

Controlling Aqueous Silica Nanoparticle Synthesis in the 10-100 nm range

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Supporting information

	Relative Polarity (E_T^N)†	Solv. solubility in water at 25°C (wt%)	Water solubility in Solv. at 25°C (wt%)	Solv. solubility in water at 70°C (wt%)	Water solubility in Solv. at 25°C (wt%)	b.p.	Reference*
Ethanol	0.654	Miscible	Miscible	Miscible	Miscible	78.4°C	-
i-Propanol	0.546	Miscible	Miscible	Miscible	Miscible	82.6°C	(Barton 2013)
1-Butanol	0.586	7.4	19.7	6.7	25	117.4°C	(Barton 2013)
1-Hexanol	0.559	0.6	7	0.56	8.8	156°C	(Barton 2013)
Cyclohexane	0.06	0.006	0.008	0.0027	0.032	80.7°	(Maczynski, Shaw et al. 2005)

Table S1 Solvent Properties

† E_T^N = obtained from (normalised values with water =1) "Solvents and Solvent Effects in Organic Chemistry". Third Edition . Christian Reichardt. Appendix, Table A1, page 472-474

* The reference relates to solubility values.

References:

Barton, A. F. (2013). Alcohols with Water: Solubility Data Series, Elsevier

Maczynski, A., et al. (2005). "IUPAC-NIST solubility data series. 81. Hydrocarbons with water and seawater—revised and updated. Part 3. C₆H₈–C₆H₁₂ hydrocarbons with water and heavy water." Journal of physical and chemical reference data 34(2): 657-708.

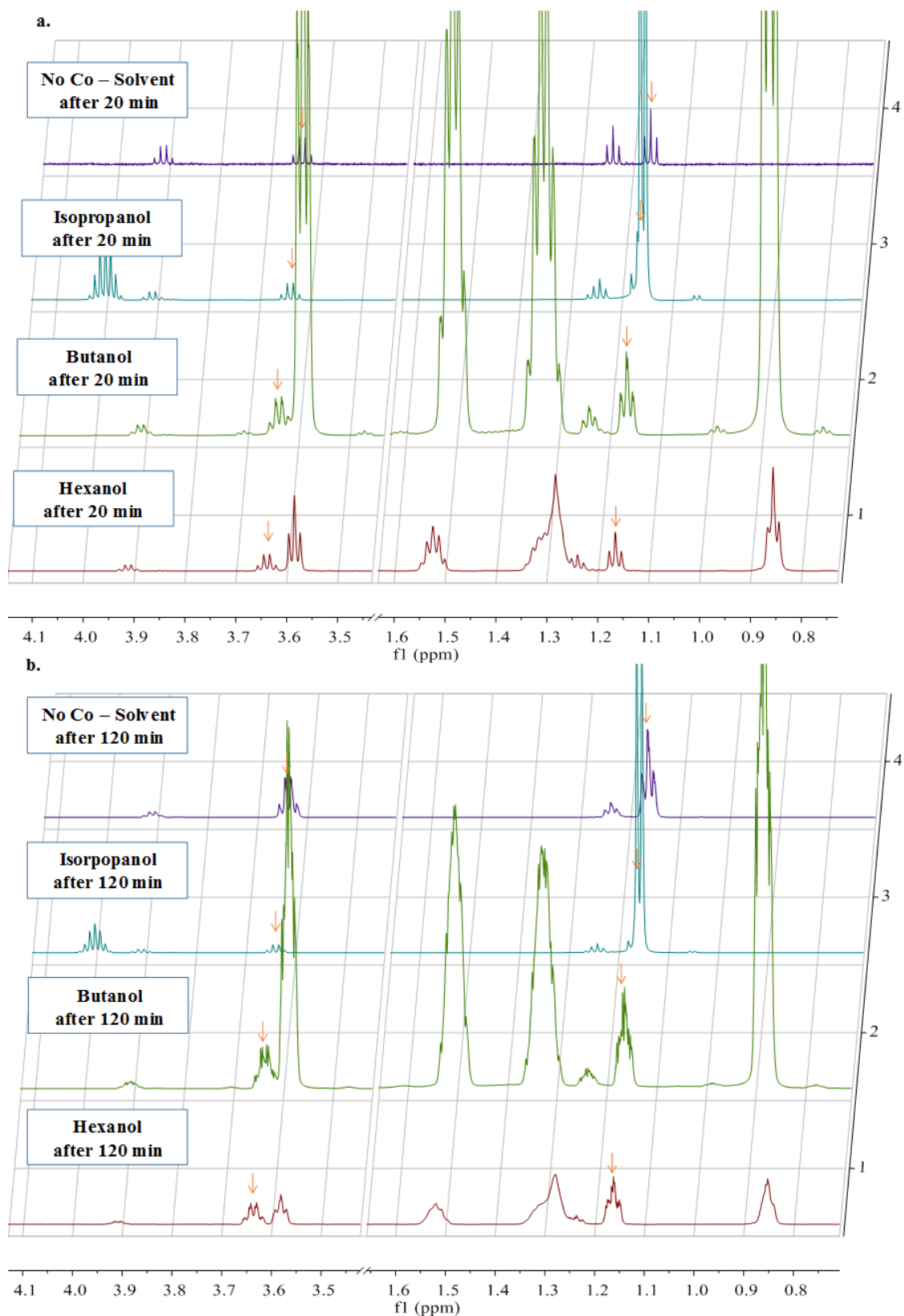


Figure S1 ^1H NMR spectra of silica synthesis solution at (a) 30 and (b) 150 minutes for TEOS only (purple), isopropanol (blue), butanol (green) and hexanol (pink) as co-solvent. The peaks at 1.17 and 3.65, indicated with an arrow are compared to an internal standard.

