Laser ablation methods for analysis of urinary calculi: Comparison study based on calibration pellets

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ABSTRACT

Methods based on laser ablation, such as Laser-Induced Breakdown Spectroscopy (LIBS) and Laser-Ablation Inductively Coupled Plasma Mass/Optical Emission Spectrometry (LA-ICP-MS/OES) are particularly suitable for urinary calculi bulk and micro analysis. Investigation of spatial distribution of matrix and trace elements can help to explain their emergence and growth. However, quantification is still very problematic and these methods are often used only for qualitative elemental mapping. There are no commercially available standards, which would correspond to the urinary calculi matrix. Internal standardization is also difficult, mainly due to different crystalline phases in one kidney stone.

The aim of this study is to demonstrate the calibration capabilities and examine the limitations of laser ablation based techniques. Calibration pellets were prepared from powdered human urinary calculi with phosphate, oxalate and urate matrix. For this comparative study, the most frequently used laser-ablation based analytical techniques were chosen, such as LIBS and LA-ICP-MS. Moreover, some alternative techniques such as simultaneous LIBS–LA-ICP-OES and laser ablation LA-LIBS were also utilized.

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1. Introduction

Laser ablation based analytical techniques, namely Laser-Induced Breakdown Spectroscopy (LIBS) and Laser-Ablation Inductively Coupled Plasma Mass/Optical Emission Spectrometry (LA-ICP-MS/OES) are the subject of increasing popularity especially in the last two decades. Some of the many benefits offered by these techniques include the ability to analyze virtually any kind of solid material, high sensitivity and high spatial resolution, which enable scanning microanalysis of material surfaces [1–6]. However, limitations due to matrix effects and calibration difficulty are well known.

In the case of LIBS, different mechanical, physical and chemical properties of samples can result in serious matrix effects because the resulting laser-induced plasma is a product of the very complicated process of laser–matter interaction. LIBS can be highly sensitive to these undesirable effects also because of the high density of material in the plasma [4,5]. For these reasons reliable calibration is possible only if homogeneous samples of very similar composition and morphology are analyzed, all experimental conditions are unchanged, and standard reference samples of very similar composition and structure are available [7,8].

LA-ICP-MS/OES techniques require the transport of the dry aerosol formed in the ablation process into inductively coupled plasma via carrier gas. Therefore, the signal can be adversely affected not only by unwanted processes during laser-ablation but also by particle fractionation during transport of aerosol from the ablation chamber to ICP source [9,10]. The so-called fractionation effect origins of non-stoichiometric analysis may arise from different processes: particle production from laser ablation, particle transport from the ablation cell to the ICP plasma and particle vaporization process in the ICP plasma [11,12]. To overcome fractionation occurring throughout the whole LA-ICP system, many solutions have been studied [13]. Nevertheless to improve accuracy and precision, internal standards and matrix matching are very often used to obtain quantitative results.

LA-LIBS utilizes two independent laser processes to analyze the sample. The first laser pulse has a similar role as the LA laser in LA-ICP techniques and in contrary to the traditional LIBS or double-pulse (DP) LIBS [1–6] this laser is not used to create the atomic emission signal. The ablation plume is then passed using a carrier gas to second, analytical laser-induced plasma (LIP). The novelty of the proposed LA-LIBS approach is in the complete decoupling of the sampling LA process from the analytical LIBS plasma. This technique was proposed as a novel analytical scheme in order to minimize of the sample matrix effects and signal nonlinearities associated with traditional LIBS [14].

To understand the formation of urinary calculi and their growth, it is important to know their mineralogical structure and elemental composition. For these purposes, many analytical methods such as X-ray fluorescence spectrometry and X-ray powder diffraction [15–18], infrared spectroscopy with Fourier transform [19–22], PIXE and PIGE [23–25], Raman spectroscopy [26] or SIMS [27] were used. Surface analysis of urinary calculi was performed using polarizing microscopy, scanning electron microscope and electron microprobe [28–30]. For the total elemental composition, atomic absorption spectrometry (AAS) was used [31]. To accomplish these techniques, methods based on laser ablation are particularly suitable for investigation of spatial distribution of matrix and trace elements. This can help to explain the emergence and growth of urinary calculi. Formation of kidney stones is basically caused by elements in abundance dissolved in the urine. Urinary calculi are divided according to matrix type into four categories, namely a) oxalates, b) phosphates c) uric acid and d) cystine. The oxalate calculi are formed by whewellite ($CaC_2O_4.H_2O$) and weddellite ($CaC_2O_4 \cdot 2 H_2O$) and accounts about 70% [32]. The phosphate kidney stones are based on struvite ($MgNH_4PO_4 \cdot 6 H_2O$) and apatite ($Ca_5(PO_4)_3(OH)$; $Ca_5(PO_4,CO_3)_3(OH)$) that constitute about 15–20% and 5–10% respectively. Occurrence of the other uroliths represents 10% uric acid ($C_5H_4N_4O_3$), and 1% cystine calculi ($C_6H_{12}N_2O_4S_2$) [33]. The kidney stones are also frequently occurring in mixture. The most commonly emerging mineral in kidney stones in Czech Republic is whewellite (about 73.2%). The second most common mineral is uric acid (19.6%). The third group is a mixture of whewellite, weddellite, apatite, struvite and/or uric acid (about 7.2%) [34].

