Identification of Trace Elements in Bovine Extracted Hydroxyapatite Using LIBS

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Abstract

Hydroxyapatite has been extensively studied being an essential part of the mammalian bone. Usually, hydroxyapatite in the bone is not present in its pure stoichiometric form, rather, it contains traces of ions of various elements like Na, Mg, Sr, Zn etc. Hydroxyapatite extracted from biological resources contains some these ions. At present EDX, XRF and ICP are being used for the elemental identification of these trace elements. Special sample preparation and more time for scanning is required in these techniques. We report LIBS as alternative technique for trace element identification in bovine extracted hydroxyapatite. The sample has been extracted from bovine bone by calcination at elevated temperature of 1000 °C. The presence trace elements (Mg, and Na) has been successfully identified using LIBS spectra.

Keywords: LIBS, hydroxyapatite, Ca/P ratio, EDX, BHA

1. Introduction

Hydroxyapatite \([\text{(Ca}_{10}\text{(PO}_4\text{)}_6\text{(OH)}_2)]\), is being used in the manufacturing of biomaterials for hard tissue repair. It is highly biocompatible and osteoconductive making it good for orthopaedic applications \textsuperscript{1}. Hydroxyapatite is major mineral component of mammalian bone. It is non-stoichiometric in nature and is mostly composed of Ca, P and O with trace amounts of various ions like \(\text{Na}^{2+}, \text{Mg}^{2+}, \text{Sr}^{2+}, \text{Ni}^{2+}, \text{F}^-, \text{SO}_4^{2-}, \text{SiO}_4^{2-}\), etc. Key role is played by these ions in the development of the hard tissue \textsuperscript{2}. Various techniques EDX \textsuperscript{3}, XRF \textsuperscript{4} and ICP \textsuperscript{5} have been employed for elemental analysis of hydroxyapatite. All these techniques require special sample preparation and most of them require vacuum conditions and are time consuming. LIBS gives the advantage of minimal sample repARATION along with rapid measurements.

In this study BHA (HA obtained from bovine bone) has been analysed using LIBS as a rapid alternative technique for trace elemental identification.

2. Experimental

2.1. Sample preparation

A fresh bovine bone was purchased from Pasar Tani (Farmers market), Sri Pulai, Johor Bahru, Malaysia. Visible flesh and tissues were removed from the bone using a kitchen knife. The bone was, boiled in deionized water for 4 hours, remaining softened tissue and fats were removed and the bone was soaked in the acetone for 1 day for deproteination. The deproteinated bone was, dried in the oven.
at 80 °C for 1 day. The bone was then calcined at 1000°C for the extraction of hydroxyapatite (BHA). A piece of BHA was used for LIBS.

2.2. LIBS Procedure

The schematic diagram of LIBS experiment is shown in figure 1. LIBS system consisted of Big Sky Q-Switched Nd:YAG laser (1064nm, 200mJ, 10ns, 10Hz), an array of seven (7) HR2000+ spectrometers covering a wide range of wavelengths, 200-1100nm, with a high resolution of 0.1nm and an interfacing, dedicated computer system was utilized in these investigations.

![Figure 1. Schematic diagram for LIBS experiment](image)

An externally Q-switched Nd:YAG laser at operating at fundamental wavelength, 1064 nm, was employed to produce plasma on the sample surface. Radiations from the plasma plume were collected through an optical fibre aligned at 45° to the incident laser beam. An array of 7 spectrographs equipped with CCD detectors recorded LIBS spectra on a computer system through an interfacing software ‘OOILIBS’ (OceanOptics Inc.).

Sample was exposed with 10 laser shots at 5 different spots on the surface after 5 cleaning laser shots at each spot. The spectra were averaged together to one representative spectrum of the sample.

3. Results and Discussion

3.1. SEM-EDX analysis

The results of the SEM-EDX are depicted in figure 2. The results show the presence of Ca, P, O and C along with the presence of trace elements Na and Mg in BHA. The presence of these trace elements has also been observed in the previous studies 6.
3.2. LIBS spectrum analysis of BHA

LIBS spectrum of BHA sample is depicted in figure 3. Multiple emission lines characteristic for Ca, P, Mg, Na and N were observed. Identification of lines is done by matching the wavelength and relative intensities with the NIST atomic spectral database (https://www.nist.gov/pml/atomic-spectra-database) and the published literature. Presence of N lines in the spectra are from the air as LIBS was carried out in air.

Figure 3. LIBS spectrum of BHA sample

Multiple emission lines (253.56 and 671.74 nm) characteristic for P were observed. And several emission lines (315.89 nm, 317.93 nm, 370.60 nm, 373.69 nm, 422.67 nm, 443.57 nm, 445.48 nm, 610.27 nm, 612.22 nm and 616.22 nm) characteristic of Ca were also observed. Emission lines (279.55 nm, 280.27 nm, 285.21 nm) characteristic of Mg were observed. Characteristic lines of Na were observed at 588.99 nm and 589.59 nm.
4. Conclusion

In this study, LIBS has been applied for the identification of trace elements in bovine extracted hydroxyapatite. LIBS spectrum has successfully identified the presence of Mg, and Na (trace elements) by their characteristic lines at (279.55 nm, 280.27 nm, 285.21 nm) and at (588.99 nm, 589.59 nm) respectively. EDX was used as reference technique for the confirmation of the presence of trace elements. Hence, LIBS can be used for fast identification of trace elements in hydroxyapatite extracted form biological resources.

5. Acknowledgement

The authors acknowledge the financial support and research facilities provided by Universiti Teknologi Malaysia through Tier 1 (16H44) and FRGS (4F891) grants.

References