Electronic Supplementary Information (ESI)

Bio-inspired synthetic approaches: From hierarchical, hybrid supramolecular assemblies to $CaCO_3$-based microspheres

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Content:

1. Syntheses
2. Single-crystal X-ray diffraction data of **1**
3. X-Ray powder data of **1**
4. IR analysis of **1** and **2**
5. EDX analysis of **2**
6. Thermogravimetric analysis of **1** and **2**
7. Additional SEM analysis of **2**
8. TEM analysis and sample preparation
9. IR and Raman analysis of **2** aged in a CO_2 atmosphere
10. X-ray diffraction analysis of **2**
11. Powder X-ray Diffraction analysis of the $CaCO_3$ precipitates obtained in the presence of C_{12}hda at different time points
12. SEM images of aragonite crystals precipitated in $CaCO_3$ crystallising mixtures at elevated temperatures
13. Additional SEM images of calcite crystals precipitated in $CaCO_3$ crystallising mixtures in the presence of C_{12}hda at different pH conditions
14. Additional SEM images of the hollow calcite spheres
15. X-Ray powder patterns of $CaCO_3$-based samples
16. EDX mapping of hollow calcite spheres

1. Syntheses

Synthesis and characterisation of had-type ligands and coordination compounds 1 and 2

All chemicals were purchased from Sigma Aldrich, Fluka or TCI Europe in a high purity grade (>97%) and were used without further purification. Solvents were of reagent grade and purchased from local solvent suppliers.

Synthesis of 2,2'-(2-hydroxy-3,5-dimethylbenzylazanediyl)diacetic acid (Me_2hda)

Me_2hda was synthesised by following a modified literature procedure.[S1] 5 g (0.041 mol) of 2,4-dimethylphenol was placed in the round bottom flask. 100 mL of a 1:1 (v/v) mixture of methanol/water was added. The reaction mixture was stirred and heated at 55 ºC. In a separate beaker 3.36 g (0.084 mol) of sodium hydroxide was dissolved in 10 mL of H_2O and 5.46 g (0.041 mol) of iminodiacetic acid was added to this solution. After the iminodiacetic acid dissolved, the content of the beaker was added to the flask that contained the phenol. 3.32 mL (0.041 mol) of a 37 wt. % solution of formaldehyde was added dropwise and the mixture was heated at 85 ºC for 4 hours. Following this, the mixture was cooled and acidified with 7.5 mL of 32% hydrochloric acid. Methanol was removed from the mixture using a rotary evaporator, which caused immediate formation of a white precipitate. This solid was washed with water and dried in an oven at 50 ºC overnight. Yield: 8.67 g (79%).

MS analysis (CH_3OH): $(M-H)^{+\cdot}$ 266.09; CHN analysis: Formula: $C_{13}H_{17}NO_5$; Calculated: C: 58.42%, H: 6.46%, N: 5.24%; Found: C: 58.16%, H: 6.39%, N: 5.17%; ^1H NMR δ_H (400 MHz, DMSO): 6.84 (s, 1H), 6.61 (s, 1H), 3.78 (s, 2H), 3.41 (s, 4H), 2.14 (s, 3H), 2.10 (s, 3H); IR/cm^{-1}: 3307 (w), 3073 (w), 1677 (s), 1608 (sh), 1488 (s), 1418 (s), 1298 (m), 1197 (s), 1016 (br), 1116 (w), 968 (s), 853 (s, br).

Synthesis of 2,2'-((5-dodecyl-2-hydroxy-3-methylbenzyl)azanediyl)diacetic acid ($C_{12}hda$)

The compound was prepared by following a modified literature procedure.[S1] 5 g. (0.018 mol) of 4-dodecyl-2-methylphenol was placed in a round-bottom flask and was dissolved in 50 mL of ethanol at 50 ºC. In a separate beaker 2.40 g (0.018 mol) of iminodiacetic acid was dissolved in 10 mL of H_2O after the addition of 1.44 g (0.036 mol) of sodium hydroxide. Then 20 mL of ethanol was poured into the beaker and 1.46 mL (0.018 mol) of an aqueous 37 wt. % formaldehyde solution was added. The solution of iminodiacetic acid was slowly added dropwise into the flask and the temperature was increased to 95 ºC. The reaction was carried out for 6 hours. After that time 3.65 mL (0.036 mol) of hydrochloric acid was added to the flask to neutralize the solution. When the flask was cooled down, a white precipitate formed. It was filtered off and washed with H_2O and ethanol. Then it was dried in the oven at 60ºC. Yield: 7.12 g (94%).

MS analysis (CH_3OH): $(M-H)^{+\cdot}$: 420.22; CHN analysis: Formula: $C_{24}H_{39}NO_5$; Calculated: C: 68.38%, H: 9.32%, N: 3.32%; Found: C: 68.17%, H: 9.36%, N: 3.37%; ^1H NMR δ_H (400 MHz, DMSO): 6.80 (s, 1H, ArH), 6.58 (s, 1H, ArH), 3.77 (s, 2H, CH_2), 3.39 (s, 4H, $(CH_2)_2$), 1.20 (m.m., 25H, n-$C_{12}H_{25}$), missing $-CH_3$ signal overlaps with signals originating form the C_{12} alkyl moiety; IR/cm^{-1}: 3294 (w, br), 3092 (w), 3009 (w), 2958 (w), 2915 (s), 2848 (m), 1677 (s), 1488 (m), 1421 (m), 1377 (w), 1196 (w), 959 (w), 834 (w, br), 719 (w).

Synthesis of [Ca(Me_2hda)(H_2O)$_3$]·½H_2O·½MeOH (1)

27 mg (0.1 mmol) of the Me_2hda ligand was dissolved in a 1:1 (v/v) mixture of H_2O and methanol. The reagents were heated to 50 ºC and stirred. Then 30 μL (0.215 mmol) of triethylamine was added. This step was followed by the addition of 50 μL of 1 M aqueous $CaCl_2$ solution. The reaction mixture was further stirred for 15 minutes; after that time it was allowed to cool and filtrated through filter paper into a sample tube. Colourless crystals of **1** formed after *ca.* 7 days of slow evaporation. Yield: 14.5 mg (44%). CHN analysis: Formula: [Ca(Me_2hda)(H_2O)$_3$]; *Calculated:* C: 43.45%, H: 5.89%, Ca: 11.15%, N: 3.90; *Found:* C: 43.22%, H: 5.96%, Ca: 11.01%, N: 3.79%; IR/cm^{-1}: 2923 (m), 2852 (m), 1586 (s), 1410 (m), 1325 (s), 1246 (s), 1221 (s), 1152 (s), 990 (s), 867 (s), 721 (m).

Synthesis of [Ca(C_{12}hda)(H_2O)$_2$]·H_2O (2)

42 mg (0.1 mmol) of the C_{12}hda ligand was dissolved in a mixture of 4.5 mL of H_2O and 5 mL of methanol. The mixture was stirred and heated. When the temperature reached 65 ºC, 30 μL (0.215 mmol) of triethylamine was added. In the next step 0.5 mL of the 0.1 M aqueous $CaCl_2$ solution was added causing instant formation of a white precipitate of **2**. The mixture was left to cool down under ambient conditions. The precipitate was filtered of and analysed. Total yield: 40 mg (84%). CHN analysis: Formula: [Ca(C_{12}hda)(H_2O)$_2$]; *Calculated*: C: 58.27%, H: 8.15%, Ca: 8.10%, N: 2.83%; *Found*: C: 57.92%, H: 8.71%, Ca: 8.05%, N: 2.81%; IR/cm-^{1}: 3853 (vw), 3225 (s, br), 2918 (s), 2848 (s), 2368 (vw), 1655 (m), 1613 (m), 1582 (s), 1489 (w), 1415 (m), 1338 (w), 1322 (w), 1241 (w), 1217 (w), 988 (w), 865 (w), 721 (m).

$CaCO_3$-based materials - General synthetic procedure: For each sample, a specific quantity of C_{12}hda (see Table S1, below) was placed in a 100 mL beaker. Then 14.75 mL of deionised water was added and the mixture was rapidly stirred. The temperature was adjusted to values listed in Table S1 below. Then, in a typical experiment 42 mg (0.5 mmol) of $NaHCO_3$ and *ca*. 30 seconds after that, 0.25 mL of 1 M $CaCl_2$ solution (0.25 mmol) was dropped into the mixture. The reaction mixture was stirred for another 1 minute. Analytical samples for the SEM analysis were usually collected after 24 hours by filtration using a hydrophilic polycarbonate membrane. Samples were then placed on aluminum stubs, coated with a ca. 3 nm thick layer of platinum and imaged in low kV mode using a Zeiss Ultra Plus Scanning Electron Microscope. X-Ray powder diffraction was performed using a Siemens D500 diffractometer with Cu-Kα_1 radiation with a wavelength of $\lambda = 1.54056$ Å.

Tab. S1. Applied synthetic conditions

Quantity mmol			Initial pH	T [°C]	
Ligand	Ca^{2+}	HCO_3^-			
C_{12}hda influence					
0.01	1	2	6.9	30	Calcite, elongated crystals
0.01	0.5	1	6.9	30	Calcite, elongated crystals, defected rhombohedra
0.01	0.25	0.5	6.9	30	Calcite, various morphologies; some microspheres with openings
0.01	0.1	0.2	6.9	30	Calcite, defected rhombohedra
0.01	0.07	0.14	6.9	30	Fibrous material similar to **2**, some rhombohedral calcite crystals
Temperature influence C_{12}hda/Ca^{2+}/HCO_3^- mole ratio 1:25:50					
0.01	**0.25**	**0.5**	**6.9**	**40**	**Microspheres with openings and elongated calcite crystals**
0.01	0.25	0.5	6.9	50	Calcite, elongated crystals, defected rhombohedra
0.01	0.25	0.5	6.9	60	Calcite, needle-like crystals, small quantity of needle-like Aragonite crystals
0.01	0.25	0.5	6.9	70	Aragonite/calcite needle-like crystals
0.01	0.25	0.5	6.9	80	Aragonite, needles
0.01	0.25	0.5	6.9	90	Aragonite, needles
0.01	0.25	0.5	6.9	100	Aragonite, needles
Addition of 0.2 M HCl					
0.01	0.25	0.5	6.5	40	Spheres, 'Crossed disks' morphology

S1. (a) Mannich, C.; Kroesche, W., *Arch. Pharm.*, **1912**, *250*, 647-667, (b) Temkina, V. Y.; Rusina, M. N.; Yaroschenko, G. F.; Branzburg, M. Z.; Timakova L. M.; Dyatlova, N. M., *Zh. Obshs. Kim.*, **1975**, *45*, 1564-1570.

SEM – micrographs of 1 and preparation of crystals suitable for single-crystal X-ray diffraction

Crystals of **1** typically exhibit needle-like morphology. To obtain crystals with diameters suitable for single crystal X-ray diffraction analysis, slow solvent evaporation is required (i.e. covering the crystallizing mixture with a perforated parafilm). Fig. S1 presents the fibrous morphology of **1** typically obtained rapid precipitation approaches.

