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Electronic supplementary information for

# Solute clustering in undersaturated solutions – systematic dependence on time, temperature and concentration

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## 1. Full Experimental Details

### 1.1 Small-Angle X-Ray Scattering and Nanoparticle Tracking

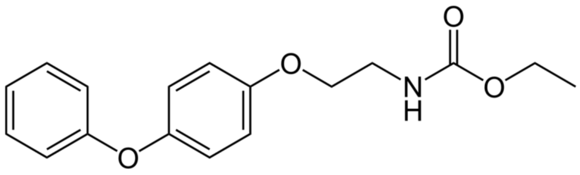


**Fig. S1.1.** Fenoxycarb chemical structure.

Fenoxycarb (Fig. S1.1) solutions with a concentration corresponding to a saturation temperature of 20.0 °C (mole fraction concentration $x = 0.050$, equalling 0.266 g/g solvent[1]) were used. The solutions were filtered at 40.0 °C in order to prevent nucleation during filtration, into capped glass vials equipped with PTFE coated magnetic stir bars. Pre-heated syringe filters (PTFE, 0.1 µm) were used for filtration in order to remove any dust particles and undissolved impurities.

For SAXS, two vials of the microfiltered solutions were brought to crystallise through quench cooling. The vials were then placed in a water bath at 40.0 °C for 15 min under agitation, leading to visibly complete dissolution of all crystals. One vial was then kept at room temperature (measured at 22.0 °C, corresponding to weakly undersaturated conditions) while the other was placed in a water bath at 55.0 °C. After 8 h, both solutions were scanned with SAXS, with bracketing scans of pure solvent. All scans were conducted at a controlled solution temperature of 20.0 °C. Prior to SAXS analysis of the vial kept at 55.0 °C, the solution was allowed 10 min to cool down to room temperature. The SAXS experiments were conducted at the I22 beamline of the Diamond Light Source, UK. A stationary brass liquid cell was used, fitted with mica windows held by PTFE spacers (thickness 2.0 mm). Temperature control was achieved by means of an external thermostatic water bath circulating water through the brass cell, and a nitrogen jet stream was used to prevent condensation at the mica window. Each sample was irradiated by monochromatic 12.4 keV X-rays during 60 repeat scans of 10 s, and scattering data was registered by a Pilatus 2M CMOS detector at a distance of 8.4 m from the sample. Data was processed and averaged using the software Dawn v. 1.4.[2] Scattering data was normalised by the total transmitted beam intensity, and the spectrum of pure isopropanol was subtracted from the solution spectra.

For NTA, two microfiltered solutions in capped vials were brought to crystallise through quench cooling. One of the suspension vials was placed in a thermostatic water bath at 25.0 °C for 10 min under agitation, leading to a visibly complete dissolution of all crystals. A sample of the clear solution was then scanned and analysed with NTA. The solution vial was then moved to a water bath at the saturation temperature, 20.0 °C. The other suspension vial was placed in a water bath at 40.0 °C, under agitation. After 22 h, the two solutions, both visibly clear, were sampled and analysed with NTA. A sample of pure isopropanol, filtered through a pre-washed 0.1 µm PTFE filter, was analysed for comparison. The NTA measurements were done using a Malvern Nanosight LM14 instrument with temperature control and a CMOS camera, at a temperature of 25.0 °C (±0.3 °C) with a laser wavelength of 635 nm. Ten repeat videos of 60 s each were recorded, with a random refocusing step in between each file. Results were collected, processed and averaged using the software NTA v. 3.0.

## 1.2 Dynamic Light Scattering and Infrared Spectroscopy

Fenoxycarb crystals were obtained by recrystallizing twice from saturation at 35.0 °C to 5 °C in isopropanol solution. The crystals were dissolved in pure isopropanol at various mole fraction concentrations ($x$) from 0.006 to 0.132 in 30 mL capped glass vials agitated with magnetic stir bars. One solution with a concentration corresponding to saturation at 25.0 °C ($x = 0.110$)[1] was prepared, and one solution with a concentration corresponding to supersaturation at 25.0 °C, with $x = 0.132$. The concentrations of the remaining solutions were all in the undersaturated range. The solvent was filtered through 0.2 µm PTFE syringe filters before solution preparation. The solids were dissolved in the vials submerged in a thermostatic bath at 25.0 °C, expect for the saturated and supersaturated solutions which were kept at 28.0 °C during dissolution. All solutions were agitated at a stirring rate of 400 rpm. The visibly clear solutions were then filtered (filtration-1) into 3 sets (I-III) of equal vials (26 x 3) using pre-heated glass syringes fitted with 0.1 µm PTFE filters, at 25.0 °C, except for saturated and supersaturated solutions which were filtered at 28.0 °C.

One set of vials (set I) was analysed directly with DLS at 25.0 °C. Another set (set II) was transferred to a thermostatic bath at 40.0 °C and kept at this temperature for 22 h prior to analysis with DLS at 25.0 °C. The third set (set III) was used to determine the concentration, and the viscosity and the refractive index of the solutions at 25.0 °C. The concentration was measured gravimetrically according to a procedure described elsewhere.[3] The viscosity was measured using a Brookfield DV3T rheometer, equipped with a water-jacketed small volume cell, a water bath and a thermocouple for temperature control. The refractive index was measured using an Abbe refractometer. Necessary care was taken during these measurements for the saturated and supersaturated solutions at 25.0 °C. Several pre-trials were made to verify that there would be no issue of rapid crystallization of the supersaturated solution at this temperature during measurements.

