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SYNTHESIS OF CALCITE NANOPARTICLES FROM BIOGENIC WASTE USING GELATIN AND ITS SPECTRAL CHARACTERIZATION

Neethumol Varghese[1*], Manjusha Harifaran[1], Dr. A. Benny Cherian[1]

[1]Department of Chemistry, Union Christian College, Aluva, Ernakulam (DIST), Kerala, India.

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***Correspondence for Author**
Neethumol Varghese
Department of Chemistry,
Union Christian College,
Aluva, Ernakulam (DIST),
Kerala, India.

ABSTRACT

Natural biogenic waste materials like eggshells have been considered as promising substitutes in medical field, owing to their biodegradability, abundance and lower price in comparison with synthetic biomaterials. The calcite polymorph of calcium carbonate was prepared from chicken egg shells employing gelatin using precipitation method. The synthesized nano particles were characterized using XRD, FTIR, UV-Visible spectroscopy and SEM. The identified particles were calcite polymorphs with particle size of 25 nm. FT-IR results confirmed the formation of calcite with characteristic absorption bands observed at 712, 876 and 1410 cm^{-1}, corresponding to bending and stretching vibrations of CO_3^{-2} ions. The observed blue shift and the calculated band gap energy from absorption studies also indicated the formation of calcite nanoparticles. The results were compared with calcium carbonate synthesized without using gelatin. The methods adopted in the synthesis of calcite polymorphs were environmentally friendly which makes it biocompatible and potentially suitable for applications in the medical, pharmaceutical, cosmetic and paint industries.

KEYWORDS: Egg shell, calcite, nanoparticles, gelatin, precipitation method.

INTRODUCTION

Nanotechnology is rapidly sweeping through all vital fields of science and technology such as electronic, aerospace, defense, medical and dental. This involves in design, synthesis, characterization and application of material and devices on the nanometer scale. At the nanoscale, physical, chemical and biological properties differ from the properties of individual atoms and molecules of bulk matter. Therefore, it provides opportunity to develop new classes of advanced materials which meet the demands from high-tech applications.[1–3] Calcium carbonate occurs in nature as limestone in various forms, such as marble, chalk and coral. Calcium carbonate is one of the main biomineral components of eggshells and seashells. It is fascinating how nature skillfully designs and precisely controls the architecture, polymorphism, chemical composition and morphology of such biominerals. $CaCO_3$ can exist in mainly four polymorphs: calcite, vaterite, aragonite and amorphous calcium carbonate, out of which calcite is the most thermodynamically stable phase under ambient conditions; aragonite, which is a high-pressure polymorph that is less stable than calcite; and vaterite, which is the least stable and has the ability to transform into one of the other two polymorphs . The amorphous phase is unstable and relatively short-lived. (Ihli et al. 2014.[4] El-Sh eikh et al., 2013.[5]).

Calcium carbonate with different morphological structures has attracted the interest of researchers in different fields and it is probably one of the most widely-used raw materials in the chemical industry. It has numerous applications, primarily to produce cement, mortars, plasters, refractories and glass as building materials. Various grades of precipitated calcium carbonate are used in several products, such as textiles, papers, paints, plastics, adhesives, sealants and cosmetics. With the present focus of interest in nanotechnology, calcium carbonate nanoparticles have been observed to be biocompatible for use in medicine, pharmaceutical industries, drug delivery systems, biosensing and protein encapsulation.[6,7] The biological behaviour of this natural material has also shown that it could be used as a bone substitute in the field of maxillofacial surgery. The calcite calcium carbonate which is totally resorbable and biocompatible shows good osteo conductivity and has been used as an effective substitute in dentistry and orthopedic.[8]

Different techniques for the preparation of calcium carbonate nanoparticles have been reported, including the precipitation of homogeneous solutions[9] water-in-oil-in-water emulsions[10] mechanochemical and sonochemical synthesis[11] and water-in-oil (W/O) microemulsions.[12] Other preparation methods include the high-pressure homogeniser (HPH) technique and high-gravity precipitation. For precipitation methods, two different routes have been reported with the only difference being the chemicals used (i) wet carbonation route, through a double decomposition reaction, where in aqueous $CaCl_2$ and Na_2CO_3, or $CaCl_2$ and $(NH_4)_2CO_3$, or $Ca(NO_3)_2$ and Na_2CO_3 are combined in an equal molar ratio; or (ii) the bubbling carbonation route, in which CO_2 gas is bubbled through an aqueous slurry of $Ca(OH)_2$ (Barhoum et al., 2014.[13] Ihli et al., 2014.[4] Ukrainczyk et al., 2007.[14] Reeder et al., 2013.[15]). The bubbling carbonation route is industrially used because of the availability of its raw materials, high yield as well as simplicity and low cost of production (Tomioka et al., 2012.[16] Garcı´a Carmona et al., 2003.[17]).

