

SELECTIVE CONTROL OF CALCIUM CARBONATE CRYSTALS MORPHOLOGIES USING SULFONATED POLYMER AS ADDITIVE

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ABSTRACT

We report two selective morphologies of CaCO₃ crystals using poly(potassium 2-acrylamido-2-methylpropanesulfonate) [poly(KAMPS)] as template through gas diffusion method. The presence of anionic sulphonate groups on poly(KAMPS) determined the kinetic crystallization and was fundamental for stabilizing oriented triangle and tubular shape of calcite. The modified CaCO₃ were followed by SEM and XRD techniques. Inorganic architectures with hierarchically complex shape and optimized properties have great potential as agents in optical, electronic, magnetic and biomedical applications.

Keywords: calcite, crystallization, gas diffusion method, polymorphs.

INTRODUCTION

Along the evolution, the living organisms have developed capacities to form intricate hybrid materials (biominerals) highly optimized through a complex mineralizing process called biomineralization⁽¹⁻⁶⁾. For example, certain bacteria produce magnetic nanocrystals, many mollusks build CaCO₃ shells, and mammals form bone and teeth, etc⁽⁷⁻⁹⁾. These biominerals often have remarkable mechanical, optical and magnetic properties related to the precise hierarchical assembly of nanoscale building blocks, formed at ambient temperature^(10,11). Therefore, the biominerals with defined microstructure consisting from mineral and organic parts, subdivided into the soluble and insoluble fraction^(1-6,12,13). The mineral generally contain a small amount of organic material, which is thought to provide a structural framework and to play an important roles in nucleation and regulation morphology and direction of crystal growth^(1-6,13-15).

In this communication, the use of sulphonated poly(KAMPS) was presented as good functionalization strategy for controlling the selectively of CaCO₃ crystals morphology. We have demonstrated that the spatial arrangement of sulphonic groups attached to poly(KAMPS) can alter the nucleation and morphology of CaCO₃ and the poly(KAMPS) can effectively induce oriented triangle crystals and tubular shape of calcite. The modified CaCO₃ were followed by SEM and XRD techniques. This communication tried to highlights how functionalized polymers provide viable approach for studying various aspect of biomineralization such as shape, morphologies and production of the unstable polymorphs of CaCO₃.

MATERIALS AND METHODS

1. Materials

All reagents and solvents were of the highest available grade. Calcium chloride, ethanol, sodium hydroxide (Analytic) and tris(hydroxymethyl) aminomethane (TRIS) were obtained from ACS-Merck; ammonium hydrogen carbonate (NH₄HCO₃) was from J.T. Baker and methylene chloride (CH₂Cl₂ - □99.5%) from Sigma-Aldrich. The distilled water was obtained from capsule filter 0.2 μm flow (U.S. Filter). Methylene chloride was purified by refluxing over lithium aluminum hydride (Aldrich-95% pellets particle size 10x6 mm) for 72 h and distilled under argon. AMPS (Aldrich Chemicals) were used without further purification. The potassium salt of AMPS was prepared by dissolving 50 g of AMPS in 145 mL of milli-QTM water, with the subsequent addition of 27.8 g of KOH, with cooling and constant stirring. The solution was titrated with KOH (1000 mol/m³) to pH 7. The total solid content of the KAMPS/ water precursor material was 58.5 wt-%. The polymer initiator, 4,4'-Azobis (4-cyanovaleric acid); ACVA (APS; Merck), and paraffin oil (Merck) were used as received. The chemical formulae for the poly(KAMPS) synthesized and studied are presented in Scheme 1.