The article of Pineda et al. was focused on elemental mapping of urinary stones and creating the basic maps of elemental distribution [24]. Correlations were observed between Mn, Fe, Cu, Zn and Sr in relation to Ca. These correlations showed two possible ways in distribution of these trace elements among whewellite and weddellite. Some trace elements, like As, Se, Br and Pb were evenly distributed.

The spatial distribution of elements in urinary calculi has been also examined by LA-ICP-MS using line scans [35,36]. LIBS technique was used for qualitative analysis of kidney stones in different locations (nucleus, outer shell and surface stone). Quantitative estimation of the trace elements as Cu, Mg, Zn, and Sr contents in different parts of the calculi by LIBS was also described. This method was unusable for Ca line scanning, because calibration curves for Ca were nonlinear due to the high Ca content. Commercially available standards were used for calibration [37,38].

The main purpose of this article is to demonstrate the calibration capabilities and limitations of LA-based techniques applied for spatially-resolved elemental mapping on urinary calculi cross sections. The calibration difficulty of these techniques due to matrix effects and lack of appropriate reference materials is generally known. However, information about spatial distribution of investigated elements is very often much more important than accurate (average) content measurements and semi-quantitative estimation is sufficient. From this perspective calibration with an acceptable uncertainty can be performed using calibration pellets prepared from urinary calculi, for which the bulk analysis was performed by another technique. For this comparative study the most frequently used LA-based analytical techniques were chosen, such as LIBS and LA combined with mass or optical ICP spectrometry. Moreover, some alternative techniques such as simultaneous LIBS–LA-ICP-OES and LA-LIBS were also utilized.

2. Experimental

2.1. Simultaneous LIBS and LA-ICP-OES

The simultaneous LIBS and LA-ICP-OES setups were designed by utilizing a modified and commercially available laser ablation system UP 266 MACRO (New Wave) that consists of a pulsed Nd:YAG laser operated at a wavelength of 266 nm. The original ablation chamber was replaced with a special laboratory made chamber, which is equipped with a window for collection of LIP emission. The radiation was transported by a fiber optic system onto the entrance slit of a Czerny-Turner monochromator (Jobin Yvon, TRIAX 320) equipped with an ICCD detector (Jobin Yvon, Horiba). This combination provides a spectral resolution about 0.1 nm. The detection delay time and gate width were optimized during preliminary experiments and were set for all measurements as 1 μs and 10 μs, respectively. The spectral data were collected using five spectral windows centered at 280, 315, 450 and 588 nm for detection of emission lines Mg I 285.2 nm, Ca II 315.9 nm, Sr I 460.6 nm, Ba I 458.0 nm and Na I 588.5 nm, respectively.

As a carrier gas for transport of ablated aerosol to laterally viewed ICP-OES spectrometer (JobinYvon, 170 Ultrace) argon was used, at a flow rate of 0.6 l.min^{-1}. The 40-MHz generator was operated at a forward power of 950 W. The ICP system was equipped with a polychromator Paschen Runge (measured emission lines Mg II 280.274 nm and Ca II 393.366 nm) and monochromator Czerny Turner (measured lines Sr II 407.771 nm, Ba II 455.403 nm and Na I 588.995 nm). Mode ON-PEAK and no background corrections were used.

The ablation experiments were performed at a laser pulse repetition rate of 10 Hz, pulse energy of 20 mJ and ablation spot size of 100 μm. The ablated area was enlarged by moving the laser beam on 1 mm diameter circle with duration of 120 s for each measurement. Due to ICCD throughput and integration time, ICP measurement signals integrated for 1 s from 100 measured values were chosen for analytical evaluation of LIBS and LA-ICP-OES techniques.

2.2. LA-LIBS

For the LA-LIBS measurements, the ablation laser was a Nd:YAG laser (Big Sky, CFR 400) operating at 355 nm, 40 mJ/pulse, with a focal lens of 100 mm, focused about 25 mm below the pellet surface.

The ablation chamber was custom made from a stainless-steel vacuum chamber of a diameter equal to 3.5 cm, and the pellets were mounted to a rotation stage, pellet rotation 0.25 s^{-1}. The laser beam was translated across the pellet surface, to produce ablation tracks of different radii, thereby sampling new material.

An approximately 3.2 mm inside diameter tube allowed the argon carrier gas to enter and exit the sample chamber on opposite sides, transporting the ablation particles directly to the free-standing LIP in the adjacent sample chamber. A 6.5 cm length of tubing was used to connect the ablation chamber to the analytical LIBS chamber, thereby minimizing any sample losses during transport. A vacuum pump was used to draw argon gas through the ablation chamber and the LIBS sample chamber, with a minimum pressure drop, leaving the LIBS chamber about 0.75 Torr below atmospheric pressure. The flow rate of Ar was 0.1 l min^{-1}.

For the analytical LIBS plasma, a Nd:YAG laser (Continuum Precision II) operating at 1064 nm, 200 mJ/pulse, and focused by lens 75 mm focal length was used to create the plasma. The plasma emission was then collected by pierced mirror 100 mm^2, and a collimation lens of 100 mm focal length was used to collect and launch the emission into a fiber optic bundle, which was subsequently coupled to a Czerny Turner spectrometer and detected with an ICCD detector (0.15 nm spectral resolution).

The ablation and analytical plasma laser pulses were synchronized in time using a digital delay generator, and the carrier gas flow rate was adjusted for optimal transport from the ablation chamber to the analytical LIBS plasma. Optimization was realized by maximizing the analyte emission signal-to-noise ratio. It is noted that with the two laser pulse synchronized to fire at the same time, the analytical