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Accurate Determination of High Field Strength Elements (HFSE) in Geological Samples by ICP-MS Using an Improved Closed Digestion With Tartaric Acid as Matrix Modifier

Huan Yang[a], Lanlan Jin*[, a], Xiuji Wang[b], Wenxia Liu[c, a], Shaowei Wu[d], and Shenghong Hu[a]
[a] School of Earth Sciences, State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan 430074, P.R. China
[b] Guangdong Medical University, Dongguan 523808, P.R. China
[c] Northwest Research Institute of Mining and Metallurgy, Baiying 730900, P.R. China
[d] College of Chemistry and Environmental Engineering, Hubei University for Nationalities, Enshi 445000, P.R. China

ABSTRACT

An accurate determination of high field strength elements (HFSE) in geological samples provides information on the diagenetic evolution of rocks and is also of great significance for revealing the formation and evolution process of the continental crust and mantle. However, currently the concentration of HFSE in rocks is usually obtained using a conventional acid closed digestion process and its corresponding analytical result is relatively poor because of irreversible hydrolysis and polymerization. Fortunately, HFSE can be stable in solution when they complex with α−hydroxy acids due to their strong coordination abilities. After comprehensive consideration of the reagent blank and the complexing ability of the three α−hydroxy acids, it was found that when the tartaric acid concentration was 20% (v/v) together with the ratio of tartaric acid to nitric acid at 1:1, the tested values of HFSE for six rock standard reference materials agreed well with the certified values. Meanwhile, the structure of the complex products for HFSE with tartaric acid were resolved using a Q Exactive mass spectrometer. The detection limits of Nb, Ta, Zr, and Hf were obtained at 0.042, 0.018, 0.011, and 0.036 μg g^{-1} for this method, respectively. In this study, 35 geological SRMs were analyzed using this proposed method, and their accurate results for HFSE confirmed that it has great potential for the analysis of most types of geological samples.

INTRODUCTION

High field strength elements (HFSE), mainly Nb, Ta, Zr, and Hf, are tracers of considerable importance for large-scale mass and energy transfer in the Earth's interior for their relatively stable geochemical properties (1-4). Interpretive studies of the diagenetic evolution of magmatic rocks in the continental crust and mantle require precise and accurate data for these elements at their abundance levels in geological materials. Although instrumental neutron activation analysis (INAA) and X-ray fluorescence spectrometry (XRF) could be used for the determination of HFSE (5-9), these two methods are inappropriate for these four elements together and lack adequate sensitivity at trace levels (10). As a sensitive and immensely powerful multi-element analytical technique (11-14), the inductively coupled plasma source mass spectrometer (ICP-MS) is equipped to develop precise analytical methods for HFSE (10, 15-19). Due to the strong tendency to hydrolyze and to occur as polymerized species in acid solution chemistry (20, 21), the concentrations of HFSE, particularly Nb and Ta with extremely low levels in mantle rocks (22), are still virtually difficult to measure precisely with conventional ICP-MS methods.

Previous studies have shown that HFSE can be stabilized in solution by adding HF to form soluble HFSE fluoro-complexes (22-25). However, some authors reported that F^- can easily form fluoride precipitates with matrix elements such as Al, Mg, and Ca, which may adsorb some trace elements (HFSE included) from the HF solution and result in the loss of trace elements (26-32). In early analytical studies, tartaric acid (TA) was generally used as one of the auxiliary complexing agents for spectrophotometric determination of niobium and tantalum (33). It has been proven that due to the strong coordination of HFSE, they easily form complexes which can be stable in the solution with TA, citric acid, oxalic acid, malic acid, or other organic compounds of alpha-hydroxy acids (34-36). It was also found that only a proper amount of TA can stabilize the complex in a wide range of pH (33). In subsequent studies, Li et al. (37) effectively overcame the hydrolysis of Nb and Ta using potassium sodium tartrate complexing, and the detection limits of Nb and Ta based on

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*Corresponding author.
E-mail: annjll@163.com
Tel: +86-027-67848602
Fax: +86-027-67883452

ICP-MS were obtained at 1.05 ng mL^{-1} and 0.13 ng mL^{-1}, respectively (38). Undoubtedly, the use of TA is effective in stabilizing HFSE in solutions. However, due to the limitation of the analytical techniques, there are few reports on the mechanism of the complexation reaction between HFSE and TA, which directly affects the combined forms and the stability conditions of HFSE in TA solution. Researchers have speculated that TA and tantalum form polynucleic acid complexes (33, 34), but their specific polymer needs to be further discussed.

In this work, a validated method was developed for the simultaneous and accurate determination of HFSE in geological samples using an improved closed digestion ICP-MS method with TA as the matrix modifier, aiming to prevent the hydrolysis and polymerization of HFSE in acid medium. On the basis of optimizing the dosage and concentration of TA, the types of complex products of high field strength elements with TA were preliminarily resolved by HPLC-MS. A series of 35 geological standard reference materials (SRMs) of different properties were evaluated by the method under study. It was found that this method works well for analyzing HFSE in various geological samples.

EXPERIMENTAL

Analytical Instrumentation

The experiments for elemental analysis were carried out using a quadrupole ICP-MS instrument, NexION® 350D (PerkinElmer, Inc., Shelton, CT, USA) (39-42). The performance of the instrument was checked daily with a NexION Setup Solution (Be, Ce, Fe, In, Li, Mg, Pb, and U, 10 µg L^{-1}, 1% HNO_3) to obtain maximum signal intensities, while keeping the minimum oxide formation rate and doubly charged ratio. The typical instrumental operating conditions and measurement parameters are listed in Table I.

Structural elucidation of the organic complexes was performed using a Q Exactive Hybrid Quadrupole-Orbitrap mass spectrometry (Thermo Scientific, Bremen, Germany) (43). The instrument control and mass spectra processing were carried out by using the Xcalibur 3.0 software package (Thermo Scientific). A 10 µL sample solution was injected into doubly distilled water containing 0.1% formic acid at a flow rate of 0.3 mL min^{-1}. The identification experiments were processed in a negative ionization full-scan mode with a mass range of 120–600. The system was operated with a sheath gas flow rate of 30 L min^{-1}, auxiliary gas flow rate of 5 L min^{-1}, spray voltage of –3.2 kV, capillary temperature of 320 °C, S-lens RF level of 50%, and mass resolution of 70,000. All other instrumental parameters were set at the default values.

Reagents and Standards

Solutions used in this study were prepared with 18.2 MΩ cm ultrapure water produced by a water purification system (90005-02, Labconco WaterPro PS, USA). All chemicals used were of at least guaranteed reagent grade and most of them were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, P.R. China), except tartaric acid and citric acid which were obtained from Sigma-Aldrich, USA. To reduce the blank testing value, commercially available nitric acid and hydrofluoric acid were purified by subboiling in a two-bottle Teflon® still (Savillex Corporation, USA). External calibrators and internal standard solutions were respectively prepared by gravimetric serial dilution from 10 µg mL^{-1} multi-element standard solutions (SPEX CertiPrep, USA) and a single-element standard solution of rhodium at 1000 µg mL^{-1} (National Center for Analysis and Testing of Steel Materials, Beijing, P.R. China). The single element standard solutions of HFSE at 100 µg mL^{-1} used in the mass spectrometric analysis were purchased from the National Center for Analysis and Testing of Steel Materials, Beijing, P.R. China.

TABLE I
Instrumental Operating Parameters

NexION 350D ICP-MS	
RF plasma power	1500 W
Plasma gas flow	18 L min^{-1}
Nebulizer gas flow	0.90 L min^{-1}
Auxiliary gas flow	1.2 L min^{-1}
Peak pattern	3 points/peak
Sampling depth	8 mm
Dwell time	50 ms
Integration time	1250
Sweeps per reading	25
Reading per replicate	3
$^{156}CeO/^{140}Ce$ ratio	< 2.0%
Ce^{2+}/Ce^{+} ratio	< 2.0%

Geological Reference Materials

Thirty-five geological SRMs were used to evaluate the accuracy of the established method. Three rock standard reference materials (SRMs) including AGV-2 (andesite), BCR-2 (basalt) and BHVO-2 (basalt) were purchased from the U.S. Geological Survey (USGS, USA), three SRMs (ORTO-1 rhyolite, OU-7 gabbro, GSM-1 gabbro) were obtained from the International Association of Geoananlysts (IAG, UK), and 29 geological SRMs (GBW07103 granite, GBW07105 basalt, GBW07106 quartz sandstone, GBW07107 shale, GBW07108 carbonate, GBW07110 trachyte, GBW07111 diorite, GBW07112 gabbro, GBW07114 dolomite, GBW07120 limestone, GBW07121 granite-gneiss, GBW07403 yellow-brown earth, GBW07404 soil, GBW07405 yellow-red earth, GBW07407 latosol, GBW07423 Hongze lake deposit, GBW07426 soil from Xinjiang, GBW07428 soil from Sichuan basin, and stream sediments GBW07302-GBW07306, GBW07307a, GBW07309-GBW07313) were from the Institute of Geophysical and Geochemical Exploration (IGGE, P.R. China) .

Sample Pretreatment

A 50 mg homogenized sample powder (<200 mesh) was weighed into a 10 mL homemade PTFE-lined stainless steel bomb and soaked with several drops of ultrapure water. Then 1 mL of purified HNO_3 and HF were successively added, and the sample was digested under high pressure at 190 °C in an electric oven for 48 hours. After cooling, the bomb was opened, and the digestion solution evaporated to incipient dryness at 120 °C on a heater. This was followed by adding 1 mL HNO_3 and evaporating it for a second round of dryness in order to remove HF thoroughly. The resultant salt was redissolved while it was hot by adding 1.5 mL of 30% (v/v) HNO_3 and 1.5 mL of 20% (m/v) TA. The final solution was diluted to ~100 g with 2% (m/v) HNO_3 for ICP-MS analysis. A reagent blank solution was prepared using the same procedure.

RESULTS AND DISCUSSION

Effect of Different Complexant

Niobium and tantalum reacting with organic carboxylic acid can be stabilized in the form of complex anions in acid medium. It has been reported that the stability of complexes formed by niobium and different compounds is in the following order: oxalic acid > hydrogen peroxide > citric acid > TA (34). Therefore, in order to investigate the overall complexation ability between HFSE and three organic complexants (including TA, oxalic acid, and citric acid), the relative errors (REs) of detection results for HFSE coupled with these reagents in six rock SRMs were compared (Figure 1). The data showed that the REs of HFSE results using TA were less than those with the other two organic complexants, which may be due to the effect of the nature of the HFSE and the difference in concentration of the matrix elements. The reagent blanks of these three complexing agents at a uniform concentration (0.2% m/v) were inspected at the same time. The levels of Nb, Ta, Zr, and Hf in TA and citric acid were both less than in oxalic acid, which are listed in Table II. Based on comprehensive consideration of the experimental results, TA was selected as the desired organic complexing agent for the present work.

Optimization of Concentration and Dosage for TA

The stability and reactivity of HFSE in the organic complex both depend on the concentration and dosage of the organic complexing reagent. Previous studies indicated that stable anionic complexes formed by TA and tantalum usually required 18 times the concentration of TA than that of Ta (34). Otherwise, uncomplexed HFSE will produce insoluble oxide precipitates in the dilute acid medium due to hydrolysis and polymerization. In our research, TA was added in the extraction stage to prevent hydrolysis of HFSE. It was found that 20% TA was sufficient to complex HFSE in rock samples, as shown in Figure 2a.

