Calcium to phosphate ratio measurements in calcium phosphates using LIBS

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Abstract. In this study, we report the measurement of Ca/P ratio of hydroxyapatite using LIBS. Hydroxyapatite was prepared by wet chemical precipitation method with different stoichiometric ratios of Ca and P (1.5, 1.6, 1.67, 1.70, 1.80). Samples were then pelletized using KBr as a binder under a hydraulic press. Samples were irradiated with a laser beam and corresponding spectra were recorded. Spectra were further analyzed to calculate the Ca/P ratio which was then compared with the EDX for validation. LIBS results showed promise with a deviation of < 5% with the Stoichiometric ratios and EDX results.

1. Introduction
Natural bone is a composite of mineral and organic phase (70% mineral and 30% organic). There is immense importance of hydroxyapatite in biomedical applications as 70% mineral phase of natural bone contains 95% hydroxyapatite [1]. Biomaterials can be prepared synthetically to function appropriately in a bio-environment. After the invention of first generation of materials during 1960–1970 for use inside a human body, synthetic biomaterials became a subject of interest [2]. Calcium phosphate ceramics are usually described by their calcium/phosphorus atomic ratio (Ca/P). Among these ceramics, hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂, HA, Ca/P = 1.667) and β-tricalcium phosphate (β-Ca₃(PO₄)₂, β-TCP, Ca/P = 1.5) have been extensively studied, and their various biological behaviours are well-known [3].

The properties of calcium phosphates of biological interest depend strongly on their calcium/phosphorus atomic ratios (Ca/P). Therefore, the precision for the determination of this ratio is crucial [3]. Calcium to phosphate ratio plays a very important role in the mechanical properties of the biomaterials. In a previous study [3] it is shown that a small variation in the Ca/P ratio of calcium deficient HA from 1.650 to 1.667 (i.e relative deviation of Ca/P ratio <1%) resulted in substantial changes in the mechanical characteristics in densified BCP ceramics. Various techniques XRD[2], EDX [4,5], XRF [6,7], ICP [8] have been employed for elemental analysis and to find the Ca/P ratio 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 or no sample preparation along with being a rapid measurement technique [9].

LIBS is a powerful and versatile analytical technique for elemental analysis [10]. It is an optical emission spectroscopy of laser induced plasma which is a source of ions and electrons [11]. In LIBS,
laser pulse interacts with a target generating plasma [12]. For chemical analysis LIBS is a highly potential diagnostic technique [13].

In this study, we have used LIBS to determine the Ca/P ratio in synthetically prepared hydroxyapatite with Ca/P ratios ranging from 1.5-1.8. Suitable peaks of Ca and P are selected for the reliable determination of Ca/P ratio.

2. Experimental

2.1. Sample Preparation for LIBS
Calcium nitrate tetrahydrate (Ca(NO3)2.4H2O, QReC) and di ammonium hydrogen phosphate ((NH4)2HPO4, QReC) were used as precursors for Calcium and Phosphate elements respectively. Corresponding molar solution of calcium nitrate tetrahydrate and diammonium hydrogen phosphate were prepared in 100 ml DDW. Solution of di ammonium hydrogen phosphate was added dropwise in calcium nitrate tetrahydrate solution with continuous stirring. The reaction mixture was then transferred to the 800 W household microwave (SHARP, model R-218LS) to reflux for 30 min [5]. Resulting precipitates were filtered, washed with distilled water, dried in the oven at 80 °C for 17 h and calcined in muffle furnace at 1000 °C for 2 h. Five samples (A, B, C, D & E) of HA were prepared with Ca/P ratio 1.5, 1.6, 1.67, 1.7 and 1.8 respectively.

After completing the calcination process, the mixture of synthetic HA and binder (KBr) of known quantities was pelletized using hydraulic press by pressing by applying 10 tons force to compress the sample as shown in Figure 1. The pellets were prepared in identical mass and dimensions.

![Image of one of synthetic HA samples in pellet form](image)

Figure 1. Image of one of synthetic HA samples in pellet form

2.2. LIBS Setup and Procedure
The LIBS experimental setup shown in Figure 2 was used for LIBS analysis of samples. Nd:YAG (1064 nm, 10 ns, 10 Hz) laser pulses of 320 mJ of energy were focused onto the target sample, a fiber optic cable was used to collect plasma radiations and transfer to Ocean Optics MayaPro spectrometer equipped with CCD detector. Laser pulse energy was constantly examined by deflecting 6% of the beam to a calibrated energy meter through quartz beam reflector.
Each sample was 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 was recorded on the computer. Ca/P ratios were calculated using peak intensities of Ca and P emission lines. Results were compared with EDX results, which were considered as reference, to calculate an approximate error in the results obtained from LIBS.

3. Results and Discussion

3.1. EDX

Energy Dispersive X-ray (EDX) spectroscopy was used for the elemental analysis of prepared samples. Figure 3 shows the EDX graph of the sample D.

