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Controlling of dielectric parameters of insulating hydroxyapatite by simulated body fluid

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ABSTRACT

Hydroxyapatite (HAp) samples were synthesized under various amounts of citric acid using the sol–gel method. Before and after immersion in simulated body fluid (SBF) for 14 and 28 days, the structural properties of HAp samples were analyzed by X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy with energy dispersive X-ray (EDX) spectroscopy and dielectric measurements. The crystallite size (D) was found to be in the range of 25.17–33.06 nm with the crystallinity percent (X_c%) of 69.53–86.09. The lattice parameters of a and c were calculated to be in the ranges of 9.373–9.434 Å and 6.828–6.896 Å, respectively. The morphology of the as-synthesized samples was changed with the amount of citric acid and soaking period in SBF. The Ca/P molar ratios indicated a decrease with increasing immersion time, and Ca-deficiency was observed. The relative permittivity (ε') and dielectric loss (ε'') were significantly affected by citric acid content and soaking period in SBF. It was seen that the alternating current conductivity (σ_{ac}) increased with increasing frequency and the σ_{ac} values changed with increasing soaking period and amount of citric acid.

1. Introduction

It is well-known that hydroxyapatite (HAp) with chemical formula $Ca_{10}(PO_4)_6(OH)_2$ is the major inorganic component of the hard tissues (e.g., bone and teeth) of human and vertebrate animals [1,2]. The molar ratio of calcium to phosphorus (Ca/P) for an ideal HAp is equal to a value of 1.67 and HAp can be produced by several techniques such as sol–gel method, chemical precipitation, spray pyrolysis, solid-state reactions, mechanochemical route, microwave synthesis, microemulsion and combustion synthesis under laboratory conditions [3–13]. Among these techniques, the sol–gel method is very suitable to prepare nano-HAp having high purity and crystallinity [14–16]. HAp is an insoluble compound in physiological pH and temperature [17]. Additionally, HAp has non-toxic, non-allergenic, osteoconductive, non-mutagenic and endophilicity properties, and has been extensively used in medical and biological applications as a material for bone-replacement and carrier for growth factors, anticancer drugs and antibiotics [18–21].

Citric acid ($C_6H_8O_7$) is a weak organic acid and inhibits the formation of calcium phosphate. The importance of citric acid in metabolisms of bones has been known for many years [22,23]. In addition to its use as a nucleating agent, the strong chelating ability of citric acid with the calcium ion is reported [24,25].

The simulated body fluid (SBF) applied for the first time by Kokubo et al. [26] is an in vitro test method and has been used for the determination of in vivo bone bioactivity and observation of apatite formation on the surfaces of different type biomaterials. The inorganic ion concentration of SBF is almost equal to human blood plasma ion concentration [26,27].

The dielectric properties have a great importance on the use of the bioceramics for bone repairing applications. One of the most desired properties of a biomaterial is its relative permittivity, which is so close to that of bone. Unfortunately, in the literature, there are only few articles related to the dielectric relaxation properties of hydroxyapatites in detail. In this regard, it is expected that the present work may be contributed to the literature.

We have evaluated that the order of interaction of hydroxyapatite (HAp) with SBF is important for biomedical applications. With this aim, we prepared HAp with various citric acid contents to control the crystallite size of the HAp. Hydroxyapatite (HAp) samples were synthesized under various amounts of citric acid using the sol–gel method. The structural properties of HAp samples were analyzed by X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, scanning electron microscopy with energy dispersive X-ray (EDX) spectroscopy and dielectric measurements before and after immersing in simulated body fluid (SBF) for 14 and 28 days.