On the other hand, when the amount of TA is excessive, high levels of Total Dissolved Solids (TDS) in sample solution will not only cause blockage of the atomizer or sampling cone, but also will lead to significant matrix effects and potential spectral interferences during the analysis process, which affects accuracy of the analytical results. We optimized the proportion of 20% TA and high purity nitric acid. Figure 2b shows that when the ratio of TA to nitric acid is 1:1 (v/v), the tested values of HFSE for the six rock SRMs agreed well with the

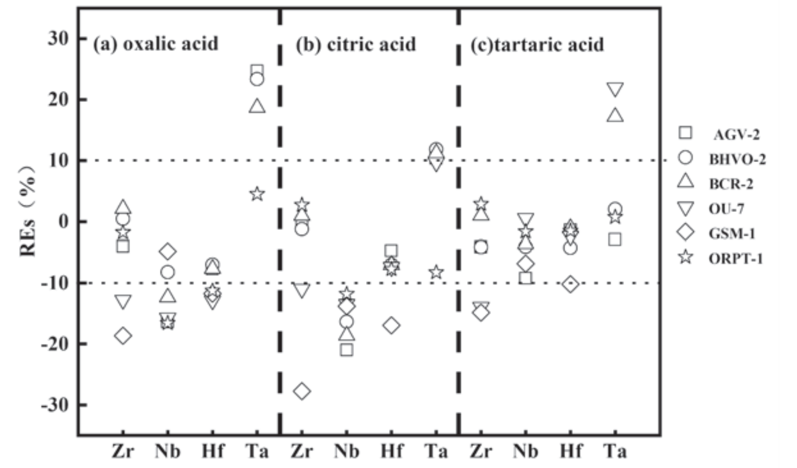

Fig. 1. Relative errors (REs) of detection results for HFSE coupled with (a) oxalic acid, (b) citric acid, and (c) TA in six rock SRMs.

TABLE II
Concentrations of Zr, Nb, Hf, and Ta in 0.2% (m/v) TA, Citric Acid, and Oxalic Acid, Respectively (ng mL^{-1})

Reagent Blank	Zr	Nb	Hf	Ta
Oxalic acid ($H_2C_2O_4$)	1.7	0.077	0.043	0.080
Citric acid ($C_6H_8O_7$)	0.036	0.0036	0.0011	0.0075
TA ($C_4H_6O_6$)	0.051	0.011	0.0038	0.011

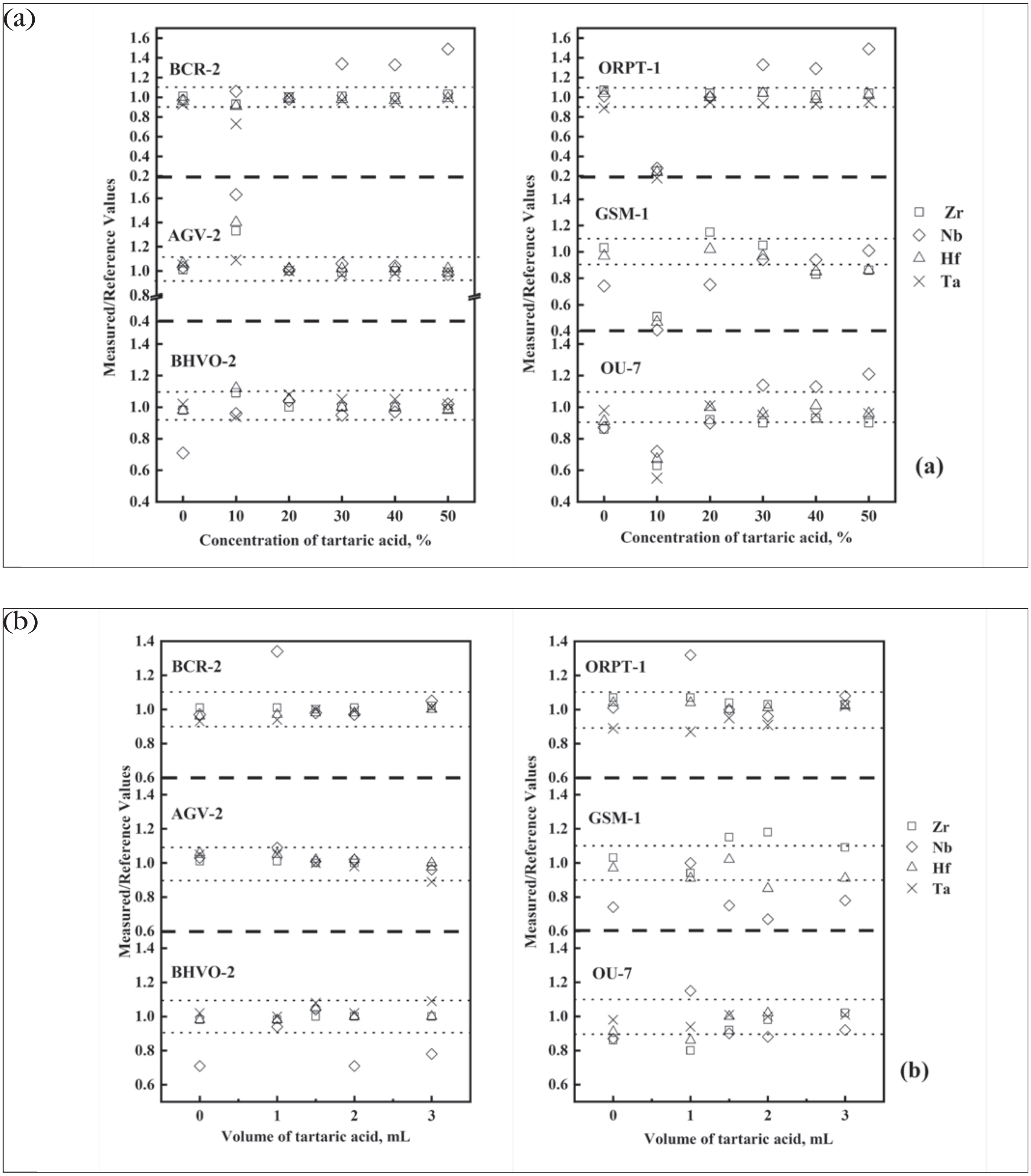

Fig. 2 Effects of concentration (a) and volume (b) of TA on accuracy of HFSE in six rock SRMs.

certified values, and at this point TA did not cause significant matrix effects in the testing process by ICP-MS.

Identification of Organic Complexes of HFSE with TA

The properties of Nb and Ta in TA solution directly affect their reactivities, reaction characteristics, and reaction rules. But there were few data on the complexes of HFSE with TA. Some researchers have speculated that the composition of the complexes of niobium, tantalum, and TA might be complex salts, such as $H[NbO(C_4H_4O_6)_2]$, $H_5[NbO_4(C_4H_4O_6)]\cdot 2.5H_2O$, $Me_2ONb_2O_5(C_4H_4O_6)_2\cdot nH_2O$, $MeH[TaO(C_4H_4O_6)]$, and so on (34). In the present study, mass spectra of possible organic complexes of HFSE with TA were analyzed using the Q Exactive mass spectrometer, and chemical formulas for these were calculated using the Xcalibur software which allowed for the calculation of the basic elements C, H, O, and HFSE atom within a 5 ppm mass tolerance.

The single element standard solutions of Nb, Ta, Zr, and Hf (100 µg mL^{-1} each) were mixed with 20% tartaric acid and 30% nitric acid, respectively, and then diluted to 1 µg mL^{-1} with deionized water to simulate the complex products of HFSE with tartaric acid in dilute nitric acid matrix. In these complexing solutions, the obtained mass spectrum was characterized by the base peak at m/z 149.0097, which is attributed to the excess deprotonated tartaric acid. Firstly, we studied the complexes of Zr and Hf, which are easy to be analyzed by mass spectrometry because both of them have multi-natural isotopes. As shown in Figure 3, five isotopic mass spectra peaks of Zr (relative abundances are 51.46, 11.23, 17.11, 17.40, and 2.80) were found at m/z 384.9001 (Figure 3a), 404.9066 (Figure 3b), and 534.9168 (Figure 3c), and the proposed molecular formulas were $[C_8H_7O_{12}Zr]^-$, $[C_8H_{17}O_7Zr_2]^-$, $[C_{13}H_{29}O_5Zr_3]^-$ or $[C_{30}H_5O_5Zr]^-$, respectively. We checked every chemical formula according to the basic chemical criteria and finally selected its valid chemical formula of $[C_8H_7O_{12}Zr]^-$, whose structure

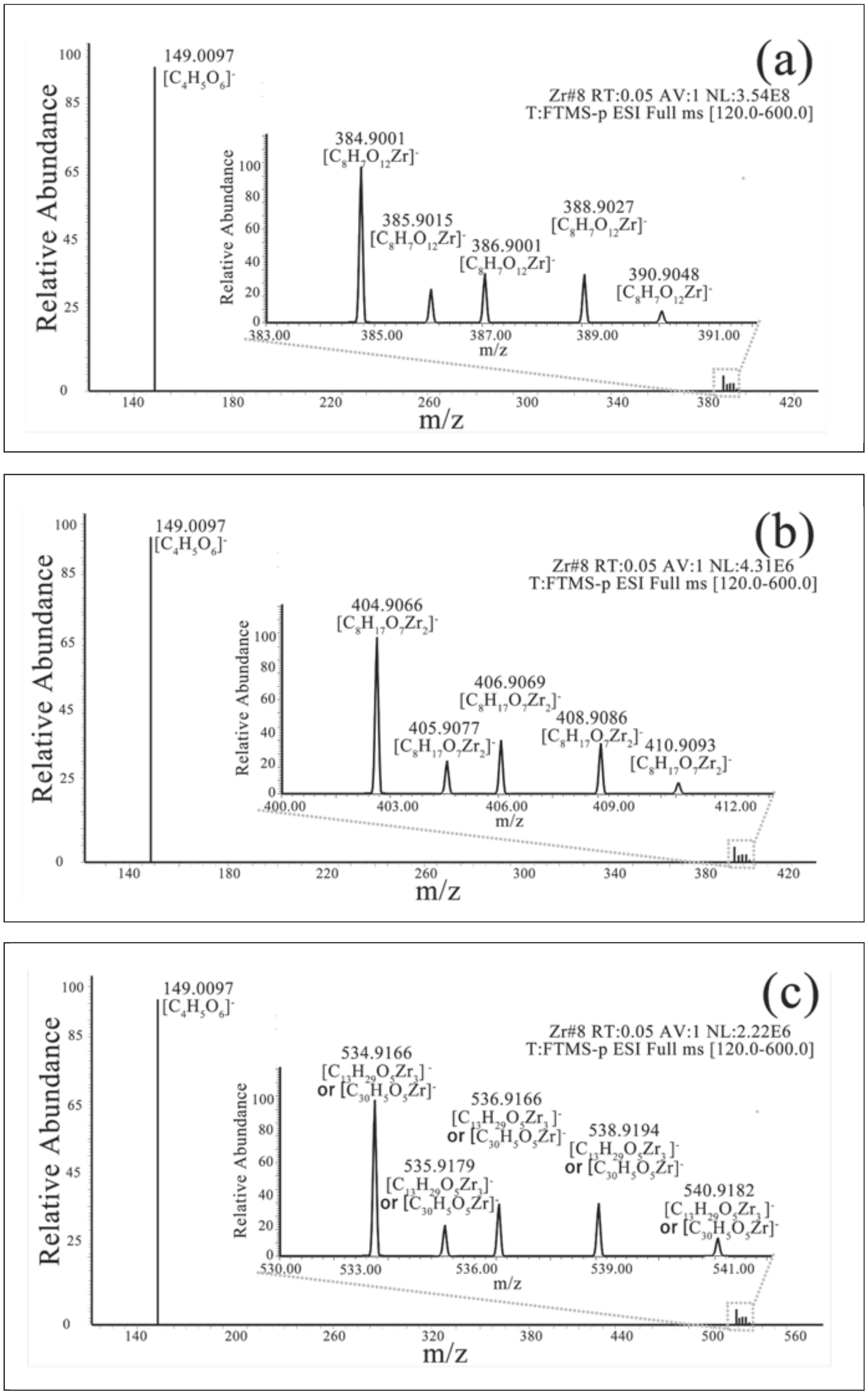

Fig. 3. Mass spectra of Zr/tartaric complex in negative mode: (a) m/z values of 384.9001; (b) m/z values of 404.9066; (c) m/z values of 534.9168.

should be $(C_4H_4O_6)_2Zr$. Unfortunately, no effective complex of Hf with tartaric acid has been found in our experiments.

