Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 120 (2014) 610–615

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Review Article

# Thermal and electron stimulated luminescence of natural bones, commercial hydroxyapatite and collagen

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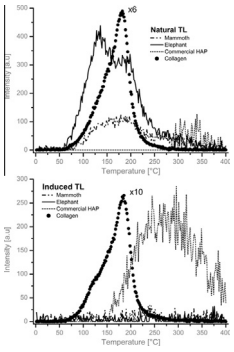
## HIGHLIGHTS

- We report the cathodoluminescence and blue thermoluminescence properties.
- We characterized the samples by X-ray diffraction and Raman spectroscopy.
- By chemical analyses were found small amounts of REEs in the natural bones.
- The CL spectra of the natural bones are clearly marked by the presence of collagen.
- The collagen sample displays higher natural and induced TL intensity.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 13 June 2013
Received in revised form 30 September 2013
Accepted 6 October 2013
Available online 22 October 2013

*Keywords:*
Cathodoluminescence
Thermoluminescence
Natural bones
Hydroxyapatite
Collagen

## ABSTRACT

The luminescence (cathodoluminescence and thermoluminescence) properties of natural bones (Siberian mammoth and adult elephant), commercial hydroxyapatite and collagen were analyzed. Chemical analyses of the natural bones were determined using by Electron Probe Micro-Analysis (EMPA). Structural, molecular and thermal characteristics were determined by X-ray Diffraction (XRD), Raman spectroscopy and Differential Thermal and Thermogravimetric analysis (DTA-TG). Cathodoluminescence (CL) spectra of natural bones and collagen showed similar intense broad bands at 440 and 490 nm related to luminescence of the tetrahedral anion $(PO_4)^{3-}$ or structural defects. A weaker luminescence exhibited at 310 nm could be attributed to small amount of rare earth elements (REEs). Four luminescent bands at 378, 424, 468 and 576 nm were observed in the commercial hydroxyapatite (HAP). Both natural bones and collagen samples exhibited natural thermoluminescence (NTL) with well-defined glow curves whereas that the induced thermoluminescence (ITL) only appears in the samples of commercial hydroxyapatite and collagen. Additional explanations for the TL anomalous fading of apatite, as a crucial difficulty performing dosimetry and dating, are also considered.



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http://dx.doi.org/10.1016/j.saa.2013.10.027

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## Introduction

The principal component of the bone, dentine and tooth enamel is the inorganic compost hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] (~70%) which is also composed by carbonate ($CO_3$) impurities incorporated into or attached surface of the crystals. Natural and synthetic hydroxyapatite has been used extensively in many applications such as material for bone replacement, for coating metal prosthesis, as a release carrier of excipient of proteins, for retrospective dosimetry using Electron Paramagnetic Resonance (EPR) and thermoluminescence methods, etc. [1–3]. Thermoluminescence retrospective dosimetry is an important application where the natural hydroxyapatite (extracted from bones and tooth enamel) could be used to estimate the dose in a radiological accident. Thermoluminescent measurements on human tooth enamel showed that the formation of the TL curve is attributed to recombination of the $CO_2^-$ radicals generated from ionization of the $CO_3$ impurities within hydroxyapatite [4]. Garcia-Guinea et al. [5] compared cathodoluminescence spectrum of different kinds of bones and collagen and they observed that collagen is much more cathodoluminescent than the calcium-phosphate phases, including hydroxyapatite. Dosimetric and dating characteristics of apatite specimens have recently been study to learn on the continuously observed anomalous fading of thermo- and optical stimulated luminescence emissions, e.g., Tsirliganis et al. [6] and Kitis et al. [7]. That anomalous fading is an unknowable attribute always poorly understood. In this work we study the chemical composition, structural characteristics and luminescent properties of natural bones, commercial hydroxyapatite and collagen using EMPA, XRD, Raman spectroscopy, thermal analysis, cathodoluminescence and thermoluminescence.

