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Research paper

Cation doped hydroxyapatite nanoparticles enhance strontium adsorption from aqueous system: A comparative study with and without calcination

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ABSTRACT

The present study reports the synthesis of a biocompatible, eco-friendly, anisotropic cation doped hydroxyapatite nanoparticles (nHAp) for strontium removal from aqueous environment. The nHAp was modified by cation doping, characterized using suitable techniques like Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM) and, high resolution transmission electron microscopy (HRTEM) attached with selected area electron diffraction (SAED) and energy dispersive X-ray spectroscopy (EDAX). nHAp based materials were investigated for its strontium adsorption property with and without calcination. Successful doping of the cations into the nHAp matrix was confirmed by X-ray photoelectron spectroscopy (XPS). The morphology and the particle size of the nHAp varied significantly with cation doping (Na, Mg and Al) and calcination. Calcination of nHAp decreased the dissolution rate when compared to uncalcined nHAp. The biocompatibility and toxicity studies of modified nHAp with human osteoblast (MG63) cell line indicated that the cation doping onto nHAp had considerable impact on its toxicity. In the initial screening studies, Al-nHAp and Mg-nHAp showed higher strontium adsorption percentage of 83.90 ± 3.03 and 70.98 ± 2.74 respectively. The adsorption capacity of the materials was much superior to many of the HAp based materials reported earlier. These studies clearly indicate that the cation doped, degradable (for efficient disposal of adsorbate saturated HAp beyond reuse), eco-friendly nHAp is suitable for removal of strontium from contaminated water.

1. Introduction

Hydroxyapatite (HAp) is a well-known ion exchanger material found naturally as inorganic constituent in human bones and teeth. They are widely used as implants for hard tissue and carriers for genes, enzymes and proteins (Yang and Zhang, 2009; Xu et al., 2009). These groups of ion exchangers have high biocompatibility and are bioactive (Mhammedi et al., 2009). HAp interact with various anions and cations through adsorption/ion exchange process (Lusvardi et al., 2002; Smiciklas et al., 2006; Sandrine et al., 2007) and hence is widely investigated for contaminant abatement.

Strontium contamination in groundwater and other water sources has been reported in various countries like USA, Jordan and Japan (Usuda et al., 2006; Casas et al., 2003). United States-Environmental Protection Agency (US-EPA) has recommended a permissible limit of 4.0 mg L^{-1} of strontium in drinking water. In India, strontium contamination in water has been reported in areas like Cuddapah, Nellore, Prakasam and Srikakulam districts in Andra Pradesh and in Hoshiarpur and Nawanshahr districts of Punjab state. Maximum concentration of 6165 μg L^{-1} was observed in Bathinda district, Punjab (Bhalla et al., 2011). Such contamination of drinking water sources may potentially lead to rise in many diseases including neurological disorders starting from learning disabilities to severe mental retardation (Hollriegl and Munchen, 2011). Strontium interacts with HAp mainly through adsorption, substitution/ion exchange or through precipitation process (Sternitzke et al., 2012). Although there are superior adsorbents such as novel carbon nanomaterial (Shawabkeh et al., 2002), TiO_2–SiO_2 mixed gel spheres (Gurboga and Tel, 2005) and silica impregnated trihexyl tetradecylphosphonium chloride (Cyphos IL-101) (Negrea et al., 2014) reported for strontium removal, higher strontium adsorption capacity along with superior biocompatibility and degradability (for efficient disposal of adsorbate saturated HAp beyond reuse) makes HAp (in bulk and nano-forms), a material of choice. HAp can be synthesized using various methods like, reverse microemulsion, hydrothermal,

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microwave-hydrothermal, solid-state reaction, sol-gel method and precipitation (Ferraz et al., 2004). Among all these methods, precipitation process has been reported to be favourable due to the ease in synthesis, cost effectiveness and eco-friendly nature (Saeri et al., 2003). The non-intermediate formation, low temperature of crystallization and possibility of ionic substitution/doping along with high yield, make the precipitation process more applicable for synthesis of various types of HAp in bulk and nanoform (Moghimian et al., 2012).

Recently, the possibility of synthesizing cation incorporated HAp has opened up a new arena for its application in various fields like biomedical, dentistry and purification systems. This cation substitution or doping in HAp can occur with calcium ions, phosphate groups or with hydroxyl groups (Tang et al., 2009). Such modifications impart new functionalities to HAp materials. Coating of Ce^{2+}/Eu^{3+} substituted HAp materials onto surgical grade stainless steel has been found to improve the antimicrobial and bioactivity (Gopi et al., 2014b). Surface modification of HAp using Zn^{2+}, In^{3+}/Bi^{3+} has increased the biological activity of HAp by promoting osteoblast adhesion and differentiation (Webster et al., 2004). Copper substituted HAp has been reported to increase the uranium adsorption onto the surface (Liu et al., 2010). All these studies clearly indicate the positive influence of cation modification in HAp towards a particular functionality. Such functionally modified HAp has been reported for its application in adsorption studies. Nie et al. (2012) has reported the synthesis of aluminium doped HAp nanoparticles for the defluorination studies. Ziani et al. (2014) reported the synthesis of magnesium doped HAp nanoparticles, synthesized via sol-gel technique. Similarly the silicon doped HAp nanoparticles were synthesized and used for cell culture studies (Marques da Silva et al., 2010). Bogya et al. (2009) used silicon doped HAp for copper adsorption studies. Despite these reports, there are very few studies which directly correlate doping of cation with varying ionic radii into HAp and its influence on strontium adsorption capacity. In the present study, we report the synthesis of modified HAp nanoparticle by wet chemical precipitation method. Its impact after the incorporation of cation such as sodium, magnesium and aluminium (selection based on ionic radii and reactivity towards strontium) on structure and morphology of nHAp were also studied. The influence of cation doping on crystallinity, elemental composition and phase purity of the materials were investigated using suitable techniques. Further the strontium adsorption capacity of the Na, Mg and Al doped nHAp were studied in the initial screening process. To our knowledge, this is the first study to report the influence of cation doping on strontium adsorption capacity of nHAp.