Nb has only one natural isotope, which makes it difficult to analyze the possible complexes by mass spectrometry. Although Ta has two natural isotopes (^{180}Ta and ^{181}Ta), the relative abundance of ^{180}Ta is only 0.012, which is often annihilated in the process of mass spectrum elucidation. Therefore, for Nb and Ta, we used mass spectrometry to validate the possible complexes, which were boldly speculated based on previous work (34). On the basic chemical criteria, namely, that there may be five products in the complexation reaction of Ta with tartaric acid. The corresponding mass numbers of the five products were calculated by software Xcalibur. The results showed that the effective ionic currents and corresponding mass spectra peak of $H_5[NbO_4(C_4H_4O_6)]$, $Me_2ONb_2O_5(C_4H_4O_6)_2$ and $MeH[NbO(C_4H_4O_6)]$ did not appear, which confirms that these are not valid chemical formulas. Nevertheless, two effective mass spectra peaks have been found for Nb at m/z 272.8986 (Figure 4a) and 404.9051 (Figure 4b), the chemical formulas $[C_8H_8O_{13}Nb]^-$ and $[C_4H_4O_8Nb]^-$ resolved by software are in accordance with our expectations. Therefore, the complex product of Nb with tartaric acid might be $H[NbO(C_4H_4O_6)_2]$ and $H[NbO_2(C_4H_4O_6)]$. A similar procedure was used for Ta. The peak of $[C_8H_{10}O_{14}Ta]^-$ obtained at m/z 510.9556 is in agreement with what we expected (Figure 5), which confirms that the complex product of Ta might be $HTa(OH)_2(C_4H_4O_6)_2$.

At the same time, we also scanned the blank solution of tartaric acid using the Q Exactive mass spectrometer. It was found that the mass spectrum peak of the HFSE complex resolved in our work did not exist in the blank solution, which excluded the influence of background interference peak.

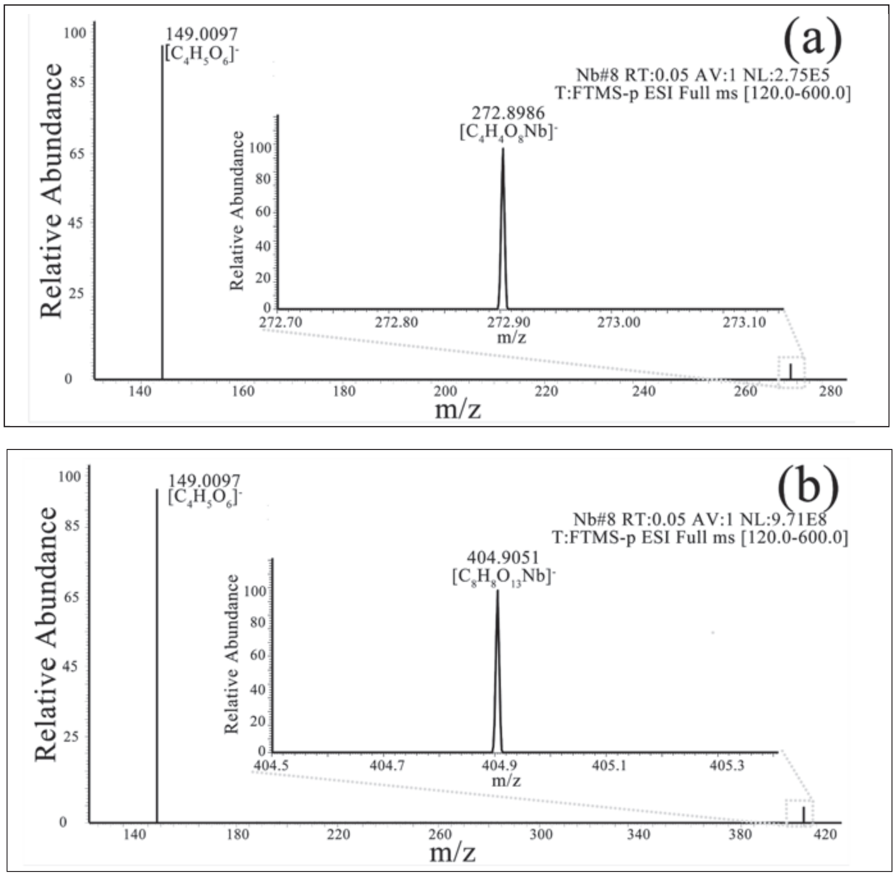

Fig. 4. Mass spectra of Nb/tartaric complex in negative mode: (a) m/z values of 272.8986; (b) m/z values of 404.9051.

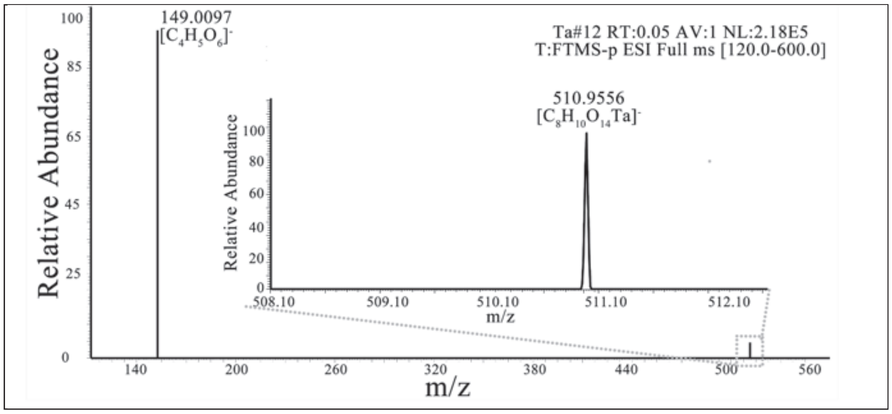

Fig. 5. Mass spectra of Ta/tartaric complex in negative mode.

Analytical Performance

The analytical performance of the proposed method, including

TABLE III
Nb, Ta, Zr, Hf Levels in Six Geological SRMs by Present Method
(n=3, µg g^{-1})

Name	Nb			Ta			Zr			Hf		
	Ref. Value	This Work	RSD (%)	Ref. Value	This Work	RSD (%)	Ref. Value	This Work	RSD (%)	Ref. Value	This Work	RSD (%)
AGV-2	14.5	14.0	4.66	0.87	0.87	6.70	230	232	1.94	5.00	5.22	2.14
BHVO-2	18.1	18.6	2.66	1.14	1.19	3.11	172	170	2.96	4.36	4.41	4.07
BCR-2	12.6	12.1	3.35	0.78	0.79	3.35	184	184	0.61	4.90	4.83	2.54
OU-7	6.35	6.71	4.41	0.38	0.47	7.00	133	115	3.42	3.17	3.00	2.44
GSM-1	0.90	0.95	10.3	-	0.20		27.0	26.5	7.63	0.83	0.83	3.13
ORPT-1	11.6	11.6	5.19	0.99	0.88	14.7	245	251	3.93	7.53	7.36	3.16

precision, accuracy, and detection limits, was evaluated under the optimal conditions. Precision was studied by calculating the relative standard deviation (RSD) of three replicate measurements, and the accuracy was determined by comparing the obtained results with the certified or information values for AGV-2, BHVO-2, BCR-2, GSM-1, OU-7, and ORPT-1 (Table III). The detection limits of Nb, Ta, Zr, and Hf in this work, defined as the concentration equivalent to 10 times the standard deviation of 11 measurements for a blank solution, were 0.018, 0.036, 0.042, and 0.011 µg g^{-1}, respectively.

Analysis of Geological SRMs

Using the optimized procedure, a series of geological SRMs comprising seven soils, 17 rocks, and 11 sediments were chosen to validate the established analytical method in which these samples cover three basic rock types containing igneous (andesite, basalt, diorite, gabbro, trachyte), sedimentary (limestone, dolomite, sandstone, shale, carbonate), and metamorphic (gneiss) rocks. Due to the difference of the HFSE content in samples of distinct lithology and the test accuracy requirements of different concentration ranges are not the same, the results of 35 SRMs were evaluated according to the analytical accuracy control rules in the specification of geochemical reconnaissance survey (1:50,000) (DZ/T 0011-2015) (44). The comparison for the logarithm values between the analytical results and the reference values for the HFSE in 35 reference materials is illustrted in Figure 6. Except for two sedimentary carbonate rocks (GBW07114 dolomite and GBW07120 limestone), whose contents of HFSE are at very low levels, the accuracy of the results in 33 geological reference materials was in good agreement with the requirements of the specification of the geochemical reconnaissance survey.

CONCLUSION

This work has demonstrated that the method of a modified closed digestion with TA as the matrix modifier based on ICP-MS analysis can precisely and accurately analyze HFSE in various geological samples at the same time. For HFSE, the irreversible hydrolysis and polymerization in acid medium and the loss due to adsorption of fluoride precipitation in HF medium were solved successfully by this proposed method. Furthermore, in order to clarify the mechanism for the stable existence of HFSE in TA, the complexing products of high field strength elements with TA were analyzed by high resolution mass spectrometry. Except for Hf, the molecular formulas of complexing products for the other three HFSE (Nb, Ta, and Zr) were obtained. It can be stated that the proposed method is valid and was employed to measure 35 geological SRMs, and that the results of 33 materials were in good agreement with the certified or reference values.

