Electronic Supplementary Information (ESI) – Antisolvent addition at extreme conditions

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S1. Large volume press (LVP)

The LVP used in this work is of the same construction as reported previously. For antisolvent addition, a glass tube was designed to hold the selected antisolvent (water). The length of the glass tube was specific to the target pressure that we wished the tube to break. Tests were performed to measure the contraction of the sample tube as a function of applied load (See Section S4). The glass tube was prepared by flame sealing a standard laboratory glass pipette approximately 25 mm from the taper (Figure S1). After cooling, the remaining piece was cut to the desired size using a ceramic cutting blade before filling with deionized water using a syringe and needle. Once filled, the antisolvent tube was sealed using epoxy glue and allowed to fully harden before use in LVP experiments. The length of the resulting sealed tube was verified to be suitable prior to experiment after checking of the sealed tube length and the length of the PTFE sample tube. Prior to use in LVP experiment, the glass tube was lightly scored around the diameter of the tube to promote breakage at the target pressure.

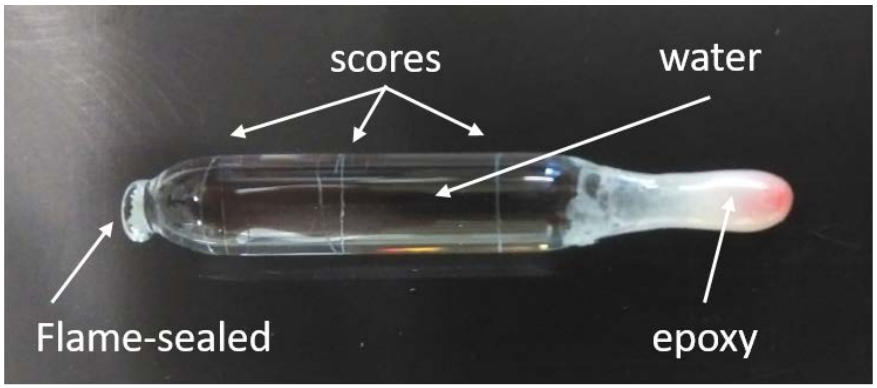

Figure S1. Prepared antisolvent glass tube. Glass tube formed by flame-sealing a laboratory glass pipette and then cutting the pulled tip to size before filling and sealing with epoxy glue

S2. UV-vis concentration determination

Spectra were obtained over the range 200-400 nm and the absorbance at 248 nm used for evaluation of PCM concentration. In order to avoid saturation of the spectrograph detector it was found that 0.2 g/g solutions required dilution by a factor of 8000 – this dilution factor was applied to all measured samples. A calibration curve was produced using standard samples of PCM dissolved in 64% w/w MeOH:H_2O with concentrations of 0.14, 0.16, 0.18, 0.20 and 0.22 g/g. A plot of the calibration curve is shown in Figure S2.

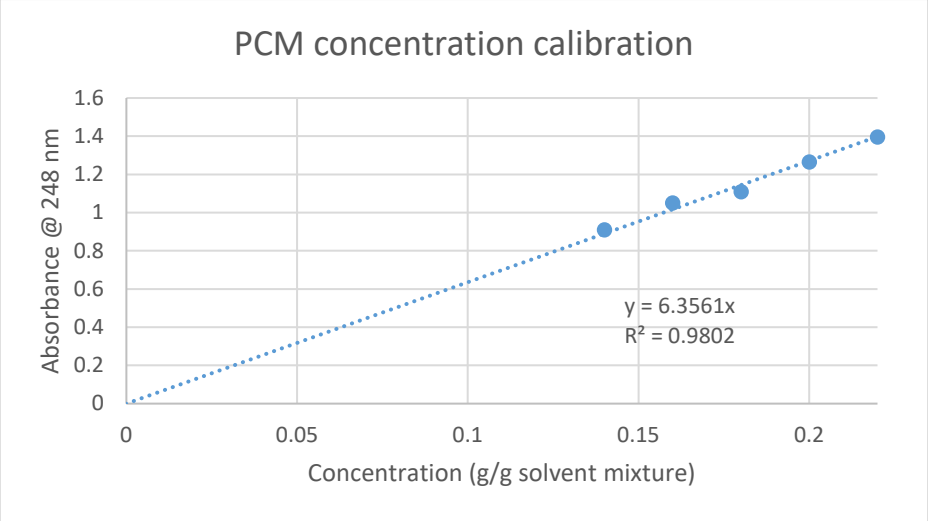

Figure S2. Calibration curve obtained for standard PCM solutions prepared in 64% w/w MeOH:H_2O. Dotted line shows the linear line of best fit with y-intercept = 0.

