VATERITE BIOCERAMICS: MONODISPERSE $CaCO_3$ BICONVEX MICROPILLS FORMING AT 70°C IN AQUEOUS $CaCl_2$-GELATIN-UREA SOLUTIONS

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ABSTRACT

Calcium carbonate microparticles with a unique biconvex pill shape were produced by simply ageing the prerefrigerated (4°C, 24 h) $CaCl_2$-gelatin-urea solutions at 70°C for 24 h in glass media bottles. Gelatin is known to be the denatured collagen. Thermal decomposition of dissolved urea was exploited to provide the Ca^{2+} ion and gelatin-containing solutions with aqueous carbonate ions. Monodisperse $CaCO_3$ micropills formed in solution had the mean particle size of 4±2.5 μm. $CaCO_3$ micropills were typically biphasic in nature and consisted of 93% vaterite and 7% calcite. Identical solutions used without prerefrigeration yielded only trigonal prismatic calcite crystals upon ageing at 70°C for 24 h. Prerefrigeration of $CaCl_2$-gelatin-urea solutions was thus shown to have an unusual effect on the particle morphology. Samples were characterized by scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR) and powder X-ray diffraction (XRD).

INTRODUCTION

$CaCO_3$ (calcium carbonate) is an important material of marine and geological biomineralization processes. $CaCO_3$ powders are widely used in rubber, plastic, paper making, printing ink, cosmetics, toothpaste, and food industries. Calcium carbonate has three anhydrous polymorphs; calcite, aragonite and vaterite. Amorphous calcium carbonate (ACC) may also be added to the polymorph list as the fourth component [1]. Calcium carbonate monohydrate and calcium carbonate hexahydrate may be regarded as the fifth and sixth $CaCO_3$ polymorphs [2]. At the ambient temperature and pressure, calcite is the most stable and abundant polymorph of calcium carbonate, while vaterite (μ-$CaCO_3$), named after Heinrich Vater [3], is known to be the least stable among the anhydrous polymorphs.

Vaterite has a higher aqueous solubility than calcite and aragonite [4]. The log(K_S) values for calcite, aragonite and vaterite were experimentally determined by De Visscher and Vanderdeelen [5]. Vaterite is rare in nature, perhaps owing to its instability, as it would readily convert into one of the more stable calcium carbonate phases [6-8]. However, Grasby [9] discovered micron-sized spheres of vaterite at a supraglacial location in the Canadian High Arctic at very low temperatures. Vaterite was known to be a mineralization product in the egg-shells of some gastropodia [10], the spicules of certain sea squirts [11], and the skeletons of woodlice [12].

By using the method of CO_2 gas bubbling through an aqueous solution of Ca-chloride (or Ca-nitrate), either well-crystallized rhombohedra of calcite or spheres of vaterite could be produced [13-15]. Han *et al.* [15] reported that the higher the concentration of CO_3^{2-}, the higher will be the tendency for formation of vaterite rather than its dissolution and gradual transformation into calcite. Using dissolved sodium carbonate (either Na_2CO_3 or $NaHCO_3$) as the CO_3^{2-} source, in place of CO_2 gas bubbling, was another practical option to produce vaterite or calcite crystals [16-18]. $CaCO_3$ spheres were also grown in a desiccator via slow diffusion of CO_2 released by the decomposition of $(NH_4)_2CO_3$ crystals placed at the bottom of the same desiccator, which also contained a glass dish with $CaCl_2$ solution [19].

Urea (NH_2CONH_2) was used (instead of CO_2 gas bubbling or Na_2CO_3, $NaHCO_3$ and $(NH_4)_2CO_3$ additions) to produce calcium carbonate powders [20-26]. Wang *et al.* [21] synthesized non-agglomerated calcite (trigonal), vaterite (spherical) and aragonite (needle-like) particles by using the decomposition of urea in $CaCl_2$-containing aqueous solutions (50 to 90°C).

Wakayama *et al.* [27] immersed chitosan-coated glass slides into a solution of Ca-acetate and polyacrylic acid (PAA) in the presence of supercritical CO_2 at 50°C and 76.5 kg/cm^2 (7.5 MPa) and observed the formation of heavily agglomerated but "pill-like" particles of vaterite deposited on the chitosan-coated glass slides.

