Available online at www.sciencedirect.com

ScienceDirect

Ceramics International 41 (2015) 3116–3127

CERAMICS INTERNATIONAL

www.elsevier.com/locate/ceramint

Novel malic acid mediated green route for the synthesis of hydroxyapatite particles and their spectral characterization

D. Gopi[a,b,*], N. Bhuvaneshwari[a,c], L. Kavitha[d], S. Ramya[a]

[a]Department of Chemistry, Periyar University, Salem 636 011, Tamilnadu, India
[b]Centre for Nanoscience and Nanotechnology, Periyar University, Salem 636 011, Tamilnadu, India
[c]Department of Chemistry, Chikkaiah Naicker College, Erode 638 004, Tamilnadu, India
[d]Department of Physics, School of Basic and Applied Sciences, Central University of Tamilnadu, Thiruvarur 610 101, Tamilnadu, India

Received 25 September 2014; received in revised form 27 October 2014; accepted 27 October 2014
Available online 6 November 2014

Abstract

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HAP], a principal inorganic constituent of bone and teeth, is widely used in the biomedical field, due to its similarity, chemically and structurally to the bones. In this paper, we have reported the synthesis of HAP particles using the malic acid mediated green template method. The malic acid used in this method has been obtained from various sources, such as natural and commercially available one. The extract of the apple fruit is taken as the natural source of malic acid. The extracted malic acid and the as-synthesized HAP particles are characterized by various spectral techniques like Fourier transform infrared spectroscopy (FT-IR), proton-1 nuclear magnetic resonance spectroscopy (^{1}H-NMR), carbon-13 nuclear magnetic resonance spectroscopy (^{13}C-NMR) and X-ray diffraction (XRD) technique for the phase identification of the HAP particles. The morphology of the HAP particles were assessed thoroughly using a scanning electron microscope (SEM) equipped with energy dispersive X-ray analysis (EDAX). Moreover, the antibacterial activity of the as-synthesized HAP particles was evaluated against the two pathogen bacteria strains. The experimental results revealed that the HAP particles synthesized in the presence of an extracted malic acid are pure, low crystalline and spherical particles with reduced size. These HAP particles also showed an enhanced antibacterial activity than that of the HAP synthesized in the presence of commercially available malic acid. Hence, the HAP particles synthesized using the extracted malic acid as a green template can act as a good biomaterial for biomedical applications.

Keywords: A. Calcination; B. Spectroscopy; B. X-ray methods; D. Apatite; E. Biomedical applications

1. Introduction

Hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$ is the main mineral constituent of vertebrate skeletal systems [1]. Natural bone can induce infection problems and antigenic reaction [2]; therefore, much attention has been paid to hydroxyapatite because it is the main mineral component of bone and teeth. HAP has been widely studied for bone replacement, dental defect filling, bone tissue engineering, drug delivery applications and also commonly used as a coating material for metallic implants due to its excellent biocompatibility, bioactivitity, osteoconductivity and bone-bonding property [3–17]. The HAP particles are used in the application of bone repair, retardation of the multiplication of cancer cells and also as efficient drug delivery agents and replacement of traumatized bone tissues [8]. Nowadays, the HAP particles at nanoscale level propose a variety of customized solutions for conventional problems. The preparation of HAP can be done by a number of methodologies such as hydrolysis [18], precipitation method [19], co-precipitation method [20] hydrothermal method [21], sol–gel method [2,22], microemulsion [23,24], emulsion technique [25], spray pyrolysis [26], mechano-chemical method [27] and the template directed method [28,29]. However these methods employed organic solvents or organic templating agents which are potentially dangerous to the environment and biological systems

*Corresponding author at: Department of Chemistry, Periyar University, Salem 636 011, Tamilnadu, India.
Tel.: +91 427 2345766; fax: +91 427 2345124.
E-mail address: dhanaraj_gopi@yahoo.com (D. Gopi).

