br
$\nu_{as}(CN_3$ double bond)	1686 s	1673 s	1682 s	1675 s
$\nu_{as}(COO^-)$	1591 vs	1584 vs	1591 vs	1586 vs
$\nu_s(COO^-)$	1393 s	1390 s	1391 s	1391 s
$\nu_{as}(CN_3$ single bond)	1049 s	1053 s	1056 s	1054 s
$\nu_s(CN_3$ single bond)	982 s	980 s	980 s	982 s

4. Geometry Optimisation using CASTEP

First-principles DFT calculations were carried out using CASTEP[1] (version 4.1) on the crystal structures of form I, II and monohydrate of CTN obtained from SA. Preparation of the input files and visualisation of the results were carried out using Material Studio (MS) software suite (version 4.4) (Accelrys©) interface to CASTEP. The lattice parameters were kept fixed at their experimental values for all the calculations. Preliminary optimisations in space group P1 confirmed the structures were close to minima and subsequent optimisations were carried out in experimentally observed space groups to facilitate comparison of the optimised structures with the experimental data. Exchange-correlation potential was described using a generalised gradient approximation (GGA-PBE)[2] with a plane-wave basis set cut-off of 435/300 eV and a single k-point for the Brillouin zone (BZ) sampling. Medium convergence criteria (tolerances of energy per atom 0.00002 eV, maximum forces 0.05 eV/Å, maximum displacement 0.002 Å) were used. Ultrasoft pseudopotentials were employed for core electrons as they allow calculations to be performed at the lowest possible cut-off energy for the plane wave basis set. CASTEP calculations were carried out by distributing on the National Grid Server (UK) facilities and optimisations converged satisfactorily within the default number of iterations. Representative example of CTN monohydrate before and after geometry optimisation is shown in Figure S2.

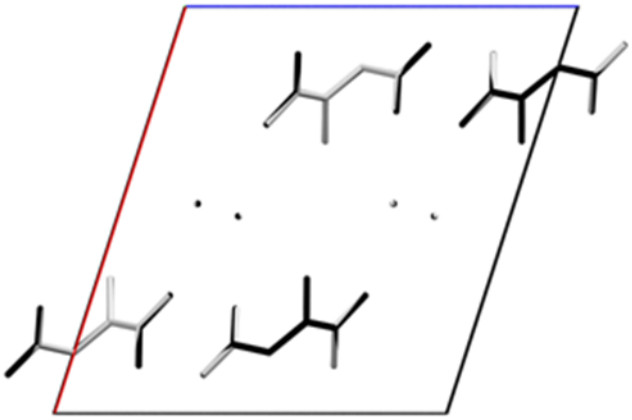

Figure S 2. Overlay of crystal structures of CTN monohydrate obtained from SXD (dark grey) and one obtained after CASTEP optimisation (black). Rmsd of heavier atoms 0.0109