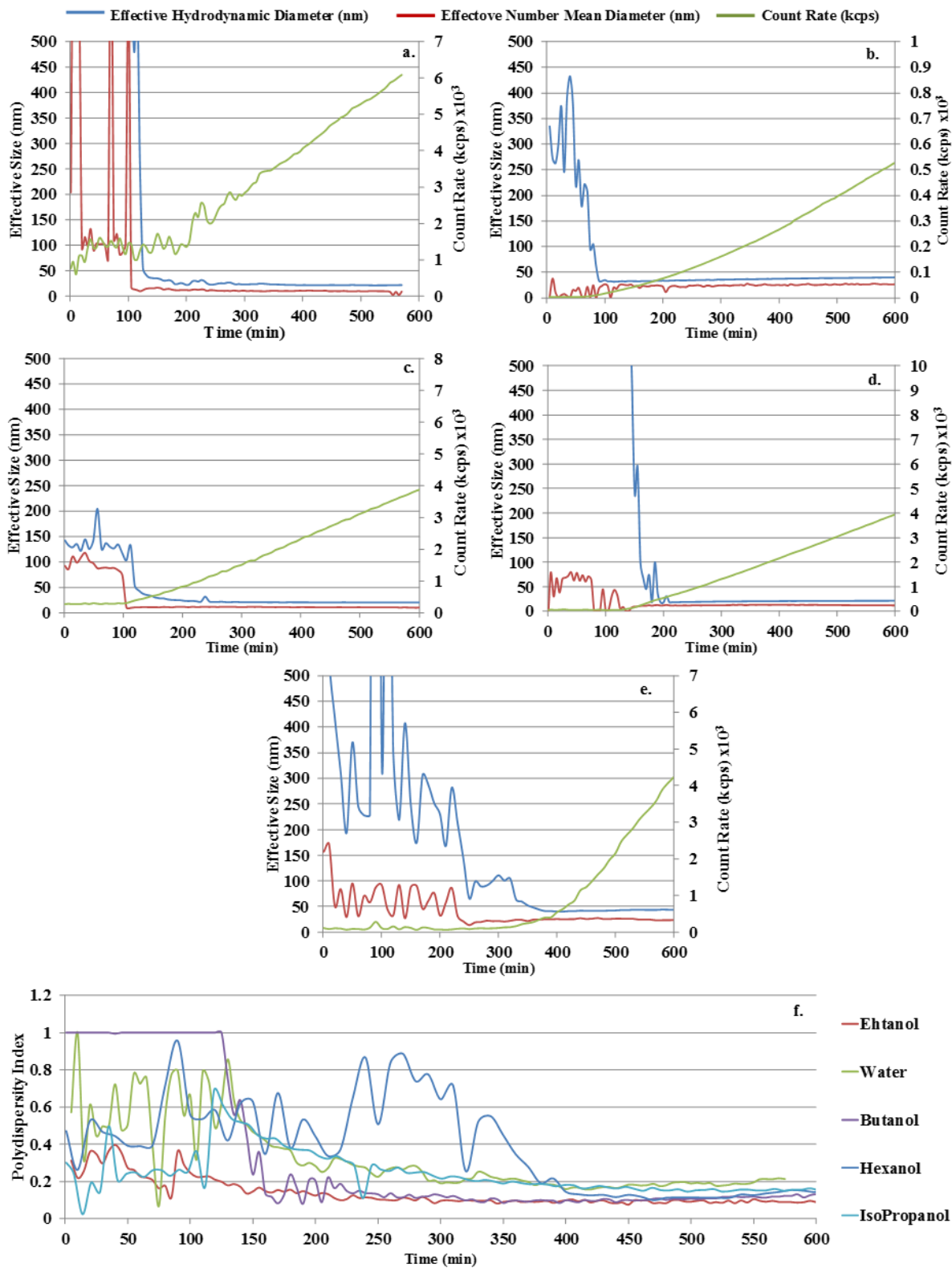


Figure S2 Dynamic light scattering monitoring, shown in a – d as effective hydrodynamic diameter(blue), effective number mean size (DLS) (red) and count rate (DLS) (green) of the synthesis of two phase particles in the (a) TEOS only, (b) ethanol, (c) isopropanol, (d) butanol and (e) hexanol co - solvent. (f) The progression of the polydispersity index of the measurements are presented.

