Analyst

Accepted Manuscript

Organic Salt, NEDC (N-Naphthylethylenediamine Dihydrochloride) Assisted Laser Desorption Ionization Mass Spectrometry for Identification of Metal Ions in Real Samples

Jian Hou[a], Suming Chen[a], Ning Zhang[a], Huihui Liu[a], Jianing Wang[a], Qing He[a], Jiyun Wang[a], Shaoxiang Xiong[b] and Zongxiu Nie[,a,b]*

[a]Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical

Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of

Sciences, Beijing 100190, China

[b]Beijing Center for Mass Spectrometry, Beijing 100190, China.

Corresponding author: Zongxiu Nie

Phone: +86-10-62652123;

E-mail: znie@iccas.ac.cn

ABSTRACT

The significance and epidemiological effects of metal to life necessitate the development of a direct, efficient, and rapid method of analysis. Matrix assisted laser desorption/ionization technique is on the horns of a dilemma of metal analysis as the conventional matrixes have high background in the low mass range. An organic salt, NEDC (N-Naphthylethylenediamine Dihydrochloride) is applied as a matrix for identification of metal ions in negative ion mode in the present work. Sixteen metal ions, Ba^{2+}, Ca^{2+}, Cd^{2+}, Ce^{3+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Sn^{2+} and Zn^{2+}, in the form of their chloride-adducted clusters were systematically tested. Mass spectra can provide unambiguous identification through accurate mass-to-charge ratio and characteristic isotope patterns. Comparing to ruthenium ICP standard solution, tris(2,2′-bipyridyl)dichlororuthenium(II) ($C_{30}H_{24}N_6Cl_2Ru$) can from the organometallic chloride adducts to discriminate from the inorganic ruthenium by this method. After evaluating the sensitivity for Ca, Cu, Mg, Mn, Pb and Zinc and plotting their quantitation curves of through the signal intensity versus concentration respectively, we determined magnesium concentration in lake water quantitatively as 5.42mg/L using the standard addition method. The result is no big difference with ICP-AES as 5.8mg/L. Human urine and blood were also detected to ascertain the multi-metal analysis ability of this strategy in complex samples. At last, we still explored its applicability to tissue slice and visualized sodium and potassium distribution by mass spectrometry imaging in the normal Kunming mouse brain.

INTRODUCTION

Driven by their significant physiological effects in most organisms and practical needs into routine lives, metals have long been a hot spot in academic research. In biological systems, metal ions are required cofactors to functional proteins and also can catalyze cytotoxic reactions.[1, 2] The trend in metal analysis is demanding fast and accurate performances. Serving as a powerful analytical platform, mass spectrometry has been regarded as an irreplaceable tool for its unambiguous identification by accurate mass-to-charge (m/z) ratio detection.

Matrix-assisted laser desorption/ionization mass spectrometry[3, 4] with its predecessor LDI[5, 6] is a high-throughput ionization technique, which provides a useful way to cut sample consumption down to one microliter. This method is qualified to handle hundreds of samples in a short period of analysis time without cross-contamination, but is seldom applied in metal analysis. Besides the high background in the low m/z range of conventional matrixes, the low desorption/ionization efficiency for metal ions seriously hinders its boom in metal analysis. LDI is used for elemental measurements in the last century, in which high laser irradiance is used and singly-charged ions are generated.[7] Its quantitation of metal isotopic ratios was reported by Koumenis *et al*[8]. However, the applications of LDI in anlytical chemistry are not prevalent. Nano materials like silicon nanopost arrays (NAPA) [9, 10] and new organic matrixes like proton sponge have been developed to solve the high background problems[11, 12]. Though they have been successfully used in targeted metabolomics studies, but not reported for metal identification. Then

Obena *et al.* demonstrated iron oxide nanomatrix to facilitate metal ionization[13]. Huang *et al.* used a functional nano-gold membrane coupled with laser desorption/ionization mass spectrometry to detect lead ion in bio-fluids[14]. But the tedious synthesis processes of these materials still hamper their wide applications. As we know, no conventional organic matrix has yet been reported for analysis of metal ions.

In the present study, we found the organic salt matrix, N-naphthylethylenediamine dihydrochloride (NEDC), could artfully achieve this goal of metal analysis. This matrix was previously reported by us for the analysis of glucose in rat micro-dialysates. [15] It is also treated by ammonia for quantitative and qualitative determination of serum free fatty acids.[16] We found that this organic hydrochloride-structured matrix could efficiently form the Cl adducts with metal ions in the laser desorption/ionization process, which provided a unique approach for metal ions detection.

Herein, sixteen representative metals, including Ba^{2+}, Ca^{2+}, Cd^{2+}, Ce^{3+}, Co^{2+}, Cu^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Pb^{2+}, Sn^{2+} and Zn^{2+} were chosen to test validity and versatility of NEDC-assisted LDI method. Chloride ion adducts of these metal ions, $[MCl_{n+1}]^-$, were formed and detected in negative ion mode, where n is the charge state of the metals. This method could be used to discriminate organic Ru complex from inorganic Ru standard solution. Besides measuring the sensitivity and quantitation ability for calcium, copper, magnesium, manganese, lead and zinc, we test lake water human urine and blood the multi-element analysis ability of this

strategy in real samples. We get a consistent quantitation result of Mg in lake water

with ICP-OES. Combining with mass spectrometry imaging technique, NEDC was

applied to visualize the sodium and potassium distribution in the normal Kunming

mouse brain. It is believed that NEDC broad the application of matrix assisted laser

desorption/ionization mass spectrometry for metal identification.

