# Determination of isotopic distribution of lead by a matrix assisted laser desorption/ionization *versus* a laser desorption/ionization time of flight mass spectrometry

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*Abstract*

In this work it has been shown that both the laser desorption/ionization mass spectrometry (LDI MS) and the matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI TOF MS) are the simple and quick methods for determination of relative natural isotopic distribution of lead. The analysis of metal salts with these approaches does not require any time-consuming preparation of samples: a single run can take only a minute, and numerous information can be obtained**.** Results obtained in this work show that chosen matrix has no negative effect on quantitative determination of lead isotopes and support once more the applicability of MALDI TOF MS for lead isotope distribution determination in the sample and accurate data are obtained. Additionally, the generation of $Pb_nO_n$ and $Pb_nO_{n-1}$ ($n$: 2–6) clusters have been successfully achieved in the positive mode, using the both LDI and MALDI methods. All stoichiometries were confirmed using isotopic pattern modelling.

*Keywords*: isotopic analysis of lead, mass spectrometry, MALDI, LDI.

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Lead has four stable isotopes, three of them, $^{206}$Pb, $^{207}$Pb and $^{208}$Pb are radiogenic, being products of radiogenic decay of uranium or thorium, while $^{204}$Pb is considered stable. Stable isotope $^{204}$Pb is rarely used for quantitative analysis, due to low natural isotopic abundance and due to isobaric overlapping with isotope $^{204}$Hg, what complicates the analysis by mass spectrometry [1,2]. The most used lead isotope for quantitative analysis is $^{208}$Pb, which is the most abundant one. Isotopic distribution of lead varies in dependence of origin of the sample, its localization and the age. The ratio of four lead isotopes is useful in tracking the source of metals in igneous rocks, the source of sediments and even origin of people via isotopic distribution of their teeth, skin and bones. Lead/lead isotopes have been used in forensic science to fingerprint bullets, *via* peculiar $^{204}$Pb/$^{206}$Pb *vs.* $^{207}$Pb/$^{208}$Pb ratio unique for each batch of ammunition. Hence, this is very useful method for history, geochemistry, archaeology research, but also for monitoring of air pollution from exhausted gasses or to define the source of lead absorbed in plants, sediments, aquatic organisms. In biochemistry, it can be used as standard, which binds to biomolecules and can be used for their quantification. In addition, reactions of lead with chelating agents, such as dimercaptosuccinic acid (DMSA), 2,3--dimercapto-1-propanesulfonic acid sodium (DMPS), or alpha lipoic acid (ALA) can be monitored in toxicology [3–12].

Mass spectrometry is widely used technique for qualitative and quantitative analysis of isotopic composition of different elements, either coupled with other techniques or applied independently [13–16]. For analysis of isotopic ratios, very sensitive mass analyzers and appropriate ionization methods are required. Thermal ionization mass spectrometry (TIMS) [17–19], inductive coupled plasma mass spectrometry (ICPMS) [20], fast atom bombardment/secondary ionization mass spectrometry (FAB/SIMS) [21–23] and resonance ionization mass spectrometry (RIMS) [24] are the methods have been used routinely for detection of metals in traces. ICP MS is the most used technique [25–29], due to its high sensitivity and precision. Inductively coupled plasma (ICP) is a very "hard" ionization, it means that metal ions, metals in coordinated states, and metalloproteins in crude biological solutions can be detected efficiently as atomic ions only [30,31]. The newest ionization methods used in the mass spectrometry are electrospray ionization (ESI) and matrix-assisted laser desorption/ionization. Electrospray ionization has already demonstrated great potential in characterizing metalloproteins, but it cannot ionize metal ions them-

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selves [32]. On the other hand, matrix assisted laser desorption/ionization time-of-flight mass spectrometry has been utilized for the analysis of biological macromolecules, polymer, routine characterization of polar organic molecules, however there are a reports of its use in the analysis of inorganic species, for example, a rapid method for determination gold ($Au^{3+}$) and platinum ($Pt^{4+}$) ions in tissues [33].