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REFERENCES

1. Y.J. Liu and L.M. Cao, Introduction to elemental geochemistry, Geologi-

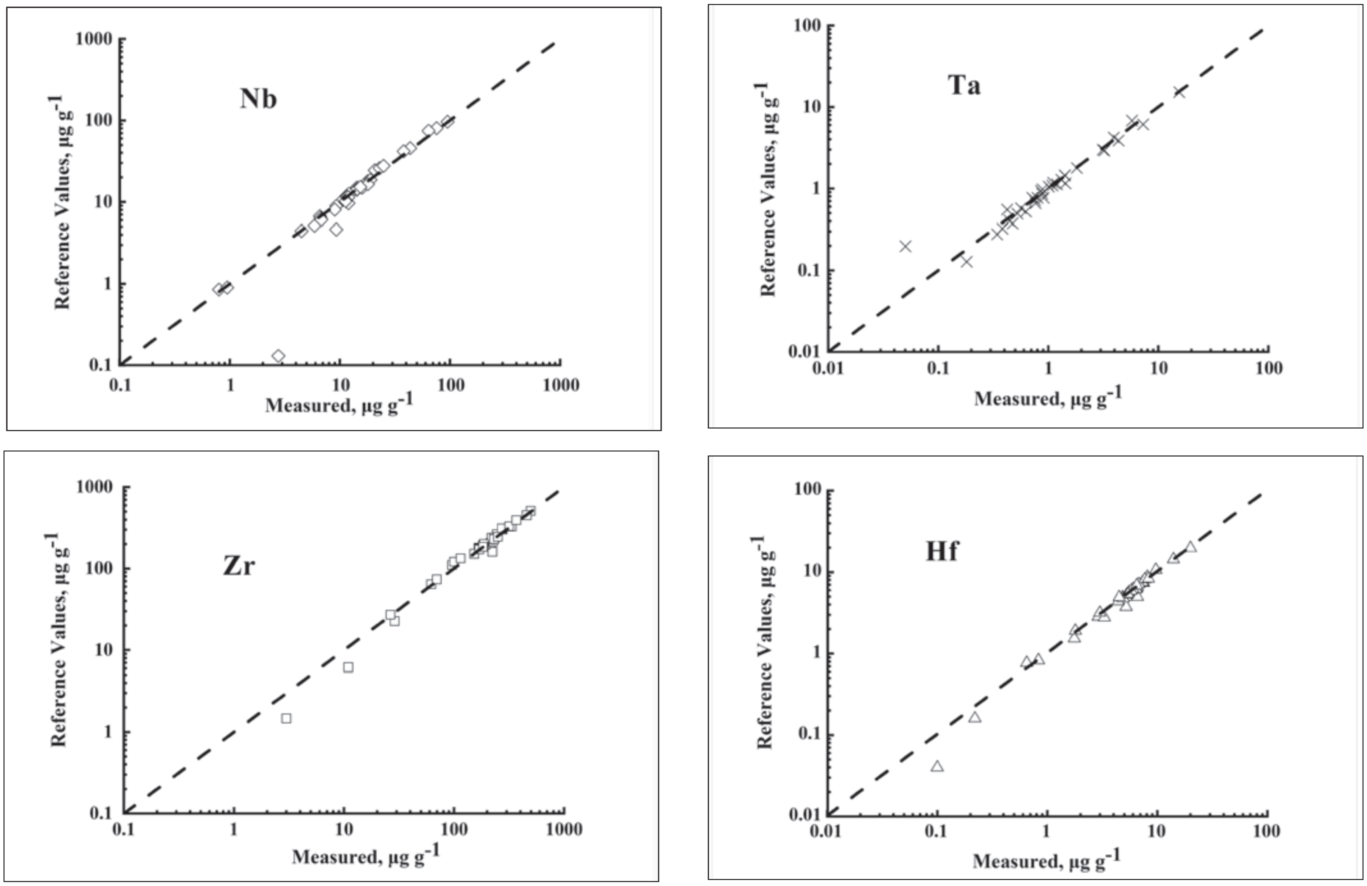

Fig. 6. Determination values of Nb, Ta, Zr, and Hf in this study vs. the reference values for 35 geological SRMs.

cal Publishing House, Beijing, P.R. China (1987).

2. M.G. Barth, W.F. McDonough, and R.L. Rudnick, Chem. Geol. 165(3-4), 197 (2000).
3. Z.C. Hu and S. Gao, Chem. Geol. 253(3-4), 205 (2008).
4. M. Kirchenbaur and C. Münker, Geochim. Cosmochim. Ac. 165, 178 (2015).
5. D. Robinson and M.C. Bennett, Geostand. Newsl. 5(2), 175 (1981).
6. R.B. Hallett and P.R. Kyle, Geostand. Newsl. 17(1), 127 (1993).
7. U. El-Ghawi, N. Vajda, and G. Patzay, J. Radioanal. Nucl. Chem. 241(3), 605 (1999).
8. E. Steinnes, J. Radioanal. Nucl. Chem. 261(3), 701 (2004).
9. S. Waheed, N. Siddique, and Y. Faiz, Geostand. Geoanal. Res. 37(2), 197 (2013).
10. X.J. Yang and C. Pin, Anal. Chim. Acta 458(2), 375 (2002).
11. Y.T. Li, W. Guo, Z.C. Hu, L.L. Jin, S.H. Hu, and Q.H. Guo, J. Agric. Food Chem. 67, 935 (2019).
12. Z.W. Wu, W. Guo, L.L. Jin, and S.H. Hu, Microchem. J. 142, 251 (2018).
13. W. Guo, L.L. Jin, S.H. Hu, and Q.H. Guo, J. Agric. Food Chem. 65, 3406 (2017).
14. D.Y. Tao, W. Guo, W.K. Xie, L.L. Jin, Q.H. Guo, and S.H. Hu, Microchem. J. 135, 221 (2017).
15. X.L. Pu, C.Z. Huang, B. Hu, and Z.C. Jiang, Geostand. Geoanal. Res. 30(2), 97 (2006).
16. G. Bayon, J.A. Barrat, J. Etoubleau, M. Benoit, C. Bollinger, and S. Revillon, Geostand. Geoanal. Res. 33(1), 51 (2009).
17. C. Xiong , M. He and B. Hu, Geostand. Geoanal. Res. 33(3), 385 (2009).
18. E.V. Smirnova, B. Flem, E.A. Anchutina, I.N. Mysovskaya, V.I. Lozhkin, and L.L. Petrov, Geostand. Geoanal. Res. 34(1), 49 (2010).
19. S.L. Sun and J. Li, Microchem. J. 119, 102 (2015).
20. Y.R. Wang, F. Gu, and Z.Q. Yuan, Geochimica (1), 55 (1992).
21. F. Poistrasson, C. Pin, P. Telouk, and J.L. Imbert, Geostand. Newsl. 17(2), 209 (1993).
22. S. Weyer, C. Münker, M. Rehkämper, and K. Mezger, Chem. Geol. 187(3-4), 295 (2002).
23. A. Makishima, E. Nakamura, and T. Nakano, Geostand. Newsl. 23(1), 7 (1999).
24. K. Nagaishi and T. Ishikawa, Geochem. J. 43, 133 (2009).
25. W. Zhang, Z.C. Hu, Y.S. Liu, L. Chen, H.H. Chen, M. Li, L.S. Zhao, S.H. Hu, and S. Gao, Geostand.

Geoanal. Res. 36(3), 271 (2012).

26. T. Yokoyama, A. Makishima, and E. Nakamura, Chem. Geol. 157(3-4), 175 (1999).
27. R. Tanaka, A. Makishima, H. Kitagawa, and E. Nakamura, J. Anal. At. Spectrom. 18, 1458 (2003).
28. A. Makishima, R.Tanaka, and E. Nakamura, Anal. Sci. 25, 1181 (2009).
29. W. Zhang, Z.C. Hu, Y.S. Liu, H.H. Chen, S. Gao, and R.M. Gaschnig, Anal. Chem. 84 (24), 10686 (2012).
30. Z.C. Hu, W. Zhang, Y.S. Liu, H.H. Chen, R.M. Gaschnig, K.Q. Zong, M. Li, S. Gao, and S.H. Hu, Chem. Geol. 355, 144 (2013).
31. W. Zhang, L.Qi, Z.C. Hu, C.J. Zheng, Y.S. Liu, H.H. Chen, S. Gao, and S.H. Hu, Geostand. Geoanal. Res. 40(2), 195 (2016).
32. O.I. Okina, S.M. Lyapunov, and A.S. Dubensky, Microchem. J. 140, 123 (2018).
33. Z.M. Luo, Acta Chimica Sinica 38(5), 433 (1980).
34. G.R. Qin, Chinese J. Anal. Chem. 6(6), 474 (1978).
35. X.J. Song, T.C. Duan, P.G. Guo, and H.T. Chen, Microchem. J. 84(1-2), 22 (2006).
36. M. Biver and M. Filella, Geostand. Geoanal. Res.42(3), 197 (2018).
37. S.M. Li, G.Z. Wang, J. Zhao, and A.X. Liu, Metall. Anal. 32(3), 48 (2012).
38. Z.Q. Li, X.Y. Li, K. Zhu, X.X. Xu, and Z.Y. Yan, Spectrosc. Spect. Anal. 35(8), 2303 (2015).
39. W. Guo, Z.W. Wu, S.H. Hu, L.L. Jin, K.Y. Qiu, Q.H. Guo, and Y.Q. Gan, RSC Adv. 6, 47394 (2017).
40. Y.E. Peng, W. Guo, J. Zhang, Q.H. Guo, L.L. Jin, and S.H. Hu, Microchem. J. 124, 127 (2016).
41. W. Guo, S.H. Hu, Z.W. Wu, G. Y. Lan, L.L. Jin, X.G. Pang, J.C. Zhan, B. Chen, and Z.Y. Tang, J. Anal. At. Spectrom. 30, 986 (2015).
42. W. Guo, S. H. Hu, Y. F. Xiao, H. F. Zhang, and X. J. Xie, Chemosph. 81, 1463 (2010).
43. L.F. Yun, Y.E. Peng, Q. Chang, Q.X. Zhu, W. Guo, and Y. X. Wang, J. Agric. Food Chem. 65, 5384 (2017).
44. Standards for Geology and Mineral Industry of the People's Republic of China: Specification of Geochemical Reconnaissance Survey (1:50000), DZ/T 0011-2015. Ministry of Land and Resources, PRC (2015).

Comparison of Modified Tessier and Revised BCR Sequential Extraction Procedures for the Fractionation of Heavy Metals in Malachite Ore Samples Using ICP-OES

Elif Varhan Oral
Dicle University, Faculty of Pharmacy, Department of Analytical Chemistry, 21280 Diyarbakır, Turkey

ABSTRACT

In this study, two sequential extraction procedures (modified Tessier procedure and revised BCR procedure) were applied for the decomposition of Fe, Cu, Co, Pb, Zn, and Mn in the structure of malachite ore from the Keban region of Turkey. The effects and efficiency of each extraction step used in these procedures were examined. Metals analysis was performed by ICP-OES. Due to the differences in the binding forms of the metals existing in the structure of the ore and the different properties of reagents used, the concentrations of the metals were different in the extraction steps of both procedures. When the results were examined, it was found that the metal concentrations passing to the solution in each extraction step of the modified Tessier procedure were much higher than of the revised BCR procedure. Thus, it was concluded that the modified Tessier procedure is more suitable for decomposing metals in malachite ore.

INTRODUCTION

Heavy metals are dangerous environmental pollutants and their toxicity is an increasing problem for ecological, evolutionary, nutritional, and environmental reasons (1). The accumulation of heavy metals in soil and waters depends on anthropogenic activities and lithogenic sources and are found to alter the geochemical distribution of heavy metals more than in lithogenic sources (2-4). Mining activities facilitate the movement and distribution of heavy metals, and according to World Health Organization (WHO) reports, it is one of the activities that can affect human health and pollute the environment (5). Contamination of the environment with potentially hazardous materials is a complex function due to factors such as hydrology, geology, geochemistry, mining, and ore processing (6). Metal mining is known to cause contamination by spreading heavy metals into the environment in excess concentrations to soil and water (7, 8). Therefore, analysis of heavy metals in the structure of ores is of great importance.

Information about the presence of heavy metals in solids, such as soil and ore, can be obtained not only from the total concentration but also by assessing their specific chemical forms, their binding states, and their elemental properties (9). Potentially toxic elements can accumulate between different phases such as water-soluble, exchangeable, carbonate-associated, Fe-Mn oxide-associated, organic-associated, and residual forms (10, 11). Therefore, when evaluating the toxic properties of trace elements in solids, determination of the total metal content provides insufficient information about bioavailability, mobility, or toxic effects (9, 12). As a result, information about the bonding force between the metals and the ore and the capacity of mobilization of heavy metals existing in the ore structure is necessary.

