Electronic Supplementary Info

In situ continuous growth formation of synthetic biominerals

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Experimental

Materials

Chitosan (CS) (Pure Science, degree of deacetylation = 88±2%, M_w = 100,000 to 300,000 g mol^{-1}), acetic acid (Univar, AR grade), poly(acrylic acid) (PAA) (Sigma-Aldrich, average M_w = 1,800 g mol^{-1}), chitosan oligosaccharide lactate (Aldrich and Carbomer, average M_w= 5,000 g mol^{-1}), $CaCl_2.2H_2O$, $NaHCO_3$ (Panreac) and $CaCO_3$ (Univar, AR grade) were used as received. Deionised water (18.2 MΩ cm resistivity, Sartorius arium 611UV purification system) was used in all solutions.

Oligomers of differing molecular weights and from various sources can be used in this method. Chitosan oligomers which have been used include chitosan oligomers prepared in the laboratory with low target molecular weights, and commercial oligomers from Sigma-Aldrich and Carbomer with molecular weights of approximately 5,000 gmol^{-1}. This variety of suitable chitosan oligomers shows that the system is robust and adaptable to a variety of components.

Chitosan and chitin templates

CS (0.400 g) was stirred in 20.0 mL H_2O, acetic acid (0.200 mL) was added and the mixture was stirred for 1 h. The 2% (wt/wt) CS solution in 1% (v/v) acetic acid solution (20 mL) was centrifuged at 3,000 rpm for 30 min, and poured into circular 9 cm diameter plastic Petri dishes and dried at 50ºC for 24 h.

The chitosan films were treated with base or acetic anhydride to give chitosan or chitin films, respectively. NaOH solution (30 mL, 1.00 M) was added and the film was left to set for 1 h. The chitosan film was subsequently washed repeatedly until the solution was neutral. The chitosan templates were reacetylated as described elsewhere[1]. Acetic anhydride (5.0 mL) dissolved in methanol (25 mL) was applied to the chitosan template and left overnight at ambient temperature. The acetylated film was rinsed with ethanol and washed thoroughly with water. The degree of deacetylation = 40 ± 10%.

Poly(electrolyte) complex formation

The formation of a PEC between the PAA and the chitosan scaffold or oligomers was investigated, and characterised by IR spectroscopy and DSC. The chitosan template was soaked overnight in 30 mL of a 13.3 mg/mL PAA solution (1:1 ratio of chitosan to PAA). The template was gently washed with water and left at ambient temperature overnight to dry. An aqueous solution of chitosan oligomers was mixed with a solution of PAA (5:1 ratio of

chitosan oligomers to PAA) and a cloudy suspension formed quickly. The suspension was left overnight and dried in a 50°C oven for 2 hours, then the solid was broken up to form a powder.

Mineralisation

The mineralisation process was performed according to previous work [2]. Briefly, the organic scaffolds were sequentially soaked in two presoaking solutions which contained $CaCl_2$ and $NaHCO_3$, respectively, for 24 h followed by a saturated calcium carbonate solution (Kitano solution[3]) for five days. The three solutions were prepared as follows: 500 mM $CaCl_2$ were bubbled with CO_2(g) at 3 L h^{-1} for 1 h, 500 mM $NaHCO_3$ were bubbled with CO_2(g) at 3 L h^{-1} for 1 h, and $CaCO_3$(s) (2.61 g) was added to 1.00 L of H_2O and bubbled with CO_2(g) for 6 h at 3 L h^{-1}. The $CaCO_3$ suspension was double filtered and CO_2(g) bubbled through the filtrate at 3 L h^{-1} for 1 h. A solution of PAA dissolved in water was added to each crystallisation solution to give final concentrations of 500 mM $CaCl_2$ and $NaHCO_3$, respectively, and 0.267 mg mL^{-1} PAA (2 wt% with respect to the polymer template). The scaffold was then exposed to the solution. After 30 min of exposure an aliquot of an aqueous chitosan oligomer solution was added and the system left undisturbed for approximately 24 h. Solutions containing various amounts of chitosan oligomers (10, 20, 30 wt% with respect to the scaffold) were trialled, and of these 10 wt% was preferred.

The alternate soaking method for mineralisation was performed with the same solutions and concentrations as described above. The polysaccharide scaffolds were soaked in calcium chloride solution followed by sodium bicarbonate solution each for 24 h, the soaking cycle of calcium chloride and sodium bicarbonate soaking was repeated three times. As for the combined alternate soaking/Kitano method, chitosan oligomers can be incorporated into the alternate soaking mineralisation method, 30 minutes after exposure of the scaffold to each of the soaking solutions.