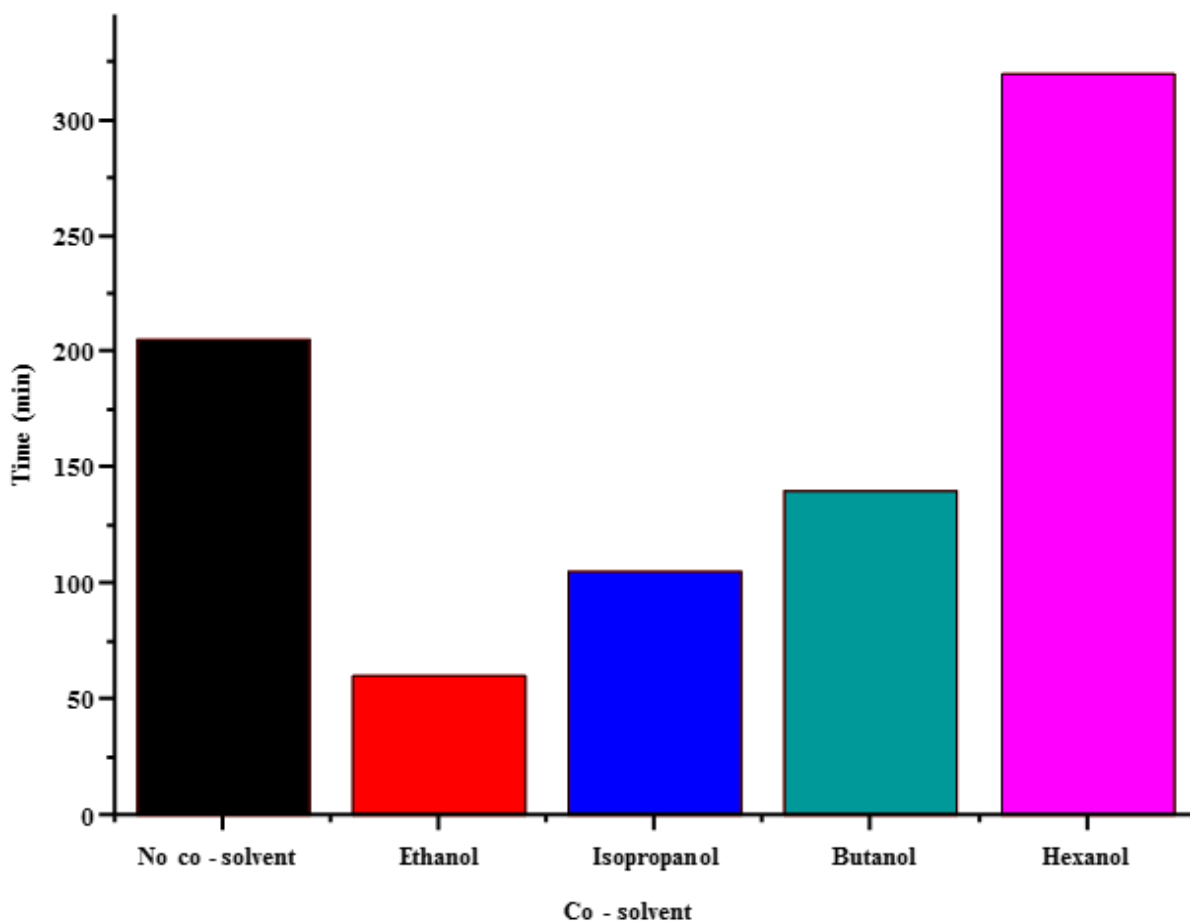


Figure S3 Nucleation times (in min) of particles determined by dynamic light scattering monitoring for different co-solvent systems.

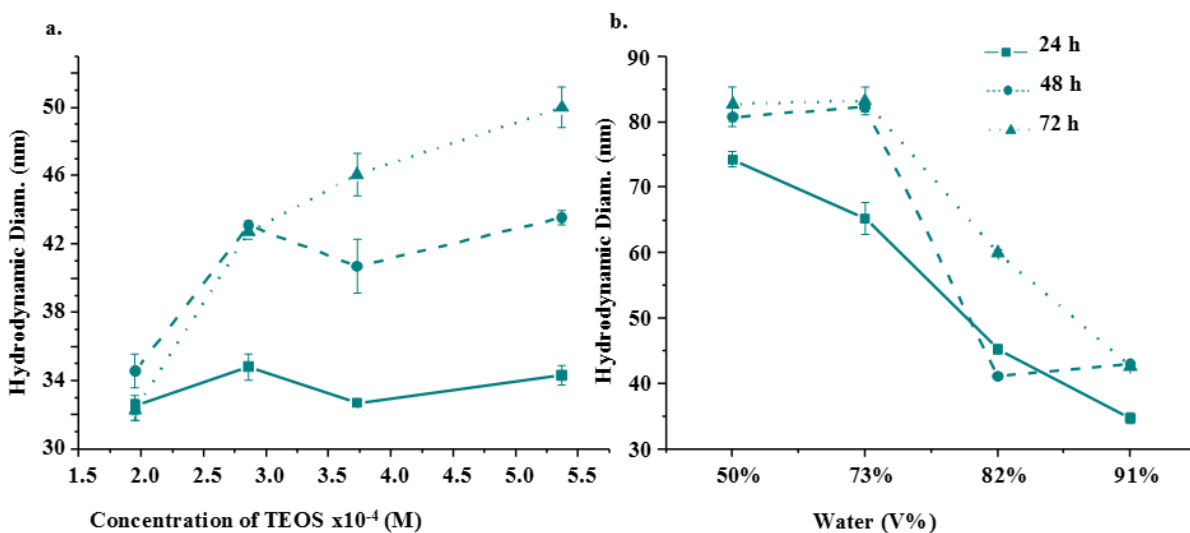


Figure S4 Monitoring, by DLS, of the growth of particles using butanol as co-solvent with variations in (a) TEOS concentration and (b) co-solvent volume percent at time points of 24, 48 and 72 hours.

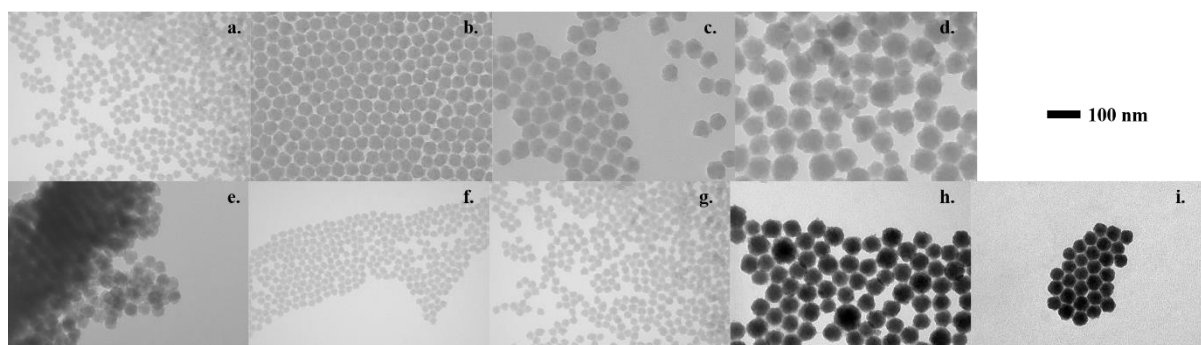


Figure S5 TEM images of size control by changing the ratio between the aqueous phase and the organic phase (butanol) as (a) 91%, (b) 82%, (c) 73% and (d) 50% and changing the polarity of the co-solvent as (e) TEOS only, (f) ethanol, (g) butanol, (h) hexanol and (i) cyclohexane at 91% V. of water