The Chibowski et al. (2010) produced calcium carbonate nano-particles in water-in-oil micro-emulsion at 20-30°C. Calcite precipitation experiments were conducted in the presence of Polyethylene Glycol (PEG).[18] Ying et al. (2010) prepared $CaCO_3$ nano-particles via micro-emulsion route at room temperature with ethanol and n-hexanol as co-surfactant.[19] Wang et al.[20] synthesized nanometer size PCC (15-40 nm) using a lime suspension in a rotating packed bed reactor and had a very narrow distribution. Tsuzuki et al.[21] synthesized calcium carbonate nanoparticles using a mechanochemical reaction followed by heat treatment. $CaCO_3$ nanoparticles have also been prepared using a microemulsion technique consisting of sodium dodecyl-sulphate (SDS)/isopentanol/cyclohexane/water. Zhang et al.[22] synthesized nanoparticles of calcium carbonate in the reaction system of $Ca(OH)_2$-H_2O-CO_2.

The concept of calcium carbonate synthesis inspired by natural processes has expanded greatly during three past decades (Mann, 2001).[23] The morphology and structural properties of calcium carbonate can be controlled by the use of specific organic additives. In the present study, calcium carbonate nanoparticles were prepared from egg shell using water-soluble gelatin via precipitation method. Eggshell is natural composite bioceramics containing 5% of organic and 95% of pure calcium carbonate, usually both in the form of calcite and aragonite (Aras and Fernandez 2003).[24] It was reported that 1,90,000 tonnes of egg shell is wasted in India and might be used as a calcium source in human nutrition (Schaafsma *et al* 2000).[25] Gelatin was used to control the size of calcium carbonate nanoparticle and dispersion due to the expansion during calcination. Similar findings were reported for alcohols like ethanol, isopropanol or diethylene glycol which also were used to influence the $CaCO_3$ morphology. Recently, dendrimers were discovered as active additive for the controlled $CaCO_3$ precipitation (Cölfen, 2003).[26] The structural and morphological properties of synthesized powder were characterized by XRD and SEM. The optical properties of the sample were investigated by UV-VIS and FT-IR spectrophotometry. The results were compared with calcium carbonate synthesized without using gelatin.

MATERIALS AND METHODS

Egg shells were collected from a nearby restaurant. Gelatin powder was supplied by s.d. fine-CHEM Ltd., Mumbai and carbonic acid was purchased from Sigma Aldrich.

Synthesis of calcium carbonate nanoparticles

Eggshells were collected and washed thoroughly, initially in tap water and later in distilled water. The adhering membrane was separated manually and shells were dried at room temperature (30^0C). Later the shells were crushed using a domestic mixer. 20 g of gelatin powder was dissolved in 50ml of water at 50^0C with constant stirring. To 25 ml of gelatin solution, 30 g of egg shell powder was added; contents were mixed well and made into paste using a mortar and pestle. The raw eggshell was calcined in an air atmosphere at 900^0C for 3hours. The thermal treatment had two parts: in the first 30 minutes most of the organic materials were burnt out, whereas in the second part the eggshell transformation into calcium oxide takes place. The calcium oxide obtained was reacted by an exothermic reaction with carbonic acid. The mixtures was stirred well using a magnetic stirrer. The obtained calcium carbonate is further calcined at 900^0C for 2 hours. The calcium carbonate powders were finally packed into a polyethylene plastic bag for further analysis.

$$CaO + H_2CO_3 \longrightarrow CaCO_3 + H_2O + \Delta$$

Characterization

The following instruments were employed for the observation and characterization of the calcium carbonate nanoparticles.

X-ray diffractometry (XRD)

The samples were analysed using PANalytical, XRD machine (DY-1656). Monochromatic CuKα radiations of wavelength, 1·5406 Å were used as a source of 40 kV/35 mA power and the pattern was recorded in the 2θ range of 3^o– 80^o with a scan step of 0.02 in a scan time of 65.6seconds.