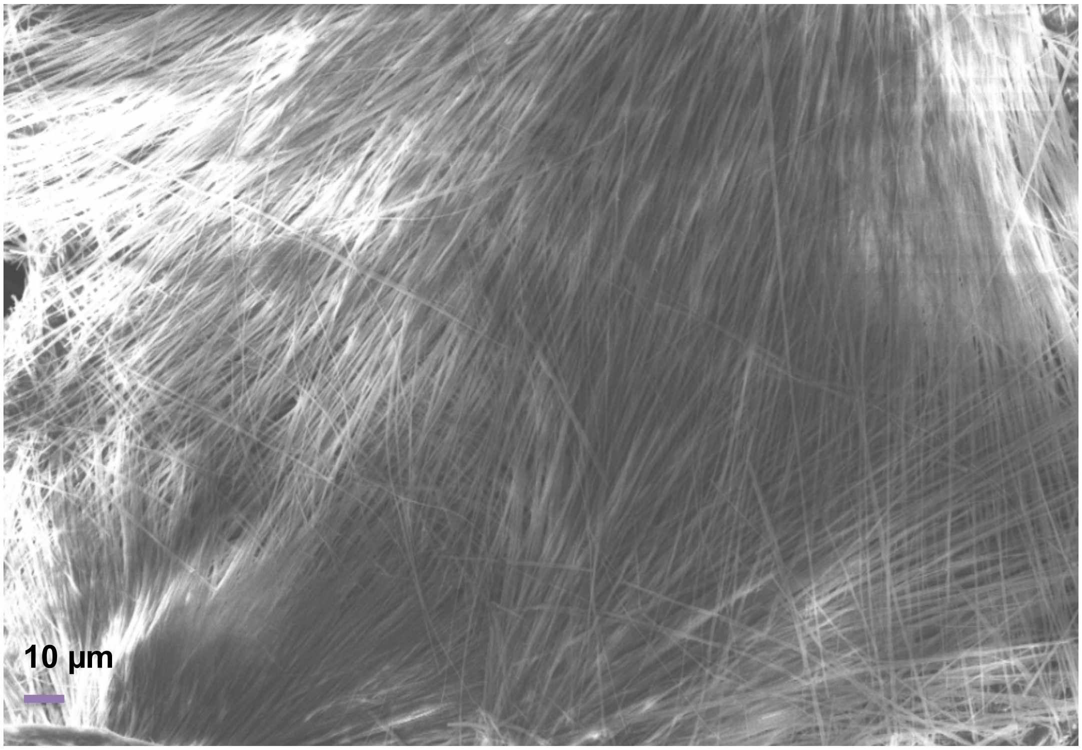


Figure S1. SEM micrograph showing the fibrous morphology of **1**.

2. Single-crystal X-ray diffraction data of 1

Single crystal X-ray structure determination of **1** was performed at 150(K) on the Bruker SMART Apex diffractometer using graphite-monochromated Mo-Kα radiation. Absorption corrections were applied using SADABS.[S2] The data sets were processed and corrected for Lorentz and polarisation effects using SMART and SAINT[S3] software packages. Structures were solved by direct method and refined by full-matrix least-squares on F^2 using *SHELXTL* and OLEX2.[S4, S5] All atoms except H atoms were refined anisotropically. Hydrogen atoms were assigned to calculated positions using a riding model with appropriately fixed isotropic thermal parameters. Crystal data and details of data collection and refinement are summarized in Table S2. Crystallographic data, CCDC **793553**, can be obtained free of charge from the Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

S2. Sheldrick, G. M. *SADABS, Program for area detector adsorption correction*. Institute for Inorganic Chemistry, University of Göttingen, Göttingen (Germany), 1996.

S3. Bruker SMART and SAINT, 1997, Bruker AXS Inc., Madison, Wisconsin.

S4. G. M. Sheldrick, Version 5.1, 1999, Bruker AXS Inc., Madison, Wisconsin.

S5. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard, H. Puschmann, H. OLEX2: A complete structure solution, refinement and analysis program. *J. Appl. Cryst.,* 2009, **42**, 339.

Table S2. Crystallographic details for **1**.

Empirical formula	$C_{13}H_{23}CaNO_9$	
Formula weight	377.4 g/mol	
Temperature	150(2) K	
Wavelength	0.71075 Å	
Crystal system	Triclinic	
Space group	*P*-1	
Unit cell dimensions	a = 6.2649(11) Å	α = 96.657(4)°
	b = 8.2062(14) Å	β = 94.771(4)°
	c = 33.649(6) Å	γ = 92.072(4)°
Volume	1710.5(5) Å^3	
Z	4	
Density (calculated)	1.466 g/cm^3	
Absorption coefficient	0.413 mm^{-1}	
F(000)	800	
Crystal size	0.60 x 0.05 x 0.05 mm^3	
Theta range for data collection	0.61 to 25.86°	
Index ranges	-7<=h<=7, -10<=k<=10, -41<=l<=41	
Reflections collected	6606	
Independent reflections	6606 [R(int) = 0.0402]	
Completeness	99.7%	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	6606 / 13 / 493	
Goodness-of-fit on F^2	1.044	
Final R indices [I>2sigma(I)]	R1 = 0.0514, wR2 = 0.1191	
R indices (all data)	R1 = 0.0677, wR2 = 0.1316	
Largest diff. peak and hole	0.433 and -0.548 e.Å^{-3}	

3. X-Ray powder diffraction data recorded for 1

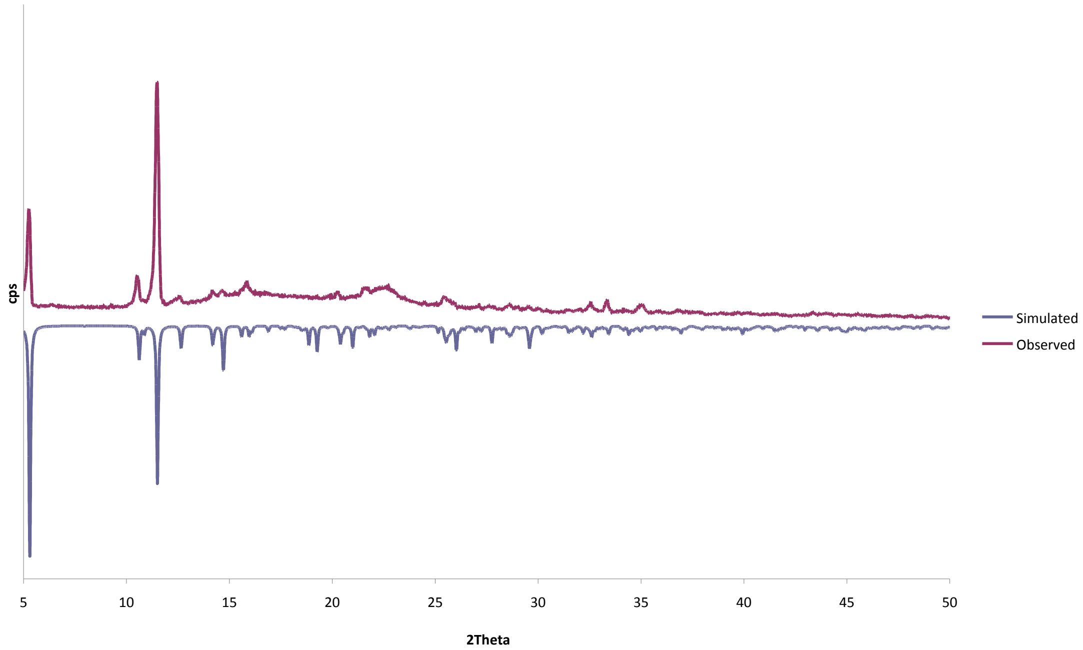
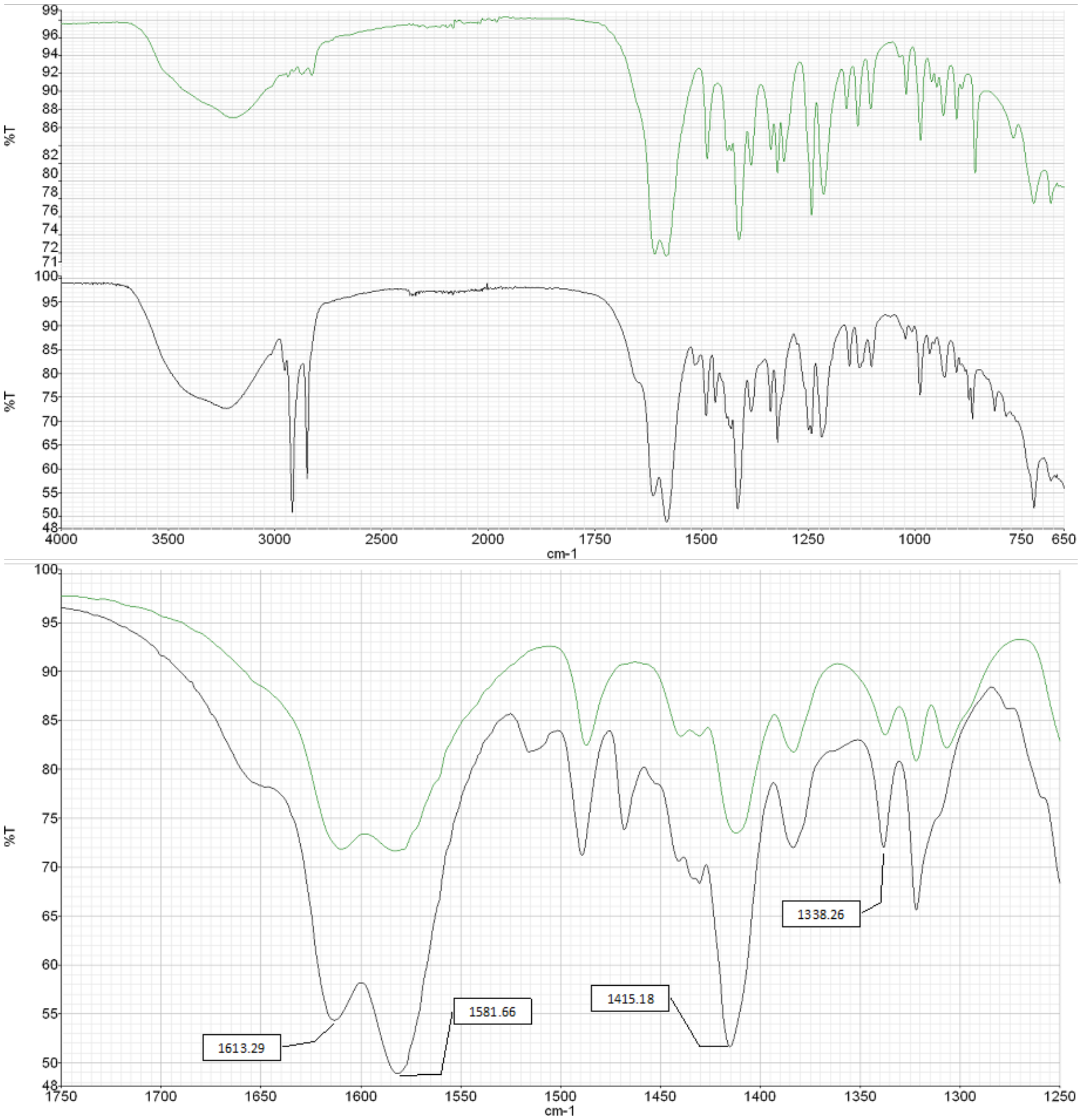


Figure S2. Experimental X-ray powder pattern of **1** (red) compared with the calculated pattern based on the single crystal measurements (blue).