5. Pawley type Refinement

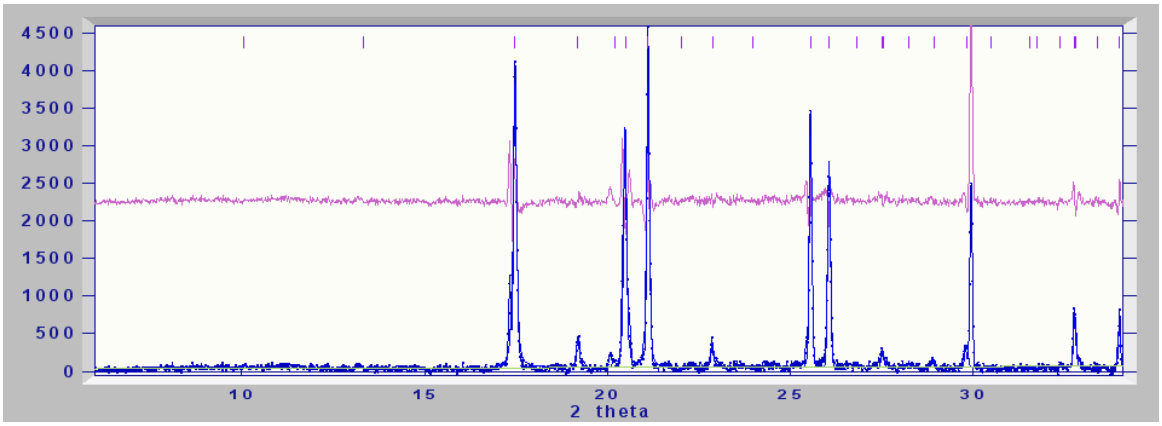

Figure S 3. Representative XRPD data of CTN formic acid solvate, collected from CTN water slurry experiment. Refined unit cell parameters in the orthorhombic space group Pna21 are: *a*, *b*, *c* (Å) = 17.588, 4.79, 10.148; α, β, γ (^{o}) 90.0, 90.0, 90.0. Pawley χ^2 = 9.14. Peaks which are present in the powder pattern with no corresponding tick marks can be attributed to the presence of small quantities of CTN monohydrate

