A novel method for the synthesis of calcium carbonate (aragonite) nanoparticles from cockle shells

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A B S T R A C T
We report a simple, novel and low-cost method for the synthesis of aragonite nanoparticles from cockle shells. Aragonite is one of the least abundant biogenic polymorphs of calcium carbonate and is widely used as a biomaterial for the repair of fractured bone, development of advanced drug delivery systems, and tissue scaffolds. The method involves a simple mechanical grinding of the micron-sized cockle shell powders in the presence of a non-toxic and non-hazardous biomineralization catalyst, dodecyl dimethyl betaine (BS-12). The method produces rod-shaped aragonite particles with a diameter of 20±5 nm with good reproducibility and without any additional impurities. This was confirmed by a combined analysis of variable pressure scanning electron microscopy (VPSEM), transmission electron microscopy (TEM), Fourier transmission infrared spectroscopy (FTIR), thermogravimetric analyzer (TGA), X-ray diffraction spectroscopy (XRD) and energy dispersive X-ray analyser (EDX). The method should find potential applications in industry for the large scale synthesis of aragonite nanoparticles from a low cost but abundant natural resource such as cockle shells.

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1. Introduction

Calcium carbonate (CaCO3) is one of the most abundant minerals in nature and has three polymorphs: calcite, aragonite and vaterite [1]. Among these three polymorphs, aragonite has been extensively studied because of its biocompatible properties [2–5]. It is denser than calcite and can be integrated, resolved and replaced by bone [4,6]. Aragonite is also being used as a biomaterial for the repair of fractured bone, development of advanced drug delivery systems, and tissue scaffolds. The method involves a simple mechanical grinding of the micron-sized cockle shell powders in the presence of a non-toxic and non-hazardous biomineralization catalyst, dodecyl dimethyl betaine (BS-12). The method produces rod-shaped aragonite particles with a diameter of 20±5 nm with good reproducibility and without any additional impurities. This was confirmed by a combined analysis of variable pressure scanning electron microscopy (VPSEM), transmission electron microscopy (TEM), Fourier transmission infrared spectroscopy (FTIR), thermogravimetric analyzer (TGA), X-ray diffraction spectroscopy (XRD) and energy dispersive X-ray analyser (EDX). The method should find potential applications in industry for the large scale synthesis of aragonite nanoparticles from a low cost but abundant natural resource such as cockle shells.

attention is paid to invent methodologies for the controlled and facile synthesis of aragonite nanoparticles (ANPs) of convenient sizes and shapes [2,3,5]. Two fundamental routes for the bottom up synthesis of ANPs are outlined: (1) the solution route in which aqueous CaCl2 and (NH4)2CO3, or CaCl2 and Na2CO3, or Ca(NO3)2 and Na2CO3 [2,3,8–12] are combined in equimolar ratio through a double decomposition reaction and (2) the carbonation route in which CO2 gas is bubbled through an aqueous slurry of Ca(OH)2 at a specified temperature in the presence of organic substrates such as polyacrylamide [5]. However, none of these routes can produce pure aragonite nanoparticles of suitable sizes and shapes. They are often mixed with calcite [3] or calcite and vaterite [2] and thus, may not be suitable for a specialized biomedical application. Although carbonation methods are industrially useful and environmentally friendly, they need stringent temperature control, purified raw materials, and laborous gas (CO2 or mixture of CO2 and N2) bubbling steps which are complicated and incur additional cost and time [5,13]. They also added additional impurities such as dodecyl dimethyl betaine (RN+(CH3)2CH2COO−) which is popularly known as BS-12, to the final products [13].

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The synthesis of ANPs from its natural reservoirs such as cockle shells or sea shells using a top down approach is greatly promising. In our last report [4], we have shown that cockle shells and its powders are a rich and naturally purified source of aragonite polymorphs of calcium carbonate. In this paper, we have described an easy-to-perform, environmentally friendly, and low-cost method that adopts a simple mechanical stirring of cockle shell powders in the presence of BS-12 as a biomineralization catalyst to synthesize ANPs of the smallest sizes so far reported in bibliographies [2–5]. The beauty of the method is that it does not add any impurities to the final products but still produces very small-sized rod-shaped ANPs in a reproducible fashion, a criterion required for industrial applications.