2. Synthesis of Poly(potassium 2-acrylamido-2-

methylpropanesulfonate)

Homopolymers of poly(potassium 2-acrylamido-2-methylpropanesulfonate), poly(KAMPS), were synthesized with free-radical suspension-polymerization using a ACVA as initiator (Scheme 1). The monomers KAMPS (15 g) and the initiator ACVA (0.0063 g) were dissolved in 22.5 mL of milli-QTM water. After the solution had been purged with nitrogen gas for 30 min, the mixture was transferred to a funnel. A suspending agent, polyvinyl alcohol (PVA; 0.6 g) was added to paraffin oil (69.6 mL) in a round-bottomed flask with three necks. The PVA solution in paraffin oil was kept at a temperature of 70 °C and the aqueous solution of the monomers was added dropwise with stirring. The polymerization reaction was allowed to proceed for 4 h, after which the reaction vessel was cooled to room temperature and the polymeric material was retrieved by filtration and precipitation with acetone. The polymeric material was washed in milli-QTM water and precipitated twice with acetone, and then dried under vacuum at a temperature of 50 °C. The KAMPS moiety in the homopolymer was identified by ¹³C nuclear magnetic resonance (NMR) spectroscopy and was in good agreement with that of the added monomer.

3. *In vitro* CaCO₃ crystallization

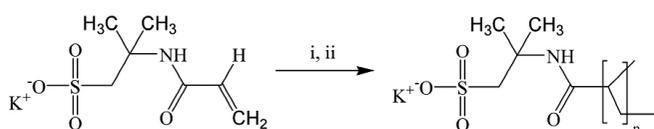
Crystallization experiments were carried out in the presence of poly(KAMPS) as additive and compared with a solution without polymer as blank control using the gas diffusion (GD) method. The GD method was performed as we described in previous works⁽¹⁶⁻²⁰⁾. All experiments were carried out inside the Petri dish using of poly(KAMPS) at different pH values from pH 7 to 12 at 1.0 mg/mL at 20 °C for 24 h. For these experiments a stock solution of 1.6 mg of poly(KAMPS) in deionized water at different pH prepared as mother solution in an Eppendorf tube. CaCO₃ results from the diffusion of CO_{2(g)} into the buffered CaCl₂ solution. The microbridges with the CaCO₃ crystals formed were rinsed with deionized water and dehydrated through solution of ethanol in growing concentrations (50%, 80% and 100%), dried at room temperature and observed the microstructure by SEM.

4. Measurements

For the *in vitro* CaCO₃ crystallization, both scanning electron microscopy (SEM) and X-ray diffraction (XRD) were applied to all resultant crystals particles. The crystals were observed in a Tesla BS 343 A scanning electron microscope (SEM) and the XRD of crystals were made in Siemens D-5000X X-ray diffractometer with CuK_α radiation (graphite monochromator). The geometric scanning Bragg-Brentano (θ-θ) and the angle range from 5°-70° (2θ) were performed. The Diffrac Plus was used as data control software. It is difficult to obtain the molecular weight of the poly(KAMPS) by GPC measurement due to the polymer aggregation on the column fillers induced by the ionic groups on the polymer side chain⁽²¹⁾.

RESULTS AND DISCUSSION

Poly(KAMPS) was synthesized according to the follow reaction (Scheme 1):



Reagent and conditions

- 4,4'-Azobis (4-cyanovaleric acid); ACVA
- PVA (suspending agent) / Paraffin oil, 70°C, 4 h

Scheme 1. Synthesis of poly(KAMPS).

Fig. 1 shows SEM images of CaCO_3 crystals grown in the presence of poly(KAMPS) as additive at 1 mg/mL varying the pH from pH7 to pH12 at 24 °C. Fig. 1 A shows a grouped flat CaCO_3 crystals with 20 μm of size with nanometric oriented triangle on the surface when 1 mg/mL of poly(KAMPS) was present at pH 7 at 20 °C. Similarly flat shape CaCO_3 morphology with poly(KAMPS) at pH 8, 9 and 10 were observed. Surprisingly, unusual and regular rugby-ball crystals with 10 μm of size were crystallized at pH 7. Another observation is that the unstable polymorphism of aragonite-like crystals was also stabilized from pH 8 to 10 (Fig. 1 B-D). Moreover, it can be seen that only oriented triangle crystals were formed from pH 7 to 10 (Fig. 1 A-D). Fig. 1 E shows regular aggregated rosette-like crystals of truncate calcite with size in the range of 5-10 μm at pH 11. The single calcite crystals, which are aggregated, are in the range of nanometric to micrometric in size. However, tubular calcite crystals with size in the range of 2-4 μm were obtained at pH 12. Here we observed {104} faces at the extreme of tubular crystals (see red arrow). At high magnification is clear to recognize the rhombohedra calcite crystals. Oriented calcite with alignment of {012} face has been also obtained by using self-assembled monolayer (SAM) functionalized with carboxylic group using Kitano crystallization method (22). In addition, XRD patterns of CaCO_3 after crystallization using poly(KAMPS) shows {104} face at $2\theta = 29.4^\circ$ as the most intense peaks confirming the calcite (data no shown). Faces of aragonite were also determined confirming the occurrence of this polymorphism at pH 10.

