

## Well-defined star-shaped calcite crystals formed in agarose gels

Dong Yang, Limin Qi\* and Jiming Ma

State Key Laboratory for Structural Chemistry of Stable and Unstable Species, College of Chemistry, Peking University, Beijing 100871, P. R. China. E-mail: liminqi@chem.pku.edu.cn

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Single crystals of calcite exhibiting a morphology of well-defined 8-armed stars, which evolved from original rhombohedral calcite crystals with their 8 points extending radially into eight arms, were produced by crystallization of  $\text{CaCO}_3$  in agarose gels.

The remarkable range of morphologies exhibited by biominerals has inspired the biomimetic synthesis of inorganic materials with complex form.<sup>1</sup> In recent years, much effort has been devoted to the biomimetic morphogenesis of calcium carbonate, one of the most abundant biominerals, in the presence of various organic substrates including soluble additives, insoluble matrices, as well as self-organized media.<sup>2,3</sup> For instance, synthetic polymers have been employed as soluble additives,<sup>4</sup> soft<sup>5</sup> and solid<sup>6,7</sup> templates for the controlled morphogenesis of  $\text{CaCO}_3$  with a variety of morphologies. On the other hand, biomacromolecules including proteins and polysaccharides have been used for the *in vitro* study of controlled crystallization of calcium carbonate since these biomolecules play key roles in biomineralization processes. In particular, collagenous matrices, such as cross-linked gelatin xerogels<sup>8</sup> and gelatin hydrogels,<sup>9</sup> have received considerable attention. It is noted that insoluble polysaccharide matrices such as chitosan/chitin have been used in combination with soluble acidic macromolecules to induce thin-film crystallization of  $\text{CaCO}_3$ ;<sup>10</sup> however, there are only few efforts made towards the controlled crystallization of  $\text{CaCO}_3$  in hydrogels formed by polysaccharides.<sup>11</sup> It is noteworthy that the silk fibroin in mollusk shell organic matrices exists in the form of a hydrogel, indicating that gel matrices play an important part in the  $\text{CaCO}_3$  crystal growth.<sup>12</sup>

Agarose is a linear polysaccharide extracted from marine red algae, and consists of alternating 1,3-linked  $\beta$ -D-galactose and 1,4-linked 3,6-anhydro- $\alpha$ -L-galactose. It forms a gel when a homogeneous solution is cooled to below the ordering temperature ( $\sim 35^\circ\text{C}$ ) and an infinite three-dimensional network of agarose fibers develops; the melting of agarose gels occurs at a comparatively higher temperature ( $\sim 85^\circ\text{C}$ ), leading to a setting–melting hysteresis behavior.<sup>13</sup> Agarose gels are widely used in the food and pharmaceutical industries and as an important support for chromatography analysis.<sup>14</sup> In this work, we show that well-defined star-shaped calcite crystals can form in agarose gels when the hydrogels doped with soluble calcium salt are mineralized by diffusion from a solution of carbonate.

In a typical synthesis,  $0.05\text{ mol L}^{-1}$   $\text{CaCl}_2$  solution was heated to  $75\text{--}80^\circ\text{C}$  and 1% (w/w) of agarose (Aldrich) was added, which was further heated to  $85\text{--}90^\circ\text{C}$  to ensure dissolution of agarose. The calcium chloride/agarose solution was poured into a culture dish and allowed to cool at the room temperature. The cooled agarose gels were cut into cubic pieces about  $1\text{ cm}^3$  in volume, which were soaked in  $0.05\text{ mol L}^{-1}$   $\text{Na}_2\text{CO}_3$  solution at  $30^\circ\text{C}$  or  $65^\circ\text{C}$ . After 24 h of incubation, the gels were rinsed in water to get rid of the residual inorganic salt. Finally, the resulting  $\text{CaCO}_3$  crystals were recovered by heating the gels containing  $\text{CaCO}_3$  crystals in hot water ( $\sim 90^\circ\text{C}$ ) to dissolve agarose followed by washing and filtration. Our preliminary experimental result showed that the short period of heating in hot water did not affect the crystal morphology

considerably. The obtained  $\text{CaCO}_3$  products were characterized by scanning electron microscopy (SEM) on an AMARY 1910FE microscope operated at 5 kV and Powder X-ray diffraction (XRD) on a Rigaku Dmax-2000 diffractometer with  $\text{Cu K}\alpha$  radiation.