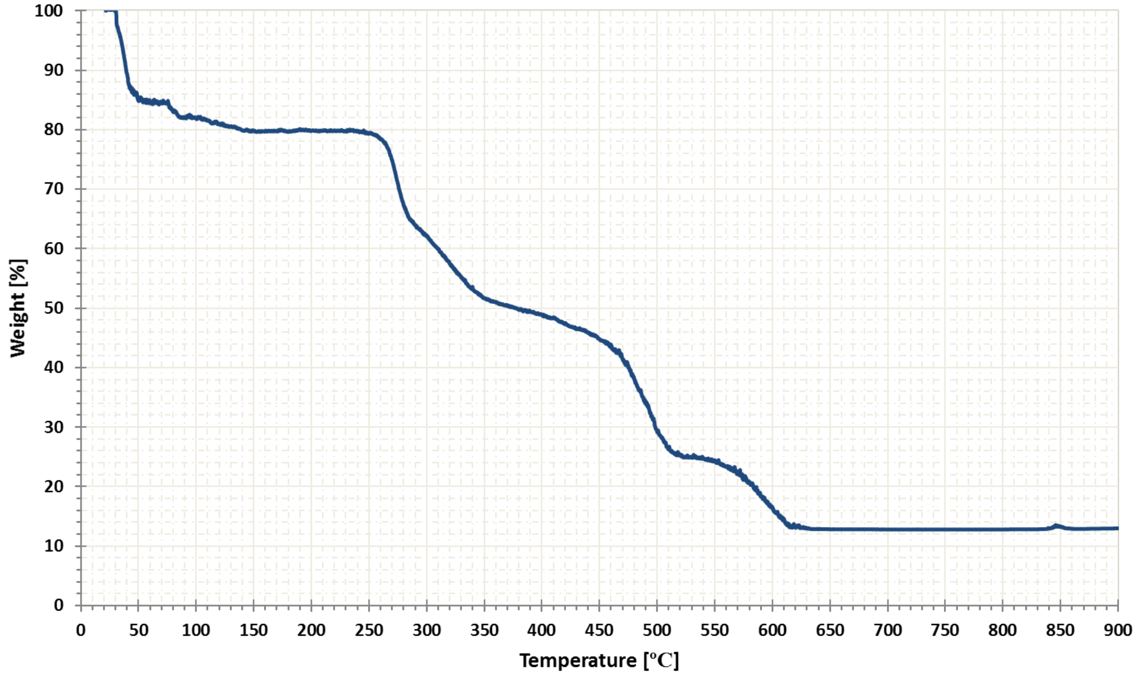
4. IR analysis of 1 and 2



Compound	ν_{as1} (COO$^-$)	ν_{as2} (COO$^-$)	$\nu_{\text{in plane bend}}$ (C-OH)	ν_{s1} (COO$^-$)
1	1610 cm^{-1}	1583 cm^{-1}	1412 cm^{-1}	1338 cm^{-1}
2	1613 cm^{-1}	1582 cm^{-1}	1415 cm^{-1}	1338 cm^{-1}

Figure S3. IR spectra of **1** (green) and **2** (black). Infrared spectra were recorded in the range 4000 – 650 cm^{-1} on a Perkin Elmer Spectrum One FTIR spectrometer using a diffuse reflectance sampling accessory. The scan rate: 8 scans/minute; resolution: 4 scans/cm^{-1}.

6. Thermogravimetric analysis of 1 and 2



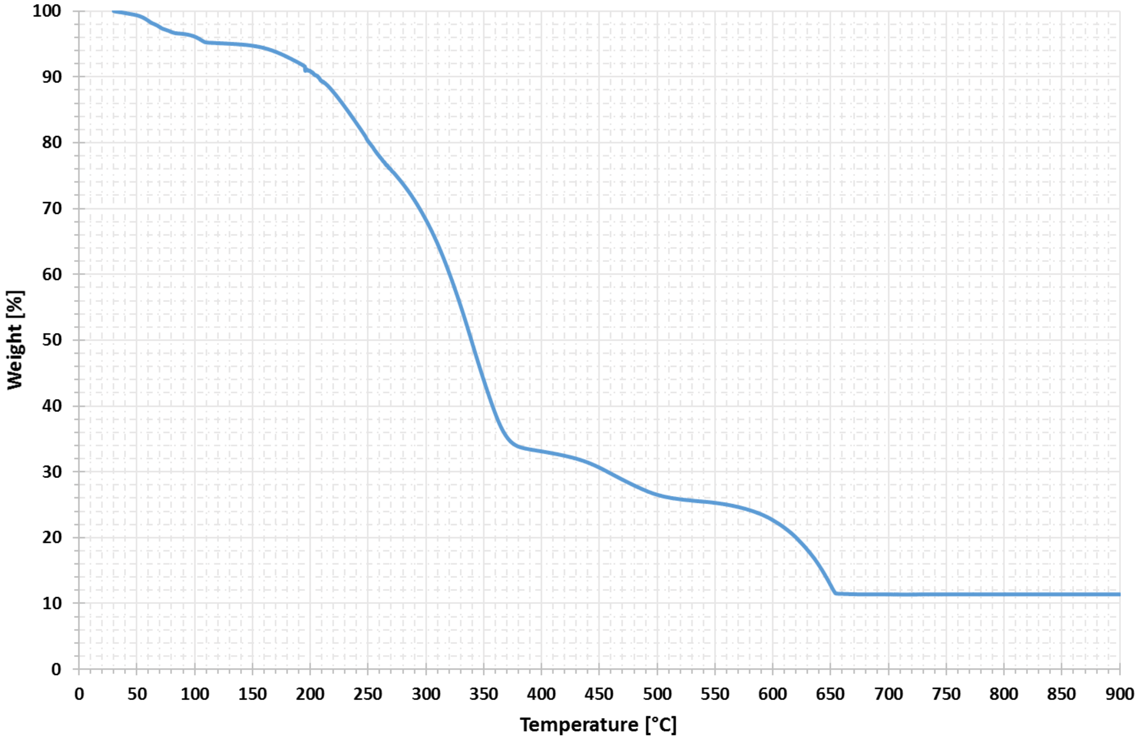


Figure S4. Thermal degradation pathway of **1** (*top*) and **2** (*bottom*). The analyses were performed in an air atmosphere. The heating rate: 1˚C/min.

7. Additional SEM analysis of 2

The additional SEM images presented in Fig. S5 show the morphology of freshly precipitated fibres of **2**. Samples were prepared by depositing about 5 mL of the reaction mixture of **2** on a silicon wafer (ca. 1 cm x 1 cm) followed by rinsing with deionised water.

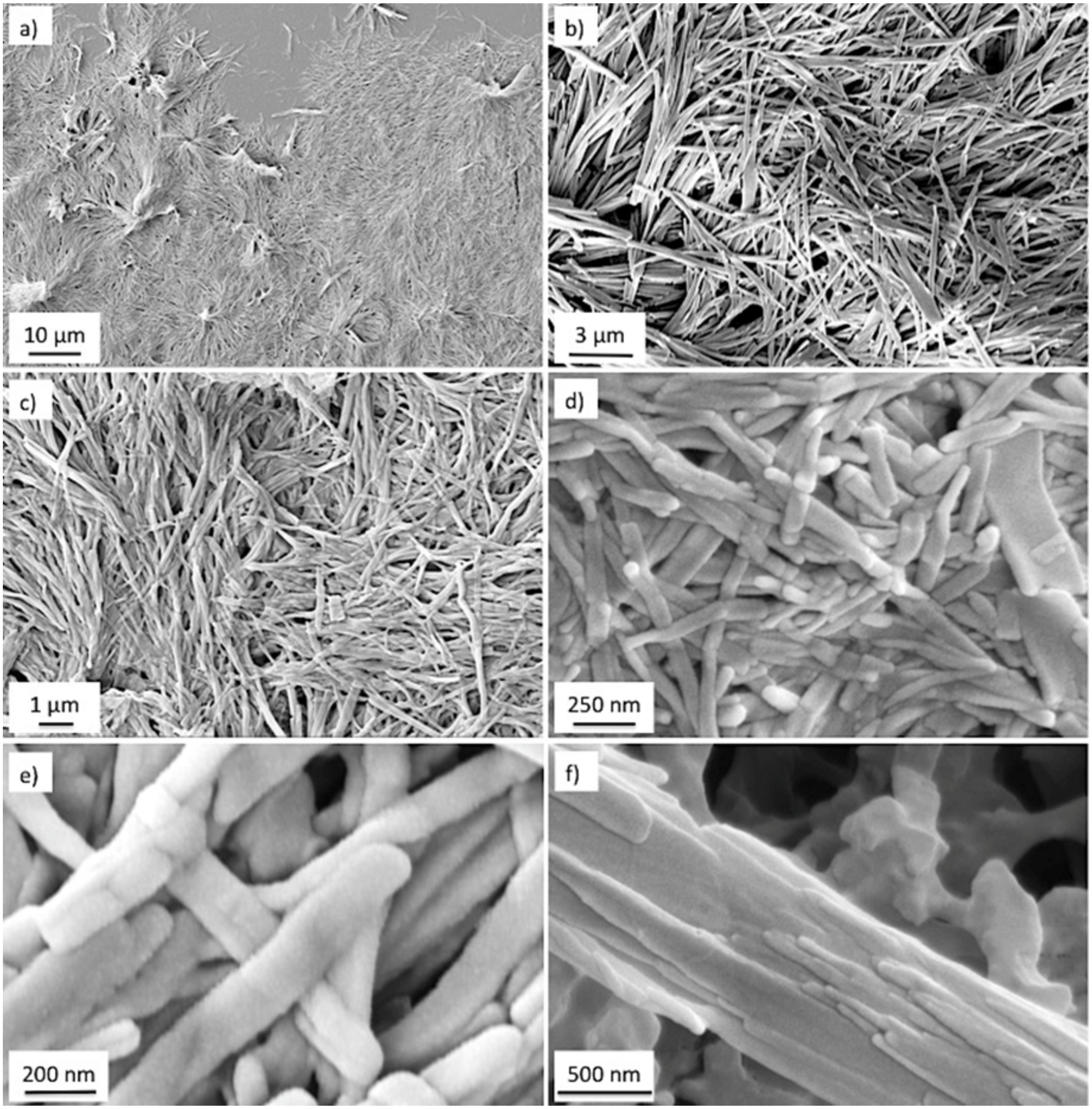


Figure S5. SEM microphotographs of **2**.

