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Effect of experimental conditions on surface hardness measurements of calcified tissues via LIBS

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Abstract This paper reports on the effects of LIBS experimental conditions on the measurement of the surface hardness of calcified tissues. The technique mainly depends on a previously demonstrated correlation between the intensity ratio of ionic to atomic spectral lines and the hardness of the target material. Three types of calcified tissues have been examined, namely enamel of human teeth, shells, and eggshells. Laser-induced breakdown spectra were obtained under two different experimental conditions. In the first nano and picoseconds, laser pulses were used in a single-pulse arrangement, while in the second, single- and double-pulse regimes with nanosecond laser excitation were utilized. The results show that the ionic to atomic spectral line intensity ratios are higher in the case of picosecond laser pulse for both Ca and Mg spectral lines. This effect has been justified in view of the repulsive force of the laser-induced shock waves which depends clearly on the target surface hardness and on the laser irradiance. The electron densities ratio (pico/nano) is shown to be strongly depending on the laser irradiance too. In the case of calcium, single-pulse ratios are higher than the double-pulse ratios, while there is no appreciable difference between both in the case of magnesium. The results obtained herein suggest that double-pulse nanosecond arrangement and the choice of a minor element such as Mg furnishes the best experimental conditions for estimating the surface hardness via LIBS spectra. To validate this method, it has been applied on two previously measured groups of teeth enamel, the first is of ancient Egyptians, and the second from Nubians and Ugandans. The results support the usefulness of this method for similar real-life applications.

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1 Introduction

Calcified tissues are important materials from the biological point of view for humans, animals, and marine life. Environmental conditions, dietary habits, and age have strong and direct effects on the physical properties of such materials, composed mainly of calcium carbonate and/or hydroxyapatite [1]. One of the important parameters is the surface hardness of the calcified tissues. Crystalline structure, density, presence of some minor and/or trace elements, and the age are the factors determining the hardness of calcified tissues such as teeth enamel, marine shells, and eggshells [2]. So, it is a useful task to measure its surface hardness in order to have a better insight about the internal and external conditions to which the investigated samples have been exposed. It is also well known that calcified tissues keep adsorbed elements in archival way, so ecological follow-up of the region in which the calcified tissues are found or grown up will be feasible via measuring a property relevant to its elemental structure, namely its surface hardness.

Conventionally, surface hardness is measured mechanically using Vickers testers. Such instruments measure directly the Vickers hardness number (VHN) for the material investigated [3]. The technique is destructive, time consuming, and relatively expensive. Abdel Salam et al. [4] demonstrated that it is possible to apply the laser-induced breakdown spectroscopy (LIBS) technique in order to measure the surface hardness of calcified tissues. It is well known that

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LIBS is originally a spectrochemical technique of elemental analysis introduced to the scientific and technological community more than twenty years ago [5, 6]. LIBS technique has been used in a wide variety of analytical applications for the qualitative, semi-quantitative, and quantitative analysis of different materials. For measurements of physical properties of materials, LIBS has been used only to assess the hardness of concrete samples of different degrees of humidity [7] and for surface hardness of calcified tissues [4]. In fact, the analytical performance of LIBS depends strongly on the choice and optimization of the proper experimental conditions [8]. The major experimental parameters of interest in any LIBS setup are:

1. Laser parameters, e.g., wavelength, pulse duration, irradiance.
2. Ambient conditions, e.g., ambient gas nature and pressure.
3. Experimental arrangements, e.g., the observation time and measurement arrangements (single and/or double pulse).

The above parameters can also affect the utilization of LIBS to measure the surface hardness, since such measurements depend mainly on the information obtained from the LIBS spectra which is the raw material of the analytical information too. It has been demonstrated that there is a remarkable correlation between the target hardness and the intensity ratio of ionic to atomic emission spectral lines [4, 7]. In case of calcified tissues CaII/CaI and MgII/MgI, intensity ratios have been estimated and used to compare between the hardness of different types, namely, enamel of human teeth, shells, and eggshells. The results of such measurements have been interpreted in view of the repulsive force of the laser-induced shockwaves that is found to depend clearly on the target surface hardness. The use of a minor element such as magnesium was found to be more advantageous than calcium, which is a major element with more than 90% abundance. This is, of course, because of the self-absorption effect, which is strongly reduced in case of minor elements, but not for major ones.