Usually, in MALDI-TOF MS the sample, prepared as a solution of the matrix and analyte, is deposited on a plate and then dried in air, before it has, as the target, been inserted into the mass spectrometer, which is operating under vacuum. MALDI matrices are organic acids that can to adsorb light at the laser wavelength, for example, three most commonly used matrices for nitrogen lasers are 3,5-dimethoxy-4-hydroxycinnamic acid (sinapinic acid), *α*-cyano-4-hydroxycinnamic acid (CHCA, alpha-cyano or alpha-matrix) and 2,5-dihydroxybenzoic acid (DHB). It is important to indicate that the desorption/ionization process in vacuum conditions and under laser irradiation is not completely understood, but the most likely, there are several simultaneous processes, which result in the formation of positive and negative ions. These processes are absorption of the laser energy by the analyte and matrix, which leads to solid-gas phase transition of the sample/ /matrix. Further, gas-phase photoionization, ion–molecule reactions, disproportionation, excited-state proton transfer, energy pooling, thermal ionization, and desorption of preformed ions result in the formation of stable ions, which reach the detector. It is believed that analyte ionization occurs in the expanding plume as the direct result of interactions between analyte neutrals, excited matrix radicals/ions and protons and cations such as sodium [34]. There is often no need for a matrix to obtain good spectra, this type of analysis can be named as laser desorption/ionization or a matrix-free LDI, as no matrix is used to assist in the desorption process [32].

To date, MALDI has been applied in a growing number of cases to simple coordination complexes, for example, MALDI TOF MS is used as powerful technique for analyzing platinum and ruthenium metal complexes, potential chemotherapeutics, where characteristic isotope patterns of complexes and their adducts could be detected [35]. Recently, Liu *et al.* [36] have developed gold nanomembrane functionalized with bovine serum albumin, as substrate for the analysis of lead in biological fluids samples, using a commercial matrix assisted laser desorption ionization-time of flight mass spectrometric instrument. However, the quantitative analysis and determination of isotopic composition by this approach were not considered.

In this work, the applicability of both matrix-free and matrix-assisted laser desorption/ionization methods for qualitative and quantitative analysis of the isotope composition of lead has been investigated. Potential applications and advantages of this approach are discussed in this work.

## EXPERIMENTAL

Standard solutions of $Pb(NO_3)_2$ of different concentrations in the range 0.001–10 mg/ml were prepared in water with 1% nitric acid. Samples were mixed with the matrix *α*-CHCA in the volume ratio 2:1 and 1.5 µl was suspended on the sample plate. The solution of matrix *α*-CHCA was made in 50% acetonitrile at the concentration of 10 mg/ml. Results in earlier work are shown that the addition of TFA (trifluoroacetic acid) to the matrix solution has an important role in detecting the signals of platinum and ruthenium metal complexes [37]. For this reason TFA was added to the final concentration of 0.01%. For testing, when metal clusters of lead and alkali ions can also be detected by MALDI-TOF MS, sodium and potassium chloride were prepared as 0.01% solutions and mixed with the sample in the ratio 1:1 before adding the matrix. Applied samples were dried under the stream of warm air. All chemicals used were purchased from Sigma Aldrich (Germany) and used without further purification. MALDI-TOF mass spectra were acquired on a Voyager Biospectrometry DE Pro Workstation (PerSeptive Biosystems, Framingham, MA, USA) MALDI-TOF mass spectrometer, equipped with a 20 Hz pulsed nitrogen laser (337 nm). All spectra were recorded in positive reflector mode (voltage 20 kV), maximal mass-to-charge ratio (*m*/*z*) range 200–300, by averaging 400 laser shots at the 27% laser power. Recorded mass spectra were calibrated by setting the peak of the protonated α-CHCA matrix to appropriate value (190.05).

## RESULTS AND DISCUSSION

The mass spectra of $Pb(NO_3)_2$ obtained using both the matrix-free and the assistance of CHCA-matrix laser desorption/ionization methods, in the *m*/*z* range 200––1400, are presented in Fig 1a and b, respectively. Conserved isotopic distribution of lead and lead adducts are presented in insets.

Identities of mass peaks which appear in both spectra, LDI and MALDI, and belong to lead adducts are given in the Table 1.