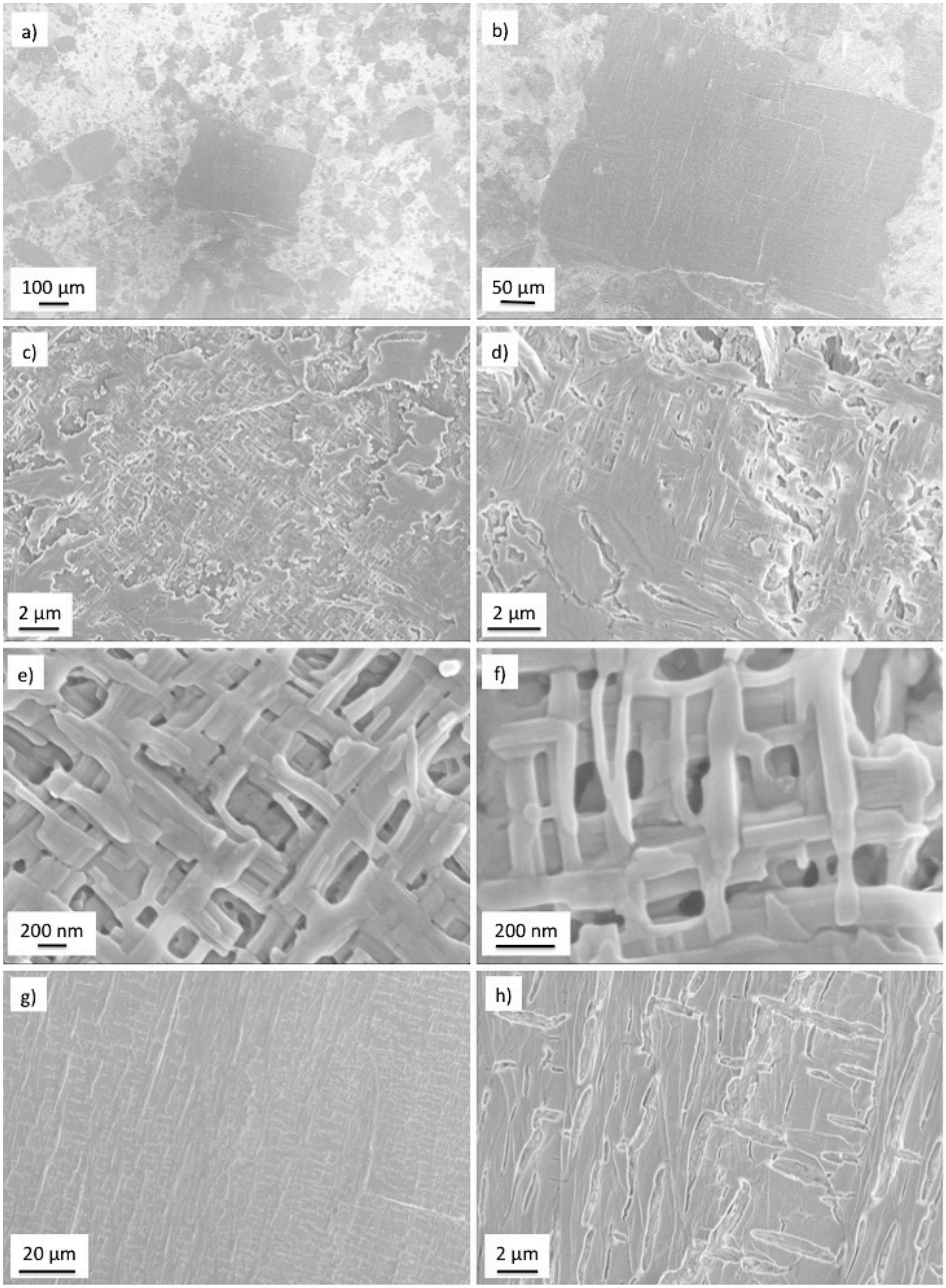


Figure S6. SEM microphotographs of **2** recorded 2 weeks after the sample deposition. 1D belts amalgam into a smooth surfaces to produce films.

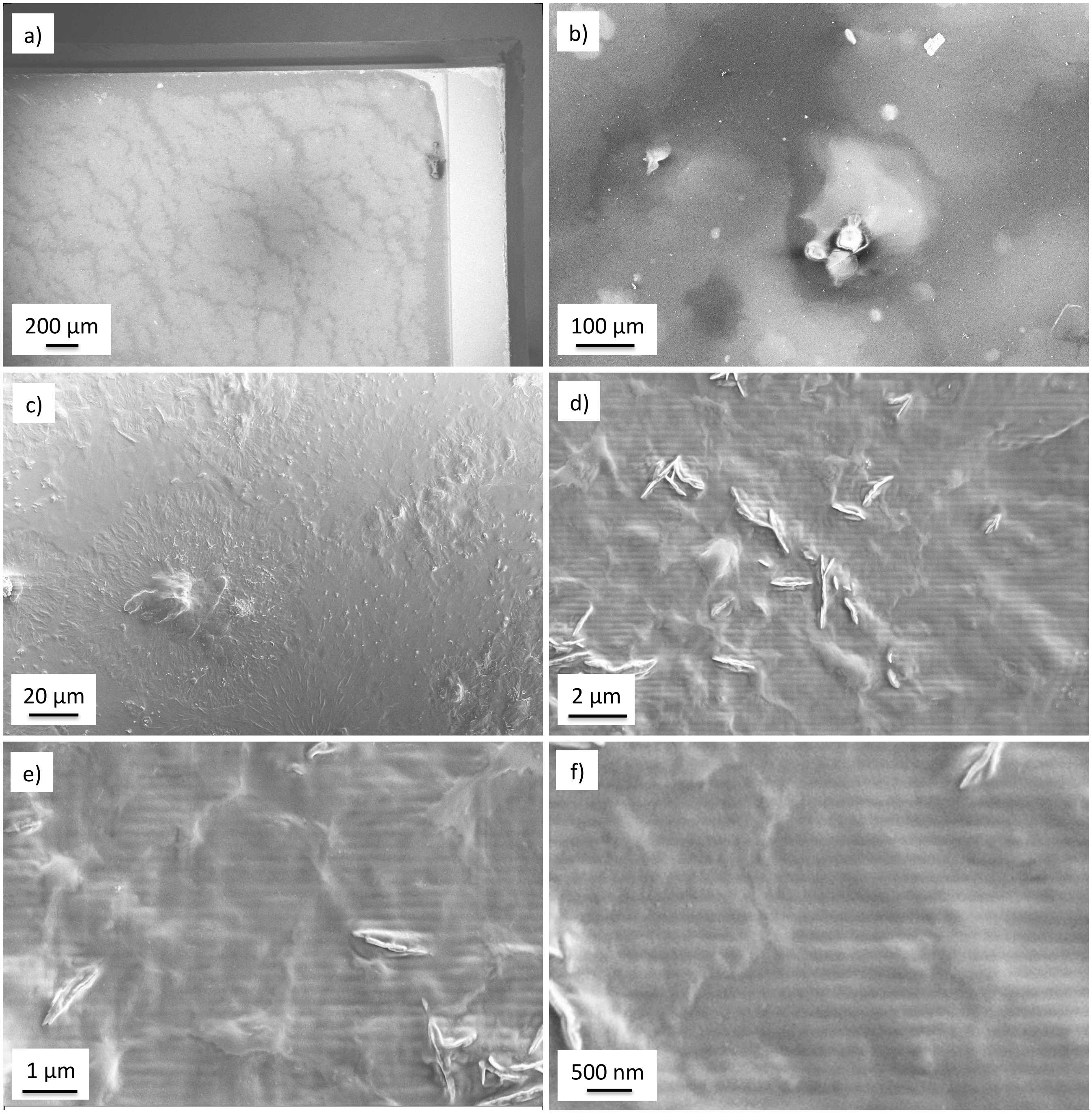


Figure S7. SEM images of compound **2** recorded after 4 weeks.

When the sample is further aged, crystalline films may break or may separate from the surface depending on the thickness and the deposited amount of sample (Fig. S8). The thickness of the crystal plates varies between 1 μm and 2 μm and is dependent on the amount of sample deposited on the surface.

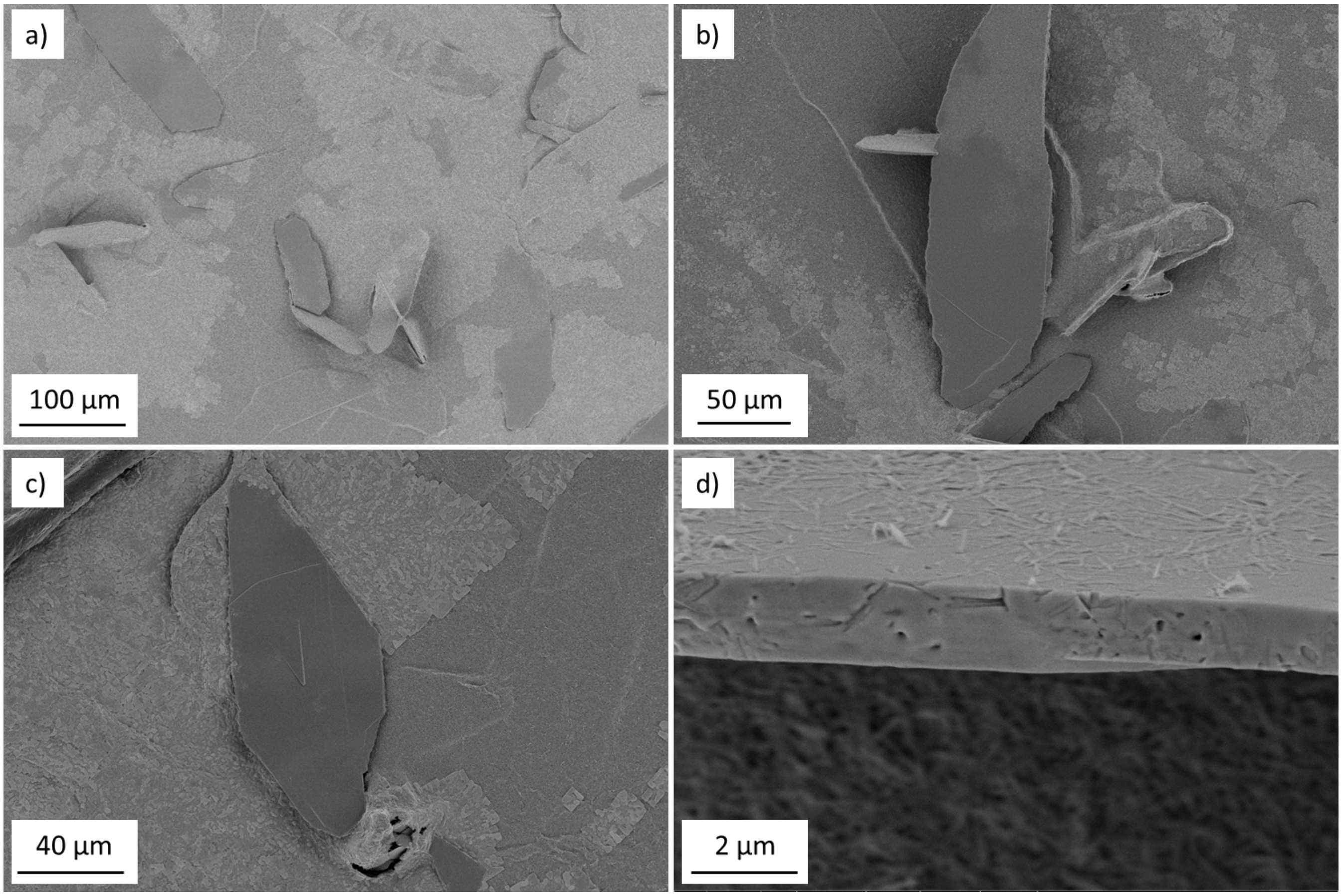


Figure S8. SEM images samples **2** deposited on a Si substrate (imaged after 4 weeks). In some places it is possible to notice thin, plate-like crystals separating from the surface.

8. TEM analysis and sample preparation

A small amount of **2** was dispersed in ethanol. Then *ca*. 200 µL of the dispersion was deposited on a Cu-200 TEM grid covered with Formvar (purchased from Agar Scientific). The imaging was performed using a FEI Titan G^3 microscope operating at 300 kV. As the sample was susceptible to electron beam damage, the images were recorded using short exposure times. Additional TEM images of the self-assembled fibres of **2** are presented in Fig. S9 and HR TEM image (Fig. S9c) shows that the separation distance between the layers is *ca*. 2.5 (±0.3) nm, which is in agreement with the expected separation distance between the neutral layers in **2**.