The solute concentration in samples following LVP compression were tested by pipetting 5 ul and diluting by a factor of 8000 with 64% w/w MeOH:H_2O solvent mixture. 3.0 mL of diluted sample was transferred to a 10 mm pathlength quartz glass cuvette for UV-vis measurement. Each sample concentration was measured in triplicate and averaged to obtain the mean absorbance for each concentration. A summary of the PCM concentration in the top portion of samples after compression/decompression in LVP (without antisolvent addition) is shown in Table S1

Table S1. Summary of PCM concentrations in upper layer of solution sample after compression/decompression in large volume press experiment determined UV-vis.

Solution concentration (g/g solvent)	Mean absorbance (248 nm)	Calculated concentration (g/g solvent)	% difference to expected concentration
0.14	0.302	0.048	66.0
0.16	0.446	0.070	56.1
0.18	0.631	0.099	44.9
0.20	0.682	0.107	46.4
0.22	0.973	0.153	30.4

S3. SC-XRD testing

S3.1 DAC samples

A Merrill-Basset DAC (600 um culet diamonds) was used with an indented tungsten foil gasket (100 um thick) that was drilled to prepare a 250 um hole for the sample chamber. Solution samples were loaded along with a small chip of ruby to provide pressure readout by the ruby fluorescence method.[1] Solution samples were taken to approximately 0.8 GPa to achieve comparable pressures to those in the LVP experiment. Crystal nucleation was only observed on decrease of pressure from this point (0.10 and 0.20 g/g solution samples) and on the application of further pressure to ca. 1.5 GPa for 0.05 g/g solution sample.

Crystals obtained by in-situ nucleation experiments were subject to single crystal X-ray diffraction (SC-XRD) to verify the solid form. Data were collected using a standard run list as shown in Table S2.

Table S2. Typical data collection strategy used for DAC samples. Frame exposure time can vary depending on the nature (quality/size) of the sample crystal

Scan	distance (mm)	2 Theta (deg)	Omega (deg)	Phi (deg)	Chi (deg)	Time (sec)	Width (deg)	Sweep (deg)	direction
Omega	70	-28	-10	0	54.726	30	0.3	30	negative
Omega	70	28	40	0	54.726	30	0.3	65	negative
Omega	70	-28	25	0	-54.726	30	0.3	65	negative
Omega	70	28	40	0	-54.726	30	0.3	30	negative
Omega	70	-28	-10	180	54.726	30	0.3	30	negative
Omega	70	28	40	180	54.726	30	0.3	65	negative
Omega	70	-28	25	180	-54.726	30	0.3	65	negative
Omega	70	28	40	180	-54.726	30	0.3	30	negative
Omega	70	28	40	0	90	30	0.3	52	negative
Omega	70	-28	29	0	-90	30	0.3	64	negative
Omega	70	28	40	180	90	30	0.3	52	negative
Omega	70	-28	29	180	-90	30	0.3	64	negative

Owing to the quality of the in-situ grown crystals, a few runs were sufficient for indexing of crystals. Several full collections were performed on samples that were later identified as a PCM:MeOH solvate. Data for these samples were reduced using Bruker, Apex3 software. Resolved structures were solved by intrinsic phasing using SHELXT using Olex2 (v1.2) software. Full-matrix least-squares refinement of data was also performed with SHELXL using Olex2 software. All non-hydrogen atoms were treated anisotropically for the Form II whilst for the methanol solvate the non-hydrogen atoms were treated isotropically due to the paucity of data. Hydrogen atoms were placed on the carbon atoms and allowed to ride on their parent atoms. The datasets from the 0.1g/g PCM solutions were not of sufficient quality to be deposited in the Cambridge Structural Database but were submitted as part of the reviewing process.

Details of the DAC samples that were tested by SC-XRD are provided in Table S3 and their crystallographic information can be found in Table S4.

Table S3. Summary of XRD data collection performed for samples in DAC.

Compound	Sample	Solution concentration (g PCM / g solvent mixture)	Pressure (GPa)	Solid form	CCDC code
	PCM_01gg_201218	0.1	0.76	MeOH:PCM	-
	PCM_01gg_040119	0.1	0.76	MeOH:PCM	-
1	PCM_02gg_070119	0.2	0.71	MeOH:PCM	1902446
2	PCM_02gg_180119	0.2	0.19	PCM II	1902444
3	PCM_02gg_ambient	0.2	Ambient	PCM II	1902442
4	PCM_005gg_250119	0.05	0.79	MeOH:PCM	1902445

Table S4: Crystallographic information for the five datasets taken at various solution concentrations and pressures. Dataset 1 crystallised from a 0.2 g/g paracetamol to methanol:water (64% w/w) solution at 0.72 GPa. Dataset 2 was performed on a crystal isolated from same loading as 1 but reduced in pressure to 0.21 GPa. The crystal grew from solution (FigureS3) after leaving the sample. Dataset 3 was the same crystal as dataset 2 but at ambient pressure. Dataset 4 was taken on a crystal isolated from a 0.05 g/g paracetamol to methanol:water (64% w/w) solution at 0.75 GPa. Dataset 5 was taken on a crystal at ambient pressure recovered from the large volume press.

