Research Article

Influence of Lead on the Interpretation of Bone Samples with Laser-Induced Breakdown Spectroscopy

Abdolhamed Shahedi,¹ Esmail Eslami,¹ and Mohammad Reza Nourani²

¹Department of Physics, Iran University of Science and Technology, Narmak, Tehran 16846-13114, Iran

²Tissue Engineering Division, Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

Correspondence should be addressed to Esmail Eslami; eeslami@iust.ac.ir

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This study is devoted to tracing and identifying the elements available in bone sample using Laser-Induced Breakdown Spectroscopy (LIBS). The bone samples were prepared from the thigh of laboratory rats, which consumed 325.29 g/mol lead acetate having 4 mM concentration in specified time duration. About 76 atomic lines have been analyzed and we found that the dominant elements are Ca I, Ca II, Mg I, Mg II, Fe I, and Fe II. Temperature curve and bar graph were drawn to compare bone elements of group B which consumed lead with normal group, group A, in the same laboratory conditions. Plasma parameters including plasma temperature and electron density were determined by considering Local Thermodynamic Equilibrium (LTE) condition in the plasma. An inverse relationship has been detected between lead absorption and elements like Calcium and Magnesium absorption comparing elemental values for both the groups.

1. Introduction

Nowadays, an increasing spread of polluting industries, the pollution caused by toxic metals, and the hazards on human health are crystal clear to everyone. Thus detecting these pollutants and their effects on biological organisms is of great importance. Laser-Induced Breakdown Spectroscopy (LIBS) method has attracted much attention of many researchers due to its properties, unique capabilities such as low cost and minimal time duration of preparing the sample, prompt and simultaneous analysis of multielements, capability of being used in all three states of materials, and its nondestructive and noncontact nature [1, 2]. This method has been successfully used to identify corpses, bones, and humans' fossils or mummies remaining from centuries. It determines characteristic backgrounds like age, sex, race, and stature of bodies [3]. Clinical application of Laser-Induced Breakdown Spectroscopy to the analysis of teeth and dental materials used the pulsed lasers for controlled material ablation. This method is suggested as an alternative to mechanical drilling for the removal of caries and in tooth modification. Spectral analysis of the ablated plasma can be exploited to monitor

precisely the laser drilling process in vivo and in real time [4, 5]. LIBS procedure proposes a new technique for diagnosing the presence or occurrence of cancer accurately and safely. It is now apparent that the malignant tumors can be predicted via detection of Calcium, Potassium, and Copper in body [6]. The method was also used to detect the elements of newborn heart muscles by reviewing elements such as Potassium, Calcium, and Sodium [7]. Analyzing biological seminal fluid [8], detecting pathogenic bacteria and pathogens [9], and so forth are among other applications of this method.

The presented technique depends on the interaction between the laser and the soft and hard biological surfaces in order to induce plasma. The plasma formation results from the matrix effects initiated by the absorption of the laser energy by the ablated material [2]. When the plasma expands in an ambient air, relaxation of the plasma plume by different processes—one of which is the recombination of the free electrons with the positive ions—occurs [10]. This recombination is a radiative process, which provides a continuum emission in addition to the bremsstrahlung. Another radiative process is the atomic emissions due to transitions between the different energy levels of an element from

FIGURE 1: Schematic of the LIBS setup.

which spectrometric measurement can provide qualitative information on the atomic composition of the investigated biological samples. In addition, LIBS can also be used to provide information about the relative quantitative elemental composition of the samples. This is achieved by investigating the ratio between the intensity levels of the atomic emission lines: high concentrations of an element yield a higher intensity of the characteristic elemental emission spectra [11, 12].

In this study, we attempted to employ this method to differentiate between two groups of rats based mainly on the statistical analysis of the collected spectra. These spectra intrinsically possess information of the different concentration levels of the elements found in both the groups.

