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Elemental and Molecular Analysis of Metal Containing Biomolecules Using Laser Induced Breakdown Spectroscopy and Sonic Spray Ionization Mass Spectrometry: A Step Towards Full Integration and Simultaneous Analysis

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Abstract

A novel methodology is proposed that combines sonic spray ionization (SSI) mass spectrometry (MS) with laser induced breakdown spectroscopy (LIBS) for analyzing metal-containing biomolecules and complexes. Focusing pulses from a nanosecond laser (Nd:YAG, $\lambda = 1064$ nm) in the microdroplet ensemble produced by a pneumatic nebulizer yielded LIBS spectra that enabled highly sensitive detection of several metal ions in aqueous and aqueous methanolic solutions. Based on the calibration curve method, LOD values at the ng/mL level were achieved for Ca (15 ng/mL), Ba (27 ng/mL), Cu (67 ng/mL) and Fe (650 ng/mL) with accuracy greater than 90%. LIBS measurements were performed for the first time on aerosols of solutions of known biomolecules such as superoxide dismutase and alpha-lactalbumin, which led to the reliable determination of the concentration of Cu and Ca, respectively, both in the range of a few $\mu\text{g/mL}$. In parallel, the relative molecular mass of the metalloproteins was determined by separate SSI-MS measurements performed using an identical pneumatic nebulizer based sample introduction system. This is a first step towards the ultimate aim of integrating the two analytical techniques by use of a single pneumatic nebulization system for simultaneous sample introduction for both LIBS and SSI-MS. Such a system is expected to greatly enhance our capabilities to simultaneously acquire molecular and atomic data.

Keywords: Laser-induced breakdown spectroscopy; Sonic Spray Ionization Mass Spectrometry, Aerosols, Metalloprotein

Introduction

The necessity to further understand the biotransformation of metals and metalloids in biological systems has promoted elemental speciation analysis to become a vital bioanalytical procedure. This has been assisted by the tremendous progress made in the development of novel analytical methods, mainly based on advanced hyphenated techniques, incorporating a separation using gas chromatography (GC) or liquid chromatography (LC) coupled on-line with an element specific detector such as inductively coupled plasma mass spectrometry (ICP-MS) and/or a molecular detector such as electrospray tandem mass spectrometry (ES-MS/MS). [1–8]

As a result, significant capabilities for the speciation of As, Se, Sb and several other elements have been developed and applied, especially for the characterization of small biomolecules[9–13]. This has allowed for relatively high throughput, almost routine elemental speciation analysis for several elements in several different sample types. However, little progress has been achieved in the detection of metals present in larger biomolecules such as peptides or proteins, especially for high throughput, high coverage analyses. Metalloproteins, for example, constitute a large group of proteins that essentially avoid direct detection of their metal content during the most popular bottom-up MS-based proteomics analysis, in which proteins are digested, peptides are detected and proteins are identified from database searches. This approach only allows for the indirect identification of metalloproteins, since their metal content is not determined and is thus not verified. For direct metal determination a separate analysis is therefore required. However, once again it is difficult to associate the determined metal content to any specific protein, unless the protein has been isolated. Molecular MS of intact proteins (native mass spectrometry) can in some cases provide this vital information. However, even in such cases, limitations exist, most importantly concerning difficulties in detecting monoisotopic elements present in proteins, or metals in high molecular weight proteins or protein complexes, especially when the mass spectrometer does not provide sufficient resolving power. In addition, quantification of the metal present in the protein is extremely difficult.

Such limitations promote the need for a more integrated approach towards acquiring molecular and atomic data during the analysis of biological systems in order to detect the presence of metal and metalloid containing biomolecules, as well as to investigate interactions of metal species with biomolecules. The objective of this study is to explore the possibility to develop a combined molecular and atomic detection system suitable for analyzing metal-containing biomolecules and complexes. To achieve this we have investigated the use of sonic spray ionization (SSI) mass spectrometry (MS) in combination with laser induced breakdown spectroscopy (LIBS), thus exploring the possibility to fully integrate them.