	(1)	(2)	(3)	(4)	(5)
Crystal data					
Chemical formula	$CH_4O{\cdot}C_8H_9NO_2$	$C_8H_9NO_2$	$C_8H_9NO_2$	$CH_4O{\cdot}C_8H_9NO_2$	$C_8H_9NO_2$
M_r	183.20	151.16	151.16	183.20	151.16
Crystal system, space group	Monoclinic, $P2_1/n$	Orthorhombic, *Pbca*	Orthorhombic, *Pbca*	Monoclinic, $P2_1/n$	Orthorhombic, *Pbca*
Temperature (K)	293	296	296	293	296
Pressure (GPa)	0.72	0.21	Ambient	0.75	Ambient
a, *b*, *c* (Å)	13.0234 (15), 17.2078 (9), 13.0925 (15)	17.1202 (17), 11.7968 (11), 7.288 (2)	17.143 (8), 11.806 (6), 7.399 (10)	12.9717 (15), 17.1881 (9), 13.0437 (19)	17.1522 (5), 11.8201 (4), 7.3985 (2)
α, β, γ (°)	90, 116.209 (7), 90	90, 90, 90	90, 90, 90	90, 116.032 (8), 90	90, 90, 90
V (Å^3)	2632.4 (5)	1471.9 (5)	1497 (2)	2613.2 (5)	1499.98 (8)
Z	12	8	8	12	8
Radiation type	Mo *K*α	Mo *K*α	Mo *K*α	Mo *K*α	Cu *K*α
μ (mm^{-1})	0.10	0.10	0.10	0.11	0.80
Crystal size (mm)	0.15 × 0.14 × 0.05	0.09 × 0.07 × 0.05	0.09 × 0.07 × 0.05	0.18 × 0.06 × 0.05	0.2 × 0.18 × 0.05
Data collection					
Diffractometer	Bruker *APEX*-II CCD	Bruker *APEX*-II CCD	Bruker *APEX*-II CCD	Bruker *SMART APEX2* area detector	Bruker *APEX*-II CCD
Absorption correction	Multi-scan *SADABS2016*/2 (Bruker,2016/2) was used for absorption correction. wR2(int) was 0.1071 before and 0.0616 after correction. The Ratio of minimum to maximum transmission is 0.8490. The λ/2 correction factor is Not present.	Multi-scan *SADABS2016*/2 (Bruker,2016/2) was used for absorption correction. wR2(int) was 0.1466 before and 0.0755 after correction. The Ratio of minimum to maximum transmission is 0.8510. The λ/2 correction factor is Not present.	Multi-scan *SADABS2016*/2 (Bruker,2016/2) was used for absorption correction. wR2(int) was 0.0969 before and 0.0605 after correction. The Ratio of minimum to maximum transmission is 0.7071. The λ/2 correction factor is Not present.	Multi-scan *SADABS2016*/2 (Bruker,2016/2) was used for absorption correction. wR2(int) was 0.0848 before and 0.0556 after correction. The Ratio of minimum to maximum transmission is 0.9145. The λ/2 correction factor is Not present.	Multi-scan *SADABS2016*/2 (Bruker,2016/2) was used for absorption correction. wR2(int) was 0.0894 before and 0.0482 after correction. The Ratio of minimum to maximum transmission is 0.8688. The λ/2 correction factor is Not present.
T_{min}, T_{max}	0.632, 0.745	0.634, 0.745	0.527, 0.745	0.681, 0.745	0.654, 0.753
No. of measured, independent and observed [*I* >	12022, 1214, 819	4019, 413, 271	866, 304, 161	9591, 1319, 765	15062, 1378, 1223

