Biomimetic synthesis of monodisperse rosette-like calcite mesocrystals regulated by carboxymethyl cellulose and the proposed mechanism: An unconventional rhombohedra-stacking route†

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Carboxymethyl cellulose (CMC) is systematically investigated for the first time in regulating the CaCO3 crystallization using a biomimetic gas-diffusion method. Monodisperse rosette-like calcite spherules in uniform size with their surfaces composed of rhombohedral subunits were synthesized in certain conditions. According to time-resolved experiment, the evolution of the crystal morphology was traced and the possible route in which rosette-like spherules form is suggested. We propose that the amorphous calcium carbonate precursors form initially and act as secondary nuclei, followed by the stacking of rhombohedral subunits in partial rather than complete superposition between each other due to the electrostatic repulsive interactions between the polyanion chains adsorbed on the blocks, which results in the final rosette-like morphology. Mineralization experiments in CMC solutions with different concentrations were also carried out and the results obtained at no higher than 1 g L⁻¹ further prove the above mechanism from the fact that the extent of the polymer influence decreases proportionally with concentration, i.e. the degree of superposition of the building blocks becomes larger by decreasing the CMC concentration. In contrast, in a higher polymer concentration of 5 g L⁻¹, another aggregation way from nanoparticles is adopted. In addition, the influence of the initial calcium ion concentration and the chain length of CMC on the crystal morphology were also investigated. The present investigation on the influence of CMC on CaCO3 crystallization not only provides for the formation mechanisms of the rosette-like calcite spherules but also leads to a new possible route to fabricate new materials which can be used in many industrial areas.

Introduction

Nature fabricates various kinds of biominerals existing in almost all of the organisms which are involved in a wide variety of important biological functions including protection, structural support, cutting and grinding etc., such as shells, bones and teeth.¹⁻³ In the past few decades, numerous studies on biomimetic mineralization have been made to control the structures and properties of the organic–inorganic complex and to explore the formation mechanism of inorganic minerals with various kinds of morphologies.⁴⁻⁸ Calcium carbonate, one of the most important biominerals existing in organisms and also a very important industrial raw material in ceramics, paper-making, cosmetics, pigments, rubber industry etc., has been intensively researched as a model compound in biomimetic mineralization as well as for its potential application.⁷,⁹ It is widely accepted that CaCO3 has three different anhydrous phases: vaterite, aragonite and calcite, with an increasing thermodynamic stability.¹ It is known that the polymorph, morphology and structural properties of CaCO3 can be effectively controlled by the use of water-soluble crystal growth modifiers such as synthetic polymers,¹⁰⁻¹⁴ proteins,¹⁵ polysaccharides as well as substrates like microemulsions,¹⁶,¹⁷ self-assemble monolayers¹⁸,¹⁹ and nanotubes²⁰ etc. However, the understanding of the mechanism how additives modulate the mineral phase and the structural relationships between organic and inorganic phase is still far from complete.

Carboxymethyl cellulose, usually used in its sodium salt (NaCMC), is a sort of water-soluble ether of cellulose with parts of its –OH side groups substituted by –CH₂COONa groups. As is well known, NaCMC is widely used in a lot of industrial sectors like adhesive industry, paper-coating, cosmetics, mineral processing, pharmaceutical and food industry.²¹ Besides, in fundamental research, it was also frequently researched as a kind of representative polyanion as well as a derivative of polysaccharide in polymeric and colloidal chemistry.²² Although CaCO₃-based biomimetic mineralization in the presence of polysaccharides has been studied by many researchers (partly because of the important role that the polysaccharides have for the storage of calcium¹), no systematic work on the mineralization regulated by NaCMC was carried out until now. Herein, we choose NaCMC as a crystal growth modifier and systematically investigate for the first time its influence on the CaCO₃ crystallization. Various kinds of morphologies of CaCO₃ were obtained depending on the reaction conditions. In certain conditions regular rosette-like
calcite spherules were obtained. Such rosette-like CaCO₃ particles have been obtained by other researchers with the use of double-hydrophilic block copolymers,²³,²⁴ heparin²⁵ and pectin.²⁶ However, the formation mechanism of such crystals is still not clear. Interestingly, by optimizing the experimental conditions, we obtained nearly monodisperse rosette-like calcite spherules. It is believed that monodisperse particles have numerous applications in ceramics, pigments and many other areas.²⁷

Recently, Colfen and Meldrum et al. reported a non-classic crystallization mechanism. They proposed that all crystalline particles, even including single crystal, can be formed in an aggregation way with a difference in the degree of orientation, followed by a fusing process of the building units, and there are no abrupt changes in the mechanism of particle formation. In our present work, based on the time-resolved experiments and the above theory, we propose the formation route of the above-mentioned rosette-like crystals, that is, through a rhombohedra-stacking-fusing way to form “mesocrystals”.²⁸,²⁹ The effect of the NaCMC concentration was also studied and the results further proved the mechanism, which shows that the effect of the polymer additive is constant and the extent of the effect of the polymer decreases proportionally to concentration.

