Radiation Physics and Chemistry 81 (2012) 118–125

Contents lists available at SciVerse ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Characterization of x-ray diffraction and electron spin resonance: Effects of sintering time and temperature on bovine hydroxyapatite

Eny Kusrini [a,*], Muhammad Sontang [b,**]

[a] Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, 16424 Depok, Indonesia
[b] Department of Chemical Sciences, Faculty of Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

ARTICLE INFO

Article history:
Received 12 April 2010
Accepted 3 October 2011
Available online 25 October 2011

Keywords:
Bovine hydroxyapatite
Electron spin resonance (ESR)
Free radicals
Rietveld analysis
Sintering

ABSTRACT

The physical and chemical properties of a hydroxyapatite produced by the sintering of bovine bone were investigated by powder x-ray diffraction (PXRD), electron spin resonance (ESR), energy dispersive x-ray spectroscopy (EDX), scanning electron microscopy (SEM), Fourier transform infrared (FTIR), and differential thermal analysis (DTA). A bovine bone powder was sintered at different temperatures ranging from 500 to 1400 °C. The influences of post-irradiation storage on the radiation ESR response of the bovine bone powder before and after sintering were also studied. The results indicate that the sintered bovine bone powder contained hydroxyapatite. Diffraction patterns were sharp and clear based on the (211), (300), and (202) reflections corresponding to bovine hydroxyapatite (BHA), which confirmed the phase purity and high crystalline grade of the BHA produced. The PXRD profile of BHA was dependent on sintering temperatures and times. The molecular formula of BHA was determined by Rietveld analysis showed a similar structure and composition to calcium hydroxyapatite in hexagonal $P6_3/m$ space group $a=b=9.435$ Å and $c=6.895$ Å. ESR data showed that the sintering process can decrease the number of free radicals in BHA; it also revealed that the number of free radicals is constant during long storage periods (75 days). The sintering technique described in this study may be used to extract hydroxyapatite from biowaste bovine bone, leading to its application as a bone filler.

1. Introduction

Bovine bone-derived hydroxyapatite is widely used in bone grafting for bone repair or replacement, and in reconstitution of dental tissues because of its excellent biocompatibility with hard tissues, bioactivity despite its low degradation rate, high osteoconductivity, non-toxicity, non-inflammatory behavior, and non-immunogenic properties (Irina et al., 2009; Zhuofan et al., 2009). BHA has neither antigenicity nor cytotoxicity (Burg et al., 2000). The morphological, structural, mechanical, and composition of hydroxyapatite (HA) with the chemical formula of $Ca_{10}(PO_4)_6(OH)_2$ is nearly identical to the mineral composition of human bone (Rogers and Daniels, 2007). Bone is a complex composite material with a mineral matrix commonly consisting of carbonated calcium hydroxyapatite (CHA) (Bigi et al., 1997), and hydroxyapatite has the ability to bind in the human bone. It is also capable of promoting bone regeneration and bonding directly to regenerated bone without intermediate connective tissue at a faster rate than other materials (Gao et al., 2006).

Xenogenous treatment is a current medical practice using bovine bones for the correction of human bone defects. In Indonesia, the bovine hydroxyapatite (BHA) is easy to obtain as a biowaste and is available in unlimited supply at a lower cost than autograft or allograft. Autograft and allograft are standard treatments for bone defects, but they are sometimes followed by problems such as donor site scarcity, rejection by recipient immune systems, resorption, and pathogen transfer (Gao et al., 2006). We noted that BHA can be isolated by powder processing (Irina et al., 2009), and variations in processing routes produce differences in morphology, crystallographic structures, density, and physical, chemical, and mechanical properties (Murry et al., 1995). Heating (annealing) and sintering processes to obtain HA from bovine bone have been extensively investigated (Juang and Hon, 1996; Gao et al., 2006; Ooi et al., 2007). The structural and chemical properties of BHA are very sensitive to sintering temperature, duration of sintering, and bovine feed material (Joschek et al., 2000; Herliansyah et al., 2009).

ESR is a very senstive method for detection of free radicals (Engin et al., 2006). Engin and Demirtas (2004) reported that thermal treatment induces the modification of the ESR signal and may lead to production of a new signal due to transformation of the organic part of an egg shell. Investigations regarding the formation of free radicals in hydroxyapatite derived from bovine

* Corresponding author. Tel.: +62 21 7863516x6207; fax: +62 21 7863515.
** Corresponding author. Tel.: +609 668 3332; fax: +609 669 4660.
E-mail addresses: ekusrini@che.ui.ac.id (E. Kusrini), msontang@umt.edu.my (M. Sontang).

0969-806X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.radphyschem.2011.10.006

bone in biowaste and the stability of hydroxyapatite have rarely been reported in conjunction with efforts to identify a suitable form of BHA for use as a bone substitute or filler. This study supports the use of ESR data to determine the quality of and the relative sensitivities of the ESR signal in BHA at various sintering times and temperatures.

The purpose of the present study is to investigate in detail the effects of sintering BHA at various temperatures ranging from 500 to 1400 °C, and at various sintering times on the FTIR, XRD, and ESR spectra. We expected that the results would provide a better understanding about ESR signal behavior and long or short-lived characteristics of free radicals produced in BHA before and after irradiation at sintering temperatures ranging from 1000 to 1400 °C for 2, 3 and 4 h in $g=2.00$ region. In this work, we also studied the influence of post-irradiation storage on the radiation ESR response of fresh bovine bone powder and sintered BHA. The effects of sintering at different temperatures ranging from 500 to 1400 °C on phase purity, as well as physicochemical and mechanical properties of BHA, were also investigated.

