SPATIALLY RESOLVED LASER INDUCED BREAKDOWN SPECTROSCOPY IN ORTHOGONAL DOUBLE PULSE CONFIGURATION AND LASER ABLATION INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY OF ARCHAEOLOGICAL FINDINGS

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Keywords: laser ablation, inductively coupled, plasma, mass spectrometry, laser induced, breakdown spectrometry, orthogonal, double pulse, archaeological, tooth

1. Introduction

Local analysis of dental calcified tissues plays important role in medicine, toxicology, environmental science, paleontology and anthropology. In these fields of action a bulk analysis of a mineralized material from a defined and well-localized volume belongs to a marginal stream. On the other hand, spatially resolved analyses of calcified tissues as teeth or bones without any previous decomposition have been routinely performed with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)[1–10], X-ray fluorescence (XRF)[11–13], particle (proton) induced X-ray emission spectroscopy (PIXE)[14,15], secondary ions mass spectrometry (SIMS)[16,17] or even formerly electron microprobe analysis (EMP)[18,19].

Nowadays, Laser Induced Breakdown Spectroscopy (LIBS) becomes a widely employable technique in analytical chemistry. It can be used for elemental analyses of samples of any state (i.e. gas, solid and liquid)[20]. In last ten years the double pulse configuration (DP) became a great importance due to some advantages as intensity enhancement of emission lines[21]. Up to now, several combinations were tested. The four basic are: *i*) two consequent coaxial laser pulses (but perpendicularly to the sample) – the first one ablates the sample and the second one even more feeds the microplasma induced by the first one, *ii*) the first one ablates the sample and the second one feeds the plasma perpendicularly, *iii*) the first pulse is collinear with the sample and induces a plasma by a breakdown of the atmosphere and the second one strikes the sample perpendicularly through the breakdown plasma, *iv*) any other spatial non-orthogonal configuration. The typical time gap between the pulses is 1–10 μs.

Due to a transient behaviour of the microplasma some non-linear effects occur at analytical measurements with LIBS and finally demonstrate themselves as nonlinear calibration curves (intensity *vs.* element content). These phenomena are matrix-dependent and limit the use of LIBS to mostly semi-quantitative precision. Nevertheless, it is commonly used for *in situ* analysis of cultural heritage objects[22]. For spatially resolved analysis of bones and teeth it has been still rarely used[23].

The main advantage of the DPLIBS is a substantial enhancement of the sensitivity against the single pulse LIBS. This approach is still rarely applied to analysis of artifacts[22]. To the best knowledge of the authors the DP variant has been never used for the analysis of calcified tissues.

Thus, one can expect that a valuable comparison of LA-ICP-MS with DPLIBS will be to some extent feasible. This is also the main goal of this work. It aims to compare whether the spatial distributions of some major and minor elements in a reindeer tooth section yielded by both the methods are equivalent.

2. Experimental

2.1. LA-ICP-MS Instrumentation

The measurements were realized using an ICP-MS Agilent 7500ce. It was operated under standard conditions with forwarded rf power of 1350 W without the collision cell. A New Wave Research (Laser Ablation Systems, USA, UK) UP 213 facility was used for laser ablation. The basic source was a Nd: YAG laser emitting the fundamental wavelength 1064 nm which is consequently frequency quintupled to the 213 nm one and the lasing beam is homogenized to a flat-top energy cross section profile. The impact laser pulse fluence 12 J cm^{-2} at the beam diameter of 100 μm was selected with respect to yield an appropriate signal levels for both major and minor elements. The produced aerosol was transported into the ICP-MS with 1 l min^{-1} helium carrier gas through a Tygon tubing (i.d. 4 mm, length about 1.5 m) and behind the ablation cell it was mixed with 0.6 l min^{-1} argon make-up gas. The flow rate was stabilized with a Brooks mass flow controller.

With the LA-ICP-MS, three rows of craters were ablated in a single-spot mode with repetition frequency of 10 Hz during 45 s so that 450 pulses were executed on each spot. The mutual distance of crater centres was 0.15 mm which was controlled with an x, y translation stage (a sub-component of the UP 213 facility). The sum signal for each crater was processed and depicted in a graph as one point (all Figs.).

2.2. Double pulse LIBS Instrumentation

The first (ablation) pulse was executed with a UP 266 Macro laser (New Wave, 266 nm flat top beam) with 8 mJ and the second one with a Quantel Brilliant (1064 nm) laser with 125 mJ pulse energy. The triggering signal from the UP 266 laser flash lamp was led to an external delay generator DG 535 to control the gap between the pulses (0.1 μs) and between the flashlamp ignition and the Q-switch of the Quantel laser to control its pulse energy. Between the pulse generator and the final destination of the delayed signal a pulse repetition rate divider was placed and each 20th pulse was used. The delay between the 2nd pulse and the spectra

measurement (1 μs) was controlled with an in-built delay generator of a Triax 320 spectrometer.

