Ethanol assisted synthesis of pure and stable amorphous calcium carbonate nanoparticles†

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Stable monodispersed amorphous calcium carbonate (ACC) nanoparticles can be synthesized in ethanol media by a facile method, and crystallization of ACC is kinetically controlled, resulting in the formation of three polymorphs in a mixed solvent of ethanol-water at different pH values.

Amorphous calcium carbonate (ACC) plays a role in the formation of many biominerals and also in the initial crystallization stage of biomimetic mineralization.¹ Recent research work in the field of non-classical crystallization has described amorphous nanoparticles as intermediates between pre-nucleation clusters and crystals in solution, for example, calcium phosphate and calcium carbonate.^{2–4} ACC is extensively found in living organisms as a precursor for mineralization or a storage phase for calcium carbonate.^{5–9} Based on the analysis of local atomic environments of ACC by Infrared spectroscopy (IR), solid-state Nuclear Magnetic Resonance (NMR) spectroscopy and extended X-ray absorption fine structures (EXAFS), the local arrangement of atoms in ACC can differ between various ACCs.^{10–12} Corresponding to the three main polymorphs of CaCO₃, ACC can be classified into proto-calcite, proto-aragonite and proto-vaterite types by the short range order of the atoms as detected by NMR and EXAFS techniques.^{13,14} Thus ACC can form polytypes.¹⁵

On the other hand, ACC can also be classified into transient and hydrated ACC by its water content.⁵ Transient ACC contains no water or less than one third water molecule per calcium carbonate molecule and has been so far only found in biominerals.^{5,6,11} Synthetic ACC is generally hydrated, consisting of at least one water

molecule per calcium carbonate.¹⁶ From the viewpoint of thermodynamics, ACC is an unstable phase and tends to crystallize. However, it was discovered that transient biogenic ACC has not only served as a precursor for subsequent non-classical crystallization,^{5–7,11,17,18} but also has been stable for the whole lifetime of some organisms,^{19–21} where magnesium cations and phosphate and silicate anions were identified to prevent crystallization of transient ACC materials.^{19,22–24}

Current methods to synthesize hydrated ACC include direct mixing of soluble calcium and carbonate salts with or without introduction of additives,^{25,26} bubbling CO₂ into calcium salts or calcium hydroxide solution,^{12,27} as well as decomposition of dimethylcarbonate in a calcium salt solution.²⁸ To prepare “stable” ACC, similar impurities found in transient ACC were introduced to delay the crystallization of synthetic ACC, such as magnesium ions,^{13,29,30} polyphosphonates,³¹ polyaspartate or hydrophilic polymers,^{13,32} and some others.^{33,34} Furthermore, in polymer mediated biomimetic mineralization,³⁵ it is frequently found that a synthetic ACC phase adsorbs hydrophilic polymers to form hybrid precursors,^{36,37} as feedstock for crystallization and formation of building blocks with different shapes³⁸ for further meso-scale transformation to complex structures under the control of the polymer,³⁹ and even formation of a large area of a poly/single-crystalline CaCO₃ film⁴⁰ or a three dimensional porous CaCO₃ network. So far, most studies involving preparation of stable ACC have introduced organic molecules or inorganic ions to prevent ACC from crystallization.

Here, we present a simple method to prepare pure stable ACC nanoparticles on a large scale without introduction of any additives (see the Experimental section in ESI†). The ACC colloids are uniform monodispersed nanoparticles with a diameter of 100–200 nm. The crystallization behaviour of these ACC nanoparticles in the water–ethanol system is then investigated.

The XRD pattern of the as-synthesized glassy blue powder presents two broad peaks at 30 and 45 degrees, respectively, indicating an amorphous phase (Fig. 1a). The Fourier transform infrared (FT-IR) spectroscopy spectrum of the powder (Fig. 1b) shows typical characteristics of an ACC phase. Bands at 864 and 1075 cm^{–1} can be attributed to out-of-plane bending (ν_2)

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Fig. 1 (a) XRD pattern and (b) FT-IR spectrum of the ACC sample.

and symmetric stretch (ν_1) in non-centrosymmetric structures.⁴¹ The band at 693 cm^{-1} can be assigned to O–C–O bending (ν_4), and the splitting peaks at 1417 and 1474 cm^{-1} can be attributed to the asymmetric stretch of the carbonate ions (ν_3), which are typical characteristics of ACC vibrations.¹¹ The peak at around 1635 and the broad band at around 3399 cm^{-1} are due to vibrations of structural water molecules of ACC.^{14,22,42} An obvious difference in IR spectra between proto-calcite and proto-vaterite ACCs is that proto-vaterite ACC has two ν_1 bands (1071 and 1026 cm^{-1}), while proto-calcite ACC only has one ν_1 band (1074 cm^{-1}).¹⁴ Hence, we regard the as-synthesized ACC as being in the proto-calcite form.