A 1500 µL sample was extracted from each solution vial and transferred to a 12 mm square glass cell with a path length of 10 mm. All measurements were carried out using a Malvern Zetasizer ZSP Nano instrument, equipped with a temperature controller. The mean solvodynamic diameter was estimated using the cumulants method.[4] The refractive indices of the crystalline solid and of the solution at the actual solute concentration were used in the calculations. Measurements were made at a forward scattering angle of $\theta = 12.8°$ using laser light of wavelength $\lambda = 632.8$ nm, with automatic cell positioning, an automatic attenuator, at a controlled temperature of 25.0 °C. All the solutions irrespective of pre-treatment and concentration were allowed to equilibrate for 120 seconds at 25.0 °C inside the DLS cell before commencement of the measurements. Each measurement consisted of 15 scans. For each sample, 14 consecutive measurements were made over a period of 72 hours. Intensity size distributions were obtained from the autocorrelation function using multiple narrow modes. Data analysis was carried out using the Malvern Zetasizer software v. 7.11.

Fourier-transform infrared (FTIR) spectra were recorded for all solutions (irrespective of pre-treatment temperature) immediately after the first DLS measurement following filtration-1 (at 0 h) and then again following the DLS measurement after 24 h. A Mettler Toledo ReactIR 10 fitted with a silver halide *in situ* probe composed of diamond composite and an MCT (mercury cadmium telluride) detector cooled with liquid nitrogen was used. The probe was inserted into a temperature-controlled glass sample vial containing solution, which was then sealed with a cap. The spectrum of the pure solvent was subtracted from that of the solution. The IR spectrum of solid fenoxycarb was recorded and used as a reference. In total, 128 scans were collected for each spectrum, with a resolution of 4 cm$^{-1}$ in the spectral region 2000-650 cm$^{-1}$ using the iC IR software v. 4.3

Immediately following the DLS measurement after 72 h, all the set I solutions were then again filtered through pre-wetted 0.1 µm PTFE syringe filters (filtration-2) In conjunction with this, the concentrations of the solutions after filtration were measured gravimetrically.[3] A 100 µL sample of each solution was collected and analysed before filtration, and another sample of 100 µL immediately after filtration. The solutions were then again repeatedly analysed with DLS at 25.0 °C over a period of 3 h. The performance of the 0.1 µm filters was evaluated using commercial NIST traceable polystyrene latex standard particles with diameters of 100 and 200 nm, as previously done by others.[5] The tested filter (taken from the same lot as used in the filtration of our solutions) entirely retained the 200 nm as well as the 100 nm latex particles. The experimental protocol used for the 15 concentrations is described in Fig. S1.2. In order to ensure sufficient statistical confidence, the entire protocol was repeated 5 times, and for a select set of solutions the process of a second filtration step with an ensuing concentration measurement was repeated an additional 5 times.

### 1.3 Molecular Dynamics Simulations

To build the model, 71 fenoxycarb and 1207 isopropanol molecules were placed randomly in a periodic cubic box with a side length of approx. 60 Å, and the system was relaxed using 1000 steps of steepest descent minimization. Subsequently, the simulation cell was pre-equilibrated for 1 ns at 25.0 °C and then equilibrated for 50 ns at 25 °C. The simulations were performed within the NAMD software,[6] using Langevin dynamics in the NPT ensemble, and with Ewald summation used for calculation of electrostatic interactions. The system was parametrized using the Charmm General Force Field (CGenFF)[7] v. 2b7. The FC molecule was built by bridging with an oxygen atom two residues available in the CGenFF parameters and topology files, *i.e.* a biphenyl (C37) and diethylcarbamate (DECB).

## 2. Supplementary results

### 2.1 SAXS and NTA results

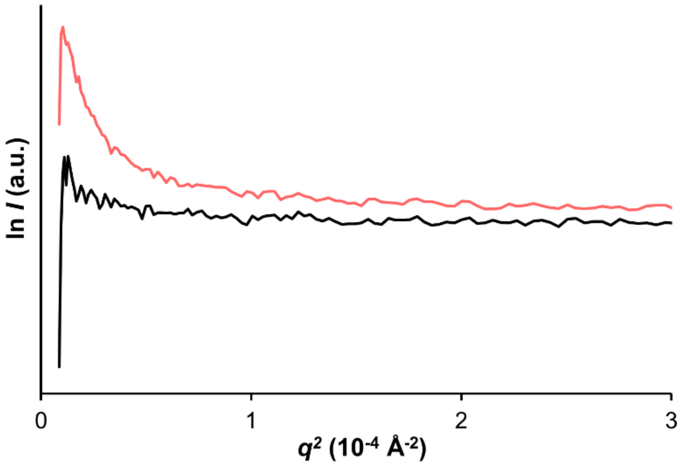


**Fig. S2.1.** SAXS Guinier plot of solutions with different pre-treatment temperature after dissolution: 22°C (red) and 55°C (black).

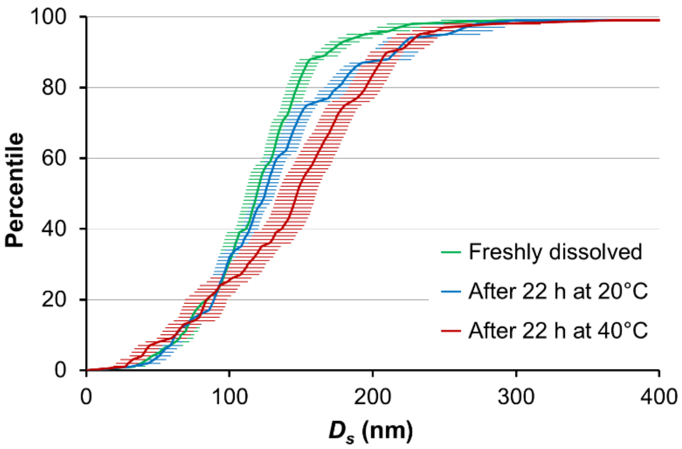


**Fig. S2.2.** NTA cumulative size distributions vs solvodynamic diameter of solutions freshly dissolved crystals (green) and after 22 h of pre-treatment at 20°C (blue) and 40°C (red).