EXPERIMENTAL SECTION

Reagents and Materials. N-Naphthylethylenediamine Dihydrochloride (NEDC,

$C_{12}H_{14}N_2$), bovine serum albumin, lauric acid and tris(2,2′-bipyridyl)

dichlororuthenium(II) were purchased from Sigma-Aldrich Chemie (St. Louis, MO,

USA). Ethanol, barium chloride, calcium chloride, cadmium acetate, cerous nitrate,

cobalt chloride, cuprous chloride, copper chloride, iron dichloride, iron chloride,

mercury bichloride, nickel chloride, magnesium chloride, manganese chloride, lead

nitrate, stannous chloride, stannic chloride, zinc chloride and acetonitrile, glutamic

acid and asparagine were purchased from Beijing Chemical Works (Beijing, China).

Triton X-100 and Ruthenium ICP Standard in 10% hydrochloric acid were bought

locally (J&K, China). All chemicals were analytical reagent-grade purity and were

used without further purification. The water used in all experiments was prepared

from a Milli-Q water purification system (Millipore, Milford, MA). $Pb(OH)_2$ has a

pK_{sp} of 16.09 and Pb^{2+} is easy to hydrolysis at high concentration in water. The stock

solution of lead nitrate must be freshly prepared and used in a week. Lauric acid was

dissolved in ethanol. Cuprous chloride was saturated in acetonitrile solution, and the

other salts were dissolved in water. NEDC matrix was prepared as a saturated solution in ethanol/water (2:1, v/v). Be cautious not to inhale the NEDC powders into lungs.

Real samples preparation. The lake water sample used for quantitation data of Mg, was collected from the Yuanmingyuan Imperial Garden in December and stored at -20 C in a temperature-monitored freezer. A urine sample was collected from a healthy adult woman (24 years old) and stored in a similar manner as the lake water. It is diluted 5-fold by deionized water before MALDI analysis. The human blood was supplied by Chaoyang Hospital (Beijing, China). It was treated with 1% Triton X-100 in a 1/4 volume before mixed with NEDC matrix. The ICP data of Mg in lake water was performed on Optima 7000 DV ICP-OES, PerkinElmer Inc. (Waltham, USA) directly without any pretreatment.

Mass Spectrometric Analysis. Sample and matrix were mixed at equal volume, and a 200nL mixture was spotted on the steel target with an Eppendorf pitette (0.05-2.50μL), dried at room temperature. When evaluating the sensitivity of this method, a plastic target from Bruker Daltonics were used to prevent intervention from the steel target. MALDI MS spectra were recorded on a Autoflex Ⅲ MALDI-TOF mass spectrometer (Bruker Daltonics, Bremen, Germany) in the negative reflector ion detection mode. The samples were irradiated using a Nd:YAG (output: 355 nm) at 100 Hz. Laser intensity was set marginally above the threshold of ionization. The accelerating voltage was 19 kV and the reflection voltage was 20 kV. Mass range of 0 to 900 Da was analysed and calibrated using oligosaccharides $C_6H_{12}O_6Cl^-$ (m/z 215), $C_{12}H_{22}O_{11}Cl^-$ (m/z 377), $C_{18}H_{32}O_{16}Cl^-$ (m/z 539) and $C_{24}H_{40}O_{21}Cl^-$ (m/z 699). Raw

data were processed with Bruker Daltonics FlexAnalysis software and the quantitation data were plotted by dynamic curve fitting using Origin 9.0 software.

MALDI Mass Spectrometry Imaging. Male Kunming mice (20~22 g) were provided by the Experimental Animal Center of Peking University. The animal experiments were performed according to the NIH Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication, No. 3040-2, revised 1999, Bethesda, MD) and were approved by the Animal Care and Use Committee of the Chinese Academy of Sciences. The brain tissues were dissected and flash-frozen in liquid nitrogen. Tissues were stored at -80 °C until further preparation. They were fixed atop a drop of saline on the cutting stage. Tissues were sectioned at 10 μm thickness using a Leica CM1950 cryostat (Leica Microsystems GmbH, Wetzlar, Germany) and thaw mounted onto indium tin oxide (ITO) coated glass slides. The glass slides were then placed into a vacuum desiccator for approximately 1 h before matrix application. The matrix solution NEDC was sprayed onto the tissue sections using an automatic matrix sprayer (ImagePrep, Bruker Daltonics) and make sure homogeneous matrix coverage over the entire tissue surface. An Ultraflextreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Billerica, MA) equipped with a 355-nm nitrogen laser operating at 2000Hz in negative reflection mode was employed for MALDI-MSI. The mass spectrometer parameters were set as manufacturer recommended and adjusted for optimal acquisition performance. The laser spot size was set at medium focus (~ 50 μm laser spot diameter), and laser power was optimized at the start of each run and then fixed for the whole MALDI-MSI experiment. The mass spectra data were acquired over a mass range of m/z 0-900 Da. Imaging spatial resolution was set to 100 μm for tissues from mice and each spectrum consists of 400 laser shots. Regions of interest were manually defined in the imaging software.

RESULTS AND DISCUSSION

Metal Ion Signal Acquired.

Electrospray ionization has been applied in element analysis[17-19] while MALDI has to deal with adversity of high background in low m/z range. We have reported N-(1-naphthyl) ethylenediamine dihydrochloride (NEDC) to be well-suited for use as a MALDI matrix for MS analysis of low molecular weight compounds, which presented high sensitivity and salt tolerance. [15] Two groups of peaks, Cl^- and HCl_2^- dominate the mass spectrum of NEDC (Figure 1). This matrix consists of an organic amino cation and two chloride anions. The salt-like structure makes this matrix co-crystallize with metal ions easier.