Attempts to crystallize $CaCO_3$ in the presence of gelatin (or collagen) were found to be rather limited [28-36]. Moreover, none of these studies utilized aqueous Ca^{2+}-gelatin "solutions" as their calcium carbonate synthesis media.

To the best of our knowledge, there was no study in the literature on the *in situ* hydrothermal synthesis of $CaCO_3$ in Ca^{2+} ion-containing aqueous solutions which simultaneously have gelatin and urea.

We have discovered that prerefrigerated $CaCl_2$-gelatin-urea solutions, when simply aged at 70°C in sealed glass bottles, produced monodisperse, biphasic vaterite-calcite biconvex micropills or micropills with a unique morphology not seen and reported before. $CaCO_3$ is used in significant amounts in the powder formulations of new orthopedic and dental cements [37, 38] designed for skeletal repair, and our interest in $CaCO_3$ stemmed from such clinical applications. This manuscript reports, for the first time, the synthesis of micropills of $CaCO_3$.

EXPERIMENTAL PROCEDURE

Preparation of $CaCl_2$-gelatin-urea solutions

Ca-containing gelatin-urea solutions were prepared as follows. 200 mL of deionized water was placed into a 250 mL glass beaker and 11.761 g of $CaCl_2 \cdot 2H_2O$ (>99%, Cat. No. C79-500, Fisher Scientific, Fairlawn, NJ) was added to it, followed by stirring on a hot-plate, with a Teflon®-coated magnetic stir bar, at room temperature (RT: 21±1°C). This solution thus contained 0.4 M Ca^{2+}. 0.30 g of gelatin powder (>99%, Cat. No. G7-500, Fisher Scientific) was then dissolved, by stirring at RT, in the above solution. Finally, 6.00 g of urea powder (>99%, NH_2CONH_2, Cat. No. U15-500, Fisher Scientific) was added to the above Ca-gelatin solution, and the solution was stirred at RT for a minute to dissolve the urea. The transparent solution, which contained 0.4 M Ca^{2+}, 0.5 M urea and 0.3 g gelatin, was then transferred into a 250 mL-capacity Pyrex® media bottle (Cat. No. 06-423-3B, Fisher Scientific). Since these solutions contained urea, and since urea starts going through a very slow decomposition process even at RT, such solutions were not stored at RT for long times; therefore, these solutions must be prepared freshly prior to each synthesis experiment.

These solutions were then used to produce $CaCO_3$ particles with two different morphologies. "As-prepared solutions" and "prerefrigerated solutions" resulted in two different particle morphologies.

Synthesis of trigonal prismatic $CaCO_3$ (calcite) crystals

Only freshly prepared $CaCl_2$-gelatin-urea solutions (prepared in the way described above) were used in this compartment of this study. 200 mL of solution was first placed into a 250 mL-capacity Pyrex® media bottle. Then, one piece of microscope cover glass (Cat. No. 12-542B, 22x22x0.15 mm, Fisher Scientific) was dropped into the bottle and made sure that it laid flat at the bottom of the bottle. The bottle was tightly capped and placed into a microprocessor-controlled oven pre-heated to 70°C, and kept there undisturbed for 24 hours. At the end of 24 h, the bottle was opened; the white-coated cover glass was removed, and washed with an ample supply of deionized water, followed by rinsing with ethanol (95%, denatured, Cat. No. S73985, Fisher Scientific). The cover glass was dried in an oven at 37°C, overnight in air.

Synthesis of $CaCO_3$ micropills

A freshly prepared portion (200 mL) of $CaCl_2$-gelatin-urea solution was placed in a 250 mL-capacity Pyrex media bottle, tightly capped and then refrigerated (at 4°C) for 24 h. The pH of the refrigerated solution was measured to be 6.5 (at 6°C). One piece of microscope cover glass was dropped into the bottle and made sure that it laid flat at the bottom of the bottle. The bottle was capped

and placed into a microprocessor-controlled oven pre-heated to 70°C, and kept there undisturbed for 24 hours. At the end of 24 h (solution pH was 7.5 at 68-69°C), the bottle was opened and the white-coated cover glass was removed, and washed with an ample supply of deionized water, followed by rinsing with ethanol. The cover glass was dried in an air atmosphere oven at 37°C, overnight. For further analyses, the white powdery material coating the cover glass was gently scraped off by using a clean and sharp razor blade. The bottom of the glass bottle was also coated with the same material.