In the present work, we report on the effect of changing some experimental conditions on hardness measurements of calcified tissues performed via estimation of the intensity ratio of ionic to atomic spectral lines in laser-induced plasma spectra. The experimental conditions investigated were the laser pulse duration and the experimental arrangements, namely single- and double-pulse LIBS. The obtained optimum experimental conditions will be used in some real-life applications.

2 Experimental

In order to confirm the assumption that the speed of expansion of the ablated atoms is proportional to the sample surface hardness, an experiment was conducted using three types of samples of different hardness, i.e., teeth enamel, shells, and eggshells. Two experimental conditions have been used. In the first conditions, laser-induced plasma is produced via picosecond and nanosecond laser pulses, while in the second conditions, double-pulse LIBS arrangement is exploited and compared with the single-pulse results in case of nanosecond laser pulses.

2.1 Picosecond experimental setup

The employed excitation source was a Q-switched Nd:YAG laser (EKSPLA Sl 312M, Lithuania) delivering 150 ps pulses each of 50 mJ energy at $\lambda = 1064$ nm. A 10 cm focal length plano-convex quartz lens was used to focus the laser beam onto the target surface. The light emitted from the plasma plume was collected via a quartz optical fiber (core diameter 0.6 mm, length 1.5 m), the free terminal of the fiber was positioned about 15 mm above the sample surface at a 45^{o} angle with respect to the surface normal, while the other terminal was coupled to an 0.32 m imaging spectrograph (TRIAX-320, ISA) equipped with a 600 grooves/mm diffraction grating (spectral resolution 0.4 nm, range 80 nm). The spectra were recorded via an intensified charge-coupled device (ICCD) detector (DH520, Andor Technology, Ireland), which is gated by means of a pulse generator (DG-535, Stanford Research, USA) in order to discriminate the atomic emission from the continuum background present at early times of the plasma evolution. The time delay between the laser firing time and triggering the ICCD was 500 ns, and the gate width was also 500 ns in all the experiments. Each spectrum is the average of five spectra obtained by accumulation of five shots taken at fresh locations on the target surface.

2.2 Single- and double-pulse experimental setup

The instrument integrates a dual-pulse double-head Nd:YAG laser (Continuum PVI, USA) operating at the fundamental wavelength (1064 nm). The laser delivers two collinear pulses of variable energy between 10 and 50 mJ per pulse at a maximum repetition rate of 10 Hz with an inter-pulse delay time which can be set from 0 to 60 μs. The pulse width was 7 ns for both laser pulses. A 10 cm focal length plano-convex quartz lens was used to focus the beam onto the sample surface. During the experimental measurements, the pulse energy was set to 50 mJ per pulse with 1 Hz repetition rate. The inter-pulse separation was varied between the values 0 (single pulse) and 2000 ns (double pulse), and both the delay time and gate width were 2 μs. Each spectrum is the average of five spectra obtained by accumulation of five shots taken at fresh locations on the target surface. The light emitted from the plasma plume is collected via a quartz optical fiber with an aperture of 200 μm and fed to an echelle spectrome-