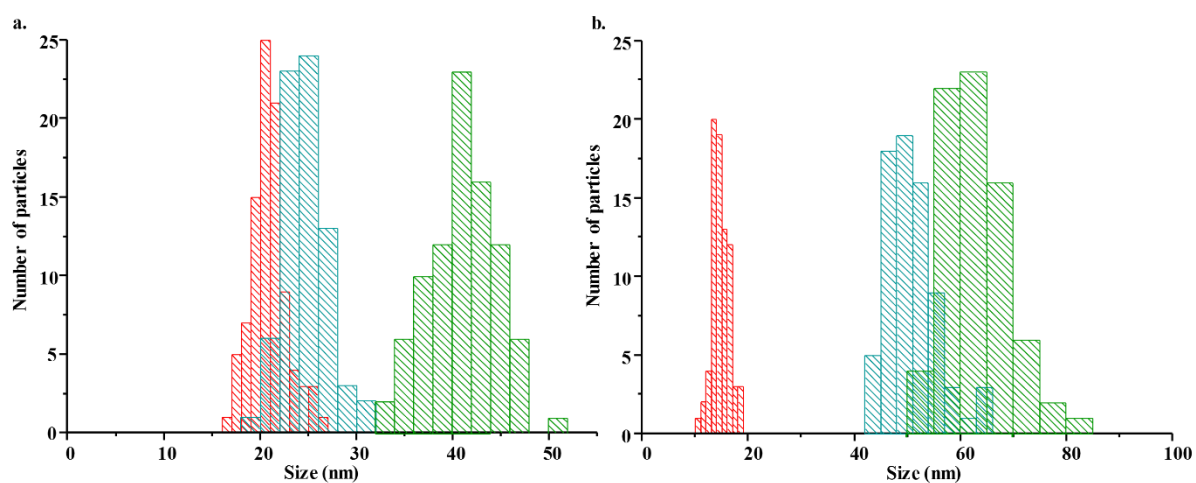


Figure S6 Comparison of the histograms obtained from TEM image analysis (Image J) of NPs synthesised by a different co-solvent: ethanol (red), butanol (dark cyan) and cyclohexane (green) at (a) 91% V. water and (b) 73% V. water.

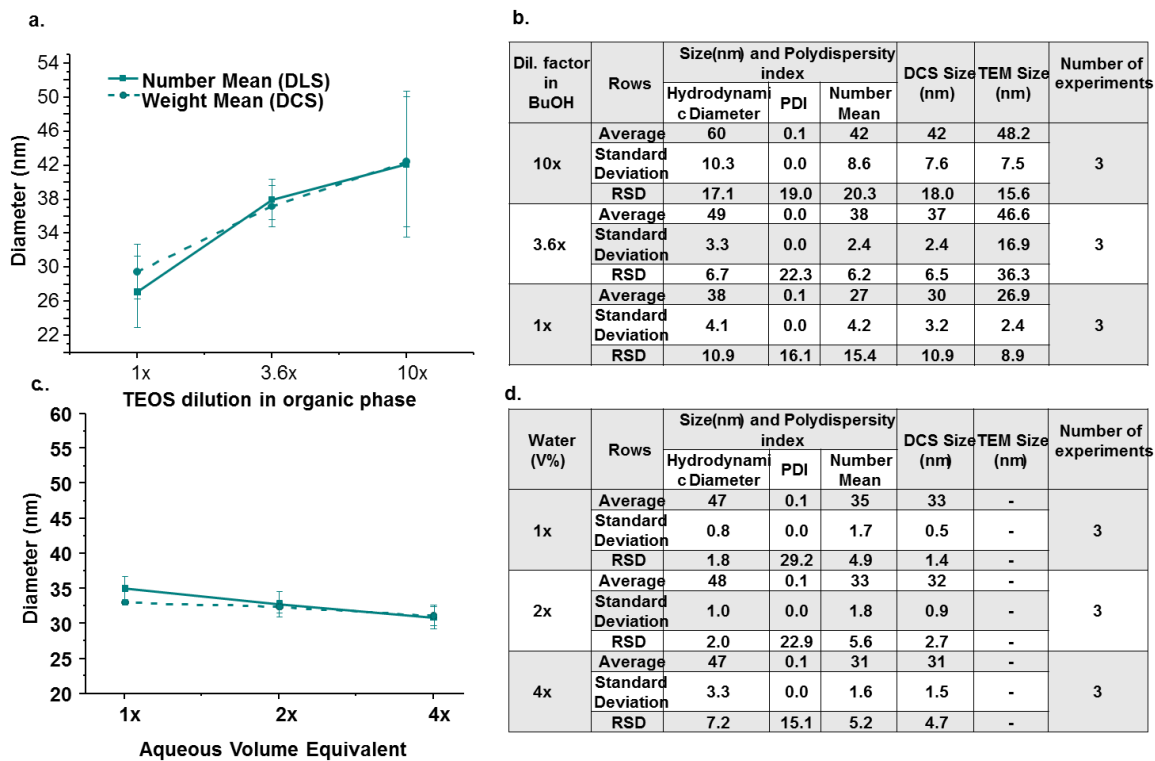


Figure S7 Size variation across particle batches synthesised by (a and b) varying organic phase volume (TEOS dilution) and (c and d) varying the aqueous (nucleation and growth) phase volume keeping other conditions constant.