Sonic Spray Ionization Mass Spectrometry was selected in this case for molecular detection, as opposed to the widely used electrospray source, because it offers many attractive features making it ideal for coupling with LIBS [14,15]. First

of all there is no need to use a pump for sample injection (because of self-aspiration) or high voltage and heat for droplet and gas-phase ion formation. The production of positive and negative charged ions occurs simultaneously, the signal-to-noise ratio is high, molecular fragmentation is absent, formation of dimers is negligible and it provides high sensitivity.

Laser induced breakdown spectroscopy, on the other hand, is a very convenient technique for detecting and monitoring metallic ions. It offers several important advantages such as the capability to analyze samples in all three phases, solids, liquids or gases with minimal or zero sample preparation, it is non-destructive, and enables multi-element detection [16–18]. The relatively straightforward experimental setup for LIBS permits development of compact, versatile units that can be easily combined with other instrumentation or even operated as mobile instruments for in situ analysis of materials [19]. While most applications focus on the analysis of solid samples, several researchers have demonstrated the benefits of using LIBS for probing liquids, aerosols, particulates and gases [20,21]. The simplicity of operation and speed of data analysis makes LIBS an attractive choice for fast elemental analysis and an excellent one for combination with SSI-MS.

Probing of aerosols by LIBS has attracted the interest of researchers since the early 1980's and was mainly motivated by the need to improve LIBS performance in liquid sample analysis. Converting a liquid to aerosol has clear benefits arising from the generation of plasma in a gaseous rather than a bulk liquid environment. Plasma quenching processes are drastically reduced while contamination of optics due to splashing is totally eliminated. It is noted that Cremers [22] and co-workers in 1983 reported the analysis of Be, Na, P, As and Hg containing aerosols in air with LOD values in the low $\mu\text{g/mL}$ range. Likewise, Omenetto [23] and co-workers studied Na containing sulfuric acid aerosols by LIBS and showed the potential of the technique for probing contaminants in industrial plants. Furthermore, Hahn and Buckley [24–26] have made systematic investigations on the mechanisms associated with plasma formation in aerosols and in particular plasma-droplet (or particle) interactions with the aim of understanding the role of key experimental parameters in enhancing the analytical performance of LIBS in aerosol monitoring.

In the present study we have investigated the combined use of SSI-MS and LIBS, and discuss the benefits of the proposed analytical approach, reporting for the first time both molecular and atomic data from the analysis of metalloproteins in water-methanol solutions. At this stage LIBS and SSI-MS were applied separately. In both systems, the sample was analyzed in the form of aerosol that was generated by means of a pneumatic nebulization system, operated under nearly identical conditions, as regards sample flow rate, gas flow and solvent matrix. SSI-MS analysis provided the relative molecular mass of the protein whereas LIBS led to the determination of the protein metal content. It is noted that optimization of experimental parameters, relevant to LIBS analysis of the aerosol, permitted the accurate determination of metal ions at the sub- $\mu\text{g/mL}$ range. This work constitutes proof of principle for the

possibility of establishing combined and simultaneous LIBS and SSI-MS analysis using a single sample introduction system.

