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Extraction of biocompatible hydroxyapatite from fish scales using novel approach of ionic liquid pretreatment

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ABSTRACT

In this study the waste fish scales (FS) were dissolved in 1-butyl-3-methylimidazolium acetate ionic liquid to obtain a valuable product of hydroxyapatite (HAp). The HAp was obtained in the yield of 32 ± 2%. The obtained HAp was characterized using Fourier Transform Infrared Spectroscopy (FTIR), Powder X-rays Diffraction (PXRD), Thermal Gravimetric Analysis (TGA), Field Emission Scanning Microscopy (FE-SEM), Energy Dispersive X-rays spectroscopy (EDX), and Brunauer–Emmett–Teller (BET). The results of FTIR and XRD showed the characteristic peaks of the HAp. The thermal degradation temperature of the extracted HAp was relatively high. Furthermore, low weight loss was measured which confirmed the removal of organic part of FS during ionic liquid treatment. The FE-SEM result showed the particles with different morphologies and EDX analysis showed a Ca/P ratio of 1.60 for the extracted HAp. The biocompatibility of the extracted HAp was assessed through MTT cell viability assay using known Human Embryonic Kidney 293 cells (HEK cells) and epidermoid carcinoma cells (A431 cells).

1. Introduction

It has been approximated that each year 18–30 million tons of fish waste is discarded which accounts for 50% of total mass of fish processing industry in the world. In the fish waste, the fish scale was estimated 4% by weight [1,2]. Fish scales consist mainly of collagen and hydroxyapatite [HAp] along with fatty acids, vitamins, antioxidant and trace elements [2,3]. Collagen is an abundant protein that has many applications in biomedical and pharmaceutical sciences [4]. HAp [$Ca_{10}(PO_4)_6(OH)_2$] having similar properties with natural bones, has gained immense importance in the field of orthopedics and in the development of dental materials [5,6]. HAp has been synthesized by various chemical methods like solid state reaction, hydrothermal reaction, co-precipitation reaction and sol–gel synthesis mechano-chemical methods [7–10]. The laser ablation technique was used to fabricate the HAp based nanoparticles while their thin film were coated using the electrostatic spray and aerosol deposition techniques [11–13].

HAp has also been extracted from various biological sources like bones and fish scales, etc. [14,15]. All these methods involve the use of acids, alkalis and heat to separate the HAp from biological sources. Along with environmental issues related to the use of acids and alkalis, the use of high temperature during the course of treatment also results in distortion of natural intact physical structure of the extracted HAp. In addition, the use of all these methods results in loss of other major constituents: collagen which has been acknowledged for various applications.

It is well documented that ionic liquids, due to their advantageous properties (negligible vapor pressure, nonflammability, non-explosiveness, electrochemical and thermal stability, easy recyclability and high conductive characters), has gained importance for the last 25 years in many fields. Likewise, ionic liquids have been reported for the dissolution of other biopolymer, like cellulose, chitosan, chitin, collagen and silk, etc. [16–20]. The novelty of this work lies in the use of ionic liquid first time for extraction of HAp from fish scales. In this study the waste fish scales were collected from the local market and dissolved in 1-butyl-3-methylimidazolium acetate ionic liquid to obtain the HAp.

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Comparatively, the fish scales was preferred to fish bones, as the bone matrix is hard and difficult to bring into small size particle as it needs more crushing and ball milling. Fish scales consist of more collagen as compare to fish bones that has been targeted to separate simultaneously with extraction of HAp during ionic liquid pretreatment. The collagen part is compiled as separate study and will be published elsewhere. The HAp was separated from the resultant mixture and its characterization was performed using FTIR, FE-SEM, XRD, TGA, particle size distribution and BET surface analyzer. The biocompatibility of extracted HAp was assessed through the cell viability assay using the known cells of HEK and A431.

2. Experimental

2.1. Materials and methods

Carp fish (*Cyprinidae*) was obtained from local market located opposite to Dalian Institute of Chemical Physics, Chinese Academy of Sciences in Dalian, China. FS was isolated from the fish and washed thoroughly with tap water followed by distilled water to ensure the removal of undesired debris attached during scales removal from fish. After washing, the FS was dried at room temperature and then ground with grinder (AISITE, China). 10.0 g of 1-butyl-3-methylimidazolium acetate (CJC, China) was charged into 30 mL reagent bottle followed by addition of 0.5 g of ground fish scales. The FS was added in two fractions during the course of pretreatment. After charging, the bottle's cape was closed and kept in the oil bath and heated for 12 h at 100 °C. After dissolution process, water of equal volume of reaction mixture was added followed by addition of NaOH solution (0.5 M) to bring the pH at 9. The HAp was obtained as precipitate by centrifugation (11,000 rpm) for 30 min. The supernatant contained the ionic liquid and other constituents of FS were further subjected to fractionation and ionic liquid was recycled. The supernatant contained the ionic liquid, organic portion etc. was collected and NaCl solution (2 M) was added followed by adding HCl solution (0.5 M) to bring the pH around 2. The precipitate of organic portion (collagen) was obtained which has been separated from the resulting mixture by centrifugation and subsequently washing at 11,000 rpm. The resultant acidic supernatant was neutralized with NaOH solution (0.5 M) and further subjected to rotary evaporator to remove the water. Ionic liquid was extracted with acetone and dichloromethane followed by subsequent filtration and evaporation.