## 2.2 DLS results for 15 concentrations

*For solutions in set I*

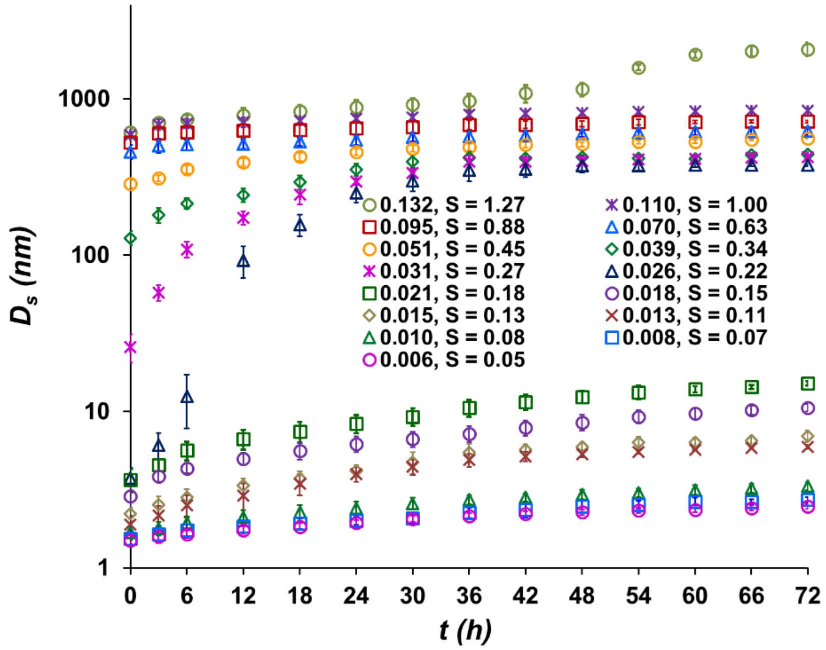


**Fig. S2.3.** DLS mean solvodynamic diameter of FC clusters *vs*. residence time at 15 different concentrations for solutions pre-treated at 25 °C. Error bars show standard errors over 5 repetitions.

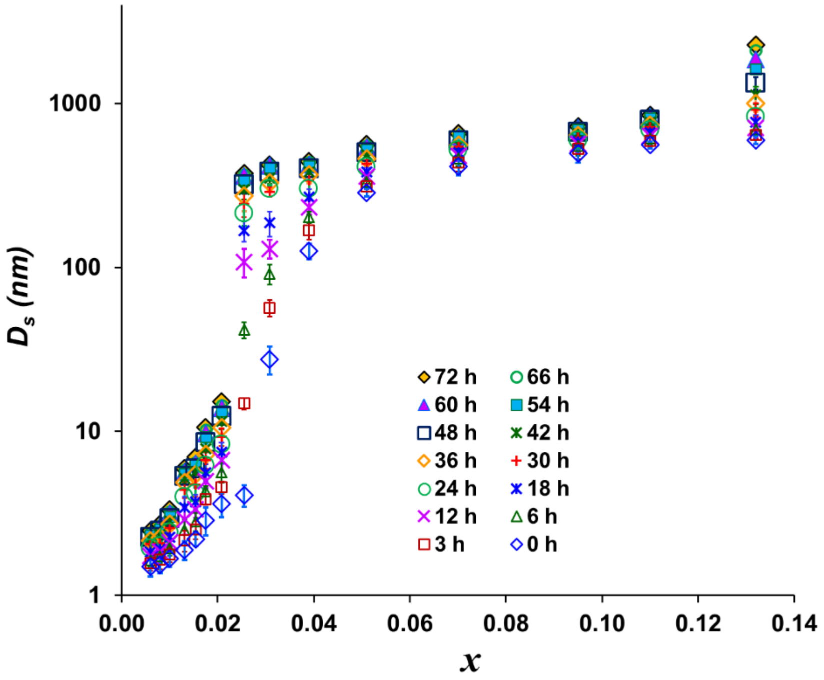


**Fig. S2.4.** DLS mean solvodynamic diameter of FC clusters *vs.* concentration for different residence time for solutions pre-treated at 25 °C. Error bars show standard errors over 5 repetitions.

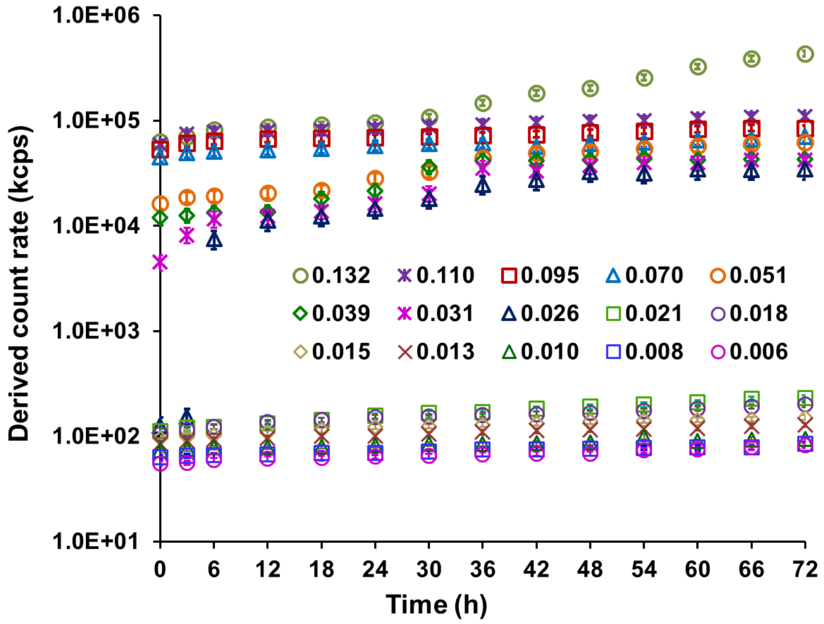


**Fig. S2.5.** Derived count rates observed at different residence time for solutions of 15 different concentrations pre-treated at 25 °C. Error bars show standard errors over 5 repetitions.