Sequential extraction procedures provide information on potential bioavailability, mobility of solid and metal bonds, and heavy metal bioavailability (11, 13). Among various and different sequential extraction procedures, the most commonly used procedures are the Tessier method and its modifications (14-20) and the Community Bureau of Reference (BCR) method and its modifications (19-28).

The five-step sequential extraction procedure was first introduced by Tessier et al. (18) and many studies have utilized this procedure since (17, 19). This five-step scheme has grouped the metals as exchangeable, weakly absorbed, hydrous oxide-bound, organic bound, and lattice material components (29-32). The modified Tessier procedure, which is another extensively utilized sequential extraction method, differs from the original Tessier method by having a four-step extraction: exchangeable, carbonates, organic matter and sulfides, and residual (19). The BCR procedure and the Tessier method greatly resemble each other, except there is a major difference in the first step of the BCR method. In the study of Ure et al. (28, 30, 32), the exchangeable and carbonate-bound steps were combined in the first part, not calculating them separately. Hence, this study proposed a three-step sequential extraction procedure and they divided the metals into acid-soluble/exchangeable, reducible, and oxidizable fractions (19, 29).

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*Corresponding author.
E-mail: evarhan@dicle.edu.tr
Tel: +90 412 248 8030/7543
Fax: +90 5056893699:

The purpose of the current study is to compare the concentration of metals in the malachite ore structure obtained from the Keban region by applying the modified Tessier and the revised BCR procedures. For the heavy metal fractions, the extraction steps and the aforementioned methods were tested.

EXPERIMENTAL

Instrumentation

An Optima™ 2100 DV inductively coupled plasma optical emission spectrometer (ICP-OES) (PerkinElmer, Inc., Shelton, CT, USA) was used for the metals analysis. The respective instrumental operating parameters are listed in Table I. The absorbance was measured at the maximum wavelength of 238.2 nm for Fe, 228.6 nm for Co, 257.6 nm for Mn, 327.4 nm for Cu, 220.4 nm for Pb, and 206.2 nm for Zn.

TABLE I
ICP-OES Instrumental Operating Conditions

Parameters	
RF power	1450 W
Plasma gas flow rate	15 L min^{-1}
Auxiliary gas flow rate	0.2 L min^{-1}
Nebulizer gas flow rate	0.8 L min^{-1}
Sample flow rate	1.5 L min^{-1}
View mode	Axial
Read	Peak area
Source equilibration time	15 s
Read delay	50 s
Replicates	3
Purge gas	Nitrogen
Shear gas	Air
Plasma gas	Argon

Metals	Maximum Wavelength
Fe	238.2 nm
Co	228.6 nm
Mn	257.6 nm
Cu	327.4 nm
Pb	220.4 nm
Zn	206.2 nm

Acid digestion of the samples was performed using a MWS-3 microwave digestion system (Berghof Co., Germany) with pressure control. A Memmert brand shaker (Memmert GmbH, KG, Germany) and a Heraeus brand Labofuge-200 model centrifuge (Thermo Fischer Scientific, Germany) were utilizied for the sequential extractions. The pH measurements and adjustments were carried out with a Mettler-Toledo pH meter (Schwerzenbach, Zürich, Switzerland). A Baysan brand jaw crusher and pulverizer (Turkey) was used for grinding the ore which was then passed through a 100 mesh sieve (Yüksel Kaya Makina, standard testing sieve, Turkey). A Milli-Q® purifier system (Millipore Corporation, USA) was used for obtaining ultrapure water.

Chemicals and Standard Solutions

All reagents used in this study were of analytical grade: hydrogen peroxide (Merck), acetic acid (Sigma), ammonium acetate (Merck), hydroxylamonium hyrochloride (Sigma), magnesium chloride, sodium acetate, hydrofluoric acid and nitric acid (Fluka). Serial dilution of the 1000 mg L^{-1} stock solution of the desired element was done in preparation of the standard solutions.

Preparation of Ore Samples

In this study, the ore samples were obtained from the Keban region in Elazığ, Turkey. First, the samples were stored in boxes, then dried in an oven at 120 °C for 2 hours andy broken with the jaw crusher and pulverizer, and finally milled and passed through a sieve of 100 mesh.

MATERIALS AND METHODS

Sequential Extraction Procedure

In this study, the heavy metal concentrations in malachite ore were calculated by using the modified Tessier and revised BCR procedures, and the data obtained from these two procedures were compared. The chemicals used in the sequential extraction procedures and the extraction conditions are listed in Tables II and III. After the extraction steps, the ore samples were washed with ultrapure water, and the supernatants separated by centrifugation. The reason for this step was to eliminate the remaining chemical residues from the previous extraction steps.

Revised BCR Procedure

The revised BCR is a 3-step sequential extraction procedure which has been proposed by gathering and validating the fractionation methods used for soils and solids (33). The original BCR procedure can also be applied to solids such as soils (34) and sediments (35). Compared with the original BCR procedure, the revised BCR procedure was studied using a high concentration of NH_2OH–HCl and low pH (33). In this procedure, approximately 1 g of the ore sample was weighed and metal extraction was carried out by adding the specified volume and concentrations of chemicals listed in Table II. The mixtures obtained after each step were centrifuged and separated from the residue. The first step of this method depends on acetic acid extraction, the second step is a hydroxylamine hydrochloride extraction, and the third step is an hydrogen peroxide oxidation and ammonium acetate extraction. In step four, the residual phase is extracted using *aqua regia* (HCl/HNO_3, 3:1). After this step, the resulting mixture was filtered through a filter paper and completed to 100 mL volume with ultrapure water (19).

Modidfied Tessier Procedure

Table III presents the modified Tessier procedure diagram. In step 1, approximately 1 g of the ore sample is extracted with 10 M 1 mL of ammonium acetate (pH = 7). The mixture is then shaken at room temperature for 1 hour at 150 rpm and centrifuged at 4,000 rpm for 10 minutes. The aqueous phase is decanted and completed to 50 mL volume with ultrapure water. In step 2, 1 M 10 mL of acetic acid is added to the residue obtained from step 1. After shaking for 1 hour at 150 rpm, the mixture is centrifuged at 4,000 rpm for 10 minutes. Then, the aqueous phase is decanted to 50 mL with ultrapure water. In step 3, 5 mL of 30% H_2O_2 is added to the residue obtained from step 2. The pH is adjusted to 2.00 with nitric acid; shaken at 150 rpm at 35 °C for 3 hours and centrifuged at 10,000 rpm, respectively. The sample is then filtered through a filter paper (10 mesh size) and completed to 100 mL volume with ultrapure water. In step 4, *aqua regia* (2.5 mL HNO_3 (65%) and 7.5 mL HCl (37%)) is added to the residue remaining from step 3; then solubilization is performed by microwave oven. The sample is then filtered through the filter paper, and finally completed to 100 mL volume with ultrapure water (19).

RESULTS AND DISCUSSION

Revised BCR Procedure

The first step was utilized for determining the solubilization ratio of the metal ions (Fe, Cu, Zn, Pb, and Mn) in the ore structure by forming acetate salts. As a result of this step, it was thought that these ions in the ore structure could pass at different rates according to their solubility in the mineral structure; this observation was supported with the experimental results as listed in Table IV.

The second step was applied to create conditions where the metal ions can pass into the solution by reduction of the hydroxyl amine present in the medium and both the proton donor and the complexing properties of HCl. When Table IV was examined, it was observed that the other metals (Cu, Zn, Pb, Mn), except for Fe, were dissolved, which may be due to the Fe precipitate as its hydroxide compounds.

The reason for the addition of H_2O_2 is that it provides decomposition of organic matter (CO_2, H_2O, NO_x) as a strong oxidant due to its high oxidizing activity which causes a reduction electrode potential of 1.70 V. This step was done for dissolution of the sulfur compounds by producing elemental S

TABLE II
Operating Conditions of Revised BCR Sequential Extraction Procedure

Fraction	Extracting Agents	Extraction Conditions Shaking Time[a]	 Temperature
F1. Exchangeable + water and acid soluble	40 mL CH_3COOH (0.11M, pH=7)	16 h	20 °C
F2. Iron and manganese oxides	40 mL NH_2OH-HCl (0.5 M, pH=5)	16 h	20 °C
F3. Organic matter and sulfides	20 mL H_2O_2 (30%, pH=2) and then 50 mL CH_3COONH_4 (1 M, pH=2)	1 h, 2 h, 16 h	20, 85, 20 °C
F4. Residual	10 mL demineralized water and 10 mL *aqua regia* (HCl/HNO_3, 3:1)	26 min	Microwave Oven

[a] Shaking was applied at 30 rpm.

TABLE III
Operating Conditions for Modified Tessier Sequential Extraction Procedure

Fraction	Extracting Agents	Extraction Conditions Shaking Time[a]	 Temperature
F1. Exchangeable	10 mL NH_4CH_3COO (1M, pH=7)	1 h	20 °C
F2. Carbonates	10 mL CH_3COOH (1 M, pH=5.5)	1 h	20 °C
F3. Organic matter and sulfides	5 mL H_2O_2 (30%, pH=2)	3 h	35 °C
F4. Residual	10 mL demineralized water and 10 mL *aqua regia* (HCl/HNO_3, 3:1)	26 min	Microwave Oven

[a] Shaking was applied at 150 rpm.

and metal ions by converting them into the highest oxidation state. Because the pH of the medium is fixed at about 2.00, the acetate compounds of these metal compounds can dissolve and enter the aqueous medium. However, when the results of Table IV were examined, it seemed that the passage of these elements (except for Mn) to the solution is prevented because they can be converted to the highest oxidation steps by oxidizing them with H_2O_2. However, in this step only the determination of Mn can be interpreted as MnS which has passed into the aqueous medium since the solubility product is too high. The last step was applied to ensure passage of the ions into the mineral structure which cannot pass to the solution in the previous steps. After addition of half-diluted HCl/HNO_3 solution (3:1) into the residue, the mixture is put into a microwave oven where the ions pass into the solution.

Modified Tessier Procedure

The first step was carried out to determine the ions which can be passed to the solution by addition of NH_4CH_3COO and effective buffering at pH 7.00. As a result, the obtained values from this step are higher than CH_3COOH addition and buffering at pH 7.00.

In order to determine the presence of carbonated compounds in the structure, the pH of the CH_3COOH medium was adjusted to 5.5, then solubilization was applied in step 2. When the results of Table IV were examined, it was observed that Fe, Cu, Co, Zn, Pb, and Mn passed to the solution.

The addition of H_2O_2 causes the decomposition of organic matter (CO_2, H_2O, NO_x) since it is a strong oxidizer by having a reduction electrode potential of 1.70 V in the third step. The third step is applied to ensure the dissolution by producing elemental S from the sulfur compounds and converting the metal ions to their highest oxidation step. Since the pH of the medium is fixed at around 2.00, it is thought that the metal ions present in this sample may dissolve and enter the aqueous medium. However, after examining the results in Table IV it can be seen that only Mn and Cu can be dissolved at the specified pH level in H_2O_2 medium, and the others cannot be converted to their highest oxidation steps.

The remaining ions in the ore structure after applying the previous procedures are dissolved in the fourth step. The *aqua regia* is added to the residue, then the solution is put into the microwave oven, and thereby it is expected that the remaining ions (Fe, Cu, Co, Pb, Zn, and Mn) are passed into solution. Our aim in implementing these resolution steps was to identify the possible ions in the sample.