6. XRPD Pattern of Form III

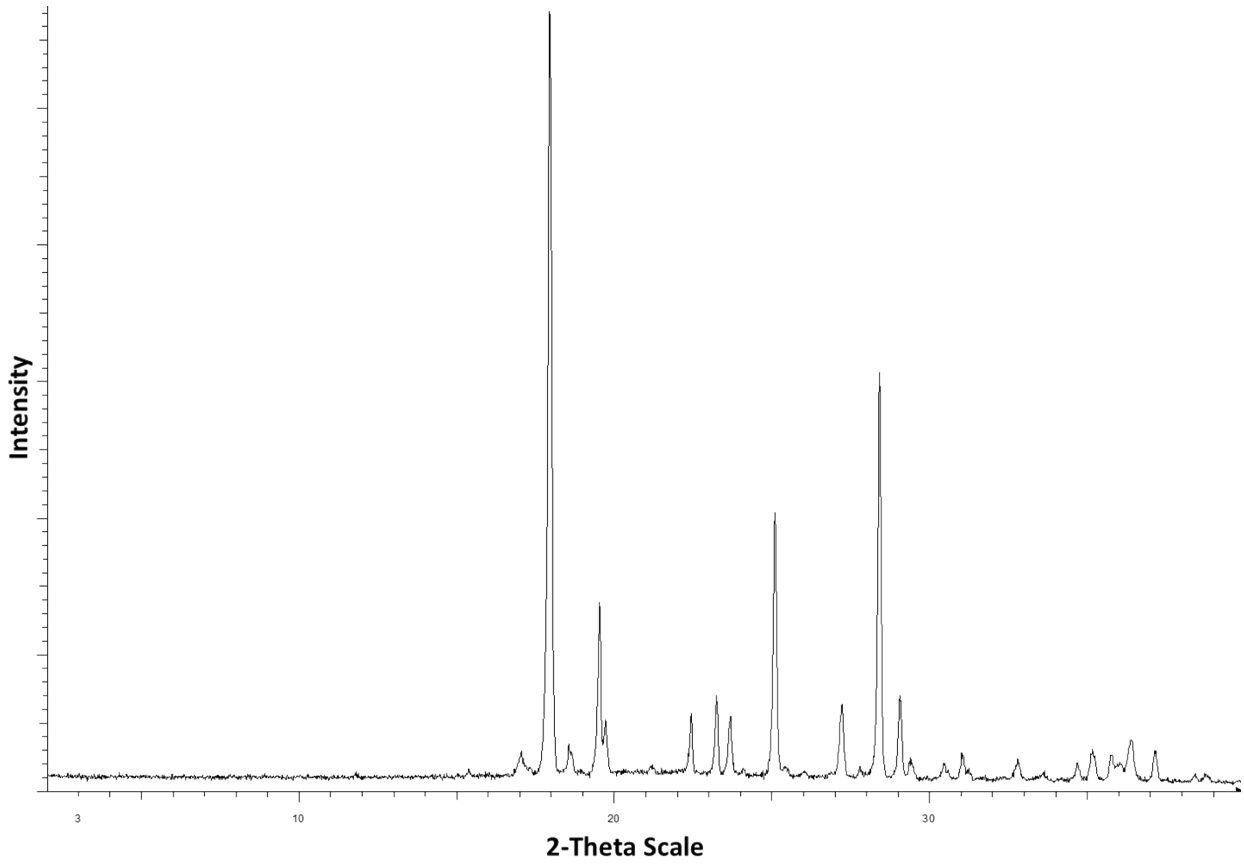

Figure S 4. XRPD pattern of CTN III sample obtained from rapid dehydration of CTN monohydrate.

7. Simultaneous Thermal Analyses

Differential Scanning Calorimetry (DSC) thermogram and thermogravimetric analysis (TGA) were simultaneously recorded with a NETZSCH STA 449C thermocouple, equipped with a NETZSCH CC 200 liquid nitrogen supply system and a NETZSCH CC 200 C control unit. Approximately 5-10 mg of sample was weighed into Al pans (10 µl) and a heating ramp was set in the range of 25-280 °C at a rate of 5 °C min^{-1}. All analyses were performed using the Netzsch Proteus data analysis package.

The TGA curve of the monohydrate shows a one-step loss of water with measured mass loss of 12.3%, which corresponds to 1 mol equivalent water and is in agreement with the values in

reported studies. Above 100 °C, CTN form II and CTN monohydrate scans are essentially superimposable. XRPD data also shows that CTN monohydrate dehydrates to CTN form II. CTN form I shows an endotherm in the same region (225-270 °C) but with only one shoulder and a peak at slightly higher temperature (263.78 °C). CTN form III also shows an endotherm in this region but with a significant difference as two peaks are observed at 248.42° C and 259.68° C (Fig. 4). The losses of weight (15.75% for CTN I, 16.56 % for CTN II and 16.47% CTN III) associated with the endotherms in the anhydrous forms approximate the expected weight loss of 13.74% associated with intramolecular cyclisation to form creatinine. The 2-3% discrepancy could be due to the melt and/or decomposition of creatinine immediately after its formation.

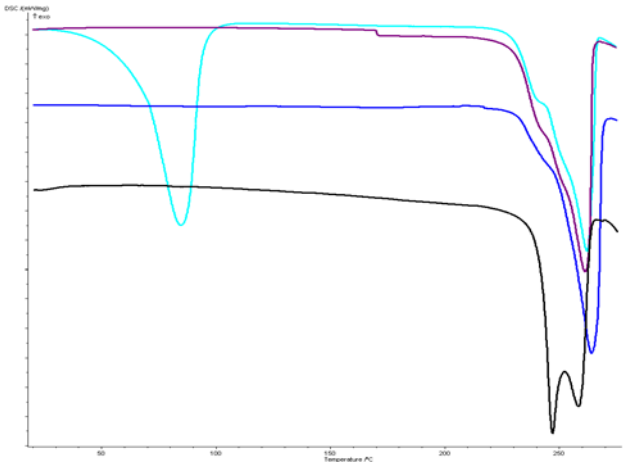

Figure S 5. DSC thermograms for monohydrate (light blue), CTN I (blue), II (violet) and III.