Infrared spectroscopy (FTIR)

The IR spectra of the samples prepared were recorded in the 4000–400 cm^{-1} range using Shimadzu, FTIR-8900 IR spectrophotometer through KBr mehod.

Ultraviolet-visible spectroscopy (UV-Vis)

The transmission spectra of the films were measured by a ultraviolet/visible spectrophotometer (Shimadzu, UV-1800) with a wavelength range 200 nm -1100 nm.

Scanning electron microscopy (SEM)

Dried samples were coated with gold ions using an ion coater (fisons sputter coater) under the following conditions: 0·1 Torr pressure, 200 mA current and 70 s coating time. Surface structure was visualized by scanning electron microscope (Hitachi, JEOL-JSM 5800) using a 20-25 kV accelerating voltage.

RESULTS AND DISCUSSIONS

XRD Analysis

X-ray diffraction is an ideal technique for the determination of crystallite size of the powder samples. The basic principle for such a determination involves precise quantification of the broadening of the peaks. XRD line broadening method of particle size estimation was chosen in this investigation for determining the crystallite size of the powder sample. The data obtained from the X-ray diffraction patterns in Figures 1(a) and 1(b) demonstrates the crystalline nature and phase composition of the calcium $CaCO_3$ synthesized without using gelatin and using gelatin respectively. Both powders exhibits characteristics peaks of calcite at 2θ values of 23^0, 29°, 35°, 39° 43° and 48° which correlate with (hkl) indices of (012), (104), (110), (113), (202) and (116). All the reflections can be attributed to the pure calcite phase of calcium carbonate, which is supported by the JCPDS file No: 05-0586.

The average crystallite sizes of synthesized samples were calculated from the full width at half maximum (FWHM) of the peaks using Debye-Scherrer formula:

$$\mathbf{D = 0.9\lambda / \beta \cos \theta}$$

where, D - crystallite size, λ - wavelength of CuKα radiation, β - corrected full width at half maximum (FWHM) of the diffraction (hkl) peak, θ – Bragg's angle of the X-ray diffraction peak. The average crystalline size of $CaCO_3$ nano particles without gelatin was in the range of 35 nm and for calcium carbonate nanoparticles synthesized using gelatin, the average crystalline size was found to be in the range of 25 nm.

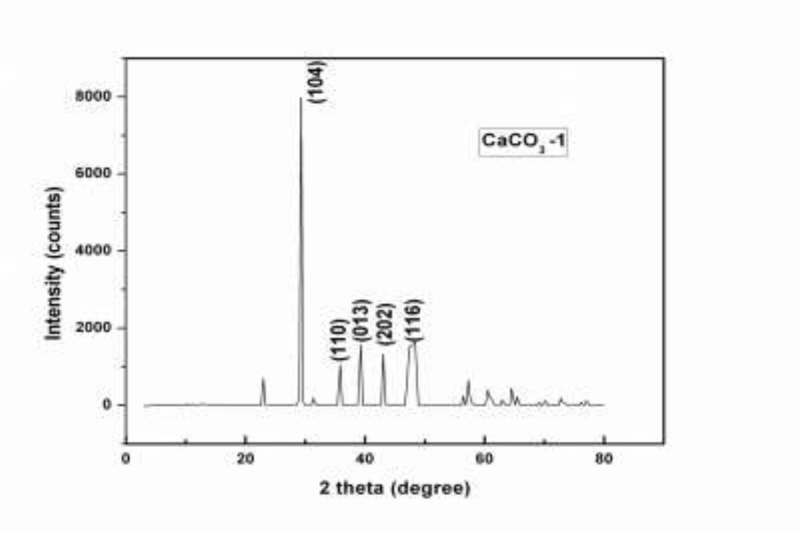

Fig. 1(a) XRD of nano CaCO$_3$ without gelatin.