Sample Characterization

Samples were characterized by powder X-ray diffraction (XRD; Model XDS 2000, Scintag, Sunnyvale, CA), scanning electron microscopy (SEM; Model S-4700, Hitachi, Tokyo, Japan), and Fourier-transform infrared spectroscopy (FTIR; Nicolet 550, Thermo-Nicolet, Woburn, MA). Powder samples for SEM and XRD analyses (scraped off of the coated cover glasses) were first gently ground in an agate mortar by using an agate pestle and then sprinkled onto ethanol-damped single-crystal quartz sample holders to form a thin layer, followed by tapping to remove the excess of powder. The X-ray diffractometer was operated at 40 kV and 30 mA with monochromated Cu K_α radiation. XRD data (over the typical range of 20 to 50° 2θ) were collected with a step size of 0.03° and a preset time of 1 sec at each step. FTIR samples were first ground in a mortar, in a manner similar to that used in the preparation of XRD and SEM samples, then mixed with KBr powder in a ratio of 1:100, followed by forming a pellet by using a uniaxial cold press. 128 scans were performed at a resolution of 3 cm^{-1}. Coated glass covers examined with the scanning electron microscope (SEM) were sputter-coated with a thin Au layer, to impart surface conductivity to the samples.

RESULTS AND DISCUSSION

Heating of as-prepared and prerefrigerated (24 h at 4°C) $CaCl_2$-gelatin-urea solutions at 70°C for 24 h resulted in the nucleation of calcium carbonate crystals with two different morphologies. While the as-prepared solutions were nucleating trigonal prismatic crystals, the prerefrigerated solutions produced monodisperse micropills.

The comparative SEM photomicrographs of Figure 1 depicted this drastic change in morphology upon prerefrigeration. Figures 1a-1b, 1c-1d and 1e-1f possessed identical magnifications. Figures 1a, 1c and 1e showed the calcium carbonate particles produced when the freshly prepared $CaCl_2$-gelatin-urea solutions were directly heated at 70°C for 24 h. On the other hand, Figures 1b, 1d, and 1f exhibited the monodisperse calcium carbonate biconvex micropills obtained when prerefrigerated (24 h at 4°C) $CaCl_2$-gelatin-urea solutions were heated at 70°C for 24 h. A biconvex tablet or pill geometrically looks like a cylinder of a given height and radius with two hemispheres glued to it at both ends. Biconvex pills (or tablets) have symmetrical top and bottom surfaces. Particle sizes were determined by using the linear intercept method directly on the SEM photomicrographs. The average particle size in powders obtained from the as-prepared solutions was 7±1.5 μm (Figs. 1a, 1c, 1e), whereas that obtained from the prerefrigerated solutions was 4±2.5 μm. The values reported here were the averages of 15 individual particle measurements along 6 lines drawn across each photomicrograph.

Some of the trigonal prismatic calcium carbonate crystals showed very flat surfaces (as shown in Figs. 1a and 1e), and these flat surfaces were considered to be created in direct contact with the glass surfaces, on which the initial phase separation occurred. Such flat surfaces on calcite crystals were also observed by Didymus *et al.* [39]. In the case of micropills forming in prerefrigerated solutions (Figures 1b, 1d, 1f), the simultaneous observation of small (1 μm in diameter) and large (5 μm) pills indicated the presence of several different nucleation events/waves in progress.