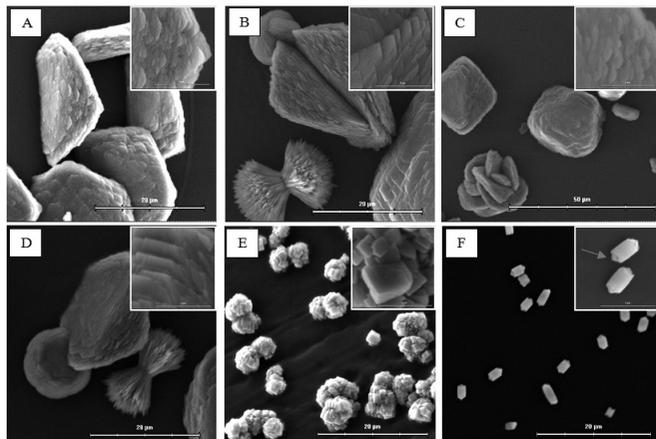


Figure 1. SEM images of CaCO_3 grown using gas diffusion with 1.0 mg/mL of poly(KAMPS) at different pH value from 7 to 12. A) pH 7, B) pH 8, C) pH 9, D) pH 10, E) pH 11 and F) pH 12 at 20 °C for 24 h.

The Fig. 2 shows a summary of the resultant oriented, aggregated of CaCO_3 morphologies obtained with 1.0 mg/mL of poly(KAMPS) and their relation with the pH values of mineralization solution.

CONCLUSIONS

The use of poly(KAMPS) as additive template could effectively control the morphogenesis of CaCO_3 crystals and its crystallographic polymorphism. The presence of sulphonate groups of poly(KAMPS) and the pH of the mineralization solution seem to be crucial during CaCO_3 nucleation, crystal growth determining the kinetic *in vitro* crystallization. The pH of poly(KAMPS) solution can undergo different degree of sulphonic group dissociation inducing different conformation of poly(KAMPS) in solution and thereby elicit changes in CaCO_3 morphology. Therefore, the resultant crystals, which is triggered by

the sulphonate moieties of poly(KAMPS) results from a local accumulation of Ca^{2+} ions, which correlates closely with the pH of the mineralization solution. SEM images showed unusual CaCO_3 crystal morphologies as a function of pH, which was viable for stabilizing oriented triangle from pH 7 to 10 and tubular shape of calcite at pH 12, respectively. The preparation of hierarchical hollow of CaCO_3 particles as inorganic complex structures have great potential in material science and anticancer therapy (23,24). XRD pattern are in good agreement of our results demonstrated the coexistence of its polymorphs. In summary, we demonstrated that the functionalization strategy on polymers provides viable approach for studying shape, controlled morphologies and production of the unstable polymorphs of CaCO_3 .

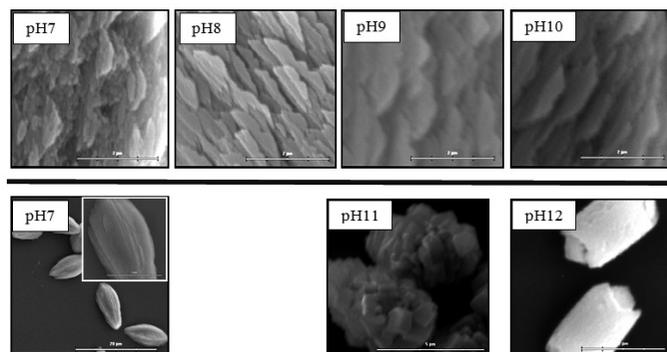


Figure 2. Scheme of oriented CaCO_3 morphologies obtained with 1.0 mg/mL of poly(KAMPS) at different pH value.

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