CONCLUSION

The modified Tessier and the revised BCR procedures were examined to reveal the two main difference between the two procedures as: (a) The way in which metals bonded to the ore and (b) the characteristics of the reactants. Therefore, the amount of metals penetrating to the solution is different for both procedures. When the obtained results were compared, the Fe, Cu, Pb and Mn concentra-

TABLE IV
Results Obtained (mean ± standard deviation, n=3) for Sequential Extraction Procedures

Revised BCR	Fe $(mg\ kg^{-1})$	Cu $(mg\ kg^{-1})$	Co $(mg\ kg^{-1})$	Pb $(mg\ kg^{-1})$	Zn $(mg\ kg^{-1})$	Mn $(mg\ kg^{-1})$
Step 1	6.10±0.15	8186±175	54±1	2.24±0.05	149±3	289±6
Step 2	Nd	5024±122	32.31±0.74	0.42±0.01	39.12±0.81	194±4
Step 3	Nd	Nd	Nd	Nd	Nd	2.73±0.06
Step 4	224,400±5432	5846±124	82±3	79±2	574±15	610±16

Modified Tessier	Fe $(mg\ kg^{-1})$	Cu $(mg\ kg^{-1})$	Co $(mg\ kg^{-1})$	Pb $(mg\ kg^{-1})$	Zn $(mg\ kg^{-1})$	Mn $(mg\ kg^{-1})$
Step 1	17,215±510	35,308±859	67±2	96±3	170±4	308±7
Step 2	343±9	73,008±1690	61±1	35.73±0.72	199±5	193±5
Step 3	Nd	2.62±0.06	Nd	Nd	Nd	47.22±1.15
Step 4	257,400±6748	6794±138	80±3	123±2	451±11	637±18

Nd: Not detected.

tions passing to the solution in each extraction step of the modified Tessier procedure were much higher than those of the revised BCR procedure. However, it was only in the fourth step of the BCR procedure when the Co and Zn concentrations passing to the solution were higher than of those in the modified Tessier procedure. It can, therefore, be stated that the modified Tessier procedure proved to be a more suitable procedure for the purposes of decomposing Fe, Cu, Co, Pb, Zn, and Mn in malachite ore. In addition, the modified Tessier procedure requires less chemicals and a shorter analysis time.

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REFERENCES

1. F. M. Hassan, M.M. Saleh, and J.M. Salman, Iraq. J. Chem. 7, 685 (2010).
2. A. Sargaonkar, and V. Deshpande, Environ. Monit. Assess. 89, 43 (2003).
3. M. Pérez-López, M.H. de Mendoza, A.L. Beceiro, and F.S. Rodríguez, Ecotox. Environ. Safe. 70, 154 (2008).
4. K.C. Beeson, W.R. Griffitts, and D.B. Milne, National Academy of Sciences, Washington, D.C. , USA, 88 (1977).
5. R. Dabiri, M. Bakshi Mazdeh, and H. Mollai, J. Min. Environ. 8(2), 277 (2017).
6. M.M. Salvarredy-Aranguren, A. Probst, M. Roulet, and M.P. Isaure, Appl. Geochem. 23, 1299 (2008).
7. M. Anju and D.K. Banerjee, Chemosphere 78, 1393 (2010).
8. M. Anju and D.K. Banerjee, Environ. Monit. Assess. 176, 67 (2011).
9. K. Nemati, N.K., Abu Bakar, M.R. Abas, and E. Sobhanzadeh, J. Hazard. Mater. 192, 402 (2011).
10. B. Krasnodebska-Ostrega, J. Paldyna, J. Kowalska, L. Jedynak, and J. Golimowski, J. Hazard. Mater. 167, 128 (2009).
11. D.L. Lake, P.W.W. Kirk, and J.N. Lester, J. Environ. Qual. 13, 175 (1984).
12. M. Ghaedi, F. Ahmadi, and M. Soylak, J. Hazard. Mater. 147, 226 (2007).
13. M. Zemberyova, J. Bartekova, and I. Hagarova, Talanta 70, 973 (2006).
14. T.O. Ferreira, X.L. Otero, P.V. Torrado, and F. Macias, Soil Sci. Soc. Am. J. 71, 484 (2007).
15. T. Huynh, A.R. Tong, B. Singh, B. J. Kennedy, Clays Clay Miner. 51, 397 (2003).
16. E. Peltier, A.L. Dahl, and J.F. Gaillard, Environ. Sci. Technol. 39, 311 (2005).
17. W. Salomons and U. Forstner, Springer-Verlag, Berlin, Germany (1984).
18. A. Tessier, P.G.C. Campbell, and M. Bisson, Anal. Chem. 51, 844 (1979).
19. E.D. van Hullebusch, S. Utomo, M.H. Zandvoort, and P.N.L. Lens, Talanta 65, 549, (2005).
20. H. Yin, N. Tan, C. Liu, J. Wang, X. Liang, M. Qu, X. Feng, G. Qiu, W. Tan, and F. Liu, Chemosphere 161, 181 (2016).
21. N.T. Basta, J.A. Ryan, and R.L. Chaney, J. Environ. Qual. 34, 49 (2005).
22. D.T. Cuong, and J.P. Obbard, Appl. Geochem. 21, 1335 (2006).
23. S. Kartal, Z. Aydin, and S. Tokalioglu, J. Hazard. Mater. 132, 80 (2006).
24. M.D. Petit, and M.I. Rucandio, Anal. Chim. Acta 401, 283 (1999).
25. P. Quevauviller, Anal. Chem. 17, 289 (1998).
26. P. Quevauviller, G. Rauret, H. Muntau, A.M. Ure, R. Rubio, J.F. Lopez-Sanchez, H.D. Fiedler, and B. Griepink, Fresenius J. Anal. Chem. 349, 808 (1994).
27. G. Rauret, J.F. Lopez, A. Sahuquillo, R. Rubio, C. Davidson, A. Ure, and P. Quevauviller, J. Environ. Monit. 1, 57 (1999).
28. A.M. Ure, P. Quevauviller, H. Muntau, and B. Griepink, Int. J. Environ. Anal. Chem. 51, 135 (1993).
29. J.L. Gomez Ariza, I. Giraldez, D. Sanchez-Rodas, and E. Morales, The Science of the Total Environ. 271 (2000).
30. A.J. Zimmerman and D.C. Weindorf, Int. J. of Anal. Chem. 1 (2010).
31. I. Maiz, I. Arambarri, R. Garcia, E. Millan, Environ. Pollut. 110, 3 (2000).
32. A. Sherry, J. Faye, M. Richards, A.M. Gallardo, K.R. Campbell, and R. Sudowe, J. Radioanal. Nucl. Chem. 312, 37 (2017).
33. K.F. Mossop and C.M. Davidson, Anal. Chim. Acta 478 , 111 (2003).
34. G. Rauret, J.F. Lopez-Sanchez, A. Sahuquillo, E. Barahona, M. Lachica, A.M. Ure, C.M. Davidson, A. Gomez, D. Luck, J. Bacon, M. Yli-Halla, H. Muntau, Ph. Quevauviller, J. Environ. Monit. 2, 228 (2000).
35. C.M. Davidson, R.P. Thomas, S.E. McVey, R. Perala, D. Littlejohn, A.M. Ure, Anal. Chim. Acta 291, 277 (1994).

ICP-AES Characterization of PHWR Irradiated Thoria Bundles for Fission Products

Arijit Sengupta*, B. Rajeswari, V.C. Adya, and R.M. Kadam
Radiochemistry Division, Bhabha Atomic Research Centre,
Mumbai, India

ABSTRACT

A methodology was optimized for the determination of fission products in irradiated thoria. Chlorinated cobalt dicarbollide (CCD) and tri-n-butyl phosphate (TBP) were employed for the separation of U and Th, respectively, for dose reduction and to avoid spectral interferences arising out of the emission rich spectra of U and Th. The tolerance level of U and Th on these analytes was found to be 100 mg L^{-1} and 125 mg L^{-1}, respectively. Five contacts of TBP were required for preferential separation of U and Th. CCD formed a Cs^+CCD^- complex through an 'ion exchange' mechanism, while TBP formed $UO_2(NO_3)_2 \cdot 2TBP$ and $Th(NO_3)_4 \cdot 3TBP$ complexes through a 'solvation' mechanism.

INTRODUCTION

The current generation of Pressurized Heavy Water Reactor (PHWR) fuels have been highly optimized for the traditional PHWR fuel cycles (1-3). Thorium-based fuels have been studied for their potential applications in almost all types of reactors including Pressurized Water Reactors (PWRs), Boiling Water Reactors (BWRs), PHWRs, High Temperature Reactor (HTRs), Fast Breeder Reactors (FBRs) and Molten Salt Reactors (MSRs), though on a small scale compared to U/U-Pu fuels (4-7). With a view to address the suitability and potential advantages of a thorium-based nuclear system to perform full actinide recycling with transuranic isotopes burning, it is necessary to characterize the irradiated thoria bundles for its fission product contents.

Thoria bundles, which have been irradiated in PHWRs, are being taken up for reprocessing in the Power Reactor Thoria Reprocessing Facility (PRTRF), Bhabha Atomic Research Centre, Mumbai, India. To generate data from this thoria fuel for understanding reactor physics, analytical data such as total U and Th content, isotopic composition of generated U, individual contents of fission products and minor actinides are inevitable for understanding the irradiation history, and thereby calculation of burn-up data of thoria bundles. In this context, efforts were put towards analyzing five solutions supplied by PRTRF for their fission product and Th contents using inductively coupled plasma atomic emission spectrometry (ICP-AES) with a glove box-adapted facility for handling radioactive samples. In view of its high sensitivity, good precision, large linear dynamic range, relative freedom from matrix effects, and its capability to provide relatively low detection limits for the simultaneous determination of common as well as rare earth impurities in different matrices, ICP-AES has been routinely used for trace metallic characterization of nuclear materials (8-15). However, the commercially available ICP emission spectrometers cannot be readily employed for the analysis of ^{239}Pu and ^{233}U based nuclear fuels due to their inadaptability to handle radiotoxic materials. Moreover, the presence of emission-rich actinides in the samples leads to erronious results due to the spectral interference and hence, required preferential chemical separation (16-20).

EXPERIMENTAL

All of the atomic emission spectrometric studies were carried out using a Jobin-Yvon Ultima high resolution ICP-AES (Jobin Yvon, Longjumeau, France). It has the practical resolution of ≤ 0.005 nm and a spectral range of 180–800 nm. The operating conditions are chosen based on our previous reports (20) and are listed in Table I.

TABLE I
Specification and Experimental Parameters for ICP-AES Instrument

Optical Design	Czerny Turner
Grating	Holographic
Groove density	2400 / mm
Range of wavelength	120 - 800 nm
Radiofrequency	40.68 MHz
Nebulizer	Pneumatic
Ar gas flow	12 L min^{-1}
Sample flow rate	1 mL min^{-1}

Reagents and Standard Solutions

Rare earth elements and other common impurity standard solutions were prepared from high purity solutions (E. Merck, Darmstadt, Germany) by suitable dilutions with 0.5M HNO_3. Suprapur® (E. Merck) HNO_3 and quartz double-distilled water were used for preparing 0.5M HNO_3. The calibration plots for each analyte were obtained by using multiple standards having analyte concentrations in the 0–100 µg/mL range. This method consisted of creating an analytical method for choosing the required fission products, profiling, peak searching, and auto-attenuation of each analyte element, followed by optimization of the working parameters, calibration of

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*Corresponding author.
E-mail: arijita@barc.gov.in
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working standards and finally, analysis of the samples in quadruplicate to establish reasonably good precision. Since the solutions received from PRTRF had a high dose radioactivity (approx. 600 mR/hr) due to the presence of ^{137}Cs, the latter was separated from these solutions using 0.1 M CCD (chlorinated cobalt dicarbollide) extractant in FS-13 (phenyl trifluoromethyl sulfone) diluent, thereby bringing down the dose of both solutions into the workable level of 20 mR/hr (21-23). Further, the major matrix elements, namely 'Th' and 'U', which are bound to cause spectral interference with the analyte detection, were separated by solvent extraction using three contacts of 30% TBP in Xylene/3M HNO_3 system (24, 25). The residual (raffinate) solution after separation of U and Th was aspirated into the plasma for the determination of potentially important fission products present in the solutions. Three solutions, which were less active and not containing U or Th, were directly analyzed by the ICP-AES. To estimate the Th content in the dissolver and 1 AF solutions (both contain Th at % level), direct aliquots were appropriately diluted and estimated in the ICP-AES.

