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LIBS analysis of artificial calcified tissues matrices

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ABSTRACT

In most laser-based analytical methods, the reproducibility of quantitative measurements strongly depends on maintaining uniform and stable experimental conditions. For LIBS analysis this means that for accurate estimation of elemental concentration, using the calibration curves obtained from reference samples, the plasma parameters have to be kept as constant as possible. In addition, calcified tissues such as bone are normally less "tough" in their texture than many samples, especially metals. Thus, the ablation process could change the sample morphological features rapidly, and result in poor reproducibility statistics. In the present work, three artificial reference sample sets have been fabricated. These samples represent three different calcium based matrices, $CaCO_3$ matrix, bone ash matrix and Ca hydroxyapatite matrix. A comparative study of UV (266 nm) and IR (1064 nm) LIBS for these three sets of samples has been performed under similar experimental conditions for the two systems (laser energy, spot size, repetition rate, irradiance, etc.) to examine the wavelength effect. The analytical results demonstrated that UV-LIBS has improved reproducibility, precision, stable plasma conditions, better linear fitting, and the reduction of matrix effects. Bone ash could be used as a suitable standard reference material for calcified tissue calibration using LIBS with a 266 nm excitation wavelength.

1. Introduction

1.1. Bone elemental analysis

Bone is composed of organic and inorganic matter, the inorganic matter is mainly a carbonate-containing hydroxyapatite (HAP) analog with a composition approximating $Ca_{10}(PO_4)_6(OH)_2$, also called bioapatite. HAP crystals are plate-like in morphology and have dimensions of approximately 35 nm × 5 nm with a thickness of about 2–3 nm [1]. Study of the elemental composition of bone has been used in the fields of archeology and anthropology for investigating the relationships between nutrition and diseases and to estimate the health effects of trace element deficiencies or excesses in human tissues. In addition, such studies are crucial in the verification of dietary habits [2], cultural, customs, and environmental levels of trace elements in soil and water [3]. Elemental analysis of bone also has been used to investigate toxic pollutants for example lead (Pb) exposure in historical populations or to explore the source of specific nutritional deficiencies among ancient communities [4]. These investigations depend mainly on the fact that once elements are incorporated in the hydroxyapatite structure of the bone and/or tooth matrix, a number of such elements leach out very slowly [5].

1.2. LIBS of biological samples

Laser induced breakdown spectroscopy (LIBS) has emerged as a very promising technique for the analysis and characterization of a broad variety of objects due to advantages such as: no need for laborious sample preparation, fast analysis and in-situ analysis capability. Laser ablation based analysis requires less amount of sample compared to sample digestion in conventional techniques (e.g. ICP, AAS, etc.) [6]. Typically laser ablation analysis requires femtograms to nanograms of materials compared to micrograms to milligrams for the other methods. High resolved spatial information is attainable as oppose to the complete loss of this type of information when traditional sample preparation like acidic dissolution is used. LIBS has been successfully used in the characterization of archeological bone samples from different historic ancient Egyptian dynasties in comparison with recent bone samples and it was shown that diagenesis or postmortem effects must be taken into consideration on studying dietary habits and/or toxicity levels via analysis of ancient bones [7]. In exploiting LIBS for the analysis of relatively soft biological samples such as bones,

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it should be taken into consideration that fast changes taking place in the target morphological features can affect the reproducibility of the results. In general biological samples are rather inhomogeneous, again influencing selectivity, statistics and reproducibility of results. To obtain reliable quantitative results using laser ablation, the experimental conditions should be maintained uniform and stable, similar to all analytical measurement technologies. For LIBS analysis this means that for accurate estimation of element concentrations, using relevant calibration curves, the plasma parameters have to be kept constant throughout the measurements [8]. For relatively soft matrices like calcified tissues this is even more important than for metallic samples. However, this is not always straight forward since drilling into the sample during repetitive single-position exposure is accompanied by a gradual change in plasma conditions. In general, a compromise has to be found in keeping the plasma conditions reasonably constant on the one hand but sampling over a sufficient number of laser pulses for statistical averaging on the other hand. At the same time, maintaining spatial resolution, if required, should be kept under control. Another difficulty in the analysis of calcified tissue samples is that suitable reference standards, required for quantification, may not be available. Quantitative analysis of trace element concentrations in calcified tissues using reference samples with a $CaCO_3$ based matrix were investigated by Samek et al. [9]. The overall physical properties of pellets pressed from $CaCO_3$ are roughly comparable to those of hydroxyapatite, but slightly more brittle than biological specimens because of the absence of the biological growth mechanism. In addition, introducing phosphorus-carrying compound was not possible because of homogeneity problems and substantial local variations in the Ca and P distribution. Also, samples based on a $CaCO_3$ matrix have shown dependence of LIBS spectra on the water content in the pellets, and it is essential to account for the water content to achieve accurate quantitative LIBS analytical results [10]. Since calcium represents the major element in the bone matrix with the highest elemental concentration, it has been used in many studies as internal standard for the calibration of elements such as Pb, Al and Sr [9]. In the present study, we had focused on studying calcium in different matrices using conventional LIBS arrangement at two different excitation wavelengths, 1064 nm and 266 nm to examine the stability of plasma conditions in different matrices, measure the extent of matrix effects, and the reproducibility and precision for calibration.