Experimental Section

Aerosol LIBS: A standard LIBS experimental arrangement was employed that was properly modified in order to accommodate a pneumatic nebulizer as a sample source in the laser-sample interaction area (Fig. 1). In brief, pulses at $\lambda=1064$ nm ($\tau=8$ ns), from a Q-switched Nd:YAG laser (Series 5000, BMI, France), focused via a plano-convex lens ($f = +50$ mm), lead to plasma formation within the volume of an aerosol spray, generated in ambient conditions by the pneumatic nebulizer (TR-30-C1, Meinhard®, Golden, CO, USA). Argon is used as a nebulization gas, at a pressure of 60 psi, producing a fine aerosol spray from an aqueous solution. The liquid microdroplets, typically formed by pneumatic nebulizers, have a relatively tight size distribution centered at approximately 10 μ m in diameter and expand in a conical geometry with a vertex angle of approximately 35 degrees [27]. In the present study the pneumatic nebulizer used was modified by inserting a 10-15 cm piece of fused silica capillary (100 μ m i.d. x 200 μ m o.d.) resulting in a free aspiration with a sample flow rate of 20 μ L/min. This is expected to result in microdroplet clouds with densities on the order of 10^6 droplets/cm³ and expansion velocities in the range of 30-80 ms⁻¹, as already reported for conventional pneumatic nebulizers [27]. The laser beam intersects the aerosol at 90 degrees with respect to the axis of the spray cone. Both the laser beam propagation and the nebulizer axes lie in the horizontal plane. Precise adjustment of the nebulizer position with respect to the laser beam focus is achieved by means of a x-y-z micrometer resolution translation stage. In the current measurements the tip of the nebulizer is positioned at a distance of $d=2$ mm from the laser beam axis, while the spray axis is shifted by a distance of $h=2$ mm in front of the laser beam focal point. The latter is critical in order to ensure optimum laser-spray interaction and avoid breakdown taking place outside the spray cone or at the edge of it. The exact location of the laser beam focal point is determined by observing plasma forming in air at near breakdown threshold conditions.

Figure 1. Schematic of the LIBS experimental setup (upper) and detail showing the position of the nebulizer and the aerosol spray expansion cone with respect to the focusing laser beam (lower).

Typical laser pulse energy values in this series of measurements have been in the range of 190-200 mJ, corresponding to an energy density of approximately $6.5 \times 10^3 \text{ J cm}^{-2}$ at the focus or equivalent power density of $8.1 \times 10^{11} \text{ W cm}^{-2}$. It is noted that, under these irradiation conditions, strong plasma emission is observed both in the presence of the aerosol and in air. The light emitted by the plasma plume is collected vertically by use of a telescope system consisting of two fused silica lenses ($f=+50$ mm) and imaged at 1:1 magnification on one end of a quartz optical fiber (diameter: 600 μm) coupled, with its other end, to a Czerny-Turner imaging spectrograph (PTI, 01-002AD). The spectrograph is equipped with two holographic diffraction gratings, 300 grooves/mm and 1200 grooves/mm, covering a spectral range of 280 nm and 70 nm with a resolution of 0.4 nm and 0.1 nm, respectively. An intensified CCD detector (ICCD, Andor iStar DH740-18F-03) attached to the spectrograph was employed for the time-resolved acquisition of the plasma spectral emission. The laser - detector synchronization was achieved via a digital delay generator (DG535, Stanford Research Systems) triggered by the output signal from a fast photodiode detecting the incoming laser pulses. The photodiode signal defined the “time zero” or start point of each measurement and the delay generator provided a trigger pulse at a variable time delay (τ_D) with respect to the laser pulse activating the ICCD and determining the signal acquisition time interval or gate window, τ_G . The influence of the temporal acquisition parameters on the quality of the data (signal-to-noise and signal-to-background ratio) was investigated and the optimal values used in this study were $\tau_D=20 \mu\text{s}$ and $\tau_G=100 \mu\text{s}$. All spectra reported herein correspond to the average of 5 sets of 200 single-laser shot spectra accumulation. The laser operates at a repetition rate of 5 Hz.