Characterisation

Samples analysed by IR spectroscopy and powder XRD were ground up in liquid nitrogen into a fine powder and dried at 50ºC for one hour. The IR spectra were acquired with a Perkin-Elmer Spectrum One FT-IR spectrometer with 8 scans using pressed KBr discs.

TGA was performed on a Shimadzu TGA-50 instrument. The samples were run as duplicates. Approximately 5 – 10 mg of sample was loaded in a platinum pan and heated from ambient temperature to 1000°C at 10°C/min in an air atmosphere with a flow rate of 50 L/min. The data were processed with Shimadzu TA-60 thermal data analysis software. The TGA traces contained a peak at approximately 72°C due to water evaporation, peaks at 308 and 546°C due to thermal degradation and decomposition of the organic polymer, and a peak at 686°C due to the decomposition of calcium carbonate to calcium oxide. Residual mass was due to the breakdown product calcium oxide. The percentage of calcium oxide was calculated for a dry sample (percentage of residual mass divided by 100 minus the percentage of water evaporation), and was calculated as a percentage of the theoretical mass of calcium oxide remaining in 100% calcium carbonate, to give the percentage inorganic material in the composite materials. The uncertainties were calculated assuming a measurement error of 0.02 mg in the mass losses in the TGA.

DSC traces of the materials were recorded with a Shimadzu DSC-60 differential scanning calorimeter. Approximately 5-10 mg of sample were sealed in a crimped aluminium pan and lid, and heated under nitrogen at 10ºC/min for an initial run (to remove water) from 50 to 160ºC, held at 160ºC for 5 minutes then cooled to 50ºC at -10ºC/min. The second run

was heated under nitrogen at 10ºC/min from 50 to 400ºC. The data were processed with Shimadzu TA-60 thermal data analysis software. The instrument was calibrated with indium.

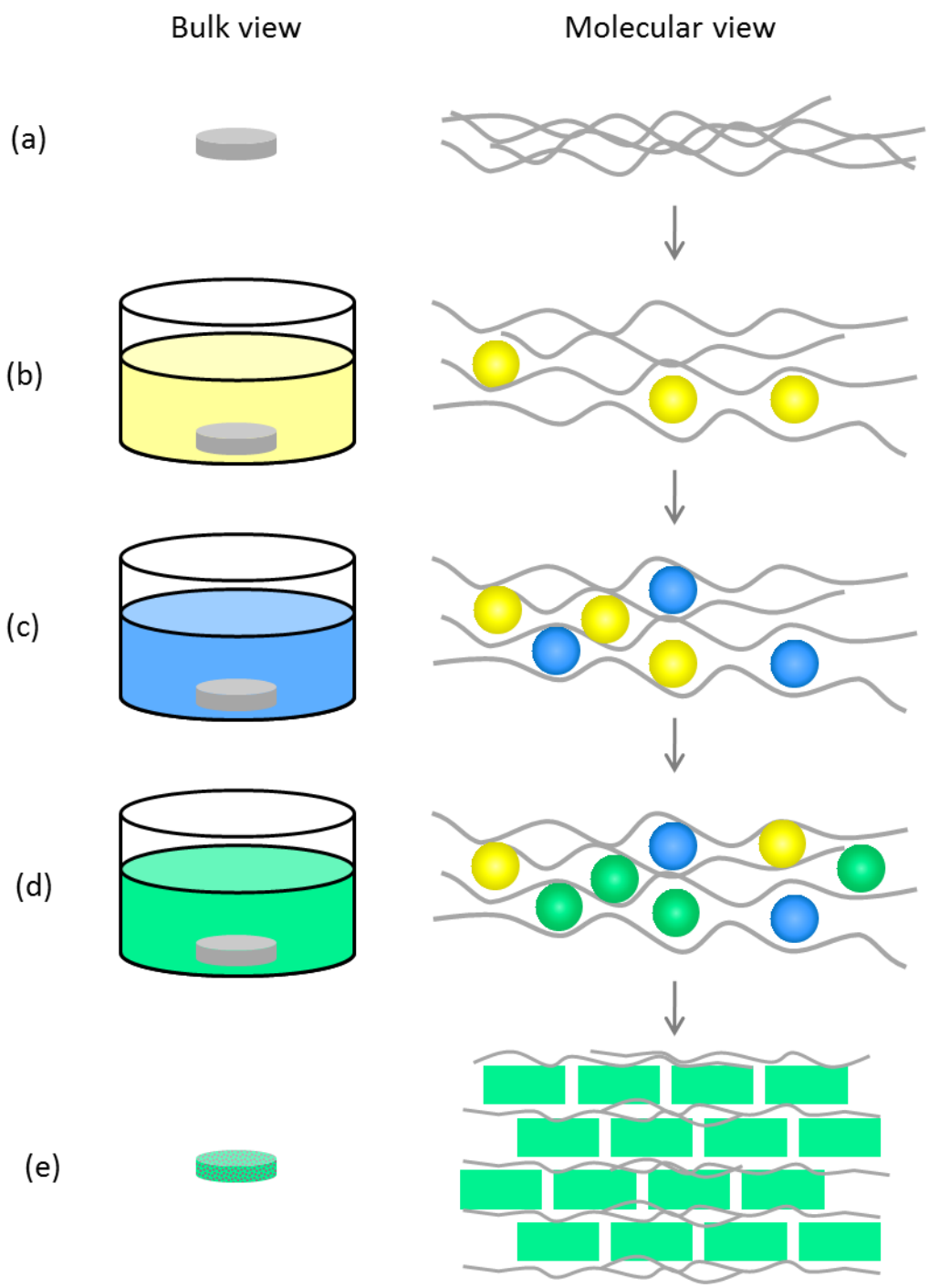
XRD patterns were acquired with a PANalytical X'Pert Pro powder difffractometer using Cu/Kα radiation. The data were obtained with generator settings of 45 kV and 40 mA, and scans were acquired with 2θ from 5° to 80°. The diffraction patterns were compared to diffraction patterns of known compounds from the database, using the software X'Pert Highscore 2.2c. Calculations of the various amounts of the three anhydrous polymorphs of calcium carbonate were performed using the software or from the peak areas for the peaks at 25.0° (vaterite), 29.5° (calcite) and 45.9° (aragonite) as described elsewhere[4].

