Pharmaceutical Crystallization with Nanocellulose Organogels

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Supplementary information

EXPERIMENTAL SECTION

Chemicals and Samples

Avicel PH-101 cellulose microcrystalline (50 μm of particle size), 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) (98%), sodium hypochlorite solution (10-15%), sodium bromide (>99%), methanol (MeOH, 99.8%), octadecylamine (≥99%), sulfapyridine (≥99%), isoniazid (≥99%), sulfamethoxazole and dimethylsulfoxide (DMSO 99.7%) were purchased from Sigma–Aldrich. Ultrapure water used throughout all experiments was purified through a Millipore system.

All reagents were used as received without further purification.

Instrumentation

Single crystal data was collected at 120(2) K on a Bruker D8Venture diffractometer (PHOTON-100 CMOS detector, IμS-microsource, focusing mirrors, MoKα λ = 0.71073Å) and processed using Bruker APEX-II software. The temperature of the samples was maintained by the Cryostream (Oxford Cryosystems) open-flow nitrogen cryostat. The structure was solved by direct method and refined by full-matrix least squares on F^2 for all data using X-seed, OLEX2 and SHELXTL software. All non-disordered non-hydrogen atoms were refined anisotropically, hydrogen atoms were placed in the calculated positions.

SEM samples were dried using vacuum drying desiccators for 2 days, coated with 3 nm of chromium using a Cressington 328 Ultra High Resolution EM Coating System, and imaged using an FEI Helios NanoLab DualBeam microscope in immersion mode, with typical beam settings of 1.5 kV and 0.17 nA. SEM images of the c-NC organogel are shown in Figures S8 and S9 and of the hydrogel in Figures S10 and S11.

DSC analysis was carried out by TA instruments DSC Q10 (V9.9 Build 303) at a heating rate of 10^{o}C per minute under a constant stream of argon at atmospheric pressure in the range of 30-175 ^{o}C.

Rheology was performed using a TA Instruments Advanced Rheometer 2000 (shear-controlled mode). Analyses of the gels were made on a 25 mm rough-surface steel plate with a gap of 1000 μm and 2 ml of sample. The measurements were carried out after stabilizing the gels for 45 min in the sample holder at room temperature (25 ºC). Stress sweep experiments were performed at a constant frequency of 1 Hz for the oscillation stress of 0.1-100.

Carboxylated nanocellulose

Synthesis**:** The preparation of the c-NC nanomaterial has been reported previously,[1] although some modifications were performed to up-scale the synthesis, as follows: microcrystalline cellulose (5.0 g), NaBr (0.0125 g) and 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) (0.145 g) were suspended in water (375 mL) and stirred at room temperature. A pH-probe was used to maintain a constant pH at 10 during all the reaction. Thus, NaOCl solution (40 mL) was added dropwise from a syringe whereas maintaining the pH of 10 with 1M NaOH. The end of the reaction was achieved when no further pH decrease are observed upon NaOCl addition. Finally, the reaction was stopped using 5 mL of ethanol. The nanomaterial was washed until a pH of 7 was achieved with water using a centrifuge at 5000 rpm. The nanomaterial was precipitated using methanol and dried under vacuum. A yield of 98% was obtained. The material was characterized by solid-state ^{13}C NMR spectroscopy (Figure S7).

Solubility studies in organic solvents: The solubility of c-NC in a variety of organic solvents (Table S1) was assessed using two methods as follows. No dissolution of the c-NC in any of the solvents tested was evident using these conditions.

a) Using as-synthesized c-NC: 10 mg of c-NC in 1mL of each organic solvent.

[1] Saito, T.; Nishiyama, Y.; Putaux, J.L.; Vignon, M.; Isogai, A. *Biomacromolecules* **2006**, *7*, 1687–1691.

b) Purification of the c-NC by gelation and re-precipitating the c-NC with methanol (see Scheme S1). The solubility of the powder obtained after this precipitation and gelation process was again assessed at 10 mg in 1ml of organic solvent.

c-NC + 500 µL NaOH → gel formation → Sonication with MeOH→ filtration (solid powder)

Scheme S1.

1,2,4 – trichlorobenzene	3 – butanone	1,2 – dibromethane
1,2 – dichlorobenzene	1,3 – dichlorobenzene	1,4 – dichlorobenzene
Ethyl pyridine	2 – picoline	Pyridine
Acetone	DMF	Nitrobenzene
Ethylene glycol	DMSO	

Table S1. Organic solvents used in solubility studies.

Gelation procedure

All gelation experiments were carried out by dissolving a low concentration of the gelator c-NC 0.3% and octadecylamine (6 mg, 22.26 µmol) in 1 mL of DMSO, and the as-prepared mixture was mixed with vortex and sonication (for few seconds) and lastly heating (for few seconds) in a vial. Afterwards, stable gels were formed following this procedure after setting aside at room temperature for few minutes.

For the crystallization process, the same amount of NC and octadecylamine was dissolved in a stock solution of the respective drug in DMSO and mixture and heating of the as-prepared suspension was keep at room temperature for a period. Gel formation was monitored by performing the “inversion test”.

Figures

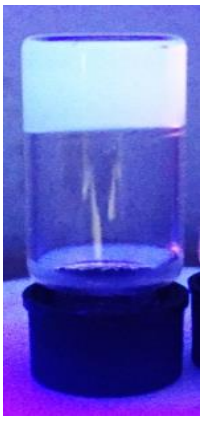


Figure S1. c-NC hydrogel at 9 wt% undergoing the inversion test.