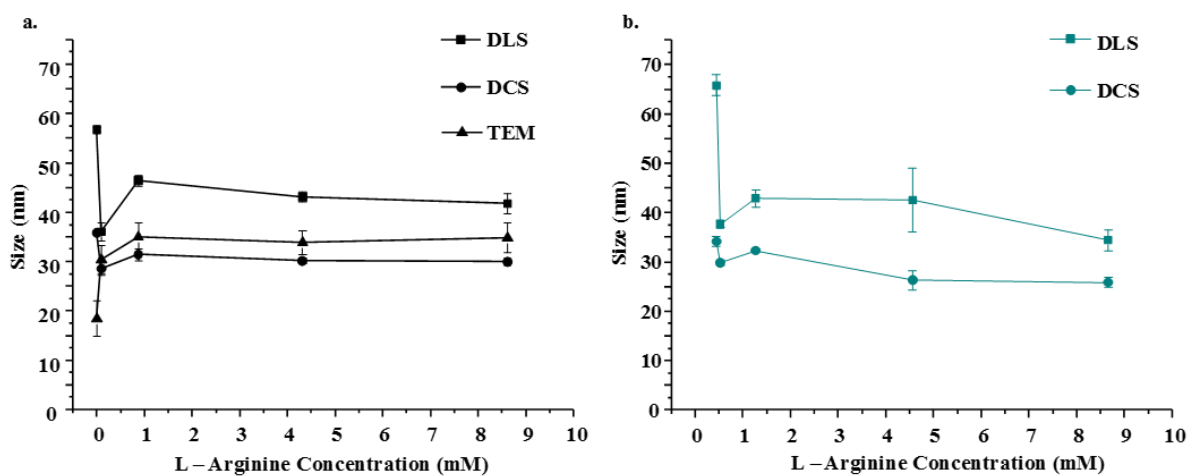


Figure S8 Control of particle size by L-arginine concentration analysed using DLS (hydrodynamic diameter), DCS (relative weight) and TEM (average size) particles synthesised using (a) TEOS only and (b) butanol as the organic phase co-solvent.

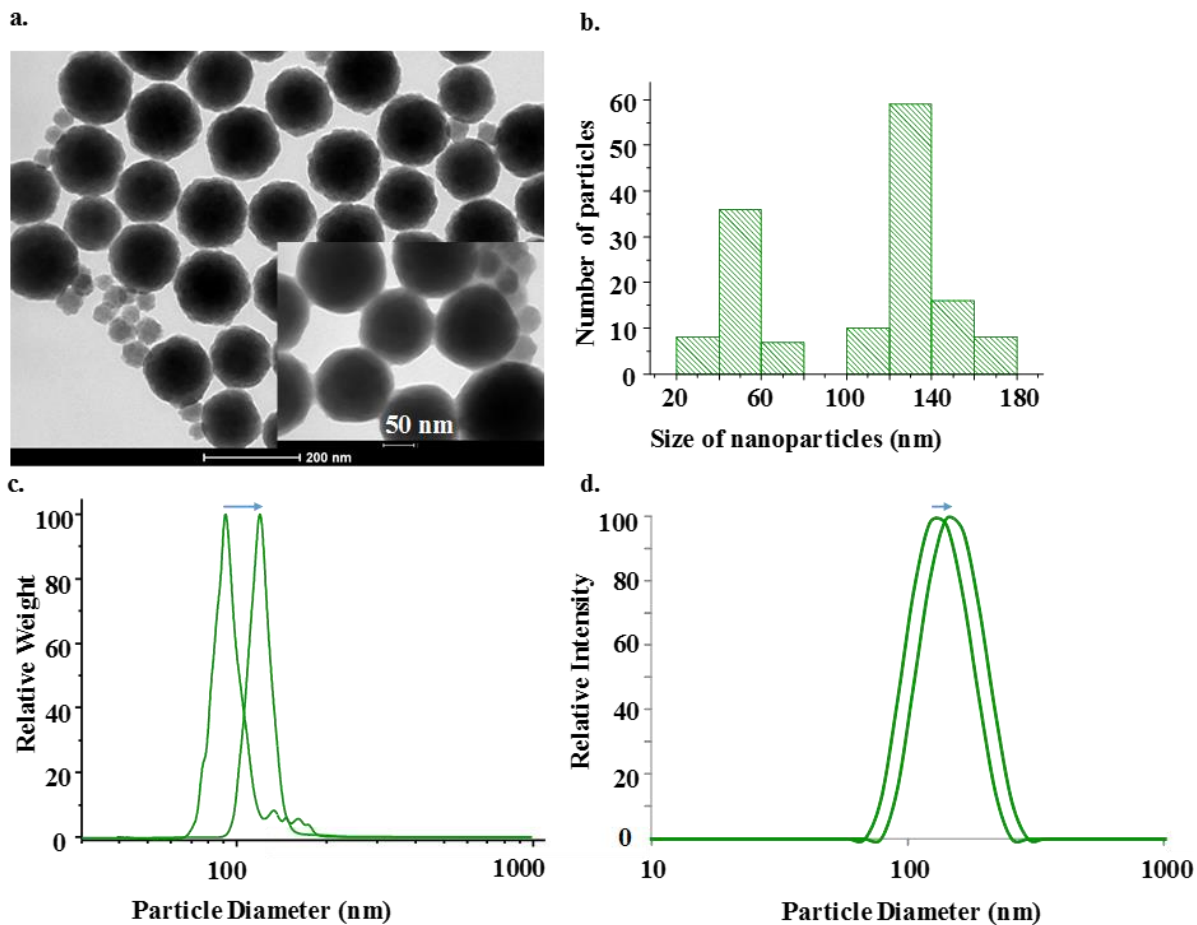


Figure S9 Dispersion size estimated by (a) TEM and (b) histogram, (c) DCS and (d) DLS of particles synthesised using cyclohexane as a co-solvent with an interfacial area/aqueous volume of 0.12 cm^{-1} . Dispersion size are obtained after 72 hours and 160 hours.

