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Control over the crystal phase, shape, size and aggregation of calcium carbonate via a L-aspartic acid inducing process

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Abstract

The acidic amino acid, such as aspartic acid (L-Asp), and glutamic acid are the primary active molecules of the glycoprotein on the organic/inorganic interface of biomineralized tissue. In this study, aspartic acid was used as the organic template in inducing the nucleation and growth of calcium carbonate. With the analysis of X-ray diffraction we investigated the relationship between the L-Asp concentration and the precipitation phase crystal structure of calcium carbonate. SEM and TEM were employed in the analysis of the morphological characteristic of the precipitation and the aggregation of the nanoscale porous phase. In order to get the direct evidence of the interaction between Ca^{2+} and L-Asp, the technique of QCM was used in the investigation of the coordinate interaction of Ca^{2+}/L-Asp. As the results have shown, L-Asp alone is adequate to switch the transformation between calcite and vaterite, and neither soluble organic additions nor metal ions are needed. Meanwhile, the morphology, size and aggregative way of the deposition are also mediated with change of L-Asp concentration. To interpret the cause of the hierarchic structure range from nanoscale to micron-scale and the formation of the porous spheres of vaterite, an assumption of limited-fusion was proposed from the view of the small biomolecules polarity that can control over the growth of the crystals and the aggregation of the micro crystals. The conclusion also provide a new material synthesize strategy.

Keywords: L-Asp; Organic template; Calcium carbonate; Polymorphs

1. Introduction

Among the crystal species that consist the polymorphs of calcium carbonate, calcite, vaterite and aragonite are extensively investigated. Vaterite and aragonite are thermodynamically unstable and can transform easily to calcite [1]. Therefore, vaterite is quite scarce in nature as natural mineral. But in biologic system, vaterite and aragonite can nucleate and grow stably and its crystal lattice can maintain stability with the existence of acidic amino acids in microenvironment formed by organic macromolecules [1,2]. In the past two decades, scientists in different fields have been interested in the mechanism of the interaction between polymorphs of calcium carbonate and specific corresponding complex matrix [3–6]. Therefore, a kind of synthetic method, which explore the effect of such templating species on structure and properties of coordination compound aggregates, has been developed [7–9]. Elementary results have shown that the concentration and the conformation of the polypeptides that are rich in acidic amino acid are entrapped onto the surface of matrices play an important role in the control of the polymorphs of calcium carbonate. The amino acid component on the interface of organic template and calcium carbonate have been tested to be rich in aspartic acid and glutamic acid. Thus, it can be assumed that the carboxyl of aspartic acid and glutamic acid provide abundant Ca^{2+} ions bonding sites [10].

In fact, small quantity of acidic macromolecule such as acidic glycoprotein exists on nearly all the organic/inorganic interface of biomineralized species (tooth, otolith, bone, pearl and mollusk shell etc.) [11,12]. These glycoprotein which contain large proportional acidic amino acid and aspartic acid account for 30–40% in atomic weight, while the mean concentration of other amino acids is 10% in atomic weight [13,14]. This may suggest that it is simple biomolecule such as aspartic acid and glutamic acid that accomplish the inducing of the nucleation and the mediating of the growth of

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minerals [5]. The common view is that the surface formed by multi-carboxyl of the acidic amino acid residues takes on the surface of heterogeneous nucleation and furthermore, plays an important role in the controlling of polymorphs. But the exact mechanism of the interaction of biomolecules to calcium ions and calcium carbonate is still unclear and further understanding of the details of the molecule recognition happened on the interface and the control that simple biomolecules cast on the polymorphs is needed.

In this study, L-Asp was used as organic template that induce the nucleation of calcium carbonate. The attention was focussed on the effect of the L-Asp/Ca^{2+} multi-carboxyl chelate on the polymorphic character, morphological modification and aggregate state (shape and size) of calcium carbonate in the presence of L-Asp with different concentration. The aim of the research is a better understanding of the principles governing biomineral formation. In order to attain the direct evidence that Ca^{2+} interacted with L-Asp, QCM was employed to investigate the complex action of Ca^{2+}/L-Asp. The structure of calcium carbonate precipitation was investigated by a wide-angle X-ray diffract meter (XRD). TEM and SEM were employed to investigate morphologic features of the nanoscale porous aggregate of the precipitation of calcium carbonate. Finally, a new fusion-limit assumption was proposed for simulating organic control crystal growth and particle aggregation.