2. Experimental

2.1. Preparation of micron-sized cockle shell powders

Approximately 250 g of cockle shells (Anadara granosa) was washed, scrubbed to remove dirt, boiled for 10 min and then cooled at room temperature. The shells were then washed thoroughly with distilled water and dried in an oven (Memmert UMS500, Germany) for 7 days at 50 °C. The cockle shells were finely ground using a blender (Blendor, HCB 550, USA). The powders were sieved using a stainless laboratory test sieve with an aperture size of 90 μm (Endecott Ltd., London, England) to obtain micron-sized (10–90 μm in diameter) powders [4]. Dodecyl dimethyl betaine (BS-12) was obtained from Sigma-Aldrich (Steinheim, Germany). The water used was HPLC-grade of resistance >18 MΩ obtained from a Milli-RÖ6 plus Milli-Q Water System (Organex).

2.2. Preparation of nano-sized cockle shell powders

Five grams of micron-sized cockle shell powders was taken into a 250-ml conical flask. A slurry was formed by adding and mixing 50 ml of distilled water. To synthesize CaCO₃ nanoparticles, 1 ml of BS-12 (as obtained from Sigma-Aldrich) was added into the flask and vigorously stirred at 1000 rpm at room temperature for 90 min using a mechanical hot plate and magnetic stirrer bar. The obtained sample was separated from the mother liquid using a double ring filter paper of size 18.0 cm (Filtres Fioroni, China). The final product was dried for 1 day in an oven (Memmert UM500) at 100 °C and was packed in a polyethylene plastic bag (JP Packaging) for further uses.

2.3. Analysis of cockle shell nanoparticles

The surface morphologies of the produced nanoparticles were studied using a variable pressure scanning electron microscope (VPSEM, LEO 1455, Germany) after coating the powder with gold. The elemental analyses were performed using an energy dispersive X-ray analyser (EDX, model 7353, England), directly connected to the VPSEM. The crystal shapes and sizes were determined using a TEM (Hitachi H-7100, Japan). For TEM study, the produced nanoparticle powder of cockle shells was mixed with absolute alcohol under ultrasonication for 30 min, the colloidal solution was dropped onto a carbon-covered copper grid, placed on a filter paper, and dried at room temperature for 1 h. The purity and crystalline properties of the powders were investigated by an X-ray diffractometer (Shimadzu XRD-6000, Japan) operated at CuKα (λ = 1.540562 Å). Chemical analyses were done using a Fourier transform infrared (FT-IR) spectrophotometer (Model 100, Perkin Elmer, 710 Bridgeport Avenue, Shelton, USA) over a range of 280 to 4000 cm⁻¹ at a resolution of 2 cm⁻¹ and at scan speed of 64/s. The decomposition compositions of cockle-shell particles were studied using a thermogravimetric analyzer (SEIKO SSC/5200, Tokyo, Japan). Initially, the sample was heated at 105 °C for 20 min to dry up the moisture. Finally, the sample was heated up from 105 °C to 900 °C at a rate of 10 °C/min under nitrogen flow (10 ml/min) and the weight loss was recorded in this process.

3. Results and discussion

3.1. The surface morphology and crystal structure of aragonite nanoparticles

The surface morphologies as obtained from VPSEM cockle shell powder before (a) and after adding BS-12 for ~85 min (b), 90 min (c) and >90 min (d) are shown in Fig. 1. While the rod-shaped aragonite crystals clearly appeared in the cockle shell powders before adding BS-12 (a), the aragonite crystals were agglomerated and clumped together to give a homogenous mesh-like appearance in the presence of BS-12 (c and d). The rod-shaped appearance of the aragonite crystals obtained from cockle shell powder was also observed in previous studies [4]. The SEM studies indicated that BS-12 can reduce the size of the crystals and enhance the particle agglomeration probably through an increment in the inter-particle adhesiveness.

