



The Role of Mg^{2+} and BSA Langmuir Monolayers in Controlling Polymorph and Morphology of $CaCO_3$ Crystal

Zhonghui Xue^{1*}, Ning Xue²

¹Shanghai Publishing and Printing College, Shanghai, China

²Northeastern University, Qinhuangdao, China

Email: hnlxzh@163.com

How to cite this paper: Xue, Z.H. and Xue, N. (2020) The Role of Mg^{2+} and BSA Langmuir Monolayers in Controlling Polymorph and Morphology of $CaCO_3$ Crystal. *Open Access Library Journal*, 7: e5797. <https://doi.org/10.4236/oalib.1105797>

Received: September 7, 2019

Accepted: April 26, 2020

Published: April 29, 2020

Copyright © 2020 by author(s) and Open Access Library Inc.

This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).

<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

Mg^{2+} and bovine serum albumin (BSA) Langmuir monolayers were used as effective crystal nucleation and growth modifiers and template to control the crystallization of $CaCO_3$. The polymorph and morphology of crystals were characterized using scanning electron microscopy (SEM), and X-ray diffraction (XRD). The results demonstrated 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 produced at the interface of film/solution. With the increasing of the molar ratio of Mg^{2+} to Ca^{2+} , the polymorph of calcium carbonate obtained undergoes an evolvement from calcite to vaterite and aragonite. The ability of Mg^{2+} 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 crystals obtained were all aragonite. In the systems of Mg^{2+} -BSA Langmuir monolayers, the presence of Mg^{2+} was helpful for the formation of aragonite. The possible formation mechanisms of $CaCO_3$ in different systems were discussed in the paper.

Subject Areas

Composite Material

Keywords

Calcium Carbonate, Langmuir Monolayers, Mg^{2+} , Bovine Serum Albumin

1. Introduction

Biom mineralization is the process of inorganic crystal growth at 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_3 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^{2+} 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 [13]. Yong-Jin Han *et al.* have investigated effect of Mg^{2+} on oriented growth of calcite on carboxylic acid functionalized self-assembled monolayers [14]. Davis *et al.* have indicated that the role of Mg^{2+} as an impurity in calcite growth [15]. 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 [16]. Yun-Feng Jiao *et al.* studied the co-effect of collagen and magnesium ions on calcium carbonate biomineralization [17]. An-jian Xie *et al.* investigate the role of Mg^{2+} and Mg^{2+} /amino acid in controlling polymorph and morphology of calcium carbonate crystal [18]. Li-Ying Zhu *et al.* synthesize star shaped calcite with Mg^{2+} without organic template in the mixed solvent of ethanol and H_2O [19]. However, the effect of different concentrations Mg^{2+} on the polymorph and morphology of CaCO_3 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] [20], it exists at inorganic-organic interface of almost all the biominerals such as teeth, bones, pearls, mollusk shells [2] [21]. They may be important factors in controlling of CaCO_3 polymorphs and morphologies. Many studies on the combined effect of organic additives and magnesium ions on calcium carbonate crystal growth have been reported recently [22] [23] [24], 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. $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$,

CaCO₃ 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 MΩ·cm⁻¹ and its pH was 7.0.

2.2. Crystallization of CaCO₃

Supersaturated solutions of calcium bicarbonate were prepared according to the procedures of *Kitano* [25]. MgCl₂·6H₂O was dissolved into Ca(HCO₃)₂ aqueous solution (5 mmol·L⁻¹) and the pH value of solution was 7.0. The spreading monolayers at the air-water interface were formed by spreading solutions of BSA (1 × 10⁻⁴ mol·L⁻¹) on the pure water surface or Supersaturated solutions of calcium bicarbonate containing Mg²⁺. 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 [26]. In the different concentration of Mg²⁺ system, Mg²⁺/Ca²⁺ 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 CaCO₃

The sizes and morphologies of CaCO₃ 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 Cu Kα₁ radiation (λ = 1.5406 Å).