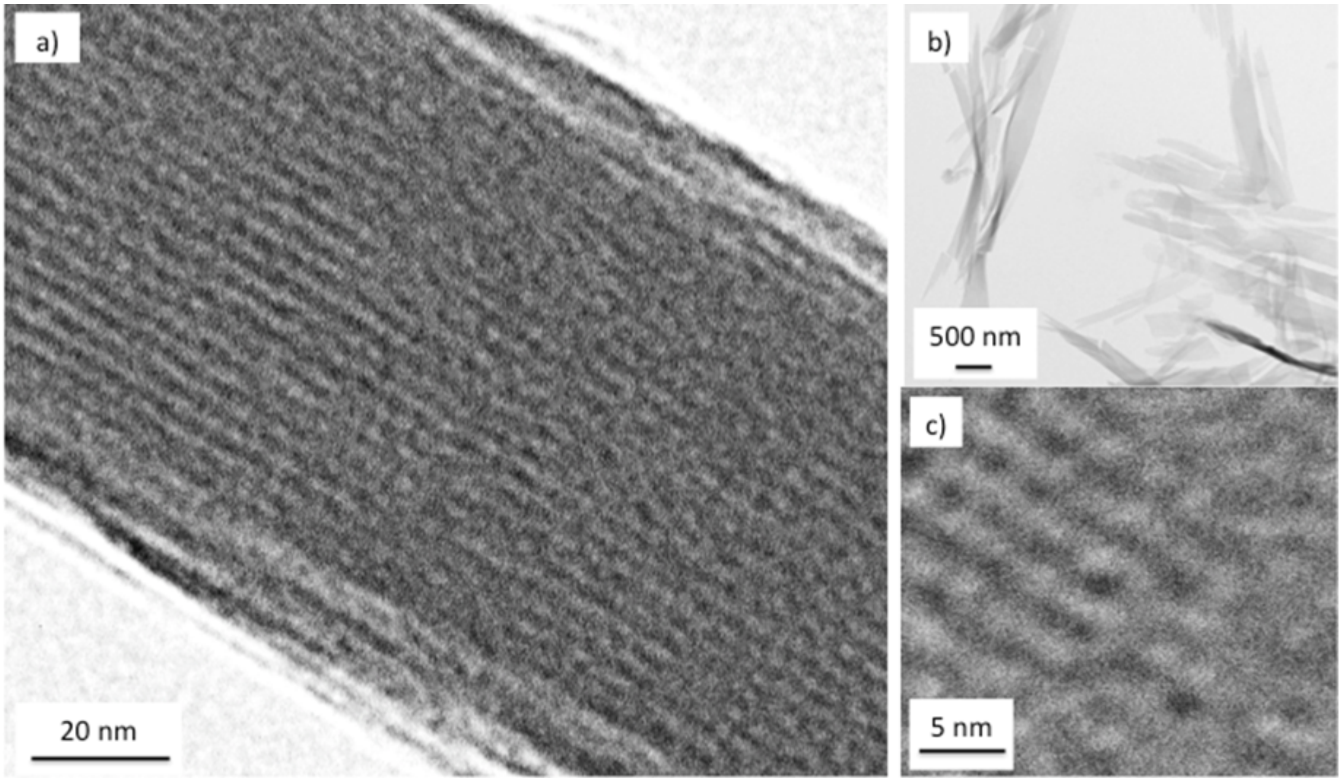


Figure S9. HR-TEM images of **2**.

9. IR and Raman analysis of 2

In order to confirm that the self-assembled fibers of **2** and the observed crystals are structurally similar, the IR spectra of an aged sample were recorded over time. As presented in Fig. S10, no significant shifts were observed, strongly suggesting that the coordination environment around Ca^{2+} ion does not depend on the morphology of the sample.

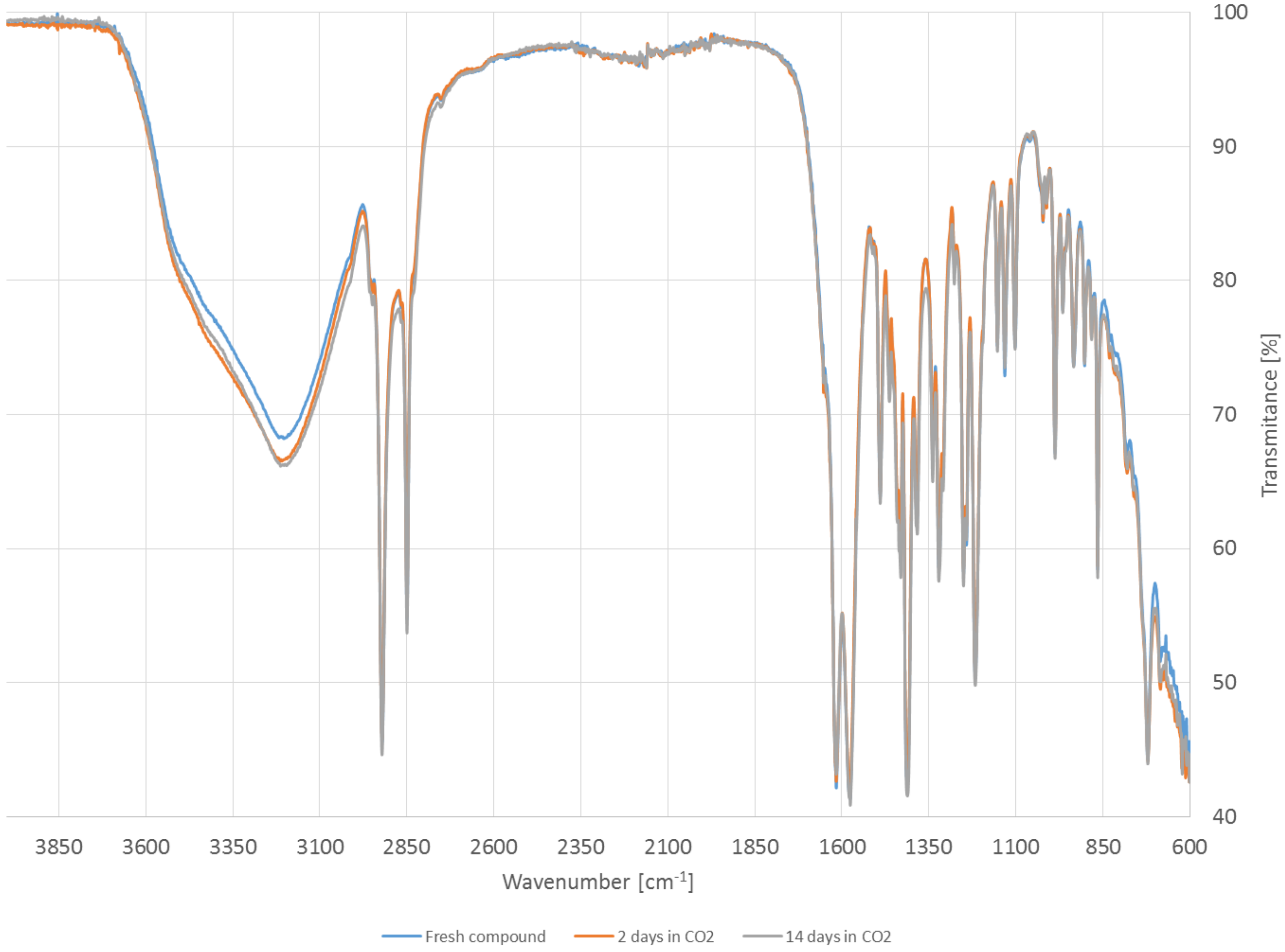


Figure S10. Comparison between FT-IR spectra recorded for a fresh sample of **2** (blue) and a sample that was aged for 2 days (orange) and 14 days (black).

Raman spectroscopy was used as a complementary analysis technique. As shown in Fig. S11, no major differences are visible in Raman spectra recorded for a fresh sample of **2** and an aged one. The strong signals observed at *ca.* 930 cm^{-1} and 510 cm^{-1} originate from the Si substrate.

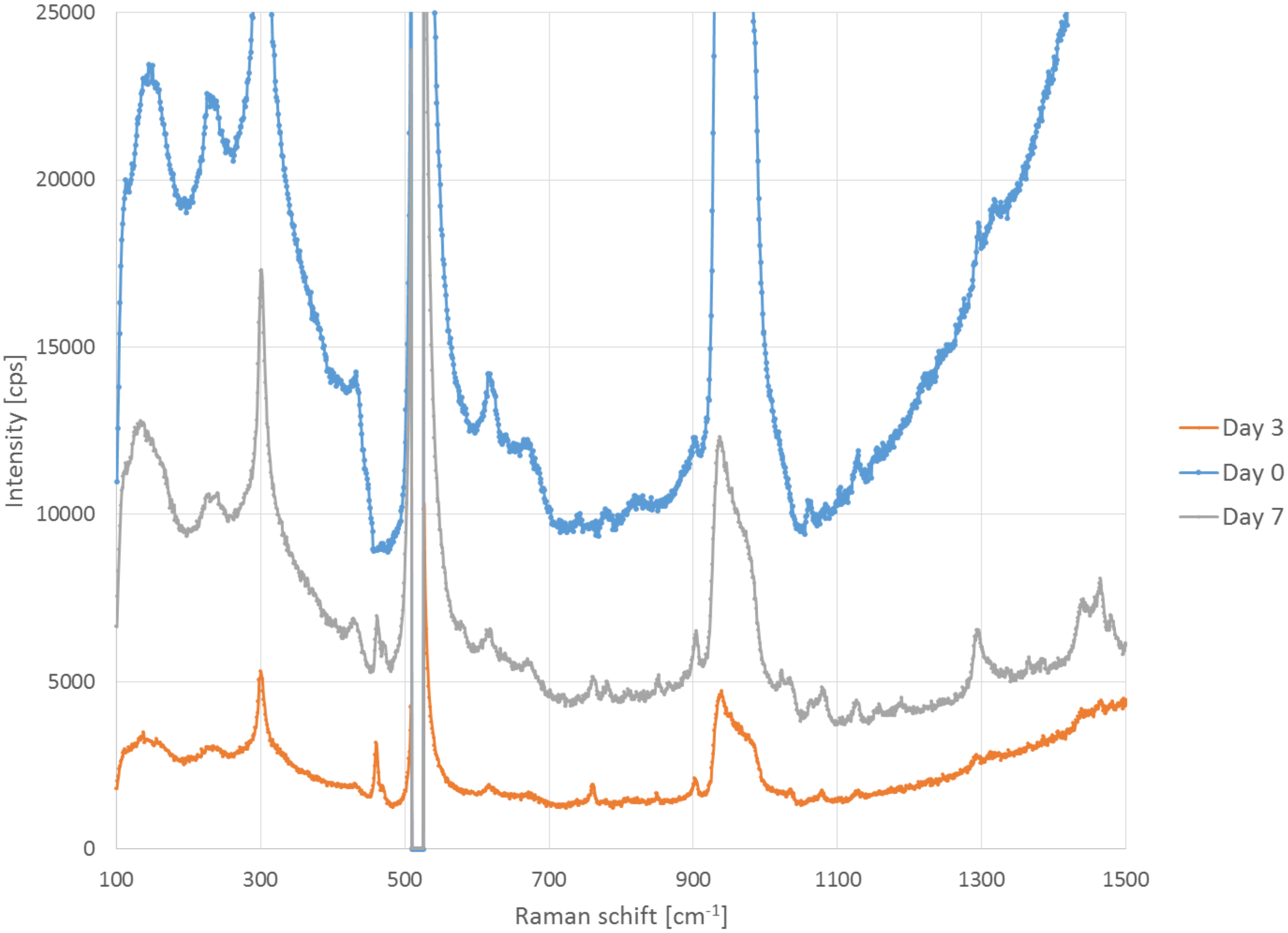


Figure S11. Raman spectra recorded for a sample of **2** deposited on a Si substrate and aged in an incubator filled with CO_2. Fresh sample (blue), spectrum after 3 days (orange) and 7 days (grey). The highly intense signals at 930 cm^{-1} and 510 cm^{-1} can be attributed to the crystalline Si-substrate.

10. Powder X-ray diffraction analysis of 2

The development of the crystalline phase can be effectively monitored using powder X-ray diffraction using CuKα radiation (λ=1.54056Å. Fig. S12 presents P-XRD patterns recorded for a freshly deposited sample of **2** and for a sample that was kept in a desiccator (CO_2 purged) for 1 week and for 2 weeks.