2. Methods and Materials

2.1. Test Layout. The experimental setup is schematically shown in Figure 1. It consists of a Nd:Yag laser having 10 ns pulse width at 1064 nm wavelength, 2 Hz pulse repetition frequency, and 160 mJ energy. To eliminate bremsstrahlung radiation in early stage when plasma is generated, and to have atomic spectrum with the highest ratio of signal to noise, different time intervals in plasma radiation are required. A delay time between laser pulse and data recording by photodiode was about 10 μ s. As illustrated in Figure 1, a part of laser light is reflected toward photodiode by a half-silvered mirror (reflects about 4% of light) which lies in a 45-degree angle in line with light and delay contributor starts the camera by sending a pulse. Laser beam is focused on the surface of the sample located at the focal length of the converging lens with $f = 10$ cm. An optical fiber locates at 45 degrees with beams in optimal distance from the location where plasma was generated (to avoid saturation) and transmits the light resulting

FIGURE 2: An example of rat's bone.

from the plasma to the 2D intensified CCD (Andor iStar, DH720) through a Czerny-Turner spectrograph (Chromex, 500 IS) in the wavelength range of 115–920 nm. LIBS spectra were collected from 10 different spots of each sample; 10 spectra were collected from each spot. These spectra enable us to analyze the sample quantitatively and qualitatively. To obtain ratio of signal to high noise, best conditions such as delay time and optical field of view were optimized.

2.2. Sample Preparation. Female rats with average age of four weeks with same race and conditions were chosen for preparing bone samples. Ten rats were divided into two equal groups. Group A received 250 mL distilled water including lead salts (lead acetate 325.29 g/mol) with 4 mM concentration daily and group B received only 250 mL distilled water with no additives daily for 50 days. Most portion of lead is absorbed in bones [13]. So the left legs of the rats were split up and cleaned. Since almost all portions of lead injected to

FIGURE 3: Typical mean LIBS spectra in both groups.

the body were absorbed in bone, they influence absorption of other elements in bone according to the procedure to prepare for laser pulse irradiation (Figure 2).

3. Plasma Characteristics

The LIBS spectra collected from the samples were processed from the raw data using the ORIGIN software. Figure 3 shows the mean spectra (mean value and standard deviation) of 10 bone spectra of both groups. To exemplify, the most prominent peaks existing in the spectra of both samples were used to show the standard deviation of all of the 10 spot-measurements. The elements with prominent emissions common to both bone types, found under the measurement conditions of this study, were Ca, Mg, and Fe. The LIBS spectra of group B exhibit more prominent atomic spectral lines than those of group A. The identification of the atomic lines is based on [14, 15]. As can be seen in this figure a significant decrease of Mg and Ca can be observed due to lead consumption. Note that during all experiments an intense line on Pb I at 405.78 nm was observed. As illustrated in Figure 4, its intensity is increased with lead consumption in bone. However, our analysis is mainly focused on the effect of Pd on other elements.

3.1. Plasma Temperature. Plasma temperatures are often determined from the measurement of ratios of the intensities of neutral to neutral lines, usually for the same element. The line intensities are combined with the Boltzmann equation to determine

$$\ln \left(\frac{I \lambda_{ki}}{A_{ki} g_k} \right) = \ln \left(\frac{hc}{4\pi} \frac{N_0}{U(T)} \right) - \frac{E_k}{KT_{\text{exc}}}, \quad (1)$$

FIGURE 4: Typical lead spectrum at 405.78 nm.

where I is the intensity of the spectral line, λ_{ki} is the wavelength of the transition from level k to i , A_{ki} is the transition probability, g_k is the statistical weight, E_k is the energy value of higher level, and T_{exc} is the excitation temperature. Thus, a plot of the above equation versus the energy of the upper level E_j yields a straight line called Boltzmann plot. Its slope equals $-(KT_{\text{exc}})^{-1}$ [16]. In this work, the plasma temperature was determined by using the total intensity of the different strong lines as Fe, Mg, and Ca in the visible region. Figure 5 shows the plasma temperature

FIGURE 5: Boltzmann plots for different elements present in group A and group B.

obtained for both the groups. The results are summarized in Table 1.

