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Synthesis of magnetic hydroxyapatite by hydrothermal–microwave technique: Dielectric, protein adsorption, blood compatibility and drug release studies

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Abstract

The hydroxyapatite (HAp) nanoparticles with some magnetic ions (cobalt (Co), iron (Fe), nickel (Ni) etc) exhibit strong ferromagnetic properties, which play an important role in the targeted drug delivery and magnetic hyperthermia treatment of the tumours. Cobalt (Co^{2+}) doped HAp (Co–HAp) was synthesized using a combinatorial methods of hydrothermal and microwave irradiation. The crystallinity was found to reduce with an increase in Co^{2+} incorporation. Pristine samples consisting of monodispersed spheres of size 121 nm were diamagnetic. The incorporation of Co^{2+} (44 ppm) leads to a significant reduction in size (55%) and change in morphology (spherical to hexagonal rods). The presence of 139 ppm of Co^{2+}, lead to the formation of a super paramagnetic porous structure consisting of hexagonal nanorods and nanospheres. There was a drastic increase in the magnetization (1.2×10^{-2} to 6.1×10^{-2} emug^{-1}) and dielectric constant (1–2476) on incorporation of cobalt. The Co–HAp exhibited better protein adsorption due to the increase in positive surface potential on addition of Co^{2+}, as confimed by the zeta potential analysis. Further, Co^{2+} ions incorporated HAp exhibited enhanced haemocompatibility and sustained release of an anticancer drug (5-fluorouracil). This cobalt ion doped HAp could be a promising material for magnetic imaging, drug delivery and hyperthermia treatment.

Keywords: Hydroxyapatite; Hyperthermia; Superparamagnetism; Haemocompatibility; 5-fluorouracil

1. Introduction

The magnetic nanoparticles play a major role in biological applications such as cell separation [1], magnetically targeted drug delivery [2], magnetic resonance imaging (MRI) [3] and as heat mediator for the hyperthermia treatment of cancers [4,5]. Osteosarcoma is one of the bone tumours which can be remedied by using magnetic nanoparticles [6]. HAp has been widely used for bone and dental reparation and in drug delivery system. HAp exhibits high osteoconductivity and osteoinduction when implanted into the human body [7,8]. The main drawback of this material is the lack of mechanical properties which lead to implant failures. In order to improve the mechanical properties of HAp, the sublattices are replaced with various ions [9]. HAp has been shown to accelerate the healing of bone fractures upon electrical stimulation [10,11]. Cobalt containing vitamin B_{12} plays a vital role in the formation of red blood cells [12,13].

In the HAp structure, divalent cation such as calcium ion (Ca^{2+}) may be substituted by metallic ions Zn^{2+}, Fe^{2+}, Cu^{2+}, Mg^{2+}, Ni^{2+}, Cr^{2+}, Mn^{2+}, Co^{2+}, Sr^{2+}, Pb^{2+} and Cd^{2+}, and the anions (phosphate $(PO_4)^{3-}$ and hydroxyl $(OH)^{-}$) could be replaced by F^{-}, Cl^{-}, B^{-}, CO_3^{2-} and VO_4^{3-} [14]. Baikie et al. [15] reported a variation in the unit cell parameters of the apatite

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structure by the incorporation of 3d metal ions such as Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}. It has been shown that upto 30 mass% of iron oxide (Fe_3O_4)–HAp could be used for the treatment of bone cancer [16]. Ajeesh et al. [17] had found that HAp containing 40 wt% of iron oxide-HAp is not detrimental to the osteoblast cell activity and has X-ray opacity compared to the lower percentage of iron oxide-HAp. Cr^{3+}doped HAp could also be used in fluorescent probes for biomedical applications [18]. Co–HAp synthesized by hydrothermal technique exhibited low crystallinity and change in morphological (rod-like to lamellae) structure; in addition, to a reduction in '*c*' cell parameters, faster than '*a*' cell parameters [19]. The Co^{2+} containing HAp could be used to catalytically remove oxygenated volatile organic compounds such as methanol [20].

Our group has already reported that La^{3+}doped HAp had enhanced crystallite size, hardness, high resistance to Gram positive and Gram negative bacteria, along with a sustained drug release property [9]. Sr^{3+}-doped HAp possessed increased surface area, better bioactivity and exhibited prolonged drug release [21]. The Fe^{3+}incorporation decreased the particle size with a morphological change from spherical to nanorods and exhibited superparamagnetic property [22].