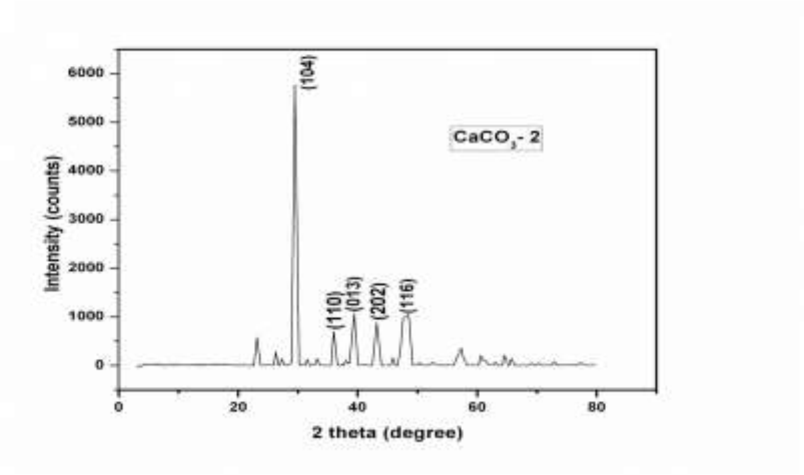

Fig. 1(b) XRD of nano CaCO$_3$ using gelatin.

FTIR Analysis

FTIR spectra of nano calcium carbonate synthesized without using gelatin and using gelatin are presented in Figures 2(a) and 2(b) respectively. FTIR spectroscopy is an important instrument used to identity different phases of organic and inorganic compounds. The spectral data obtained for the samples reveal the common characteristic features of the carbonate ions in calcium carbonate and are the fundamental modes of vibration for this molecule. The broad absorption peak at approximately 1410 cm^{-1} is due to the stretching vibrations of the C–O and the peak at around 876 cm^{-1}and 712 cm^{-1} are due to the bending vibrations of the C–O bond. The combination of the three peaks at 1410 cm^{-1} , 876 cm^{-1} and 710 cm^{-1} appears at 2515 cm^{-1} and is called overtone or combination band (Forbes et al., 2011) [27]. The broad absorption peaks around 3500 cm^{-1} are assigned to stretching vibrations of the O–H bond and it can be attributed to the presence of absorbed water and hydroxyl groups on the surface of $CaCO_3$ particles. Both samples of $CaCO_3$ exhibit similar vibration patterns. However, the only difference between the two kinds of powders was in the depth of the bands. For the nano powders synthesized using gelatin, the peaks are much sharper than the sample synthesized without using gelatin, indicating more crystalline nature.

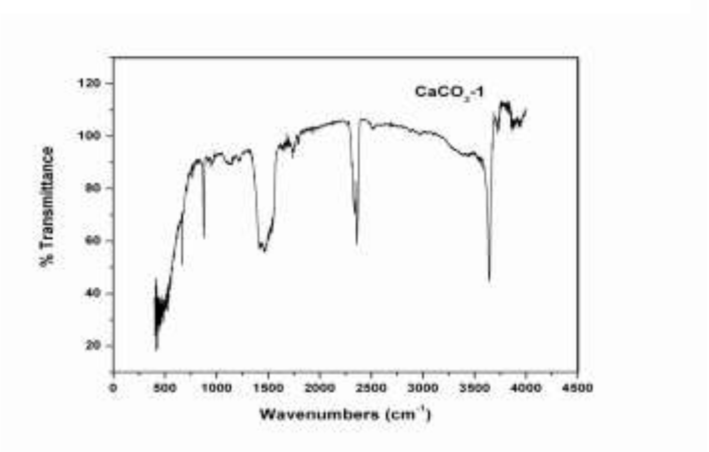

Fig. 2(a) FTIR spectrum of nano $CaCO_3$ without gelatin.

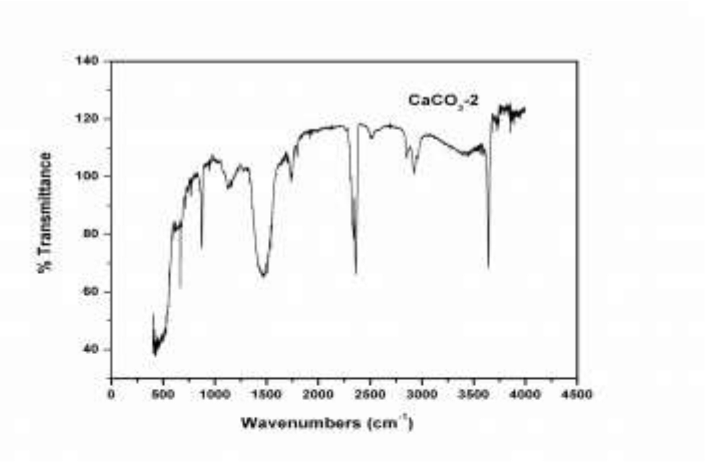

Fig. 2(b) FTIR spectrum of nano $CaCO_3$ using gelatin.