Mass Spectrometry: The experimental setup employed for the SSI-MS measurements (Figure 2), consisted of a prototype SSI source (Elemental Scientific Inc., Omaha, USA) fitted with the same pneumatic nebulizer as the one used in the LIBS experiments, mounted onto an ion trap mass spectrometer (LCQ Advantage, Thermo Scientific). The nebulizer was modified as stated for the LIBS experiments, i.e. insertion of fused silica capillary, and operated using nitrogen gas at a pressure of

60 psi corresponding to a gas velocity higher than 1 Mach. The use of this SSI source for molecular mass spectrometric characterization of metalloproteins, peptides and coordination compounds has been reported in previous studies. [28,29] The ion transfer capillary temperature was set to 300 °C and the acceleration potential, which directs the ions to the mass analyzers was set to 20 V. The mass spectrometer covers a range from 100 to 2000 m/z with a resolution of 1 m/z. Due to the relatively narrow mass detection range of the spectrometer, extensive protonation of the protein is necessary so as to reduce the m/z ratio and for this reason, acetic acid was used as a proton source at a concentration of 2-5% v/v. The ion trapping time was typically set to 400 ms, and integration was conducted on 3 scan events.

Figure 2. Detail from the SSI-MS system showing the nebulizer positioned in front of the mass spectrometer inlet.

ICP-MS: The concentration of calcium and copper in protein solutions was also determined using ICP-MS as a reference method to check the validity of LIBS measurements. For this, a quadrupole ICP-MS (NexION 300xx, PerkinElmer, Shelton, CT, USA) was used to measure isotopes ^{43}Ca , ^{63}Cu and ^{65}Cu . Protein solutions containing these metals were introduced into the ICP-MS using a peristaltic pump and analyzed. Concentrations were determined using external calibration.

Materials

Calibration standards: Aqueous stock solutions were prepared by dissolving appropriate amounts of analytical grade chloride, nitrate or sulfate salts of metal ions (CaCl_2 , $\text{Ba}(\text{NO}_3)_2$, CuSO_4 , FeCl_3 purchased from Aldrich) in 18 M Ω ·cm ultrapure water or a 1:1 mixture of ultrapure water-methanol, a more favorable solvent system for molecular mass spectrometry measurements. Standard solutions were prepared fresh by dilution with ultrapure water from the stock solutions.

Metalloprotein standards: Superoxide dismutase (SOD) was purchased from Aldrich (S7571-75KU). The protein consists of two identical subunits and is known to bind two copper(II) ions per molecule one on each subunit [30]. SOD standards were prepared by dilution of an appropriate amount of the protein in water or 1:1 water methanol mixture. α -Lactalbumin (LALBA) was purchased from Aldrich (L5385-100MG) and used without any further purification. The protein contains calcium (II) ions in 1:1 stoichiometry [31]. Solutions of the proteins for analysis were prepared by dissolving appropriate amounts of solid protein in a 1:1 mixture of ultrapure water-methanol.

Results and Discussion

In order to ensure optimum sensitivity and reliability for the LIBS analysis of metal containing proteins, a series of experiments, investigating the influence of several critical parameters on the signal strength and spectra quality, were conducted. Considering the demands for reaching low LOD values for metal ion analysis, compatible with typical sample concentrations used in molecular mass spectrometry, maximum exploitation of the analyte mass in the aerosol droplets is of critical importance. This was achieved by employing laser pulses of relatively high energy, in the range of 190-200 mJ, for plasma formation. Under these conditions, a strong continuum emission is produced immediately upon arrival of the laser pulse at the aerosol spray, which persists for approximately 10 μ s, when atomic emission lines start growing (Fig. 3). It is noted that emissions from the metal analyte survive until *ca* 60-80 μ s after the laser pulse. The signal-to-background ratio (S/B) was found to be optimal for $\tau_D > 15$ μ s. Thus LIBS spectra were collected with a time delay, $\tau_D = 20$ μ s, and for a time gate, $\tau_G = 100$ μ s, in an effort to exclude contribution of the continuum at early times and exploit as much as possible the atomic emission signal in order to enhance the sensitivity of the analysis.