NEDC assisted LDI can yield chloride-metal clusters, $[MCl_{n+1}]^-$, which can be detected by mass spectrometer. The ionized process may be similar to MALDI with ammonium chloride added[20]. To verify that this method is partly a universal method for metal analysis, not only essential metal ions Ca^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Sn^{2+} and Zn^{2+} with toxic metal ions Ba^{2+}, Cd^{2+}, Hg^{2+} and Pb^{2+}, but also transition metal ions Ce^{3+} were detected in the form of $CaCl_3^-$, $CoCl_3^-$, $CuCl_3^-$, $FeCl_4^-$, KCl_2^-, $MgCl_3^-$, $MnCl_3^-$, $NaCl_2^-$, $NiCl_3^-$, $SnCl_3^-$, $ZnCl_3^-$, $BaCl_3^-$, $CdCl_3^-$, $HgCl_3^-$, $PbCl_3^-$ and $CeCl_4^-$ respectively in the current experiment. The observed metal-chloride patterns in typical MALDI-TOF mass spectra recorded are shown in Figure 2 in an alphabet order. The relative abundance of each metal-chloride isotopes strikingly resemble the theoretical patterns from simulation by Bruker Isotopepattern in Figure 2 insets. The isotopic envelope is a unique trait attributing to the combinations of ^{35}Cl, ^{37}Cl and the metal isotopes. The characteristic isotopic pattern of metal-chloride

clusters helps to discriminate the signal from the irrelevant interferences. Plus,

negative mode operation has less MALDI background, which is also an auxiliary

advantage to ensure the certain identification of metal ions.

To elucidate the ability of this method to differentiate inorganic and organic metal

compounds, we analysed 1mmol/L $RuCl_3$ in Ru standard solution in 10% HCl and

tris(2,2′-bipyridyl)dichlororuthenium(II) $(C_{30}H_{24}N_6Cl_2Ru)$ water solution with mass

spectra in Figure 3. The isotope peaks from $RuCl_3^-$ (the most abundant peak m/z 208)

and $RuCl_4^-$ (the most abundant peak m/z 244) are the strongest peaks in the mass

spectrum   of   inorganic   ruthenium.   Organic   ligands   of

tris(2,2′-bipyridyl)dichlororuthenium(II) are partly replaced by Cl^- forming $RuCl_3^-$

and $RuCl_4^-$ peaks. We can see tris(2,2′-bipyridyl)dichlororuthenium(II) forms

$[C_{30}H_{24}N_6Cl_2Ru+Cl]^-$ with the most abundant of peak at m/z 677 by NEDC-assisted

LDI mass spectrometry. This method can be applied to analysis organometallics and

to discriminate organic metals from inorganic metal ions.

We measured the detection limits of six metals, calcium, copper, magnesium,

manganese, lead and zinc according to the most abundant peak of each element is at

S/N=3 (Table 1). It should be pointed here we found reduction of Cu^{2+} to Cu^+ in the

mass spectrum and the mechanism has been delineated by zhang *et. al.*.[21] Peak of

$CuCl_2^-$ were chosen to evaluation the sensitivity and the dynamic range of copper

since $CuCl_2^-$ is more stable at gas-phase. The isotope distribution of metal-chloride

adducts can be quite complex. The resolution of mass spectrometer can have a great

impact on the isotope pattern. For example, in high resolution, all the isotopes are

totally isolated and $^{63}Cu^{35}Cl_2$ (m/z 134.8655) is the most abundant peak. But in low resolution, $^{63}Cu^{35}Cl^{37}Cl$ (m/z 134.86436) and $^{63}Cu^{35}Cl^{37}Cl$ and $^{65}Cu^{35}Cl_2$ cannot be discriminated and they form one peak which is higher than $^{63}Cu^{35}Cl_2$. Considering the resolution of TOF analyser, we get 135 of the two overlapped isotopes as the most abundant peak. The relative abundance of $CuCl_2$, $MgCl_3$, $PbCl_3$ and $ZnCl_3$ in high resolution and low resolution are all listed in Table S1. We then evaluate the quantitation ability of this method. The linearity response of signal of each elements vs their concentration was shown in Figure 4, respectively. The dynamic ranges are different for these metals. It is may be due to the different sensitivity and ionization efficiency of each metal ion.

To study the anti-interference ability of this method, gradient concentration of bovine serum albumin (BSA, M.W. 66kDa), glutamic acid (Glu, $C_5H_9NO_4$, M.W. 146) and lauric acid ($C_{12}H_{24}O_2$, M.W. 200) were added to 100 μmol/L Mg^{2+} solution (Figure S2). The signal of Mg^{2+} is severely suppressed by the 5 mg/mL BSA and 0.1 mg/mL BSA has no impact on the signals. When the concentration of glutamic acid is under 1 mmol/L, there is no interference to Mg peak. Lauric acid dose not suppress the magnesium signals even when it is above 20 mmol/L. Then we added amino acid asparagine (Asn, $C_4H_8N_2O_3$, M.W. 132) to the Mg^{2+} solution. The Asn peak (m/z 131) is covered with the most abundant peak of $MgCl_3^-$ (Figure S3). It is hardly to find Mg peak when Asn is above 20 mmol/L. When Asn is 5 mmol/L, the existence of Mg can be confirmed by isotope peak of 129 and 133. We can recognize all three Mg peak when Asn is below 1 mmol/L.