The microplasma emission was delivered with an optical bundle to the Triax 320 monochromator (Horiba JY, ICCD, entrance slit 50 and 100 μm, grating 2400 mm^{-1}). The spectral range in the used setting was about 19 nm for simultaneous measurements.

The sample was ablated vertically and the very small primary microplasma was excited horizontally (orthogonal configuration) using a quartz lens with 80 mm focal length. The produced craters were also about 0.1 mm in diameter and spaced 0.15 mm. 4 pulses per crater were executed and the sum signal processed and depicted in graphs (all Figs.). After one crater line was finished the measured spectral interval was changed and the same crater line was ablated again. As a result, each crater was made by 4×7 pulses because 7 spectral intervals were necessary to cover all the requested spectral lines.

2.3. Sample

A reindeer tooth (right 2nd upper molar, era: upper paleolithic, 29-21 000 BP) from Moravany-Lopata, Western Slovakia, was chosen due to a well-defined structure with several lamellas. It was embedded with a polyester resin, cut lengthways and polished (all Figs. background).

3. Results and discussion

Spatial distributions of four key elements are presented: Ca, Mg, Sr, Zn. They are biologically very important. Some isotope ratios: Ca 44/Mg 25, Ca 44/P 31 and Sr 88/Ca 44 were calculated from the LA-ICP-MS calibration measurement using a NIST 1486 bone meal standard pressed in a pellet (Fig. 1).

The other Figs. 2–4 depict maximum-normalized intensities in order to highlight relative changes in an appropriate scale. All the scans are fitted to the structure of the analyzed tooth (Figs. 1–4). The structural description of the tooth is marked in Fig. 1.

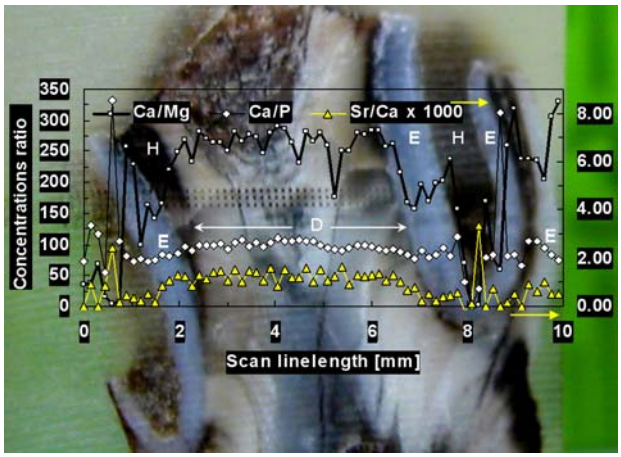

Fig. 1. **LA-ICP-MS line scan. Concentration ratios of selected elements are depicted. The Sr/Ca ratio refers to the right axis.** Additionally, the tooth structure is marked: E…enamel; H…hole, empty area with resin; D…dentin. The depicted scans refer to the upper crater line

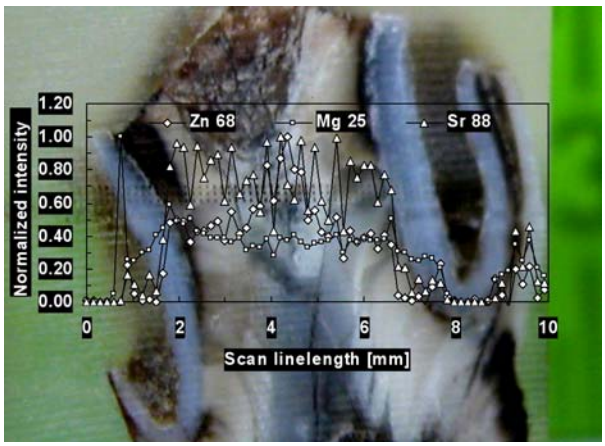

Fig. 2. **LA-ICP-MS line scan.** Maximum normalized intensities of the selected elements are depicted

3.1. Calcium and strontium

The major element is calcium. It is a component of hydroxyapatite which is a main constituent of any calcified tissue. It is also a main matrix element which can be used as an internal standard.

In the tooth structure, a slight increase of strontium is observable from the outer part of the enamel towards the dentine. In the dentine, its content gradually decreases towards the pulp[24–26]. These facts are confirmed by the results of our analysis except for the pulp which was out of the scanned area (Figs. 1, 3, 4). Strontium is not an essential element and has no specific biochemical or physiological function in organisms. Its metabolism is analogical to the calcium one. More than 90 % of calcium is accumulated in calcified tissues. In contrast to the calcium its content in a body is not under a homeostatic control[7]. Strontium may substitute calcium and thus the ratio Sr/Ca increases in case of calcium deficiency. The ratio Sr/Ca in bone reflects the Sr/Ca in consumed food and consequently the bone Sr/Ca ratio is higher for herbivores than for carnivores.