UV–Visible Analysis

Figure 3(a) and 3(b) shows the UV spectrum of nano $CaCO_3$ synthesis without using gelatin and using gelatin respectively. By employing gelatin, the absorption was blue shifted from 375nm to 250nm which is a clear evidence for decrease in particle size in case of calcite synthesized using gelatin. Band gap energy was calculated using the Tauc plot. As calcium carbonate is an indirect band gap material, Tauc plot was plotted between photon energy (hν) on the X axis and a quantity $(\alpha h\nu)^{1/2}$ on the Y axis and extrapolating the linear portion of the curve to the X axis yields the energy of the material which is found to be in eV. This value is higher for $CaCO_3$ synthesized using gelatin (5.9 eV) than the calcite nano particles synthesized without using gelatin (4.8 eV). Increase in energy indicated decrease in particle size.

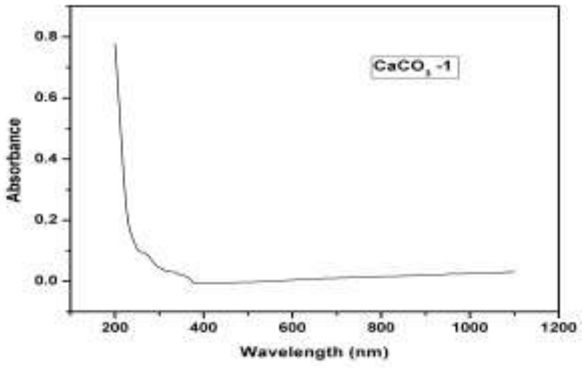

Fig. 3(a) UV spectrum of nano $CaCO_3$ without gelatin.

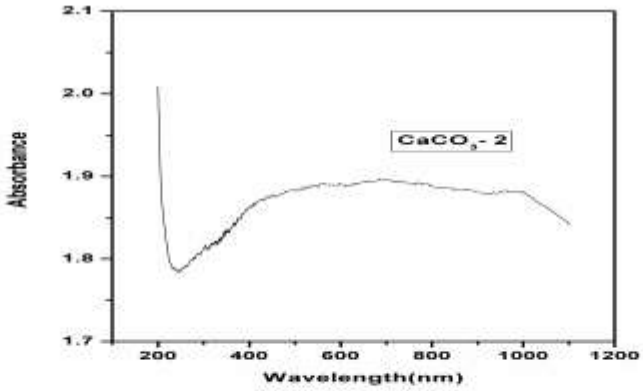

Fig. 3(b) UV spectrum of nano $CaCO_3$ using gelatin.

SEM Analysis

Scanning electron microscopy was used to analyze the morphology and size of the synthesized calcium carbonate nanoparticles. Figure 4(a) and 4(b) shows the SEM images of the calcium carbonate nanoparticles synthesized without using gelatin and using gelatin respectively. The SEM images of calcium carbonate nanoparticles show particles with cubical morphology for $CaCO_3$ synthesized without using gelatin. By employing gelatin in the synthesis, the particles are distributed with less agglomeration and the surface morphology has changed slightly. Large particles are seen due to the high temperature of calcination. Use of gelatin provides long-term stability for nanoparticles by preventing particles agglomeration.

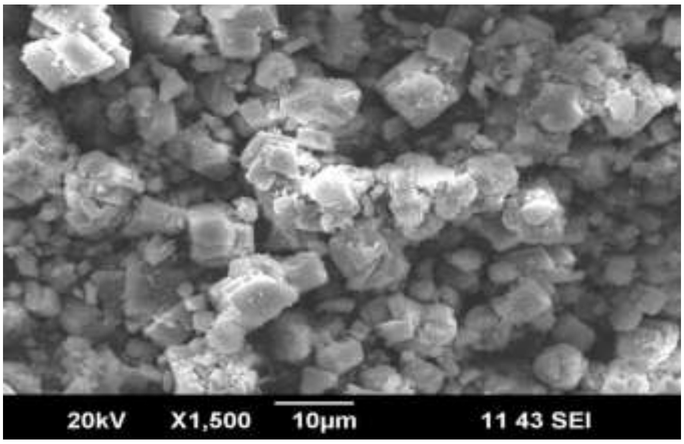

Fig. 4(a) SEM of nano $CaCO_3$ without gelatin.

