

SYNTHESIS AND CHARACTERIZATION OF CALCITE NANO-PARTICLE DERIVED FROM COCKLE SHELL FOR CLINICAL APPLICATION

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Abstract

Calcite nano-particle is suggested as an extremely effective material in the biomedical due to their shape, non-toxic properties and being easily metabolized by cells. In clinical application, calcite nano-particle has to be useful for recovering the surface of tooth. The surface of tooth was often damaged due to wrong tooth care treatment, dental caries and periodontal disease. Because of tooth surface destruction, it could lead to dental hypersensitivity. It is desirable to find a suitable source for supplying calcium to fill in dentinal tubules and later regenerate the tooth surface. This study demonstrated the synthesis and characterization of calcite nano-particle for its application in dental hypersensitivity treatment. Cockleshell was found to be a suitable source to synthesize calcite. Using cockleshell as raw materials to produce calcite nanoparticle is meaningful for both environmental protection and biomedical application. Therefore, in this study, calcite nano-particle was synthesized using cockleshell as a reproducibility source of calcium carbonate (CaCO_3) and chitosan as a size-reducing agent. X-ray Diffraction (XRD) was used to characterize phase composition of raw materials, as-prepared powder and calcined powder. Transmission Electron Microscopy (TEM) was used to evaluate the size and morphology of powder. Results indicated that calcite nano-particle with size about 50-100 nm could be obtained. It would be expected to consider for further studies in clinical application.

Keywords: Calcite, Hypersensitivity, Nano-particles

Introduction

Dentine hypersensitivity is a wide spread painful dental problem that can be found in people with tooth sensitivity having their dentinal tubules exposed. These microscopic tubes are perpendicular to the surface of the tooth, and extend to the inner pulp chamber. It is necessary to find a material to supply calcium that could recover the surface of tooth. This material should be in the form of nano-particles so that they could spontaneously spread over every surface of the mouth, both teeth and gums. If they encounter any crevices or holes, they will migrate into them. Whenever the nano-particles encounter a solid surface, they stick there by virtue of the omnipresent molecular attractive forces, such as van der Waal's forces, and electrostatic attraction.

Calcium carbonate (CaCO_3), is one of the potential sources to supply Calcium, has three polymorphs in nature such as calcite, aragonite and vaterite. Aragonite is the polymorph researched the most among them because of being biocompatible [1]. However, concerning about the thermodynamic stability, calcite is suggested [2]. Calcite nano-particle is considered as an extremely effective material in the biomedical due to their

shape, non-toxic properties and being easily metabolized by all cells. Calcite with nanostructure could supply a calcium sources to retain this ion in supersaturating state in the enamel minerals. Hence, calcite has great potential in preventing of tooth erosion and it could be used for dental hypersensitive treatment.

The bio-system research has been showing many ways to synthesize calcite under different forms and structure from various sources. The cockle belonging to the species of *Anadara granosa*, a type of sea molluscan is a favourite seafood in South East Asian area. Cockle shell is abundantly available as a by-product from seafood industry after the mussels were consumed. Cockleshell is one of the abundant sources in CaCO_3 . Some of previous reports stated that 95-99% amount of cockle shell is CaCO_3 in the form of aragonite or calcite [3-6].

In this study, calcite nano-particle was synthesized by dissolution- precipitation method using cockleshell as a reproducibility source of CaCO_3 and chitosan as a size-reducing agent. In this way, it was possible to control the properties of calcite nano-particle via adjusting of synthesis temperature and calcination process.

Materials and Method

Materials Preparation

Cockleshells were firstly washed to remove dirt. Then, approximately 100 grams cockleshells were boiled at 100°C for 30 minutes and dried for 2 days at 110°C in the oven. After that, the shells were grounded using agate mortar. Finally, the powder with size approximately $90\ \mu\text{m}$ were collected by a stainless laboratory test sieve. The powder then was kept in a glass bottle for further reaction.

Cockleshell powder was dissolved with hydrochloric acid (HCl) 37%. Chitosan was dissolved in Acetic Acid and mixed with calcium chloride (CaCl_2). The mixture was then reacted with sodium carbonate (Na_2CO_3). The reaction was kept overnight at 80°C and 90°C under stirring with speed 1000 rpm. The solution was filtered several times to obtain as-prepared powder. The as-prepared powder was washed with distilled water then dried and calcined in the furnace at 550°C for 6 hours, 600°C for 6 hours and 8 hours, and 650°C for 2 hours and 6 hours. The calcination temperatures were chosen to remove organic completely and to prevent decomposition reaction. After calcination, obtained powder was ground using agate mortar and characterized for phase composition and morphology.