2.2. Characterization

FT-IR spectrometer (Bruker Tensor 27) was used to characterize the sample. The samples were prepared as a compressed KBr discs and measurements were performed in the range of 450–4000 cm^{-1}. Powder XRD (X-rays diffraction model; PANalytical X'Pert Pro Multi-Purpose Diffractometer) was used to confirm the phase composition of extracted HAp. The XRD spectra were recorded from 5° to 80° and step size of 0.1°. Thermal behavior of the samples was analyzed using thermal analyzer (NETZSCH, STA 449) over a temperature range of 42–780 °C and heating rate of 10 °C min^{-1} in nitrogen atmosphere. The morphology of the samples was investigated using Field Emission Scanning Microscopy (FE-SEM, Jeol JSM-7800F, Japan). Energy dispersive X-rays spectrometry (EDX) was used for elemental analysis. The particle size distributions were measured using Zetarsizer nanoseries (Malvern Ltd., UK). For particles size distribution measurement, 0.02 g of extracted HAp was dispersed in the 2 mL of ethanol to make a 1% solution. Brunauer–Emmett–Teller (BET) method was used to measure the surface area for the samples at 77 K of liquid N_2. Prior to adsorption and desorption of N_2 gas, the samples were degassed

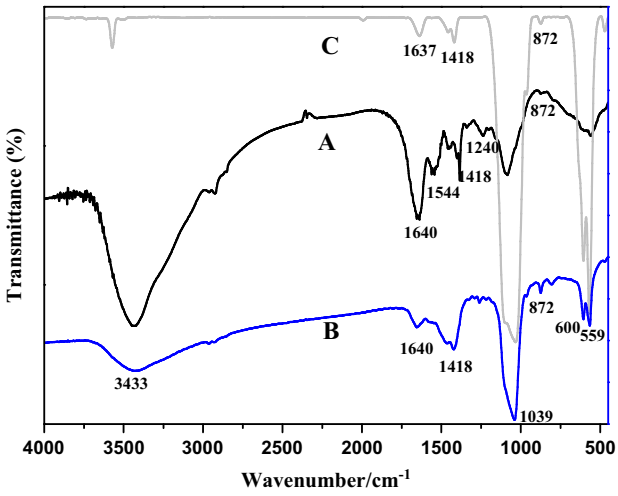

Fig. 1. FTIR spectra of fish scale (A), extracted hydroxyapatite (B) and commercial synthetic hydroxyapatite (C).

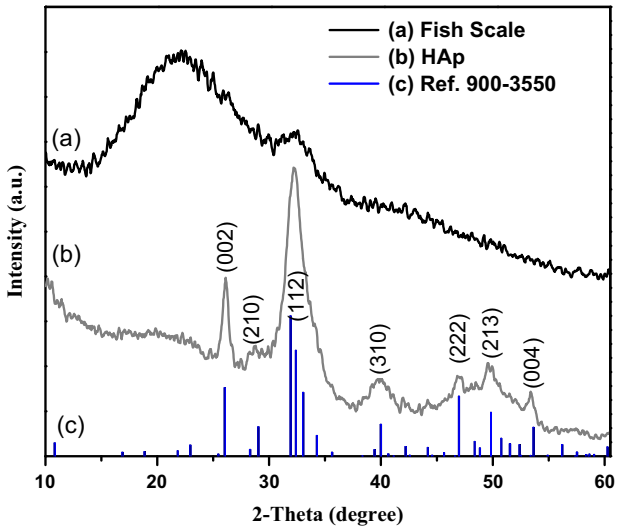

Fig. 2. XRD analysis of fish scale (a), extracted hydroxyapatite (b) and hydroxyapatite Reference card 9003550 HAp (c).

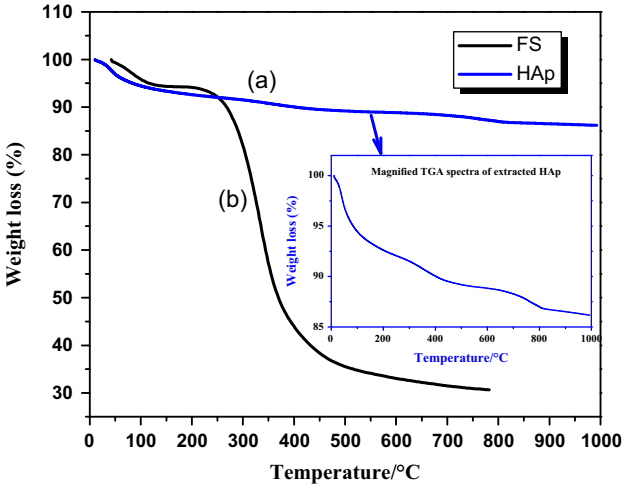

Fig. 3. TGA of fish scale (FS, black; a) and extracted hydroxyapatite (HAp, blue; b). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)