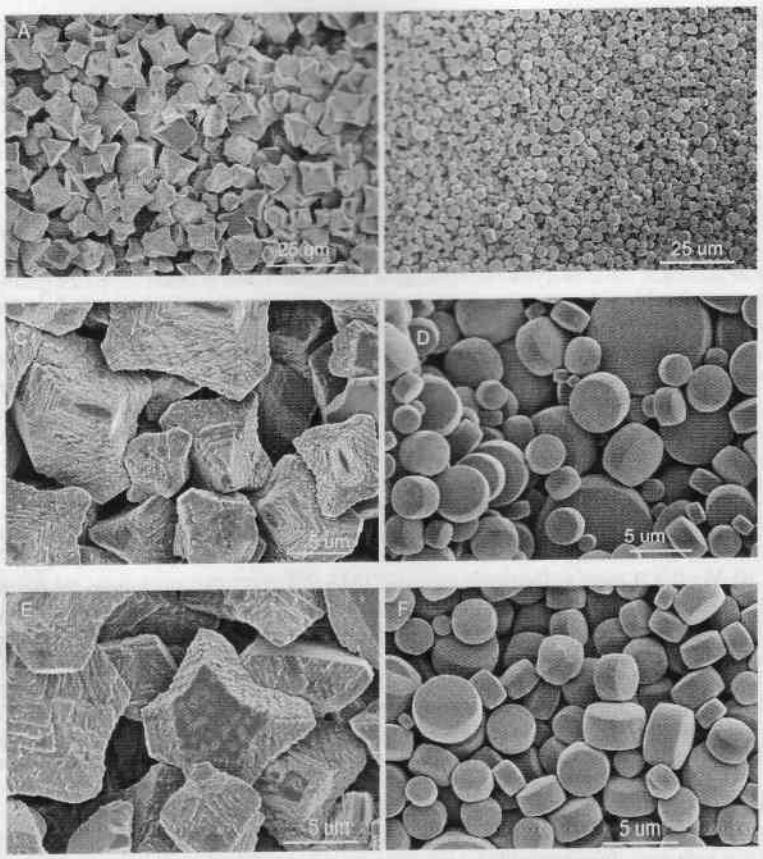

Fig. 1 SEM photomicrographs of $CaCO_3$ particles produced after ageing at 70°C for 24 h;
(a), (c) and (e): from "as-prepared" $CaCl_2$-gelatin-urea solutions
(b), (d) and (f): from "prerefrigerated" (4°C, 24 h) $CaCl_2$-gelatin-urea solutions

The powder XRD traces of samples obtained from both the as-prepared and prerefrigerated solutions were shown in Figure 2. As-prepared solutions, upon ageing at 70°C for 24 h, produced single-phase trigonal prismatic calcite crystals, conforming to the ICDD PDF 5-0586 [40]. Prerefrigerated solutions, on the other hand, produced vaterite [41] micropills contaminated with a minor amount of the calcite phase. The experimental XRD data of the vaterite micropills conformed well to that given in ICDD PDF 72-0506. The only calcite peak appeared in the XRD spectrum of vaterite micropills was indicated by the letter *C* in the Figure 2b trace. That peak corresponded to the strongest reflection of the calcite phase, i.e., (104).

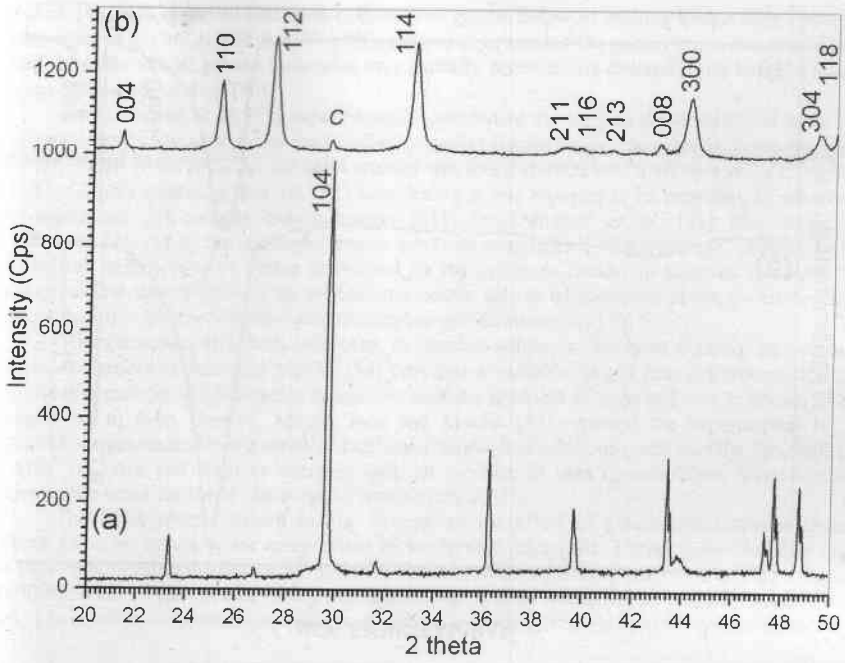

Fig. 2 XRD data of $CaCO_3$ particles produced after ageing at 70°C for 24 h;
(a) from "as-prepared" $CaCl_2$-gelatin-urea solutions (single-phase calcite)
(b) from "prerefrigerated" (4°C, 24 h) $CaCl_2$-gelatin-urea solutions (biphasic vaterite-calcite, the only calcite peak was indicated by *C*)