$2\sigma(I)$] reflections					
R_{int}	0.078	0.118	0.129	0.081	0.038
θ_{max} (°)	23.3	23.3	23.4	23.3	68.3
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	0.556	0.557	0.558	0.556	0.603
Refinement					
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.094, 0.272, 1.07	0.058, 0.147, 1.08	0.068, 0.207, 1.08	0.129, 0.417, 1.55	0.045, 0.122, 1.07
No. of reflections	1214	413	304	1319	1378
No. of parameters	130	90	90	130	91
No. of restraints	0	75	75	0	75
$\Delta\rangle_{max}$, $\Delta\rangle_{min}$ (e Å^{-3})	0.31, -0.23	0.13, -0.15	0.14, -0.13	0.56, -0.36	0.30, -0.18

S3.2 Ambient pressure PCM II

Following LVP experiments, the sample material recovered was tested by SC-XRD to verify its contents. Crystals were dispersed in silicone oil and mounted on a low-background Kapton microloop (200 µm). Data was collected on a Bruker D8 Venture diffractometer equipped with a Photon 100 detector. Crystals were indexed in order to assess their solid form, in order to do this a 'fast scan' experimental method was employed. This method is also used for screening crystals ahead of determining the strategy for a full collection for structural solution.

A full collection was performed on a particle of PCM II in order to assess the quality of the crystallized material. A summary of the collected data is shown in Table S4 Compound 5.

S4. Axial compression measurement

For large volume press antisolvent experiments it was necessary to establish the resulting length of the PTFE tube as a function of pressure. This would in turn allow us to produce a glass tube of suitable length such that it breaks at the target pressure (internal length of the PTFE sample tube). Contraction of the sample tube was monitored by video microscopy during compression of 2 solvent systems – water and methanol:water (64% w/w).

PTFE sample tubes were assembled as per normal procedures and filled only with the chosen solvent before capping and assembling in the copper beryllium cell. Sample pressure is generated by use of a pneumatic actuator to apply load to the top of the sample tube. A camera (Basler acA1920—40uc) and zoom lens was used to image the actuator and to monitor its translation as a function of applied load. Still images were obtained at each pressure point and analysed using ImageJ to calculate the translation of the actuator.

S5. Video monitoring

Video monitoring of DAC samples was performed using solution concentrations of 0.05, 0.10 and 0.20 g/g PCM/solvent mixture. Solution samples were loaded in a DAC for compression and decompression studies. Video microscope was used to monitor crystallized material during decompression to aid identification of the dissolution point of the crystalline phase

S5.1 Dissolution point monitoring

Once a sample had nucleated and a suitable crystal was obtained the sample pressure was gradually decreased and monitored at each point by video microscopy. Sample pressure was established by the ruby fluorescence technique using an Almax Optiprexx PLS spectrometer equipped with a 532 nm excitation laser (20 mW). At each pressure point the sample would be monitored over at least a 30

minute period with images recorded every minute. For longer monitoring periods (over night or weekend during experiments) a 5 minute or 30 minute interval would be used.

Table S5. Summary of dissolution points (pressure) recorded for PCM:MeOH solvate in solutions of 0.05, 0.10 and 0.20 g/g concentration in 64% w/w MeOH:H2O solvent mixture.

Solution Concentration (g/g solvent)	Lowest pressure crystal observed (GPa)	Highest pressure solution phase (GPa)
0.05	0.30	0.26
0.10	0.14	0.13
0.20	0.22	0.19

For each sample concentration the pressure at which the crystal completely dissolved was recorded. Only one sample was used for this determination for each of the solution concentrations, the obtained solubility points are summarized in Table S5.

S5.2 Nucleation of PCM II

During monitoring of the 0.2 g/g sample dissolution of a MeOH solvate crystal (verified by SC-XRD) was observed and simultaneously nucleation of a crystal at the gasket edge was observed. This process is summarized in Figure S3a-d that shows frames during the transformation over a 4-hour period. The nucleated crystal was subsequently identified as PCM II by SC-XRD (Table S4; 2)