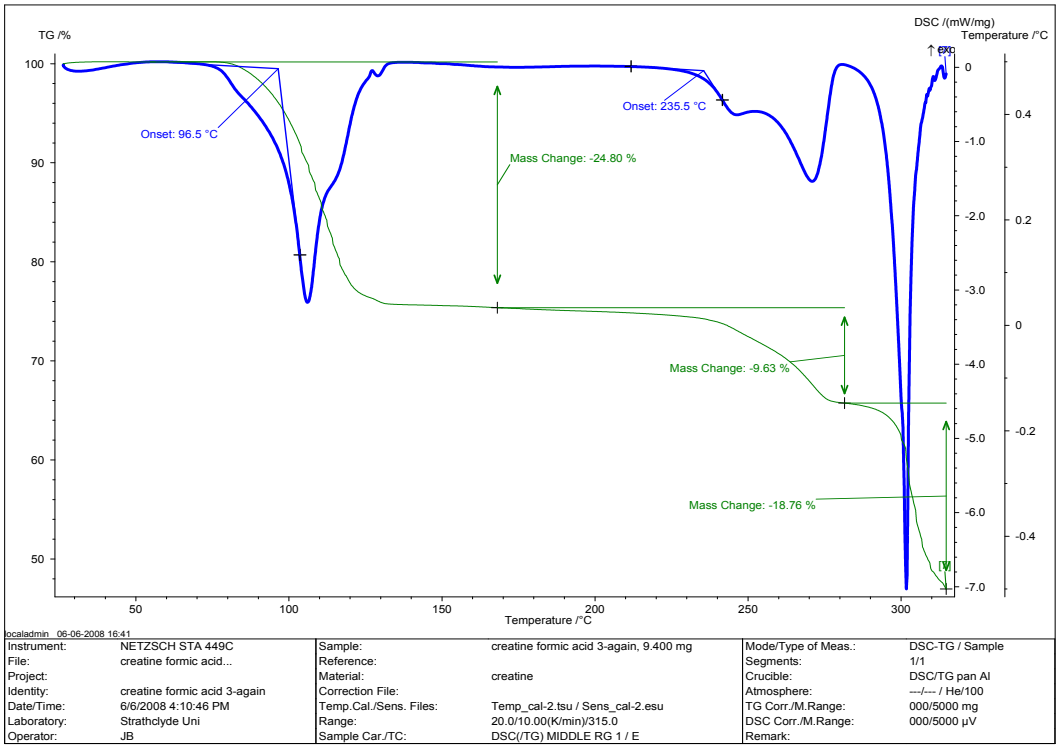

Figure S 6. STA data with marked thermal events for CTN formic acid solvate.

8. Relative Stability under Ambient Conditions

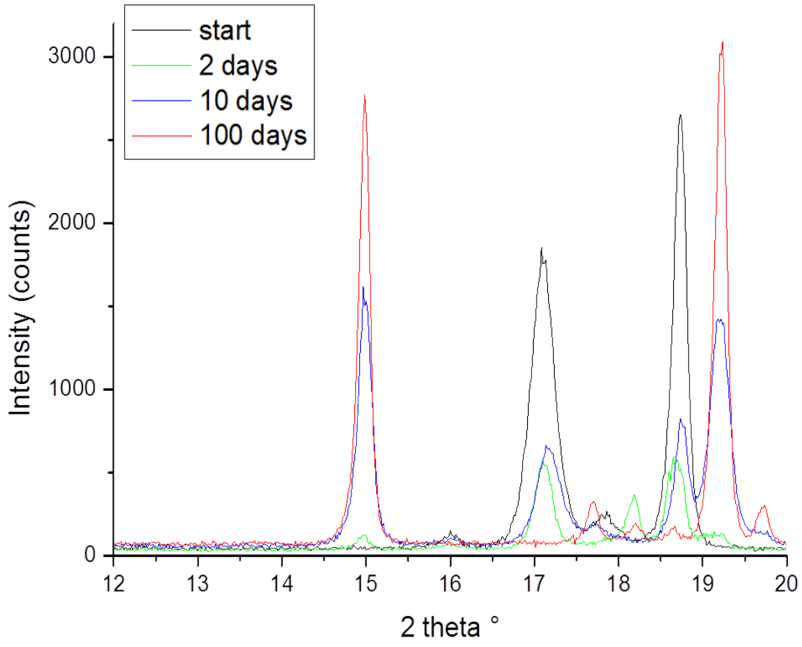

Figure S 7. Overlay of XRPD patterns of CTN II samples kept under ambient conditions.