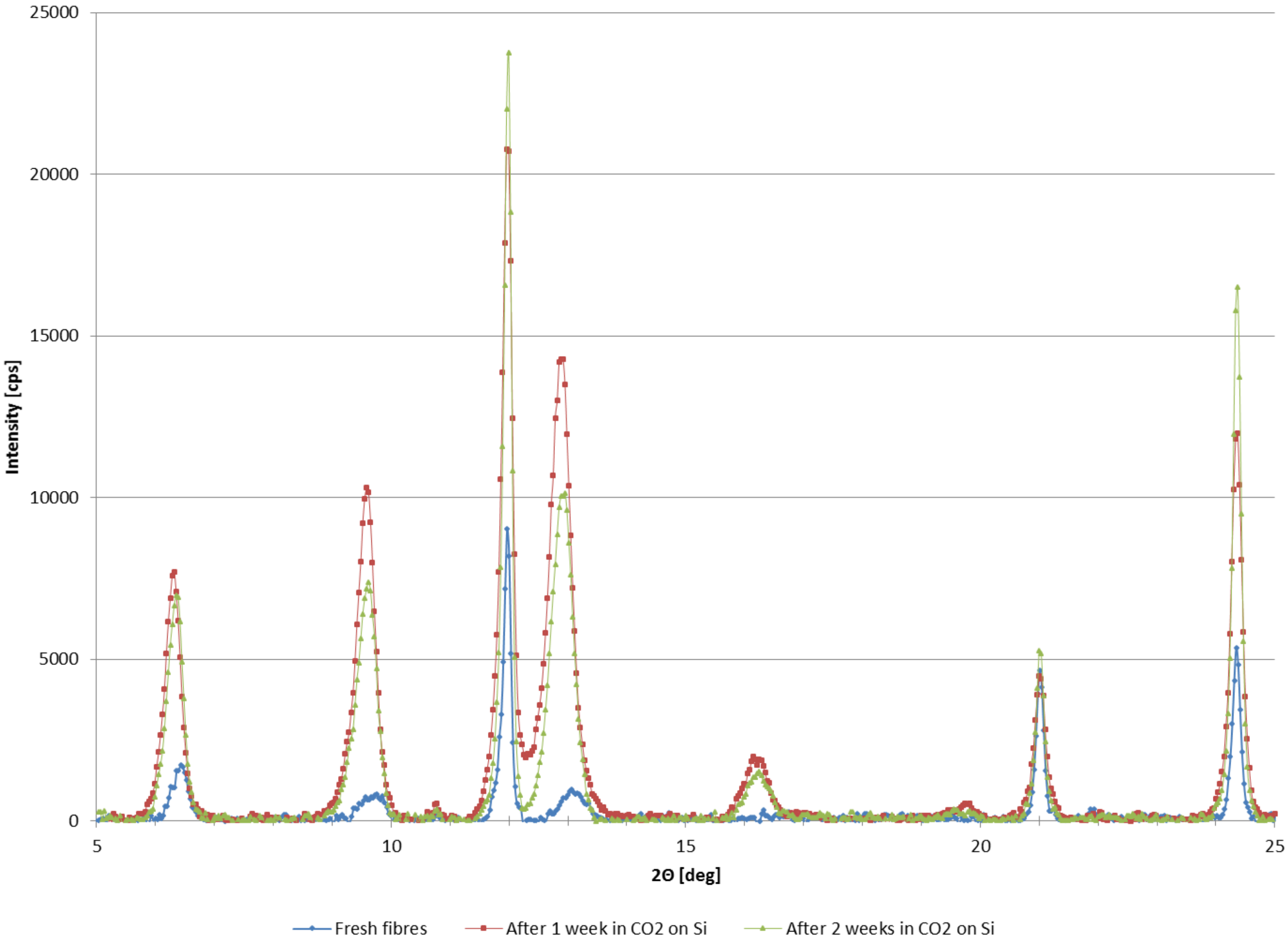


Figure S12. Powder X-ray diffraction patterns recorded for a sample of **2** deposited on a cut off-axis Si substrate. Pattern recorded immediately after deposition: blue; sample aged for 1 week: red, sample aged for 2 weeks: green.

11. Powder X-ray Diffraction analysis of the $CaCO_3$ precipitates obtained in the presence of C_{12}hda at different time points

The development of crystallinity in a $CaCO_3$ growth mixture composed of $CaCl_2$ and $NaHCO_3$ and also containing C_{12}hda was monitored by powder X-ray diffraction. The mole ratio between Ca^{2+} ions and the habit modifier was kept at mole ratio of Ca^{2+} : C_{12}hda = 25:1 (see Table S1). The temperature of the reaction mixture was kept at 40 °C and the initial pH was 6.9. As shown in Fig. S14, after 5 minutes from the moment in which the reagents were mixed the precipitate was clearly amorphous, suggesting that the initial polymorph obtained in the investigated reaction was amorphous calcium carbonate (ACC). After 30 minutes a fraction of ACC crystallised to yield vaterite. After 60 minutes some of the vaterite tranformed to form calcite – the thermodynamically stable polymorph. After 24 h calcite was the only crystalline polymorph detected in the precipitate.

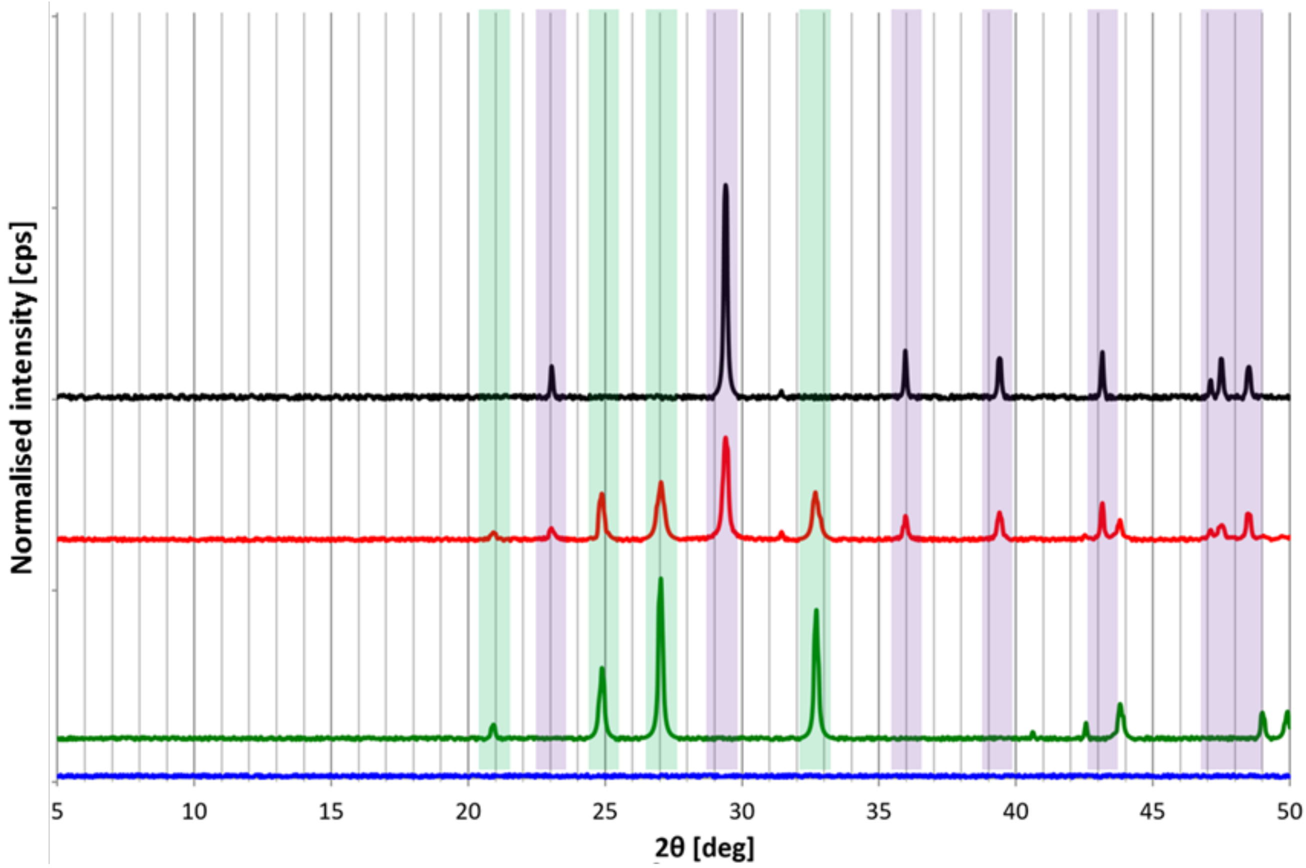


Figure S13. Powder X-ray diffraction patterns recorded for the precipitate forming in a 16.7 mM Ca^{2+} reaction crystallisation mixture (C_{12}hda/Ca^{2+}/HCO_3^- mole ratio 1:25:50, see Table S1); after 5 minutes (blue), 30 minutes (green), 60 minutes (red) and 24 hours (black). The highlighted areas show the typical positions of calcite (violet) and vaterite (green) reflections.

13. SEM images of aragonite crystals precipitated in $CaCO_3$ crystallising mixtures at elevated temperatures

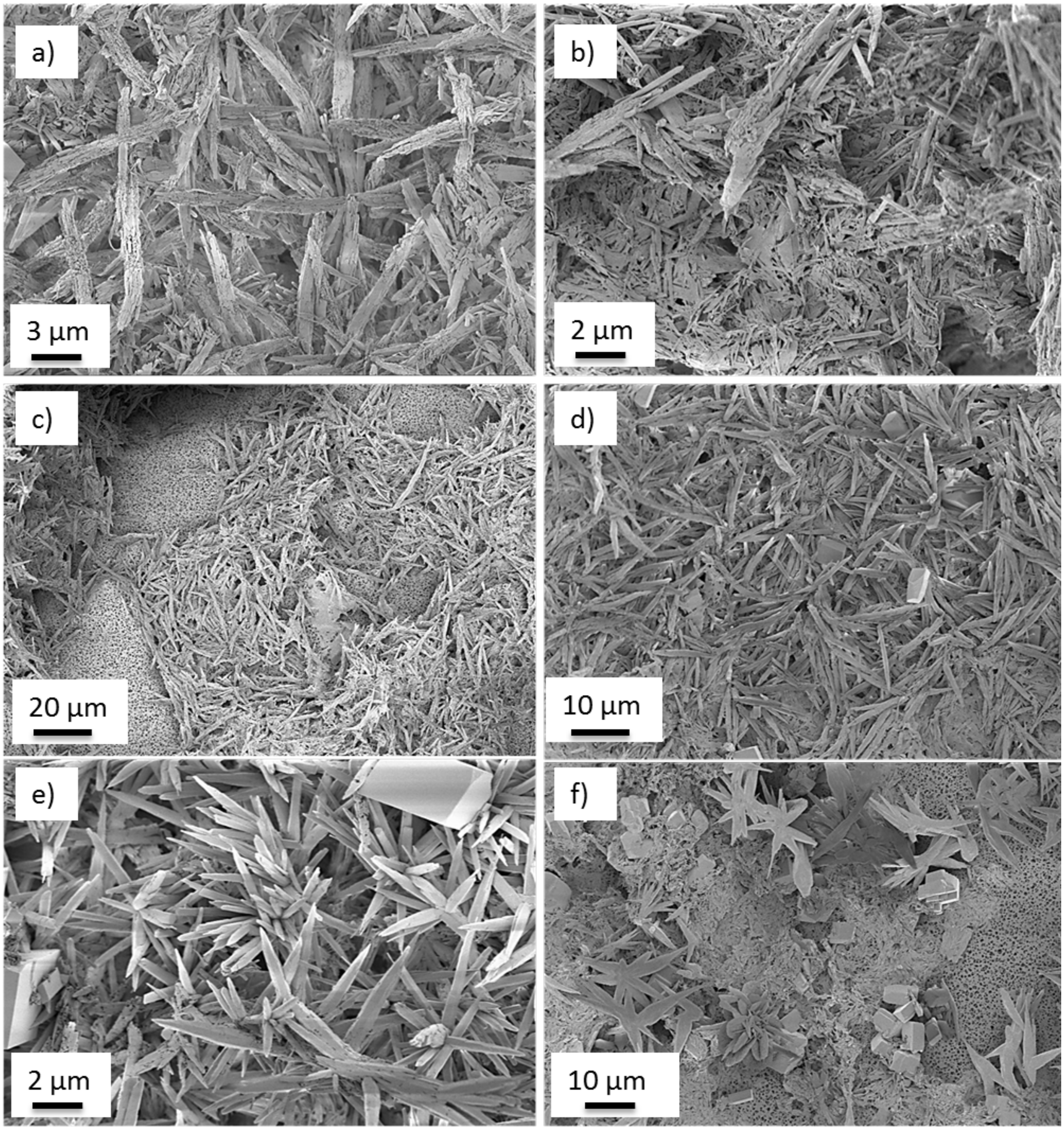


Figure S14. SEM images of calcium carbonate crystals precipitated from a 16.7 mM Ca^{2+} crystallisation mixture (C_{12}hda/Ca^{2+}/HCO_3^- mole ratio 1:25:50, see Table S1); at 90°C (a-b), 80°C (c), 70°C (d), 60°C (e) and 50°C (f). The needle-like aragonite crystals are clearly visible.