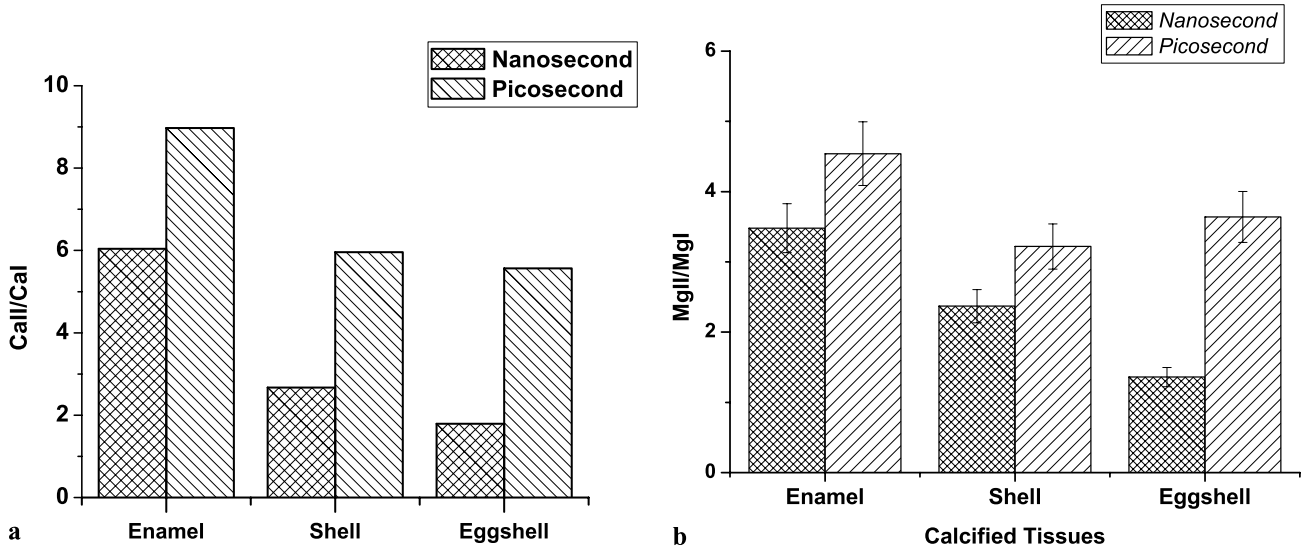

Fig. 1 **a** The ratio of the ionic calcium line intensities at 373.69 nm to the neutral line at 428.9 nm CaII/CaI for the three types of calcified tissues for both nanoseconds and picoseconds laser pulses. **b** The ratio of the magnesium ionic line intensities at 280.26 nm to the neutral line at 285.22 nm MgII/MgI for the three types of calcified tissues for both nanoseconds and picoseconds laser pulses

ter (Princeton Instrument, SE200PI-HO) covering the wavelength range 190–1100 nm with a constant spectral resolution (CSR) of 3100. The spectrometer is coupled to an ICCD camera (Princeton, I MAX). Data acquisition and analysis are performed using the data analysis spectroscopic software Grams version 8 (Galactic industrial, Salem, NH, USA).

3 Results and discussion

3.1 Picoseconds and nanoseconds laser pulse experiments

The intensity ratios between the ionic calcium line at 373.69 nm and the neutral line at 428.9 nm and between the magnesium ionic line at 280.26 nm and the neutral line at 285.22 nm are obtained for human teeth enamel, shells, and eggshells LIBS spectra. The chosen calcium lines are to a great extent self-absorption free since they are nonresonant and have low partial self-absorption coefficients $K_R(\lambda)$ that can be obtained by multiplying the self-absorption cross-section of such lines (SL in m^2) by the lower-energy level population (C in s^{-1}) [9, 10], $K_R(\lambda) = 2.5001 \times 10^{-7}$ $m^2\,s^{-1}$ and 5.0078×10^{-7} $m^2\,s^{-1}$, respectively, as calculated in a previous work [4]. For magnesium, self-absorption is minimal, since this element has low concentration in the three investigated target materials. The histograms in Figs. 1a and 1b depict the ratio CaII/CaI and MgII/MgI for the three types of calcified tissues for both nanosecond and picosecond laser pulses. Though the trend is the same for the two elements and for both laser pulse durations, the ratio is systematically higher in case of ultrashort laser pulses. This is mainly because the repulsive force of the picosecond laser-induced shockwaves for each calcified tissue target type is higher than that produced by the nanosecond laser pulses. Such a mechanism, in turn, leads to an appreciable increase in the ionic species on the account of the neutral ones and consequently raises the ratio of CaII/CaI and MgII/MgI in case of the ultrashort laser pulses with respect to the ratios in case of nanoseconds laser pulses. These higher ratio values can be also justified in view of the difference in the laser irradiance incident onto the target surface and the propagation velocity of the consequently produced shockwaves. In the picosecond case, the incident laser irradiance on the focal spot ($144\mu^2$) was 2.31×10^{14} W/cm^2, which was about 80 times the nanosecond laser irradiance, 2.89×10^{12} W/cm^2. The relation between the propagation velocity V of the induced shockwave and the laser irradiance I_0 is given by [13]:

$$V = \left[\frac{2(\gamma^2 - 1)I_0}{\rho_0}\right]^{\frac{1}{3}},$$

where $\rho_0 = 1.161$ g/L is the density of the ambient gas, I_0 is the incident laser irradiance, and $\gamma = 1.4$ is the specific heat ratio in the plasma [2]. The estimated velocity at normal temperature and pressure (NTP) in case of picoseconds laser is 3367.9 m/s (9.79 Mach), while its value in case of nanosecond at the same environmental conditions is 781.84 m/s (2.27 Mach). The effect of the target type on the plasma electron density n_e has been also investigated. n_e is estimated from the Stark-broadening of the spectral emission lines. The other mechanisms contributing to the line broadening (such as Doppler, natural, and Van der Waals broadenings) are negligible at the densities of interest [11].

The histograms in Fig. 2 depict the electron number density of the three calcified tissue obtained using nano-and picoseconds laser pulses. The ratio between the velocities (V_{pico}/V_{nano}) is nearly the same as that between the electron densities of the pico-and nanosecond lasers (Table 1). This shows that the electron density ratio (pico/nano) is laser irradiance-dependent in case of calcified tissues. However, Fig. 2 also shows that the harder the target material, the lower the electron density due to the fast expansion of the plasma plume because of the stronger repulsive force of the laser-induced shockwaves, as has been proven experimentally via the direct measurement of the shockwave velocities described in [4]. The same idea was previously proposed in [7].

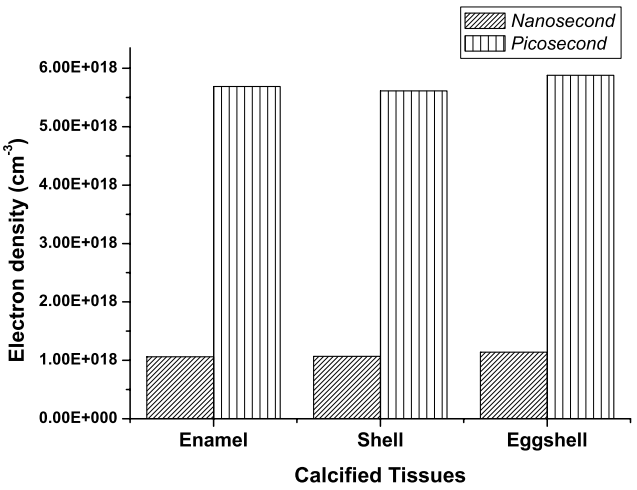

Fig. 2 The electron number density of the three calcified tissues in case of nano- and picoseconds laser pulses

Figures 3a and b respectively show the relation between the CaII/CaI and MgII/MgI ratios and the corresponding surface hardnesses (Vickers Hardness Number, VHN) of the investigated sample materials. The relation is linear and in good agreement with the results published for double-pulse experiments [4].