Characterization

Composition of the specimen was characterized using powder X-ray diffraction (XRD) analysis. Sample was ground to fine powder and the XRD patterns were recorded using a diffractometer system (D2 Phaser, Bruker AXS GmbH, Karlsruhe, Germany). The size and shape of the particle were observed using Transmission Electron Microscopy (JEM-1400, JEOL, Japan).

Results and Discussion

The XRD patterns of cockleshell powder (Figure 1a), samples synthesized at different reaction temperatures (Figure 1b), samples calcined at different temperatures within 6 hours (Figure 1c), samples calcined at 600°C at different soaking time (Figure 1d), and samples calcined at 650°C at different soaking time (Figure 1e) were demonstrated in Figure 1.

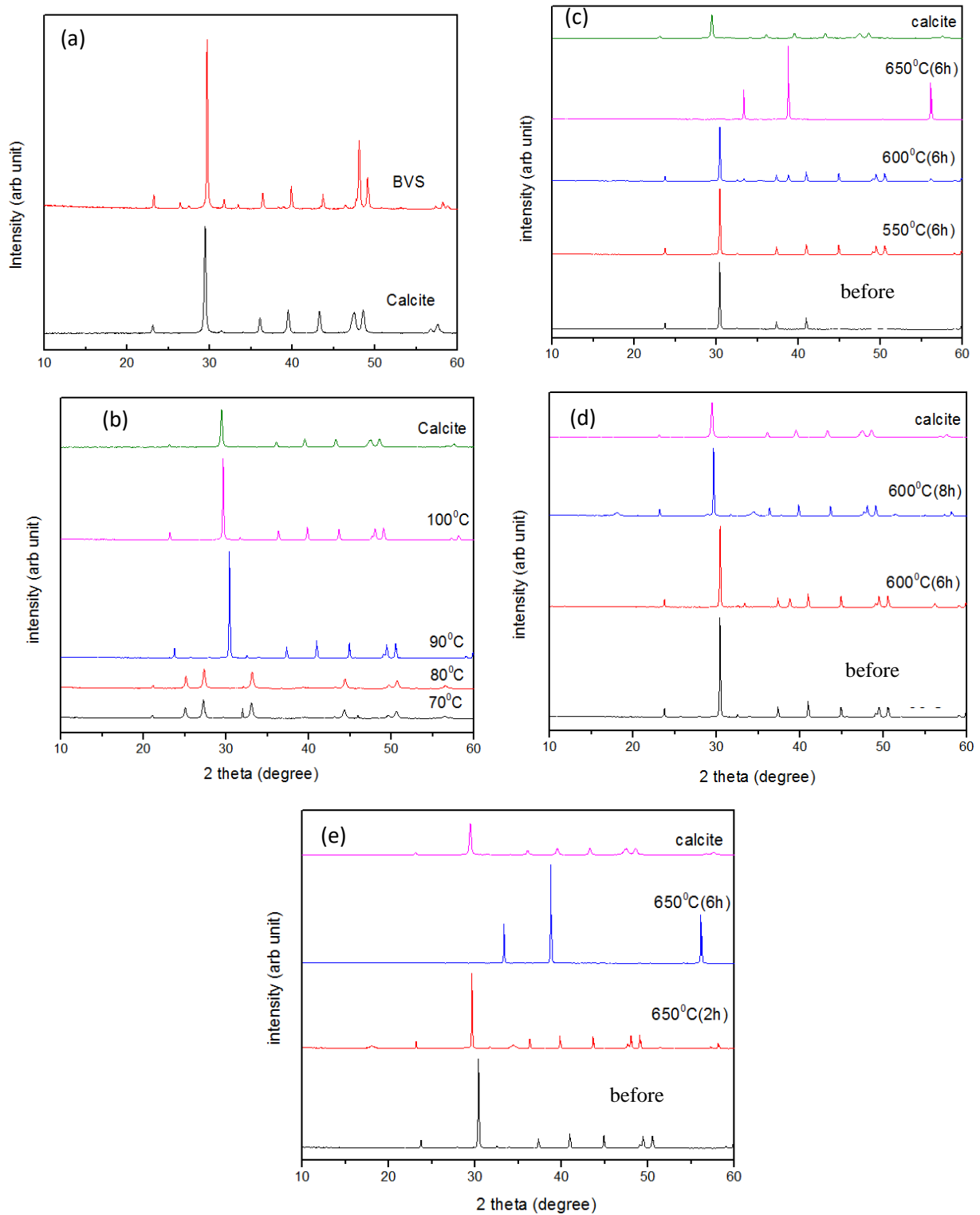


Figure 1. XRD patterns of cockle shells powders (a), samples synthesized at different reaction temperatures (b), samples calcined at different temperatures for 6 hours (c), samples calcined at 600°C with different soaking time (d), and samples calcined at 650°C with different soaking time (e)

