Surface and Physicochemical Properties of Calcium Phosphate from Bovine Bone

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Abstract

A prominent calcium phosphate, belonging to the apatite group is the hydroxyapatite, it has been used for many application, besides it has a lot of particular properties, between those are the sorption of metallic ions, then it can be used for the treatment of contaminated groundwater and soil decontamination, from the different methods of synthesis, the chipset is from bovine bone due to this is a waste. In this work is described the obtaining method of the calcium phosphate from bovine bone, the purification of this material and characterization of the calcium phosphate from X-ray diffraction, Infrared analysis, thermogravimetric and differential scanning calorimetric analysis, scanning electron micrography, and surface area by the BET method. The physicochemical characteristics are described from the hydroxyapatite from bovine bone, and preliminary results are shown in order to determine if the hydroxyapatite is ideal as a barrier reactive permeable in the treatment of metallic and radionuclides contaminants.

1. INTRODUCTION

Among the potential materials under investigations, phosphate compounds are expected to play an important role in the safety of underground radwaste repositories because they could be used for engineered barrier [1]. The management of radioactive wastes has become a major concern, particularly with regards of the release of radioactive materials to the environment. migration of uranium in water-rock systems is largely controlled by uranium solution-mineral equilibrium and sorption reactions [2]. A large number of sorption data for various radionuclides on minerals have been compiled [3]. The zirconium, thorium and iron phosphates have been studied in great detail

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[4]; however, very little attention has been paid to the sorption properties of other metal phosphates such as those calcium and aluminum. Calcium phosphate has been considered one of the most promising support materials.

One of the main characteristics of the calcium phosphate compounds is their great stability and their capacity to retain a large variety of elements, those was due to their particular structure that allows substitution in different points and diffusion phenomena, besides the complexation reaction with functional groups in the surface of the compound, and the formation of insoluble compounds via dissolution processes [5].

One of the most popular phosphate is an apatite named hydroxylapatite (HAp), its chemical structure is $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, the commun crystal phase is the hexagonal [6], but the monoclinic can be present too [7]. The HAp has a lot of applications for example in bone surgery, due to the biocompatibility properties in the body [8]; in chromatography to separate and purify proteins [9]; in the solid state ionics [10]; catalysis [11]; drug delivery systems [12]; fuel cells [13]; and chemical gas sensor [14]. The HAp in laboratory can be synthesized by different methods as; sol-gel process [15]; hydrothermal [16]; microwave synthesis [17]; ultrasonic spray pyrolysis [18]; wet precipitation [19]; emulsion system [20]; and sonochemical synthesis [21]. The HAp has been found in bone and teeth, it has several impurities but the main impurities are the carbonates (among 5-6% of dry weight) [22]. Between 60-70% of the total weight of the bone is HAp, In the case of bovine bone is a waste product because the bone is not use for human consume, then this material could be used for obtaining HAp, thus the aim this work is to evaluate the chemical and surface properties of calcium phosphate toward use it like prominent reactive material to sorbs metals ions from aqueous solutions.

2.EXPERIMENTAL

2.1. Bovine Hydroxyapatite Preparation

A bovine femur was got from a slaughterhouse, afterwards this was cleaned, cut, milled, and washed with deionized water and hydrogen peroxide in water. Once the fat was removed the bone particles were annealed at 900°C during 24 hours.

2.2. Initial pH Determination from Bovine Hydroxyapatite in Deionized Water

An experiment was carried out in order to determine the initial pH in deionized water, in base of previous results about the carbonate content in hydroxyapatite from vertebrate source [22], then the annealed bone particles were centrifuged 3 minutes with deionized water, afterwards the pH from the original deionized water and the residual from bone washed were taken.

2.3. Purification of Bovine Hydroxyapatite

The annealed hydroxyapatite was washed with 2,000 mL of deionized water until the pH of the remaining solution was similar to the pH of the deionized water.

2.4. Physicochemical and Surface Properties Characterization of Samples
2.4.1. Scanning Electron Microscopy, SEM
The bovine bone samples were placed on a holder of aluminium, adhered with a conductive tape of carbon, later the samples were covered with a golden cap of approximately 200 Å of thickness. For this purpose, an equipment of sputtering mark Dentrum Vacuum model Desk II was used and the samples were analyzed in a scanning electronic microscope PHILLIPS XL-30 in an empty high place to 25kV. The material was analyzed in order to observe the morphology of materials, and their behavior under the electron beam. In all cases, the images were taken with the backscattered electrons detector. Elemental composition was also studied by Energy Dispersion Spectroscopy (EDS) with an EDAX spectrometer. The probe mark EDAX model DX-4, with a detector of count from 2000 to 2500 cps, dead times of 25-30 %, 150 seconds of time of acquisition was used for X-ray spectra; obtaining in this way the chemical elementary composition of every sample.

2.4.2. X ray diffraction, XRD
A mineralogical analysis by X-ray diffraction was also studied for the bovine bone samples. The samples were placed in a holder of lucita (zone of interaction with the electronic beam), later, they were placed on the goniometer of the diffractometer. The solid sample was characterized by X-ray diffraction using a Siemens D-5000 diffractometer coupled to a copper anode X-ray tube. The Kα radiation was selected with a diffracted beam monochromator to 25 kV and a step size of 0.02° during 50 minutes to acquire X-ray spectrum with sufficiently high intensities to achieve the lines to identify in angle 2θ of the present minerals; scan rate of 2° min⁻¹, and scan range from 0 to 80° 2θ. Compounds were identified comparing with the JCPDS cards in the conventional way.