TBP and n-dodecane were procured from Koch-Light Laboratories, USA, and Lancaster, UK, respectively. The TBP solution was washed with 5% Na_2CO_3 to remove the acidic impurities. The FS-13 and CCD were obtained from Marshallton Research Laboratories (King, NC, USA). Figure 1 represents the structures of CCD, FS 13, and TBP used in the present investigation.

RESULTS AND DISCUSSION

Optimization of Separation Procedure

The distribution ratio values for Cs^+ (D_{Cs}) were varied as a function of aqueous feed acidity because the feed acidity plays a significant role in the extraction efficiency of different metal ions. In the present case, the D_{Cs} value was found to be ~ 800 from 0.01 M HNO_3 solution and this D_{Cs} value was found to decrease drastically up to 3 M HNO_3, followed by only moderate decrease. This type of extraction profile revealed the predominance of the ion exchange mechanism shown by the following equation:

(Eq. 1)

$$Cs^+_{aq} + nH^+CCD^-_{org} =$$
$$Cs^+.nCCD^-_{org} + nH^+_{aq}$$

where 'aq' and 'org' represented the aqueous and organic phase, respectively. At higher feed acidity, the backward reaction is favored, therefore the D_{Cs} value reduced. 'n' is the number of CCD moieties attached to the Cs^+ ion. The equilibrium constant for the above equation can be expressed as:

(Eq. 2)

$$K_{ex} = \frac{[Cs^+.nCCD^-_{org}][H^+_{ag}]^n}{[Cs^+_{aq}][H^+CCD^-_{org}]^n}$$

At a particular temperature and feed acidity the equation can be simplified as:

(Eq. 3)

$$K'_{ex} = \frac{D_{cs}}{[H^+CCD^-_{org}]^n}$$

K'_{ex} is the conditional extraction constant. After taking the logarithm of both sides of Equation 3, followed by rearrangement led to the equation as follows:

(Eq. 4)

$$\log D_{Cs}=\log K'_{ex}+m \log[H^+CCD^-_{org}]$$

A plot of log D_{Cs} vs log CCD concentration gave a straight line with a slope ~ 1 suggesting that one CCD molecule is attached to the Cs^+ ion (Figure 2). The change in Gibb's free energy (26-27) during the extraction was evaluated as –18.84 kJ mol^{-1} revealing the spontaneity of the extraction process (Table I). This study revealed that higher feed acidity was not suitable

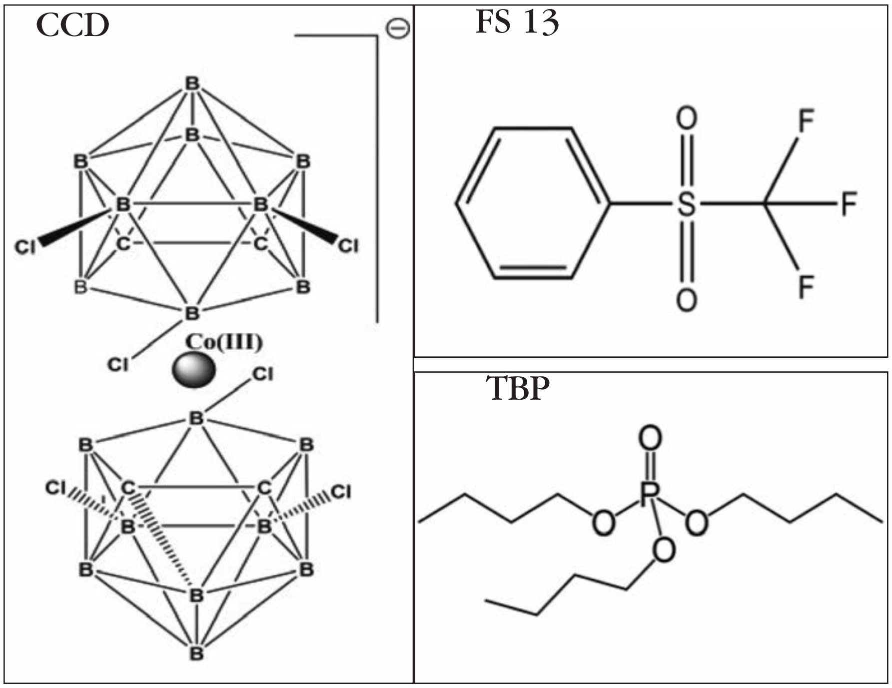

Fig. 1.Structure of CCD, FS 13, and TBP.

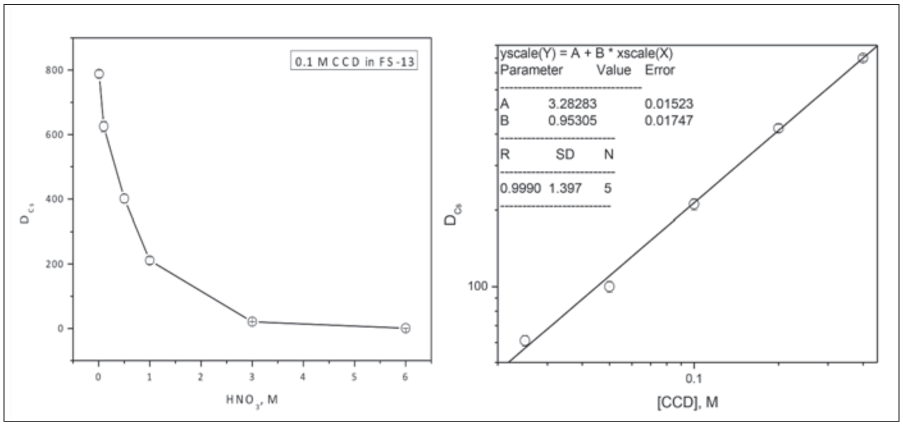

Fig. 2. The variation of D_{Cs} value as a function of (a) feed acidity and (b) concentration of CCD.

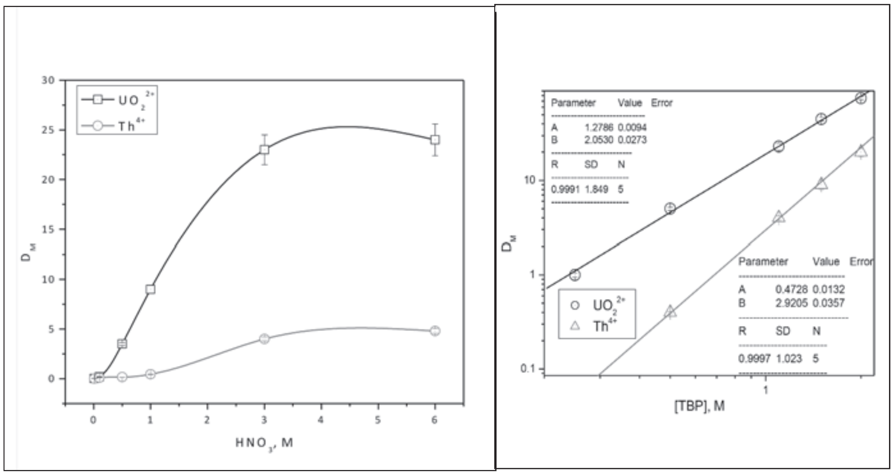

Fig. 3. Variation of D_U and D_{Th} value as a function of (a) feed acidity and (b) concentration of TBP.

for the extraction of Cs; therefore, 1 M HNO_3 was taken to serve the purpose with a D_{Cs} ~ 200.

Similarly, to optimize the method, the D values for Th^{4+} and UO_2^{2+} were varied as a function of aqueous feed acidity. Generally, actinides are multi-electronic systems and, therefore, have multiple lines in their emission spectra. The presence of these elements thus may lead to erroneous determination of other analytes present at the trace level due to their spectral interference. To avoid such circumstances, U and Th need to be separated from the samples without loss of other impurities. To serve the purpose, 30% TBP in xylene was employed. The D_U and D_{Th} values were found to increase with an increase in aqueous feed acidity, followed by a plateau, suggesting the predominance of a solvation mechanism.

A similar approach was adopted to find out the metal-ligand stoichiometries for the present case. The UO_2^{2+} was found to form a 1:2 complex with TBP in xylene, whereas Th^{4+} formed a 1:3 complex (Figure 3). Both extraction processes were found to be thermodynamically favorable as suggested by the negative ΛG values. Based on the studies, it was decided that though the 1 M HNO_3 feed was found to be sufficient for the selective and efficient removal of Cs^+, the feed acidity was to be readjusted to 3 M HNO_3 for the selective separation of U and Th.

TABLE II
Determination of Metal-Ligand Stoichiometry, Conditional Extraction Constants, and Change in Gibb's Free Energy for the Extraction Processes

Metal	M:L	K'_{ex}	ΛG (kJ mol^{-1})
UO_2^{2+}	1:2	19.1	-7.35
Th^{4+}	1:3	2.95	-2.70
Cs^+	1:1	1905	-18.84

(Eq. 5)

$$UO_2^{2+}{}_{aq} + 2TBP_{org} + 2NO_3^-{}_{aq} = UO_2(NO_3)_2.2TBP$$

(Eq. 6)

$$Th^{4+}{}_{aq} + 3TBP_{org} + 4NO_3^-{}_{aq} = Th(NO_3)_3.4TBP$$

Evaluation of Analytical Performance for the Considered Analytical Lines in ICP-AES

Before analyzing the actual samples, it was necessary to choose the analytical lines of the elements, which shows the desired analytical performance such as lower detection limits, higher sensitivity, and good precision. For the present investigation, the Ba 455.403 nm, Ce 413.765 nm, Eu 420.505 nm, Gd 379.639 nm, La 408.671 nm, Mo 202.030 nm, Nd 401.225 nm, Pr 414.311 nm, Sm 359.262 nm, Sr 407.771 nm, Y 371.029 nm, Th 401.913 nm, and U 385.958 nm analytical lines were chosen for their determination. The slope values for the calibration curves were determined as the sensitivity of the analytical lines. The concentration corresponding to the intensity equivalent to the blank + three times the relative standard deviation was evaluated as the detection limits for the analytes using the mentioned lines. The % RSD values were found to be well below 5% revealing the reproducibility of the results due to the highly stable excitation source. The detection limits for all these analytical lines were found to be below 0.1 mg L^{-1}, while the sensitivity values were found to be more than E+02. Table III summarizes the analytical performance of the emission lines of the different analytes.

Evaluation of Number of Contacts

In optimizing the separation process, it is required to know the number of contacts of TBP-xylene required to reduce the U and Th concentration below a certain level (U: 100 mg L^{-1}, Th: 125 mg L^{-1}) to avoid spectral interference. Obviously, the number of steps depends on the initial concentration of U and Th. The initial concentrations for Th and U were chosen as ~ 25000 mg L^{-1} and 1000 mg L^{-1}, respectively. It was observed that only after five contacts, the Th concentration on the raffinate became less than the tolerance level, while for U after two contacts the same can be achieved. Therefore, based on this study, five contacts were chosen for the simultaneous removal of U and Th from the analytes even at the trace level. Figure 4 shows the concentration of U and Th after each contact of TBP-xylene.