2. Methodology

2.1. Samples

Three sets of reference samples representing three different calcium based matrices have been fabricated. Each sample was mixed with paraffin binder for 10 min using an automatic mixer machine (Spex Sample Prep, Mixer/Mill 8000M) and then pressed into a pellet using an automatic press (Spex Sample Prep, X-press 3630). The samples were pressed into pellets using an automatic program to assure reproducibility set at 25 t of pressure for 1 min for 5 g pellet, and 7 t of pressure for 2 min for 1 g pellet. The 1st sample set was a calcium carbonate matrix; 10 samples were prepared by mixing pure powder of $CaCO_3$ with different amounts (by weight) of $MgCO_3$ and paraffin (binder). Samples with a calcium concentration in the range of 0.1–10% were obtained while the Mg content was fixed at 1%. The 2nd set was a hydroxyapatite matrix; 7 samples were prepared using a similar procedure but this time by mixing the paraffin with high purity powder of Ca-Hydroxyapatite "solid dilutions" calcium concentration was set in the range 1–9%. Ca-Hydroxyapatite was obtained from the National Institute of Standards and Technologies (NIST2910). It is a high purity powder formed of crystalline calcium hydroxyapatites of about 0.1–0.5 μm sizes. One unit of SRM2910 consists of 2 g of material synthesized at the NIST by solution reaction of calcium oxide and phosphoric acid. It has certified values (Ca 39.15%, P 18.18%, Ca/p 2.15) and reference values (hydrogen phosphate 0.592%, carbonate content 0.032%, water content 1.5%) and is used to simulate the molecular structure of bone apatite. Lastly, the 3rd set was of bone ash matrix; 8 samples were prepared using the same procedure by mixing the paraffin with the high purity powder bone ash "solid dilutions" calcium concentration was set in the range 1–10%. Bone ash was obtained from the National Institute of Standards and Technologies (NIST 1400). It consists of bone ash that was blended to a high degree of homogeneity. It has certified values (Ca 38.18%, P 17.9%, Mg 0.68%, Sr 249 μg/g, Fe 660 μg/g, Pb 9.07 μg/g, K 186 μg/g, Zn 181 μg/g) and non-certified concentrations (Si 0.13%, Na 0.6%, Al 530 μg/g, Ar 0.4 μg/g, Cd 0.03%, Cu 2.3%, F 1250 μg/g, Mn 17 μg/g, Se 0.08%).

2.2. LIBS arrangements

Two LIBS experimental systems based on an RT100 design (Applied Spectra, Inc.) with different laser excitation wavelengths (1064 nm, 266 nm) were used throughout the measurements. The experimental conditions (laser energy, spot size, repetition rate, irradiance, etc.) for the two systems (Table 1) were kept as close as possible to investigate the wavelength effect.

In the 1st setup the laser source was a Q-switched Nd:YAG laser (New wave, Minilase II, USA), operating at its fundamental wavelength ($\lambda = 1064$ nm), with a pulse duration of 6–8 ns. The pulse energy was set to 22 mJ and the repetition rate to 5 Hz. The laser beam was tightly focused vertically onto the target surface using an objective lens (LmH-5x-1064, Thorlab, USA). High resolution camera (USB 2.0 CMOS Camera, Thorlab, USA) was used for viewing the sample surface and adjusting the laser focus. The samples were mounted on an *X*–*Y*–*Z* motorized translational stage. LIBS spectra were collected from 10 replicates from fresh spots, 100 accumulative shots were recorded for each spot to optimize the signal-to-noise ratio and establish reproducibility.

The plasma optical emission was collected through a plano-convex lens with 4 cm focal length to fiber-optics bundle for light coupling to the spectrometer entrance slit. The spectrometer (LIBS Aurora spectrometer, 6 channel CCD, Applied spectra, Inc., USA) covers the spectral coverage (190–1040 nm), with spectral resolution < 0.1 nm for UV to VIS, and < 0.12 nm for VIS to NIR. The detector type was a CCD linear array; the spectrometer gating control is achieved through fully integrated electronic pulse generator gate delay adjustment from 50 ns to 10^6 ns with 25 ns step resolution, with integration time 1.04 ms–10^4 ms. To obtain the optimum delay time an optimization procedure has been performed where the delay time has been changed in steps

Table 1
LIBS measurement parameters.

Experimental parameters	LIBS-1064 nm	LIBS-266 nm
Wavelength (nm)	1064	266
Pulse width (ns)	~7	~5
Energy (mJ)	~22	~14.3
Laser repetition rate (Hz)	5	5
Number of shots	100	100
Irradiance (W/cm^2)	1.778×10^{10}	2.154×10^{10}
Fluence (J/cm^2)	124	107
Replicates	10	10
Spot size diameter (μm)	~150	~130
Delay time (μs)	1	1
Integration time (ms)	1.04	1.04
Spectrometer	6-Channels CCD, Aurora	6-Channels CCD, Aurora