The FTIR spectra of both samples (trigonal prismatic calcite and vaterite micropills) are depicted in Figure 3. The trigonal prismatic calcite particles obtained from the as-prepared solutions contained some surface adsorbed water (at least at the moment of IR data collection) and this was indicated by the broad water band extending over the range of 3600 and 3100 cm^{-1} (Figure 2a). The H-O-H band observed at 1650 cm^{-1} in Figure 3a was also pinpointing to this fact. The band observed at 1080 cm^{-1}, in both Figures 3a and 3b, was assigned to the symmetric stretching, v_1, and lattice mode vibration. The strong carbonate band seen at 873 cm^{-1} (out-of-plane bending, v_2) was common to both calcite and vaterite. However, based on the IR spectra, it is quite an easy task to distinguish between vaterite and calcite polymorphs. The absorption band at 713 cm^{-1} is characteristic for calcite, whereas in vaterite the same band (in-plane bending, v_4) is characteristically shifted to 744 cm^{-1} [42]. Moreover, in vaterite, the main carbonate band (i.e., asymmetric stretching, v_3) is split into two at 1450 and 1407 cm^{-1} (indicated by an arrow in Figure 3b). This carbonate band splitting was not seen in phase-pure calcite, and the asymmetric stretching band for calcite was observed at 1405 cm^{-1}.

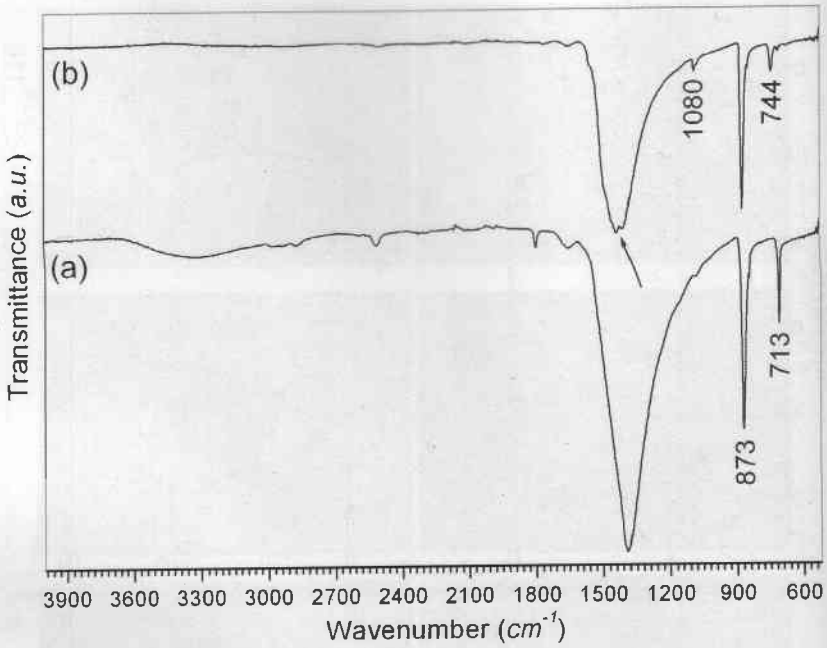

Fig. 3 FTIR spectra of $CaCO_3$ particles produced after ageing at 70°C for 24 h;
(a) from "as-prepared" $CaCl_2$-gelatin-urea solutions
(b) from "prerefrigerated" (4°C, 24 h) $CaCl_2$-gelatin-urea solutions (arrow indicates the characteristic splitting for vaterite)

The amount of calcite phase present in the monodisperse micropills was determined by using both the XRD and the FTIR data according to the methods suggested by Rao [13, 43] and Andersen and Kralj [44], respectively, and the calcite phase was present at about 7±1%. Therefore, the monodisperse micropills were biphasic in nature, i.e., 93% vaterite-7% calcite.