In [4] it has been demonstrated that in order to minimize the self absorption effect, it is better to avoid the host element (Ca) with its very high abundance of more than 95% in the three calcified tissues and adopt a minor element such as magnesium (<1.5%). The magnesium ionic and atomic lines at 280.26 and 285.22 nm, respectively, have been chosen, in view of their expected optical thinness [12]. The two lines of magnesium are resonant lines; however, the expected self-absorption was negligible because of the element's low concentration as we mentioned before. Comparing Figs. 3a and 3b, it is clear that the quality of the linear proportionality in case of magnesium is less than that of the calcium. This can be attributed to the fact that, in the case of picosecond laser pulses, the plasma plume is expanding faster, and the ionic species density is less than in the nanosecond case. This fast expansion phenomenon affects MgII/MgI ratio more than CaII/CaI ratio because of the originally much higher concentration of the calcium atoms.

Table 1 Ratio of the Shockwave (SW) velocity and Electron density (ne) for the case of picosecond and nanosecond laser pulse irradiation of different types of calcified tissues

SW velocity	ne_{pico}/ne_{nano}		
V_{pico}/V_{nano}	Enamel	Shell	Eggshell
4.3	5.13	4.01	4.01

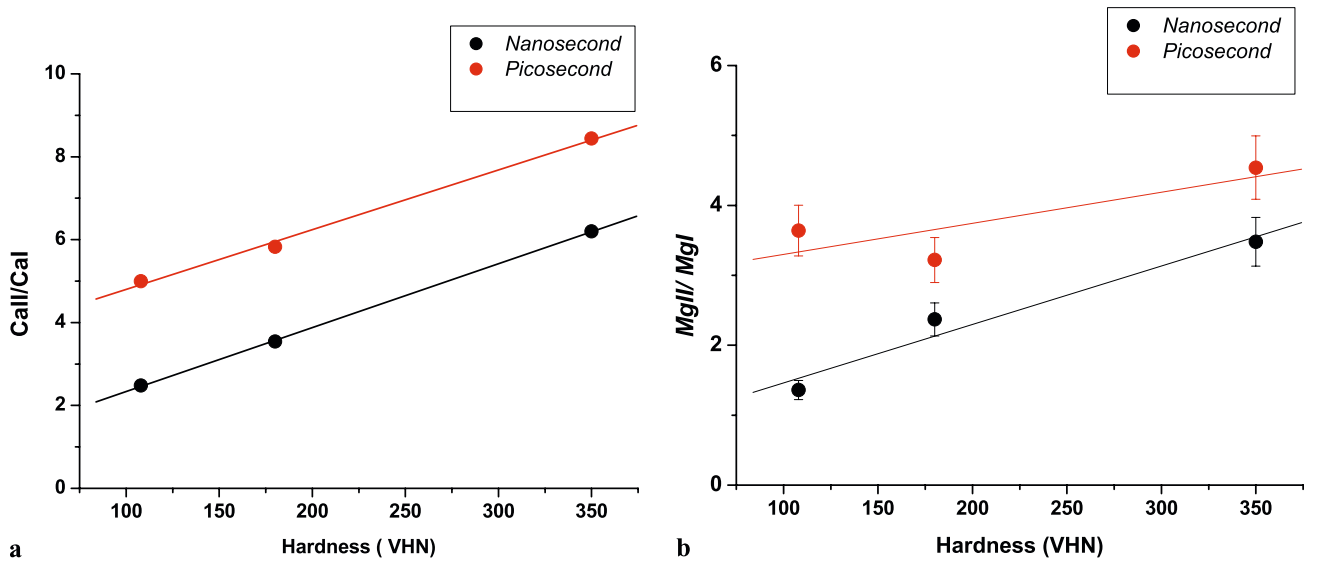

Fig. 3 **a** The relation between the CaII/CaI ratios (same spectral lines used in Fig. 1a) and the corresponding surface hardness. **b** The relation between the MgII/MgI ratios (same spectral lines used in Fig. 1b) and the corresponding surface hardness