From these experimental results the following tendency can be observed. At first glance, it can be concluded that laser desorption/ionization approach without matrix gives simpler spectra, better signal to noise ratio due to the absence of the additional peaks originating from matrix and matrix adducts. Lead clusters of the type $Pb_nO_n$ and $Pb_nO_{n-1}$ have been obtained in the mass range *m*/*z* 400–1400 using both LDI and MALDI

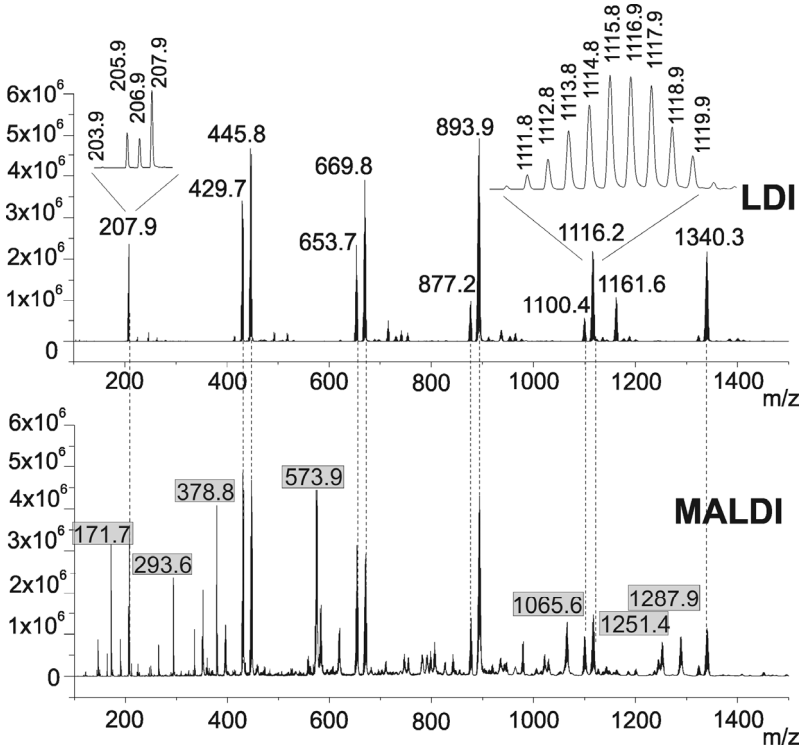


*Figure 1. The positive mass spectra of lead standard solution (5 mg/mL) obtained using LDI method (a) and MALDI method (b).*

*Table 1. List of ions observed in the positive mode from LDI and MALDI mass spectra of lead standard solution*

| MALDI | | MALDI and LDI | |
|---|---|---|---|
| *m/z* | Ion | *m/z* | Ion |
| 207.9 | $Pb^+$ | 207.9 | $Pb^+$ |
| 212.5 | Matrix adduct | 429.7 | $Pb_2^+(H_2O)$ |
| 224.9 | $PbOH^+$ | 445.8 | $Pb_2O_2$ |
| 239.4 | Matrix adduct | 653.7 | $Pb_3O_2$ |
| 240.3 | Matrix adduct | 669.8 | $Pb_3O_3$ |
| 246.9 | $PbONa^+$ | 877.2 | $Pb_4O_3$ |
| | | 893.9 | $Pb_4O_4$ |
| | | 1100.4 | $Pb_5O_4$ |
| | | 1116.2 | $Pb_5O_5$ |
| | | 1161.6 | $Pb_5O_4(NO_3)$ |
| | | 1340.3 | $Pb_6O_6$ |

methods (cf. Table 1 for their identity). This fact suggests that the matrix is not the limiting factor for their detection. The mass spectra in Fig. 1a and b show that MALDI method provides determination of isotopic distribution of lead, as well as LDI method. It has been mentioned that the main advantage of MALDI approach is the possibility to detect simultaneously inorganic species as biomolecules in the single run [38]. Moreover, for some inorganic molecules, the sensitivity of the ion detection is higher in the presence of organic matrices [39]. In addition to the signals arising from the Pb, which are detectable at identical position as in the LDI mass spectra, there are additional signal, which arise from matrix (indicated by their *m/z* and highlighted in Fig 1b). They stem from the monomer, dimmer, and higher adducts of CHCA molecule, generated by the loss of one or more $H_2O$ molecules and/or $CO_2$. Their identity is given only to demonstrate that there are no overlapping with signals of interest.

For this reason, in the next part, the test whether MALDI-TOF MS can be used for determination of isotopic distribution of lead is presented. In the Figure 2 we present positive MALDI-TOF MS spectra of lead with CHCA matrix in the *m/z* range 200–250 (Fig. 2a) and theoretical mass spectra of lead (left) and lead adduct $PbOH^+$ (the graph on the right, Fig. 2b). Theoretical mass spectra were delivered using the free software for calculating isotopic distribution of different elements – Selket.