**Experimental**

Calcium chloride, ammonium carbonate and ethylenediamine tetraacetic acid were purchased from Sinopharm Chemical Reagent Co. Ltd. The two kinds of sodium salts of carboxymethyl cellulose (NaCMC) were both purchased from Acros with a molecular weight of 90 000 and a degree of carboxyl substitution (DS) of 0.7 and the other 700 000 and DS of 0.9, respectively. All of the above chemicals are of analytical grade and used without further purification. The water used in all experiments is deionized water (DIW).

All glassware (glass bottles and small pieces of glass substrates) was cleaned and sonicated in ethanol for 15 min, then rinsed with DIW, further soaked in a HNO₃–H₂O₂–DIW(1 : 1 : 1) solution, then rinsed with DIW, and finally dried with acetone. The mineralization experiments were carried out with a biomimetic CO₂-diffusion method described by Addadi et al.⁰ Briefly, the mineralization experiment was performed in a vessel with a volume of 15 ml which was placed in a closed desiccator at room temperature (24 ± 2 °C). For a typical mineralization process, 10 ml 0.5 g L⁻¹ NaCMC solution with 0.01M CaCl₂ was freshly prepared in a 15 ml vessel and covered with acetone. The mineralization experiments were carried out in a DIW(1 : 1 : 1) solution, then rinsed with DIW, and finally dried at ambient condition. Time-resolved experiments were performed by taking out the glass pieces at a set interval to stop the reaction. The residual calcium ion concentration in the reaction solution was determined by titration with calibrated ethylenediamine tetraacetic acid (EDTA) aqueous solution.

The transmission electron microscopy (TEM) images were taken with a JEOL JEM 2011 at 200KV equipped with electron diffraction. Scanning electron microscopy (SEM) observations were performed on a TS5136MM microscope with gold coated. X-Ray powder diffraction data were recorded on an X’pert Pro with Cu Kα radiation. For IR analysis, the CaCO₃ powders were pressed with KBr into pellets before being examined in transmission mode by a Nicolet Nexus 470 with a resolution of 4 cm⁻¹. Thermal gravimetric analysis of the powder was carried out on a Perkin-Elmer Pyris-1 TGA to investigate the contents of organic additives. The pH values of the solution were monitored by a PHS-3C(A) acidimeter purchased from Shanghai Dapu Instruments.

**Results**

**Effect of NaCMC concentration on the morphology of the CaCO₃ Crystals**

We first adopt 0.01M [Ca²⁺] solution to prepare CaCO₃. A series of concentration of NaCMC aqueous solution from 5 to 0.01 g L⁻¹ was taken to investigate the effect of NaCMC concentration on the formation of the crystal. SEM images of CaCO₃ crystals after 4 d reaction are shown in Fig. 1. The crystals grown in 5 g L⁻¹ solution are approximately 10 µm spheres with relatively smooth surfaces composed of nanoparticles with a size of tens of nanometers. When the polymer concentration decreased to 1 g L⁻¹, a more complex superstructure (shown in Fig. 1b), which was the so-called rosette-like spherule with its surface composed of rhombohedral subunits, was obtained. Such rosette-like crystals are 10–20 µm, with their surfaces composed of 1–3 µm rhombohedral subunits and have been reported by some researchers including Yu and Arias et al. In contrast, a small amount of other morphologies, including a stacked structure made up of thin crystal sheets as well as imperfect rhombohedra with a rough surface composed of nanoparticles (see Fig. 1c and ESI, Fig. S1a†) is almost the same as those obtained in control Fig. 1d, e and Fig. S1b†). The cross-section of the spherule broken by supersonic, as shown in the inset image of Fig. 1e, depicts a radial structure of the inner part of the spherule. Regulated by a widely used industrial stuff NaCMC, this kind of monodisperse spheres may find certain use in many industrial aspects like ceramics and pigments industry.²⁷ Noticeably, after 15 d of reaction, we can find that around some former spherules grows a circle of rhombohedral units with a size of 3–4 µm, shown in Fig. 1f. The rhombohedral units stack not in complete superposition between each other to surround the former spherule. The sample obtained in 0.1 g L⁻¹ solution, as shown in Fig. 1g, shows an irregular rhombohedral morphology with a curved surface. The crystals obtained from 0.05 g L⁻¹ solution (Fig. 1h) have much smoother surfaces but rounded angles, which is similar to the crystals obtained in Colfen’s recent work.²⁸ When the concentration decreased to 0.01 g L⁻¹, the influence of the polyanions is so slight that the morphologies of a large number of crystals (shown in Fig. 1i and Fig. S1c†) is almost the same as those obtained in control
conditions (no additives, Fig. S1d†), but there is still a small amount of crystals (Fig. 1i) modulated by NaCMC, which show carved surfaces.

