Freeze-Drying Yields Stable and Pure Amorphous Calcium Carbonate (ACC)

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Experimental Methods

Materials: The chemical reagents used in the experiments were either purchased from Fischer Scientific (hydrogen peroxide 30% w/v, nitric acid in aqueous solution 70% w/v and Sodium Sulfate anhydrous 99%) or were ordered from Sigma Aldrich (sodium carbonate anhydrous 99.8% w/w, calcium chloride dihydrate 99% w/w and sodium phosphate dibasic heptahydrate 98.0-102.0%). Reagents were used without further purification. Solutions were prepared using Milli-Q Water (18.2 M Ω cm).

Glassware Preparation: To ensure that no impurities were introduced into the synthesised ACC, the glassware was cleaned as described here. Glassware used to store reagents (e.g. ball flasks, Shott bottles and volumetric flasks) were soaked overnight in 10% w/v sodium hydroxide and were then rinsed with diluted hydrochloric acid, before finally washing with Milli-Q water. Glassware used to hold the saturated solutions of minerals was placed overnight in Piranha solution (70:30% wt sulfuric acid: hydrogen peroxide) and were then washed copiously with Milli-Q water, before sonicating for 15 min, re-washing with Milli-Q water and drying under Nitrogen.

Synthesis of Amorphous Calcium Carbonate: ACC produced by freeze-drying requires the production of "counter-ion free", saturated CaCO₃ solutions. "Pure" solid CaCO₃ was formed by repeated dissolution and re-precipitation of CaCO₃ which had been obtained by combining 1 M CaCl₂ and 1 M Na₂CO₃. Saturated solutions were then prepared by adding 2.5 g of "pure" CaCO₃ to 500 ml Milli-Q water, and were stored for 24 hours at room temperature before centrifuging to remove the majority of any remaining un-dissolved calcium carbonate. This saturated solution was then cooled to remove possible ghost nuclei, and was then filtered through a 0.2 µm membrane filter. Freezing of the prepared statured solutions (1 - 400 ml volumes) was achieved by slow immersion into a liquid nitrogen bath, followed by a 10 minute annealing period to strengthen the ice structure. As an example of reaction conditions, immersion in liquid nitrogen of 30 ml of saturated CaCO₃ solution held in a $10 \times 2.5 \times 2.5$ cm Teflon holder at a rate of ~0.5 cm min⁻¹ resulted in solidification in ≤ 15 min and returned ACC. ACC formation occurs during the freezing process and is based on rapid water removal due to solvent crystallisation. The reacting ions, Ca^{2+} and " CO_3^{2-} " are pushed away from the ice front into smaller and smaller liquid reservoirs as freezing progresses, creating highly supersaturated environments in which ACC ultimately precipitates. The ACC is then stabilised against crystallisation due to the complete solidification of the solvent. Subsequent sublimation (Labcono FreezeZone 1, 50mBar, -49°C) of the excess solvent delivers the freeze-dried ACC. Slower immersion giving solidification in \geq 30min resulted in calcite.

Synthesis of Amorphous Calcium Phosphate: A counter-ion free, saturated solution of calcium phosphate was prepared by repeated dissolution and re-precipitation of calcium phosphate formed by mixing 200 mM CaCl₂.2H₂O with 200 mM Na₂HPO₄-7H₂O. Amorphous calcium phosphate (ACP) was then produced from this solution using the method described for ACC.

Characterisation: Raman (Renishaw invia Raman Microscope, 785nm diode laser as excitation source, focused onto the sample using a 50x (NA ¹/₄ 0.75) objective) and FTIR spectroscopy (Perkin Elmer Spectrum 100 FT-IR Spectrometer equipped with a universal ATR sampling accessory) were used to characterise the phases of the minerals formed. Imaging was performed using a LEO 1530 Gemini FEG-SEM operating at 3 kV using an in-lens detector mode. TEM was carried out using a FEI Tecnai TF20 FEG-TEM fitted with an HAADF detector and a Gatan Orius SC600A CCD camera, operating at 200 kV. That the solids produced were fully amorphous (as judged by the absence of sharp reflections corresponding to crystalline polymorphs) was confirmed using Powder X-ray Diffraction (PXRD), carried out using a Bruker D8 Advanced diffractometer equipped with an X-ray source emitting Cu K α_1 radiation. Samples were placed on a piece of silicon wafer, and XRD data were collected in an angular range between 5° and 70° in intervals of 0.02°. Thermogravimetric analysis (TGA) (TA Instruments STD Q600, 5°C/min, 100ml/min N₂) was used to obtain information on the sample composition of the amorphous solids.

Figure S1. Analysis of particle size distribution of amorphous calcium carbonate (ACC) particles produced using freeze-drying, using dynamic light scattering (DLS) of particles dispersed in ethanol.



Figure S2. (A) Powder XRD and (B) EDX spectra of amorphous calcium carbonate (ACC) formed via freeze-drying. (B) Energy Dispersive X-ray spectrum (EDX) of ACC formed via freeze-drying, where the sample is supported on a Ni TEM grid. The spectra confirm the amorphous character of the ACC and demonstrate the absence of secondary ions.



Figure S3. Raman spectra of freeze-dried ACC, held under normal atmospheric conditions, over a time-frame of 6 weeks. The ACC is still amorphous after 4 weeks, but has crystallised to calcite after 6 weeks.



Figure S4. (A) Raman and (B) FTIR Spectra of amorphous calcium phosphate (ACP) obtained via rapid freeze-drying (---) and by direct combination of 200 mM $CaCl_2.2H_2O$ and 200 mM $Na_2HPO_4.7H_2O$ (-). (C) Powder XRD of ACP obtained via freeze-drying.



Figure S5. Thermogravimetric analysis (TGA) of ACP obtained via rapid freeze-drying, obtained at an applied heating rate of 10 °C min⁻¹ and a nitrogen flow of 100 ml min⁻¹. The data reveals a calcium to phosphate ratio of ~ 1.70 .¹



References

1. L. Sun, L. C. Chow, S. A. Frukhtbeyn and J. E. Bonevich, *Journal of Research of the National Institute of Standards and Technology*, 2010, **115**, 243-255.