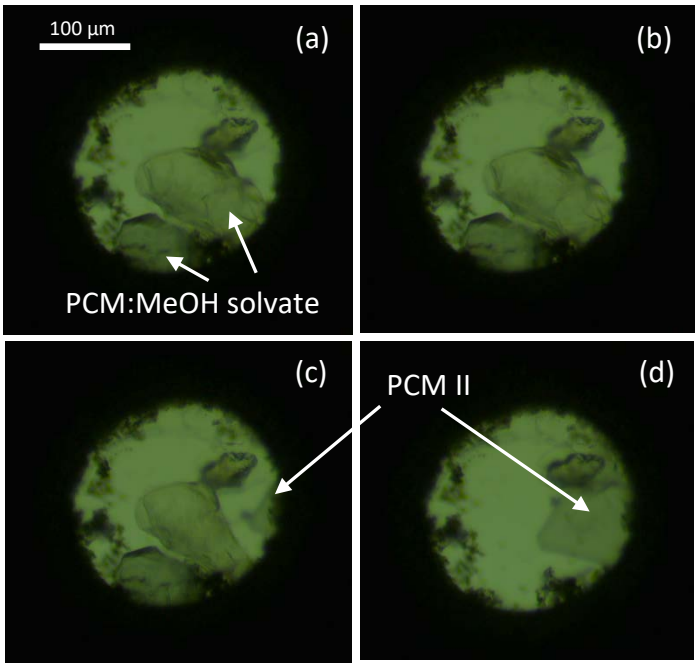

Figure S3. Still images taken from video monitoring of MeOH solvate in 0.2 g/g solution at ca. 0.21 GPa. The consecutive frames are recorded every 30 minutes and show the dissolution of PCM:MeOH and the nucleation and growth of a new crystal subsequently identified as PCM II by SC-XRD (compound 2). The scale bar in the image represents 100 µm.

S5.3 Solution mediated transformation (PCM II -> I)

Following a LVP antisolvent addition experiment, sample was taken immediately for monitoring using a Leica DM6000M microscope. Solids were not isolated from the supernatant but were left in the mother liquor to monitor for suspected solution mediated phase transformation. The sample was monitored over a 30 minute period with an image recorded every 60 seconds. The recorded images show dissolution of PCM II (needles) and simultaneous growth and nucleation of PCM I (blocks), Figure S4. An animated version of this timelapse, which show the transformation more clearly, are available in the ESI for this paper.

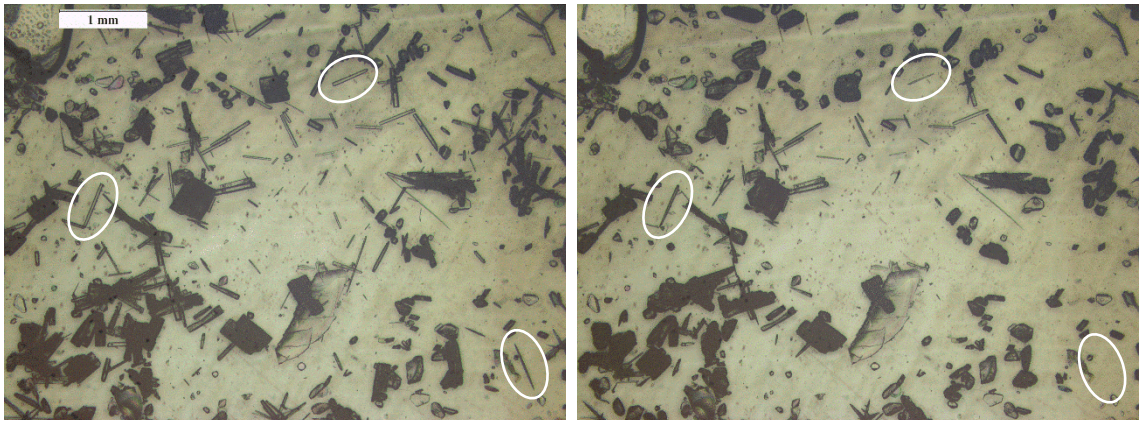

Figure S4. The first and last image recorded during a 30 minute monitoring period of the sample material obtained from LVP experiment. The particles were not isolated from the supernatant, but remain in contact with the solution phase. Images were recorded every 60 seconds and show the rapid dissolution of PCM II particles (rods) and growth of PCM I (blocks). The scale bar represents 1 mm. Dissolution of PCMII particles has been highlighted (white circle)

S6. X-ray powder diffraction

XRPD data was collected on samples isolated after LVP antisolvent addition experiment. The same sample was repeatedly collected 5 times over 3 hours (approx. 35 min/collection). The XRPD patterns obtained in this test are shown in Figure S5. The patterns show no change with time and indicate that after isolation from the supernatant, no further solid form change occurs (over a monitoring period on XRPD of ca 3 hours).