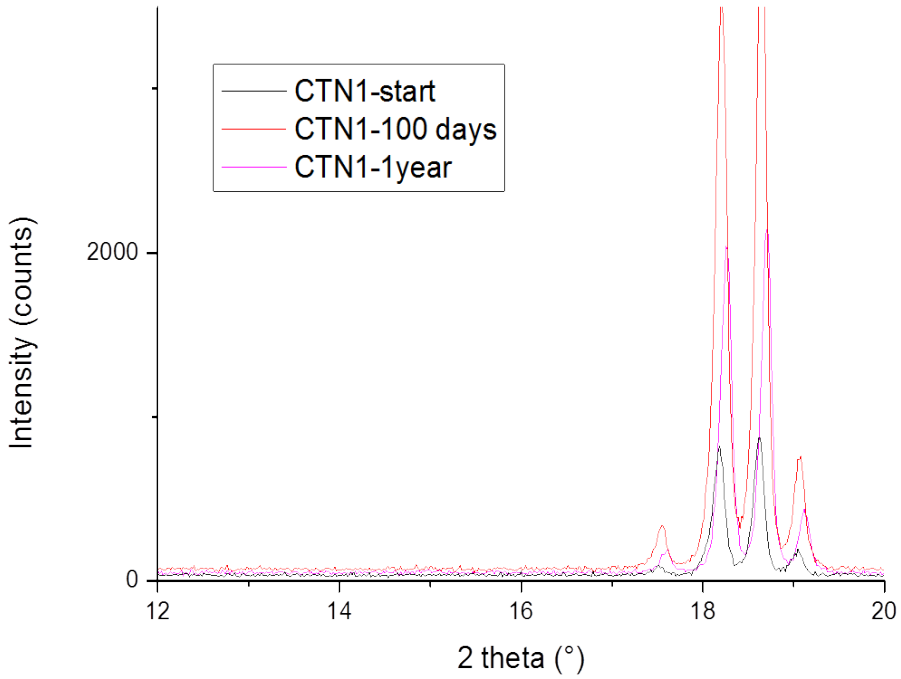

Figure S 8. Overlay of XRPD patterns of CTN I samples kept under ambient conditions.

9. Competitive Slurry Experiments

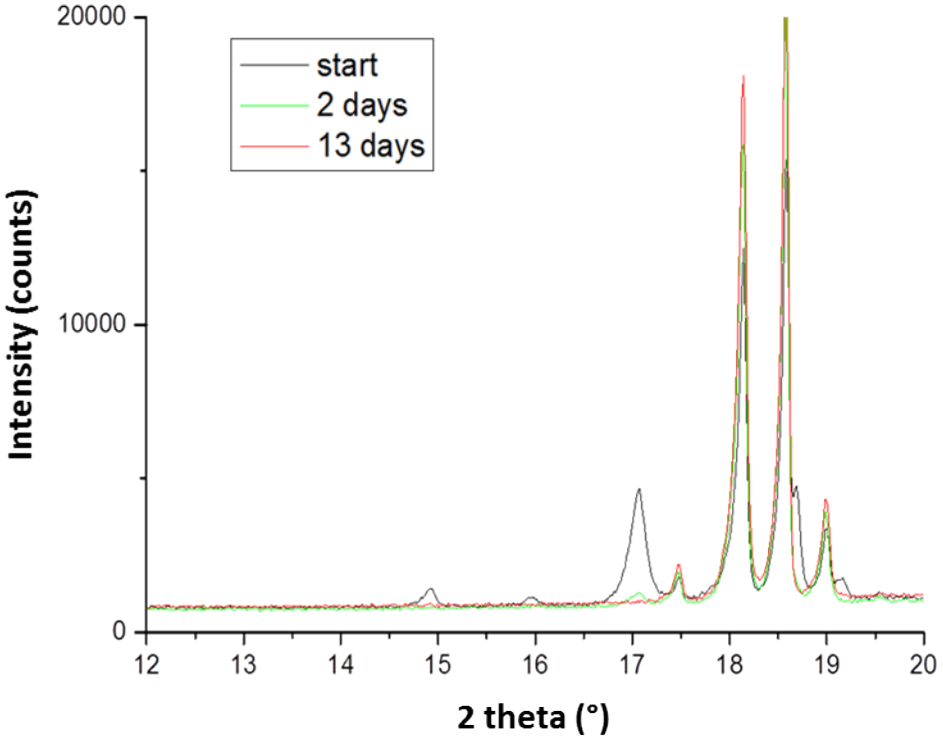

Figure S 9. Overlay of XRPD patterns of samples obtained from competitive slurry of a 1:1 mixture of CTN I and II in isoamyl alchol