3. Results and Discussion

3.1. The Influence of BSA Langmuir Monolayers and Magnesium on Polymorph of CaCO₃

From XRD it can be seen that the polymorph of CaCO₃, 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 attain 3 almost the crystals are aragonite, only a few are calcite crystals (**Figure 1(c)**). While all crystals are aragonite when the ratio of Mg/Ca is 4 and 5 (**Figure 1(d)**),

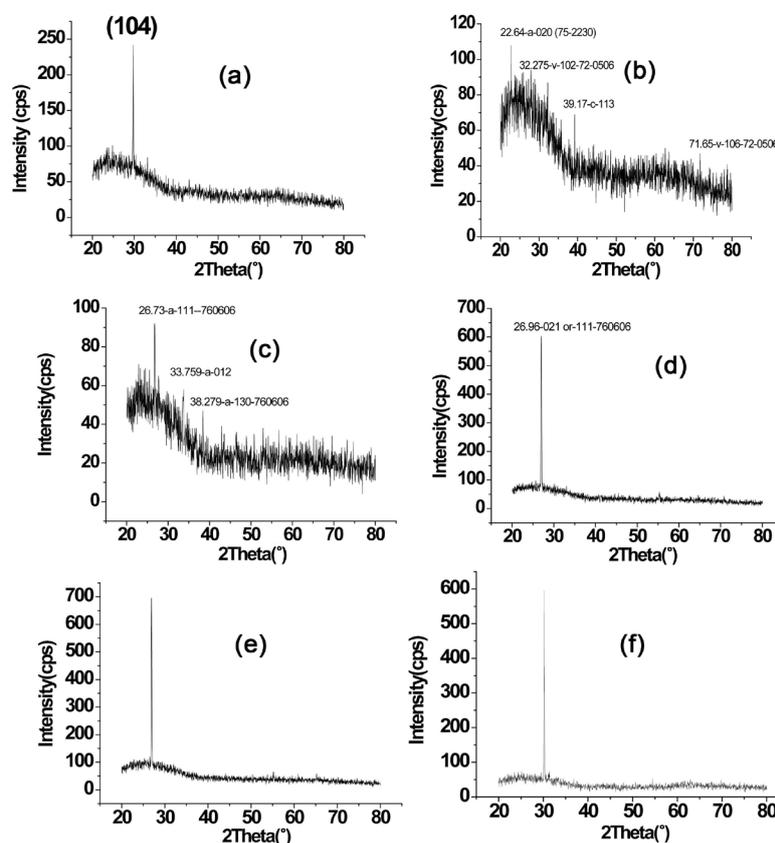


Figure 1. XRD patterns of CaCO_3 particles obtained from different $\text{Mg}^{2+}/\text{Ca}^{2+}$ molar ratio systems. (a) 1:1; (b) 2:1; (c) 3:1; (d) 4:1; (e) 5:1. (f) without Mg^{2+} in solutions.

Figure 1(e). A lot of reports indicate that only at ratio of Mg/Ca of 4 or above that 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 ratio of Mg/Ca equal to or greater than 4, while at lower Mg/Ca ratio mostly calcite and magnesian calcite are formed [27]. 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_3 crystals.

3.2. The Influence of BSA Langmuir Monolayers and Magnesium on Morphology of CaCO_3

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 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

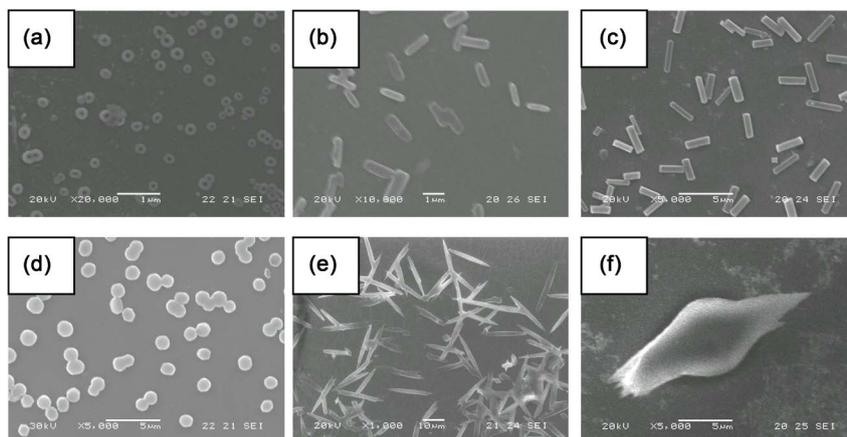


Figure 2. SEM images of CaCO_3 particles obtained from different $\text{Mg}^{2+}/\text{Ca}^{2+}$ molar ratio systems. (a) 1:1; (b) 2:1; (c) 3:1; (d) 4:1; (e) 5:1. (f) without Mg^{2+} in solutions.