3.2. Magnesium

The magnesium content is substantially higher in the dentine than in the enamel[17–19,29,30] which is also in accordance with the presented results (Figs. 1–3). Possible significant increase of magnesium at the beginning of the scan can indicate the presence of the tartar (Fig. 2). This increase is commonly not observable, e.g. in the DPLIBS profile (Fig. 3) it is absent. This fact probably signalizes some local differences in the tartar presence. Magnesium is an important constituent of calcified tissues and plays a key role in the early phase of the development of the mineral component[27,28]. Its content slightly increases from the enamel surface and the increase continues also in the dentine.

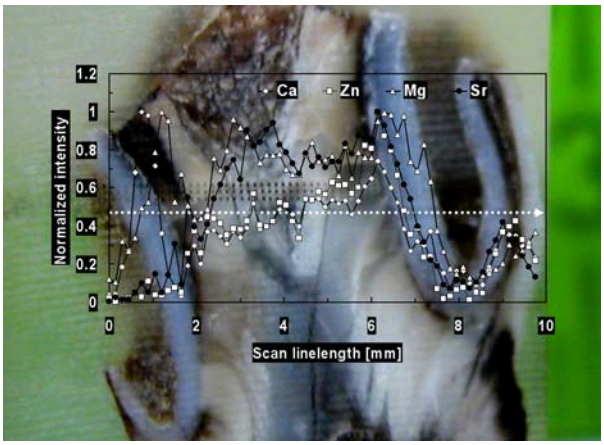

Fig. 3. **DPLIBS line scan.** The crater line is marked with the dotted arrow. The craters are not very deep and thus they are invisible. Maximum normalized intensities of the selected elements are depicted. Used spectral lines [nm]: Ca I 335.020, 335.035; Mg I 285.21; Sr II 407.77; Zn I 330.29

3.3. Zinc

The initial increase of zinc is well observable in the LA-ICP-MS profile (Fig. 2) while the DPLIBS scan yielded a substantially weaker and nearly negligible peak (Fig. 3). Again, this fact can be attributed to the different position of the LA-ICP-MS and DPLIBS scans (see and compare the calcium distributions by LA-ICP-MS and DPLIBS in Figs. 1 and 4). The initial zinc increase is, however, significantly lower in comparison with the magnesium maximum (Fig. 2). Zinc is an essential element which is extremely important for the growth and the mineralization of bones and teeth[32,33] and may be also related to sanative processes. It is preferentially accumulated in surface layers of the enamel[7,9,12,24,25,26,34], as well as in the dentine[9]. Besides, the zinc content increases from the boundary enamel-dentine towards the pulp[8,24].

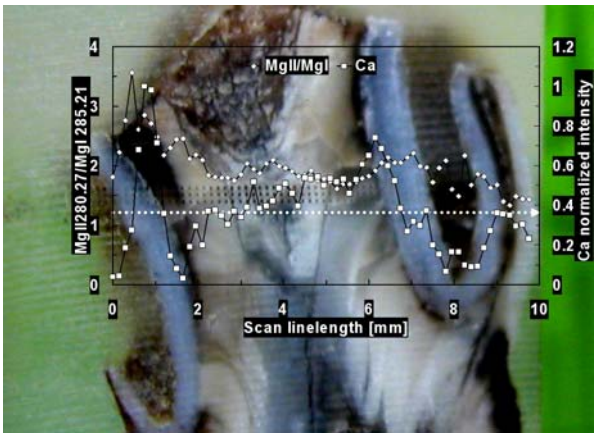

Fig. 4. **DPLIBS line scan.Tooth hardness local changes across the ablated path**

3.4. Tooth hardness monitoring

Both these methods are based on LA sampling and thus they may suffer from some matrix effects. The scan line crosses all parts of the tooth and these have various hardnesses. In this aspect the leader is the enamel, the second is the dentine and the softest is the pulp. The ablation can then be more efficient in the dentin thus delivering more material into the excitation source. The consequent precise quantification of the elemental contents in various parts of the tooth would be quite problematic. This difficulty can be partially solved by use of the Mg II 280.26 nm / Mg I 285.22 nm ratio or by a calcium ionic to atomic line ratio[35]. This ratio increases with the hardness of the tissue. In our case it is applicable only to the DPLIBS method. It really shows that the highest ratio well corresponds with the enamel position (Fig. 4). The other variations are not so much significant.

4. Conclusion

The presented comparison of DPLIBS with LA-ICP-MS and with already published results showed that the DPLIBS method in the presented particular orthogonal configuration can be successfully used for the mapping of the spatial distributions of biologically important elements as Ca, Mg, Sr, Zn. It can be applied as a suitable alternative to the routinely used LA-ICP-MS method due to the sufficient spatial resolution and sensitivity to the minor elements. These properties are not obtainable for single pulse LIBS.

The authors wish to thank Miriam Fišáková from the Institute of Archaeology Brno for samples and the Czech Ministry of Education, Youths and Sports for the projects MSM0021622411 and ME08002.

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