Figure 3. Time evolution of the LIBS signal originating from Cu I emission ($\lambda = 324.754$ nm) and from the continuum background collected at 323.2 nm (dashed line indicates change of scale for the continuum emission data from the left to the right intensity axis). Spectra were collected with $\tau_G = 500$ ns. Sample: aqueous solution, $[\text{Cu}^{2+}] = 1.0 \times 10^{-4}$ M).

Positioning the laser pulse focus with respect to the spray axis is also critical for achieving efficient sampling of the aerosol droplets and for maximizing the laser-spray interaction. Examining the longitudinal position (d) of the laser pulse focus with respect to the nebulizer tip, it was found that signal intensity decreased with increasing d , due to the reduction of droplet density arising from the conical geometry of the spray expansion (Fig. 4). A short value of d , approximately 2 mm from the tip, was selected for all measurements. Considering next the transverse arrangement, it is noted that because of the high pulse energy of the laser, plasma starts forming slightly in front of the actual focal point of the laser beam. Therefore, in order to ensure that the laser pulse interacted optimally with the aerosol and symmetrically with respect to

its cone axis, the nebulizer was adjusted in a way that the laser focal point was located at $h=2$ mm past the spray axis. (See Fig. 1, lower)

Figure 4. Emission intensity from Cu I ($\lambda = 324.754$ nm) as a function of the distance, d , between the tip of the nebulizer and the laser beam propagation axis (dashed line is a guide to the eye). Sample: aqueous solution, $[\text{Cu}^{2+}] = 2.0 \times 10^{-4}$ M.

Several test measurements were carried out under this geometry and single-shot LIBS spectra were collected with aqueous solutions of CuSO_4 . Each spectrum exhibited distinct emission lines of the metal analyte demonstrating that each and every laser pulse samples the spray. The relative standard deviation of such single-shot measurements was found to be on the order of 30-80%, in sets of 200 spectra, with the higher variance observed in samples with concentration near the LOD. However the %RSD of the emission intensity corresponding to the mean of 5 different measurements, representing averages of 200 spectra each, was less than 5%. This was further verified by measuring a wide range of aqueous and/or methanolic (1:1 $\text{H}_2\text{O}:\text{CH}_3\text{OH}$) solutions of metal ion salts. As a result, the present experimental geometry and signal acquisition parameters were maintained throughout the experiments reported in this study.

Following this LIBS methodology, spectra were collected from solutions containing Ca, Ba, Fe and Cu ions at different concentrations (Fig. 5) and the appropriate emission lines were selected for analysis (Table 1). In all spectra, besides the sharp atomic lines, intense broad band emission features are evident arising from excited diatomic species such as CN ($\text{B}^2\Sigma^+ \rightarrow \text{X}^2\Sigma^+$), NH ($\text{A}^3\Pi \rightarrow \text{X}^3\Sigma^-$) or OH ($\text{A}^2\Sigma^+ \rightarrow \text{X}^2\Pi$) forming in the plasma plume [32],[33]. These bands can be a source of interference with the atomic line signals particularly at low levels of analyte concentration. Calibration curves (Fig. 5) obtained from these metal ion solutions exhibit excellent linearity ($R^2 > 0.99$). LOD values, determined on the basis of the 3σ method [34], were found to be in the tens of ng/mL range (Table 2) for Ca, Ba and Cu and about an order of magnitude higher for Fe the latter mainly due to the low intensity of the Fe emission lines. These LOD values demonstrate the high analytical sensitivity of the current methodology. Finally, validation measurements led to determination of metal ion concentration in aqueous and/or methanolic solutions with accuracy (% Recovery) greater than 95%. In comparison with the present work, Almirall [35] and co-workers, working with a similar nebulizer, have reported LOD

values of 0.6 and 0.7 µg/mL for Ca and Ba, respectively, using a double-pulse LIBS approach (two laser pulses at 532 nm with 65 mJ of energy each, temporally separated by 50 ns with respect to one another). Also, Yalcin [36] and co-workers employed an ultrasonic nebulizer coupled with a membrane dryer that fed the droplets (diameter < 1.5 µm) into a small chamber where they were probed by single-pulse LIBS (laser pulse energy at 532 nm, in the range of 20-150 mJ). LOD values for several elements were found to be in the µg/mL range. Similarly, Groh et al [37] performing LIBS measurements on single droplets (diameter: 40-50 µm) have achieved LOD values as low as 0.05 µg/mL for the case of Ca ions in H₂O.