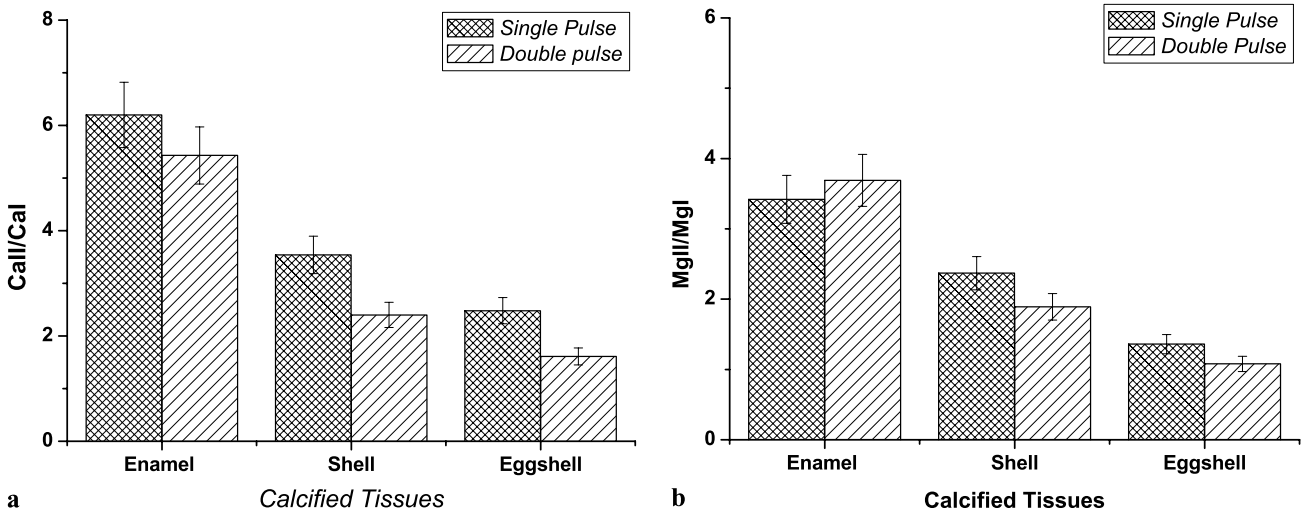

Fig. 4 **a** The ratio of CaII/CaI obtained for the different materials using single and double pulse. **b** The ratio of MgII/MgI obtained for the different materials using single and double pulse

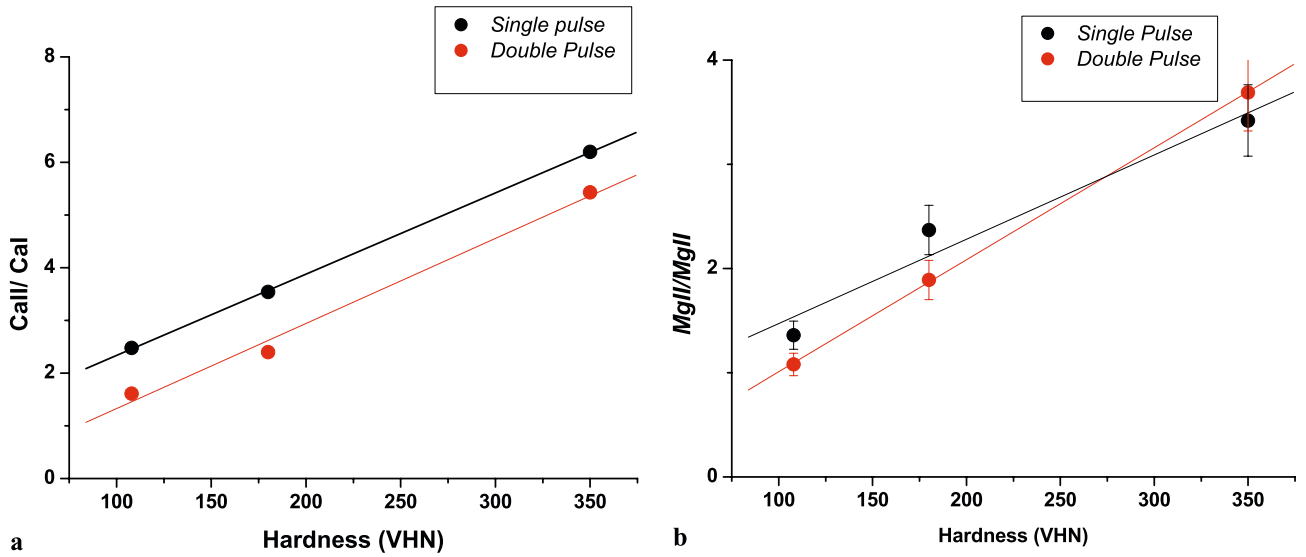

Fig. 5 The relation between the target material hardness and the intensity ratios of the ionic to atomic spectral lines in case of **a** calcium and **b** magnesium

