

# The Role of Mg<sup>2+</sup> and BSA Langmuir Monolayers in Controlling Polymorph and Morphology of CaCO<sub>3</sub> Crystal

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## Abstract

Mg<sup>2+</sup> and bovine serum albumin (BSA) Langmuir monolayers were used as effective crystal nucleation, growth modifiers and template to control the crystallization of CaCO<sub>3</sub>. Scanning electron microscopy (SEM), and X-ray diffraction (XRD) were used to characterize the polymorph and morphology of crystals obtained at different experimental conditions, respectively. The results indicated that various morphologies such as abacus-bead-like particles, spherical-shaped particles, wood block-like particles, pignut-shell-like particles and the rolling pole shaped particles have been formed at the interface of air-solution. The polymorph of calcium carbonate obtained undergo an evolvement from calcite to vaterite and aragonite with increasing of the molar ratio of Mg<sup>2+</sup> to Ca<sup>2+</sup>, which indicated that the ability of Mg<sup>2+</sup> to induce the formation of aragonite was enhanced as the molar ratio of  $Mg^{2+}$  to  $Ca^{2+}$ increased. When the molar ratio reached 3, the samples obtained were all aragonite phase of calcium carbonate, which suggests that the presence of Mg<sup>2+</sup> of subphase solutions was helpful for the formation of aragonite phase in the systems of Mg<sup>2+</sup>-BSA Langmuir monolayers. The possible formation mechanisms of CaCO<sub>3</sub> in different systems were discussed in the paper.

#### **Subject Areas**

Analytical Chemistry

#### **Keywords**

Calcium Carbonate, Langmuir Monolayers, Mg<sup>2+</sup>, Bovine Serum Albumin

#### **1. Introduction**

Biomineralization is the process of inorganic crystal growth at the inorganic-

organic interface [1] [2] [3], which exerts a significant influence on the growth of life. Therefore, the researches about the biomineralization have aroused many interests [4]-[9]. From the number of carbonate minerals, CaCO<sub>3</sub> is the most important biominerals in various industrial fields, such as additives in medicine, foods, plastics, printing ink, cosmetic, etc. Its final application is determined by its purity, particle size and distribution, crystal structure and morphology [10]. Some studies indicated that high-density Mg<sup>2+</sup> was found in bio-environment and it took an important part in the biomineralization of calcium carbonate [11] [12]. Eva Loste *et al.* have studied the role of magnesium in stabilizing amorphous calcium carbonate and controlling calcite morphologies [11]. Yong-Jin Han et al. have investigated the effect of Mg2+ on oriented growth of calcite on carboxylic acid functionalized self-assembled monolayers [13]. Davis et al. have indicated that the role of Mg<sup>2+</sup> as an impurity in calcite growth [14]. Norio Wada et al. have demonstrated the effects of divalent cations upon nucleation, growth and transformation of calcium carbonate polymorphs under conditions of double diffusion [15]. Yun-feng Jiao et al. studied the co-effect of collagen and magnesium ions on calcium carbonate biomineralization [16]. An-jian Xie et al. investigate the role of Mg<sup>2+</sup> and Mg<sup>2+</sup>/amino acid in controlling polymorph and morphology of calcium carbonate crystal [17]. Li-ying Zhu et al. synthesize star-shaped calcite with Mg<sup>2+</sup> without an organic template in the mixed solvent of ethanol and  $H_2O$  [18]. However, the effect of different concentration  $Mg^{2+}$  on the polymorph and morphology of CaCO<sub>3</sub> crystal using protein Langmuir monolayers method was not described systemically so far. Further, protein as an organic matrix also plays a critical role in the biomineralization of the  $CaCO_3$  [2] [19], it exists at inorganic-organic interface of almost all the biominerals such as teeth, bones, pearls, mollusk shells [2] [20]. They may be important factors in controlling of CaCO<sub>3</sub> polymorphs and morphologies. Many studies on the combined effect of organic additives and magnesium ions on calcium carbonate crystal growth have been reported recently [21] [22] [23], but little research has been carried out on the cooperative influence of protein Langmuir monolayers and magnesium ions on calcium carbonate precipitation.

This paper mainly discusses the cooperative influence of BSA Langmuir monolayers and magnesium ions on calcium carbonate precipitation. The aim of these experiments is to find the effect on the polymorph and morphology of calcium carbonate by combined BSA Langmuir monolayers and magnesium ions. Simultaneously, the possible formation mechanism of  $CaCO_3$  crystals on the basis of the principle of biomineralization was discussed. It is significant to understand the mechanism of biomineralization and synthesizing new and special functional materials.