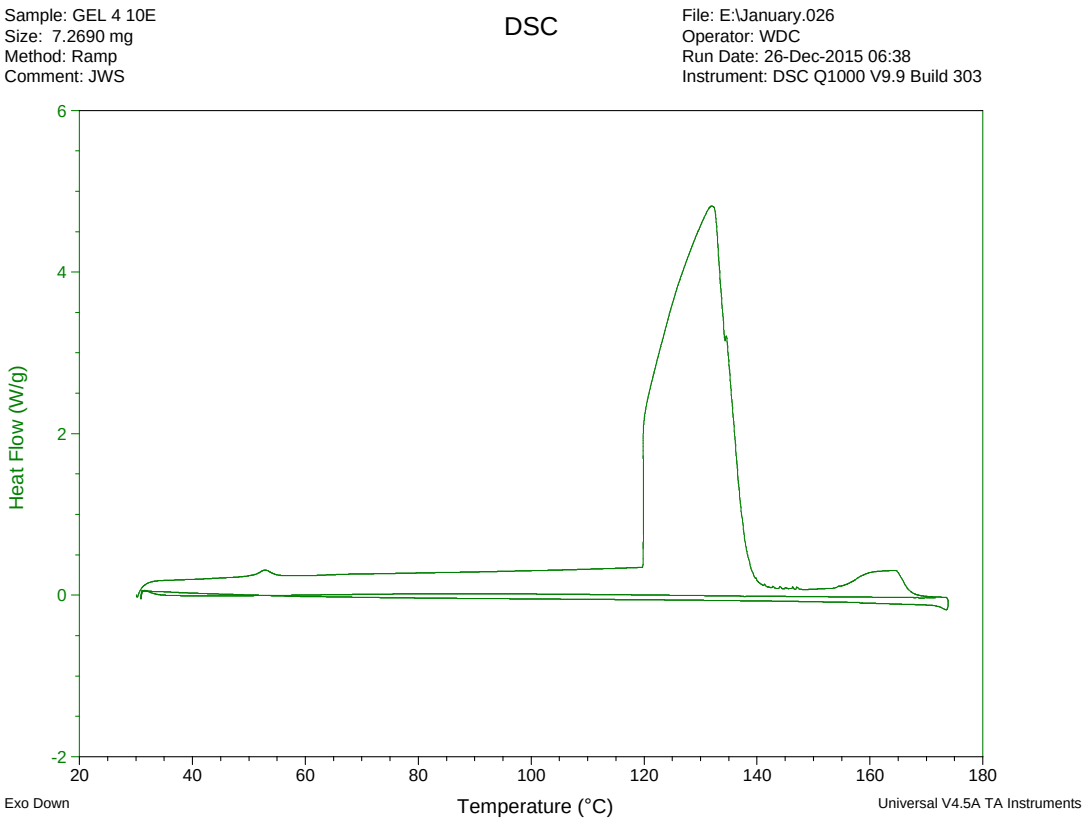


Figure S2. DSC thermogram of an OD/c-NC DMSO gel showing the gel-sol transition at 55 ºC.