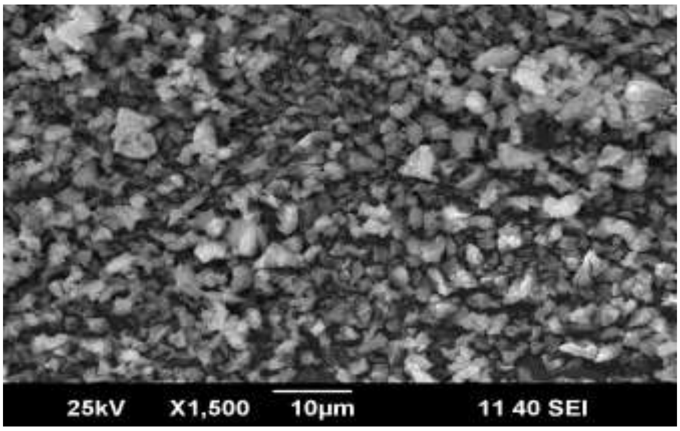

Fig. 4(b) SEM of nano $CaCO_3$ using gelatin.

CONCLUSION

Calcite nanoparticles were synthesized from chicken egg shells employing water-soluble gelatin using precipitation method. From XRD and FTIR results, it is clear that synthesized calcium carbonate nanoparticles exhibited the calcite structure. The crystalline particle size was calculated from the XRD data using Debye – Scherer equation and was found to be about 25 nm for calcite synthesized using gelatin and for the $CaCO_3$ synthesized without using gelatin, the particle size was 35nm. XRD and SEM results indicated that using gelatin solution as a matrix improves the crystallinity and decreases the size of particles. FTIR The broad absorption peak at 1410 cm^{-1} and the peak at around 876 $cm^{-1}$and 712 cm^{-1} are due to the stretching and bending vibrations of the C–O bond of carbonate ion respectively. The effect of gelatin on the surface morphology of calcite nano particles is clear from the SEM analysis. Optical studies were carried out and the band gap energy was determined. The values of the energy band gap obtained for calcite synthesized using gelatin and without using gelatin are 5.9 eV and 4.8 eV respectively. The blue shift observed in the spectrum is a clear indication of decrease in particle size on employing gelatin. The results confirm that gelatin is an appropriate particle size controlling agent in the synthesis of calcite nanoparticles. The method adopted is simple, cost-effective and environmental friendly and can be extended to prepare nanoparticles of other interesting materials.

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REFERENCES

1. K. J. Klabunde, Nanoscale Materials in Chemistry, Wiley-Interscience, New York, NY, USA, 2001.
2. G. Kickelbick, Concepts for the incorporation of inorganic building blocks into organic polymers on a nanoscale, Progress in Polymer Science, 2003; 28: 83–114.
3. Q. H. Zeng, D. Z. Wang, A. B. Yu, and G. Q. Lu, Synthesis of polymer-montmorillonite nanocomposites by in situ intercalative polymerization, Nanotechnology, 2002; 13: 549–553.
4. Ihli, J., Wong, W.C., Noel, E.H., Kim, Y.Y., Kulak, A.N., Christen-son, H.K., Duer, M.J., Meldrum, F.C., Dehydration and crystallization of amorphous calcium carbonate in solution and in air, Nat. Commun, 2014; 5: 1–10.
5. El-Sheikh, S.M., El-Sherbiny, S., Barhoum, A., Deng, Y., Effects of cationic surfactant during the precipitation of calcium carbonate, Colloids Surf. A, 2013; 422: 44–49.

a. Xu, Y. Ma and H. Colfen, Biomimetic mineralization, Journal of Material Chemistry, 2006; 17: 415–449. View at Google Scholar.

6. Y. Fukui and K. Fujimoto, Bioinspired nanoreactor based on miniemulsion system to create organic-inorganic hybrid nanoparticle and nanofilm, Journal of Material Chemistry, 2012; 22: 3493–3499. View at Google Scholar.
7. Park JW, Bae SR, Suh JY, Lee DH, Kim SH, Kim H, Lee CS, Evaluation of bone healing with eggshell-derived bone graft substitutes in rat calvaria: A pilot study, Journal of Biomedical Materials Research, 2007; 87(1): 203-214. Return to text.