*For solutions in set II*

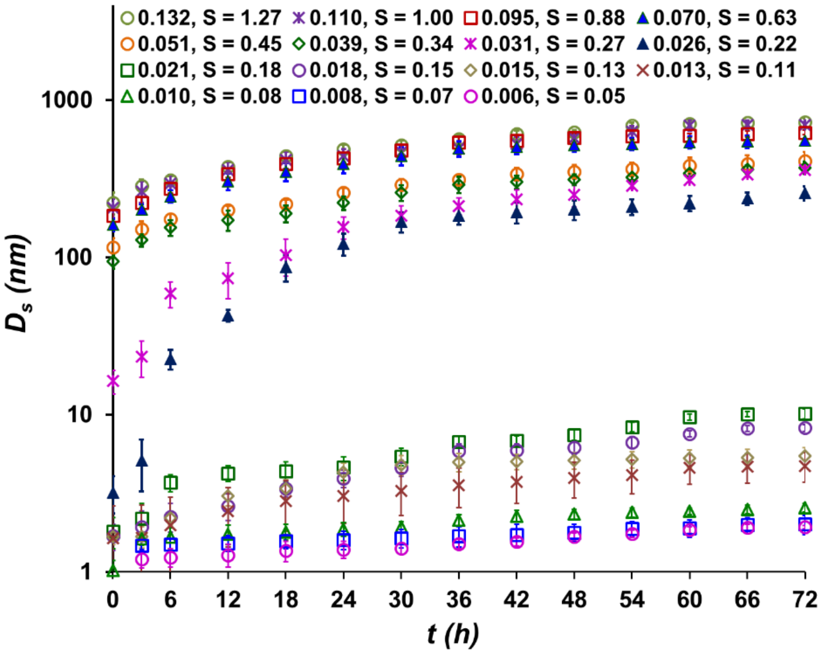


**Fig. S2.6.** DLS mean solvodynamic diameter of FC clusters *vs*. residence time at 15 different concentrations for solutions pre-treated at 40 °C. Error bars show standard errors over 5 repetitions.

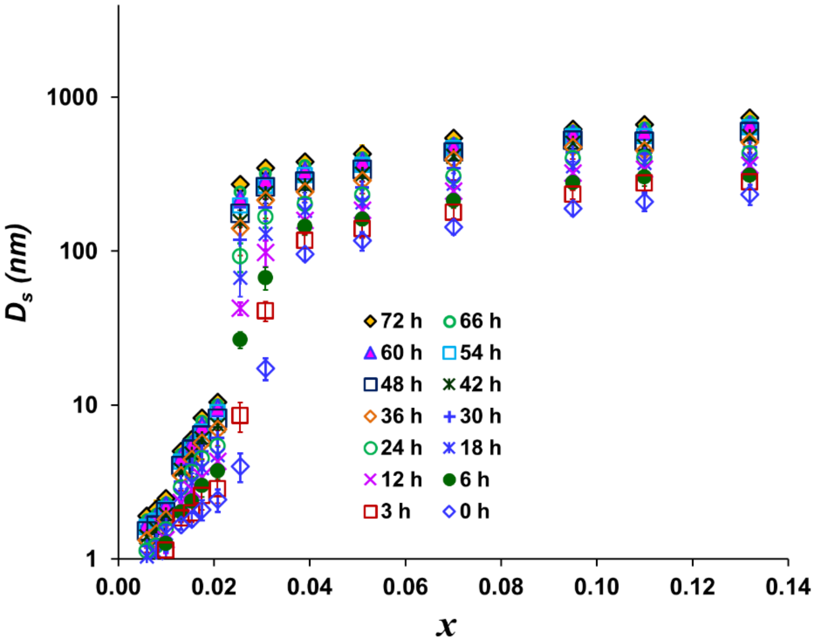


**Fig. S2.7.** DLS mean solvodynamic diameter of FC clusters *vs.* concentration for different residence time for solutions pre-treated at 40 °C. Error bars show standard errors over 5 repetitions.

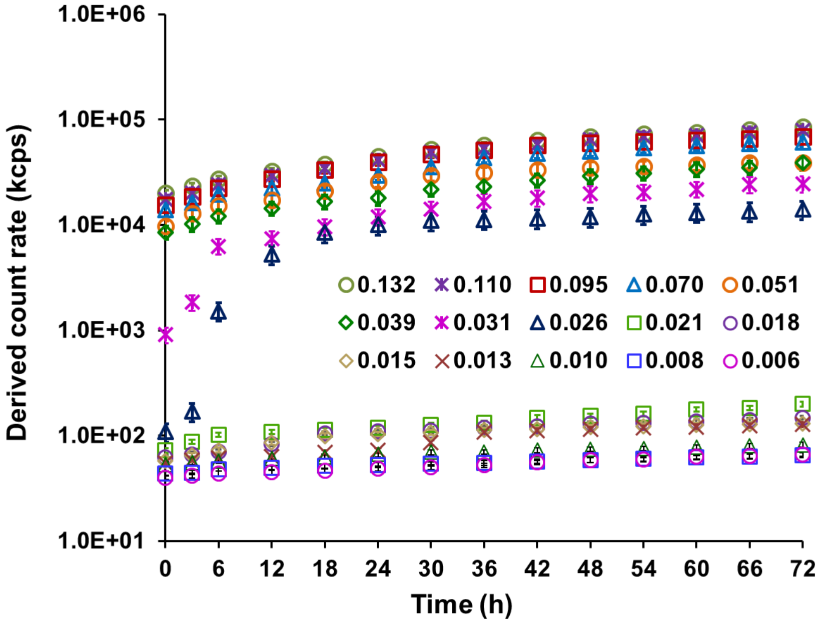


**Fig. S2.8** Derived count rates observed at different residence time for solutions of 15 different concentrations pre-treated at 40 °C. Error bars show standard errors over 5 repetitions.