From comparison Fig. 2a and Fig. 2b it can be seen that the mass peaks obtained in the experiment, show the characteristic pattern and intensity ratios matching the theoretical mass spectra of lead. These isotopes are: $^{204}Pb$, $^{206}Pb$, $^{207}Pb$ and $^{208}Pb$, each appearing at *m/z* values: 203.95, 205.95, 206.95 and 207.95, respectively. The presence of CHCA matrix, *i.e.*, MALDI method provides slightly better condition for detection of the isotope patterns of lead than LDI method (compare Figs. 1a and 2a). Group of mass peaks appearing at *m/z* 222.95, 223.95 and 224.95 have the same isotopic distribution as lead belong to isotope cluster $PbOH^+$, according to literature [40]. Peaks at somewhat higher values, *i.e.*, at *m/z* 244.95, 245.95 and 246.95, match

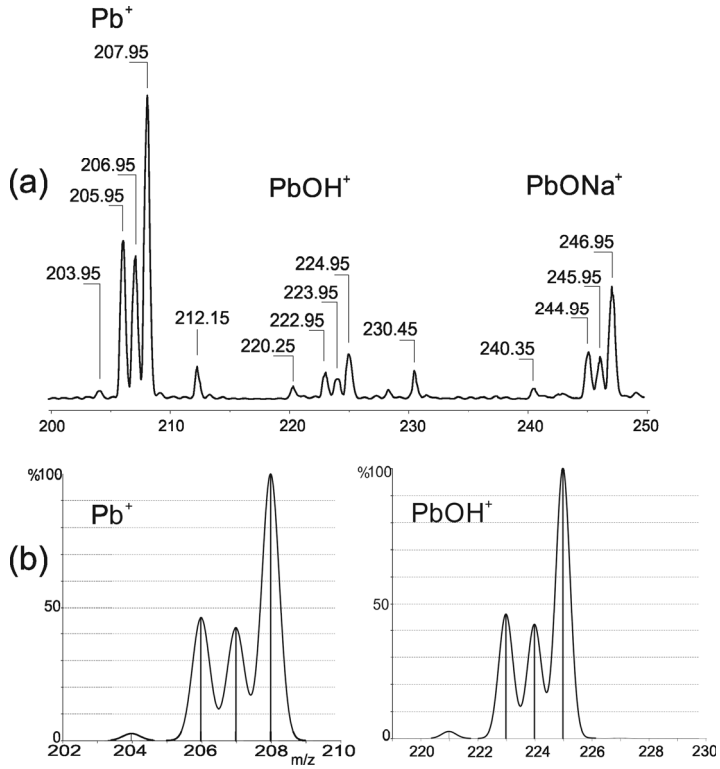


*Figure 2. MALDI mass spectra of lead standard solution (5 mg/mL) in the matrix CHCA recorded in the positive ion mode (a). Theoretical mass spectra of $Pb^+$ and $PbOH^+$ ions, obtained by free software Selket Caption (b).*

the isotopic pattern of lead and *m*/*z* values indicate the isotopic cluster of lead with sodium ion, $PbONa^+$. It is worth mentioning that group of mass peaks in the *m*/*z* area 204–208 belong to single positive Pb, while the lead in $PbOH^+$ adduct is double positively charged. First ionization potential of lead is 7.4167 eV and second ionization potential is 31.943 eV; both lead species are, however, detected in the MALDI TOF mass spectra simultaneously. Due to their low abundance, the corresponding adducts of $^{204}Pb$, which should give signals at *m*/*z* 220.95 and 242.95, are not detectable in the spectra. This result shows that there are no interferences of matrix mass peaks with mass peaks of lead isotopic and that the mass spectrum of lead obtained by MALDI-TOF MS qualitatively correspond to its theoretical presentation of the spectrum.