2. Materials and methods

2.1. Material

Polyethylene glycol (PEG) was purchased from Sigma. L-Asp was obtained from Institute of Biological Products of Shanghai (Shanghai, China). $CaCl_2$ and Na_2CO_3 are all of analytical reagent grade. Deionized ultrapure water was used throughout the experiment.

The piezoelectric crystals used in this study were gold deposited AT-cut with 9 MHz basic resonance frequency and was purchased from Beijing Chengguang Co. (Beijing, China). The crystal consists of a $12.5 \cdot 0.2$ mm (diameter, thickness) quartz wafer, placed between 6 mm gold electrode, the oscillator circuit was constructed from a transistor–transistor logic integrated circuit (TTL-IC). The frequency was monitored by a high resolution frequency counter (CN3165, resolution ± 1 Hz, Sampo Co., Taiwan, China).

2.2. Experimental procedure

2.2.1. QCM measurements

Let the PEG-bound wafers dipped into the sample pools containing L-Asp solution of various concentration for 30 min at 37°C and at the same time surveillanced the change of frequency. When the change of frequency retained stable which showed L-Asp reached its saturation of absorption, noted down this frequency (F_1). Dripped the same quantities of Ca^{2+} solution (0.01 mmol/ml) into those sample pools mentioned above and noted down the frequency (F_2) after 30 min, thus changes of frequency $F_2-F_1=\Delta F$ were attained and these changes were related to the amounts of Ca^{2+} absorbed on the L-Asp-bound wafers.

2.2.2. The synthesis of calcium carbonate

The effect of L-Asp on crystal polymorphs was studied by adding various amounts of L-Asp (0, 5, 10, l5, 20, 30, 40 mg) into $CaCl_2$ solutions (20 ml, 0.5 mol/l) (dissolved in ultrasonic) and plugging them slowly into Na_2CO_3 solutions (20 ml, 0.5 mol/l) at 37°C. A compare of precipitation of calcium carbonate formed with the presence of different amounts of L-Asp including the sample without L-Asp could be made. Then the $CaCO_3$ precipitation was rinsed with deionized ultrapure water, centrifugalized, dried and glued to SEM, TEM and XRD stubs.

2.2.3. Characterization of crystal phase and morphology of precipitation

Morphological investigations were carried out by using a SEM (X-650 Hitachi, Japan) and a TEM (100-CX JOEL, Japan). The crystal phase was characterized by wide angle X-ray diffraction analysis (XRD, RIGAKU, Japan). The work condition of XRD was CuK_0 radiation via a rotating anode at 40 kV and 50 mA. The data were collected in step of 0.05° 10^{-1} s and range of scattering angles (2θ) from 10° to 60°. The following $h\,k\,l$ family reflections were monitored: for calcite (1 0 4), vaterite (1 1 2)(1 1 4).

3. Results and discussion

3.1. Investigation of Ca^{2+}/L-Asp complexation with QCM

A QCM has been used as a mass sensor for a wide field of applications in chemistry, biology and environmental, food and clinical analysis [15,16]. Its resonant frequency decreases with the increase of mass on the QCM electrode in a nanogram level. Previous work has proved that soluble organic matrix of mollusc shell contains large proportions of acidic amino acids [13,14]. A number of studies of this organic matrix have indicated that electrostatic binding or association, close geometric match and stereo-chemical correspondence at inorganic/organic interface are important for controlled crystallization in biological systems, but the exact nature of the molecular interaction between

the interface of biomolecular and organic crystals could not be comprehended well. In the present work, by means of QCM, we have investigated the complex interaction between Ca^{2+} and L-Asp, which is the largest proportions of the amino acid composition on organic matrix. Fig. 1 shows the change of frequency shift (ΔF) depend on the complex time of L-Asp/Ca^{2+}. ΔF increases with the increases of the complex time. After 30 min, ΔF becomes stable which shows that the complexation reach its saturation. According to this curve we can determine the optimum time for recording stable data. Fig. 2 reveals the ΔF appears as a function of L-Asp concentration when the concentration of Ca^{2+} remain stable. ΔF takes monotone increase as the concentration of L-Asp increases at [Ca^{2+}] 0.01 mmol/ml. These results realizingly proved the existence of strong interaction between Ca^{2+} and L-Asp and the double carboxyls on the side chains of L-Asp provide proper binding sites for Ca^{2+}, thus a complexation of $[Asp\text{-}Ca]_x^y$ that was important for the inducement of biomineralization was formed. It is that complexation that acts as the organic template to induce the nucleation of calcium carbonate crystal.