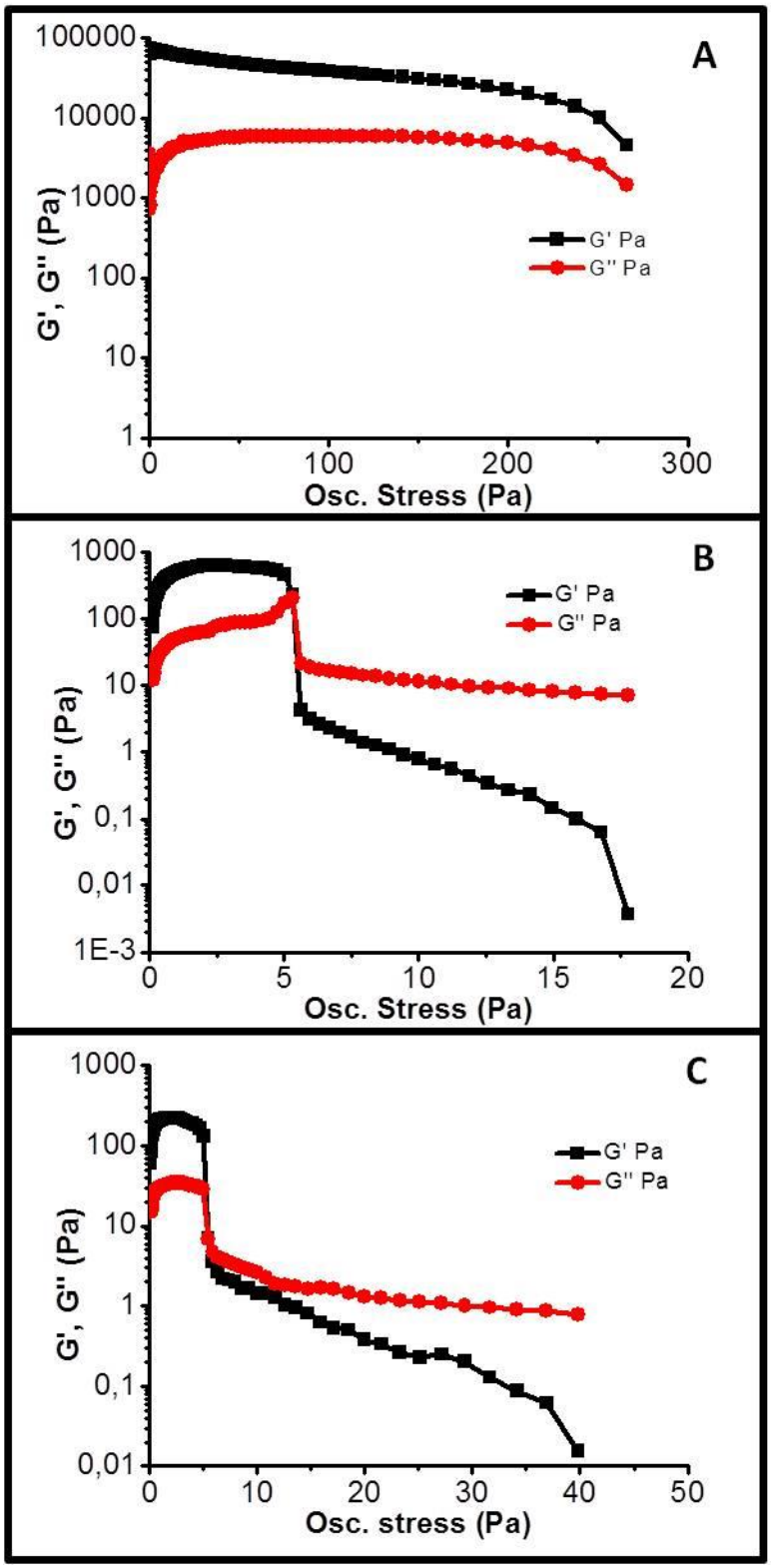


Figure S3. Stress sweep rheometry of A) OA/c-NC gel in DMSO, and the corresponding gels containing 200mg mL^{-1} sulfapyridine (B) and sulfamethoxazole (C).

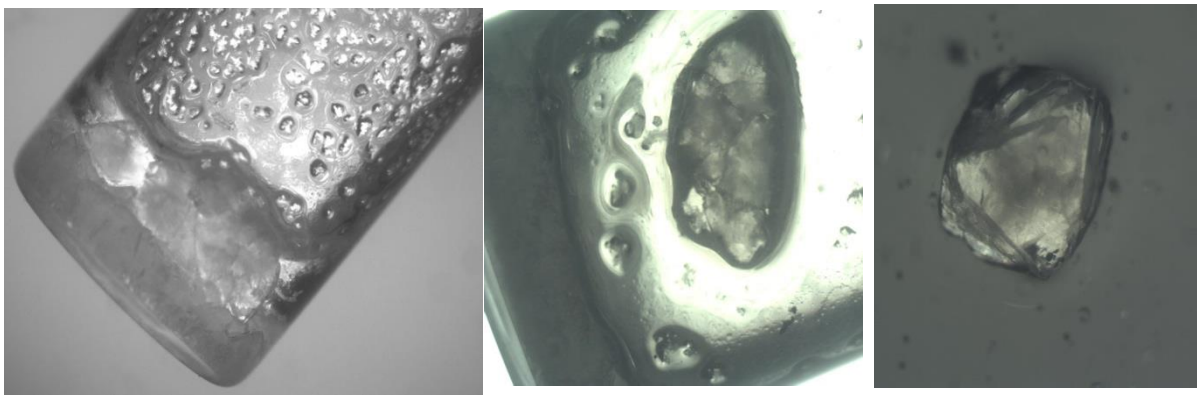


Figure S4. Crystals of sulfapyridine 1:1 DMSO solvate obtained in c-NC gels. The crystals pictures are from the 500 mg mL^{-1} sample. Upon cooling all samples containing 10, 20, 50, 100 and 500 mg mL^{-1} gelation was observed, followed by crystallization in the case of the 100 and 500 mg mL^{-1} samples. The crystals formed within the gel. Mechanical agitation of the 500 mg mL^{-1} sample during isolation of the crystals caused the gel to partially break down.

Figure S5. Crystals of the octadecylammonium salt of sulfamethoxazole 1:2 DMSO solvate obtained in OD/c-NC gels.

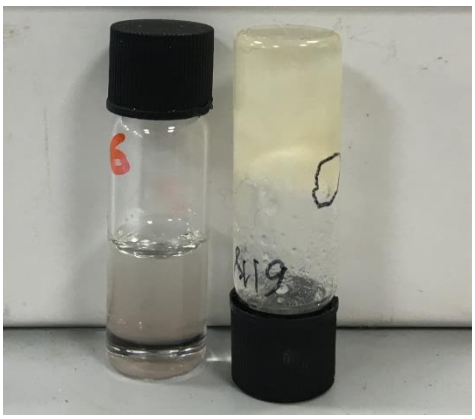
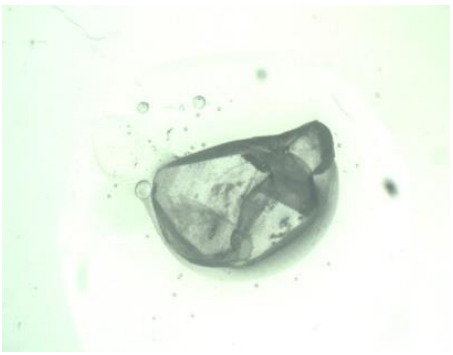
(a)  (b) 

Figure S6. (a) Sulfonated-NC gels containing sulfapyridine DMSO solvate crystals (right) and control solution crystallization (left) in DMSO. (b) An isolated crystal sulfapyridine DMSO solvate obtained from the gel.