SEM samples were mounted on aluminium stubs with double sided carbon tape. Cross-sectional samples were prepared by mounting a piece of the sample against a carbon-tape coated cut stub, with the sample protruding above the top of the stub. A folded over piece of carbon tape was applied, level to the top of the stub. A second piece of carbon tape was pressed onto the tape and the sample. The whole stub was frozen in liquid nitrogen, and the tape and sample were fractured while frozen, then thawed. The samples were coated with three coats of carbon using a Quorum Q150T coater. SEM images were acquired using a JEOL JSM-6500F field emission SEM in SEI and BEI mode. An accelerating voltage of 10.00 kV and a probe current of 9.0 μA were typically used. The instrument was generally operated at a working distance of approximately 10 mm.

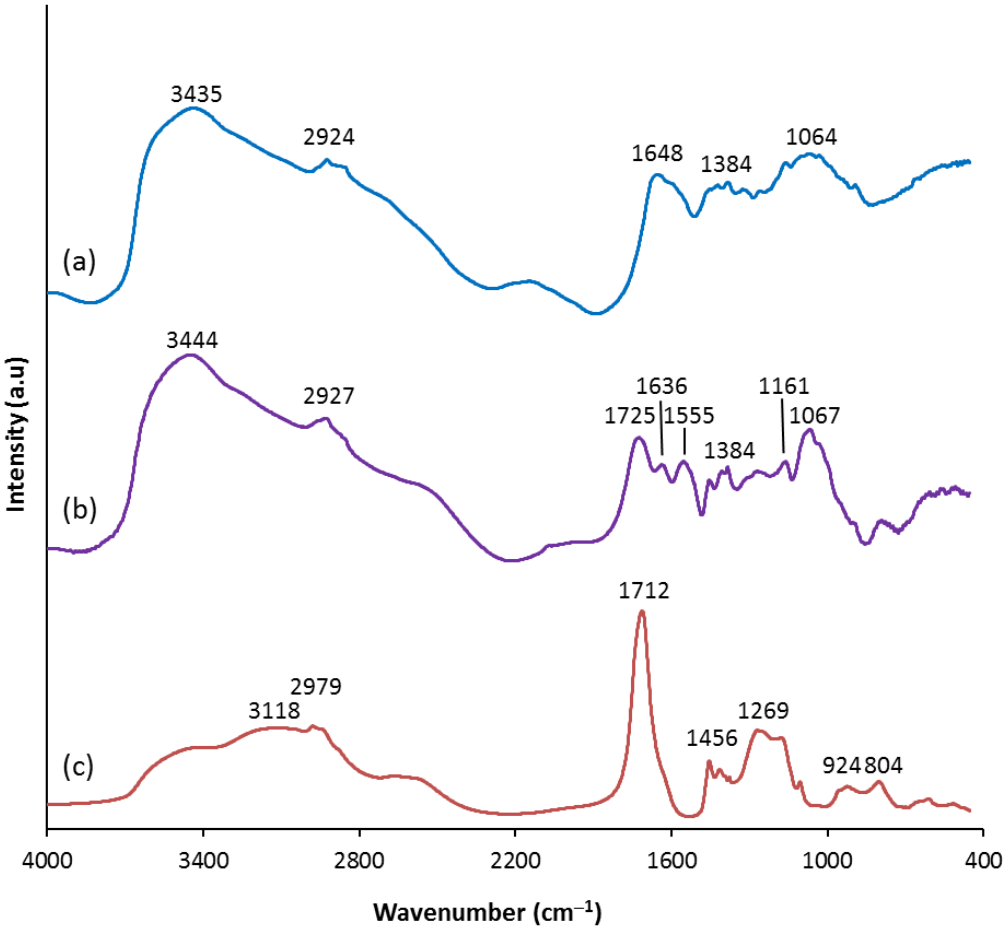
Tables and Figures

ESI Table 1 – TGA data of chitosan and chitin composites prepared with or without oligomers

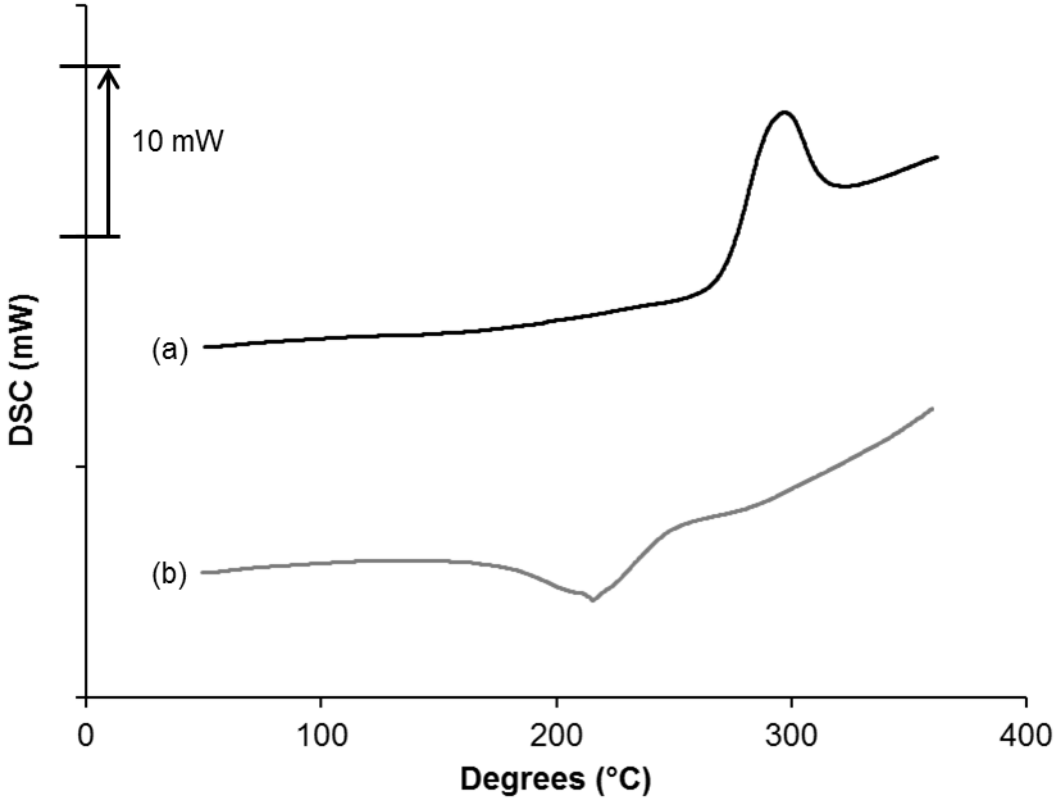
Scaffold	*Oligomers?*	*% Organic*	*% Inorganic*
Chitosan	No	79 ± 4	21 ± 4
Chitosan	Yes	71 ± 7	29 ± 7
Chitin	No	73 ± 8	27 ± 8
Chitin	Yes	83 ± 7	17 ± 7