Though this method still has a long way to catch up with the most powerful element

identification technique Inductively-coupled plasma mass spectrometry (ICP-MS)[22] in

sensitivity and quantitation ability, MALDI is a potential tool for metal identification

with its advantages over other methods.

Metal Identification and Mg Quantitation in Real Samples.

NEDC assisted LDI is a multi-element analysis method. We inspect its ability for

metal identification in complex samples. As the metal species in environmental water

are important influence factors to the survival and proliferation of aquatic

organisms,[23] we adopted the technique to detect metal ions in lake water (pH=7). The

mass spectra of lake water samples are present in Figure 5a. Several essential metal

elements, $[NaCl_2]^-$, $[KCl_2]^-$, $[MgCl_3]^-$, $[CuCl_2]^-$, $[CaCl_3]^-$, $[FeCl_3]^-$ and $[ZnCl_3]^-$ are

identified with NEDC, as well as some fundamental anions like $[HSO_4]^-$ or $[H_2PO_3]^-$

(m/z 97), proving that this method can be used to get practical information. Then we

determined the sensitivity of spiked lead in lake water as 2.5 μmol/L which is about

five times higher than lead in water at 0.5μmol/L. Figure 5b is the linearity of lead

peak vs the spiked lead concentration in lake water. This method has also been used to

quantitate Mg^{2+} in lake water using standard addition method. We got a good linearity

between the magnesium signal (m/z 131) and the additional concentration with

$R^2=0.9954$. The result of Mg^{2+} is 5.4 mg/L, no significant difference with the ICP

result of 5.8 mg/L (Figure 5c). We also applied NEDC to ionize metal ions in bio-fluid,

human urine and blood (Figure 6). Some vital elements such as Na^+, K^+ and Ca^{2+} are

identified, proving its ability in complex matrix. The results for real samples verify

the possibility of metal analysis based on NEDC assisted MALDI mass spectrometry in environmental and biological territory.

NEDC-assisted laser desorption/ionization mass spectrometer points to free metal ion identification and is capable of multi-element analysis. These features integrated its bio-compatibility suggest this instrument to be a promising way to image free ions and monitor their distribution in tissues and can be a complementary implement for the total metal imaging of laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)[24, 25]. In the present work, with mass spectrometry imaging technique, we used this method to obtain the distribution of Na and K in the 10 μm-tissue slice by this method (Figure 7b and Figure 7c, respectively). In the normal brain, the two metal ions are both homogeneously distributed. Sodium and potassium are the primary ions and electrolytes within the body.[26, 27] They are required for sending electrical impulses through neurons and maintaining osmotic pressure and water homeostasis. This method will be applied to map sodium and potassium concentration in brains compared between animal models of diseases and the healthy control groups in our future research.

CONCLUSION

The matrix NEDC (N-naphthylethylenediamine dihydrochloride) and its derivative are qualified matrix for small organic molecules[28]. In this study, the organic salt (NEDC) has proved as a good matrix for the MALDI MS analysis of metal ions. The easily formed chloride metal clusters with their unique isotopic pattern facilitate the

detection and identification of various metals. The qualitative and quantitative analysis was successfully applied in real samples. Additionally, we have shown the distribution of Na^+ and K^+ in the mouse brain, providing the possibility for metal imaging by matrix assisted laser desorption/ionization mass spectrometry. NEDC is a promising matrix be potentially applied in biological researches about metal and organic compounds.

ACKONWLEDGEMENTS

REFERENCES

1.   L. A. Finney and T. V. O'Halloran, *Science*, 2003, **300**, 931-936.

2.   K. H. Thompson and C. Orvig, *Science*, 2003, **300**, 936-939.

3.   Karas and Hillenkamp, *Anal. Chem*, 1988, **60**, 2299-2301.

4.   K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida and T. Yoshida, *Rapid Commun. Mass Spectrom.*, 1988, **2**, 151-153.

5.   R. E. Honig and J. R. Woolston, *Appl. Phys. Lett.*, 1963, **2**, 138.

6.   X. Y. Zhang, S. C. Zhu, Y. Xiong, C. H. Deng and X. M. Zhang, *Angew. Chem. Int. Ed.*, 2013, **52**, 6055-6058.

7.   Q. Yu, L. Z. Chen, R. F. Huang, W. Hang, B. L. Huang and J. He, *Trac-Trends Anal. Chem.*, 2009, **28**, 1174-1185.

8.   I. L. Koumenis, M. L. Vestal, A. L. Yergey, S. Abrams, S. N. Deming and T. W. Hutchens, *Anal. Chem.*, 1995, **67**, 4557-4564.

9.   B. N. Walker, J. A. Stolee and A. Vertes, *Anal. Chem.*, 2012, **84**, 7756-7762.

10.   B. N. Walker, C. Antonakos, S. T. Retterer and A. Vertes, *Angew. Chem. Int. Ed. Engl.*, 2013, **52**, 3650-3653.

11.   R. Shroff and A. Svatosˇ, *Ana. Chem.*, 2009, **81**, 7954-7959.

12. R. Shroff, L. Rulisek, J. Doubsky and A. Svatosˇ, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 10092-10096.

13. R. P. Obena, P. C. Lin, Y. W. Lu, I. C. Li, F. Del Mundo, S. D. Arco, G. M. Nuesca, C. C. Lin and Y. J. Chen, *Anal. Chem.*, 2011, **83**, 9337-9343.