13. Additional SEM images of calcite crystals precipitated in $CaCO_3$ crystallising mixtures in the presence of C_{12}hda at different pH values

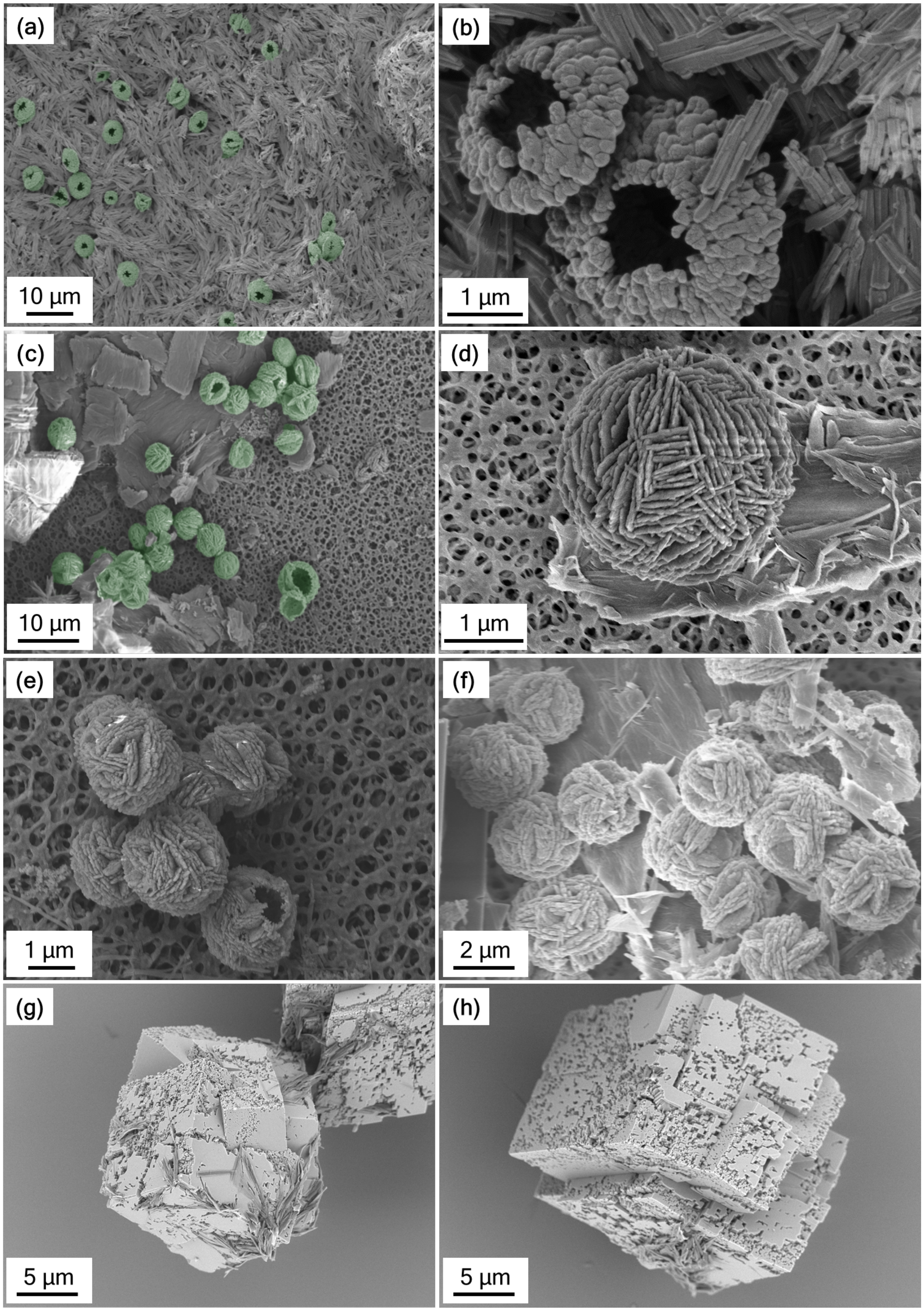


Figure S15. SEM images of calcium carbonate assemblies precipitated from a 16.7 mM Ca^{2+} crystallisation mixture (C_{12}hda/Ca^{2+}/HCO_3^- mole ratio 1:25:50, see Table S1) at different pH values; pH = 6.9 (a-b), pH = 6.5 (c-f) and pH at *ca.* 8.0 (g-h) using $NaOH_{aq}$ (1M) as base.

14. Additional SEM images of the hollow calcite spheres

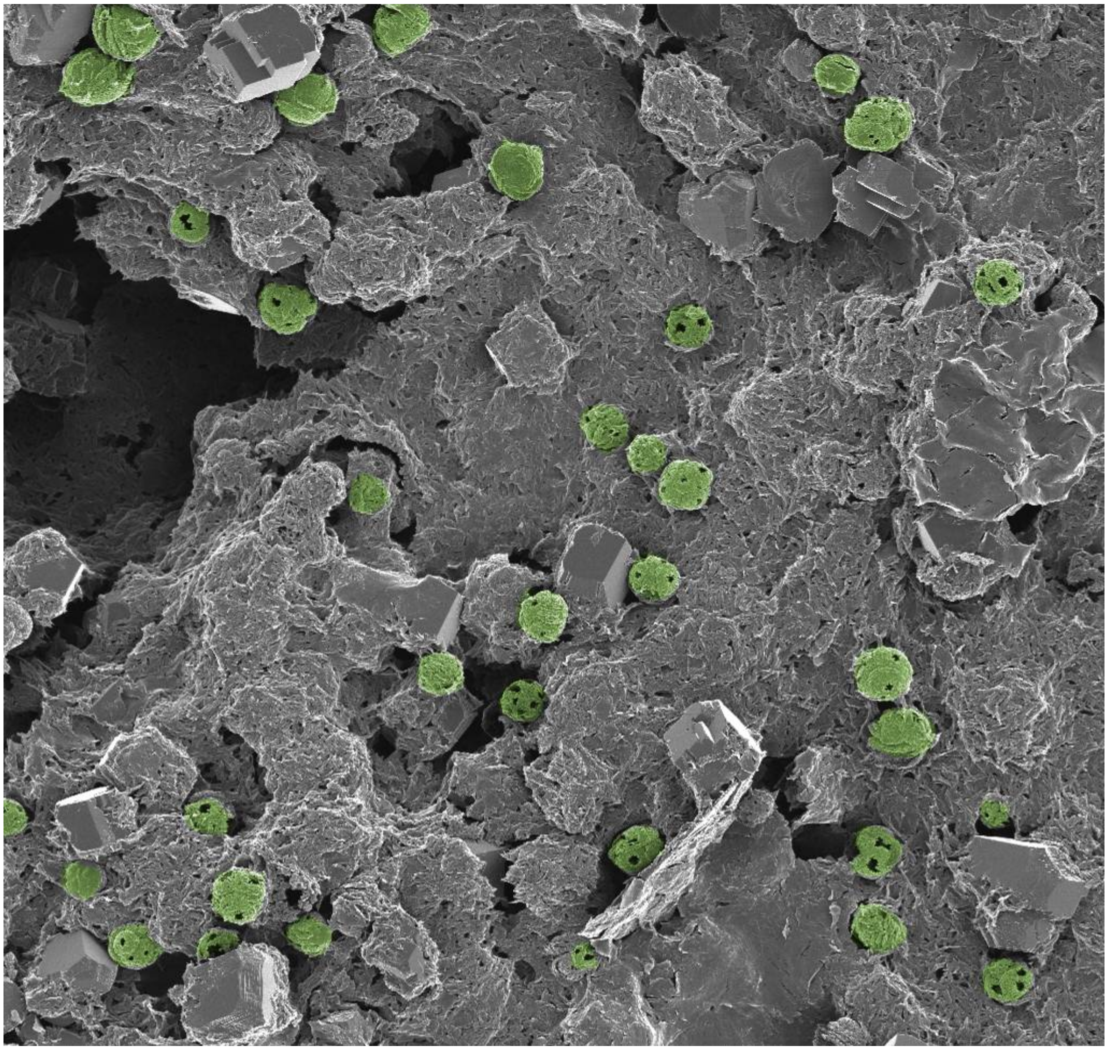


Figure S16. Overview SEM image of hollow calcite spheres; 16.7 mM Ca^{2+} crystallisation mixture (C_{12}hda/Ca^{2+}/HCO_3^- mole ratio 1:25:50, 40 °C, see Table S1).