Thermogravimetry (TG) analysis (ESI,† Fig. S1) proves that this kind of ACC is hydrated, giving a form of $\text{CaCO}_3 \cdot \text{H}_2\text{O}$. The size distribution of ACC particles measured by analytical ultracentrifugation (AUC) is shown in Fig. 2. It is observed that the size distribution of ACC particles is narrowly distributed at around 125 nm when the reaction time is 2 days. The size distribution of ACC particles increases to a narrow distribution at around 175 nm when the reaction time is prolonged to 3 days. Besides the distributions centered at 125 or 175 nm , there are small shoulders at the small diameter zone of the AUC curves. It is worth noting that centrifugation and then re-dispersion of the as-synthesized ACC nanoparticles (after reaction for 2 or 3 days, dashed lines) do not change their size distribution, indicating the formation of stable, monodispersed nanoparticles. In addition, ACC nanoparticles after a longer reaction time (7 days) show two different distribution bands at 20 – 100 and 125 – 200 nm . The formation of ACC nanoparticles is a continuous process, in which the size of initially formed ACC nanoparticles increases and new secondary small ACC nanoparticles form, due to the lack of decomposed gas (NH_3 , CO_2 , H_2O) from NH_4HCO_3 at the beginning of synthesis. Hence, the size distribution by AUC always has a small shoulder which is located at the small diameter zone. Two clear bands of size distribution formed when the reaction time was long enough (7 days).

Currently, the size distribution of ACC nanoparticles depends on the synthesis process. For example, ACC nanoparticles synthesized by rapidly mixing CaCl_2 and NaCO_3

Fig. 2 Particle size distribution of as-synthesized ACC using Analytical Ultracentrifugation.

Fig. 3 (a) ACC as synthesized, after reaction for 3 days; (b) Tyndall light scattering of diluted ACC ethanol dispersion from ACC solution shown in (a); (c) TEM image of ACC nanoparticles after reaction for 2 days; and (d) electron diffraction pattern (ED) of as-synthesized ACC.

aqueous solutions have a size distribution of 50 – 400 nm ,⁴² while the size would decrease to about 2 – 3 nm if poly(acrylic acid) is used as a stabilizer.²⁵ In another case, the size distribution of ACC ranges from 400 to 1000 nm , depending on the decomposition temperature of dialkyl carbonate.²⁸ Fig. 3a presents a bottle of ACC colloidal suspension in ethanol after reaction for 3 days. The ACC nanoparticles are well suspending in ethanol. The Tyndall effect was observed in a diluted ACC ethanol suspension by an incident light beam, demonstrating a stable dispersed nanoparticle system (Fig. 3b). The TEM image (Fig. 3c) presents ACC nanoparticles with size ranging from 40 to 140 nm (see statistical analysis in ESI,† Fig. S2), which corresponds to the size distribution from AUC analysis shown in Fig. 2. Furthermore, the absence of signals in the ED pattern indicates that the nanoparticles shown in Fig. 3 are amorphous.

Crystallization of ACC is kinetically controlled. The intrinsic structure of ACC has little influence on the final crystalline polymorph.¹⁴ Dried synthetic ACC powders would crystallize and form a calcite phase in moist air on the time scale of 4 – 12 hours. When dried ACC powder was immersed in acidic (pH 5) or neutral water, uniform rhombohedral calcite crystals precipitated quickly.

In a mixed solvent of ethanol and water, in which the water content is fixed at 15% , a subsequent polymorph transition from a thermodynamic stable calcite to a metastable vaterite phase was observed by increasing the pH value of water (Fig. 4a–d; ESI,† Fig. S3). Pure calcite with typical rhombohedral shape was synthesized by introduction of acidic water (pH 5, Fig. 4a). Increasing the pH value to 8 – 12 , metastable vaterite and aragonite phases were captured (Fig. 4b and c), stably co-existing with the dominant calcite phase in its mother solution for at least one month.

Further increasing the pH value to 12.5 , nearly a pure vaterite phase was captured, with trace amounts of the calcite phase (Fig. 4d). If the pH value was increased to 14 (1 M NaOH), crystallization of ACC was inhibited, and thus no crystalline peaks were observed when the mixture was kept for 3 days at room temperature (XRD data not shown here).

The crystallization and polymorph selection of CaCO_3 from Ca^{2+} and CO_3^{2-} ions in solution strongly depend on pH values.⁴³ In the case of crystallization of ACC, furthermore, the pH value of water in a mixed solvent of water and ethanol affected solubility of ACC, and thus supersaturation degree of Ca^{2+} and CO_3^{2-} ions. It is well known that CaCO_3 as a salt tends

Fig. 4 XRD patterns of the samples: (a) 1.5 mL water (pH 5); (b) 1.5 mL water (pH 8); (c) 1.5 mL water (pH 12); (d) 1.5 mL water (pH 12.5); (e) 2.5 mL 1 M NaOH. The inserted column diagram in (b) aragonite phase (JCPDS no. 41-1475, labeled by an asterisk), in (d) vaterite phase (JCPDS no. 33-0268, labeled by a plus sign), in (e) calcite phase (JCPDS no. 05-0586, labeled by a triangle), are standard data.

to dissolve in acidic solution rather than in alkaline solution. Similarly, the increase in the pH value of water from 5 to 12.5 would decrease solubility of ACC, and affect the crystallization and polymorph selection of soluble Ca^{2+} and CO_3^{2-} ions.

However, when the content of alkaline solution (pH 14, 1 M NaOH) in a mixed solvent of ethanol and water was increased from 15% to 25%, thus promoting the dissolution of ACC, the inhibited crystallization of ACC dramatically changed. The calcite phase formed quickly (Fig. 4e). Hence, kinetic factors have great effect on crystallization behaviour of the ACC phase.

In summary, we have reported a new method to synthesize pure and stable ACC nanoparticles without the use of any additive on a large scale. As-synthesized ACC nanoparticles are monodispersed, even after redispersion following condensation by centrifugal separation. It has been demonstrated that crystallization of ACC in a mixed solvent of ethanol and water is affected by the pH value of the solution. The available pure and stable ACC can provide an ideal pristine precursor for further understanding the role of amorphous phases in biomimetic mineralization.

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