The readers who would be more enthusiastic in learning the decomposition kinetics of urea, in aqueous solutions containing metal ions, are hereby referred to the detailed works of Willard and Tang [45] and Mavis and Akinc [46], which also gave the stepwise decomposition reactions written in full. The ageing temperature was deliberately maintained low at 70°C in this study (in contrast to the use of more common 90°C [46]) to avoid the instantaneous and rapid decomposition of urea, and to provide a much slower supply of HCO_3^- ions to the Ca-gelatin solutions.

The experimental amino acid compositions of mammalian (i.e., seal, whale, porcine and bovine) and cod-skin gelatins were reported by Arnesen and Gildberg [47]. The native conformation of collagen molecules is a triple helix; however gelatin, as denatured collagen, is water soluble and forms random coils in solution [48]. Yoshioka *et al.* [49] experimentally determined that in the case of gelatin-water system the conformational coil-helix transition of the protein chains was responsible for the gel formation, and the helix formation was enhanced by lowering the temperature to about 5°C.

Guo *et al.* [48] also observed that upon cooling pure gelatin below its melting temperature (where the melting point of bovine gelatin is 36°C [47]), ordered structures of the gelatin molecules would be reformed. In other words, gelatin molecules may partially revert to the ordered triple helical collagen-like sequences upon cooling [50].

Joly-Duhamel *et al.* [51] experimentally determined the random coil-to-refolded triple helix transformation percentage in a number of gelatin samples (including bovine gelatin). When the gelatin sols were cooled to around 5°C, the helix amount was found to increase (from zero at 35°C) to about 65% [51, 52]. An annealing time (at 5°C) of at least 6 h was reported to be necessary to achieve the above-mentioned coil-to-helix transformation [51]. Joly-Duhamel *et al.* [51] also stated that renaturation (achieved by the cooling of gelatin sols) was essentially a "nonreversible" process, and the triple helical sequences were stable (stabilized by the hydrogen bonds) in aqueous solutions. This would mean that upon reheating the refrigerated gelatin sols to temperatures above its melting point not all of the triple helices formed would decompose into random coils [53].

The interaction of gelatin with urea, in aqueous solutions, has been a hardly studied topic, however, the article of Jana and Moulik [54] provided a valuable insight into the process disclosed here. The dissociation of amino acids in aqueous solution produces H^+ ions and urea is known to bind hydrogen ion to form Urea-H^+ adduct. Jana and Moulik [54] reported the experimental H^+ ion concentrations generated from a series of individual amino acid solutions (such as, Gly, Pro, Val, Gln, Ser, His, Trp, Arg and Asp) to decrease with an increase in urea concentration. Dissolved urea competes with water for the H^+ ion forming uranium ion (UH^+).

The FTIR spectra shown in Fig. 4 depicted the effect of gelatin concentration used in synthesis solutions on the phase composition of the formed micropills. The samples shown in Fig. 4 were produced by using the same Ca^{2+} and urea concentrations with those presented in Figs. 1 through 3. The gelatin concentration was slowly decreased in the synthesis solutions to produce the samples of Fig. 4. The solution concentrations were given in the figure caption of Fig. 4. 0.1 g gelatin-containing

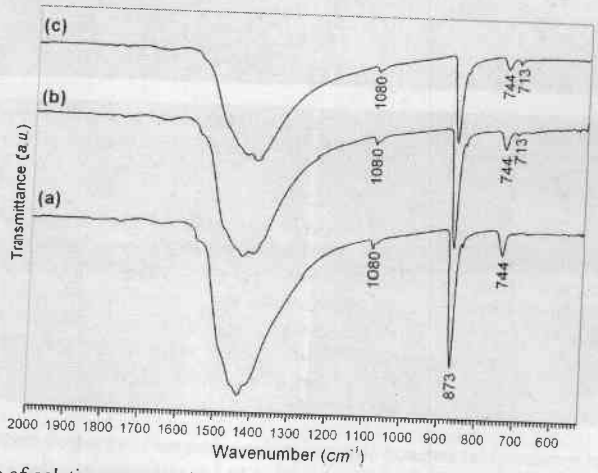

Fig. 4 Influence of gelatin concentration on the phase composition of $CaCO_3$ micropills
(a) 0.4 M Ca^{2+}, 0.5 M urea, 0.10 g gelatin, prerefrigerate at 4°C, 70°C, 24 h
(b) 0.4 M Ca^{2+}, 0.5 M urea, 0.15 g gelatin, prerefrigerate at 4°C, 70°C, 24 h
(c) 0.4 M Ca^{2+}, 0.5 M urea, 0.25 g gelatin, prerefrigerate at 4°C, 70°C, 24 h

resulted in much purer vaterite powders. The SEM photomicrographs given in Fig. 5 depicted the morphology of biconvex vaterite micropills obtained by using a solution of 0.4 M Ca^{2+}, 0.5 M urea and 0.1 g gelatin (in a total water volume of 200 mL).