2. Materials and method

The synthesis of the citric acid assisted hydroxyapatites was carried out using the sol–gel technique. Calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$, CN), diammonium hydrogen phosphate ($(NH_4)_2HPO_4$,

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DAP), citric acid ($C_6H_8O_7$) and nitric acid (HNO_3) purchased from Sigma-Aldrich were used as starting chemicals, and distilled water was used as the solvent. A solution of 0.5 M CN (100 ml) was added drop-wise to 0.3 M DAP (100 ml) solution, and a new opaque mixture was obtained. Four of the same solution were prepared with different amounts of 0.5 M citric acid solution (e.g., 0, 2, 4 and 6 ml) and were added to these mixtures. The new mixtures were named as CHA0, CHA2, CHA4 and CHA6, respectively. The pH of each mixture was adjusted to the value of 2 with HNO_3 solution. These were stirred using a magnetic stirrer at 85 °C for 5 h, and dried in an oven at 160 °C for 16 h. Then the dried samples were heated in an electrical furnace at 900 °C for 1.5 h, and the hydroxyapatite samples with white color were obtained.

In vitro tests of the samples were performed in simulated body fluid (SBF) prepared according to the earlier report by Kokubo [28]. All the samples were converted into disks of 13 mm diameter and 2 mm thickness under pressure of 10 MPa, and placed in polyethylene bottles. Then, the bottles were filled with SBF and sealed with caps. The samples in the bottles were placed into an oven at the body temperature (37 °C) for 14 and 28 days. After the treatment, all the samples were filtered and washed three times by ultra-pure water to remove the residual ions.

X-ray diffraction (XRD) patterns of the as-synthesized samples were recorded using a Bruker D8 Advance diffractometer operated at 40 kV and 40 mA within the 2θ range of 20–55°. Fourier transform infrared (FTIR) spectra were collected by a PerkinElmer Spectrum One spectrometer in the spectral range of 450–4000 cm^{-1} using the KBr pellet method. The investigation of the morphologies and elemental compositions of the as-synthesized hydroxyapatites was carried out by a JEOL JSM 7001F scanning electron microscope (SEM) equipped with an energy dispersive X-ray (EDX, Oxford Instruments INCA energy 350) spectrometer. The dielectric properties were investigated by a HIOKI 3532-50 LCR meter at room temperature.

3. Results and discussion

The XRD patterns of the as-synthesized hydroxyapatites before and after immersing in SBF for 14 and 28 days are shown in Fig. 1. All the observed peaks on the XRD patterns indicate pure hydroxyapatite phase (JCPDS card No: 09-0432) having hexagonal crystal structure because there is no peak belonging to the secondary phases such as β-TCP (tricalcium phosphate) and CaO. This indicates that the purity of the hydroxyapatite phase for all the samples is 100%. Furthermore, the formation of any new phase was not detected after immersing in SBF for 14 and 28 days for all the samples.

The average crystallite size (D) is calculated using the well-known Scherrer equation [29]. The crystallinity percent (X_C%) is calculated using the following relation reported by Landi et al. [30]

$$X_C\% = \left(1 - \frac{V_{112/300}}{I_{300}}\right) \times 100 \quad (1)$$

where $V_{112/300}$ is the intensity of the hollow between (112) and (300) planes, and I_{300} is the intensity of the peak belonging to (300) plane. To determine these values, the XPowder software was used [31]. The lattice parameters of a and c were calculated from peaks belonging to (300) and (002) planes, respectively [29]:

$$\frac{1}{d^2} = \frac{4}{3}\left(\frac{h^2 + hk + k^2}{a^2}\right) + \frac{l^2}{c^2} \quad (2)$$

where d is the distance for two adjacent planes and h, k and l are the Miller indices. The volume (V) of unit cell was computed using the obtained unit cell parameters [29]. The calculated values of the above-mentioned parameters (e.g., D, X_C%, a, c and V) for each sample before and after soaking in SBF are given in Table 1. The plots of these parameters as a function of the amount of citric acid are also shown in Fig. 2. As

