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Novel banana peel pectin mediated green route for the synthesis of hydroxyapatite nanoparticles and their spectral characterization

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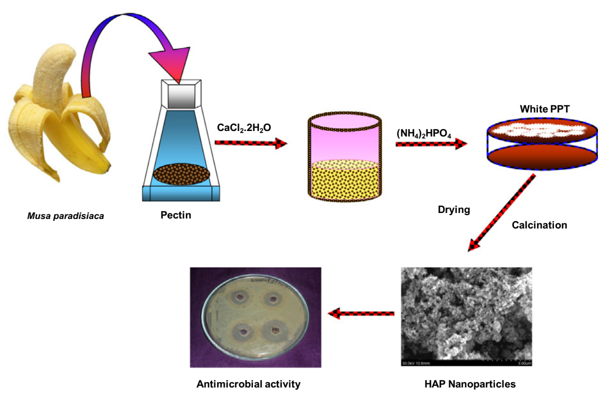
HIGHLIGHTS

- Hydroxyapatite (HAP) nanoparticles synthesis using banana peel pectin as template.
- Biological synthesis is inexpensive, ecofriendly and uses non-hazardous chemicals.
- Nano-HAP is pure, low crystalline, reduced size at high concentration of pectin.
- HAP nanoparticles showed antimicrobial activity used for biomedical applications.

GRAPHICAL ABSTRACT



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ABSTRACT

Hydroxyapatite [HAP, $Ca_{10}(PO_4)_6(OH)_2$] is the main inorganic component of natural bone and is widely used in various biomedical applications. In this paper, we have reported the synthesis of HAP nanoparticles by banana peel pectin mediated green template method. The pectin extracted from the peels of banana and its various concentrations were exploited in our study to achieve a controlled crystallinity, particle size as well as uniform morphology of HAP. The extracted pectin was characterized by spectral techniques like Fourier transform infrared spectroscopy (FTIR) for the functional group analysis, proton-1 nuclear magnetic resonance spectroscopy (^{1}H NMR) and carbon-13 nuclear magnetic resonance spectroscopy (^{13}C NMR) for the identification of H and C atoms in the extracted pectin, respectively. The HAP nanoparticles were synthesized using different concentrations of the as-extracted pectin. The purity, crystallinity and morphology of the as-synthesized HAP nanoparticles were evaluated by FTIR, X-ray diffraction (XRD) and scanning electron microscopy (SEM) with energy dispersive X-ray analysis (EDAX) and transmission electron microscopy (TEM), respectively. Moreover the antibacterial activity of HAP nanoparticles was evaluated against the gram positive and negative bacteria like *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*), respectively. The experimental results revealed that the HAP nanoparticles synthesized in the presence of an optimized concentration of pectin are pure, low crystalline, spherical and discrete particles with reduced size. Also, the HAP sample derived in the presence of pectin showed an enhanced antibacterial activity than that of the HAP synthesized in the absence of pectin. Hence, the HAP nanoparticles synthesized using pectin as a green template can act as a good biomaterial for biomedical applications.

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Introduction

Hydroxyapatite has been widely used in various biomedical fields for several decades due to its chemical composition which is very close to that of human hard tissues, such as living bone and teeth [1,2]. It is the most attractive bioactive ceramic material that can directly bond with the natural bone. Because of its excellent biocompatibility, bioactivity and osteoconductivity, it has been widely used in dental implants, alveolar bridge augmentation, orthopedics, maxillofacial surgery, scaffold materials, and drug delivery agents [3,4]. Nowadays the HAP particles with nanoscale level have great impact since their small size and large specific surface area enable a homogenous resorption by osteoclasts [5]. The HAP particles were used in the application of bone repair, replacement of damaged or traumatized bone tissues [6], retard the multiplication of cancer cells [7] and also as an efficient drug delivery agents [8].

Nanoparticles with specific sizes and morphologies can be readily synthesized by a variety of methods such as co-precipitation [9], hydrothermal [10], sol–gel [11–13], freezing [14,15], ultrasonic irradiation [16], reverse microemulsion [17] and template assisted synthesis [18,19]. However, these methods employed organic solvents or organic templating agents which are potentially dangerous to the environment and biological systems [20]. Therefore, in recent years many researchers have tried to synthesize nanoparticles with the naturally abundant green materials, since they are renewable, cost-effective and environmentally benign when compared to other synthetic organic templates [21–25]. These natural biological materials contain carbohydrates, proteins, fibers and polysaccharides in significant amounts.