## 2.3 Pre- vs. post-*filtration-2* results for 15 different concentrations

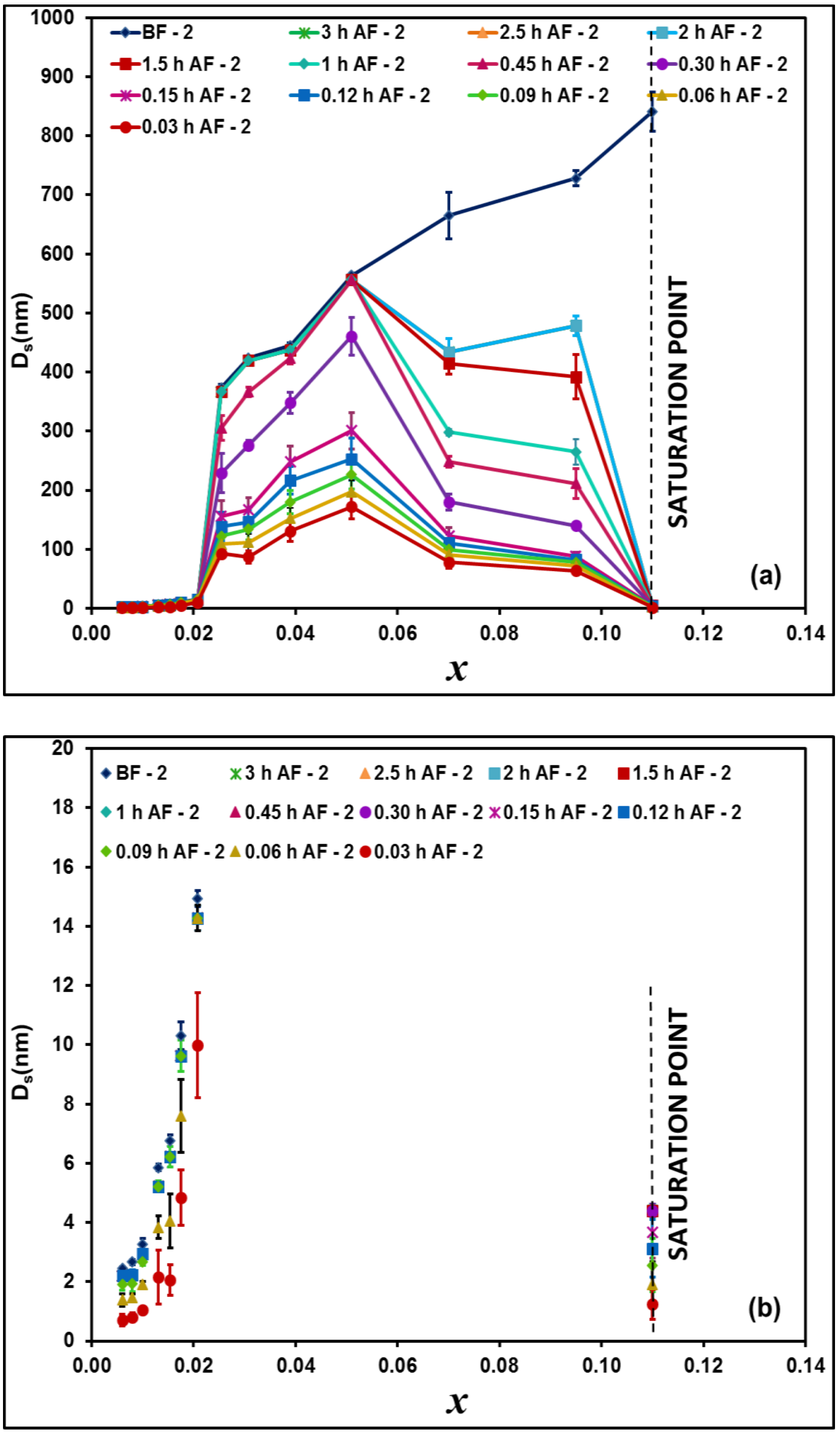


**Fig. S2.9.** (a) DLS mean solvodynamic diameter of fenoxycarb clusters in isopropanol solutions of 15 different concentrations after 3 h and 72 h during growth before filtration-2, and after different times following filtration-2, and (b) magnified view of the cluster sizes at lower concentrations. Error bars show standard errors over 5 repetitions.