Based on the XRD patterns, it showed that the cockleshell powder (BVS) had the same peaks as the calcite; specific spectra at 24, 29, 36, 39, 44, 48 and 57 degree. It was indicated that the cockleshell powder composition is mainly calcite. After chemical treatment, CaCl_2 solution reacted with Na_2CO_3 completely at 90°C and 100°C , showing with peaks coinciding with the peak of calcite. Conversely, when synthesis was carried out at 70°C and 80°C , reaction between CaCl_2 and Na_2CO_3 was incomplete. Due to incomplete reaction and other phase transformation, it also showed some extra peaks belonging to other minerals Na_2CO_3 (ICDD 18-1208) or vaterite (ICDD 74-1867). The obtained sample 70°C and 80°C had only Calcite peaks at 23, 44 and 50 degree (as showed in Figure1b). The samples of calcined powder at 550°C , 600°C soaking for 6h and 650°C soaking for 2h had the characteristic peaks of calcite. On the contrary, samples of calcined powder at 650°C soaking for 6h appeared peaks of other minerals such as lime (CaO ICDD 37-1497). This could involve to the decomposition of CaCO_3 into CaO and CO_2 when calcined at higher temperatures for longer periods of soaking time. Figure 2 illustrated the peaks of pure CaO powder [6]. It could be seen that the 650°C -calcined powder had peaks that coincided with the peak of the XRD pattern of CaO (as shown in Figure 1e).

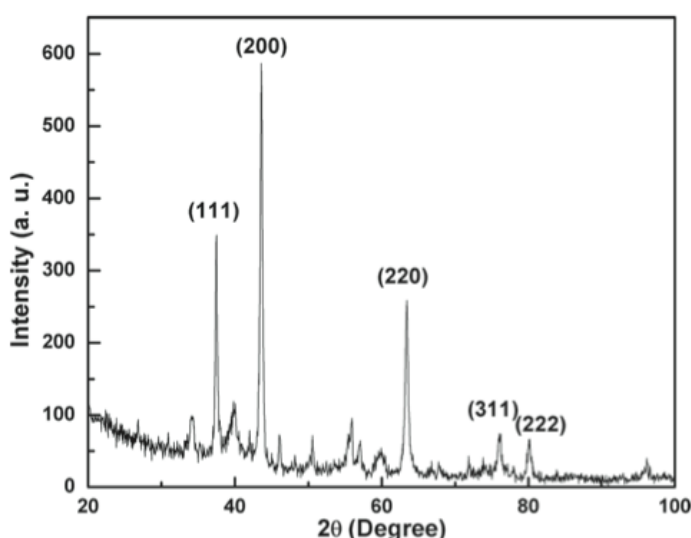


Figure 2. XRD pattern of of nano particle CaO [7]. Adapted from “Antimicrobial activity of CaO nanoparticles,” by R. Arup, S.S. Gauri, M. Bhattacharya, and J. Bhattacharya, 2013, *Journal of Biomedical Nanotechnology*, Vol. 9 (9), pp. 1570-1578. Copyright 2013 by the American Scientific Publishers.

Transmission Electron Microscopy was used to observed morphology of nano-powder. Figure 3 is the TEM images of the raw cockleshell powder (named as BVS) at different magnification and the TEM images of powder calcined at 650°C soaking for 2 hours (650°C -2h) at different magnification. It could be seen that raw cockleshell powder has particle around size of 500 nm. Calcite crystals of the raw cockleshell powder are oriented bars with a width of 500 nm and a length of 600-1700 nm.

Calcite nano-particle (650°C -2h) with size about 50-100nm could be obtained after synthesis and heat treatment process (as shown in Figure 4). For recovering tooth surface, nano particles were found to be useful to spread over every surface and migrate into it effectively [8-9]. Therefore, calcite nano particle synthesizing in this study would be advantageous for tooth enamel restoration to treat enamel hypersensitivity.