2. Experimental

2.1. Materials

All the chemicals were of analytical grade. Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) (purity > 99%), ammonium dihydrogen phosphate ($(NH_4)H_2PO_4$) (purity > 99%), sodium nitrate ($NaNO_3$) (purity > 99%), magnesium chloride ($MgCl_2 \cdot 6H_2O$) (purity > 99%), aluminium nitrate ($Al(NO_3)_2 \cdot 9H_2O$) (purity > 99%), and sodium fluoride (NaF) (purity > 99%), were procured from Merck, India. Strontium nitrate ($Sr(NO_3)_2$) was used to prepare standard strontium solution. Oxalic acid and ethylene diamine used for eluent preparation were procured from Sigma Aldrich. All chemicals were used without further purification. Ultrapure water was used throughout the experiment.

2.2. Synthesis of native and cation doped HAp

Hydroxyapatite nanoparticles (nHAp) were prepared by precipitation method (Moghimian et al., 2012). In brief, 0.4 M solution of ammonium dihydrogen phosphate (maintained at a pH > 9 by adding 1 M of sodium hydroxide solution), was added under constant stirring into 0.6 M solution of calcium nitrate. The suspension was refluxed for 2 h. The white precipitate formed after 2 h of incubation was filtered, washed with copious amount of ultra-pure water and dried overnight at 50 °C. The dried precipitates were considered as uncalcined HAp (nHAp) and further calcined in air at 800 °C for 2 h to obtain calcined HAp (C-nHAp).

Cation doped nHAp's were prepared as per the following procedure. Three different cations viz, sodium, magnesium and aluminium were used for doping HAp. The cation selection was based on atomic radii and its ability to form ionic bonds with strontium. The molar ratio of cations doped into HAp was maintained as 1.67. Respective salts (Na: $NaNO_3$; Mg: $MgCl_2 \cdot 6H_2O$ and Al: $Al(NO_3)_2$) at 0.2 M ratio was incorporated into 0.6 M solution of calcium nitrate individually. The mixtures containing both the solutions were stirred for 1 h. The solution was then added drop wise into 0.4 M solution of ammonium dihydrogen phosphate. The reaction was performed at pH > 9 (as mentioned above) with constant stirring for 2 h. The precipitate was filtered and washed with copious amount of ultra-pure water; dried overnight at 50 °C to obtain uncalcined cation doped HAp. The dry powders were further calcined in air at 800 °C for 2 h to obtain calcined cation doped nHAp.

2.3. Characterization studies

The synthesized nHAp materials were characterized using multiple techniques. The surface functional groups were analyzed using Fourier transform infrared spectroscopy (FTIR) (Nicolet Avathar-320 FTIR spectrometer, Madison) at a scan range of 400–4000 cm^{-1}. KBr was used along with milligrams of synthesized nano-material (100:1) to make pellets. The pellets were analyzed at a scanning speed of 2 mm s^{-1} at a resolution of 4 cm^{-1}. The crystal structure of nHAp and their phases were analyzed using X-ray diffractometer (XRD-600, Shimadzu, Japan) with generator settings of 30 mA, 40 kV; step size 0.001 (2 theta) with scan step time of 3.2 s in continuous mode. The morphology of the synthesized materials were analyzed using field emission scanning electron microscopy (FESEM) (Sigma, Carl Zeiss, Germany) and high resolution transmission electron microscopy (HRTEM) (JEOL-JEM 2100, Japan) at an operating voltage of 4 to 20 kV and 200 kV respectively. The selected area electron diffraction (SAED) and energy dispersive X-ray spectroscopy (EDAX) of the HAp nanoparticles were also performed. The chemical composition of the HAp was analyzed using X-ray photoelectron spectroscopy (XPS) (SPECS SAGE) with a Phoibos 150 hemispherical analyzer and an MCD-9 detector at a take-off angle of 90°. The analysis area was circular with a diameter of 3 mm. Mg Kα ($h\nu = 1253.6$ eV), was used as X-ray source operated at 10 kV and 20 mA (200 W) with a background pressure of 1.5×10^{-5} mTorr. The binding energies were referenced to the C 1s neutral carbon peak at 285 eV. Pass energy of 20 to 100 eV and energy steps of 0.1 to 0.5 eV were used to obtain the spectra. Spectra were analyzed using CASAXPS software (Neil Fairley, U.K.). Dissolution of synthesized material was investigated as per Singh (2012). 0.2 g of HAp based material was taken and soaked in 50 mL of 0.05 M tris-HCl buffer solution (pH 7.4) for 12 days. The samples were filtered and dried at 100 °C. The % weight loss of sample was calculated by the formula (Eq. (1)) as given below:

$$\%\text{Weight loss} = W1 - W2/W1 * 100 \quad (1)$$

where, W1 is the initial weight of sample; W2 is the final weight of sample after soaking in tris-HCl solution. The toxicity of the synthesized HAp materials was estimated using 3-(4, 5-dimethyl thiazolyl-2)-2,5-diphenyl tetrazolium bromide (MTT) assay against MG-63 human osteoblast cell lines. A known concentration (1–3 mg mL^{-1}) of the sterilized nHAp material was prepared in PBS buffer at pH 7.4 and sterilized by autoclaving at 121 °C. MG-63 human osteoblast cell lines were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and incubated in CO_2 incubator (Eppendorf, Germany) till complete confluency was achieved. The cells after reaching confluency (100%) were trypsinized and centrifuged at

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