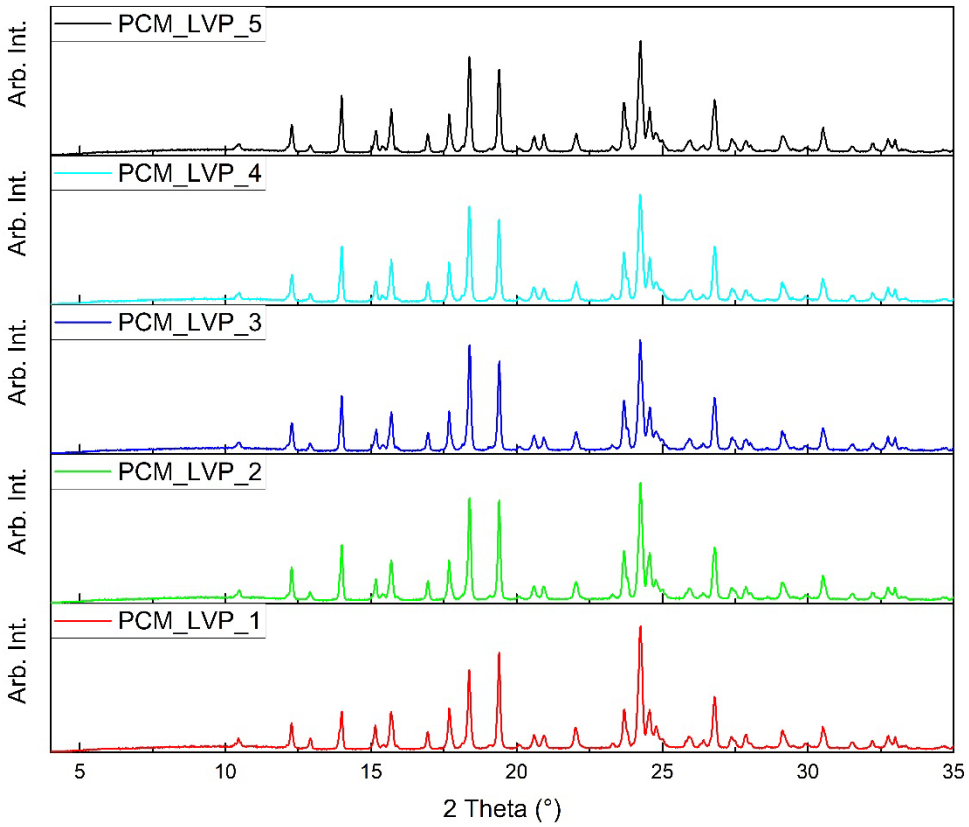

Figure S5. XRPD patterns obtained from the same sample after isolation from the supernatant following LVP experiment. Each pattern was recorded over a 35 minute period and no change in pattern is observed.

The same sample was then collected over a longer period (7 hours) to obtain a pattern with improved signal:background for Rietveld refinement. The XRPD pattern obtained is shown in Figure S6. This pattern is again unchanged from the pattern first obtained after isolation of the sample and therefore demonstrates that the isolated sample remains unchanged for at least 10 hours once removed from the supernatant.

With a view to investigating the role of solution mediated transformation of PCM II → PCM I, the LVP anti-solvent addition experiment was performed with 0.5 mL of perfluorinated oil (Perfluoropolyether fluid, Galden SV110, Solvay, Italy) in the sample tube. The aim of this was to establish if precipitated particles could be 'protected' from the solution phase by being trapped in or by being coated by the hydrophobic oil phase. Solids isolated from this test were tested by XRPD, the obtained pattern is shown in Figure S6.

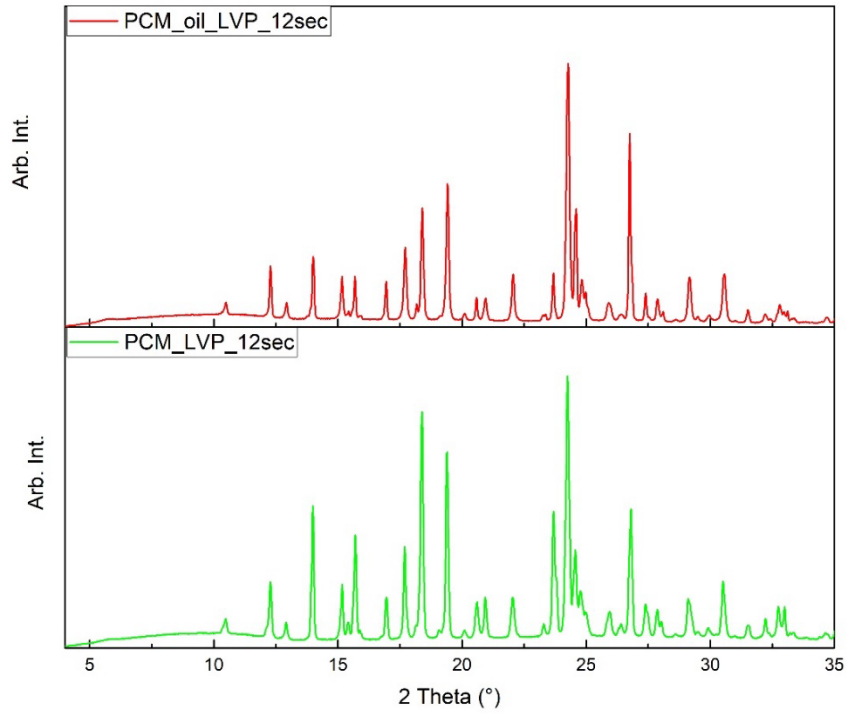

Figure S6. XRPD patterns obtained from samples produced by LVP experiment with (upper, red) and without (lower, green) inclusion of 500 µl perfluorinated oil. Subtle difference in the patterns indicate differences in the relative amounts of PCM I and II in the sample mixtures.