14. Y.-C. Liu, C.-K. Chiang, H.-T. Chang, Y.-F. Lee and C.-C. Huang, *Adv. Funct. Mater.*, 2011, **21**, 4448-4455.

15. R. Chen, W. Xu, C. Xiong, X. Zhou, S. Xiong, Z. Nie, L. Mao, Y. Chen and H. C. Chang, *Anal. Chem.*, 2012, **84**, 465-469.

16. Y. Zhang, Y. Wang, S. Guo, Y. Guo, H. Liu and Z. Li, *Analytica chimica acta*, 2013, **794**, 82-89.

17. M. B. Luo, B. Hu, X. Zhang, D. F. Peng, H. W. Chen, L. L. Zhang and Y. F. Huan, *Anal. Chem.*, 2010, **82**, 282-289.

18. J. H. Zhu and R. B. Cole, *J. Am. Soc. Mass Spectrom.*, 2000, **11**, 932-941.

19. S. Mollah, A. D. Pris, S. K. Johnson, A. B. I. Gwizdala and R. S. Houk, *Anal. Chem.*, 2000, **72**, 985-991.

20. Y. Cai, Y. J. Jiang and R. B. Cole, *Anal. Chem.*, 2003, **75**, 1638-1644.

21. J. Zhang, V. Frankevich, R. Knochenmuss, S. D. Friess and R. Zenobi, *J. Am. Soc. Mass Spectrom.*, 2003, **14**, 42-50.

22. A. R. Timerbaev, *Chemical reviews*, 2013, **113**, 778-812.

23. D. J. Spry and J. G. Wiener, *Environ. Pollut.*, 1991, **71**, 243-304.

24. J. Lear, D. J. Hare, F. Fryer, P. A. Adlard, D. I. Finkelstein and P. A. Doble, *Anal. Chem.*, 2012, **84**, 6707-6714.

25. J. S. Becker, M. Zoriy, A. Matusch, B. Wu, D. Salber and C. Palm, *Mass Spectrom. Rev.*, 2010, **29**, 156-175.

26. J. A. Filosa, A. D. Bonev, S. V. Straub, A. L. Meredith, M. K. Wilkerson, R. W. Aldrich and M. T. Nelson, *Nat. Neurosci.*, 2006, **9**, 1397-1403.

27. G. B. Dawe, M. Musgaard, E. D. Andrews, B. A. Daniels, M. R. P. Aurousseau, P. C. Biggin and D. Bowie, *Nat. Struct. Mol. Biol.*, 2013, **20**, 1054-1061.

28. R. Chen, S. Chen, C. Xiong, X. Ding, C. C. Wu, H. C. Chang, S. Xiong and Z. Nie, *J. Am. Soc. Mass Spectrom.*, 2012, **23**, 1454-1460.
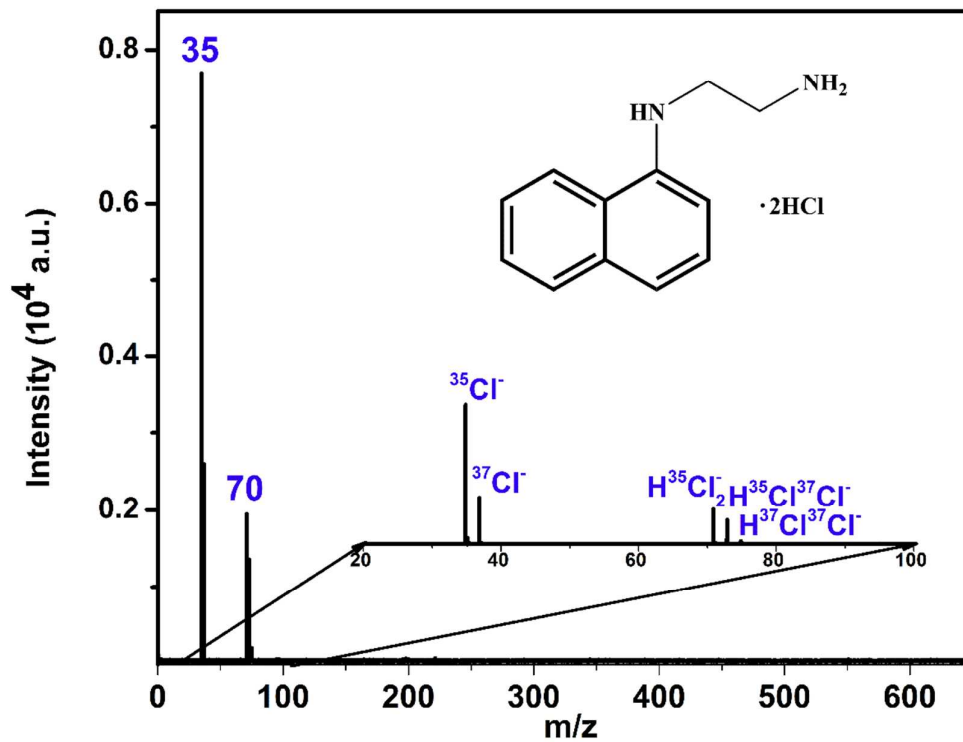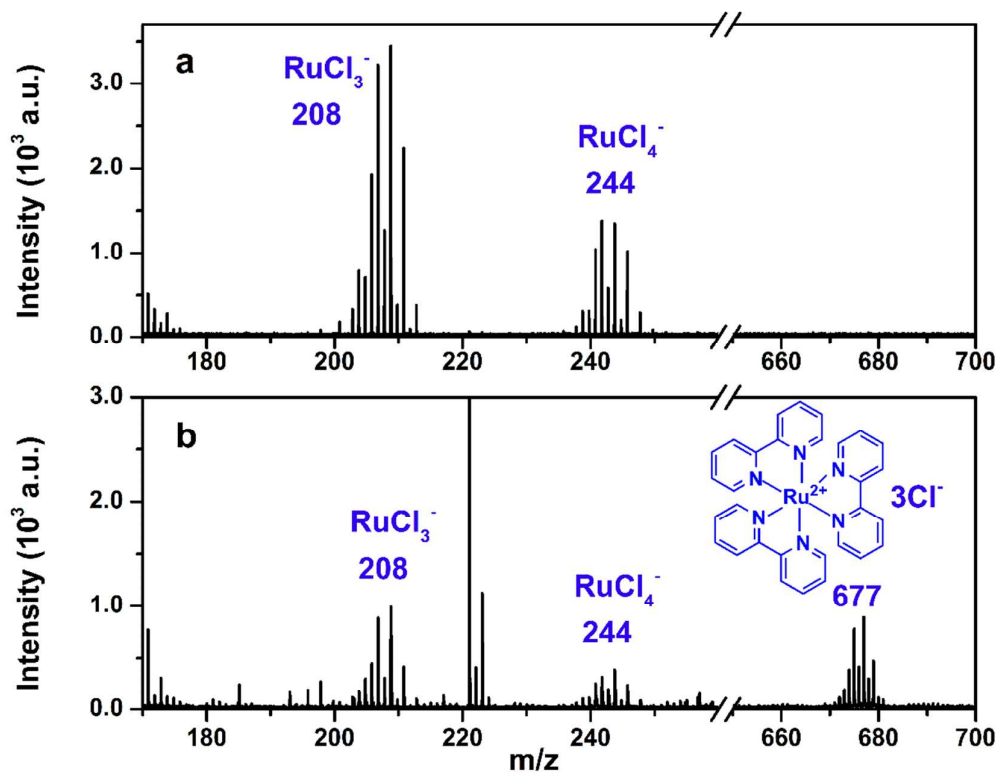
Figure 1. The mass spectrum of the organic salt, N-Naphthylethylenediamine Dihydrochloride