Table 1. Emission lines used in the LIBS analysis of metal ions^a

Element	Emission line (nm)	Transitions	Energy (cm ⁻¹)
Cu I	324.754	$3d^{10}4p^1 \rightarrow 3d^{10}4s^1$ $^2P_{3/2} \rightarrow ^2S_{1/2}$	30783.70–0.00
Fe I	302.064	$3d^74p^1 \rightarrow 3d^64s^2$ (y^5D_4) → (a^5D_4)	33095.94–0.00
Ca I	422.673	$(3p)^6(4s)^1(4p)^1 \rightarrow (3p)^6(4s)^2$ (1P_1) → (1S_0)	23652.30–0.00
Ba II	455.403	$(5p)^6(6p)^1 \rightarrow (5p)^6(6s)^1$ ($^2P_{3/2}$) → ($^2S_{1/2}$)	21952.40–0.00

a Data from NIST Atomic Spectra Database [38]

Figure 5. Calibration curves and indicative LIBS spectra for a) Ca²⁺ in H₂O), b) Ba²⁺ in H₂O), c) Cu²⁺ in 1:1 CH₃OH-H₂O, and d) Fe³⁺ in 1:1 CH₃OH-H₂O.

Table 2. LOD values and results of validation study for metal ions analyzed by LIBS.

Eleme nt	Emission line (nm)	LOD (μg/mL)				Test sample concentration		% Recovery ^d
		Curren t work	[35]	[36]	[37]	(M)	(μg/mL)	
Cu I	324.754	0.033	---	1.9 9	---	4x10 ⁻⁵	2.5	100.3
Fe I	302.064	0.65	---	---	---	5x10 ⁻⁵	2.8	95.6
Ca I	422.673	0.015	0.6 b	1.8 3 ^a	0.0 5 ^a	1x10 ⁻⁵	0.4	101.2
Ba II	455.403	0.027	0.7 c	---	---	7x10 ⁻⁶	0.1	100.7

^a value determined on the basis of the 393.366 Ca II emission line

^b value determined on the basis of the 396.847 Ca II emission line

^c value determined on the basis of the 493.409 Ba II emission line

^d % Recovery = [Recovered (Measured) Concentration / True Concentration]x100%

Metalloprotein analysis

Two metal containing biomolecules, superoxide dismutase (SOD) and α -lactalbumin (LALBA), were studied using both LIBS and SSI-MS to demonstrate the complementarity of the information obtained by the two methods. In addition, it was our objective to show that both detection systems could be operated efficiently using an identical microflow pneumatic nebulizer for sample introduction. Solution concentrations were the same for both analyses.

LIBS measurements were performed in solutions of SOD (1.66 mg/mL) and LALBA (0.62 mg/mL) in ultrapure H₂O - CH₃OH matrix (50% v/v), a solvent system commonly used in mass spectrometric studies. The concentration of the analyzed protein is in the range of a few mg/mL, which is a typical concentration used in mass spectrometry experiments. Prior to MS analysis, solutions were acidified by addition of acetic acid at 5% v/v.