3.2 LIBS single and double pulse enhancement

The histograms in Figs. 4 a and b show respectively the values of the ratio CaII/CaI and MgII/MgI obtained for the different materials using single- and double-pulse LIBS. The trend of the ratios is highest for enamel and lowest for eggshells for both elements. It is noticeable that single-pulse ratios are higher than the double-pulse ones in case of calcium, while there is no appreciable difference between both in case of magnesium. This is also clear in Figs. 5a and 5b, which depict the relation between the target material hardness and the intensity ratios of the ionic to atomic spectral lines in the case of calcium and magnesium. The relation between CaII/CaI and corresponding hardness of the measured sample materials shows that the single-pulse ratios of CaII/CaI are higher than that of the double-pulse ratios, while both are increasing linearly with the hardness. In the case of magnesium (Fig. 5b), the proportionality relation still holds, but there are no differences between the ratios of MgII/MgI for double- and single-pulse within the range of the experimental error (10%).

In [13] it has been shown experimentally that, in case of double-pulse mode, the second laser pulse, coming two microseconds after the first pulse (similar to our case), initiates a new plasma at the sample surface, which very rapidly propagates moving away from the laser focusing point on

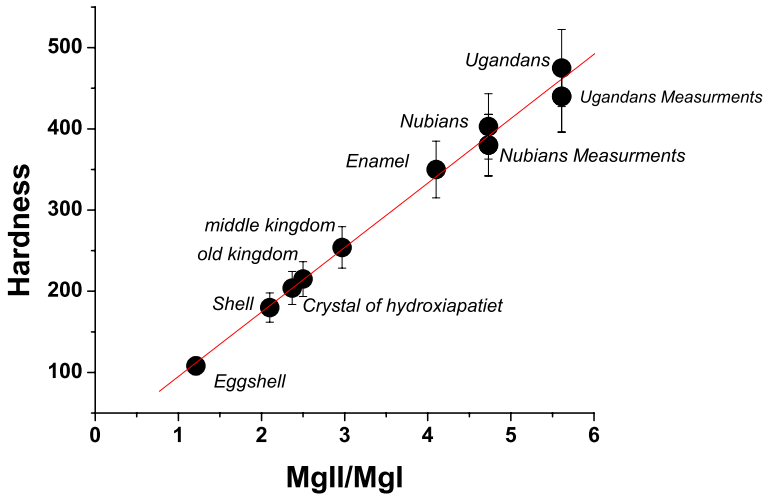

Fig. 6 The values of the ionic to atomic magnesium spectral lines of some samples of unknown hardness (Crystal of hydroxyapatite, Ancient Egyptian teeth enamel, and Nubian and Ugandan teeth enamel) have been displayed on the linear relation between MgII/MgI versus the hardness

the target surface and almost fills the rarefied volume behind the shock wave front formed by the first pulse. The evolved plasma plume in this case is nearly three times larger than that obtained in the single-pulse case [14]. This means that the repulsive force affecting the laser induced plasma in case of the double pulse is a strong function of the target hardness, contrary to the case of single pulse in which the shock wave plays a major role in the plume expansion. This effect pronounced in the double-pulse case is, as mentioned above, caused by the rarefied surrounding gas present in the bubble produced by the first pulse, as described by the point strong explosion theory, which depicts a sharp decrease of the ambient gas density behind the shock-wave front [15]. So the second plasma expands during the first few microseconds at high velocity because of the low ambient gas density. The momentum P of the ablated mass in the plasma plume is given by $P = m(t)V$ with m given by [16]