evolution of calcium carbonate, the higher $\text{Mg}^{2+}/\text{Ca}^{2+}$ ratio (4:1 and 5:1) was observed. The results indicated that the pignut-shell-like and rolling pole shaped aragonite crystals were produced at ratio of $\text{Mg}^{2+}/\text{Ca}^{2+}$ 4:1 and 5:1, respectively (**Figure 2(d)**, **Figure 2(e)**). **Figure 2(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 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 of this kinetic phenomenon is considered to contribute 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 [28], and inhibits subsequent growth. At the same time, the BSA acts with magnesium and increases the magnesium hydrate absorbed in calcite nucleus. On the other hand, owing to the polymorph of calcium carbonate is relate to its energy state [29], 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 [30] [31] [32], to form a new nucleus, the activation energy (ΔG_N) must be overcome. ΔG_N can be expressed as:

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

where ΔG_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 ΔG_i 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 aqueous solution, the negative charges on the surface of BSA were surplus, they could attract Ca^{2+} strongly because of electrostatic interactions, it aroused the concentration of Ca^{2+} was rich in this region. At the same time, the present 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 [33], such conditions enable the formation of metastable phases in general [34]; meanwhile BSA that offered nucleation sites for the growth of $CaCO_3$ particles could reduce the surface energy, so ΔG_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^{2+}/Ca^{2+} has very important effect on the morphology of $CaCO_3$. 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 cooperation of BSA Langmuir monolayers and magnesium ions in different concentrations, 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 act in combination with magnesium ions to inhibit calcite crystal growth, while favors the formation of aragonite crystals. It indicated that both Mg^{2+} and proteins played an essential importance role in the process of biomineralization of $CaCO_3$.

Acknowledgements

This work was supported by the National Natural Science Foundation of China, Grant No. 21203055.

Conflicts of Interest

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

References

- [1] Donners, J.J.J., Nolte, R.J.M. and Sommerdijk, N.A.J.M. (2002) A Shape-Persistent Polymeric Crystallization Template for $CaCO_3$. *Journal of the American Chemical Society*, **124**, 9700-9701. <https://doi.org/10.1021/ja0267573>
- [2] Fu, G., Valiyaveetil, S., Wopenka, B. and Morse, D.E. (2005) $CaCO_3$ Biomineraliza-