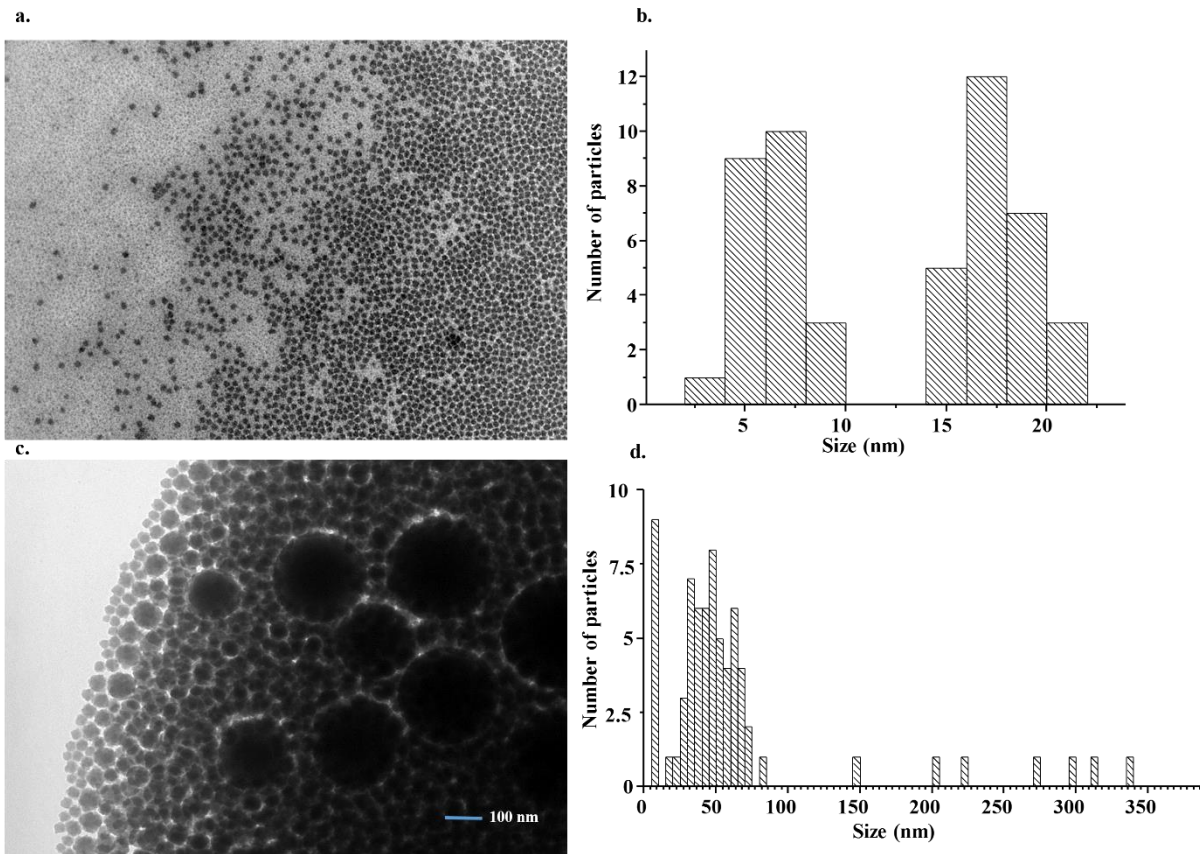


Figure S10 Characterisation of the particle dispersion synthesised at 37°C (a) TEM and (b) histogram and at dynamic temperature by increasing the temperature by 0.5°C/min (a) TEM and (b) histogram.

	Concentration of TEOS [M]	Rows	DLS Size (nm) and Polydispersity index			DCS Size (nm)	TEM Size (nm)	Number of experiments
			Z Average	PDI	Number			
H2O	0.22	Average	38	0.08	27	29		
		St Dev	3	0.01	3	0	-	10
		RSD (%)	7	15.40	10	1		
	0.34	Average	40	0.07	30	32	32	
		St Dev	3	0.02	3	0	3	15
		RSD	8	26.24	11	0	10	
	0.45	Average	47	0.07	34	34		
		St Dev	3	0.02	3	0	-	9
		RSD	6	24.77	8	1		
	0.67	Average	52	0.07	38	38		
		St Dev	4	0.02	4	1	-	9
		RSD	7	25.32	10	2		
Ethanol	0.22	Average	22	0.17	11			
		St Dev	6	0.08	5	-	-	10
		RSD	25	44.18	45			
	0.34	Average	22	0.17	11		21	
		St Dev	3	0.04	4	-	2	15
		RSD	12	20.87	31		9	
	0.45	Average	30	0.16	17			
		St Dev	5	0.03	5	-	-	9
		RSD	16	17.67	27			
	0.67	Average	32	0.17	17			
		St Dev	6	0.04	8	-	-	9
		RSD	18	25.00	44			
Butanol	0.22	Average	32	0.11	21	23	25	
		St Dev	3	0.02	3	1	3	11
		RSD	8	20.53	13	3	10	
	0.34	Average	40	0.12	25	28	24	
		St Dev	3	0.03	4	1	2	15
		RSD	7	24.81	14	4	10	
	0.45	Average	46	0.10	30	32	33	
		St Dev	2	0.03	3	1	4	13
		RSD	4	26.90	9	3	11	
	0.67	Average	51	0.10	34	34	47	
		St Dev	2	0.03	4	1	5	11
		RSD	4	28.58	11	4	10	
Hexanol	0.22	Average	55	0.03	44	45	55	
		St Dev	3	0.01	3	2	7	9
		RSD	6	35.28	6	3	12	
	0.34	Average	57	0.03	45	43	55	
		St Dev	2	0.01	2	3	8	15
		RSD	4	37.59	5	7	14	
	0.45	Average	57	0.04	45	43	55	
		St Dev	2	0.01	2	1	6	9
		RSD	3	20.90	4	2	11	
	0.67	Average	54	0.05	42	45	47	
		St Dev	4	0.01	4	1	7	9
		RSD	8	25.36	9	1	14	
Cyclohexane	0.22	Average	44	0.05	33	29		
		St Dev	3	0.01	2	0	-	16
		RSD	6	18.63	6	1		
	0.34	Average	50	0.05	38	32	41	
		St Dev	4	0.01	4	1	4	11
		RSD	8	22.95	10	4	9	
	0.45	Average	58	0.05	45			
		St Dev	3	0.01	3		-	10
		RSD	5	27.04	7			
	0.67	Average	63	0.04	49		-	13

Table S2 DLS, DCS and TEM size results averaged for all experiments done in with different co – solvents and different initial TEOS concentrations.

