Analytical Methods

Cite this: *Anal. Methods*, 2012, **4**, 1160

www.rsc.org/methods

TECHNICAL NOTE

Determination of divalent trace metals in a soil sample using electrospray ionization mass spectrometry

Hiroki Hotta,*[a] Yuta Kogure[b] and Kin-ichi Tsunoda[b]

Received 14th December 2011, Accepted 15th February 2012
DOI: 10.1039/c2ay05898g

A novel pre-separation technique for the determination of divalent trace heavy metals in a soil sample was developed, in which deferoxamine was used as a masking agent for trivalent metal ions, Al^{3+} and Fe^{3+}, and a chelate column was applied to collect target heavy metal ions and to eliminate alkali metal and alkaline earth metal ions. Deferoxamine had such a strong and selective masking effect on Al^{3+} and Fe^{3+} that divalent trace heavy metals in the soil sample were well separated from considerably greater amounts of Al^{3+} and Fe^{3+}. Consequently, the determination of four trace metals (Ni, Cu, Zn, and Pb) in a certified soil reference material (JSAC-0401) was successfully performed using electrospray ionization mass spectrometry (ESIMS).

Introduction

Electrospray ionization (ESI) is a soft ionization technique used with mass spectrometry (MS). Thus, ESIMS is a very effective tool for the detection of metal–organic or metal–inorganic ligand complexes dissolved in a solution.[1,2] Metal complexes can be detected as protonated (positive ion mode) or deprotonated (negative ion mode) molecular ions with little fragmentation. On the other hand, the, ESIMS signal intensities, or the ionization efficiencies of analytes, are greatly affected by the spray conditions; hence, the technique had been thought unsuitable for quantitative analysis. Signal intensities are often suppressed or enhanced by co-existing compounds and solvents (matrix effects).[3–15] Such matrix effects have a negative impact on quantitative analyses. Thus far, several papers[7–15] have reported ways to avoid the degradation of quantitative performance owing to matrix effects. Sample pretreatment using chromatographic techniques[7–9] and the addition of internal standard materials[10–13] are popular techniques to minimize the matrix effects. The use of nanospray[14] and the addition of a high concentration of volatile salt to a sample solution[15] have also been reported. However, these techniques are not always effective, because the matrix effects strongly depend on the chemical nature and the solution environment of target ions. Recently, we reported the application of ESIMS to the determination of trace metals and halogens in biological or environmental samples.[16] The metals were detected as very stable complexes with *trans*-1,2-diaminocyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid (CyDTA), and the complexes were detected as monovalent anions with a negative ion mode. The tested sample solutions, which were prepared by acid digestion of solid samples, were passed through an on-line size-exclusion column to separate the target complexes from other low-molecular-weight salts. Because metal complexes and an excess amount of free ligand eluted at nearly the same retention time, there was no concern regarding the dissociation of complexes through the column separation. The highly stable complex, $[Co^{III}(EDTA)]^-$, which also elutes at the same retention time, was used as an internal standard to minimize the response variation. The ESIMS signal intensities of metal–CyDTA complexes were corrected using that of the internal standard. Thus, the stable and reproducible detection of metals was demonstrated, and a quantitative method with limits of detection (LOD) of several to several tens of nmol dm^{-3} levels for metals was established using ESIMS. Although the LOD values were three orders of magnitude larger than those of ICPMS, they were almost the same as or slightly smaller than those of ICPAES and AAS. The quantitative performance of this technique was also demonstrated using certified biological reference materials.[16]

However, the same technique could not be applied to the analysis of a soil sample, because very large amounts of interfering chemicals, Al, Fe, alkali metals, alkaline earth metals, silicate, *etc.*, are present in soil. In this study, we have established a preparation, or pre-separation, procedure for the determination of divalent trace metals in a soil sample using ESIMS analysis. The quantitative analysis of trace metals in a certified soil reference material was performed using the method.

Experimental

Materials

CyDTA (Dojindo Laboratories, Kumamoto, Japan) was used as a chelating agent. Deferoxamine mesylate (desferrioxamine B), a masking agent for Fe^{3+} and Al^{3+}, was purchased from Sigma (St Louis, MO, USA). A certified soil reference material (CRM),