The  $\text{CaCO}_3$  crystals obtained in agarose gels after incubation at  $30^\circ\text{C}$  for 24 h are pure calcite crystals according to the related XRD pattern. Fig. 1 presents typical SEM images of the obtained calcite crystals, which exhibit a unique star-shaped morphology. As shown in Fig. 1a, the crystals range from 30 to  $60\text{ }\mu\text{m}$  in size and look like stars with eight arms extending radially, each of which exhibits three concave channels showing a trident-like morphology. This morphology reflects the hexagonal symmetry of calcite, indicating that they are calcite single crystals evolved from typical rhombohedral calcite crystals. An enlarged image of a trident-like arm (Fig. 1b) shows the obvious calcite  $\{104\}$  macrosteps, strongly indicating the single-crystalline nature of the star-shaped crystals. To the best of our knowledge, this is the first time that the novel star-shaped morphology of calcite crystals has been observed.

The growth process of the star-shaped calcite crystals in agarose gels was investigated by examining the early stages of the crystal growth (Fig. 2). As shown in Fig. 2a, small calcite crystals ( $\sim 5\text{ }\mu\text{m}$ ) with a typical rhombohedral morphology were obtained after incubated in the gels for 1 min. After an incubation time of 10 min, the crystals grew larger with a size about  $20\text{ }\mu\text{m}$  and a terraced excavation on each  $\{104\}$  face

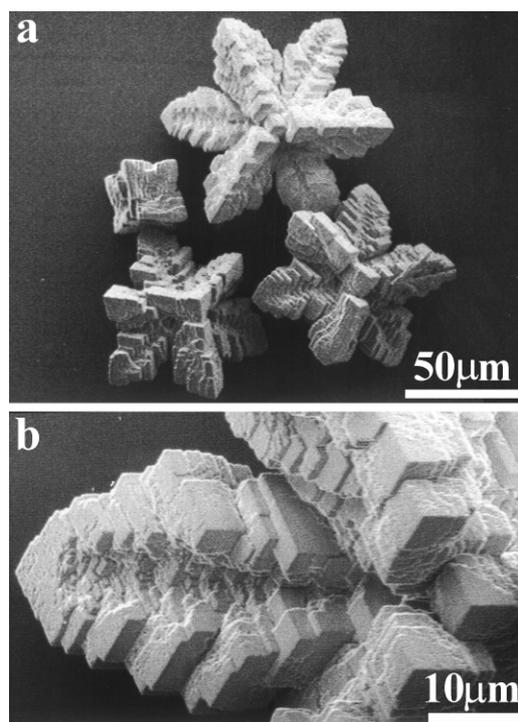
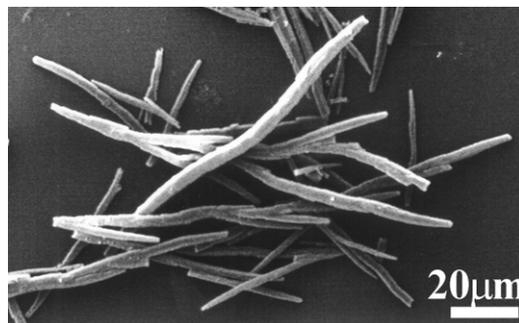


Fig. 1 SEM images of  $\text{CaCO}_3$  crystals obtained in agarose gels after incubation at  $30^\circ\text{C}$  for 24 h.

appeared, showing an incipient stage of the final star-shaped crystals (Fig. 2b). This result indicates that in the agarose gel matrix, the eight points of the initial calcite rhombohedron grew the fastest whereas the central points of the six {104} faces grew the slowest. Thereby, the eight points gradually extruded, resulting in eight distinct arms extending radially, and so the well-defined star-shaped calcite crystals as shown in Fig. 1 formed finally.