## 2.4 IR spectra for solutions of 15 different concentrations

*For solutions in set I*

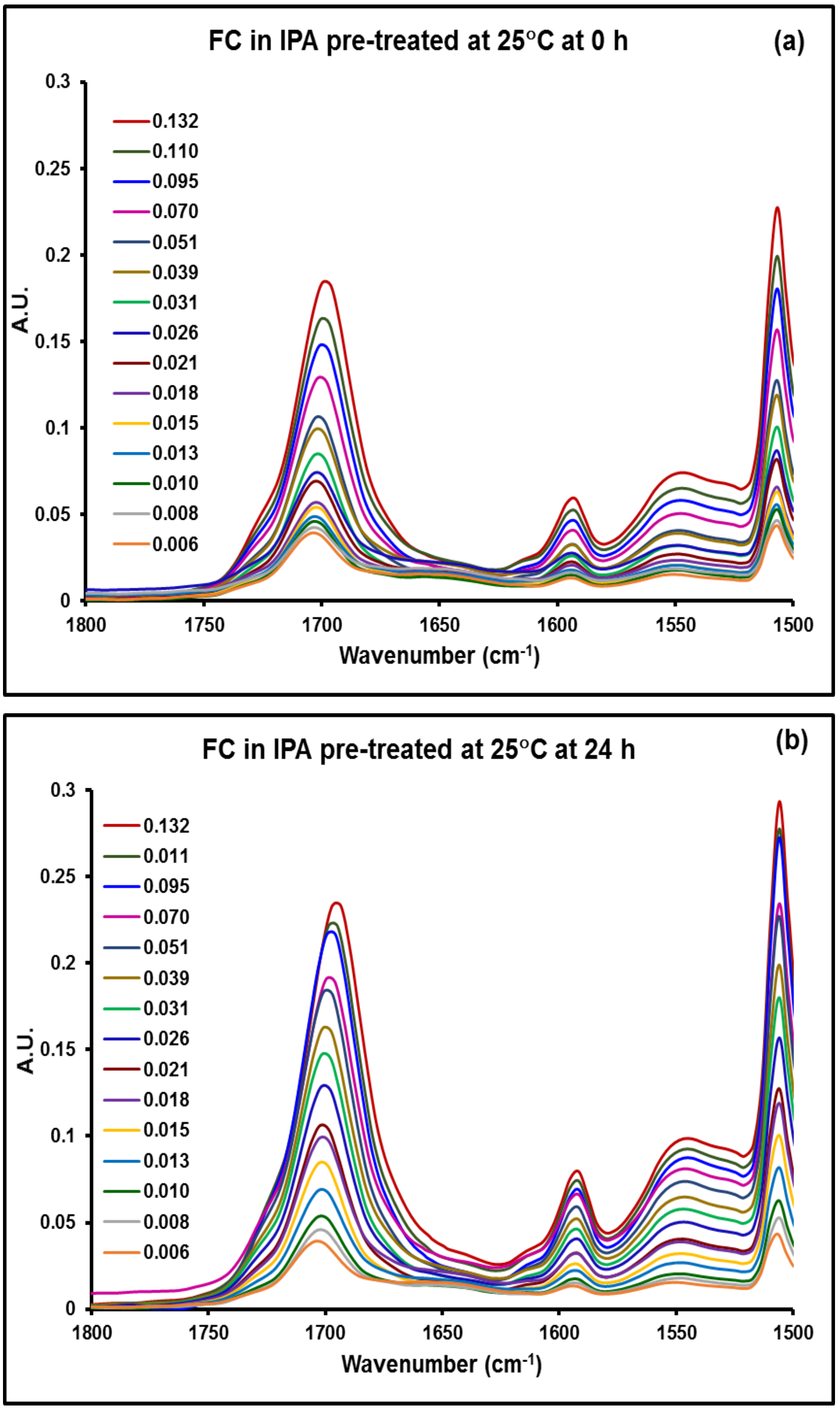
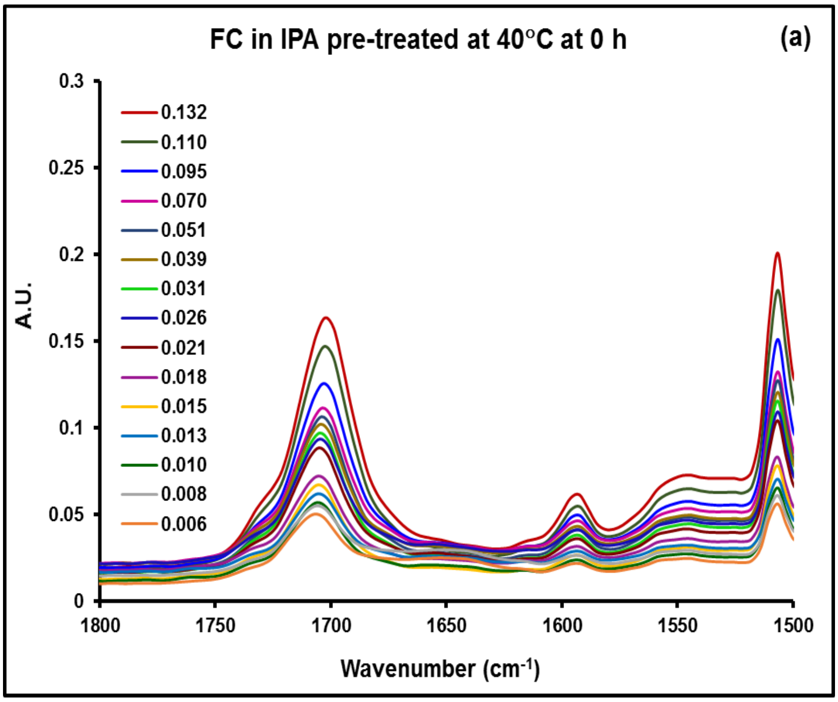


**Fig. S2.10.** IR spectra of fenoxycarb solutions of 15 different concentration pre-treated at 25 °C after (a) 0 h, and (b) 24 h.