The X-ray diffraction patterns depict the polymorph of the CaCO₃ crystals grown from the NaCMC solution ranging from 1 and 0.1 g L⁻¹ is exclusively calcite (1 and 0.1 g L⁻¹ shown in Fig. 2c and 2d are taken as representative), while those obtained in 5 g L⁻¹ solution (Fig. 2e) are a mixture of calcite and vaterite phase. According to Scherrer’s equation calculated at 2θ = 29.5 and 27.2°, the size of nanoparticles obtained in 5 g L⁻¹ is about 35 nm.

The calcite polymorph is further confirmed by IR analysis (Fig. 3b) with the feature peak of calcite phase at 712 cm⁻¹. Meanwhile, compared to the spectrum of pure NaCMC (Fig. 3a), we conclude that the polymer chains are involved in the final product, from the peak of the carboxyl groups at 1601 cm⁻¹ and the peak originated from C–O groups at 1160, 1110 and 1064 cm⁻¹, respectively. The involvement of polymer chains is also confirmed by thermal gravimetric analysis (TGA) which tells a content of ~5% and ~2% organic additives in the 0.5 g L⁻¹ sample (Fig. S2†) and 0.1 g L⁻¹ sample, respectively (Fig. S3†).

As we have examined in pure NaCMC (Fig. S4†), the decomposition temperature of pure NaCMC powder is about 240 °C, consistent with the temperature of weight loss peak in the hybrid product we obtained.

The time-resolved experiment was performed on the mineralization system with 0.01M [Ca²⁺] and 0.5g L⁻¹ NaCMC. The pH value was monitored and the residual calcium ion concentration
in the reaction solution after a different interval was determined. The curve of open circles in Fig. 4 reflecting the variation of the pH value versus time shows that the pH value of the reaction system increases to 9.5 in only 20 min and retains this value during the first 8 h and then undergoes a minor increase from 8 to 12 h when the final value is 9.68. In contrast, [Ca2+] representative by the solid circles decreases slowly during the first 40 min and after 40 min begins to decrease quickly until about 8 h, when the calcium ions in the solution are nearly depleted, to a concentration of approximately 0.0003 M.

The evolution of the CaCO3 morphology was recorded by electron microscope images taken at different intervals, as shown in Fig. 5. It was found that the initially formed CaCO3, after 10 min of reaction (Fig. 5a), is amorphous, which is affirmed by an electron diffraction pattern (the inset of Fig. 5a). The size of the amorphous calcium carbonate (ACC) product is several hundreds of nanometers and its structure is loose in which amounts of water molecules may be contained. After another 10 min, the morphology of the ACC sample changed little
(Fig. 5b) but it should be noted that many regions in small sizes (several nanometers) of crystalline grains with different orientations formed (indicated by arrows in Fig. 5c). After 40 min of reaction, larger crystalline regions with a size of tens of nanometers formed and the electron diffraction pattern performed in this area confirms the single crystal phase of vaterite with a crystal plane index of (110) and (112). The d-spacing shown in Fig. 5d is 4.20 Å, corresponding to the (004) face of vaterite. Ellipsoidal particles were obtained at 1 h interval, with a size of 3–5 µm and a zigzag edge, which indicates a surface composed of rhombohedral subunits, as shown in Fig. 5e. It should be mentioned that the crystal is solid, in consistence with the cross-section SEM image (inset of Fig. 1e). In the 2 h sample (Fig. 5f), we can see from the SEM images that 8–10 µm spheres form and a proportion of the particles are twinned spheres. The building blocks are several hundreds of nanometers. When the time extended to 4 h, rosette-like spheres were formed with a size of 12–15 µm. We also captured a few incomplete spherules (Fig. S6†), from which we can see that the inner parts of the spheres are composed of rhombohedral units with a size of several hundreds of nanometers. In 8 h, when the calcium ions are nearly depleted, the final crystals are basically formed, that is, the ~20 µm rosette-like spherules was obtained. The size of building rhombohedral blocks increases from several hundreds of nanometers to 2–3 µm. After 8 h, the morphology and the size of the particles stayed nearly unchanged. Generally, we can define that the process after 2 h is a process when the crystals grow bigger.