It is known that agarose is a neutral polysaccharide that mainly carries hydroxy functional groups. It has been documented that whereas the matrices of acid proteins including gelatin can exert significant effects on the  $\text{CaCO}_3$  crystallization,<sup>8,9</sup> the matrices of polysaccharides such as chitosan/chitin usually do not solely exert direct effects on  $\text{CaCO}_3$  crystallization.<sup>10</sup> Therefore, it is expected that in the present case, agarose molecules do not directly affect the crystallization of  $\text{CaCO}_3$  and agarose gels just play a role of reaction media that control the diffusion process of ions in the media and hinder the crystal growth when the crystal size reaches the pore size of the agarose network. Considering that the pore size for the 1% agarose gels is around 140 nm,<sup>14</sup> considerably smaller than the initial rhombohedral calcite crystals, the main role the agarose gels play on the  $\text{CaCO}_3$  crystallization could be the control on the diffusion process of reactant ions rather than the hindering of crystal growth. Therefore, a possible mechanism for the formation of the star-shaped calcite crystals is as follows: At first,  $\text{Ca}^{2+}$  ions were distributed uniformly in the agarose gel matrix. When  $\text{CO}_3^{2-}$  ions diffused into the gel, many small rhombohedral calcite crystals formed suddenly, leading to a quick decrease of the reactant ion concentration nearby. With gradual diffusion of  $\text{CO}_3^{2-}$  ions into the gel, the eight points of the rhombohedral calcite nuclei grew the fastest since the agarose network causes a stagnant diffusion of ions and the eight points had the highest probability to accumulate reactant ions for spatial reasons. With the prolongation of time, eight arms gradually extruded and radially extended, leading to the formation of the final star-shaped calcite crystals. Thus, the formation of the star-shaped calcite crystals could result from a diffusion-controlled crystallization in the agarose gel matrix. This result implies that ion transport controlled by biological matrices may play an important role on biomineralization.

The crystallization of  $\text{CaCO}_3$  in agarose gels was also carried out at an elevated temperature, *i.e.*, 65 °C, where the crystallization of aragonite, a metastable polymorph of  $\text{CaCO}_3$ ,



**Fig. 3** SEM image of  $\text{CaCO}_3$  crystals obtained in agarose gels after incubation at 65 °C for 24 h.

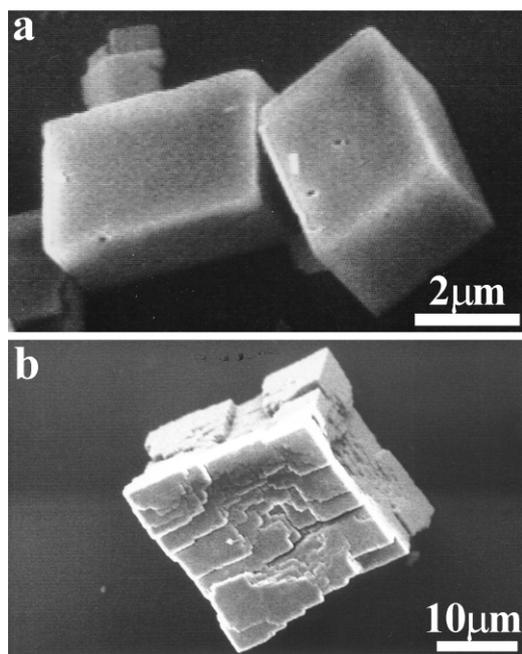
is favored.<sup>15</sup> A typical SEM image of the product is presented in Fig. 3, which shows rod-like crystals as long as 90  $\mu\text{m}$ . The corresponding XRD pattern suggests the product is a mixture of calcite and aragonite with a calcite fraction about 84%, which was calculated from the diffraction peak area using a known equation derived from a calibration curve.<sup>16</sup> It is interesting to note that the rod-like morphology typical for aragonite was completely preserved after the majority of aragonite has transformed into calcite. It is worth mentioning that when the reaction took place in water instead of agarose gels, a complete transition from aragonite to calcite was observed after 24 h of aging at 65 °C and all the final calcite crystals exhibit a rhombohedral morphology. These results imply that the normal variation in crystal morphology accompanying the phase transition from aragonite to calcite could be prevented by agarose gel matrix, which is another interesting finding and worth studying in more detail.

In conclusion, for the first time, calcite single crystals exhibiting a morphology of well-defined 8-armed stars have been produced by crystallization of  $\text{CaCO}_3$  in agarose gels. It has been revealed that these star-shaped calcite crystals evolved from initial rhombohedral calcite crystals with their 8 points extending radially into eight arms. It is speculated that agarose gels mainly play a role of control on the diffusion process of reactant ions, which might have relevant biological implications.

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**Fig. 2** SEM images of  $\text{CaCO}_3$  crystals obtained in agarose gels after incubation at 30 °C for: a) 1 min and b) 10 min.