3.2. Electron Density. Plasma near the LTE condition could be characterized by a single temperature describing the distribution of species in energy levels, the population of ionization stages, or the kinetic energy of electrons, ions, and atoms. In this case, the excitation temperature would be the same as the plasma temperature. According to the Saha-Boltzmann equation the electron density can be derived from

TABLE 1: Plasma temperature and electron density for both groups.

Sample	Plasma temperature (K)	Electron density (cm^{-3})
With Pb	$17613 \pm (14\%)$	9×10^{15}
Without Pb	$16140 \pm (18\%)$	10×10^{15}

the intensity ratio of two lines corresponding to different ionization stages of the same element [17, 18]:

$$n_e = \frac{2(2\pi m_e kT)^{3/2}}{h^3} \frac{I_{mn}^I A_{jk} g_j^{\text{II}}}{I_{jk}^{\text{II}} A_{mn} g_m^{\text{I}}} e^{-(E_{\text{ion}} + E_j^{\text{II}} - E_m^{\text{I}})/KT}, \quad (2)$$

where E_m and E_j are the upper level energies of neutral and single ionized transitions. E_{ion} is the ionization energy and n_e is the electron density. In practice, thermodynamic equilibrium is really hard to achieve in the whole area while it can exist in fine regions of plasma which are referred to as Local Thermodynamic Equilibrium (LTE). This condition is normally reached during adequate number of collisions where the energy is distributed more equally between colliding particles with the same masses. Thus, LTE may exist at high electron density limit given by McWhirter criterion [19, 20]:

$$n_e (\text{cm}^{-3}) \geq 1.6 \times 10^{12} [T_{\text{exc}} (\text{K})]^{1/2} [\Delta E (\text{eV})]^3, \quad (3)$$

where ΔE is the energy difference between the two levels [21]. Table 1 shows an estimation of the electron density for both groups. The electron density was calculated using the above standard relation. We based our estimate on the Ca line at 452.69 nm; its spectroscopic parameters are tabulated in [19]. An equality expression related to the electron density was used to estimate whether LTE is likely to prevail in our measurements. The value $4.5 \times 10^{15} (\text{cm}^{-3})$, which, according to [12, 19], is needed for LTE to exist, is well below the values encountered in our experiments. This suggests that the analytical measurements in our study were most likely carried out under LTE conditions.

4. Qualitative Analysis of Elements

The following are the dominant elements available in both samples. The elements with prominent emissions common to both bone types are Ca, Mg, and Fe. The content of these elements in bone is illustrated in Figure 6 for both groups.

Previous study shows that the Calcium (Ca I, Ca II) is the main source for the consumption of lead in bone [22]. This effect is clearly observed in Figure 3. Although the lead concentration is augmented itself, the portion of lead is consumed via chemical reactions to reduce Calcium. The results for Magnesium (Mg I, Mg II) are similar to Calcium. Magnesium like Calcium competes with lead for absorption in bone. So as expected, Mg I and Mg II decreased in sample of group A [23]. There is irregular reduction process for absorption in Fe I and Fe II. Unlike Ca and Mg, Iron element increased in some recorded wavelengths when the rats consumed lead. This behavior was observed in the

FIGURE 6: Comparison of relative intensity of different element presented in both groups.

previous work [24]. However, a decrease in Fe element at some lines is observed for group A.

In conclusion, results revealed that temperature and electron density of plasma resulting from laser beam are similar for both lead and nonlead groups and they obey Local Thermodynamic Equilibrium (LTE) conditions. The effect of its absorption in elements such as Calcium, Magnesium, and Fe had been recognized, although Laser-Induced Breakdown Spectroscopy (LIBS) method for measuring absorbed lead having such concentration in bone lacks proper performance. Qualitative comparison in value of elements in both the groups revealed that values of Ca I and Ca II and Mg I and Mg II were decreased by absorbing lead. Although there is an indirect relation for Fe concentration with absorption of lead at various wavelengths, it revealed that values of minerals such as Fe I and Fe II were globally decreased.

Competing Interests

The authors declare that they have no competing interests.

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