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Determination of calcium to phosphate elemental ratio in natural hydroxypatite using LIBS

Elham Mazalan^{*1}, Kashif Chaudhary^{1,2}, Zuhaib Haider², Siti Fatimah Abd Hadi¹ and Jalil Ali^{1,2}

¹Department of Physics, Faculty of Science, Universiti Teknologi Malaysia, Johor Bahru 81310, Malaysia

²Laser Centre, Ibnu Sina Institute for Scientific and Industrial Research, Universiti Teknologi Malaysia, Johor Bahru 81310, Malaysia

Corresponding Author: elham.mazalan@gmail.com

Abstract. In this work, laser induced breakdown spectroscopy (LIBS) is employed for determination of calcium to phosphate (Ca/P) ratio in hydroxyapatite (HA) extracted from animal bones. HA was extracted from the biological bones of lamb, bovine, and fish separately. Fresh bones were boiled in deuterium-depleted water (DDW) for 4 hours and immersed it in acetone for 15 hours followed by drying process in the oven. Dried bones were heated at 600 ^oC and crushed into powder form which was then mixed with potassium bromide (KBr) in a known quantity. The mixture for each sample was palletized into pellets of identical mass using a hydraulic press. The palletized samples of HA extracted from the bones were ablated with 10 ns Q-switched Nd-YAG laser pulses having an energy of 320 mJ/pulse. The optical emissions produced from the laser induced plasma were recorded. Ca and P emission lines are identified and the Ca/P is calculated. Ca/P ratio by LIBS analysis has been verified by EDX measurements and are found in good agreement.

1. Introduction

LIBS is based on the generation of laser induced breakdown plasma on a sample by focusing a high power pulsed laser beam onto the sample. It has many advantages, which include no requirement of pre-preparation of samples, rapid and low cost analysis [1,2]. From these advantages, LIBS is expected to be the analysis tool in the field of environmental monitoring such as for monitoring the metal element in the polluted soils [3] and analysis of geological materials containing uranium [4]. The LIBS technique also could give information on the different application process such as pulse laser deposition [5] or direct induced plasma [6] by analyzed the properties of the plasma formed. The main advantage of LIBS is that, it is quasi non-destructive, has high throughput and does multielemental analysis. However, LIBS is inferior to the conventional element analysis methods such as atomic absorption spectroscopy (AAS), induced coupled plasma-optical emission spectroscopy (ICP-OES), x-ray fluorescence analysis (XRF), and energy-dispersive spectroscopy (EDX) in sensitivity and accuracy.

Hydroxyapatite (HA) is an important bio-medical compound, especially for the preparation of bone cement. The matching Ca/P ratio in HA with human bones provides bonding to generate and fill up missing part of the broken bone without intermediate connective tissue formation. The Ca/P ratio in HA thus is a vital indicator of its compatibility with human bones as bone cement [7]. Studies show

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that human bone samples and animal meat can be studied using a LIBS technique [8]. The extraction of natural HA from bovine femur bones performed via calcination method at various temperatures affect the composition, crystallinity, and crystallizes size [9]. Hosseinzadeh et al. [10] performed an experiment to derive natural HA from bovine bones with thermal decomposition method by different sample preparation approach. The raw bone is washed and crushed to fine powder. The bone powder was immersed in acetone, then dried in the oven. They mixed the dried bone powder with liquid and pressed before sintering in the furnace. The optimum temperature of 750 °C gives the particle size in the range 420–500 μ m.

Another study investigated natural hydroxyapatite and determine the possibility of hydroxyapatite obtained from bovine cortical bone ashes at different thermal conditions [11]. Different sample preparation was used. The bone was cleaned using direct flame thus the organic components were burned. The black powder (bone ash) was placed in an air furnace to remove the remaining char due to the burn. The sample was cooled down inside the furnace until the black ash turned into white granular powder. FTIR analysis showed the existence of organic species and also comprehend the degree of probable dehydroxylation of HA. This paper is focused to determine Ca/P ratio in HA material extracted from animal bones. EDX analysis is performed on an extracted sample of HA from animal bones, (lamb, bovine, and fish) to compare and validate LIBS results.

2. Experimental Setup

This work consists of two main stages, extraction of HA from animal bones and LIBS analysis of extracted HA samples. To extract HA, the fresh bovine, lamb, and fish bones are collected from the local market in Johor Bahru. As shown in Figure 1 (a) bone samples are cut into small pieces. The attached meat, bone marrow and other soft tissues with the bones are removed using a knife. After that, bone samples are boiled in Deuterium-depleted water (DDW) for 4 hours for defatting and easier removal of soft tissues and fats from bones.



Figure 1. Bone samples bovine (top), lamb (middle), and fish (bottom). Raw bone sample from market (a), calcined bone sample (b), and pallets of calcined sample (c).

Bone samples are then immersed in acetone for 15 hours for deprotonation followed by drying in oven at 80 $^{\circ}$ C for 17 hours and then at 600 $^{\circ}$ C in a furnace for 2 hours. In the final stage, bone samples are crushed into powder using mortar and pestle and calcined at 1000 $^{\circ}$ C for 2 hours to acquire

hydroxyapatite (HA). The calcined samples of bones are shown in Figure 1 (b). The final powder form of the calcined bone samples of all three different types are palletized by mixing a calcined powder sample with KBr by weight percentage 1:10 grams respectively. HA in final pallets form are shown in Figure 1(c).