[a]*Department of Chemistry, Nara University of Education, Takabatake, Nara 630-8528, Japan. E-mail: hotta@nara-edu.ac.jp; Fax: +81-742-27-9510; Tel: +81-742-27-9510*
[b]*Department of Chemistry and Chemical Biology, Gunma University, Tenjin, Kiryu 376-8515, Japan. E-mail: tsunoda@gunma-u.ac.jp; Fax: +81-277-30-1251; Tel: +81-277-30-1250*

brown forest soil (JSAC-0401) from the Japan Society for Analytical Chemistry (JSAC), was used for the evaluation of the method. Four divalent heavy metals in the soil CRM (Ni, Cu, Zn, and Pb) were used as targets. Manganese was excluded from the list of target metals, because the Mn^{II}CyDTA complex, $[^{55}Mn(Hcydta)]^-$, had the same *m/z* value (*m/z* 398) as that of the Fe^{III}CyDTA complex, $[^{56}Fe(cydta)]^-$. Other components, *i.e.*, V, Cr, and As, were also not determined because these metals were oxidized to oxometalate complexes through the acid digestion. Several concomitant elements (Al, Fe, Si, and alkali metals) were included in the CRM. Metal standard solutions and other reagents of analytical grade were obtained from Wako Pure Chemicals (Osaka, Japan). A chelate column, Nobias Chelate-PA1 (resin amount, 190 mg; Hitachi High-Tech Fielding Co., Japan), was used for off-line sample pre-separation. Water was purified using a Millipore Milli-Q system (Millipore Corp., Billerica, MA, USA). A Co^{III}EDTA complex (K salt) was synthesized according to the literature,[17] and was used as an internal standard material.

Instruments

An LC-ESI-quadrupole MS system (LCMS-2010, Shimadzu Co., Kyoto, Japan) with an on-line size-exclusion column (4.6 mm I.D. × 250 mm long) packed with Sephadex G-10 (Sigma) was used for the separation of the target complexes from the concomitant low-molecular-weight salts. A previously reported column preparation procedure was used.[16] The inlet pressure of the flow was checked throughout the measurement and was nearly constant at approximately 1.5 MPa at 0.1 cm^3 min^{-1}. The retention times of the target complexes were constant, and the column was used for more than six months without any deterioration. The negative ion mode (electrospray voltage: −3.5 kV) at a selected ion monitoring (SIM) mode was used for all quantification experiments in this study. CH_3COONH_4 aqueous solution (1 mmol dm^{-3}) was used as a carrier solution (flow rate: 0.1 cm^3 min^{-1}). The same concentration of CH_3COONH_4 was also added to all test solutions. Test solutions were introduced by a flow injection method with a six-way loop injector whose sample loop volume was 30 mm^3. The standard addition method was used for the quantification of metals in order to eliminate the matrix effect. The other instrumental conditions were the same as previously reported.[16]

The validity of the quantitative results obtained by ESIMS was carefully confirmed by a conventional reference method using an ICP emission spectrometer (ICPS-1000III, Shimadzu Co.).

Soil sample preparation

First, acid digestion of the soil CRM (JSAC-0401) was performed as follows. Mixed acid (2 cm^3 of HNO_3 and 2 cm^3 of HF) was poured into a PTFE vessel (internal volume, 27 cm^3). 0.1 g of the sample solid was poured into a small PTFE cup (internal volume, 8 cm^3) that was placed in the PTFE vessel. The vessel was tightly packed into a stainless steel container to withstand high pressure and heated at 160 °C for 5 hours. After cooling, the small PTFE cup containing the sample liquid was removed from the container and placed on a hot plate (180 °C) to allow the sample liquid to evaporate. Then, a mixed acid (1 cm^3 of HNO_3 and 1 cm^3 of HF) was added to the residue and again dried on the hot plate (180 °C). This residue was dissolved in an appropriate volume (5 cm^3) of ammonium acetate solution (pH 5.5). Then, a masking agent (deferoxamine) was added as a solid to 1 cm^3 of the solution, and the solution was diluted to 2 cm^3 using ammonium acetate aqueous solution (the final concentration of deferoxamine was 63 mmol dm^{-3}). The solution was passed through the chelate column (Nobias Chelate PA1) as follows. The column was conditioned in advance according to the literature,[18] *i.e.*, 10 cm^3 of acetone, 10 cm^3 of 3 mol dm^{-3} HNO_3, 15 cm^3 of purified water, and 10 cm^3 of 0.1 mol dm^{-3} ammonium acetate (pH adjusted to 5.5 by adding HNO_3) were added sequentially to the chelate column by gravity. After loading the sample solution onto the column, it was washed with purified water (15 cm^3) and an elution of bound metals using 3 mol dm^{-3} HNO_3 (4 cm^3) was performed. The final eluted solution was dried at 180 °C to remove nitric acid. For the ICPAES measurement, the residue was dissolved in water; however, for the ESIMS measurement, the residue was dissolved in a solution (10 cm^3) including 0.4 mmol dm^{-3} of CyDTA, 20 μmol dm^{-3} of Co^{III}EDTA complex (as an internal standard), and 1 mmol dm^{-3} of ammonium acetate. The final pH was adjusted to 3.5. Finally, appropriate volumes of metal standard solutions (Ni, Cu, Zn, and Pb) were added to the sample solutions for standard addition analysis.