Quantitative determination of relative natural isotopic abundance of lead is also possible using the appropriate peak areas or relative intensities. The isotopic natural abundance for each lead isotope was calculated from selected mass spectra, using mass peaks for pure $Pb^+$. Obtained values are compared with theoretical values. Relative natural isotopic abundances from (MA)LDI TOF mass spectra were calculated using two methods and two sets of data: peak areas and relative intensities. According to first method, we calculated the isotopic distribution as percentage of selected isotope peak area in the area of the whole isotopic fingerprint of lead. Another method considered using relative intensities of isotope peaks, given in compare to the highest mass peak on *m*/*z* 172.15 which belongs to protonated CHCA matrix, which has lost one neutral molecule of water. Obtained results for relative natural isotopic abundances for each lead isotope via described methods (area and relative intensities) are given in the Table 2 and compared with theoretical

*Table 2. Experimental and theoretical values of relative natural isotopic abundances (Rel. nat. Is. Abund.) of lead obtained by MALDI and LDI methods. Calculated values were obtained in two ways, comparing isotope peak areas (peak area, Exp.) and relative intensities (relative intensities, Exp.)*

| Source | Calculation | $^{204}Pb$ | $^{206}Pb$ | $^{207}Pb$ | $^{208}Pb$ |
|---|---|---|---|---|---|
| MALDI | Peak area, Exp. | 1.54±1.06 | 26.21±1.64 | 21.39±1.89 | 50.87±2.43 |
| | Relative intensities, Exp. | 1.49±1.27 | 25.81±1.55 | 23.35±1.07 | 49.36±1.30 |
| LDI | Peak area, Exp. | 1.27±0.97 | 24.93±1.27 | 21.78±1.73 | 51.32±1.94 |
| | Relative intensities, Exp. | 1.47±0.92 | 25.73±1.43 | 22.43±1.06 | 53.76±1.28 |
| Selket | Relative intensities, Exp. | 1.40 | 24.10 | 22.10 | 52.40 |

values for relative natural isotopic abundances of lead obtained by Selket. Standard deviation was calculated from at least three sets of data (Table 2).

From the results presented in the Table 2 it is obvious that both sets of obtained results, comparing peak areas and relative intensities, show evident deviation from theoretical values, when calculated for the isotope with lowest abundance, *i.e.*, signal at around *m*/*z* 204. This is not surprising, because this isotope is of rather low abundance in comparison to other signals, and most probably due to matrix signals in close vicinity, its peak is in most cases difficult to obtain (*i.e.*, it might be suppressed by much higher matrix signals). On the other hand, values obtained for other lead isotopes are in a good agreement with theoretical values, indicating that this approach can be used for determination of isotope composition of metals, but most probably also for other compounds.

Slight deviations of the experimentally determined values from those calculated theoretically can be additionally corrected if the ratios $^{204}Pb/^{206}Pb$, $^{207}Pb/^{208}Pb$ and $^{204}Pb/^{206}Pb$ *vs.* $^{207}Pb/^{208}Pb$ are determined from both LDI TOF and MALDI TOF mass spectra. This approach is widely applicable for the estimation of the sample age and origin in the archeological studies [3]. Obtained results are presented in the Table 3 and compared with theoretical data. Results were obtained by comparing the peak area (*i.e.*, area under the selected isotope) or the signal intensities, as it has been described for the Table 2.

relative signal intensities) gave good agreement with theoretical values. These results were obtained by both approaches, LDI and MALDI. This can be due to higher sensitivity and precision for detection of small molecules [41]. Under those circumstances, it seems that only the nature of the sample determines which approach will be uses (matrix-free or matrix-assisted).

## CONCLUSION

In this work, we put an accent on the possibilities of metal identification and quantitative determination of lead isotope composition based on the MALDI-TOF mass spectra. Keeping in mind that MALDI method is still primarily used for analysis of molecules of biological origin and with higher masses, this work represents one of rare applications of this soft ionization technique for the acquisition of spectra of low-mass and inorganic compounds. This soft ionisation technique enables detection of all four isotopes of lead as well as precise determination of their relative abundances. It is emphasized that MALDI approach considers higher number of mass peaks originating from matrix adducts, but the matrix is not the limiting factor for detection of isotopic distribution of lead. The analysis of inorganic materials, including lead salts by LDI approach, *i.e.*, without the assistance of any matrix, is however, possible. These results can be used for determination of origin and transport traces of lead materials in a fast and simple way. Additional, these results