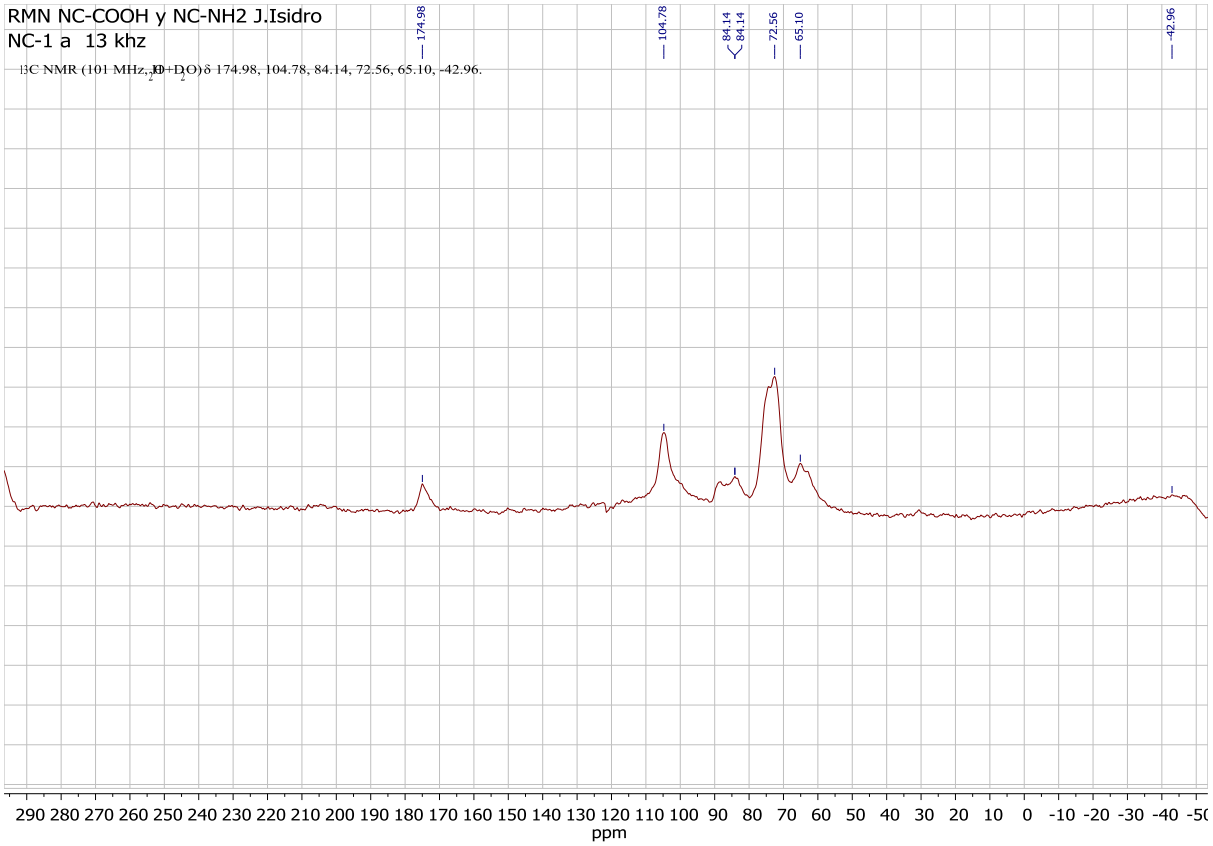


Figure S7. Solid state ^{13}C NMR spectrum of c-NC showing the presence of the carboxyl carbon atom at 174.98 ppm. The region between 70 and 80 ppm is attributed to C2, C3, and C5 of disordered cellulose. The peaks around 84 and 86 ppm are assigned to C4. The peak at 65 ppm is due to the hydroxymethyl C6 cabon atom and the peak 104.78 ppm is ascribed to C1. (Bruker Avance 400 WB).