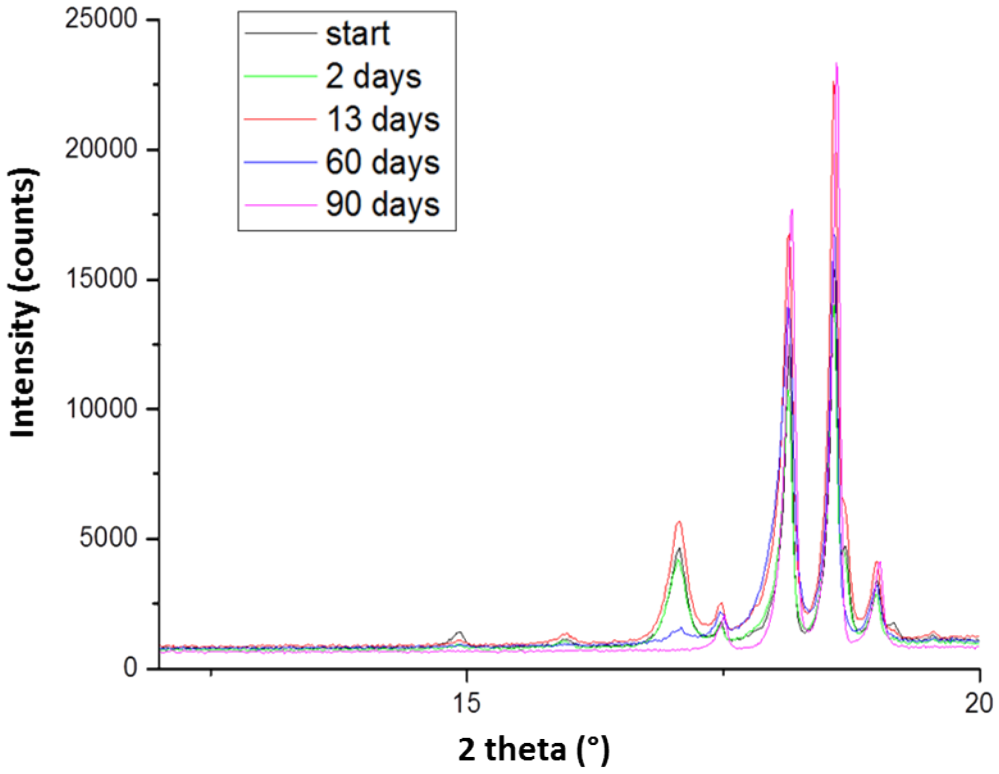

Figure S 10. Overlay of XRPD patterns of samples obtained from competitive slurry of a 1:1 mixture of CTN I and II in butyl acetate

Table S 4. Data for base vectors t in SCs $\mathbf{A_0}$ and $\mathbf{A_1}$.

	t_1	d_1	t_2	d_2	$<t_1,t_2$
Formic acid	0-1-1	11.183 Å	-	-	-
Monohydrate	001	12.075 Å	010	4.987 Å	90°
CTN I	-100	11.945 Å	001	5.344 Å	90°
CTN II	001	11.670 Å	010	5.837 Å	90°

10. References

1. S. J. Clark, M. D. Segall, C. J. Pickard, P. J. Hasnip, M. J. Probert, K. Refson, M. C. Payne, *Zeitschrift Fur Kristallographie* 2005, **220,** 567-570.

2. J. P. Perdew, K. Burke, M. Ernzerhof, *Physical Review Letters,* 1996, **77**, 3865-3868.