2. Experimental

2.1. Sample preparation

The bovine bone was obtained from a femur (age 2 years 9 months), which was procured from Darma Jaya (Jakarta, Indonesia). The bone samples were cleaned to remove visible tissues and substances on the bone's surface. The clean bovine bone samples were defatted in boiling water followed with sun drying to remove the organic substances and avoid soot formation in the material during the heating treatment. Dried bovine bone samples were cut into rectangular prism shapes, with each measuring $10\times10\times10$ mm^3. Bovine bone samples were sintered in vacuum by an electric Bohler furnace with an automatic digital and rotary vacuum pump; maximum pressure was 10^{-3} Torr. The sintering temperature was set in the range 500–1400 °C with heating rates of 5 °C/min for 2, 3, and 4 h. After sintering, the bovine bone pieces were cooled to room temperature in a slow furnace, followed by grinding into powder (60 mesh) using a mortar pestle.

2.2. Characterizations

Six different investigative techniques were used in this study: scanning electron microscopy (SEM), energy dispersive x-ray spectroscopy (EDX), differential thermal analysis (DTA), Fourier transform infrared (FTIR), powder x-ray diffraction (PXRD) analysis, and electron spin resonance (ESR) spectroscopy. The microstructure of the sintered bovine bone powder was examined using a SEM Philips XL 30 series. Chemical analysis was carried out using an EDX at 20 kV. Thermal analysis of bovine bone was conducted with DTA Harrop 716 at a heating rate of 10 °C/min. IR spectra were recorded on a Perkin-Elmer system 2000 FTIR spectrophotometer in the region of 4000–400 cm^{-1}; KBr pellets were utilized as solid samples.

A powder specimen was spread on a non-reflective quartz specimen holder with a well depth of 0.2 ram. XRD intensity patterns of the powder for Rietveld refinement were obtained from an automated XRD Shimadzu ZD 610 diffractometer with CuKα as the radiation source at a scan speed of 7°/min and a step scan of 0.05°. The crystalline phase compositions were identified based on the standard references of JCPDS data available in the system software (XRD JCPDS file no. 9-432, 1996). Crystallographic parameters of HA were determined based on the refinement of the XRD data by the Rietveld analysis and a computer program developed by Izumi (Izumi, 1989) and Rigaku Limited Company. The unit cell parameters were refined together with several non-structural parameters; a zero correction of the 2θ experimental scale; and the U, V, W, and m profile function parameters defining the full width at half maximum (FWHM). The values of the refined non-structural parameters and the unit cell parameters are shown in Table 1. The refined fractional atomic coordinates and the occupation for the resulting bovine HA upon sintering at 1000 °C for 3 h are summarized in Table 2.

The ESR measurements based on the detection of radio-induced radicals of irradiated samples also have the potential

Table 1
Summary of crystallographic data for bovine hydroxyapatite after sintering for 3 h at 1000 °C.

Recording conditions	Label column
Radiation	Cu Kα
Divergence aperture (°)	1
Receiving aperture (°)	0.2
Beam width (mm)	10
Angular range (°, 2θ)	20–60
Step width (°, 2θ)	0.02
Count time (s per step)	30
Temperature	Room
Crystal data	Label column
Molecular formula	$Ca_{10}(PO_4)_6(OH)_2$
System	Hexagonal
Space group	$P6_3/m$
$a=b$ (Å)	9.43505
c (Å)	6.89557
$\alpha=\beta$ (°)	90
γ (°)	120
Z	1
Rwp and Rp	6.53 and 5.06

Table 2
Refined structural parameters and generalized coordinates of bovine hydroxyapatite after sintering for 3 h at 1000 °C.

Atom	m	x	y	z	B (Å^2)
O (1)	6	0.33	0.47(7)	0.25	2.02
O (2)	6	0.59(9)	0.45(7)	0.25	1.84
O (3)	12	0.33(5)	0.25(5)	0.25	0.0
OH	2	0.00	0.00	0.00	1.0
P	6	0.40(4)	0.36(7)	0.06(5)	0.2
Ca (1)	4	0.3(3)	0.66(7)	0.25	0.6
Ca (2)	6	0.24(1)	0.00(5)	0.66(7)	1.2

Note: m=number of atom; x, y, z=structure parameters; B=magnetic induction.

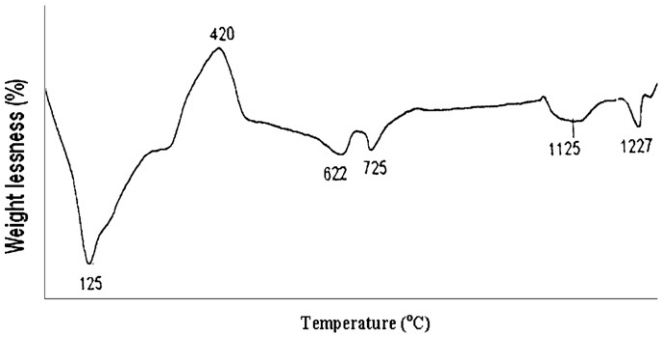

Fig. 1. Differential thermal analysis (DTA) pattern of the bovine bone powder at heating rate 10 °C/min. The endothermic peak indicates dissociation of bovine bone into the hydroxyapatite and organic matrices.