#### 2. Experimental Section

#### 2.1. Materials

BSA was purchased from Sigma and used without further purification. MgCl<sub>2</sub>·6H<sub>2</sub>O,

 $CaCO_3$  and Amyl alcohol were all analytical purity. All the aqueous solutions in our experiment were prepared by using the triply deionized water, its conductivity was a resistance of 18.2 M $\Omega$ ·cm<sup>-1</sup> and its pH was 7.0.

#### 2.2. Crystallization of CaCO<sub>3</sub>

Supersaturated solutions of calcium bicarbonate were prepared according to the procedures of *Kitano* [24]. MgCl<sub>2</sub>·6H<sub>2</sub>O was dissolved into Ca(HCO<sub>3</sub>)<sub>2</sub> aqueous solution (5 mmol $\cdot$ L<sup>-1</sup>) and the pH value of the solution was 7.0. The spreading monolayers at the air-water interface were formed by spreading solutions of BSA  $(1 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1})$  on the pure water surface or Supersaturated solutions of calcium bicarbonate containing Mg<sup>2+</sup>. A 30-min lapse time was estimated to be sufficient to equilibrate the protein monolayers before compression. We used very low compression rates and choose 15 mN/m as a target surface pressure, which has been shown to be appropriate to obtain reproducible isotherms and stable BSA Langmuir monolayers [25]. In the different concentration of Mg<sup>2+</sup> system, Mg<sup>2+</sup>/Ca<sup>2+</sup> molar ratio was 1:1; 2:1; 3:1; 4:1; 5:1 respectively. The prepared supersaturated calcium bicarbonate solutions were poured into a Langmuir trough and the air-water interface was swept and aspirated before deposition of the surfactant solution. The surfactant solution (10 µL) was carefully deposited onto the solution surface. Each experiment was repeated three times with the same condition. Crystals as-grown in association with the monolayers were respectively removed after 4 h by carefully horizontally dipping hydrophilic glass slides through the air-water interface. The crystal face growing into the solution is therefore directly deposited on the glass slide.

#### 2.3. Characterization of Crystal Phase and Morphology of CaCO<sub>3</sub>

The sizes and morphologies of CaCO<sub>3</sub> were characterized by using SEM on JSM-5600LV scanning electron microscopy (Jeol. Ltd. Japan) with operating at 30 kV. The slides supporting the crystals were mounted on copper sample stubs with conducting carbon tape and were sputter-coated with gold prior to viewing. The XRD measurements were performed by a (Philips X'Pert Pro) X-ray powder diffractometer using a monochromatized CuK $\alpha_1$  radiation ( $\lambda = 1.5406$  Å).

### 3. Results and Discussion

# 3.1. The Influence of BSA Langmuir Monolayers and Magnesium on Polymorph of CaCO<sub>3</sub>

From XRD it can be seen that the polymorph of CaCO<sub>3</sub>, obtained at the interface of film/solution changed with increasing the concentration of magnesium in the solution. When the ratio of Mg/Ca is 1 only calcite crystals are formed (**Figure 1(A)**), and when the ratio of Mg/Ca is 2 some vaterite and aragonite crystals are precipitated besides calcite (**Figure 1(B)**). And when the ratio of Mg/Ca attains 3 almost the crystals are aragonite, only a few are calcite crystals (**Figure 1(C)**). All crystals are aragonite when the ratio of Mg/Ca is 4 and 5 (**Figure 1(D)**, **Figure 1(E)**). A lot of reports indicate that only at a ratio of Mg/Ca of 4 or above that



**Figure 1.** XRD patterns of CaCO<sub>3</sub> particles obtained from different  $Mg^{2+}/Ca^{2+}$  molar ratio systems. (A) 1:1; (B) 2:1; (C) 3:1; (D) 4:1; (E) 5:1. (F) without  $Mg^{2+}$  in solutions.

aragonite can be seen if there is no organic additive or biopolymer present, in other instance only calcite can be seen, which is in agreement with conventional theoretical results. When BSA Langmuir monolayers are present alone, only calcite crystals are formed (**Figure 1(F)**). Magnesium is known to induce aragonite formation from sea water and in vitro at a ratio of Mg/Ca equal to or greater than 4, while at lower Mg/Ca ratio mostly calcite and magnesian calcite are formed [24]. The above results show that when BSA Langmuir monolayers and magnesium are both present, aragonite crystals precipitated at a lower Mg/Ca ratio of 2. This indicates that BSA Langmuir monolayers have a promotional effect on magnesium ions in controlling the polymorph of CaCO<sub>3</sub> crystals.