	Volume of water	Rows	DLS Size (nm) and Polydispersity index			DCS Size (nm)	TEM Size (nm)	Number of experiments
			Z Average	PDI	Number			
Ethanol	50%	Average	19	0.30	10			
		St Dev	5	0.09	3	-	-	9
		RSD (%)	23	29.90	28			
	73%	Average	16	0.26	8		15	
		St Dev	2	0.10	1	-	2	9
		RSD (%)	12	38.18	10		11	
	82%	Average	21	0.17	11			
		St Dev	7	0.04	5	-	-	9
		RSD (%)	33	24.96	45			
	91%	Average	22	0.17	11		21	
		St Dev	3	0.04	4	-	2	15
		RSD (%)	12	20.87	31		9	
Butanol	50%	Average	74	0.17	50	64	69	
		St Dev	16	0.19	11	3	14	13
		RSD (%)	21	112.85	22	5	21	
	73%	Average	56	0.05	43	48	51	
		St Dev	8	0.03	8	4	5	15
		RSD (%)	14	59.33	19	8	9	
	82%	Average	48	0.08	33	36	39	
		St Dev	3	0.03	5	1	3	9
		RSD (%)	6	39.38	16	3	7	
	91%	Average	40	0.12	25	28	24	
		St Dev	3	0.03	4	1	2	15
		RSD (%)	7	24.81	14	4	10	
Hexanol	50%	Average	73	0.03	59	58		
		St Dev	8	0.01	6	0	-	10
		RSD (%)	11	41.51	10	1		
	73%	Average	64	0.04	51	49	53	
		St Dev	2	0.01	1	3	5	8
		RSD (%)	4	41.35	3	6	9	
	82%	Average	57	0.03	45	48		
		St Dev	5	0.01	3	3	-	9
		RSD (%)	9	43.96	7	6		
	91%	Average	55	0.03	43	43		
		St Dev	5	0.01	4	3	-	15
		RSD (%)	8	34.81	9	7		
Cyclohexane	50%	Average	79	0.02	65	63		
		St Dev	4	0.01	4	4	-	7
		RSD (%)	5	50.67	6	7		
	73%	Average	72	0.02	58	55	63	
		St Dev	5	0.01	5	5	6	7
		RSD (%)	7	50.79	8	8	10	
	82%	Average	67	0.03	54	52		
		St Dev	4	0.02	4	4	-	8
		RSD (%)	6	59.19	8	7		
	91%	Average	52	0.05	40	40		
		St Dev	4	0.02	4	3	-	7
		RSD (%)	7	32.43	9	8		

Table S3 DLS, DCS and TEM size results averaged for all experiments done in with different co – solvents and different initial aqueous phase volume %.

Materials and methods

Materials

Tetraethyl orthosilicate (TEOS)(cat. no. 86578), 3-Aminopropyltrimethoxysilane (APTMS)(cat. no.281778), Fluorescein Isothiocyanate (Isomer I) (FITC) (cat. no.F7250) and L-Arginine (cat. no. A5006), 2 – Prop (Isopropanol) (cat. no. 34863), 1 – butanol (butanol) (cat. no. 281549), 1 – hexanol (hexanol) (cat. no. H13303) and cyclohexane (cat. no. 34855) were purchased from Sigma-Aldrich and used as received. Commercially sourced silica NPs were purchased from Kisker. Ethanol (cat. no. 111727) was purchased from Merck and used as received. Milli-Q water was obtained from an in house system. TEM grids were bought from Agar Scientific.

Two phase method for silica nanoparticle synthesis

In a typical synthesis, of L – Arginine is added to 1 mL of Milli-Q water in a safe lock eppendorf so that the final concentration is 0.86 mM, and it's placed in a preheated (70°C) incubator, the co – solvent is added and the system is left to equilibrate for 15 minutes. After this time an appropriate amount of TEOS is added carefully so as to minimize mixing with the water phase. The eppendorf is closed and left, without stirring, for some time depending on the experiment (most experiments were left for 72 hours).

Determination of molecular silicate by molybdate assay

In a clean 25 mL round bottom flask L – Arginine (0.86 mM) was dissolved in 10 mL of Milli-Q water after which co – solvent was added on top (so that the final system is 91% water), the flask was sealed using a rubber stopper through which a long needle was inserted. The system was adjusted so that the needle goes through the organic layer and into the aqueous layer almost to the bottom of the flask. The system is then placed in a preheated oil bath (70°C) and left to equilibrate for 20 minutes. TEOS (0.31 M) is finally added.

50 μ L aliquots are taken with a syringe every minute for the first ten minutes, after which every five minutes until the one hour mark, the last aliquot is taken on the two hour mark (120 minutes). The volume at the end of experiment is 10.6 mL.