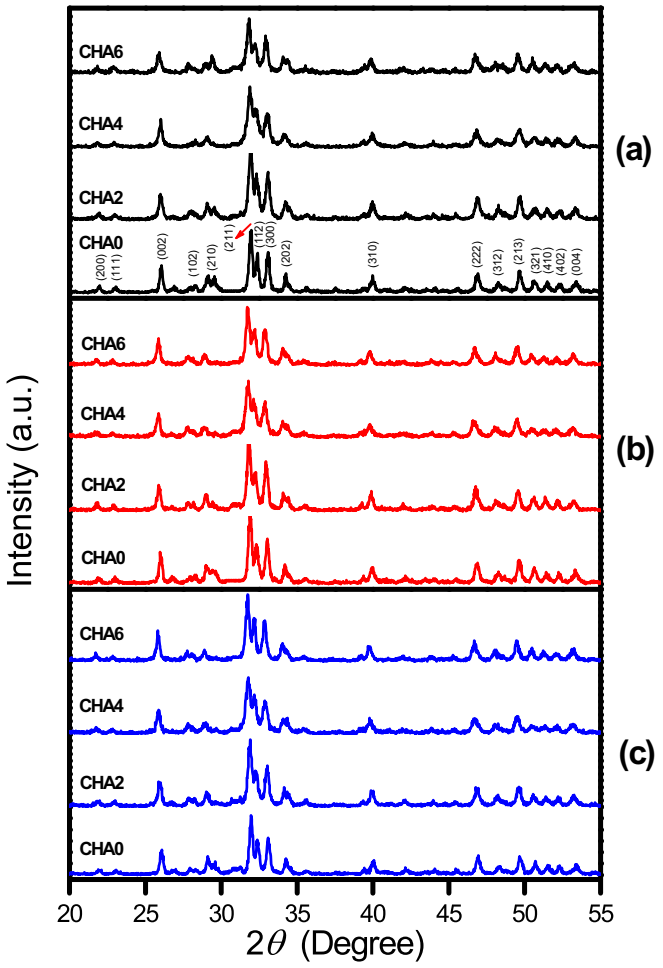

Fig. 1. XRD patterns of the citric acid-assisted hydroxyapatite samples: (a) before immersing in SBF, (b) after immersing in SBF for 14 days, and (c) after immersing in SBF for 28 days.

seen in Table 1 and Fig. 2, the crystallite size of D is changed with the addition of citric acid. The immersion period in SBF affects the crystallite size. Once the immersion period is increased, a decrease and then an increase are observed for the crystallite size for CHA2, CHA4 and CHA6, whereas the value of D is decreased gradually for CHA0. Before immersing in SBF, the crystallinity percent is decreased gradually with increasing citric acid content. After soaking in SBF for 14 and 28 days, the crystallinity percent is decreased for each sample, but it does not decrease gradually with increasing amount of citric acid. Not only the amount of citric acid but also the immersion period affects the lattice

Table 1
The calculated values of crystallite size (D), crystallinity percent (X_C%), lattice parameters (a and c) and volume of unit cell for all samples before and after soaking in SBF for 14 and 28 days.

	Sample	D (nm)	X_C%	a (Å)	c (Å)	V (Å^3)
Before SBF	CHA0	33.06	86.09	9.373	6.833	519.86
	CHA2	28.06	81.72	9.373	6.849	521.08
	CHA4	26.81	78.85	9.384	6.843	521.84
	CHA6	28.26	78.45	9.418	6.869	527.63
After SBF (14 days)	CHA0	28.61	85.12	9.384	6.854	522.68
	CHA2	28.27	81.67	9.412	6.885	528.18
	CHA4	25.17	70.31	9.429	6.880	529.71
	CHA6	25.97	75.86	9.423	6.880	529.04
After SBF (28 days)	CHA0	26.51	82.21	9.373	6.828	519.48
	CHA2	27.01	80.25	9.384	6.849	522.30
	CHA4	25.72	69.53	9.434	6.875	529.89
	CHA6	29.78	75.33	9.429	6.896	530.94