X-Ray diffraction patterns show that the polymorph of crystals obtained after 2 h of reaction are all calcite phase with an obvious (104) face. Before 2 h, XRD analysis could be hardly performed because the amount of the sample is too small (Fig. 6).

Mineralization experiments with a higher initial calcium ion concentration

To investigate the influence of the initial calcium ion concentration in the solution, we also used the 0.1 M CaCl₂ solution to synthesize CaCO₃. Fig. 7a and b show the SEM images of CaCO₃ particles obtained in 0.1 M CaCl₂ and 1 or 0.1 g L⁻¹ NaCMC aqueous solution, respectively. We find that the morphology of crystals is almost the same under the two different polymer concentrations, that is, in both conditions, spheres with a size of 6–10 µm formed and the size distribution is not very homogeneous. Similar particles were obtained at a polymer concentration of 0.5 g L⁻¹ (Fig. S1e and Fig. S1f†). From the enlarged pictures (inset of Fig. 7b) it could be found that the spheres are composed of nanoparticles in a size of tens of nanometers.

The X-ray diffraction data is shown in Fig. 2a and 2b, respectively, in which we can find that the crystals obtained under the two different conditions are all mixtures of vaterite and calcite. The results are also confirmed by infrared analysis (Fig. 3c). Scherrer’s equation is used to calculate the size of the nanoparticles. Taking crystals obtained from 0.1 g L⁻¹ NaCMC solution for instance, the size of the nanoparticles is about 25 nm, which agrees well with the results we could observe from SEM images (20–30 nm, the inset image of Fig. 7b). Besides, the TGA results show that there are 5% organic additives in the final products (Fig. S5†), which implies that the NaCMC chains are...
involved in the nucleation and growth process although the speed of nucleation and growth is rapid due to the high \([\text{Ca}^{2+}]\). The results are also confirmed by IR (Fig. 3c), which reveals peaks at 1601 cm\(^{-1}\) of asymmetric stretching of carboxyl groups and 1160, 1112 and 1064 cm\(^{-1}\) of C–O stretching mode, compared to the spectrum of the pure NaCMC.

**Effect of chain length of NaCMC**

To investigate the effect of molecular weight \(\left( M_w \right) \) of the polymer, we used NaCMC with a higher \( M_w \) of 700 000 instead of NaCMC of 90 000 to perform the same experiment. The procedure of the experiment was completely the same as that of 90 000. Fig. 7c–f shows the SEM pictures of crystals obtained from various concentrations. When the NaCMC concentration is 1 g L\(^{-1}\), the spheres consisting of rhombohedral subunits are obtained, but it is worth noting that more crystals are ellipsoidal, and a number of twinned spheres are also found, which is a little different from those obtained in the 1 g L\(^{-1}\) concentration NaCMC solution with \( M_w \) of 90 000. When the concentration decreased to 0.5 g L\(^{-1}\), it shows no big difference between the products obtained in the two kinds of NaCMC solutions with different \( M_w \), except that the size distribution of the crystals obtained in higher \( M_w \) is not as good as those obtained in the lower one. When concentration decreases to 0.1 g L\(^{-1}\), the influence of the organic additives is weakened even though some crystals also show rough surfaces.