8. R. Gupter, Synthesis of Precipitated Calcium Carbonate Nanoparticles Using Modified Emulsion Membranes [M.S. thesis], Georgia Institute of Technology, Atlanta, USA, 2004.
9. G. X. Wu, J. Ding, and J. M. Xue, Synthesis of calcium carbonate capsules in water-in-oil-in-water double emulsions, Journal of Materials Research, 2008; 23: 140–149. View at Publisher. View at Google Scholar. View at Scopus.
10. T. A. Hassan, V. K. Rangari, V. Fallon, Y. Farooq and S. Jeelani, Mechanochemical and sonochemical synthesis of bio-based nanoparticles, Proceedings of the Nanotechnology Conference, 2010; 278–281. View at Scopus.
11. Georgieva, B. Georgieva, Z. Bogdanov, and D. K. Stefanov, Microemulsion water-in-oil (W/O)—microreactor for synthesis of ultrafine carbonate nanostructures, University of Ruse Union of Scientists-Ruse, 2011; 50: 34–38. View at Google Scholar.
12. Barhoum, A., Rahier, H., Esmail Abou-Zaied, R., Rehan, M., Dufour, T., Hill, G., Dufresne, A., Effect of cationic and anionic surfactants on the application of calcium carbonate nanoparticles in paper coating, ACS Appl. Mater. Interfaces, 2014; 6: 2734–2744.
13. Ukrainczyk, M., Kontrec, J., Babic-Ivancic, V., Brecevic, L., Kralj, D., Experimental design approach to calcium carbonate precip-itation in a semicontinuous process, Powder Technol., 2007; 171: 192–199.
14. Reeder, R.J., Tang, Y.Z., Schmidt, M.P., Kubista, L.M., Cowan, D.F., Phillips, B.L., Characterization of structure in biogenic amorphous calcium carbonate: pair distribution function and nuclear magnetic resonance studies of lobster gastrolith, Cryst. Growth Des., 2013; 13: 1905–1914.
15. Tomioka, T., Fuji, M., Takahashi, M., Takai, C., Utsuno, M., Hollow structure formation mechanism of calcium carbonate particles synthesized by the CO_2 bubbling method, Cryst. Growth Des., 2012; 12: 771–776.
16. Garcı´a Carmona, J., Morales, J.G., Clemente, R.R., Rhombo-hedral–scalenohedral calcite transition produced by adjusting the solution electrical conductivity in the system of $Ca(OH)_2$–CO_2– H_2O, J. Colloid Interface Sci., 2003; 261: 434–440.
17. Sadowski Z., Polowczyk I., Fr ckowiak A., Ko lecki T., Chibowski S., Bioinspired Synthesis of Calcium Carbonate Colloid Particles, Physicochem. Probl. Miner. Process, 2010; 44: 205-209.

18. Yu D., Chu Y., Dong L.H., Zhuo Y.J., Controllable Synthesis of CaCO3 Micro/Nanocrystals with Different Morphologies in Microemulsion, Chem. Research Chinese Universities, 2010; 26: 678-683.
19. Wang, Yuhong, Chen, Jianfeng, Jia, Zhiqian, Zheng, Chong, Synthesis and Applications of Nanoparticles by Multiphase reactive precipitation in a high gravity reactor, Proceedings of the International Workshop on Materials Processing, 2001; 4th: 19-28.
20. Takayu Tsuzuki, Kellie Pethick and Paul McCormick, Synthesis of Calcium Carbonate nanoparticles by Mechanochemical processing, Journal of Nanoparticle Research, 2000; 2: 375 – 380.
21. Zhang, Shi-cheng, Han, Yue-xin, Jiang, Jun-hua Wang, Hong-kuan, Studies on thecrystallization of nano calcium carbonate in the reaction system $Ca(OH)_2/-H_2OCO_2$, Dongbei Daxue Xuebao, 2000; 21: 169-172.
22. Mann S., Biomineralization principles and concepts in bioinorganic materials, 2001.
23. Aras J.L., Fernandez S.M., Biomimetic process through the study of mineralized shells, Materials Characterization, 2003; 50: 189-195.
24. Schaafsma A, Pakan I, Hofstede G J H, Muskiet F A J, Van Der Veer E and De Vries P J F, Poultry Sci., 2000; 79: 1833.
25. Colfen H., Precipitation of carbonates: recent progress in controlled production of complex shapes, Current Opinion Colloid Inter. Sci., 2003; 8: 23-31.
26. Forbes, T.Z., Radha, A.V., Navrotsky, A., The energetics of nanophase calcite, Geochim. Cosmochim. Acta, 2011; 75: 7893–7905.