The experimental setup for real-time LIBS analysis is shown in Figure 2. The first harmonic beam (1064 nm) of 320 mJ energy from Nd:YAG laser was focused onto the sample. An optical fiber cable connected to Maya2000 spectrometer was placed in front of the sample to collect the plasma radiation with an optical response from 200 nm to 650 nm. The spectrometer was connected to the computer and measurement was monitored and recorded using SpectraSuite software. Laser pulse energy was constantly examined by deflecting 6% of the beam to an energy meter through the quartz beam splitter. Each sample is exposed at 10 distinct spots on the surface to 10 laser shots at each spot. For each laser shot an emission spectrum from the plasma is recorded on the computer which were then averaged out. Ca/P ratio is calculated using peak intensities of Ca and P emission lines for all possible combinations of lines and the best one was selected. EDX analysis of the extracted HA from different sources is also performed to calculate the Ca to P ratio. Ca/P ratio calculated by LIBS is with those obtained from EDX.



Figure 2. Block experimental set up for LIBS measurement.

3. Results and Discussion

3.1 Element Identification

Figure 3 shows the LIBS spectrum of HA samples for bovine, lamb, and fish samples. Identification of lines is done by matching spectral lines with the NIST atomic spectral database [12]. Identified Ca and P species are listed in the Table 1. Ca and P atomic spectral lines at 442.54 nm and 534.59 nm, respectively, are selected for determining the Ca/P in extracting HA bone samples. Extracted HA from lamb sample has stronger emissions than bovine and fish samples. Each HA sample shows identical spectra of Ca and P.

3.2 LIBS Analysis

Figure 4 and Figure 5 show spectral lines of Ca (Ca I 442.54 nm) and P (P I 534.59 nm) for bovine, lamb, and fish samples. The Ca/P is calculated for each extracted HA bone sample and listed in Table 2. Ca/P of lamb samples has the highest ratio due to the high Ca content.

Element	Species (I – Neutral, II – 1 st Ionic)	Wavelength, nm
Ca	Ι	442.54
		422.67
		443.50
		445.48
		610.27
		612.22
		616.22
		315.89
	Π	317.93
		370.60
		373.69
		393.37
		396.85
Р	Ι	253.40
		534.59

Table 1. Element identification of lines as comparison with NIST atomic spectral database.

Table 2. Ca/P ratio for bovine, lamb, and fish bone based on LIBS analysis.

	Int	Intensity	
Sample	Ca (442.54 nm)	P (534.59 nm)	Ca/P ratio
Bovine	2860.80	1493.43	1.92
Lamb	3657.24	1857.63	1.97
Fish	1881.74	1013.49	1.86



Figure 3. Optical emission spectra of extracted bovine, lamb, and fish samples.



Figure 4. Ca emission spectra of extracted bovine, lamb, and fish samples at 442.54 nm.



Figure 5. P emission spectra of extracted bovine, lamb, and fish samples at 534.59 nm.

3.3 EDX Analysis

Figure 6 shows the typical EDX spectrum of the extracted HA fish bone samples giving weight percent of Ca and P among other element. The values of Ca/P ratio based on EDX information on extracted HA samples from bovine, lamb and fish bones are given in table 3. The EDX detected carbon (C), Magnesium (Mg), and Oxygen (O) elements, where are Ca and P are present in dominant quantities. All HA bone samples show a higher ratio than the stoichiometric value of HA, which is 1.67 [13].

Sample	Average Ca wt.%	Average P wt.%	Ca/P rati
Bovine	39.98	16.62	1.86
Lamb	42.28	17.98	1.82
Fish	42.62	17.70	1.88
	Ģ		W1% o a 43.0 0.6 31.1 0.6 17.9 a 1.0 0.4 1.7.9 0.4 1.0 6.1 0.5 0.5 a 1.1 0.1 lg 0.9 0.1
			<u></u>

Table 3. Ca/P ratio for bovine, lamb, and fish bone based on EDX analysis.

Figure 6. Typical EDX spectra of HA extracted from Fish sample.



Figure 7. Comparison of LIBS and EDX analysis for lamb, bovine and fish HA bone samples.

3.4 LIBS and EDX Analysis Comparison

The average Ca/P ratio obtained from LIBS analysis were compared with EDX measurements. Figure 7 shows the graph Ca/P ratio for both techniques for each HA bone sample. These values are within the accepted range (1.86-1.97) for bovine, lamb and fish HA bone.

4. Conclusion

In this work, Ca/P ratio in HA extracted from animal sources is determined using LIBS. HA is extracted by the calcination process from bovine, lamb and fish bones. Extracted HA from each animal source was palletized in KBr matrix for LIBS analysis. The pelleted samples of HA were ablated by Q-switched Nd:YAG laser and optical emission in form of spectra are recorded using the optical spectrometer. To verify the LIBS results, EDX analysis of the extracted HA samples has been performed. Ca/P ratios were estimated via the intensity ratio of the selected pair Ca and P lines from the emission spectra. LIBS results were found in good agreement with EDX results. It can therefore be concluded that, LIBS can be used effectively to estimate the Ca/P ratios in HA samples.

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