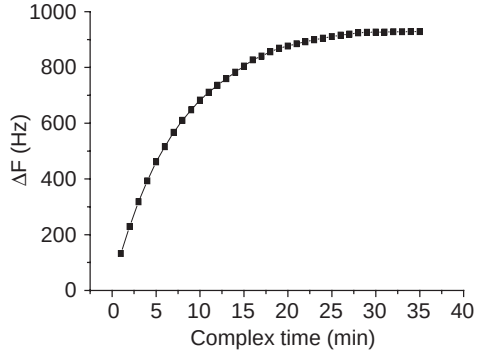

Fig. 1. Frequency shifts (ΔF) as a function of the Asp/Ca^{2+} complex time.

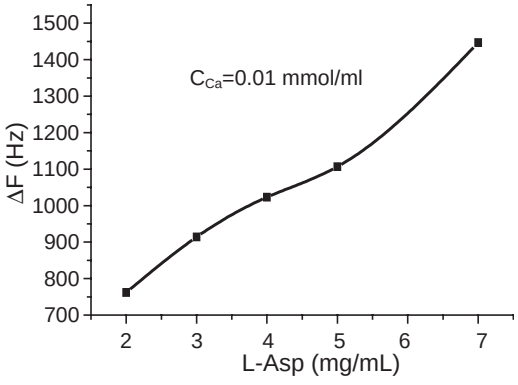

Fig. 2. Frequency shifts (ΔF) as a function of the L-Asp concentration at Ca^{2+} concentration 0.01 mmol/ml.

3.2. Control of the polymorphs of $CaCO_3$ by L-Asp

The control of particle shape and size is a complex process requiring a fundamental comprehension of the interactions at interface. These include the fluid/nuclear, fluid/matrix and nuclear/matrix. To characterize the effect of organic molecules on the interface on the structure of biominerals by proper experimental ways is critical for further comprehension of the mechanism of interface interaction related to the process of crystallization. The XRD results that show the effect of L-Asp on the crystallization of calcium carbonate polymorphs in the presence of different concentration of L-Asp are summarized in Fig. 3. Fig. 4 shows the dependence of the ratio of the strongest peak of vaterite to the strongest peak of calcite on the concentration of L-Asp. The content of vaterite in the precipitation is proportional to the quantity of L-Asp additive.

Also, the morphology and aggregates of precipitate crystal take dramatic changes as the quantity of L-Asp additive changing (Fig. 5). When there is no L-Asp added into the reaction system, only calcite can be observed and the precipitate is bulky amorphous crystal (Figs. 3a and 5a). A small quantity of L-Asp can induce the formation of vaterite. When the L-Asp concentration is within 0.25–0.5 mg/ml, the precipitate contains a small amount of vaterite besides the majority of calcite which appears layered and rhombohedral shape (Figs. 3b and 5b). As the concentration increase to more than 0.5 mg/ml and less than 1.0 mg/ml the precipitate is a mixture of spherical vaterite crystal and layered, rhombohedral calcite crystal (Figs. 3c and 5c). When the concentration increases to more than 1.0 mg/ml, nearly all the precipitate becomes spherical vaterite crystal (Figs. 3d, 5e, and f). These results indicate obviously that L-Asp plays an important role in the control of crystal polymorphs. L-Asp alone is sufficient to control the transformation between calcite and vaterite and no insoluble organic matrix nor exotic metal ion is needed.

According to classical Gibbs free energy formula, the driving force for the formation of stable vaterite (ΔG_v) is given by the equation as follows [17]:

$$\Delta G_v = -R\ T_g/n\ S, \tag{1}$$

where R, T_g and S are gas constant, absolute temperature and supersaturation, respectively. From this equation, it can be conclude that the higher the supersaturation is, the higher the tendency for the formation of vaterite will be. The negatively charged carboxyls of monodisperse L-Asp molecules in the reaction system can bond Ca^{2+} strongly and thus forms a large scale of local supersaturation microenvironment. On the other hand, strong electric field resulted from high concentration of negatively charged carboxyls is in