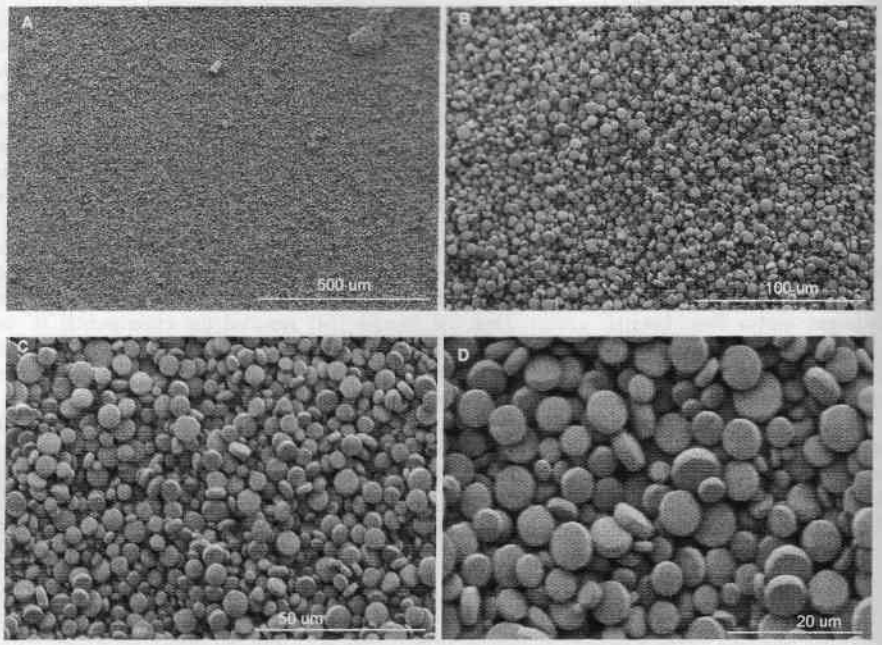

Fig. 5 SEM photomicrographs of vaterite biconvex pills (at increasing magnifications) formed in a solution containing 0.4 M Ca^{2+}, 0.5 M urea and 0.1 g gelatin, following prerefrigeration at 4°C and heating at 70°C for 24 h

It was previously reported that it would be possible to synthesize aragonite rods in $CaCl_2$-urea solutions free of gelatin [21, 55]. In this study, we have also observed the formation of needle-like aragonite particles, containing rectangular-prismatic calcite crystals, when we simply eliminated gelatin from the synthesis solutions which produced the above-mentioned micropills. These solutions were again heated at 70°C for 24 h. Obviously, prerefrigeration of these solutions had no effect on the observed morphology of the aragonite rods *in situ* formed. Fig. 6 depicted the characteristic FTIR spectrum of the aragonite-calcite biphasic powders of this study, whereas the SEM photomicrographs of the same aragonite-calcite biphasics were reproduced in Fig. 7.

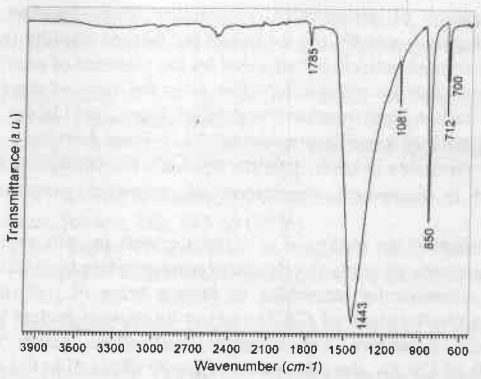

Fig. 6 FTIR spectra of aragonite-calcite biphasics formed at 70°C in 0.4 M Ca^{2+}, 0.5 M urea sols