Analysis of the patterns by Rietveld refinement (performed using Topas 5.0 academic version[2]) show an increased proportion of PCM II in the test performed using oil. Reference structures for PCM I and II were retrieved from the CSD database[3] (HXACAN04[4] and HXACAN08[5]) and used for refinement. Plots showing the output of Rietveld refinement for samples obtained without and with oil in LVP experiment are shown in Figure S7 and Figure S8, respectively.

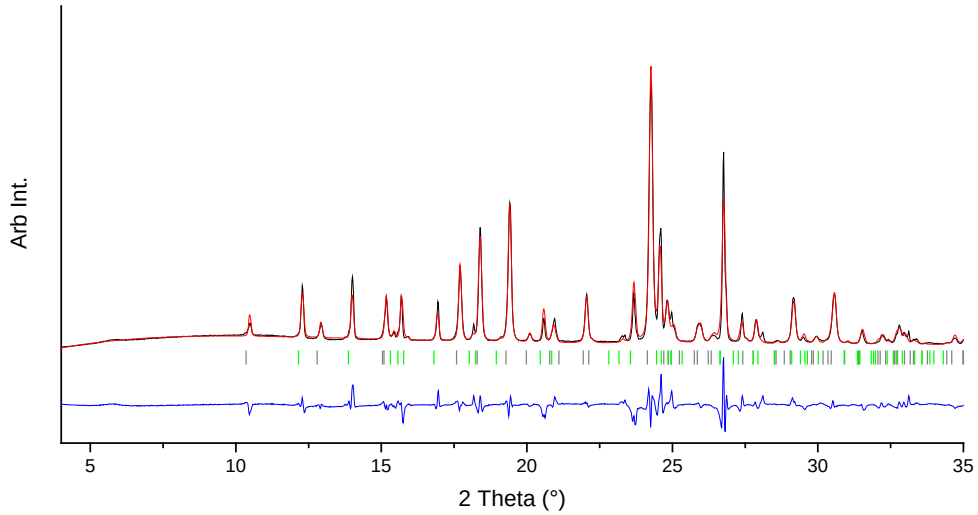

Figure S7. Results plot from Rietveld refinement of XRPD data obtained in LVP experiment without use of perfluorinated oil. Rietveld refinement indicates ca. 58 % content of PCM II in the sample mixture. Experimental data is represented by the black line, fitted data shown by the red line and the difference shown in blue. Green and grey tick marks represent reflections attributed to PCM forms I and II, respectively.

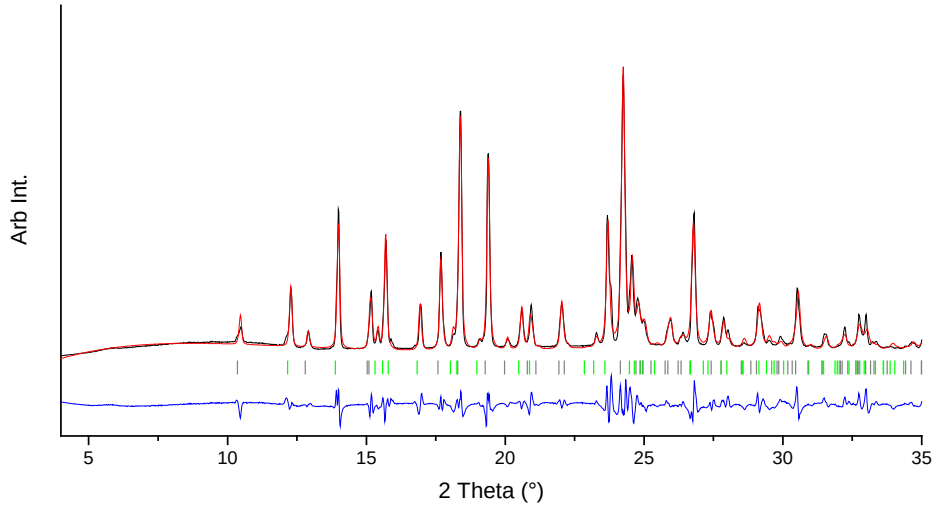

Figure S8. Results plot from Rietveld refinement of XRPD data obtained in LVP experiment without use of perfluorinated oil. Rietveld refinement indicates ca. 65 % content of PCM II in the sample mixture. Experimental data is represented by the black line, fitted data shown by the red line and the difference shown in blue. Green and grey tick marks represent reflections attributed to PCM forms I and II, respectively.