The TEM images of cockle shell powders before (a) and after adding BS-12 (b) are shown in Fig. 2. While the micron sized rod-like aragonite crystals of diameter 10–90 μm were observed in the cockle shell powders without adding any organic surfactant or catalyst (a), the rod-shaped aragonite crystals of diameter 20 ± 5 nm clearly appeared in the presence of BS-12 (b). The particle diameter was assigned according to Ali et al. [14] by counting the diameter of 300 particles. The reproducibility of the method was confirmed through the triplicate preparations and triplicate analyses. Repeated experiments demonstrated that proximately 50% particles were 20 ± 1 nm, 35% were 20 ± 3 nm, 10% were 20 ± 5 nm and the rest 5% were outside the range of 20 ± 5 nm. A number of previous studies reflected that the rod-like aragonite crystals were synthesized in the laboratory using a bottom up approach in the presence of polyacrylamide [5] or in the absence of any organic substrates such as polyacrylamide or BS-12 [3]. However, none of these studies obtained pure aragonite crystals of homogeneous sizes and shapes. In most of the cases, the aragonite crystals were obtained as a mixture of calcite and aragonite [3,5] or calcite, vaterite and aragonite [2]. For the first time, this study reports the preparation of rod-shaped aragonite crystals of the smallest and homogeneous sizes and shapes from a low cost natural resource i.e. cockle shells using a top down approach in the presence of BS-12. BS-12 is extensively reported in the bottom up approach in the presence of polyacrylamide [5] or in the absence of any organic substrates such as polyacrylamide or BS-12 [3]. However, none of these studies obtained pure aragonite crystals of homogeneous sizes and shapes. In most of the cases, the aragonite crystals were obtained as a mixture of calcite and aragonite [3,5] or calcite, vaterite and aragonite [2]. For the first time, this study reports the preparation of rod-shaped aragonite crystals of the smallest and homogeneous sizes and shapes from a low cost natural resource i.e. cockle shells using a top down approach in the presence of BS-12. BS-12 is extensively reported in the bottom up approach in the presence of BS-12. The precise role of BS-12 in the bottom down synthesis of CaCO₃ is not available in literatures. In the bottom up approaches, organic additives retard the nucleation and growth pattern of the calcite particles. However, all the reported methods added a detectable amount of BS-12 impurities in the final products which may not be suitable for biological applications [13]. However, in this study, FT-IR, XRD and TGA analyses confirmed that there was no BS-12 contamination in the final aragonite particles synthesized in the presence of BS-12. The precise role of BS-12 in the top down synthesis of CaCO₃ is not available in literatures. In the bottom up approaches, organic additives retard the nucleation and growth of CaCO₃ crystals and thus modify the shapes of the crystals [13]. Here we assume that BS-12 probably acts as a catalyst to facilitate the formation of rod-shaped aragonite crystals which might be present in large bundles in the original cockle shells as well as its powder form.

3.2. Optimization of temperature and reaction time

In this study, we selected room temperature because it does not need any temperature control which is not only inconvenient but also required specialized devices and thus incur additional cost and not feasible for industrial applications. No significant change in the size and shape of the synthesized CaCO₃ particles was found when the reaction was done between 20 °C and 30 °C (Fig. 1a). However, significant changes both in the morphology and shape of CaCO₃ particles were observed when reaction time was varied from 90 min. The rod shaped morphology was broken down into a round shaped one when the reaction was carried out for >95 min (Fig. 1d). On
the other hand, larger particles were obtained when the reaction was performed for <85 min (Fig. 1b). Thus 90 min was taken as the optimum time for the synthesis of rod shaped aragonite nanoparticles of 20 nm in diameter (Fig. 1c).