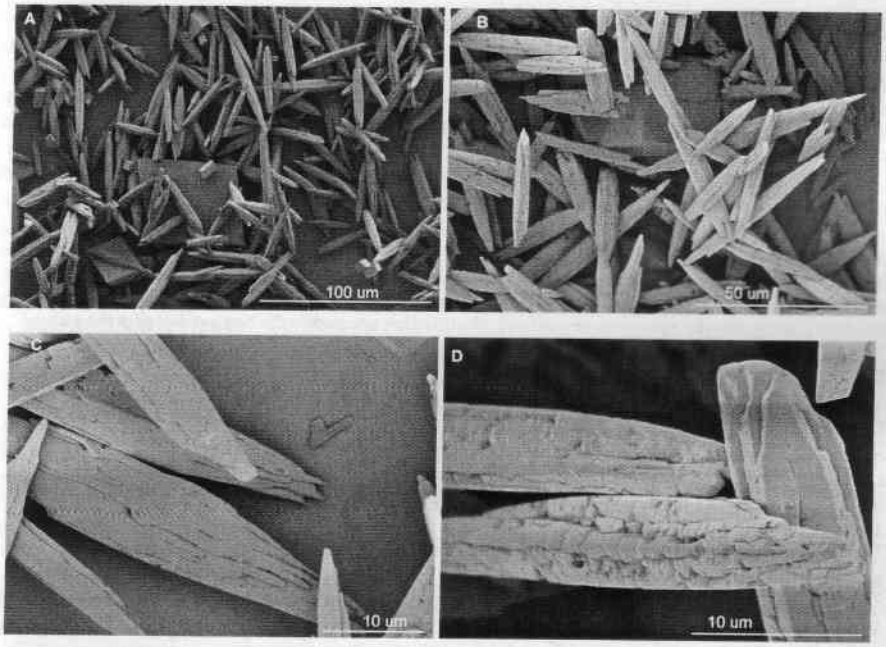

Fig. 7 SEM photomicrographs of aragonite-calcite biphasic powders (at increasing magnifications) formed in a solution containing 0.4 M Ca^{2+} and 0.5 M urea, following prerefrigeration at 4°C and heating at 70°C for 24 h

Would the extent of renaturation of gelatin from "random coils"-to-"triple helix" conformation (by prerefrigeration at 4°C for 24 h) and the thermal stability (while ageing the solutions at 70°C for 24 h) of the formed helices be enhanced by the presence of urea? If so, would this place a light on the formation of vaterite micropills? How does the ratio of random coil to triple helical conformation affect the carbonyl environments in gelatin? These could be the topics of future research.

Monodisperse $CaCO_3$ micropills presented here, besides forming a practical example for *in vitro* biomineralization processes in urea-, gelatin- and Ca^{2+} ion-containing matrices, may also find a number of applications in biomedical, pharmaceutical, cosmetics, polymer, rubber, paper and ink industries.

Birefringence refers to the ability of a mineral crystal to split an incident beam of linearly polarized light into two beams of unequal velocities (corresponding to two different refractive indices of the crystal) which subsequently recombine to form a beam of light that is no longer linearly polarized. The extreme birefringence of $CaCO_3$ makes its crystals appear to light up or glow when viewed through crossed polarizers. For technical applications which would fully exploit the birefringence properties of $CaCO_3$, the changes to be easily obtained in the particle morphology from spherical to biconvex tablets/pills or even to trigonal prisms are, therefore, extremely important.

CONCLUSIONS

(1) $CaCl_2$-gelatin-urea solutions were prepared at room temperature. These solutions nucleated trigonal prismatic calcite particles upon ageing at 70°C, in glass media bottles, for 24 h.

(2) The same $CaCl_2$-gelatin-urea solutions were first refrigerated at 4°C for 24 h and then aged in glass media bottles at 70°C for 24 h. Such solutions nucleated monodisperse, biphasic vaterite-calcite micropills. Such a biconvex pill morphology for $CaCO_3$ was not reported before.

(3) $CaCl_2$-urea solutions free of gelatin formed biphasic mixtures of needle-like aragonite and rectangular-prismatic calcite crystals following ageing at 70°C for 24 h.

NOTES

The names and models of certain commercial equipment, instruments or materials are identified in this paper to enhance understanding. Such identification does not imply any recommendation or endorsement by the author, nor does it imply that the equipment or materials identified are necessarily the best available for the purpose.

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