The SSI mass spectrum of SOD obtained in water (Fig. 6A) shows a series of peaks in the m/z range of 1000 – 2000 corresponding to the protein in different charge states. Deconvolution of these spectra revealed the molecular mass of SOD to be 15598.2 Da. This in fact represents the molar mass of one of the two identical subunits of the protein, which is known to dissociate into its subunits in an acidic environment. The concentration of Cu²⁺ ions in the analyzed SOD solution was measured by LIBS and was found to be 4.91×10^{-5} M, which was also confirmed by ICP-MS analysis. On the basis of the molecular mass measured and the mass to volume concentration of the SOD solution, the molar concentration of the SOD (dimer) solution is calculated to be 5.3×10^{-5} M. Taking into account that each SOD subunit can bind one Cu²⁺ ion [30], the bound Cu concentration of the protein solution, provided that each SOD dimer contains 2 Cu atoms, would be expected to be 10.6×10^{-5} M. This turns out to be approximately a factor of 2 higher compared to what was actually measured by LIBS and ICP-MS. This discrepancy may be due to a number of factors such as protein purity or loss of metal during protein isolation and

purification. Unfortunately, the supplier does not provide any information regarding metal – protein stoichiometry for their product. Therefore the data obtained from the complimentary use of LIBS and SSI-MS reveals the molecular mass of the protein, and that not all available protein sites are occupied by Cu ions.

Similarly MS analysis of LALBA resulted in a molecular mass of 14,180 Da. Based on this value the molar concentration of LALBA, in the solution analyzed, was calculated to be 4.37×10^{-5} M and, given that the metal to protein stoichiometry is 1:1 [31], the concentration of Ca ions in the solution is expected to be the same. Analysis by LIBS determined the concentration of Ca ions to be 8.17×10^{-5} M, which was also confirmed by ICP-MS analysis. The deviation may be attributed to the use of excess of Ca^{2+} during the preparation of this reagent in order to ensure ion saturation of the protein. In the product specification sheet it is stated that the molar ratio of Ca^{2+} /LALBA is ≥ 1 .

Figure 6. Mass and LIBS spectra obtained in the analysis of a) SOD and b) LALBA solutions. In the MS analysis the solvent matrix was 5% acetic acid/water while in LIBS analysis a H_2O - CH_3OH matrix was used.

Conclusions

We have demonstrated, for the first time, the use of sonic spray ionization mass spectrometry (SSI-MS) in combination with laser induced breakdown spectroscopy (LIBS) for the analysis of metalloprotein solutions. Considering the LIBS technique,

it is evident that highly sensitive detection of several metal ions can be achieved probing the aerosol produced by a pneumatic nebulizer and this holds also for the analysis of the metalloprotein solutions. SSI-MS on the other hand permits the measurement of the molar mass of the metal-containing biomolecules under native conditions. The information about the elemental composition of the metalloproteins provided by LIBS is complementary to molar mass data obtained with SSI-MS. It permits for the improved characterization of large biomolecules using a low resolution MS or even large MDa protein complexes when using high resolution MS.

A number of technical aspects are to be further investigated, such as for example the use of a high repetition rate laser for probing the aerosol that would increase data collection rate and consequently reduce analysis time, permitting the efficient use of LIBS as a liquid chromatography detector. Such an improvement would enable high throughput analysis both at the molecular and atomic level and could open up prospects for efficient screening of metal-containing biomolecules in mixtures. Additional research is needed in order to investigate the applicability of the proposed methodology for the characterization of different types of metal-organic complexes and a broader spectrum of metal-containing biomolecules. The next step is to implement the simultaneous use of SSI-MS and LIBS, namely to employ a single pneumatic nebulization system as a sample source and carry out LIBS analysis just in the space between the nebulizer tip and the mass spectrometer inlet.

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HIGHLIGHTS

Sonic-spray ionization (SSI) mass spectrometry (MS) and laser induced breakdown spectroscopy (LIBS) were used in the analysis of metal-containing biomolecules.

LIBS measurements were performed for the first time on aerosols of biomolecule containing solutions provided reliable determination of the biomolecule metal ion content.

The results strongly suggest that LIBS can be integrated with SSI-MS leading to a fast method for obtaining simultaneously atomic and molecular data from biomolecules.