Results and discussion

Selective separation of trace metals

In a previous study,[16] test solutions of biological CRMs were prepared by adding CyDTA, ammonium acetate, and an internal standard (Co^{III}EDTA) to the sample solutions after acid digestion by HNO_3. However, in the analysis of the soil sample, no reproducible quantitative results were obtained with the same procedure. This was because large amounts of interfering ions such as Al^{3+}, Fe^{3+}, silicate, alkali metal and alkaline earth metal ions were present in the sample solution after the acid digestion. Thus, we planned to develop a pre-separation method in which the combination of a chelate column and a masking agent for Al^{3+} and Fe^{3+} was applied. Thus, the chelate column would be used to collect target divalent heavy metals and to eliminate alkali metal and alkaline earth metal ions. Then the masking agent would prevent Al^{3+} and Fe^{3+} from collecting on the chelate column. We first searched for an appropriate masking agent: several masking agents for Fe and/or Al have been reported.[19–23] Citrate and Tiron (4,5-dihydroxybenzene-1,3-disulfonic acid) were used for the solvent extraction of trace metals from an Fe-rich aqueous solution.[19] CyDTA[20] and deferoxamine[21] were also used as masking agents for Al and Fe in order to detect fluoride ions. Fluoride ions are used as a masking agent for Fe^{3+} in steel analyses.[22] Moreover, several chelators including deferoxamine that are used for therapy to remove overloaded iron from the human body were also investigated.[23] In this study, we chose deferoxamine as a masking agent, which is a high-affinity iron(III) chelating agent usually used for medical applications.[24] It has a high selectivity for Fe^{3+} and Al^{3+}; the stability constants for Fe^{3+} and Al^{3+} were reported to be 10^{31} and 10^{25}, respectively, whereas those for Ni, Cu, and Zn were reported to be 10^{10}, 10^{14}, and 10^{11}, respectively.[23] The stability constant of the Fe^{3+} complex (10^{31}) is higher than that of the Fe^{III}–EDTA complex (10^{25}), whereas the stability constants of M^{II}–deferoxamine complexes are generally much lower than those of M^{II}–EDTA complexes. These conditions should be suitable for

the purposes of separation and analysis. We selected a hydrophilic polymethacrylate-based chelate column, Nobias Chelate-PA1. The Nobias column has ethylenediaminetriacetic acid and iminodiacetic acid (IDA) as chelating moieties. Thus, it shows higher affinity to divalent heavy metal ions than the usual IDA-based chelate resins. Considering the relatively high stability constants of the target divalent heavy metal ions with the Nobias column compared with deferoxamine, we supposed that using the Nobias column would help the quantitative recovery of the target ions. We chose a pH of 5.5 as an elution condition because, at that pH, the column has no affinity for alkali metals and alkaline earth metals, and these metals were removed from the target metals.[18] In the preliminary study, the effects of the masking agent and the chelate column were examined using solutions including 1 μg cm^{-3} of the four target metals, Ni, Cu, Zn, and Pb, and 40 μg cm^{-3} of Al^{3+} and Fe^{3+}. The 40 μg cm^{-3} concentration of Al and Fe was nearly the same as that of the real sample. The solution including 63 mmol dm^{-3} of deferoxamine buffered to a pH of 5.5 was added to the chelate column to immobilize the target metals onto the column. The column conditions and the elution are described in the experimental section. The sample solution was analyzed using ICPAES. Table 1 shows the result for the determination of the four divalent heavy metals. As shown in Table 1, Ni, Cu, Zn, and Pb were quantitatively recovered, whereas Al^{3+} and Fe^{3+} were almost removed. This result shows that deferoxamine is an excellent masking agent for Al^{3+} and Fe^{3+}. Furthermore, fluoride and citrate, which are known as strong masking agents for Al^{3+} and Fe^{3+},[19,22] were also tested. For fluoride, all metals including Al^{3+} and Fe^{3+} were bound to the chelate column even in a 0.2 mol dm^{-3} solution of NaF. When citrate (ammonium salt) was used, Al^{3+} and Fe^{3+} were not sufficiently removed and some of the target metals were masked. Thus, currently the combination of deferoxamine and the Nobias chelate column is the best choice for the separation of the target heavy metals from Al^{3+} and Fe^{3+} and other ions such as alkali metal and alkaline earth metal ions.

Determination of metals in soil CRM using ESIMS

The method was evaluated by the analysis of the soil CRM (JSAC-401). The CRM was treated and analyzed using ESIMS as described in the experimental section. Linear relationships were observed in all standard addition plots. The quantification results are shown in Table 2. These values were converted to the concentrations in the original solid sample. The results agree well with the certified values, confirming the reliability of the current method. The limits of detection (LOD) calculated from 3σ values of the blank solution are also listed in Table 2.