(NEDC) in negative mode with its structures in the inset.
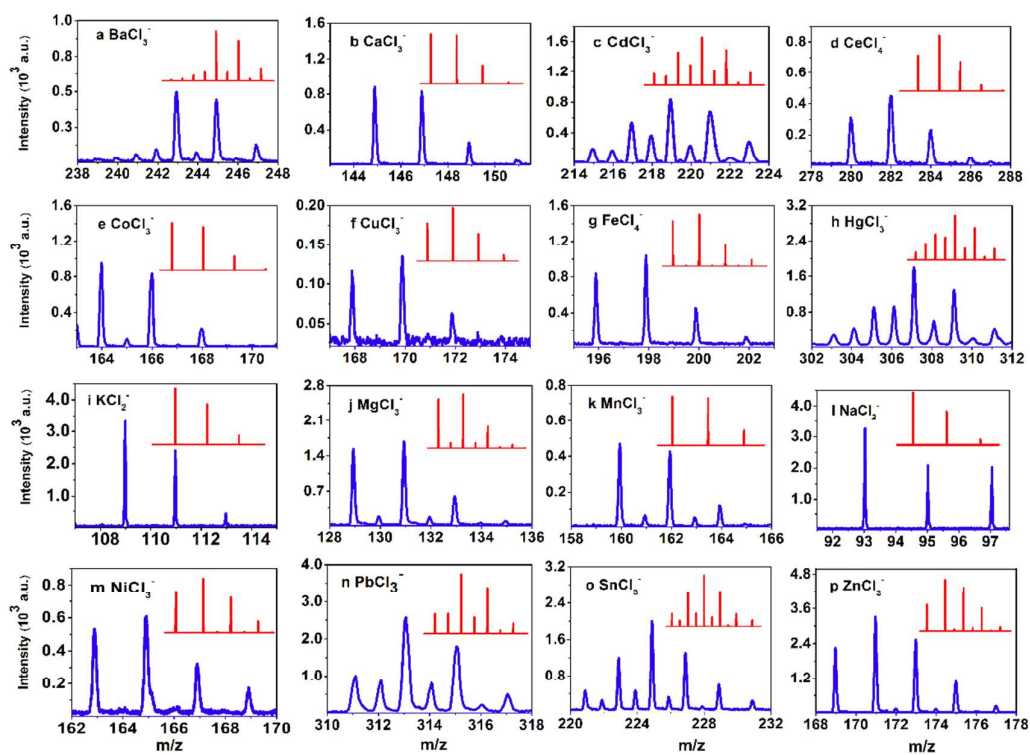
Figure 2. MALDI mass spectra of some important industrial, environmental, and biological metals and their theoretical isotope patterns (inset). The spectra were obtained with metal ions at 1 mmol/L in negative mode.

Figure 3. Compared mass spectra of inorganic RuCl$_3$ from Ru ICP standard solution a) and

organic Ru complex, tris(2,2′-bipyridyl)dichlororuthenium(II) b) by MALDI. Both solutions were

at 1 mmol/L.