*For solutions in set II*



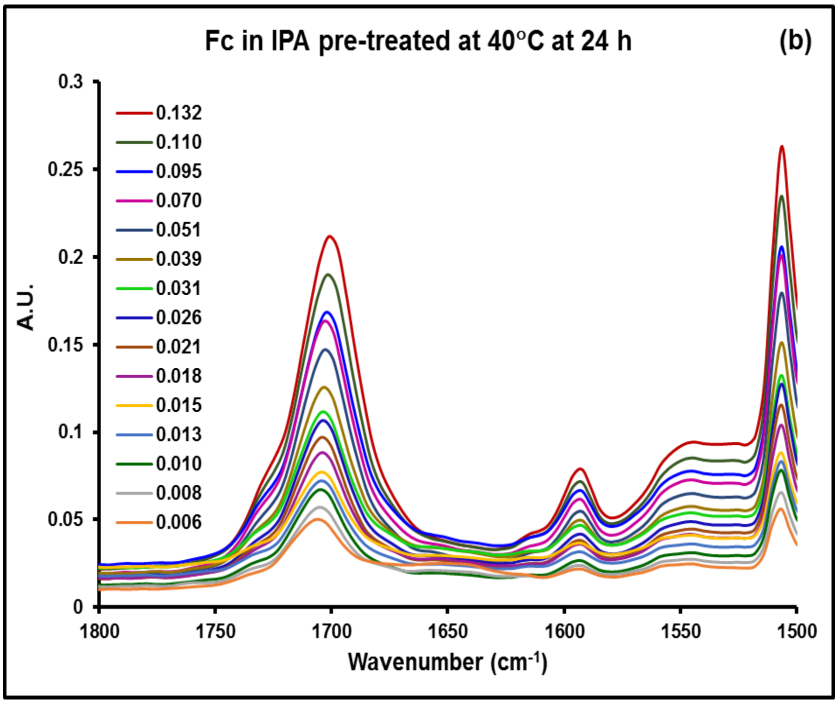


**Fig. S2.11.** IR spectra of fenoxycarb solutions of 15 different concentration pre-treated at 40 °C after (a) 0 h, and (b) 24 h.

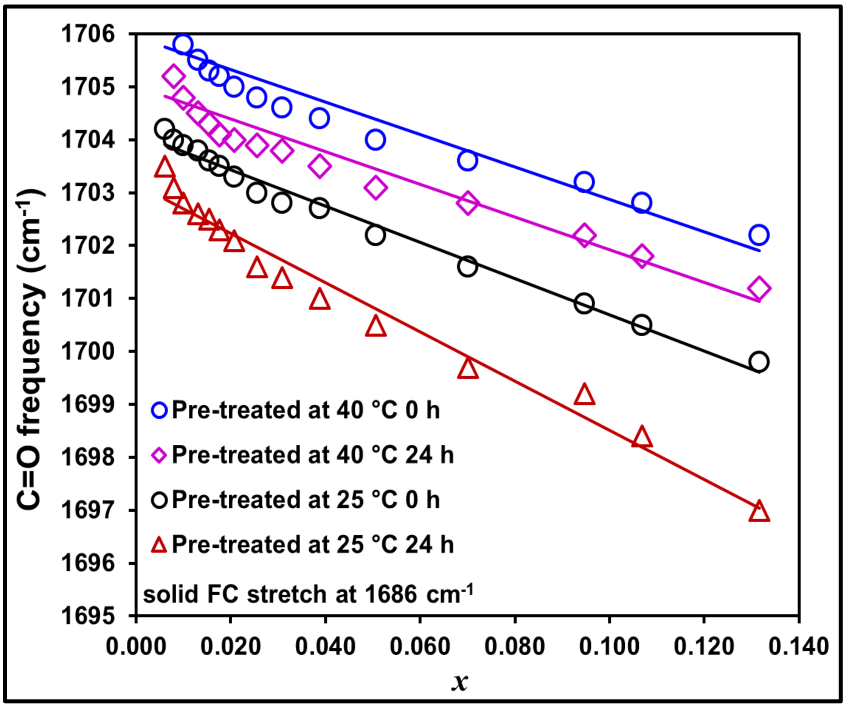


**Fig. S2.12.** ν(C=O) peak shift in solutions pre-treated at 25 °C and 40 °C with increasing solute concentrations, after 0 h and 24, together with linear fits.

# 3. Other supplementary data

**Table S3.1.** Mole fraction concentrations ($x$) of fenoxycarb in isopropanol solutions after preparation, after filtration-1 and after filtration-2, respectively, measured gravimetrically based on averaging samples from $N$ solutions, given together with standard errors. The final column lists the percentage decrease in $x$ after filtration-2 compared to after filtration-1.