## Materials and methods

Chemical composition, structural characteristics and luminescent properties of natural bones from Siberian mammoth and African elephant, commercial Fluka HAP and collagen were studied. These four samples have the following characteristics: (1) SIBERIAN MAMMOTH (Mammuthus primigenius): The woolly mammoth could be extinct in the Pleistocene–Holocene boundary (c. 12 000–9000). In the 90s decade a little transversal section, sized, $1 \times 1 \times 1$ $cm^3$ of Siberia mammoth tusk of white color exhibiting incremental growth lines or bands seen in tooth enamel, i.e., striae of Retzius, was brought here by the Russian geologist Egor Gavrilenko, nowadays working in Spain. The sample has been stored during this time at room temperature in a simple polyester box. (2) AFRICAN ELEPHANT (Loxodonta Africana): A tiny portion of Loxodonta Africana tusk sized $1 \times 2 \times 1$ $cm^3$ was kindly provided to be studied for the paleontologist researcher Ana Mazo. She is the specialist on the fossil Proboscidea group of our working centre (Museo Nacional Ciencias Naturales, Madrid). The sample looks a clean ivory sample very probably collected several decades ago and stored at room temperature conditions in an office of this Madrid museum. (3) COLLAGEN: We use powdered marketable Collagen 234184 of MERCK Co. with purity $\geqslant$95% isolated from the triple helical domain of guanidine-HCl-extracted and pepsin-digested bovine joint cartilage collagen Type II. The collagen container was previously stored 15 days in our laboratory under standard cold conditions, i.e., −5 °C. (4) HYDROXYAPATITE FLUKA 21221 of Sigma–Aldrich Co. Bio-Ultra $\geqslant$99.0%. Formula: $Ca_5(OH)(PO_4)_3$ with $\leqslant$1.5% loss on ignition up to 800 °C anion traces Cl and $SO_4^{2-}$. PubChem Substance ID 24857195. The four samples were analyzed as received in the solid state without pre-treatments. In the TL case, samples were pulverized into a pestle-mortar.

Nondestructive chemical analyses of minor and major elements presented in the natural bones were determined using a data series of electron microprobe analysis (EMPA) model Jeol Superprobe JXA-8900 M, bulk and channel-selected (TAP, PETJ, LIF, PETH) X-ray spectra search and by identification routines in the "Servicio de Microscopia Electrónica Luis Bru," Universidad Complutense de Madrid. The samples were previously coated with graphite (20 mm) in a Bio-RadSC515 sputter-coating unit. The spot diameter of the probe was circa 5 μm and the operating conditions were 20 kV and 20 nA. The structural characteristics were performed using XRD (powder method), Raman spectroscopy and thermal analysis such as differential thermal analysis (DTA) and thermogravimetry (TG). The XRD were measurement in a Phillips PW1710/00 diffractometer with a Cu Kα (1.54 Å) radiation source, equipped with a graphite monochromator. The XRD measurements were obtained by step scanning from 3 to 63 ($2\theta$ in steps of $0.060\theta$; with 4 s per step). The XRD patterns were compared with the XRD PDF2 card files of the Joint Committee on Powder Diffraction Standards using the LBX Powder diffraction software. The Raman spectroscopy was performed in a Thermo Fisher DXR Raman microscope, using the 20X objective of the confocal microscope and point-and-shoot Raman capability of 1 μm spatial resolution. The Raman microscope is provided with a source laser at 532 nm of 10 mW in mode laser power at 100%. The Raman spectra were collected using a resolution spectral of 0.96 $cm^{-1}$ from 200 up to 3200 $cm^{-1}$. Thermal analysis were recorded with a thermal analyzer Model 4851e Mettler Toledo using 55 ± 0.25 mg of each sample and alumina as reference material. Thermal treatments were performed in nitrogen atmosphere with a heating rate of 10 °C/min from room temperature up to 950 °C. The samples were held in an alumina sample crucible. The luminescent properties of the natural bones, commercial hydroxyapatite and the collagen were characterized by Cathodoluminescence (CL) and Thermoluminescence (TL). The CL spectra were measured using a Gatan MonoCL3 detector with a PA-3 photomultiplier tube attached to the ESEM model XLS30. The detector covers a spectral range of 250–850 nm and is most sensitive in the blue parts of the spectrum. The samples were placed on polished slabs, at low-vacuum mode without coating to keep open way out to the CL emission. The luminescence of the samples was collected and amplified using a retractable parabolic diamond mirror and a photomultiplier tube. The distance between the sample and the bottom of the CL mirror