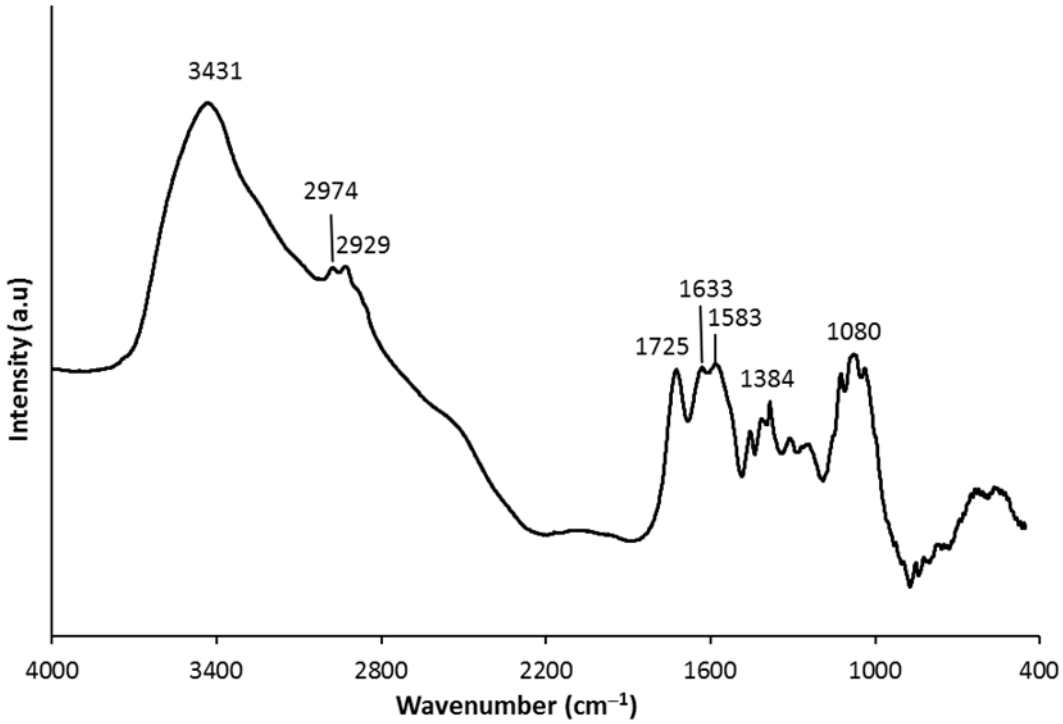
ESI Scheme 1 – A schematic showing the (a) chitosan gel which was soaked in (b) calcium chloride and (c) sodium bicarbonate solutions to preconcentrate the ions of interest, followed by (d) saturated calcium carbonate solution to form (e) a mineralised chitosan composite material. The same mineralisation technique was also used for chitin. All three solutions contained PAA, and chitosan oligomers were added 30 minutes after the chitosan gel was placed in each solution.