Table 1. The Lowest Concentration of Six Elements at S/N = 3

| Element	Most Abundant Isotope	m/z	S/N=3	
			µmol/L	fmol
Calcium	$^{40}Ca^{35}Cl_3$	145	0.25	25
Copper	$^{63}Cu^{35}Cl^{37}Cl$, $^{65}Cu\,^{35}Cl_2$	135	1	100
Magnesium	$^{24}Mg^{35}Cl_2^{37}Cl$ $^{26}Mg\,^{35}Cl_3$	131	0.1	10
Manganese	$^{55}Mn^{35}Cl_3$	160	2	200
Lead	$^{206}Pb^{35}Cl_2^{37}Cl$ $^{208}Pb\,^{35}Cl_3$	313	0.5	50
Zinc	$^{64}Zn^{35}Cl_2^{37}Cl$ $^{66}Zn^{35}Cl_3$	171	0.5	50
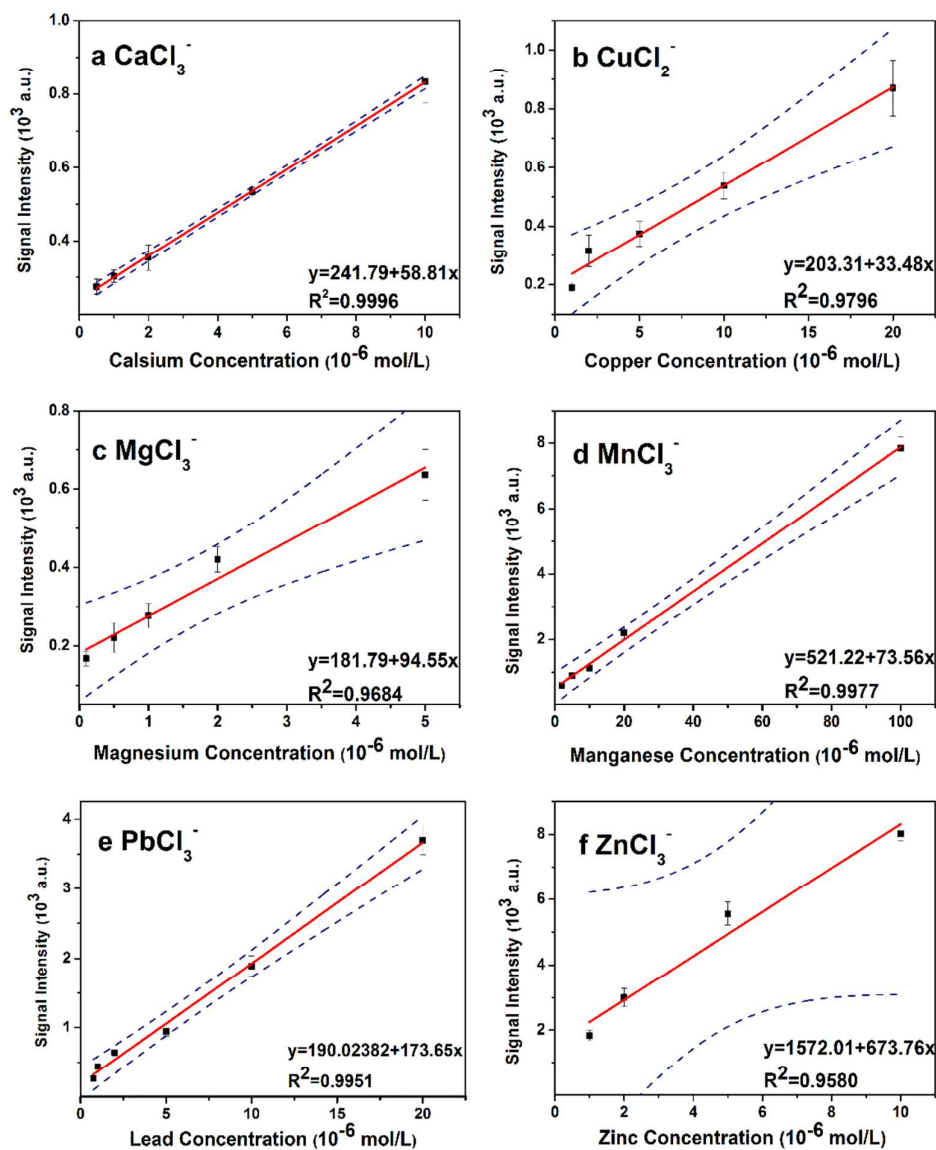
Figure 4. The most abundant peak intensity of calcium a), copper b), magnesium c), manganese d),

lead e) and zinc f) versus their concentration, repectively. The measurements were repeated five

times. The blue lines above and below the lines of the best linear fit are 99% confidence bands for