2.4.3. The Infrared Spectroscopy was measured from 400 to 4000 cm⁻¹ in order to determine the chemical information from the HAp.

2.4.4. A thermogravimetric analysis (TGA) coupled at differential scanning calorimetry (DSC) was performed on HAp from in the temperature range of 25°C to 1200°C (rate 10/min) in a N₂ atmosphere.

2.4.5. Surface Area Determination
The Brunauer, Emmet and Teller (BET) method was employed to determine the surface area of bovine bone by nitrogen adsorption. Samples are first prepared using a Micromeritics Gemini 2360. The dry and degassed samples were then analyzed using a multipoint adsorption method for measuring the surface area (m²/g).

3. RESULTS AND DISCUSSION

3.1. Bovine Hydroxyapatite Purified
The initial pH from the centrifuged solution was 10.02, for this reason it was performed the washed of the material in order to eliminate the most possible the carbonates content. Once the HAp was washed the remaining solution from the final wash was pH=5.9, meanwhile the pH of the deionized water was 5.2.
3.2. Physicochemical Characterization of Hydroxyapatite

3.2.1. Scanning Electron Microscopy

The morphology and elementary chemical composition of the bovine bone was determined by means of SEM, taking in consideration the physical characteristics of the particles of the material. The Figure 1 shows the morphologic characteristics of the particles of bone which indicates that the material is a homogeneous powder. The HAp particles consist of crystalline hard agglomerates. The size of the particles of hydroxyapatite is similar, ranges from 1 µm to 100 µm. Besides this technique allowed to realize elementary and punctual chemical analyses of the material, which indicates a composition of O, P and Ca.
Figure 1. SEM micrographs of hydroxyapatite powders sintered at 900°C and washed with deionized water: (a) 1,000X; (b) 5,000X; (c) 10,000X; (d) 20,000X; (e) 15,000X

3.2.2. Mineralogic characterization by X ray diffraction

The identification of the mineralogical components of the hydroxyapatite was realized by X ray diffraction. Figure 2 shows the diffractogram of the bovine bone. The principal minerals found in the sample are the hydroxylapatite, $\text{Ca}_3(\text{PO}_4)_5\text{OH}$ (JCPDS card D-0432), calcium carbonate, $\text{CaCO}_3$ (JCPDS card 29-0305) confronted by the Joint Committee on Powder Diffraction Standard (JCPDS) cards. Every JCPDS card (Bayliss, 1986) contains the mineralogical information of the chemical compound, as well as a table that correlates the distances interplanar in a $2\theta$ angle versus relative intensity. It is possible to estimate in the diffractogram of the bovine bone that the majority of materials present are crystalline. The diffractogram of reference of synthetic hydroxyapatite show that the sample is an hexagonal hydroxyapatite, Figure 2.

Figure 2. XDR patterns of hydroxyapatite powder sintered at 900°C

3.2.3. Infrared Spectroscopy

Infrared Spectroscopy gives the chemical information of the bovine bone, is also particularly useful for the characterization of hydroxyapatite. The infrared spectrum is shown in the Figure 3. The phosphate groups are the biggest, and those are between 1094-1045 cm$^{-1}$. The OH group appears on 3572, 3460, 1736 cm$^{-1}$. The presence of the carbonate group slightly appears between 1459-1413 cm$^{-1}$. 
3.2.4. Thermogravimetric analysis and differential scanning calorimetry analysis of the HAp is in Figure 4, corresponding to the powder sintered at 900°C. There is one stage of mass loss, it start about 700°C, Although the mass loss is very small (1.75%), it is probably that the carbonate is transformed to carbon dioxide.

3.2.5. Nitrogen absorption isotherm obtained for HAp from bovine bone is shown in Figure 5. The isotherm was type II in the Brunauer, Deming and Teller classification, associated with solid macroporous (more than 50 nm). The anterior is corroborated with SEM micrograph as shown in Figure 6, with porous more than 100 nm. The surface area for HAp from bovine bone was 3.31 $m^2/g$, and the pore volume was 0.0065 $cm^3/g$. 

Figure 3. Infrared spectrum of the hydroxyapatite powder sintered at 900°C

Figure 4. Thermogravimetric analysis coupled with Differential Scanning Calorimetry of the hydroxyapatite powder sintered at 900°C

Figure 5. Nitrogen absorption isotherm for HAp from bovine bone.

Figure 6. SEM micrograph of the hydroxyapatite powder sintered at 900°C.
Figura 5. Nitrogen adsorption isotherm obtained at liquid nitrogen temperature for hydroxyapatite powder sintered at 900°C and purified with deionized water

Figura 6. SEM micrograph of hydroxyapatite powders sintered at 900°C and washed with deionized water at 20000X

4. CONCLUSIONS

The main objective of this work was the surface and physicochemical characterization of the calcium phosphate obtained from bovine bone. The elemental chemical composition determined by EDS in the material based on Ca, P and O. The minerals identified by XRD were hydroxylapatite and calcium carbonate. For this purpose, we focused our efforts on obtaining the hydroxyapatite from the raw material, the purification and finally the characterization of this material, the next step will be to evaluate the sorption parameters on hydroxyapatite from bovine bone as the determination of the isoelectric point, sorption kinetics, hydrate kinetics, and metals and radionuclides sorption kinetics.

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