**Discussion**

**The growth mechanism of the rosette-like calcite spherules**

From the results of the above time-resolved experiments, we can trace the possible route of nucleation and growth of the rosette-like crystals. First of all, the NaCMC polyanion in aqueous solution was proven to adopt an extended conformation by Radeva *et al.*\(^{31}\) After the CaCl\(_2\) was added, the calcium ions could interact with the polyanion chains at the location of \(-\text{COO}^-\) groups.\(^{32–34}\) According to Pai’s work, it is assumed that the introduction of Ca\(^{2+}\) to some polyanion solutions would induce the formation of a complex with negatively charged groups (in their work it is –SO\(_3\)) and organize the polyanion backbone into a spherically orientated state.\(^{35}\) Calcium ions bind strongly with the carboxyl groups all along the polymer chains, as Scheme 1A shows. The strong interaction between Ca\(^{2+}\) and polyanions can retard the nucleation and growth of CaCO\(_3\), that is, it will go through a period of induction time at the very early stage of mineralization.\(^{26}\) With the CO\(_2\) and NH\(_3\) continuing to enter the reaction system, the pH value undergoes a rapid increase and the more and more CO\(_3^{2-}\) groups gradually grab the calcium ions against the competition with the carboxyl groups, thus CaCO\(_3\) begins to form and exists predominantly as ACC with a loose structure and a lot of water molecules involved. Generally, the process of primary nucleation is slow and so the calcium ion concentration decreases slowly at the very beginning. As is well known, ACC is a very unstable phase and will quickly transform into other anhydrous crystalline phases like vaterite and calcite.\(^{12,13,36}\) The transition route may be direct to calcite or through a metastable intermediate phase like vaterite or aragonite.\(^{36}\) So, small regions of crystalline grains form via the transformation process, which grows by time to larger regions of crystalline grains like the single crystal region of vaterite we have observed (Fig. 5d). At the same time, as the continual entering of CO\(_2\) occurs, the Ca\(^{2+}\) gathering around the preformed ACC particles, together with the nanoparticles which formed in the solution, begins to nucleate and grow on the surface of ACC particles. That is to say, the ACC particles are taken as secondary

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**Scheme 1** Schematic illustration of the evolution of CaCO\(_3\) of 0.01 M [Ca\(^{2+}\)], and 0.5 g L\(^{-1}\) NaCMC(A-B-C-D) or 0.1 g L\(^{-1}\) NaCMC(A-B-E) or 5 g L\(^{-1}\) NaCMC(F-G) solution. The relative size of the different species present in solution is not drawn to scale. (A) Complex formed by Ca\(^{2+}\) and NaCMC polyanions. (B) The ACC precursor was taken as second nuclei, while species in solution including Ca\(^{2+}\), CaCO\(_3\) nanoparticles as well as polyanions attach onto the surface of ACC particle. Meanwhile, some regions in ACC begin to transform into a crystalline phase. (C) The rhombohedral units stack around the nuclei with a certain angle due to the repulsive interaction between polymers adsorbed on the block surfaces. (D) The final rosette-like morphology of CaCO\(_3\) with the surface composed of rhombohedral subunits. (E) Some of the crystals obtained in 0.1 g L\(^{-1}\) exhibit a carved surface because the units stack nearly but not completely in superposition. (F) At higher polyanion concentration, [Ca\(^{2+}\)]/[COO\(^-\)] is low and the interaction between Ca\(^{2+}\) and –COO\(^-\) groups is much stronger. (G) The spheres formed by aggregation in which polymer chains are involved. See the text for more details.
nuclei (shown in Scheme 1B). ACC nuclei are soon transformed into a crystalline phase in a dissolution-recrystallization process, which would cause cavities in the core of the spherules. However, the size of primary ACC nuclei is just 200–300 nm, so it is hard to detect the cavities which are no larger than 100 nm, especially compared to the 20 μm spherules. During this period, the calcium ions start to decrease quickly because the growth speed of the crystal turns to be fast as soon as the nuclei have formed.

However, due to the existence of CMC chains in the system, the new crystal phase cannot grow in the simple classic ion-by-ion route and the process of adsorption and growth of calcium ions must be influenced by the polyanion chains. When the blocks on the nuclei surface grow bigger, the polyanions adsorbed on the surface of the blocks would interact with each other, to repulse from each other due to the electrostatic repulsive interactions. Because of the repulsive interaction between polymers adsorbed on the block surfaces, the building blocks made up of nanoparticles do not stack in complete superposition but in partial superposition between each other to reduce the repulsive interaction, as shown in Scheme 1C.