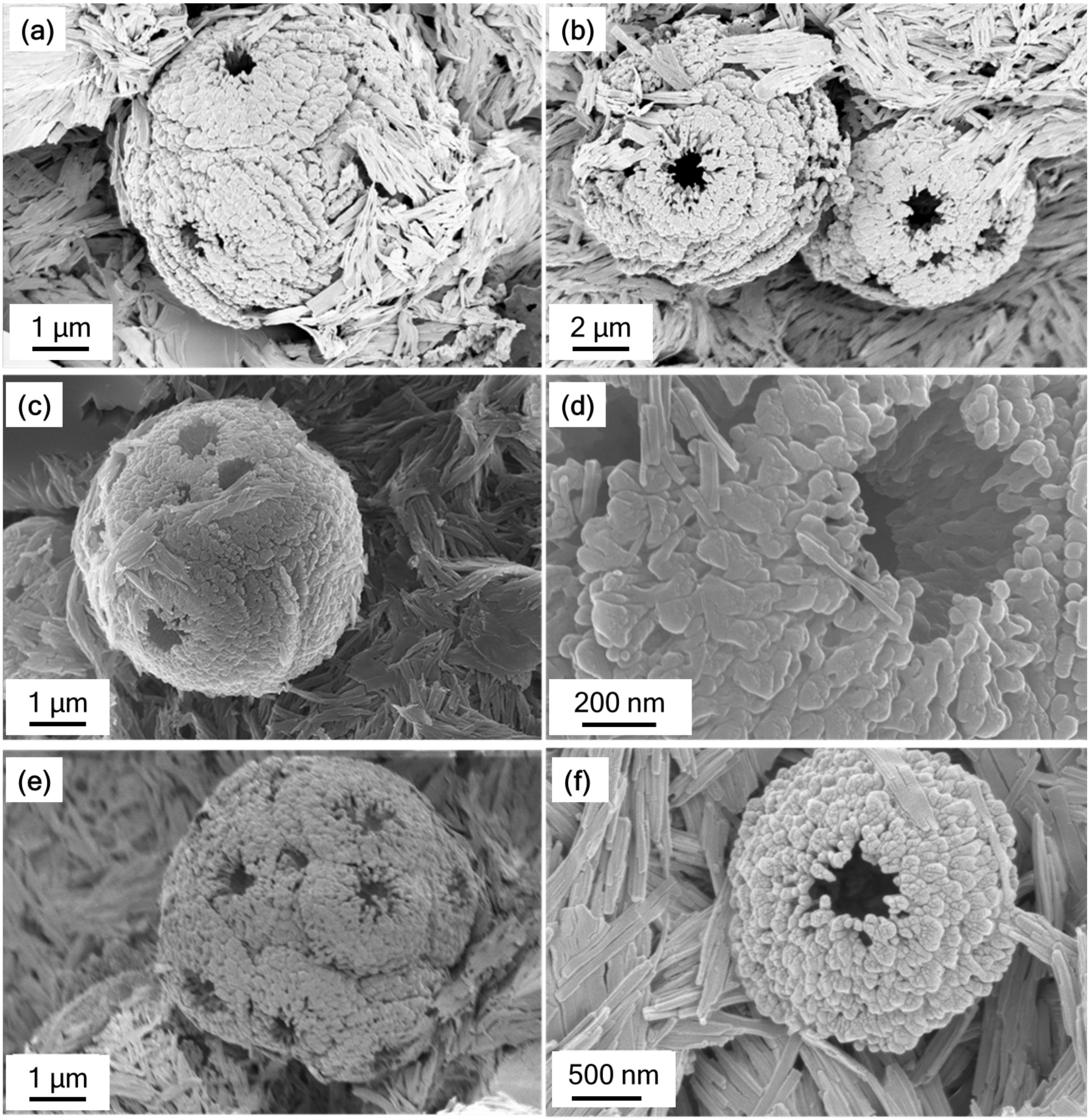


Figure S17. SEM images of hollow calcite spheres composed of organised disks. 16.7 mM Ca^{2+} crystallisation mixture (C_{12}hda/Ca^{2+}/HCO_3^- mole ratio 1:25:50, 40 °C, see Table S1).

15. X-Ray powder patterns of CaCO$_3$-based samples

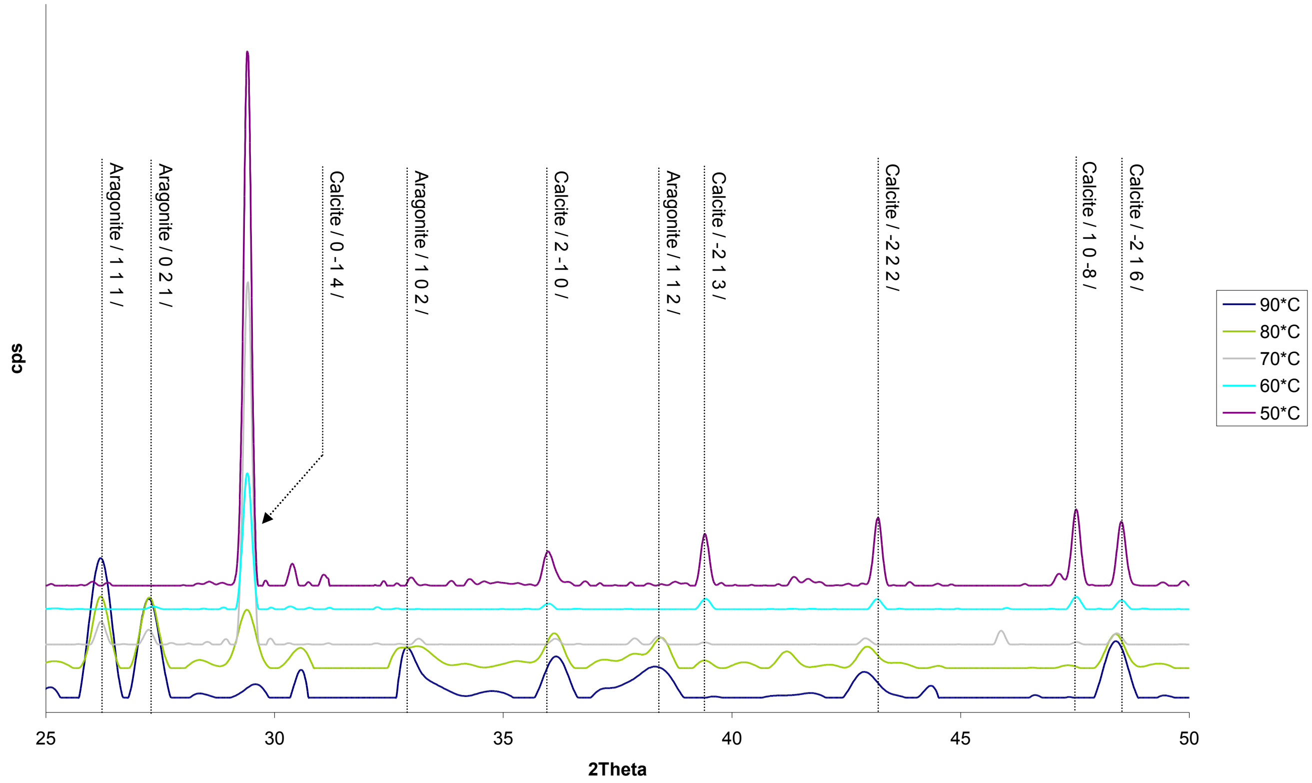


Figure S18. X-ray powder patterns of samples prepared at different temperatures at the C$_{12}$hda/Ca^{2+}/HCO$_3^-$ mole ratio of 1:25:50 (compare Table 1).

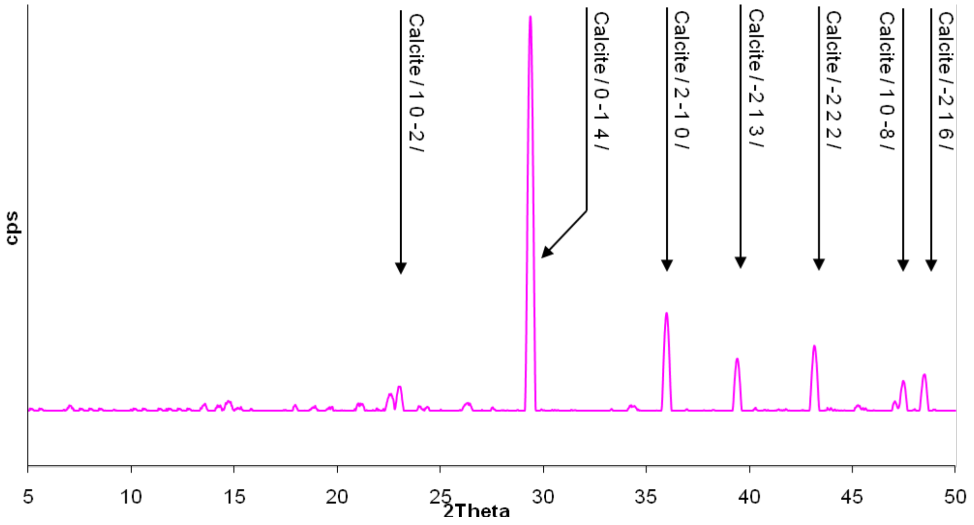


Figure S19. X-ray powder pattern of samples that contain hollow calcite spheres composed of organised disks; 16.7 mM Ca^{2+} crystallisation mixture (C$_{12}$hda/Ca^{2+}/HCO$_3^-$ mole ratio 1:25:50, 40 ^{o}C, see Table S1).

16. EDX mapping of hollow calcite spheres

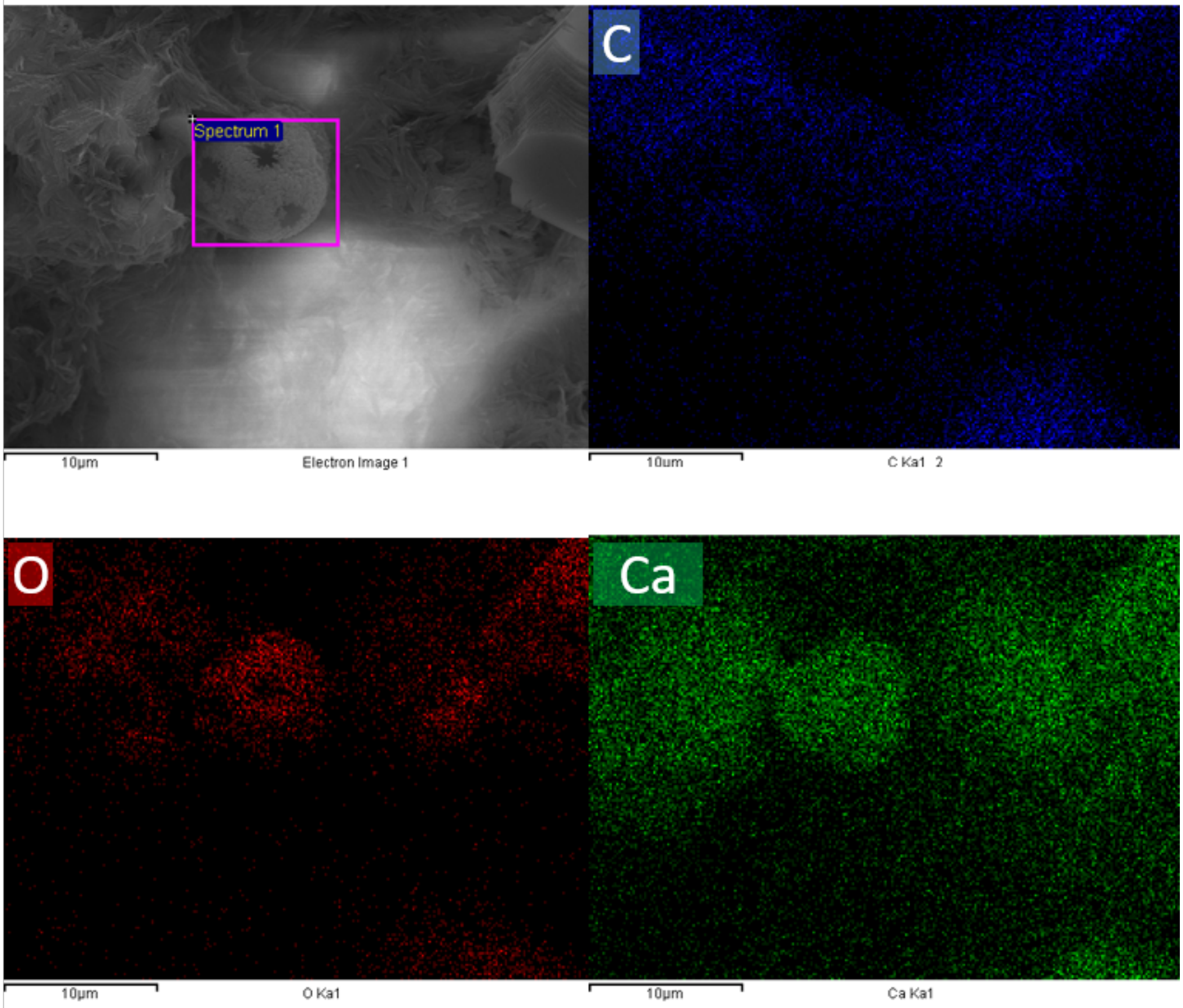


Figure S20. EDX mapping of hollow calcite spheres obtained at a C_{12}hda/Ca^{2+} ratio 1:25 at 40˚C. a) SEM micrograph; blue: carbon distribution; red: oxygen distribution; green: calcium distribution.