Molybdenum blue colorimetric method for silicate determination

Dissolved SiO_2 was measured by the colorimetric silicomolybdate method measuring dissolved silicate species, monomeric (H_4SiO_4), dimeric ($\text{H}_6\text{Si}_2\text{O}_7$), and trimeric ($\text{H}_8\text{Si}_3\text{O}_{10}$). Following desired incubation time (as outlined in the previous section) the resulting particle dispersion was centrifuged at 14,000 rpm for 60 minutes after which 500 μ L of the supernatant was analysed. 250 μ L of 1M HCl, 0.27 M Na_2EDTA and 0.265 M Ammonium molybdate were added in sequence and the obtained yellow solution was left for ten minutes at room temperature (not the solution was not always yellow), after the waiting period 250 μ L of 0.67 M Tartaric acid was added and the solution was left for further five minutes. 500 μ L of 1.35 M Sodium Sulfite was then added and solutions were left to fully react for 30 minutes. The so prepared samples were measured in a Varian 6000i UV/Vis Spectrophotometer with absorption at 715 nm taken.

Dynamic light scattering monitoring of silica nanoparticle growth

The appropriate co – solvent was added to 1000 μ L of L – Arginine (0.86 mM) in MilliQ water in a well-insulated quartz cuvette the cuvette, heated to 70°C and left for 20 minutes, TEOS (0.31 M) was added. A size measurement (11 runs, 10 accumulations per run) was automatically set up every 5 minutes for over 10 hours. The attenuator during measurement was set to 11.

Proton NMR as a method for the study of ethanol generation over time

In a Safe Lock eppendorf in which 1 mL of aqueous L – Arginine solution (0.86 mM) was placed 10%, by volume, co – solvents were added and the mixture was heated to 70°C and left to equilibrate for five minutes after which TEOS (0.34 M) was added to the organic fraction and the solution was left for the appropriate time.

300 µL of the aqueous phase were pipetted out and 300 µL of deuterium oxide containing dimethyl sulfoxide (20 mM final concentration) were added. A 5mm thin wall 8" NMR tube was used in a 600 MHz liquid NMR.

NMR spectra Processing

MestReNova 8.0 software was used for chemical shift predictions. All NMR spectra were processed in the following fashion: the obtained spectra was chemical shift referenced using the internal standard (DMSO) and standard solvent peak, an exponential apodization of 0.3 Hz was then applied, the phase of the spectra was corrected manually (if required). Spectra were then baselined using Whittaker smoother in all cases. Peak picking, integration and peak fitting were done in manual mode using MNova software.

Size determination by DLS

A Malvern Zetasizer ZS series with an autotitrator accessory was used in all measurements. 10 µL clean particles at a concentration of 10 mg/mL were taken and dispersed in 990 µL 1mM NaCl or PBS in a plastic low volume cuvette (PLASTIBRAND, semi-micro, PMMA, l = 1 cm) where the final concentration was 0.1 mg/mL. Particles were measured twice, both measurements consisted of two manual measurements each eleven runs for a total of forty four accumulations. The number presented is an average of those measurements.

Size determination by DCS

DCS was performed using a CPS Disk Centrifuge DC 24 000. The disc speed of 18 500 rpm was used and an 8% - 24% PBS based sucrose gradient was injected (settings optimized for size range analysis 0.03 – 1 µm). A 476 nm PVC (Analytik UK) commercial standard was used to calibrate the instrument before each measurement. Each gradient was checked by running the PVC standard as a sample and comparing to a database control. 100 µL of standard was injected before each measurement to calibrate the instrument followed by 100 µL of the undiluted particle dispersion.

TEM of SiO₂ particles

Silica particles were diluted to a final concentration of 0.1 mg/mL with water and 10 µL were transferred on a Formvar carbon 400 mesh copper TEM grid (Agar Scientific) and left to dry in air overnight. The grid was imaged using FEI Tecnai 120 instrument using 120keV.

Determination of time to monomer depletion

The synthesis was carried out as outlined above however the time three time points were taken, 24 hours, 48 hours and 72 hours. Size was determined by DLS.

Dilution effect experiment

TEOS Dilution Effect

In a Safe Lock eppendorf 500 μL of L – Arginine (0.86 mM) in Milli-Q water were pipetted and to it 50 μL , 180 μL and 500 μL butanol were added for a final water volume of 90%, 73% and 50% respectively. The system was left to equilibrate to 70°C for 15 min and finally TEOS (0.34 M) was added and the mixture was left stationary for 72 hours.

Nucleation phase Dilution Effect

In a Safe Lock eppendorf 1000 μL , 500 μL or 250 μL of L – Arginine (0.86 mM) in Milli-Q water were pipetted and to it 100 μL Butanol were added for a final water volume % of 91%, 83% and 72%. The system was left to equilibrate to 70°C for 15 min and finally TEOS (0.34 M) was added and the mixture was left stationary for 72 hours.

Determining the contribution of the L – Arginine concentration

The synthesis was carried out as outlined above however the L – Arginine concentration was varied from 8.6×10^{-2} mM to 8.6 mM. Particle size and distribution obtained from DLS, DCS and TEM.

Surface area experiments

Experiments were conducted in glass round bottom flasks with volumes of 50, 100 and 500 mL and interface surface areas of 20.5, 33.5 and 48.2 cm^2 respectively. In the flasks to a 40 mL of aqueous L – Arginine (0.86 mM) solution 15 mL of butanol were added and left to equilibrate at 70°C for 20 minutes. Finally 3.75 mL TEOS (0.42 M) was added to the organic phase and the resulting two phase solution was left to react for 72 hours at 70°C.

Assumptions and simplifications

As all processes are between two phases where the concentration of precursor is controlled by the speed at which it crosses a phase boundary we've making the following simplifications: The volume of the reaction is the sum of the volume of the organic and aqueous phases. As such the V% of a phase can be calculated as the V of the phase over the total volume.

The concentration of the reagents (TEOS, arginine, etc.) are calculated from the total volume of the reaction as often in the case of TEOS there is transfer across the phase boundary at any one moment and it's impossible to determine the precise concentration in either phase.