$$m(t) = A(aIt) + B(aI)^2 t^{3/2},$$

where A and B are constants related to the thermal properties of the target material, a is an energy coupling factor, I is the laser irradiance, and t is the laser pulse duration. This equation shows that m can be considered the same for the three types of calcified tissues since they have nearly the same thermal properties. Therefore, the momentum of the ablated mass will be a function of the plasma expansion velocity V, which depends in this case mostly on the target hardness. Due to the fact that magnesium is a minor element in the three calcified tissues, its mass in the expanding plume will be much less than that of calcium, however, there will be no big difference between the numbers of Mg atoms in the plume in cases of single and double pulse, contrary to Ca. This effect interprets the behavior of the ionic to atomic line intensity ratios in Figs. 4, 5.

It is clear that the results confirm what is previously concluded [4] that the double pulse nanosecond experimental arrangement is the best for estimating the surface hardness and choosing a minor element such as magnesium is better.

3.3 Applications

The above-described technique can be used to estimate the unknown surface hardness of any calcified tissue sample using a constructed calibration curve of a number of calcified samples of known (measured) hardness. This is illustrated in the following application. The idea is to make use of the above investigations in differentiating between calcified tissues according to their age or according to the race and/or the environment it has been exposed to since all these parameters can affect the surface hardness. In a previous research work [17], a detailed LIBS investigation has been performed on the enamel of groups of teeth of ancient Egyptians from the middle and old dynasties (2800 and 3400 BC, respectively). We just made use of the relevant spectra to estimate the average of MgII/MgI in 50 spectra in each case in addition to the same ratio for teeth samples of recent Egyptians living in the same area (Sakkara archaeological, 35 km south of Cairo). On the other hand, there was another detailed LIBS study performed on the enamel of the teeth of groups of Nubian (living in quasi desert environment south of Egypt) and Ugandan (living in tropical environment) [2]. Again we estimated the average of MgII/MgI ratios from the LIBS spectra of both groups. In Fig. 6 the values of the ionic to atomic magnesium spectral lines of the unknown samples have been displayed on the linear relation between MgII/MgI versus the hardness. All the points fit the linear relation very well, giving reasonable values for the hardness of the unknown samples. This shows the feasibility of determining the surface hardness of unknown calcified tissues adopting a standard calibration line.

4 Conclusion

In the present work, effects of experimental parameters on the surface hardness estimation of three calcified tissues via LIBS have been studied. The investigated experimental conditions were the laser pulse duration (nanosecond and picosecond) and the experimental arrangements, namely single- and double-pulse LIBS setups. A detailed discussion of the experimental results revealed that the ratio between the intensity of the ionic to atomic spectral lines and the surface hardness depends on the laser pulse duration and the experimental arrangements (double- or single-pulse setup). The repulsive force of the laser-induced shock wave plays an important role in both nanosecond and picosecond laser excitation. However, due to the higher irradiance of the ultrashort laser pulses, the intensity ratios CaII/CaI and MgII/MgI are found to be higher than those of the short laser pulses. The electron densities ratio (pico/nano) is similarly dependent on the laser irradiance in the studied cases. On the other hand, comparison of the single- and double-pulse results showed that in the case of single pulse, the ratios of CaII/CaI are higher than in case of double pulse, while there is no appreciable difference between both in case of magnesium. This effect is mainly due to the fact that the momentum of the ablated mass will be a function of the plasma expansion velocity. In general it has been shown that it is feasible to make use of the double-pulse nanosecond experimental arrangement with minor element to estimate the surface hardness via LIBS spectra. In addition, the proposed method has been used successfully to evaluate the surface hardness of naturally aged teeth enamel of ancient Egyptians from two dynasties (middle and old) and of the enamel hardness of Egyptian Nubians and Ugandans. The results are reasonable and in a very good agreement with hardness measurements performed on calcified tissues.

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