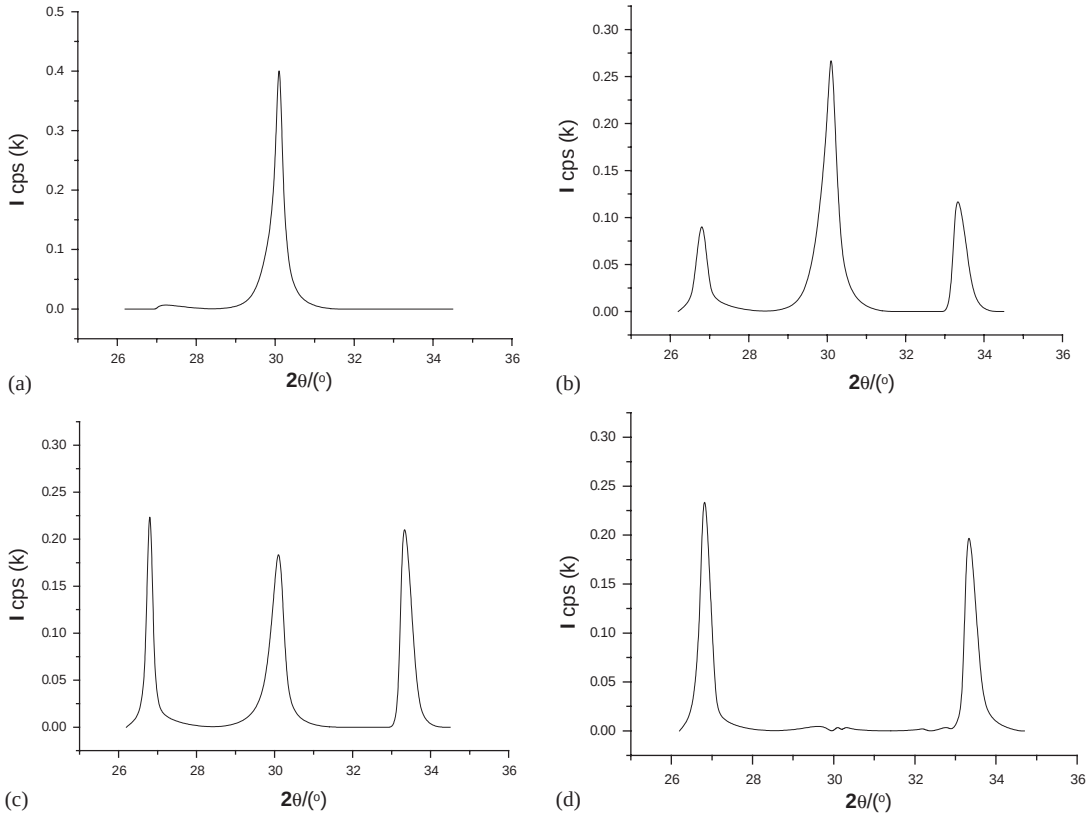

Fig. 3. XRD spectrum of $CaCO_3$ in the presence of Asp with different concentration: ( ),- - - - diffraction peak of calcite; ( )*,- - - - diffraction peak of vaterite. (a) C_{Asp} = 0.0 mg/ml; (b) C_{Asp} = 0.25 mg/ml; (c) C_{Asp} = 0.5 mg/ml; (d) C_{Asp} = 1.5 mg/ml.

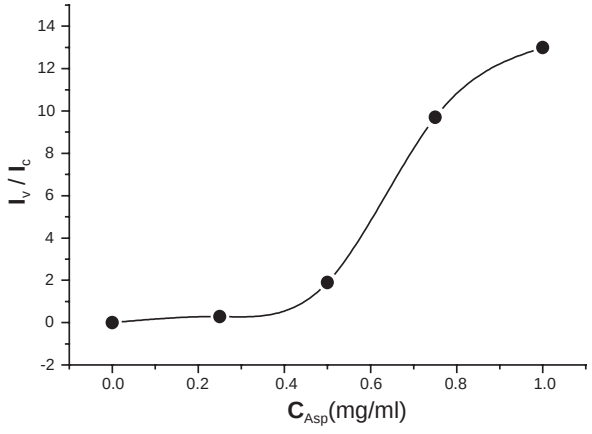

Fig. 4. The ratio of the intensities for the 104 calcite peak I_c, and 110 vaterite peak I_v as a function of the Asp concentration.

favor of the interaction that with the most positively charged crystalline plane. Vaterite has two homo-charged calcium plane (0 0 1) and (1 0 0) with a charge density of about 6.7 calcium ions/nm^2 comparing with those of the (0 0 1) calcium planes of calcite (4.5 calcium ions/nm^2) [18,19].