Techniques such as sol–gel [23], mechano-chemical [24], wet precipitate [25], gel [26], flux [27], hydrothermal [28] and microwave irradiation [29] were used to synthesize HAp. Among these, hydrothermal method has better control over particle size and shape [30]. Similarly, synthesis of HAp by the microwave irradiation method is a convenient, rapid and efficient method for the preparation of nanostructured HAp [31]. Microwave irradiation and hydrothermal techniques were applied together by Han et al. [32] to synthesize needles and spherulites of HAp.

Here, we report the rapid synthesis of HAp and Co^{2+} doped HAp (Co–HAp) by hydrothermal and microwave irradiation treatment. Synthesized samples were characterized to study its physico-chemical properties and in vitro biological performance.

2. Experimental procedure

2.1. Preparation of pristine and Co–HAp

The pristine and Co–HAp powders were synthesized using calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, Merck), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$, Merck), cobalt (II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$, Merck) and ammonia solution (analar grade). 1.0 M of $Ca(NO_3)_2 \cdot 4H_2O$ solution was prepared along with the various concentration of $CoCl_2 \cdot 6H_2O$ (0.01 M, 0.05 M, 0.1 M and 0.2 M). This solution was added drop wise into 0.6 M of $(NH_4)_2HPO_4$ solution with vigorous stirring for 2 h at room temperature. During the reaction, the pH of the solution was maintained at 10 using ammonia solution. After mixing, the precipitated solution was subjected to hydrothermal treatment at 150 °C for 3 h (pressure developed inside the Teflon lined chamber was 120 psi). After the hydrothermal treatment, the precipitated solution was further subjected to microwave irradiation (house-hold microwave oven – 900 W and 2.45 GHz) for about 30 min. Then the samples were washed with deionized water and dried at 70 °C in a hot air oven. The pristine sample was prepared by the same experimental procedure without $CoCl_2 \cdot 6H_2O$. Hereafter, pristine and 0.01 M, 0.05 M, 0.1 M and 0.2 M Co doped HAp samples were labelled as 0Co, 0.01Co, 0.05Co, 0.1Co and 0.2Co respectively. The powder samples were prepared as pellets of 8 mm diameter and 1 mm thickness by applying a pressure of 2 tons using hydraulic press for physico-chemical and biological analysis.

2.2. Structural and elemental analysis

Powder XRD analysis was carried out on pristine and Co–HAp samples (Co–HAp) using Rigaku Ultima(III) at a scan speed 0.5°/min and step rate 0.02 per second with CuKα radiation ($\lambda = 1.5406$ Å). The two theta range was carried out between 20 and 55°. The crystallinity (X_c) was determined by an empirical relation between X_c and $\beta_{(002)}$ i.e. $\beta_{(002)} \times 3\sqrt{X_c} = K_A$ where, $\beta_{(002)}$ is FWHM of (002) in degree and K_A is a constant (0.24) [33]. The XRD powder patterns of all the specimens were obtained using silicon powder as an internal standard for the refinement of lattice parameters. The lattice parameters were determined by XRDA software [34]. Fourier transform infrared (FT-IR) spectroscopy was used to identify the functional groups of the samples using PERKIN ELMER Spectrum RX1 by KBr pellet technique. The spectra were recorded from 4000 to 400 cm^{-1} in transmission mode. The Ca, P and Co content was determined by Inductively Coupled Plasma – Optical Emission Spectrometer (ICP-OES) using an Optima 5300 DV (Perkin-Elmer) instrument. The samples of about 0.1 g were dissolved in 1 mL of concentrated HCl, and then made up to 50 mL of triple distilled water and analysed.

2.3. Surface analysis

The surface topologies of the samples were visualized under a Field Emission Scanning Electron Microscope (FE-SEM) equipped with energy dispersive X-ray (EDAX) analysis (Hitachi, SU6600) at an operating voltage of 15.0 kV. The samples were ultrasonically dispersed in acetone for 5 min. A small drop of supernatant solution was dispersed and dried on a titanium substrate for FESEM analysis. The size and shape of pristine and Co–HAp were analysed under Transmission Electron Microscopy (TEM) (Philips-CM200, operating voltage of 200 kV). A small drop from the supernatant was dispersed and dried on carbon coated copper grids for TEM analysis. The particle size was measured using Image J software.

2.4. Dynamic light scattering (DLS)

Particle size and zeta potential of the samples were determined by Zetasizer nano-ZS (Malvern Instruments, UK). A He–Ne diode laser (633 nm) as the source was scattered at a fixed angle of 90° at 27 °C. The powdered samples of 1 mg in 100 mL were suspended in triply distilled water followed by ultrasonic treatment for 1 h. The sonicated