After then it is a process that the blocks fuse together and the former crystals grow bigger by rhombohedra-stacking, from several micrometers to about 20 micrometers. Calcium ion concentration decreases quickly during this period. The particle size increases from 4–5 μm in 60 min to 8–10 μm in 2 h, then 12–15 μm in 4 h, finally the 20 μm rosette-like particles (shown in Scheme 1D). The building blocks grow from several hundreds of nanometers to 2–3 μm. In addition, the slight increase of the pH value after 8 h is due to the depletion of Ca2+ and the constant diffusing of NH3. The crystals obtained after 15 d further provide evidence that the growth of the rosette-like crystal undergoes a stacked mechanism described above (Fig. 1f).

It should be pointed out that the time-resolved experiment in a strict sense is hard to perform because the in situ monitoring is hard to carry out and for in vitro experiments the intermediate phase is hard to seize. Recently, Sommerdijk et al. have successfully used the method of cyroTEM to freeze the intermediate phase and perform a quasi-time-resolved experiment, and found that the ACC can transform into vaterite or calcite in several minutes. So our present experiment can be seen as an in vitro quasi-time-resolved experiment.

### The effect of polymer concentration

Table 1 shows some parameters in the solution we used in the experiment including the overlap concentration of NaCMC and the ratio of Ca2+ to COO− groups along NaCMC polyanions ([Ca2+] /[COO−]). According to the method supplied by Radeva et al., we calculated the overlap concentration for 90 000 Mw NaCMC as ca. 0.71 g L−1. It is worth noting that only in the concentration of 1 and 0.5 g L−1 which is close to the overlap concentration could we obtain the rosette-like crystals. From the table we can conclude that polymer concentration or, to be exactly, the ratio of [Ca2+]/[COO−] plays a significant role in fabricating such rosette-like crystals. It is obvious when the NaCMC concentration is high at 5 g L−1 ([Ca2+]/[COO−] is 1/1.6), the formation route of the CaCO3 is different. As we analyzed in a high polymer concentration, the number of COO− groups is larger and the interaction between Ca2+ and −COO− groups is much stronger due to the low [Ca2+]/[COO−]. Consequently, Ca2+ and preformed nanoparticles could not be easily transported and so could just form crystals via an aggregation way described by Colfen et al. as shown in Scheme 1F and 1G. And due to the strong interaction between the polymers and nanoparticles, the vaterite phase can be retained at least for 4 d. Recently Yu et al. obtained similar spheres in a relatively high polymer concentration with a mixture phase of vaterite and calcite, and it is believed that high polymer concentration is favorable for the formation of the vaterite phase.

When the polymer concentration is 1 g L−1, the rosette-like spherules formed together with small amounts of stacked crystals and imperfect rhombohedra composed of nanoparticles (Fig. 1c). Because 1 g L−1 lies between high (5 g L−1) and medium concentration (0.5 g L−1), just as we analyzed, the rosette-like and stacked crystals form through the ACC-nucleation route, while the crystals with rough surfaces form in the aggregation way. Monodisperse rosette-like calcite spherules formed only in a concentration of 0.5 g L−1, which proves to be the optimized condition to synthesize the rosette-like crystals. As concentration decreases to 0.1 g L−1, [Ca2+] /[COO−] increases and the effect of the NaCMC becomes much smaller than the 0.5 g L−1 sample. The amount of residual CMC chains contained in the 0.1 g L−1 sample is about 2% (Fig. S3†), smaller than 0.5 g L−1 sample (~5%, Fig. S2†), which partially indicates the less influence of NaCMC in 0.1 g L−1 sample. Despite the repulsive interaction still existing among the building blocks, they could stack nearly in superposition, fuse together in the same orientation and form a bigger rhombohedron, as Colfen et al. depicts (Scheme 1E). The 0.05 g L−1 sample, which is very similar to the sample obtained by Colfen et al. also has a close ratio of [Ca2+]/[polymer] with them. The influence of the polymer in 0.01 g L−1 solution is so slight that the crystals obtained are very similar to those obtained in solutions without any organic additives (control sample, Fig. S1d†), except for a small proportion of particles which exhibit carved surfaces. The continuous, non-abrupt change in the evolution of morphology with the variation of polymer concentration confirms the mechanism we proposed.