3.3. Superfine, porous and aggregate of precipitate

It is worth paying attention to the fact that the spherical vaterite induced by L-Asp is porous (Figs. 5e,f, and 6). These spheres are aggregations of smaller microcrystals of vaterite and both the aperture and particle diameter are all in nanoscale (3 ~ 150 nm).

According to the Gibbs–Thomson formula of classical nucleation theory

$$J = A \exp[-B(\ln S)^{-2}], \quad (2)$$

where J and S are nucleation rate and supersaturation, A and B are constants, respectively. This equation indicates that the rate of nucleation increases along with the increase of supersaturation. Keeping a state of local supersaturate microenvironment is in favor of increasing the number of crystal nucleus. Furthermore, large amount of crystal nucleus will contribute to the refinement of precipitate particles. Due to that the aggregates have a structure of quite high porosity and that the crystal growth and fusion process among particles will directly control the aggregation process, we proposed an assumption *growth- and fusion-limited aggregation* to describe this type of aggregation. The illustration of this aggregate process is shown in Fig. 7.

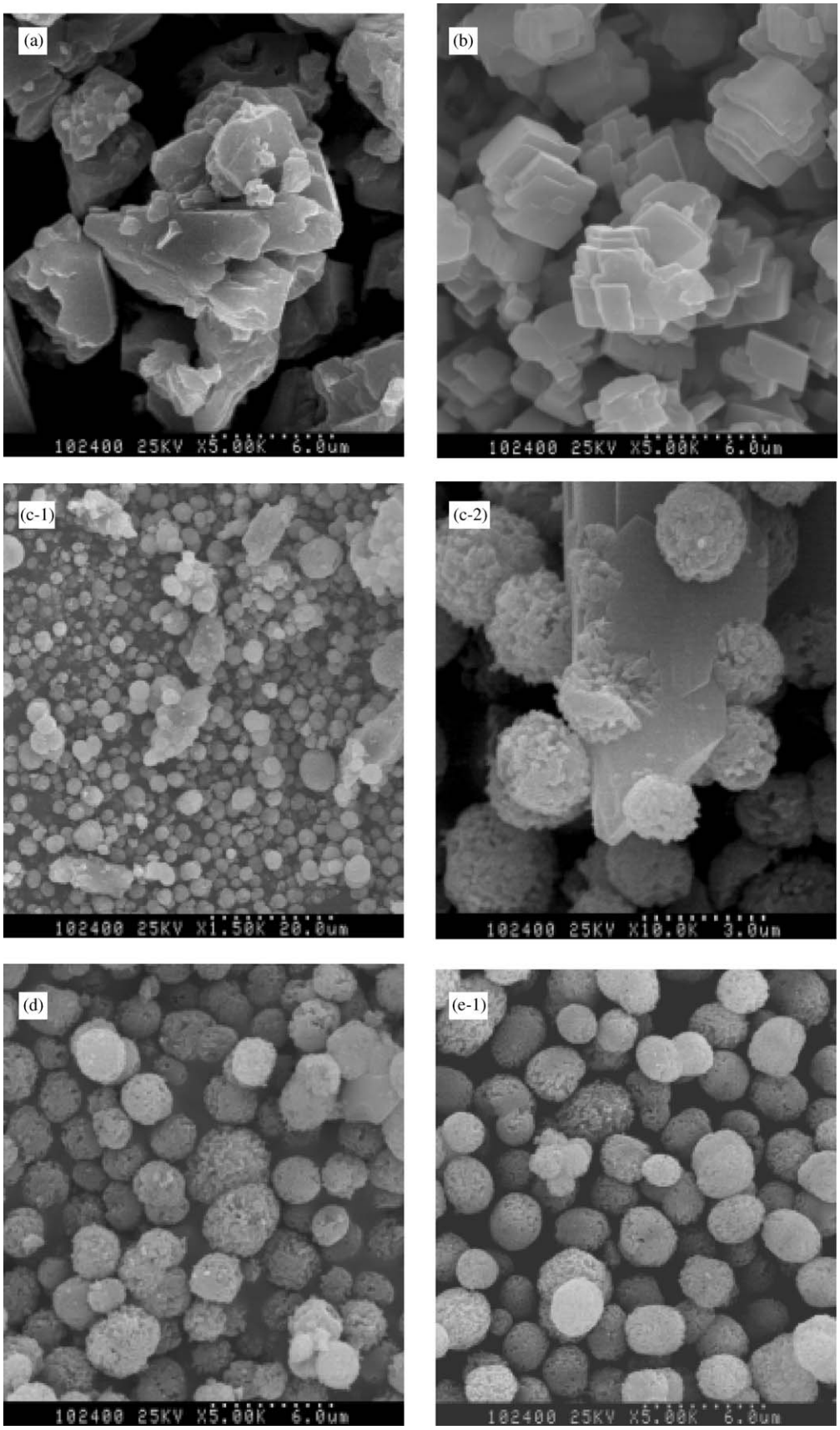

Fig. 5. SEM images of calcium carbonate polymorphs crystals grown in the presence of different concentration L-Asp: (a) amorphous calcite crystals grown in reaction solution without Asp; (b) layered and rhombohedral calcite crystals grown in reaction solution containing 0.25 mg of Asp per ml; (c) vaterite sphere and layered and rhombohedral calcite crystals grown in reaction solution containing 0.5 mg of Asp per ml; (d) vaterite spheral crystal and a few calcite crystal grown in reaction solution containing 0.75 mg of Asp per ml; (e) all vaterite spheral crystals grown in reaction solution containing 1.0 mg of Asp per ml; and (f) vaterite spheral crystals grown in reaction solution containing 1.5 mg of Asp per ml.

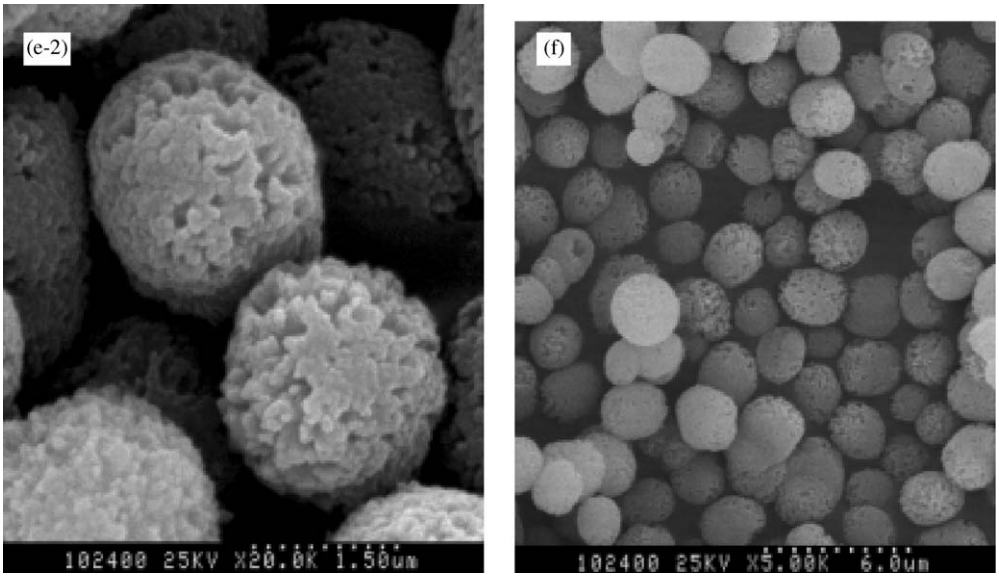

Fig. 5 (*continued*).