3.3. FT-IR patterns of the aragonite nanoparticles

The FT-IR spectra of cockle shell powders before (b) and after the addition of BS-12 (c) are demonstrated in Fig. 3. The FT-IR spectrum
of pure BS-12 is shown in Fig. 3a. The prominent peaks at 3368 and 1631 cm$^{-1}$ in the spectrum of BS-12 were assigned to OH absorption and CO stretching vibration, respectively [15]. The cluster of peaks between 2980 and 2850 cm$^{-1}$ was due to the sp3-hybridized C–H bonds [15]. Two peaks of moderate densities at 1457 cm$^{-1}$ and 1350 cm$^{-1}$ were given by C–H bending and C–H rocking vibration [15]. The two other peaks that appeared at 1295 and 1072 cm$^{-1}$ were because of the C–N stretching vibration [15]. The most prominent absorption peaks of calcium carbonate were at 1455 cm$^{-1}$ in the cockle shell powders in all conditions (b) and (c). No positional shift of these peaks was observed before (b) and after adding BS-12 (c). These peaks were attributed to the alkyl group which is present in the aragonite polymorphs [4]. Two other peaks that appeared at 1794 cm$^{-1}$ and 2520 cm$^{-1}$ can refer to the amide and carboxylic stretching vibration [4]. The characteristic peaks which represent CO$_2^-$ of aragonite polymorphs were observed at 1082 cm$^{-1}$, 857 cm$^{-1}$ and 698 cm$^{-1}$ [4,11,16]. No positional shifts of these peaks were seen before and after adding BS-12. Wang et al. [13] obtained two peaks at 2875 and 2993 cm$^{-1}$ in the calcite polymorph of calcium carbonate nanoparticles synthesized by carbonation method in the presence of BS-12 as an organic surfactant. They attributed these peaks to the surface embedded BS-12. However, such a peak that can define the presence or contamination of BS-12 was totally absent in the FTIR spectra of the synthesized aragonite nanoparticles. No features of the BS-12 were observed in the FT-IR spectra of aragonite nanoparticles synthesized in the presence of BS-12, strongly suggesting that ANPs were synthesized without any added impurities.

3.4. XRD patterns of aragonite nanoparticles

The XRD patterns of cockle shell powders before (b) and after adding BS-12 (c) are demonstrated in Fig. 4. The XRD patterns of cockle shell powders synthesized without BS-12 (b) and with BS-12 (c) completely matched with the aragonite phase of JCPDS file no.
No additional influence of BS-12 was found in any of these XRD profiles. The XRD patterns of cockle shell powders did not show any peaks of mixed polymorphs such as calcite and aragonite [3,5] or calcite, aragonite, and vaterite [2], demonstrating that pure crystalline aragonite nanoparticles were synthesized in the presence of BS-12 which has most likely acted as a biomineralization catalyst.

3.5. Elemental analysis of the aragonite nanoparticles

The elemental compositions of the cockle shell powders before and after adding BS-12 are shown in Table 1. While the cockle shell powders in the absence of BS-12 contain 20.99% carbon, 28.88% oxygen, 0.92% aluminum, 45.08% calcium, 2.88% copper, and 1.22% tellurium [4], the cockle shell powders in the presence of BS-12 contain 20.90% carbon, 28.91% oxygen, 0.93% aluminum, 45.17% calcium, 2.85% copper, and 1.22% tellurium. No significant change in the elemental compositions of the obtained cockle shell nanoparticles was found after the addition of BS-12, reflecting the catalytic role of BS-12 in the breakdown of larger cockle shell powders into the rod shaped aragonite nanoparticles.

3.6. TGA curves of the aragonite nanoparticles

The TGA curves for cockle shell powder in the absence (a) and in the presence (b) of BS-12 are represented in Fig. 5. Both types of cockle shell powders generated similar TGA curves, reflecting no significant difference in the decomposition pattern of CaCO₃ synthesized with and without BS-12. The peak temperatures of both curves were found at 780 °C. Wang et al. [13] studied the decomposition of CaCO₃ synthesized with and without BS-12 using a carbonation method. They found two distinct phases in the decomposition pattern of CaCO₃ synthesized in the presence of BS-12. The first phase started at 230 °C and was due to the removal of water and alkyl group. The second phase that started at 580 °C and ended at 815 °C was due to the removal of CaCO₃. Thus the study established that the first phase decomposition of CaCO₃ was absent, therefore the presence of BS-12 was ruled out. Thus it could be inferred that here CaCO₃ was synthesized without any addition of BS-12.

4. Conclusions

A simple, low-cost, and environmentally friendly method for the synthesis of calcium carbonate nanoparticles (aragonite) from a low-cost and abundant natural resource, cockle shells is developed. The method is a top down approach which involves minimum procedural steps, does not need any stringent temperature management, expensive and hazardous chemicals or any carbonation step of expensive and time consuming gas bubbling. It is simply a mechanical grinding using very simple, easily available, and low-cost instrument in the presence of a biomineralization catalyst, dodecyl dimethyl betaine. The method produces very small-sized rod-shaped and pure aragonite nanoparticles with an average diameter of 20 nm in a reproducible manner without any additional impurities. The method has a great potential in industry for the large scale production of calcium carbonate nanoparticles for biomedical applications.
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