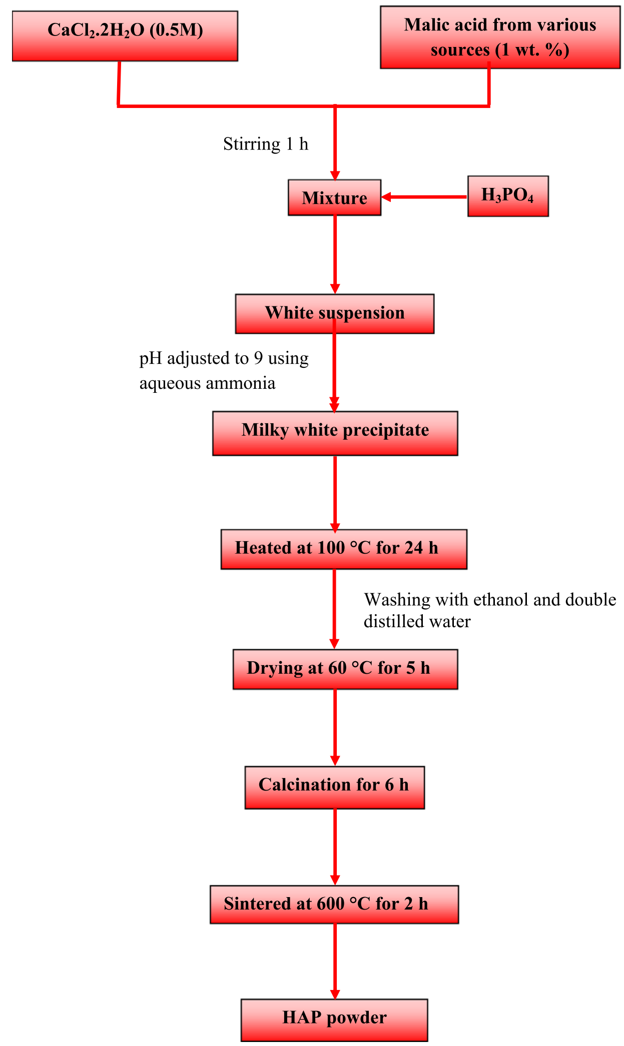
http://dx.doi.org/10.1016/j.ceramint.2014.10.156
0272-8842/

[30]. Therefore, the current design strategy is focussed on the synthesis of HAP with controlled morphology, size and structure by using abundant green materials since they are renewable, cost-effective and environmentally benign when compared with other synthetic organic templates [31–35]. These natural biological materials contain carbohydrates, dihydroxy-succinic acid, monohydroxy-carboxylic acids, proteins, fibres and polysaccharides in a significant amount.

The synthesis protocols for HAP particles involving environmentally mediated materials like plant extract offer numerous benefits of eco-friendliness and compatibility for the pharmaceutical as well as biomedical applications. Further, the green template method proves to be the best method rather than the chemical and physical methods, as it is economical, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. Very recently Gopi et al., successfully synthesized HAP particles using sucrose, tartaric acid and pectin derived from the natural sources, as green templates [36–38].

So, we aimed to synthesize the HAP particles using a green chelating agent as template under atmospheric pressure and to investigate their morphology, crystallinity and particle size. This method involves the use of commercially available malic acid and extracted natural malic acid as chelating agents. Malic acid is a natural substance present in the fruit apple. It is also known as hydroxyl succinic acid and is biodegradable, biocompatible, water soluble and polar in nature.

As far as our literature survey is concerned, there are no reports on the synthesis of HAP particles using the naturally occurring malic acid. Hence, the present work deals with the synthesis of HAP particles using malic acid extracted from natural source of apple fruit as green template. For comparison, HAP particles have also been synthesized using commercially available malic acid. Hence, we propose this method as a simple and effective route to produce the bulk ceramic material.



Scheme 1. Scheme for the synthesis of HAP using malic acid as green template.

2. Experimental

2.1. Materials and methods

Chemicals like calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), phosphoric acid (H_3PO_4), malic acid ($C_4H_6O_5$) and aqueous ammonia solution were purchased from Sigma-Aldrich. All the chemicals were of analar grade and used without any further purification. The aqueous solutions were made by dissolving them in deionized water. In order to get the malic acid from natural source, fruit apples (*Malus domestica*) were purchased from the market at Tamil Nadu, India.

2.2. Plant material and preparation of the juice extract

Green ripe apple fruits were used to make the extract. After removing the outer layer of the apple fruits, they were thoroughly washed in double distilled water. About 1Kg of inner fleshy part was weighed and cut into fine pieces. The extracts were prepared according to the previous report by Richmond et al. [39] and the malic acid was extracted from the apple juice using the high performance liquid chromatography (HPLC) as that of the procedure done by Blanco et al. [40]. The HPLC columns were prepared by using a cation exchange resin in the hydrogen ion form and the mobile phase was potassium dihydrogen phosphate. Before injection, the extract was filtered through a 0.45 μm Millex membrane to remove the impurities that might be present in the extract. Further, the standard malic acid solution of 5–100 μg/ml was prepared and then injected into the HPLC system.

2.3. Chromatographic separation of malic acid from apple juice extract

The separation of malic acid from the natural apple juice extract was done by HPLC columns of 200 × 7.8 mm i.d (interactior ION-300) packed with a cation exchange resin in the hydrogen ion form; a 250 × 4 mm i.d. 5 μm Spherisorb NH_2 and a 250 × 4 mm i.d. Spherisorb ODS-2 (C 18), using a LKB Model 2140 rapid spectral detector and an IBM data station [40]. A LKB (Bromma, Sweden) liquid chromatograph was