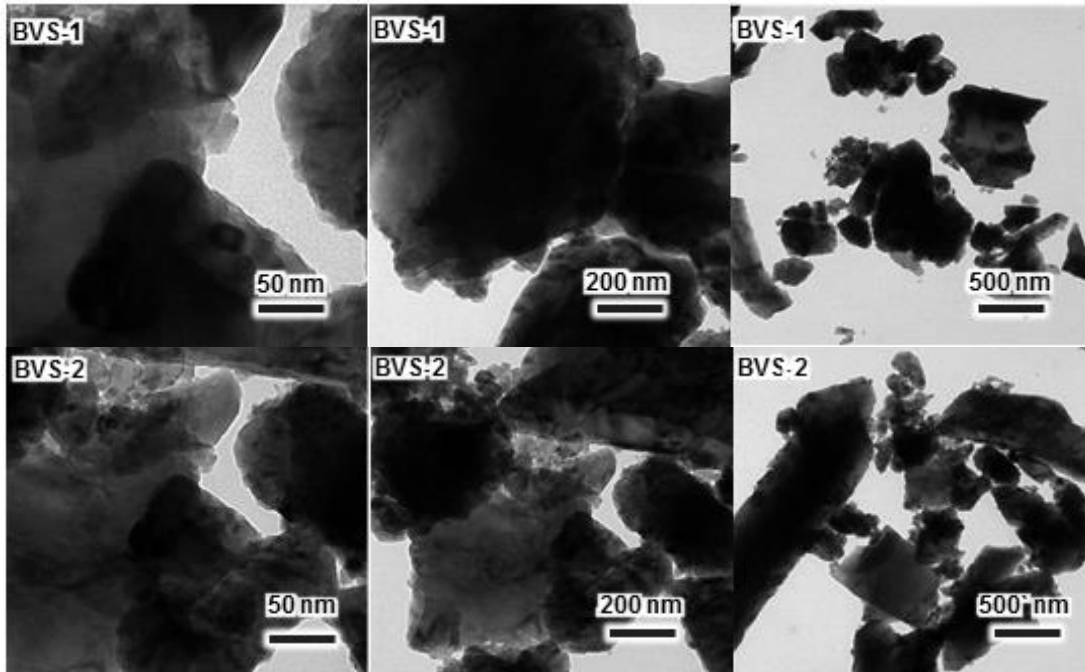


Figure 3. TEM images of the raw cockleshell powder (BVS) at different magnifications

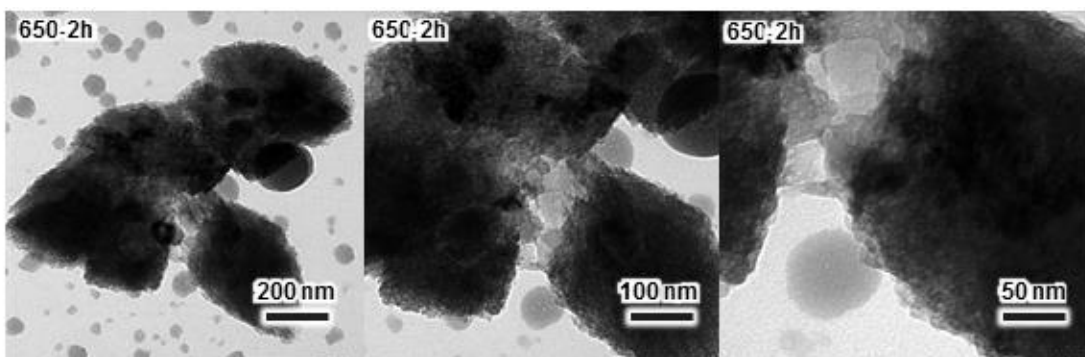


Figure 4. TEM images of powder calcined at 650°C soaking for 2 hours at different magnifications (650°C-2h)

Conclusions

Based on the results, the synthesis of nano-calcite for the purpose of tooth enamel restoration to treat enamel hypersensitivity could be optimized as synthesis reaction was complete when it was carried out at 90°C and 100°C. Next, pure calcite could be obtained when as-prepared powders were at 550°C, 600°C soaking for 6h and 650°C soaking for 2h. Finally, under the heating conditions at 650°C for 2 hours, calcite nano-particle with size about 50-100 nm could be obtained. For the next studies, *in vitro* test need to be done to evaluate the dentinal tubule occlusion and surface mineralization effect.

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