the data to be linear, p <0.0001 for Ca,Cu, Mn, Pb, p<0.002 for Mg and p<0.02 for Zn.
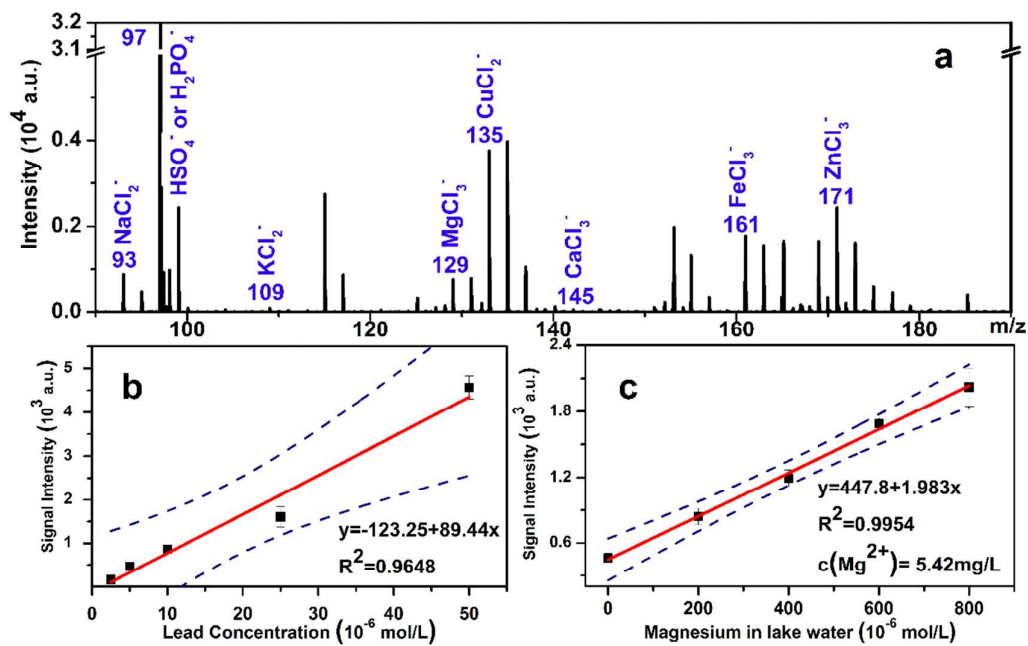
Figure 5. a) MALDI mass spectra of the metal ions in lake water; b) Linearity of spiked lead in

lake water. The measurements were repeated five times. The blue lines above and below the lines

of the best linear fit are 99% confidence bands for the data to be linear, p <0.002; c) Quantitation

data of Mg^{2+} in lake water with standard addition method. The blue lines above and below the

lines of the best linear fit are 99% confidence bands for the data to be linear, p<0.0001.
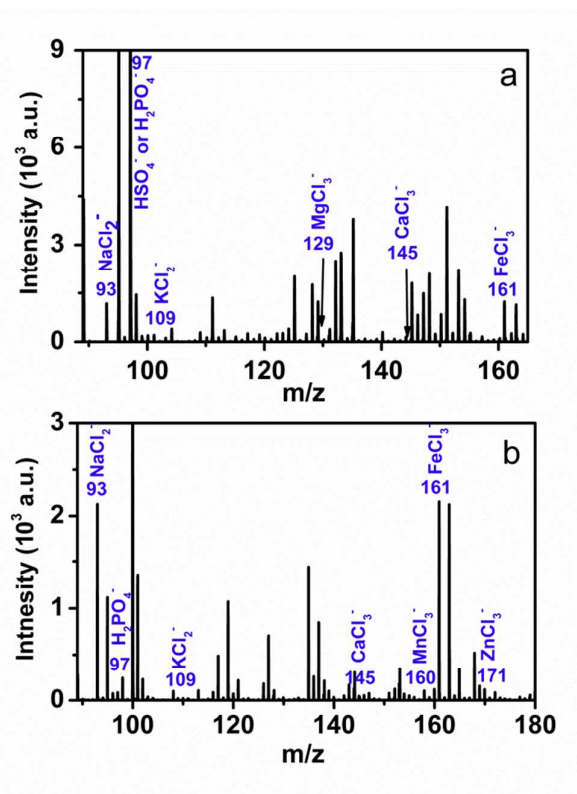
Figure 6. a) The mass spectra of five diluted human urine. Na^+, K^+, Mg^{2+}, Ca^{2+} and Fe^{2+} can be

identified; b) The mass spectra of triton X-100 treated human whole blood. Na^+, K^+, Ca^{2+}, Fe^{2+}

and Zn^{2+} were identified.
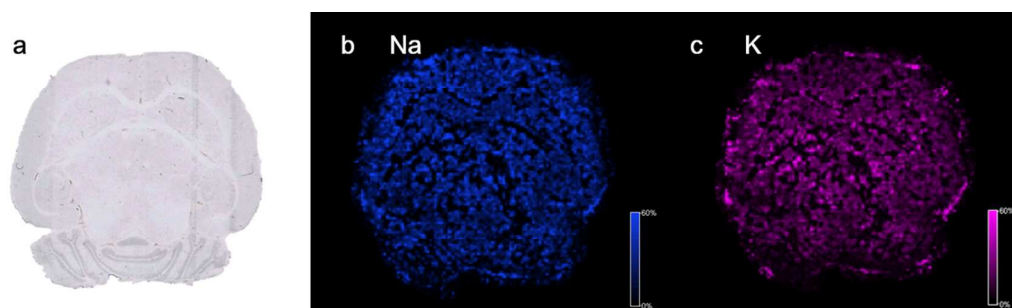
Figure 7. a) The scanning image of a 10 μm thick normal mouse brain slice on ITO glass; b) and

KCl_2^- c) map on the tissue slice at 100 μm resolution from MSI experiment performed on the

Ultraflex Bruker Daltonics instrument.
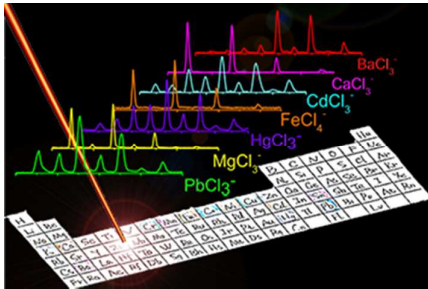
Organic salt (NEDC) has qualified as a good matrix for the MALDI MS analysis of

metal ions.