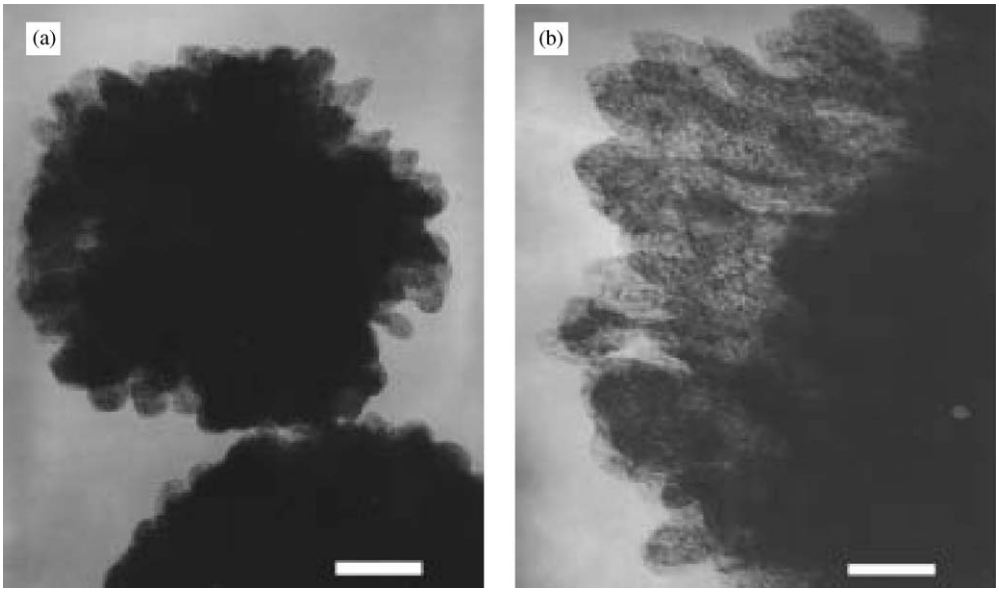

Fig. 6. TEM images of vaterite spheral crystals grown in reaction solution containing 1.5 mg of Asp per ml. (a) Scale bar 300.0 nm (b) Scale bar 50.0 nm.

When the nucleophilic substitution reaction takes place, the L-Asp in the complexation ($[\text{L-Asp-Ca}]_x^y$) will be replaced by CO_3^{2-} contain in the reaction solution. Then the replaced L-Asp can bind to the specific crystal plane with its double negatively charged carboxyls and thereby inhibit the crystal growth. Due to the steric effect, L-Asp molecules absorbed in the surface of crystal grains will inhibit the solution of vaterite and complete fusion among particles.

These results suggest that the aggregation of the precipitate is stepwise. The first step is the formation of primary particles through limited growth after nucleation. Then, by *fusion-limited* mechanism, these particles collide with each other to form secondary particles which are hard aggregates by partial fusion. To meet the rule of lowest energy, unstable solid phase frequently form spherical aggregates of small crystallites while porous vaterite spheres of micron-scale are the result of third aggregation.

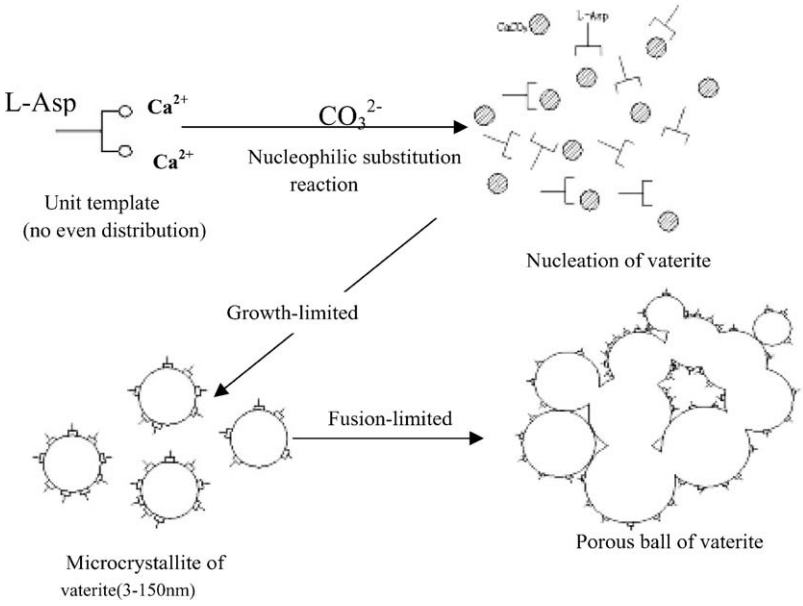

Fig. 7. The scheme of growth- and fusion-limited assumption.

4. Conclusion

1. There is strong interaction between Ca^{2+} and L-Asp and Ca^{2+}/L-Asp complexation acts as the organic template to induce the nucleation of calcium carbonate crystal.
2. With the control of L-Asp, vaterite can be formed and is able to maintain its stability compared with the crystals formed in the absence of L-Asp.
3. L-Asp realizes its control over the nucleation and the growth of crystallization in terms of increasing the rate of nucleation and limiting the growth of crystal. The assumption of *growth- and fusion-limited aggregation* mechanism can interpret the formation of nanoscale hierarchic structure and the porous sphere of vaterite.

Acknowledgements

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