| **Nominal** | **Before filtration-1** | | **After filtration-1** | | **After filtration-2** | | |
|---|---|---|---|---|---|---|---|
| | **mean [*N*]** | **$10^3 \times$ SE** | **mean [*N*]** | **$10^3 \times$ SE** | **mean [*N*]** | **$10^3 \times$ SE** | **decrease [%]** |
| 0.0060 | 0.0062 [5] | 0.0074 | 0.0062 [5] | 0.0032 | 0.0061 [10] | 0.0101 | 1.02 |
| 0.0080 | 0.0080 [5] | 0.0013 | 0.0079 [5] | 0.0082 | 0.0079 [5] | 0.0169 | 0.94 |
| 0.0100 | 0.0101 [5] | 0.0014 | 0.0101 [5] | 0.0058 | 0.0100 [5] | 0.0272 | 1.01 |
| 0.0131 | 0.0133 [5] | 0.0024 | 0.0131 [5] | 0.0580 | 0.0131 [5] | 0.0625 | 1.29 |
| 0.0154 | 0.0155 [5] | 0.0054 | 0.0155 [5] | 0.0217 | 0.0153 [10] | 0.0270 | 1.34 |
| 0.0175 | 0.0177 [5] | 0.0016 | 0.0176 [5] | 0.0292 | 0.0175 [5] | 0.0268 | 1.07 |
| 0.0208 | 0.0208 [5] | 0.0020 | 0.0207 [5] | 0.0407 | 0.0204 [5] | 0.1412 | 1.92 |
| 0.0255 | 0.0255 [5] | 0.0028 | 0.0254 [5] | 0.0056 | 0.0250 [5] | 0.1001 | 1.76 |
| 0.0308 | 0.0308 [5] | 0.0016 | 0.0307 [5] | 0.0353 | 0.0301 [10] | 0.0495 | 2.22 |
| 0.0390 | 0.0389 [5] | 0.0019 | 0.0389 [5] | 0.0117 | 0.0374 [5] | 0.2984 | 3.99 |
| 0.0510 | 0.0505 [5] | 0.0080 | 0.0504 [5] | 0.0190 | 0.0466 [10] | 0.4081 | 7.92 |
| 0.0701 | 0.0702 [5] | 0.0021 | 0.0701 [5] | 0.0146 | 0.0467 [5] | 0.6162 | 33.48 |
| 0.0950 | 0.0948 [5] | 0.0039 | 0.0946 [5] | 0.0281 | 0.0577 [10] | 0.8679 | 39.28 |
| 0.1100 | 0.1069 [5] | 0.0018 | 0.1067 [5] | 0.0475 | 0.0194 [10] | 4.2112 | 82.34 |
| 0.1320 | 0.1316 [5] | 0.0022 | 0.1314 [5] | 0.0528 | | | |

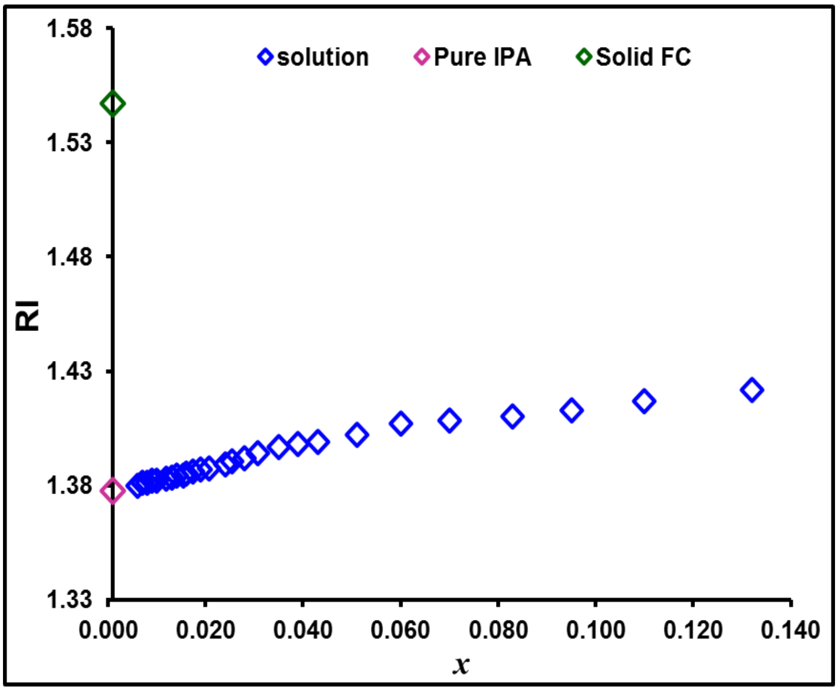


**Fig. S3.1.** Refractive index of fenoxycarb solutions of different solute mole fraction concentration.

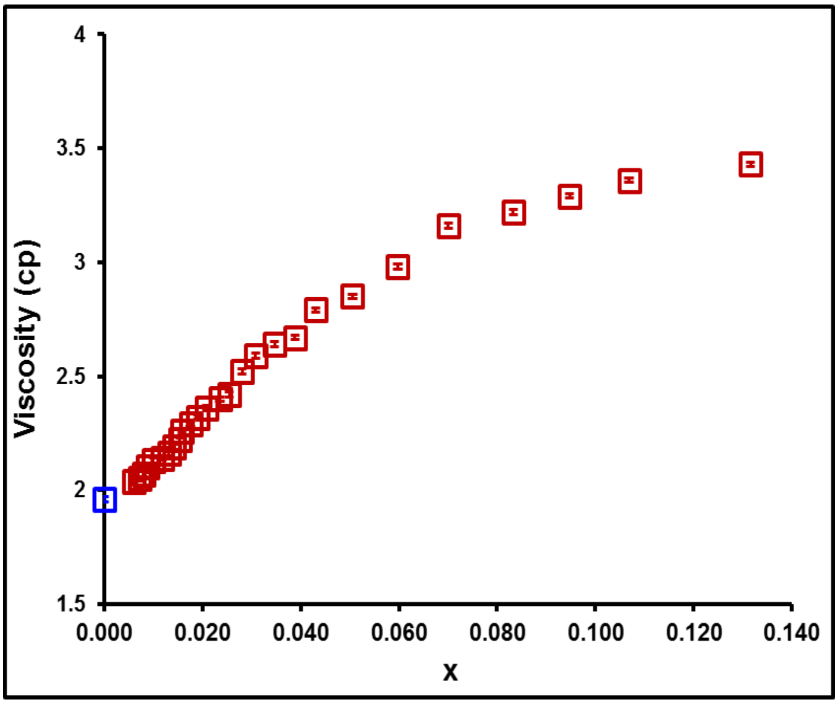


**Fig. S3.2.** Viscosity of fenoxycarb solutions of different solute mole fraction concentration.

## Supporting Information References

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