- tion: Acidic 8-kDa Proteins Isolated from Aragonitic Abalone Shell Nacre Can Specifically Modify Calcite Crystal Morphology. *Biomacromolecules*, **6**, 1289-1298. <https://doi.org/10.1021/bm049314v>
- [3] Liang, P., Shen, Q., Zhao, Y., Zhou, Y., Wei, H., Lieberwirth, I., Huang, Y., Wang, D. and Xu, D. (2004) Petunia-Shaped Superstructures of CaCO₃ Aggregates Modulated by Modified Chitosan. *Langmuir*, **20**, 10444-10448. <https://doi.org/10.1021/la0481602>
- [4] Macaskie, L.E., Yong, P., Paterson-Beedle, M., Thackray, A.C., Marquis, P.M., Sammons, R.L., Nott, K.P. and Hall, L.D. (2005) A Novel Non Line-of-Sight Method for Coating Hydroxyapatite onto the Surfaces of Support Materials by Biomineralization. *Journal of Biotechnology*, **118**, 187-200. <https://doi.org/10.1016/j.jbiotec.2005.03.006>
- [5] Kokubo, T. (2005) Design of Bioactive Bone Substitutes Based on Biomineralization Process. *Materials Science and Engineering: C*, **25**, 97-104. <https://doi.org/10.1016/j.msec.2005.01.002>
- [6] Fan, Y.W., Duan, K. and Wang, R.Z. (2005) A Composite Coating by Electrolysis-Induced Collagen Self-Assembly and Calcium Phosphate Mineralization. *Biomaterials*, **26**, 1623-1632. <https://doi.org/10.1016/j.biomaterials.2004.06.019>
- [7] Nancollas, G.H. and Wu, W.J. (2000) Biomineralization Mechanisms: A Kinetics and Interfacial Energy Approach. *Journal of Crystal Growth*, **211**, 137-142. [https://doi.org/10.1016/S0022-0248\(99\)00816-7](https://doi.org/10.1016/S0022-0248(99)00816-7)
- [8] Eiden-Aßmann, S., Viertelhaus, M., Heiß, A., Hoetzer, K.A. and Felsche, J. (2002) The Influence of Amino Acids on the Biomineralization of Hydroxyapatite in Gelatin. *Journal of Inorganic Biochemistry*, **91**, 481-486. [https://doi.org/10.1016/S0162-0134\(02\)00481-6](https://doi.org/10.1016/S0162-0134(02)00481-6)
- [9] Falini, G., Gazzano, M. and Ripamonti, A. (1996) Magnesium Calcite Crystallization from Water-Alcohol Mixtures. *Chemical Communications*, No. 9, 1037-1038. <https://doi.org/10.1039/CC9960001037>
- [10] Xie, A.J., Yuan, Z.W. and Shen, Y.H.J. (2005) Biomimetic Morphogenesis of Calcium Carbonate in the Presence of a New Amino-Carboxyl-Chelating-Agent. *Journal of Crystal Growth*, **276**, 265-274. <https://doi.org/10.1016/j.jcrysgro.2004.11.376>
- [11] Nebel, H. and Eppele, M. (2008) Continuous Preparation of Calcite, Aragonite and Vaterite, and of Magnesium-Substituted Amorphous Calcium Carbonate (Mg-ACC). *Zeitschrift für anorganische Chemie*, **634**, 1439-1443. <https://doi.org/10.1002/zaac.200800134>
- [12] Han, Y.J. and Aizenberg, J. (2003) Effect of Magnesium Ions on Oriented Growth of Calcite on Carboxylic Acid Functionalized Self-Assembled Monolayer. *Journal of the American Chemical Society*, **125**, 4032-4033. <https://doi.org/10.1021/ja034094z>
- [13] Loste, E., Wilson, R.M., Seshadri, R. and Meldrum, F.C. (2003) The Role of Magnesium in Stabilising Amorphous Calcium Carbonate and Controlling Calcite Morphologies. *Journal of Crystal Growth*, **254**, 206-218. [https://doi.org/10.1016/S0022-0248\(03\)01153-9](https://doi.org/10.1016/S0022-0248(03)01153-9)
- [14] Han, Y.J. and Aizenberg, J. (2003) Effect of Magnesium Ions on Oriented Growth of Calcite on Carboxylic Acid Functionalized Self-Assembled Monolayer. *Journal of the American Chemical Society*, **125**, 4032-4033.
- [15] Davis, K.J., Dove, P.M. and De Yoreo, J.J. (2000) The Role of Mg²⁺ as an Impurity in Calcite Growth. *Science*, **290**, 1134-1137. <https://doi.org/10.1126/science.290.5494.1134>