# 3.2. The Influence of BSA Langmuir Monolayers and Magnesium on Morphology of CaCO<sub>3</sub>

A much wide range of calcium carbonate morphologies is generated in the presence of both BSA Langmuir monolayers and magnesium ions. At low magnesium concentration (Mg/Ca ratio is 1), regular abacus-bead-like calcite crystals are formed (**Figure 2(A)**). When the concentration of magnesium is high



**Figure 2.** SEM images of CaCO<sub>3</sub> particles obtained from different  $Mg^{2+}/Ca^{2+}$  molar ratio systems. (A) 1:1; (B) 2:1; (C) 3:1; (D) 4:1; (E) 5:1. (F) without  $Mg^{2+}$  in solutions.

enough (Mg/Ca ratio attains to 2 and 3), wood-block-like aragonite crystals are precipitated (**Figure 2(B)**, **Figure 2(C)**). In order to further study the morphology evolvement of calcium carbonate, the higher  $Mg^{2+}/Ca^{2+}$  ratio (4:1 and 5:1) were observed. The results indicated that the pignut-shell-like and rolling pole shaped aragonite crystals were produced at a ratio of  $Mg^{2+}/Ca^{2+}$  4:1 and 5:1, respectively (**Figures 2(D)-(F)**) is the SEM image of calcite obtained without  $Mg^{2+}$ , it shows that the calcite took jujube-nucleus-like morphology, which is different from the results obtained at a solution containing  $Mg^{2+}$ .

Above results showed that in the presence of BSA Langmuir monolayers, when magnesium is added, the morphology of crystals changed greatly, from abacus-bead to wood-block, pignut-shell and rolling pole. The morphological changes of calcium carbonate crystals reveal that in the presence of both BSA Langmuir monolayers and magnesium ions, we tend to obtain various morphological aragonite. Especially when the concentration of magnesium is high, the aragonite crystals with more regular shapes could be obtained.

The reason for this kinetic phenomenon is considered as contributing to two aspects: on the one hand, magnesium ions inhibit the growth of calcite. The partially dehydrated magnesium ions attach to the surface of the nascent calcite nucleus, the strongly bound residual hydration sphere poisons the surface [25], and inhibits subsequent growth. At the same time, the BSA acts with magnesium and increase the magnesium hydrate absorbed in calcite nucleus. On the other hand, owing to the polymorph of calcium carbonate is related to its energy state [26], magnesium is likely also to react with BSA and to change the secondary structure of BSA molecules, and thus induces the aragonite or vaterite with higher energy in high energy state structure.

According to the nucleation and growth theory [19] [27] [28], to form a new nucleus, the activation energy  $(\Delta G_N)$  must be overcome.  $\Delta G_N$  can be expressed as:

$$\Delta G_N = 16 \pi (\Delta G_1)^3 / 3 (kT \ln S)^2$$

where  $\Delta G_1$  is the surface energy that was needed to form the new interface and maintain the crystal growth, k is the Boltzmann constant, T is the temperature, and S is the supersaturation of area. The decrease of the surface energy  $\Delta G_1$  or the increase of the *S* can reduce the activation energy for crystal nucleation. The isoelectric points (pI = 4.7) of BSA were both lower than the pH (7.0) of an aqueous solution, the negative charges on the surface of BSA were surplus, they could attract Ca<sup>2+</sup> strongly because of electrostatic interactions, it aroused the concentration of Ca<sup>2+</sup> was rich in this region. At the same time, the presence of magnesium ions during the calcium carbonate precipitation in vitro is known to inhibit the growth of calcium carbonate and consequently increase the degree of supersaturation [29], such conditions enable the formation of metastable phases in general [30]; meanwhile BSA that offered nucleation sites for the growth of CaCO<sub>3</sub> particles could reduce the surface energy, so  $\Delta G_N$  declined, which was helpful for the formation of the high-energy aragonite. From these results, we could see that the molar ratio of Mg<sup>2+</sup>/Ca<sup>2+</sup> has a very important effect on the morphology of CaCO<sub>3</sub>. However, the precise role of magnesium ions in the stabilization of the metastable phases remains enigmatic.

### 4. Conclusion

The present paper mainly studied the cooperative effect of BSA Langmuir monolayers and magnesium ions on polymorph and morphology of calcium carbonate crystal. The results indicated that BSA Langmuir monolayers and magnesium ions have a promotional effect on magnesium ions in controlling the polymorph of  $CaCO_3$  crystals. By the cooperation of BSA Langmuir monolayers and magnesium ions in different concentration, crystals showed a sequence of morphology changes and especially, aragonite crystals with various unusual morphology were precipitated at high  $Mg^{2+}/Ca^{2+}$  ions concentration ratio. The experiments suggested that BSA Langmuir monolayers acts in combination with magnesium ions to inhibit calcite crystal growth, while favoring the formation of aragonite crystals. It indicated that both  $Mg^{2+}$  and proteins played an essential important role in the process of biomineralization of  $CaCO_3$ .

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## **Conflicts of Interest**

The authors declare no conflicts of interest regarding the publication of this paper.

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