Table 1 Separation efficiency of the chelate column using deferoxamine as a masking agent

Metals	Added/μg cm^{-3}	Measured[a]/μg cm^{-3}	Recovery (%)
Ni	1.0	0.99	99
Cu	1.0	1.01	101
Zn	1.0	1.00	100
Pb	1.0	1.10	110
Al	40	0.068	0.17
Fe	40	0.021	0.05

[a] Measured using ICPAES.

Table 2 Quantification results of trace metals in soil CRM

	Concentration of metals/μg g^{-1}		
	This work[a]	Certified value	LOD/ng g^{-1}
^{60}Ni	17.7 ± 3.5	18.9 ± 1.3	1.9
^{63}Cu	14.6 ± 2.1	15.3 ± 1.3	4.6
^{66}Zn	66.1 ± 6.6	66.8 ± 2.7	3.0
^{208}Pb	29.4 ± 6.0	26.0 ± 4.0	5.0

[a] Average of three measurements.

Conclusion

The determination of trace metals in a soil sample using electrospray ionization mass spectrometry (ESIMS) was successfully demonstrated. Deferoxamine was an effective masking agent for eliminating Fe and Al from other trace metals in the soil sample. Thus, the separation of divalent trace metal ions from Fe and Al was achieved using the chelate column, and the matrix effect for the ESIMS measurements was minimized.

Acknowledgements

We thank Prof. Tajima, Department of Medicine, Graduate School of Gunma University for his support. We are grateful for the partial support provided by the Steel Foundation for the Environmental Protection Technology (SEPT) (no. C-33-26 and C-30-25) to H.H.

References

1 R. Colton, A. D'Agostino and J. C. Traeger, *Mass Spectrom. Rev.*, 1995, **14**, 79.
2 V. B. Di Marco and G. G. Bombi, *Mass Spectrom. Rev.*, 2006, **25**, 347.
3 P. J. Taylor, *Clin. Biochem.*, 2005, **38**, 328.
4 P. Kebarle and L. Tang, *Anal. Chem.*, 1993, **65**, 972A.
5 L. Tang and P. Kebarle, *Anal. Chem.*, 1993, **65**, 3654.
6 T. L. Constantopoulos, G. S. Jackson and C. G. Enke, *J. Am. Soc. Mass Spectrom.*, 1999, **10**, 625.
7 R. Pascoe, J. P. Foley and A. I. Gusev, *Anal. Chem.*, 2001, **73**, 6014.
8 H. Sasaki, J. Yonekubo and K. Hayakawa, *Anal. Sci.*, 2006, **22**, 835.
9 S. Souverain, S. Rudaz and J.-L. Veuthey, *J. Chromatogr., A*, 2004, **1058**, 61.
10 B. K. Matuszewski, M. L. Constanzer and C. M. Chavez-Eng, *Anal. Chem.*, 1998, **70**, 882.
11 B. K. Matuszewski, M. L. Constanzer and C. M. Chavez-Eng, *Anal. Chem.*, 2003, **75**, 3019.
12 M. J. Avery, *Rapid Commun. Mass Spectrom.*, 2003, **17**, 197.
13 H. R. Liang, R. L. Foltz, M. Meng and P. Bennett, *Rapid Commun. Mass Spectrom.*, 2003, **17**, 2815.
14 U. Bahr, A. Pfenninger and M. Karas, *Anal. Chem.*, 1997, **69**, 4530.
15 A. T. Iavarone, O. A. Udekwu and E. R. Williams, *Anal. Chem.*, 2004, **76**, 3944.
16 H. Hotta, T. Mori, A. Takahashi, Y. Kogure, K. Johno, T. Umemura and K. Tsunoda, *Anal. Chem.*, 2009, **81**, 6357.
17 F. P. Dwyer, E. C. Gyarfas and D. P. Mellor, *J. Phys. Chem.*, 1955, **59**, 296.
18 Y. Sohrin, S. Urushihara, S. Nakatsuka, T. Kono, E. Higo, T. Minami, K. Norisue and S. Umetani, *Anal. Chem.*, 2008, **80**, 6267.
19 M. C. Williams, E. J. Cokal and T. M. Niemczyk, *Anal. Chem.*, 1986, **58**, 1541.
20 Y. Okabayashi, M. Ikeda, T. Nakagawa, H. Tanaka and M. Chikuma, *J. Fluorine Chem.*, 1988, **41**, 25.
21 M. Tabata, T. Yamada and J. Nishimoto, *Anal. Sci.*, 2001, **17**, 599.
22 S. Hasegawa, *Tetsu to Hagane*, 2011, **97**, 90.
23 Z. D. Liu and R. C. Hider, *Coord. Chem. Rev.*, 2002, **232**, 151.
24 H. Keberle, *Ann. N. Y. Acad. Sci.*, 1964, **119**, 758.