*Table 3. Experimental values of ratios of relative natural isotopic abundances (Rel. nat. Is. Abund.) for MALDI and LDI methodes and theoretical values. Measured values were obtained in two ways, comparing isotope peak areas (peak area, Exp.) and relative intensities (relative intensities, Exp.)*

| Source | Calculation | $^{204}Pb/^{206}Pb$ | $^{207}Pb/^{208}Pb$ | $^{204}Pb/^{206}Pb$ *vs.* $^{207}Pb/^{208}Pb$ |
|---|---|---|---|---|
| MALDI | Peak area, Exp. | 0.0586 | 0.4206 | 0.1393 |
| | Relative intensities, Exp. | 0.0578 | 0.4731 | 0.1222 |
| LDI | Peak area, Exp. | 0.0509 | 0.4244 | 0.1200 |
| | Relative intensities, Exp. | 0.0571 | 0.4172 | 0.1369 |
| *Selket* | Ratios of Rel. Nat. Is. Abund. (calculated fromtheoretical values) | 0.0581 | 0.4218 | 0.1377 |

Both, LDI and MALDI methods gave good agreement of experimental and theoretical data for relative natural isotope abundance and their ratios. Irrespectively on the approach used for determination of isotope ratio of lead, the deviation of the corresponding theoretical values did not exceed 12%. Comparing both the spectra and quantitative parameters obtained experimentally and theoretically, we confirmed the possibility of application of (MA)LDI-TOF MS for isotopic distribution of lead. Apparently, it is irrelevant which measure will be used for determination of the lead isotope ratio, since both (the peak area and the also shown that lead clusters of the type $Pb_nO_n$ and $Pb_nO_{n-1}$ has been obtained using the both LDI and MALDI methods. All stoichiometries were confirmed using isotopic pattern modelling.

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## IZVOD

### ODREĐIVANJE IZOTOPA OLOVA SPEKTROMETRIJOM MASA UZ POMOĆ LASERSKE DESORPCIJE/JONIZACIJE U PRISUSTVU I BEZ UPOTREBE „MATRICE“

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U ovom radu prikazane su mogućnosti detekcije i određivanja izotopskog sastava olova metodom laserske desorpcije i jonizacije u prisustvu „matrice“ (MALDI) i bez upotrebe „matrice“ (LDI). Laserska desorpcija i jonizacija pomoću „matrice“, spada u tzv. „meke“ metode spektrometrije masa, a primarno se koristi za analizu organskih molekula velikih masa. Uprkos velikim mogućnostima u analizi malih molekula, kao što su metali, koji imaju značajnu ulogu u živim sistemima, ova metoda se veoma retko primenjuje za njihovu detekciju. U biološkim uzorcima često postoji potreba ne samo za detekcijom metalnih jona već i za saznanjima o aktivnosti pojedinih izotopa ispitivanih metala. Identifikacija i određivanja izotopskog sastava olova urađeni su okviru komercijalnog MALDI masenog spektrometra. Analiza svih dobijenih jona izvršena je na bazi vremena slobodnog preleta jona (*time of fligt*). Rezultati su pokazali da korišćenje organskih kiselina, koje imaju ulogu „matrice“ kod MALDI metode i neophodne su za analizu organskih molekula, nisu limitirajući faktor za detekciju i precizno određivanje relativnog odnosa izotopa olova. Navedeni podatak otvara mogućnost istovremene detekcije biološki važnih molekula i metalnih jona u okviru jednog seta merenja i iz jednog uzorka. Osim ovoga, analiza izotopskog satava olova jednako efikasno može biti urađena i bez prisustva „matrice“, ova vrsta metode naziva se laserska desorpcija/jonizacija (LDI). Obe metode, LDI i MALDI, su jednostavne i brze metode koje ne zahtevaju dodatnu pripremu uzorka, niti njegovu modifikaciju za izotopsku analizu olova. Sa druge strane, oba pristupa daju brojne informacije o sistemu koji se analizira. Pored izotopske analize olova, u radu pokazano je da lasererska desorpcija i jonizacija izaziva stvaranje pozitivnih jona klastera olova tipa $Pb_nO_n$ i $Pb_nO_{n-1}$ ($n$: 2–6). Takođe, u navedenim eksperimentalnim uslovima, upotreba “matrice” ne dovodi do suzbijanja stvaranja klastera. Stehiometrija navedenih klastera potvrđena je na osnovu njihovih izotopskih satava koji su teorijski izračunati.

*Ključne reči*: izotopska analiza olova • spektrometrija masa • MALDI • LDI