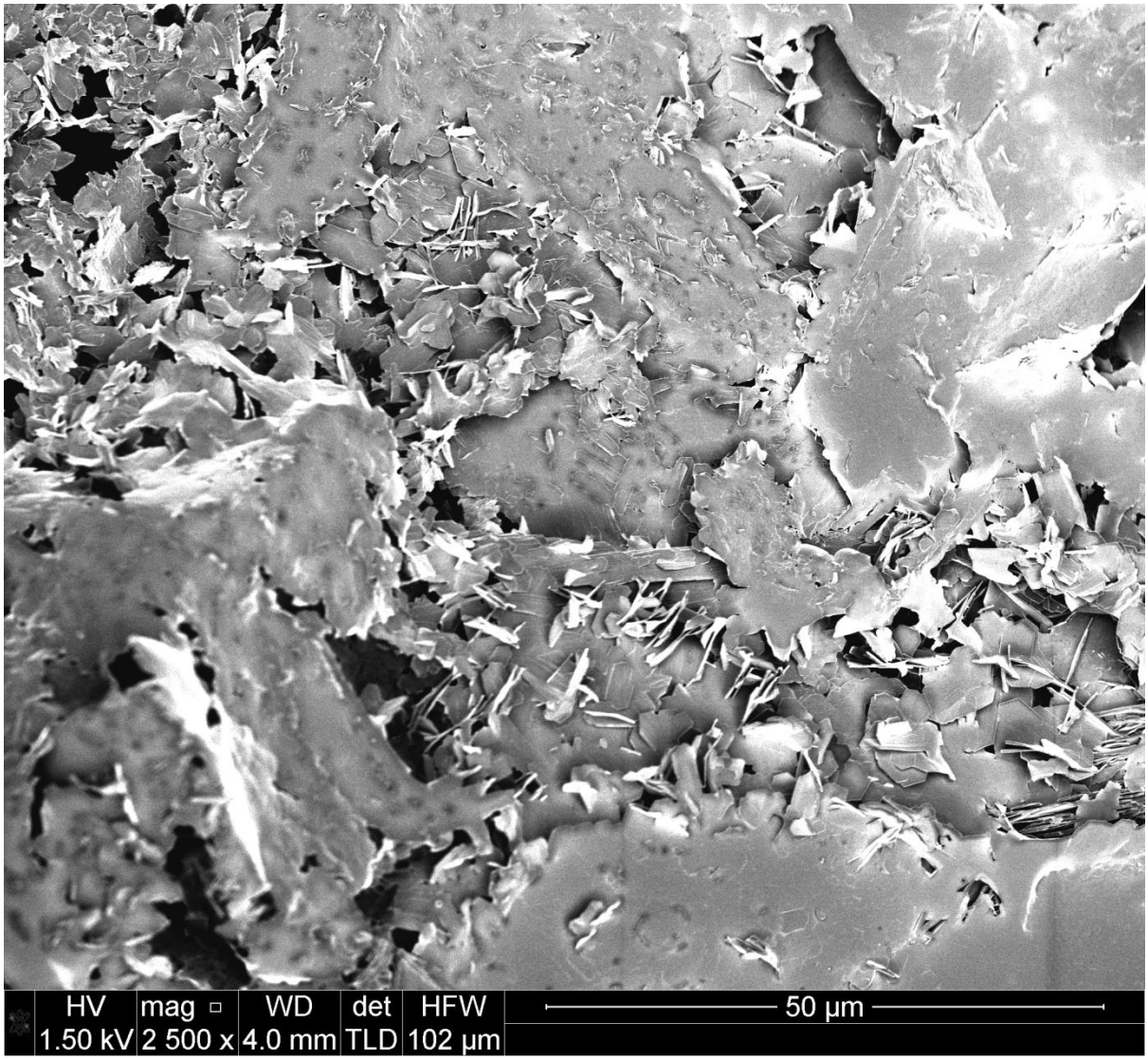


Figure S8. SEM image of the OD/c-NC xerogel from DMSO showing the layered structure.

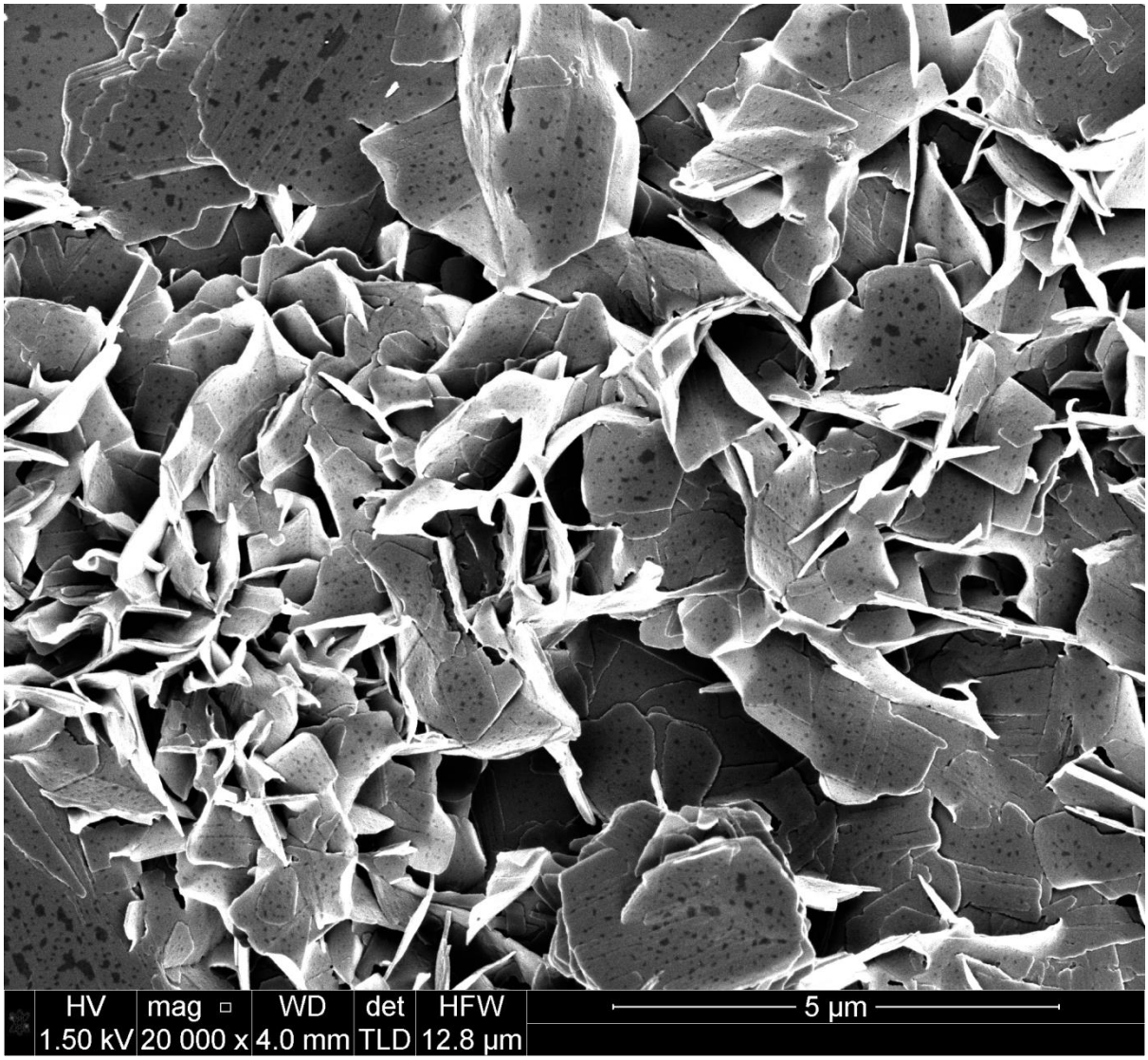


Figure S9. Further SEM image of the OD/c-NC xerogel from DMSO showing the layered structure.