![Figure 3. EDX graph of sample D](image)

Four spots on each sample were used for the EDX analysis. Ca/P ratio of each sample was calculated as an average of these four spots. EDX analysis showed that the Ca/P ratio of the samples investigated in this study were close to their stoichiometric ratio. This confirmed that the samples are appropriately prepared in desired Ca/P ratios. Table 1 shows Ca/P ratios in the samples obtained from EDX analysis.
Table 1. Results of calculation of Ca/P ratio based on EDX analysis.

| Sample | Stoichiometric Ca/P ratio | Average of weight percentage of Ca (%) | Average of weight percentage of P (%) | EDX Ca/P ratio |
|--------|---------------------------|----------------------------------------|----------------------------------------|----------------|
| A      | 1.50                      | 33.30                                  | 17.00                                  | 1.52           |
| B      | 1.60                      | 37.73                                  | 18.10                                  | 1.62           |
| C      | 1.67                      | 41.63                                  | 18.93                                  | 1.70           |
| D      | 1.70                      | 42.50                                  | 19.05                                  | 1.73           |
| E      | 1.80                      | 42.93                                  | 18.20                                  | 1.83           |

3.2. LIBS Analysis
LIBS spectrum of the sample D is shown in figure 4. Theoretically, intensity of spectral lines in emission spectra is indicative of elemental concentration. However, different spectral lines of same element do not respond equally to a similar change in the element’s concentration. Ca/P ratio was therefore calculated by intensity ratio of all possible combinations of Ca and P lines identified in the LIBS spectra. Lines of Ca, P, K and Br were identified in the spectrum. The identification of the spectral lines is performed using NIST atomic spectra database [http://physics.nist.gov/PhysRefData/ASD/lines_form.html].

![LIBS spectra of sample D](image_url)

**Figure 4.** LIBS spectra of sample D.

Of all the combinations of Ca and P lines used for calculating Ca/P ratio, Ca I 610.27 nm and P I 550.72 nm gave closed values to the stoichiometric values (also verified by EDX). Same pair of lines
is used to determine Ca/P ratio in all five samples. Emission lines are authenticated from NIST database [14] and the published literature [15]. The calculation of Ca/P ratio based on LIBS results for all samples are given in Table 2.

Table 2. Results calculation of Ca/P ratio based on LIBS results.

| Sample | Stoichiometric Ca/P ratio | Average intensity of Ca line | Average intensity of P line | LIBS Ca/P ratio |
|--------|---------------------------|-----------------------------|-----------------------------|-----------------|
| A      | 1.50                      | 1815.02                     | 1184.68                     | 1.53            |
| B      | 1.60                      | 4951.48                     | 3080.92                     | 1.61            |
| C      | 1.67                      | 3371.64                     | 1984.78                     | 1.70            |
| D      | 1.70                      | 2996.38                     | 1744.17                     | 1.72            |
| E      | 1.80                      | 3963.22                     | 2179.09                     | 1.82            |

EDX analysis has been used as the reference to LIBS analysis since EDX technique has already commercialized technique for such analysis. Table 3 shows comparison of Ca/P ratio for all five samples that are analysed using LIBS and EDX techniques.

Table 3. Comparison of Ca/P ratio for all five samples that are analyzed using LIBS and EDX techniques

| Sample | Stoichiometric Ca/P ratio | LIBS Ca/P ratio | EDX Ca/P ratio |
|--------|---------------------------|-----------------|----------------|
| A      | 1.50                      | 1.53            | 1.52            |
| B      | 1.60                      | 1.61            | 1.62            |
| C      | 1.67                      | 1.70            | 1.70            |
| D      | 1.70                      | 1.72            | 1.73            |
| E      | 1.80                      | 1.82            | 1.83            |

Results from the table 3 show that, the estimation of LIBS results are comparable with EDX results. Figure 5 shows the graph of Ca/P ratio calculated by EDX and LIBS in comparison with stoichiometric values.

Samples A, B, C, D and E are synthetic HA samples with Ca/P ratio of 1.50, 1.60, 1.67, 1.70 and 1.80 respectively. From graph, LIBS Ca/P ratio (red line) is close to EDX Ca/P ratio (blue line). The difference between LIBS and EDX Ca/P ratio is only ±0.01. These shows that Ca/P ratio of synthetic HA can be calculated using LIBS technique and the results could be nearly as accurate as those obtained from EDX.
4. Conclusion
In this study LIBS has been successfully utilized as a fast and reliable technique to find the Ca/P ratio of synthetic hydroxyapatite. The comparison of results of LIBS and EDX have demonstrated that it is a potential candidate to be used as an alternative technique for the elemental qualitative as well as quantitative study of calcium phosphates. To verify the authenticity of this further studies are required to replicate these results.

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