### Table 1

Some parameters in the solution with CaCl2 and NaCMC

<table>
<thead>
<tr>
<th>MW</th>
<th>DSa</th>
<th>DCs/nm</th>
<th>C*/g L−1</th>
<th>[Ca2+] /[COO−] with 0.01 M [Ca2+] and 5 g L−1 NaCMC</th>
<th>[Ca2+] /[COO−] with 0.01 M [Ca2+] and 0.5 g L−1 NaCMC</th>
<th>[Ca2+] /[COO−] with 0.1 M [Ca2+] and 0.5 g L−1 NaCMC</th>
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<tbody>
<tr>
<td>90K</td>
<td>0.7</td>
<td>0.71</td>
<td>0.71</td>
<td>1/1.6</td>
<td>6.2/1</td>
<td>62/1</td>
</tr>
<tr>
<td>700K</td>
<td>0.9</td>
<td>0.56</td>
<td>0.02</td>
<td>1/2</td>
<td>5.2/1</td>
<td></td>
</tr>
</tbody>
</table>

a Degree of substitution. b Distance of charges. c Overlap concentration.
The growth of the crystals at no higher than 1 g L\(^{-1}\) is led by the stacking of building rhombohedral blocks and the extent of the polymer influence decreases proportionally with concentration, i.e. the degree of superposition of the building blocks becomes larger with the decrease in polymer concentration.

**Effect of initial calcium ion concentration**

It is believed that the nucleation and growth of the crystals are mainly controlled by the kinetic regime due to the high calcium ion concentration.\(^{44}\) At a high supersaturation, the nucleation and growth of the crystal are so fast that the influence of the organic additives is not as obvious as that in the 0.01 M [Ca\(^{2+}\)] solution. It can be easily understood that at low supersaturation the nucleation and growth of the crystals are relatively slow and so yield a relatively regular morphology. Many researchers\(^{32,44}\) have reported that in relatively higher [Ca\(^{2+}\)] it was preferred to yield a vaterite phase. So in our present work, the morphology and the polymorph of crystals obtained in 0.1 M [Ca\(^{2+}\)] are largely different from those obtained in 0.01 M [Ca\(^{2+}\)]. Spheres composed of nanoparticles form and the approximate size of such nanoparticles was calculated as 25 nm using Scherrer’s equation, in good agreement with the results we can directly obtain from the SEM images. The results reveal that the sphere is probably formed by an aggregation way.

**Effect of chain length of NaCMC**

To investigate the effect of the chain length of NaCMC on the mineralization process, we used NaCMC with \(M_w\) of 700 000 instead of that of 90 000 to perform the same experiment. However, the difference between the influence of the two polymers with different \(M_w\) does not seem so obvious. Jada\(^{49}\) et al. studied the effect of the \(M_w\) of NaPAA and concluded that polymers with smaller \(M_w\) values absorb more efficiently on crystal surfaces and give smaller particle sizes compared to the polymers having higher \(M_w\). However in our present study, the morphology of the final crystals and the particle size shows no obvious difference between two polymers with different molecular weights. The reason is probably that the monomer concentration is the same for two polymers with different molecular weights. Besides, more or less, the different conformation of the two polymers in aqueous solution and the different viscosity of the solutions may influence the interactions between polymer and the particles in the solution.

**Conclusions**

In summary, we systematically investigate the influence on the CaCO\(_3\) crystallization of NaCMC, a sort of cellulose ether frequently used in fundamental research and industrial aspects. The effect of polymer concentration, the molecular weight of the polymer and the initial calcium ion concentration was studied. Especially when the polymer concentration was 0.5 g L\(^{-1}\), monodisperse rosette-like calcite spherules were obtained, with their surfaces composed of rhombohedral calcite subunits. Based on the quasi-time-resolved experiments, we propose that the ACC precursor form initially which soon transforms into vaterite and calcite crystalline grains, meanwhile, the calcium ions quickly attach onto the surfaces of ACC nuclei, continuing to grow. During the growing process, the calcite building blocks made up of CaCO\(_3\) nanoparticles stacks on the ACC nuclei, not in complete but partial superposition between each other due to the electrostatic repulsive interactions between the polyanion chains adsorbed on the blocks. The mechanism could also be used to interpret the formation of crystals obtained in concentrations no higher than 1 g L\(^{-1}\). At a higher polymer concentration, the crystal formed in another aggregation way aggregated by nanoparticles. The influence of the initial calcium ion concentration and the molecular weight of the polymer were also discussed. We demonstrate here not only an investigation on the formation mechanism of rosette-like CaCO\(_3\) crystals in biomimetic mineralization but also propose a new route to fabricate new materials which can be used in many industrial areas.

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