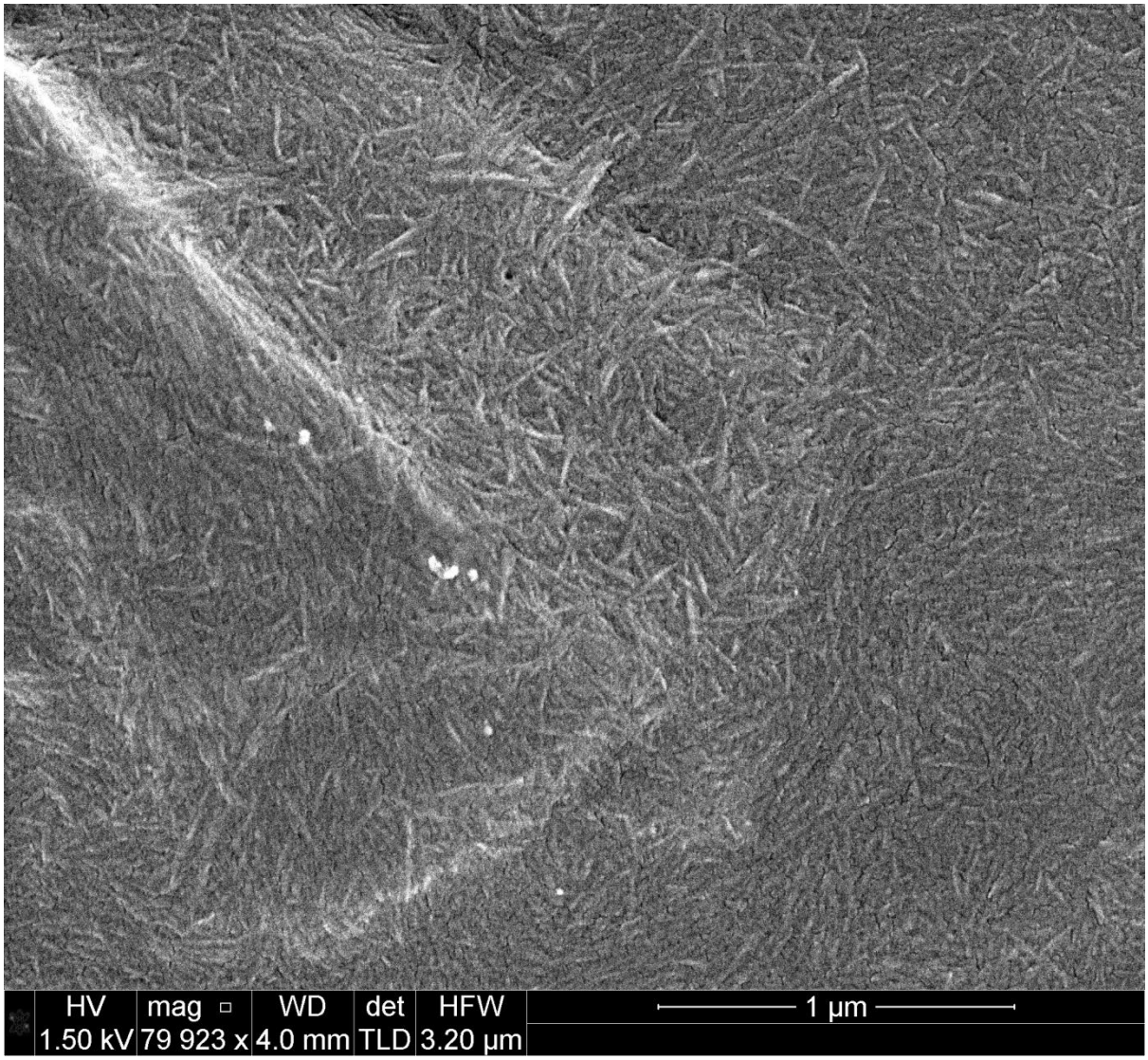


Figure S10. SEM image of the c-NC dried hydrogel showing the filamentous structure.