- [16] Wada, N., Yamashita, K. and Umegaki, T. (1999) Effects of Carboxylic Acids on Calcite Formation in the Presence of Mg^{2+} Ions. *Journal of Colloid and Interface Science*, **212**, 357-364. <https://doi.org/10.1006/jcis.1998.6067>
- [17] Jiao, Y.F., Feng, Q.L. and Li, X.M. (2006) The Co-Effect of Collagen and Magnesium Ions on Calcium Carbonate Biomineralization. *Materials Science and Engineering: C*, **26**, 648-652. <https://doi.org/10.1016/j.msec.2005.08.038>
- [18] Xie, A.J., Shen, Y.H., Li, X.Y., Yuan, Z.W., Qiu, L.G., Zhang, C.Y. and Yang, Y.F. (2007) The Role of Mg^{2+} and Mg^{2+} /Amino Acid in Controlling Polymorph and Morphology of Calcium Carbonate Crystal. *Materials Chemistry and Physics*, **101**, 87-92. <https://doi.org/10.1016/j.matchemphys.2006.02.019>
- [19] Zhu, L.Y., Zhao, Q.R., Zheng, X.W. and Xie, Y. (2006) Formation of Star-Shaped Calcite Crystals with Mg^{2+} Inorganic Mineralizer without Organic Template. *Journal of Solid State Chemistry*, **179**, 1247-1252. <https://doi.org/10.1016/j.jssc.2006.01.036>
- [20] Mann, K. (2010) Identification of the Major Proteins of the Organic Matrix of Emu (*Dromaius novaehollandiae*) and Rhea (*Rhea americana*) Eggshell Calcified Layer. *British Poultry Science*, **45**, 483-490. <https://doi.org/10.1080/00071660400001157>
- [21] Falini, G., Albeck, S., Weiner, S. and Addadi, L. (1996) Control of Aragonite or Calcite Polymorphism by Mollusk Shell Macromolecules. *Science*, **271**, 67-69. <https://doi.org/10.1126/science.271.5245.67>
- [22] Falini, G., Gazzano, M. and Ripamonti, A. (1994) Crystallization of Calcium Carbonate in Presence of Magnesium and Polyelectrolytes. *Journal of Crystal Growth*, **137**, 577-584. [https://doi.org/10.1016/0022-0248\(94\)91001-4](https://doi.org/10.1016/0022-0248(94)91001-4)
- [23] Sugawara, A. and Kato, T. (2000) Aragonite $CaCO_3$ Thin-Film Formation by Cooperation of Mg^{2+} and Organic Polymer Matrices. *Chemical Communications*, No. 6, 487-488. <https://doi.org/10.1039/a909566g>
- [24] Meldrum, F.C. and Hyde, S.T. (2001) Morphological Influence of Magnesium and Organic Additives on the Precipitation of Calcite. *Journal of Crystal Growth*, **231**, 544-558. [https://doi.org/10.1016/S0022-0248\(01\)01519-6](https://doi.org/10.1016/S0022-0248(01)01519-6)
- [25] Kitano, Y. (1962) The Behavior of Various Inorganic Ions in the Separation of Calcium Carbonate from a Bicarbonate Solution. *Bulletin of the Chemical Society of Japan*, **35**, 1973-1980. <https://doi.org/10.1246/bcsj.35.1973>
- [26] Xue, Z.H., Hu, B.B., Dai, S.X. and Du, Z.L. (2012) Crystallization and Self-Assembly of Flowerlike Superstructures of Calcium Carbonate Regulated by Pepsin Langmuir Monolayers. *Materials Chemistry and Physics*, **136**, 771-777. <https://doi.org/10.1016/j.matchemphys.2012.07.054>
- [27] Xue, Z.H., Hu, B.B., Dai, S.X. and Du, Z.L. (2015) Transformation of Amorphous Calcium Carbonate to Rod-Like Single Crystal Calcite via "Copying" Collagen Template. *Materials Science and Engineering: C*, **55**, 506-511. <https://doi.org/10.1016/j.msec.2015.05.079>
- [28] Noyes, R.M. (1962) Thermodynamics of Ion Hydration as a Measure of Effective Dielectric Properties of Water. *Journal of the American Chemical Society*, **84**, 513-522. <https://doi.org/10.1021/ja00863a002>
- [29] McCanley, J.W. and Roy, R. (1974) Controlled Nucleation and Crystal Growth of Various $CaCO_3$ Phases by the Silica Gel Technique. *American Mineralogist*, **59**, 947-963.
- [30] Yu, J.G., Tang, H., Cheng, B. and Zhao, X.J. (2004) Morphological Control of Calcium Oxalate Particles in the Presence of Poly-(styrene-alt-maleic acid). *Journal of*

Solid State Chemistry, **177**, 3368-3374. <https://doi.org/10.1016/j.jssc.2004.06.007>

- [31] Zhang, D.B., Qi, L.M., Ma, J.M. and Cheng, H.M. (2002) Morphological Control of Calcium Oxalate Dihydrate by a Double-Hydrophilic Block Copolymer. *Chemistry of Materials*, **14**, 2450-2457. <https://doi.org/10.1021/cm010768y>
- [32] Tong, H., Ma, W.T., Wang, L.L., Wan, P., Hu, J.M. and Cao, L.X. (2004) Control over the Crystal Phase, Shape, Size and Aggregation of Calcium Carbonate via a L-Aspartic Acid Inducing Process. *Biomaterials*, **25**, 3923-3929. <https://doi.org/10.1016/j.biomaterials.2003.10.038>
- [33] Fernandez-Diaz, L., Putnis, A., Prieto, M. and Putnis, C.V. (1996) The Role of Magnesium in the Crystallization of Calcite and Aragonite in a Porous Medium. *Journal of Sedimentary Research*, **66**, 482.
- [34] Raz, S., Hamilton, P.C., Wilt, F.H., Weiner, S. and Addadi, L. (2003) The Transient Phase of Amorphous Calcium Carbonate in Sea Urchin Larval Spicules: The Involvement of Proteins and Magnesium Ions in Its Formation and Stabilization. *Advanced Functional Materials*, **13**, 480-486. <https://doi.org/10.1002/adfm.200304285>