Among the various naturally available resources, agricultural wastes are renewable resources which have the potential applications in biotechnological aspect because they are non-toxic, abundant, easily available, totally regenerable, non-exotic, cheap and able to support the rapid growth [26]. The peels of the banana fruit is one of the agro-wastes which are left without proper disposal mechanism. Banana peels are classical examples of naturally available agricultural waste. Generally banana pulp is consumed and the peels are mostly dumped as solid waste. The potential applications of banana peels depend on its chemical composition. It is mainly composed of natural biopolymers in plant cell walls such as cellulose, hemicelluloses, pectin, lignin and proteins which could be used in the synthesis of nanoparticles [27–29]. Among them, pectin is a gifted, eco-friendly, biocompatible as well as biodegradable polysaccharide material and it is remarkably used as antimicrobial, anticoagulant, anti-inflammatory agent. It is also used as wound healing substance to tissue sites and as a composite material to alter the mechanical properties [30] and the degree of swelling [31,32]. Pectin also has an extensive application in the field of surface modification of medical devices [33]. Since pectin improves the proliferation of osteoblast, it has been recently attracting much attention as a novel biomaterial [34,35]. In extracellular mineralization, such polysaccharides generally rich in carboxyl and hydroxyl groups can promote the binding of calcium ions (Ca^{2+}) from the solution to carboxylate ions. This initiates the crystal nucleation and growth [36] and hence helps in bone fixation thereby promoting bone regeneration [37–39]. Very recently, Gopi et al. [40,41] successfully synthesized HAP nanoparticles using sucrose and tartaric acid derived from the natural sources as green templates.

As far as our knowledge is concerned, there are no reports on the synthesis of HAP nanoparticles using naturally occurring banana peel pectin. In the present work, we have explored a facile and green route to synthesize HAP nanoparticles using a non-toxic, renewable natural banana peel mediated pectin as template. The influence of pectin concentration on the size, morphology, purity and crystallinity of the as-synthesized HAP nanoparticles were studied elaborately using various analytical techniques. The antibacterial activity of the as-synthesized HAP nanoparticles was also investigated.

Experimental methods

Materials

Calcium chloride dihydrate ($CaCl_2{\cdot}2H_2O$), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$), ethanol, toluene and aqueous ammonia were purchased from sigma Aldrich. All the chemicals were of analytical-grade and used without further purification.

Preparation of plant material

Bananas (*Musa paradisiaca*) were purchased from the local market of Tamilnadu, India. The fruits were washed with double distilled water, rinsed with acetone and separated into pulps and peels. The peels were cut into pieces using kitchen knife and kept in oven at 60 °C for 24 h. Then it was ground into fine powder by using laboratory grinder and stored in refrigerator.

Extraction of pectin from the banana peel powder

Preparation of cell wall material (CWM)

Ground rinds were heated with 1:2 ratio of toluene-ethanol for 5 h and filtered using vacuum pump. The residue was extensively washed with 60% of aqueous ethanol to remove impurities, pigments, and free sugars until the filtrate was colorless. Then it was dried by solvent exchange with 95% ethanol and acetone then finally, dried in an oven at 40 °C for 24 h. The dried CWM were packed in a sealed polyethylene bag and stored under airless conditions at room temperature.

Fractional extraction of pectin from CWM

The dried CWM was suspended in double distilled water (solid–liquid ratio 1:25, w/v), then the suspension was stirred at 60 °C for 4 h. The resultant slurry was cooled to room temperature and filtered through Whatmann No.1 filter paper. The residue was resuspended in double distilled water (solid–liquid ratio 1:25, w/v), extracted and managed as before. The supernatant from the second extraction was added to the first extract and precipitated with four volumes of ethanol to precipitate the water soluble pectin. The obtained precipitate was filtered and dried at 40 °C and used for further experiments.

Synthesis of HAP nanoparticles

In a typical HAP synthesis process, $CaCl_2{\cdot}2H_2O$ and $(NH_4)_2HPO_4$ were taken as precursors for Ca and P with a molar ratio of 1.67 and dissolved in double distilled water to form 0.05 M and 0.03 M solution, respectively. The general procedure is as follows: 0.01 wt.% of pectin was dissolved in 50 ml of double distilled water and heated to 60 °C. Then 0.05 M $CaCl_2{\cdot}2H_2O$ was added to the pectin solution and stirred for 1 h to ensure the cooperative interaction and self-assembly process. Subsequently, 0.03 M $(NH_4)_2HPO_4$ solution was added drop wise into the above mixed solution under continuous and vigorous magnetic stirring for 3 h and thus yielded a dirty white suspension. The pH of the above suspension was maintained at 9 by using aqueous ammonia solution and then