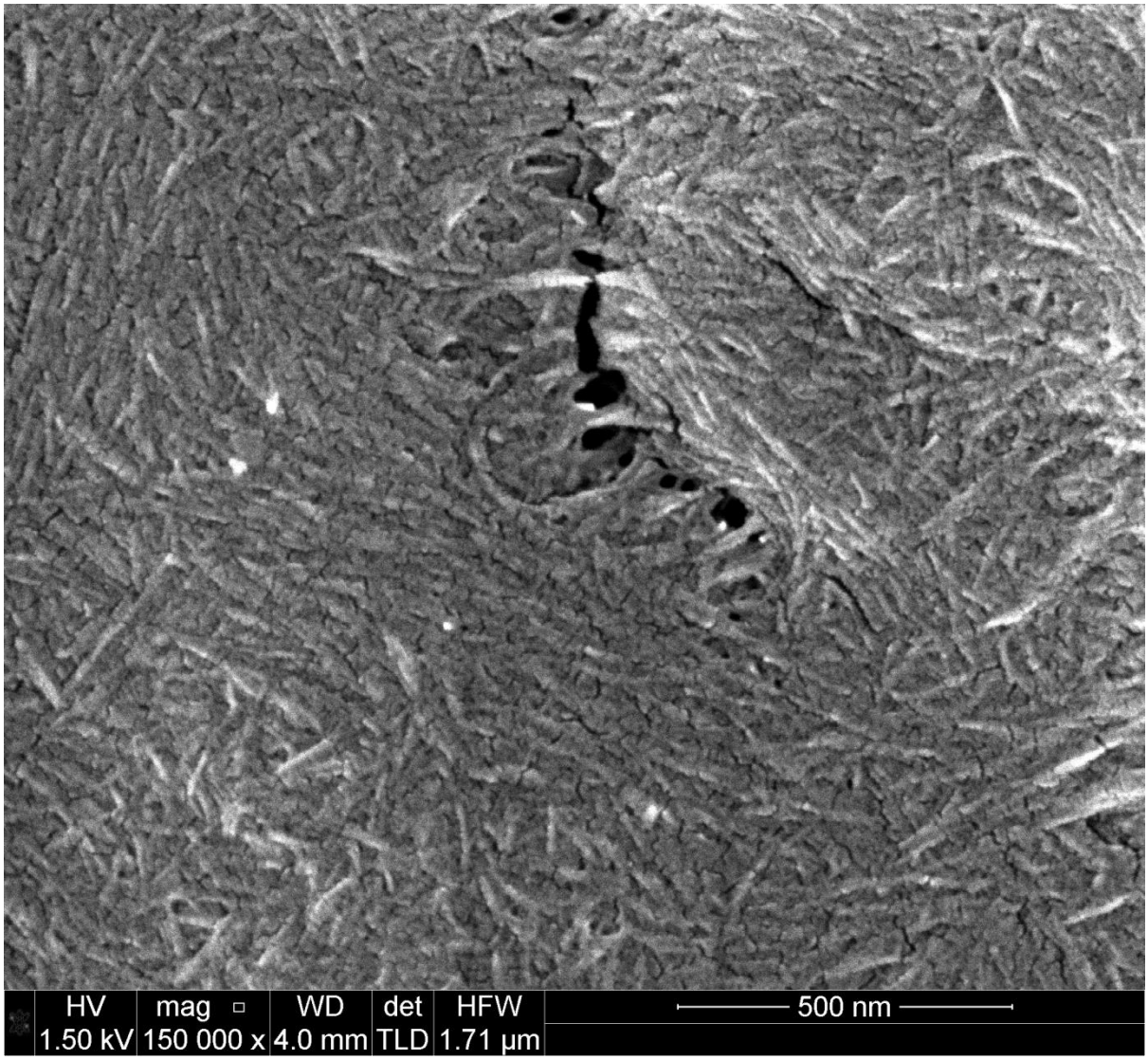


Figure S11. Further SEM image of the c-NC dried hydrogel showing the filamentous structure.

Crystallization studies of drugs in nanocellulose gels

Different drugs were introduced in the c-NC gels to evaluate their crystallization behaviour. An amount of drug (in the range of 10-500 mg) was dissolved in 1ml of DMSO and mixed with c-NC prior to gelation. Control experiments were also prepared at the same concentrations (drugs dissolved in DMSO) for each drug, as depicted in Table S2.

	10 mg	50 mg	100 mg	200 mg	500 mg
Dopamine hydrochloride	*	*	*	*	
Mexiletine hydrochloride	*	*	*		*
p-aminohippuric acid	*	*	*	*	
Benzocaine	*	*	*		*
Sulfapyridine	*	*	*		*
L-valine	*	*	*	*	
Hydrochlorothiazide	*	*	*	*	
4-aminopyridine	*	*	*	*	
5-aminosalicilyc acid	*	*	*		*
Carisodropoll C-IV	*	*	*		*
Isoniazid	*	*	*		*
Ethionamide	*	*	*	*	
Sulfamethazine				*	*
Sulfamerazine				*	*
Sulfamethoxazole				*	*
Sulfadiazine				*	*
Sulfathiazole				*	*

Table S2. Drugs used in the c-NC gel crystallization experiments. The asterisk represents the tested examples.

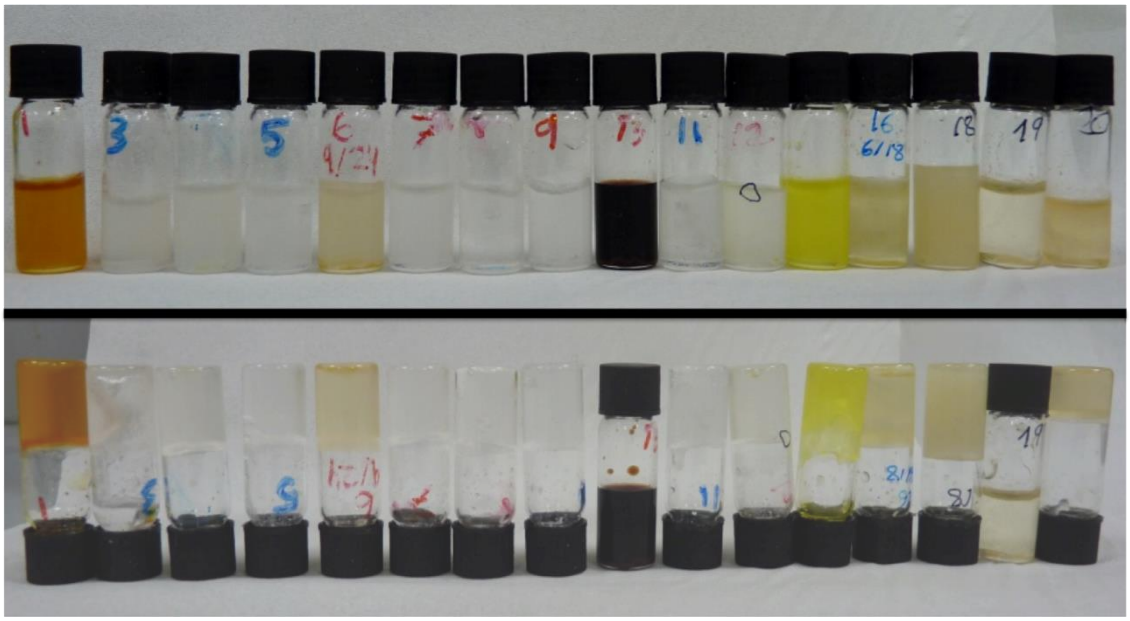


Figure S12. c-NC gels of the drugs using 0.3% w/v of the gelator (from left to right are the drugs listed in Table S2).

Only for 5-aminosalicilyc acid and sulfathiazole no gel formation was observed (see the bottom image of Fig. S12).

Individual pictures of each drug were taken (Figures S13 – S17), in which the left vial represents the control experiment (first vial of each image) whereas the other vials on his right are those involving different drug concentrations (increasing drug concentrations from left to the right). Some vials were photographed upside down in order to demonstare the formation of stable gels via the "inversion test".