S7. Raman spectra, MeOH solvate

As part of characterization of the identified MeOH solvate of PCM, Raman spectrum was collected of the crystal obtained from 0.05 g/g solvent mixture (64:36 w/w, MeOH:H_2O). The recorded spectrum is shown in Figure S9.

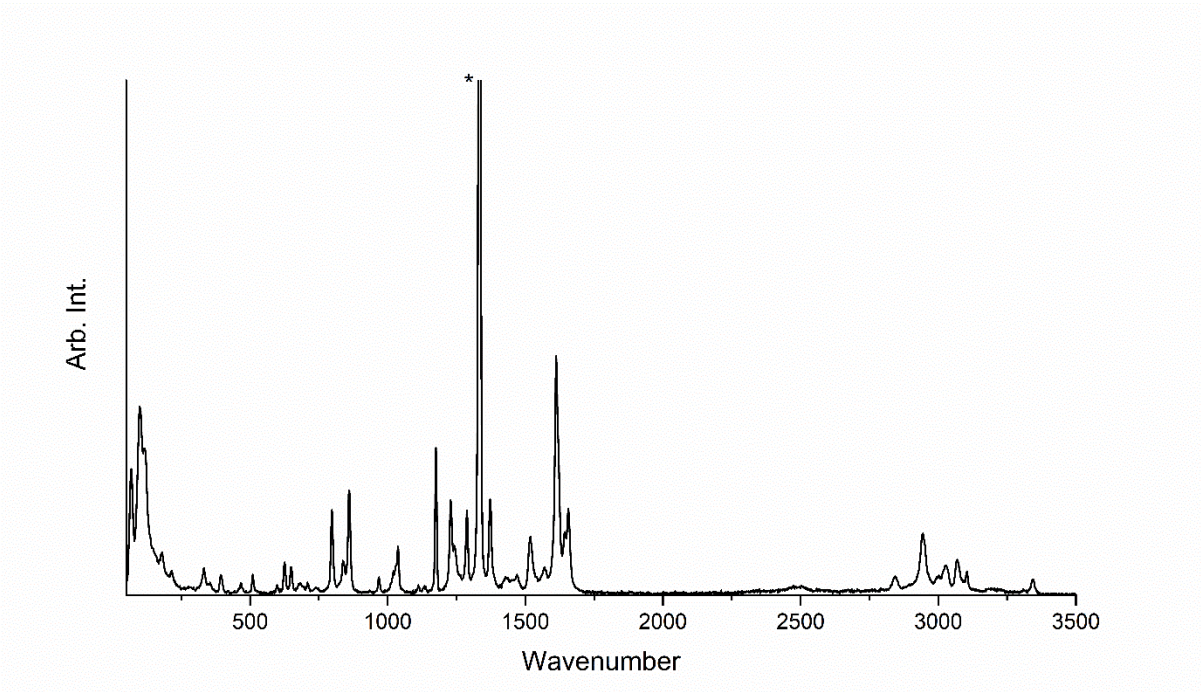

Figure S9. Raman spectrum obtained from MeOH solvate of PCM obtained from 0.05 g/g solvent mixture solution at 0.2 GPa in diamond anvil cell. Peak at approx. 1330 cm^{-1}, marked with asterisk, attributed to diamond.

References:

1 G. J. Piermarini, S. Block, J. D. Barnett and R. A. Forman, *J. Appl. Phys.*, 1975, **46**, 2774–2780.

2 A. Coelho, *TOPAS – Acad. Gen. Profile Struct. Anal. Softw. Powder Diffr. Data*, 2012.

3 C. R. Groom, I. J. Bruno, M. P. Lightfoot and S. C. Ward, *Acta Crystallogr. Sect. B*, 2016, **72**, 171–179.

4 D. Y. Naumov, M. A. Vasilchenko, J. A. K. Howard and IUCr, *Acta Crystallogr. Sect. C Cryst. Struct. Commun.*, 1998, **54**, 653–655.

5 G. Nichols and C. S. Frampton, *J. Pharm. Sci.*, 1998, **87**, 684–693.