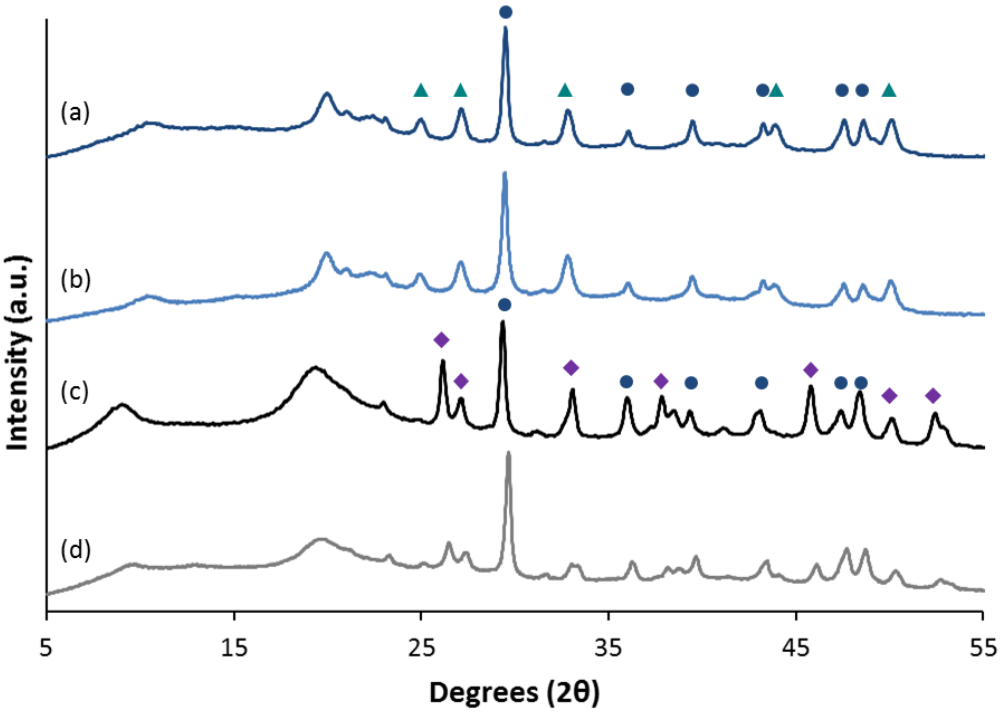
ESI Figure 1 – IR spectra of (a) chitosan, (b) chitosan/PAA poly(electrolyte complex) and (c) PAA. The spectrum of the PEC (b) has differences in the peak intensities compared to the chitosan spectrum (a), and the appearance of a new peak at 1725 cm^{-1}, which are due to the formation of the PEC. If the PAA stayed in solution and did not interact with the chitosan scaffold, the spectrum (b) would remain the same as (a).



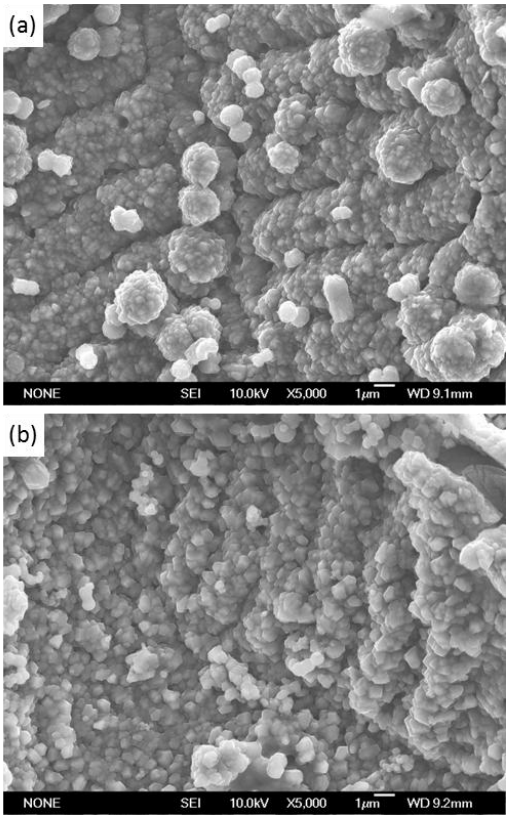
ESI Figure 2 – DSC traces of (a) chitosan and (b) a chitosan/PAA poly(electrolyte) complex. The exothermic peak of chitosan at approximately 297ºC is attributed to the oxidative degradation of the chitosan chains[5] . The DSC trace of PAA powder (not shown) displayed an endothermic peak at approximately 260ºC attributed to thermal degradation[6]. An endothermic peak centred at approximately 210ºC is present in the chitosan/PAA complex trace. Previous work has attributed this endothermic peak in chitosan/PAA complexes to cleavage of the electrostatic interactions between the oppositely charged polymers[6]. These results confirm that a polyelectrolyte complex was formed between the chitosan and PAA.



ESI Figure 3 – IR spectrum of the precipitate formed upon mixing aqueous solutions of chitosan oligomers and PAA. The similarities in this spectrum to the spectrum given in ESI Figure 1b and in particular the peak at 1725 cm^{-1} confirm that the precipitate formed is a poly(electrolyte complex) formed between the PAA and chitosan oligomers.



ESI Figure 4 – XRD of calcium carbonate composites prepared with (a) chitosan, (b) chitosan and oligomers, (c) chitin and (d) chitin and oligomers. The peaks for each polymorph are shown by circles (calcite), triangles (vaterite) and diamonds (aragonite).



ESI Figure 5 – SEM of chitin/PAA/calcium carbonate composite materials prepared with three cycles of alternate soaking applied in the (a) absence and (b) presence of chitosan oligomers (20% oligomers with respect to the initial film mass).

References

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