A B C

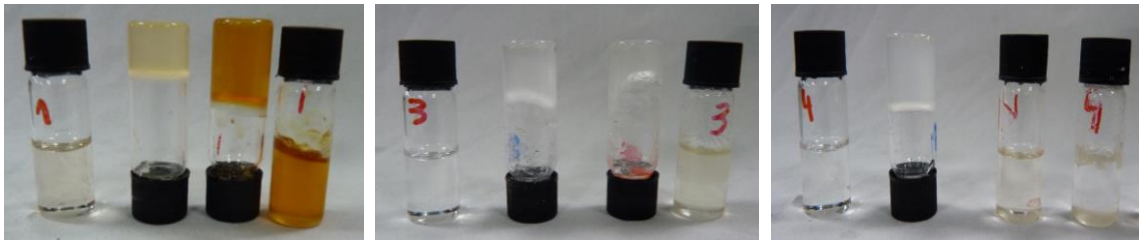


Figure S13. c-NC gels of A) dopamine hydrochloride, B) mexiletine hydrochloride and C) p-aminohippuric acid.

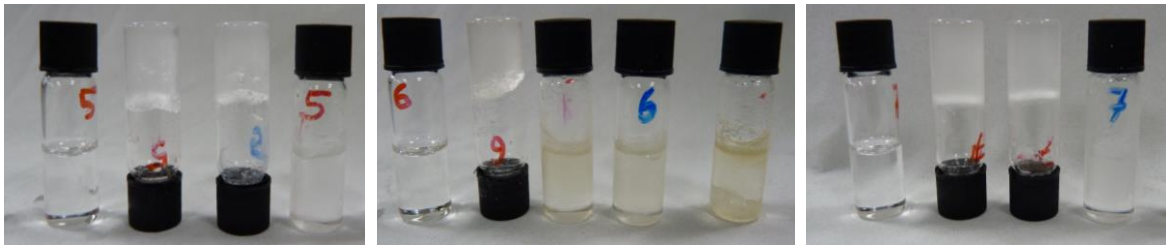


Figure S14. c-NC gels of A) benzocaine, B) sulfapyridine and C) L-valine methyl ester hydrochloride

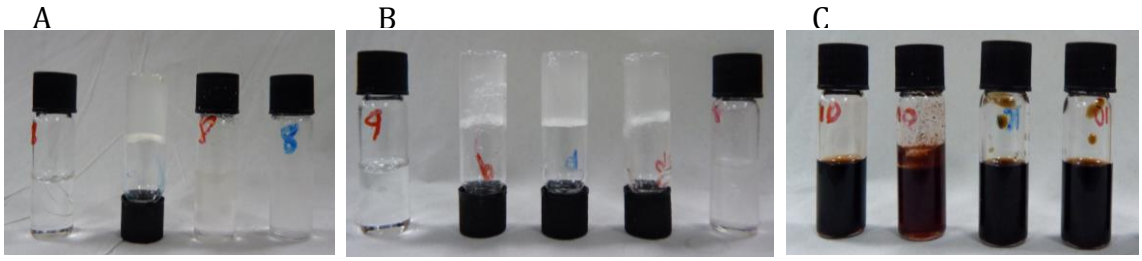


Figure S15. NC gels of A) hydrochlorothiazide, B) 4-aminopyridine and C) 5-aminosalicilyc acid.

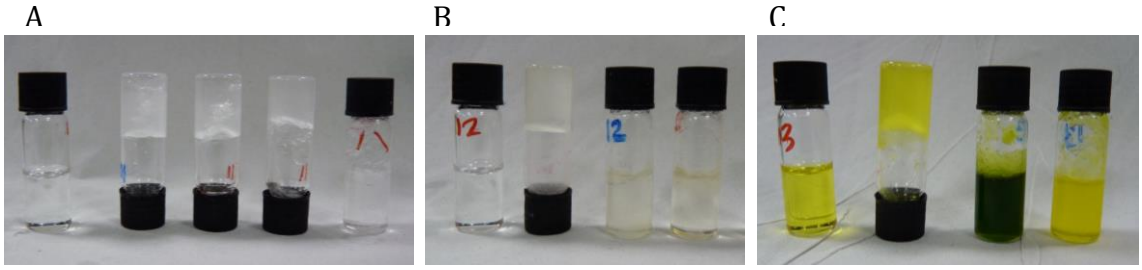


Figure S16. c-NC gels of A) carisodropoll C-IV, B) isoniazid and C) ethionamide.

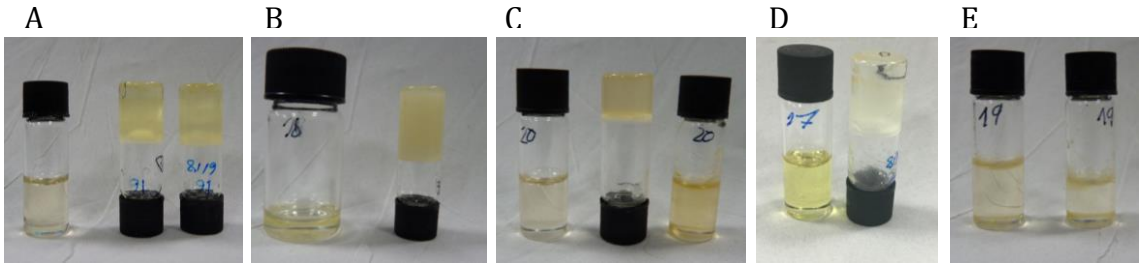


Figure S17. c-NC gels of A) sulfamethazine, B) sulfamerazine, C) sulfamethoxazole, D) sulfadiazine and E) sulfathiazole.