Table III
Analytical Performance of the Emission Lines of Different Analytes

Element	Analytical Lines (nm)	Detection Limits (mg L^{-1})	Sensitivity (Counts mg^{-1} L)	RSD (%)
Ba	455.403	0.04	1.03E+04	1.95
Ce	413.765	0.1	9.90E+02	2.94
Eu	420.505	0.07	7.36E+02	1.18
Gd	379.639	0.08	1.11E+03	0.88
La	408.671	0.09	9.67E+02	3.09
Mo	202.03	0.06	1.13E+03	4.73
Nd	401.225	0.07	9.55E+02	0.44
Pr	414.311	0.05	9.47E+02	2.1
Sm	359.262	0.07	9.14E+02	1.98
Sr	407.771	0.05	9.84E+03	2.48
Y	371.029	0.04	1.15E+04	0.23
Th	401.913	0.07	2.45E+03	3.39
U	385.958	0.1	1.17E+03	2.74

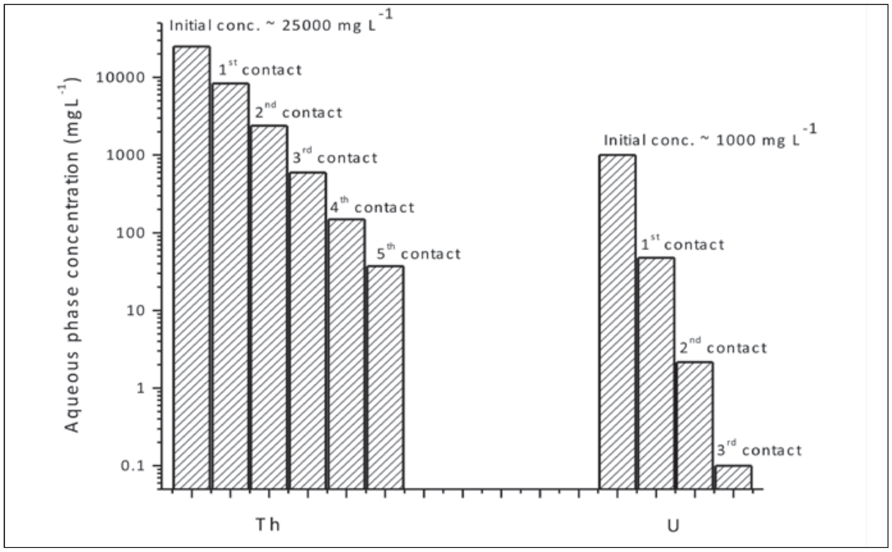

Fig. 4. Concentration of U and Th in the raffinate after each contact of TBP-xylene.

Determination of the Analytes in Dissolver Solutions of PHWR Irradiated Thoria

Five different samples (named as dissolver, 1CP, 1AF, UST1, and UST2) of dissolver solution, generated from PHWR irradiated thoria bundles, were analyzed using the following scheme as optimized earlier (Figure 5). The dissolver solution is the solution obtained after dissolving the irradiated thoria fuel assembly in HF-HNO_3 mixture. 1CP, 1AF, UST1, and UST2 refer either to different positions of irradiation pertaining to a different neutron flux in the reactor, or it can be at different times of irradiation. The exact source cannot be revealed due to the confidential nature.

The very high gamma dose of the samples was reduced by preferential separation of ^{137}Cs by 0.1 M CCD in FS 13 from 1 M HNO_3 feed acidity. Then the sample acidity was adjusted to 3 M HNO_3 for preferential separation of U and Th using 30% TBP in xylene. Table IV summarizes the analytical results obtained. The method was validated using synthetic samples. Since standard reference materials for such radioactive origin is not available commercially in national or international levels, a synthetic sample is one of the few choices to validate the method. Since these samples involve high level of radioactivity, other suitable analytical techniques for the determination of such multi-elements in one stretch is practically impossible. The Th and U contents were determined by stripping the organic phase with 5% Na_2CO_3 and feeding the same into the plasma. A 1CP solution was found to contain Ce and Gd along with Th and U, while the samples identified as dissolver and 1AF contained all the specified analytes. UST1 and UST2 samples were found to contain Ce, Gd, Nd, Pr, Sm, along with U and Th. The analytical data obtained from these studies revealed that the Nd and La concentration values, which form the basis for computing burn-up data of the fuel bundles, were in close agreement with those obtained by the ID-TIMS technique.

Dissolver solutions from
irradiated thoria bundles
from PHWR origin
↓
Cs separation by CCD in
FS 13 from 1 M HNO_3
↓
U and Th separation
by TBP-xylene after adjustment
of feed to 3M HNO_3
↓
Raffinate fed to plasma
for ICP-AES analysis

Fig. 5. Scheme for analysis of dissolver solutions from irradiated thoria bundles from PHWR.

CONCLUSION

An ICP-AES based method was developed for the determination of fission products in the dissolver solutions originating from irradiated thoria bundles from PHWR origin. In the first step, the ^{137}Cs, which was responsible for the major gamma dose of the samples, was removed from 1 M HNO_3 using 0.1 M CCD in FS 13. In the second step to avoid their spectral interference, U and Th were removed from the samples using TBP-xylene. The ion exchange mechanism was found to be predominating with metal-ligand stoichiometry of 1:1 for Cs with CCD, whereas the solvation mechanism was found to be operative with metal - ligand stoichiometry of 1:2 and 1:3 for UO_2^{2+} and Th^{4+}, respectively. All the extraction processes were found to be thermodynamically favorable as revealed from the negative ΔG values. The U and Th concentrations were determined by stripping the loaded TBP-xylene using Na_2CO_3 solution, followed by feeding into the plasma.

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REFERENCES

1. H. Eccles, Sol. Extr. Ion Exch. 18(4), 633 (2000).
2. J.N. Wilson, A. Bidaud, N.Capellan, R. Chambon, S. David, P. Guillemin, E. Ivanov, A. Nuttin and O. Meplan, Annals Nucl. Energy 36(3), 404 (2009).
3. C. Pereira and E. M.Leite, Annals

TABLE IV
Analytical Data Obtained by ICP-AES Analysis of Dissolver Solution

Elements	Synthetic Samples		Dissolver (mg L^{-1})	1CP (mg L^{-1})	1AF (mg L^{-1})	UST1 (mg L^{-1})	UST2 (mg L^{-1})
	Actual Amount (mg L^{-1})	Amount Estimated (mg L^{-1})					
Ba	5	4.6±0.2	3.9±0.04	< 0.1	5.7±0.6	< 0.1	< 0.1
Ce	3	3.1±0.1	6.4±0.1	0.16±0.003	9.0±0.3	0.36±0.07	0.84±0.04
Eu	0.2	0.17±0.01	0.2±0.04	< 0.1	0.28±0.01	< 0.1	< 0.1
Gd	0.5	0.52±0.03	0.12±0.05	0.44±0.08	0.32±0.05	1.4±0.04	3.2±0.06
La	2	1.8±0.2	3.3±0.03	< 0.1	4.8±0.04	< 0.1	< 0.1
Mo	2	2.0±0.2	2.5±0.5	< 0.1	9.4±0.4	0.32±0.02	< 0.1
Nd	5	5.1±0.4	9.6±0.2	< 0.1	15±0.7	< 0.1	0.24±0.03
Pr	2	1.9±0.1	3.0±0.1	< 0.1	4.9±0.09	0.24±0.1	0.24±0.1
Sm	1	1.08±0.08	1.8±0.05	< 0.1	2.6±0.1	0.8±0.05	1.6±0.02
Sr	2	2.07±0.08	3.4±0.06	< 0.1	5.1±0.1	< 0.1	< 0.1
Y	2	1.92±0.05	1.8±0.1	< 0.1	2.9±0.1	< 0.1	< 0.1
Th	5	4.8±0.2	12350±180	70±1	23450±700	16±0.8	9.0±0.7
U	10	9.5±0.6	133.21±0.07	109.8±0.3	167.6±0.8	300.6±0.6	600±2

Nucl. Energy 25(12), 937 (1998).

4. P. R. Kastena, Disarmament and Nonproliferation Initiatives 7(3), 237 (1998).
5. M. Lung and O. Gremm, Nucl. Eng. Design 180(2), 133 (1998).
6. A. Nuttin, D.Heuer, A. Billebaud, R. Brissot, C. Le Brun, E.Liatard, J. M. Loiseaux, L. Mathieu, O. Meplan, E. Merle-Lucotte, H. Nifenecker, F. Perdu and S. David, Prog. Nucl. Energy 46(1), 77 (2005).
7. A. Galperin, P. Reichert and A. Radkowsky, Disarmament and Nonproliferation Initiatives 6(3), 265 (1997).
8. V. C. Adya, A. Sengupta, S. K. Thulasidas and V. Natarajan, J Radioanal. Nucl. Chem. DOI: 10.1007/s10967-015-4222-2 (2015).
9. A. Sengupta, S. K. Thulasidas and V. Natarajan, ,J. Radioanal. Nucl. Chem. DOI: 10.1007/s10967-014-3679-8.(2014).
10. T. G. Bangia, B. A.Dhawale, V. C. Adya and M. D.Sastry, Fresenius J. Anal. Chem, 332, 802 (1988)
11. A. Sengupta, V. C. Adya and S. V. Godbole, J. Radioanal. Nucl. Chem. 292(3), 1259 (2012).
12. A. Sengupta, V. C. Adya, T. K. Seshagiri and S. V. Godbole, At. Spectrosc. 34(2), 53 (2013).
13. R. K. Malhotra and K. Satyanarayana, Talanta 50(3), 601(1999).
14. S. Marin, S. Cornejo, C. Jara and N. Duran, Fresenius' J. Anal. Chem. 355(5-6), 680 (1996).
15. A. Sengupta, T. Ippili, Sk. Jayabun, M. Singh and S. K. Thulasidas, J. Radioanal. Nucl. Chem., DOI: 10.1007/s10967-016-4790-9 (2016).
16. C. Mahan, S. Bonchin, D. Figg, D. Gcrth and C. Collier, J. Anal. At. Spec. 15(8), 929 (2000).
17. M. Gopalkrishnan, K. Radhakrishnan, P. S. Dhami, V. T. Kulkarni, M. V. Joshi, A. B. Patwardhan and J. N. Mathur, Talanta 44(2), 169 (1997)
18. R. Ko, Appl. Spectrosc. 38(6), 909 (1984)
19. A. Sengupta, M. J. Kulkarni, S. V. Godbole, V. Natarajan and P. N. Pathak, At. Spectrosc. 35(2), 60 (2014).
20. A. Sengupta, M. J. Kulkarni and S. V. Godbole, J. Radioanal. Nucl. Chem. 289 (3), 961 (2011).
21. V. N. Romanovskiy, I. V. Smirnov, V. A. Babain, T. A. Todd, R. S. Herbst, J. D. Law and K. N. Brewer, Sol. Extr. Ion Exch. 19(1), 1 (2001).
22. P. K. Mohapatra, A. Bhattacharyya and V. K. Manchanda, J. Hazard. Mat. 181 (1–3), 679 (2010).
23. P. R. Zalupskia, R. S.Herbst, L. Del Martin, D. R. Peterman and K. L. Nash, Solv. Extr. Ion Exch. 28 (2), 161 (2010).
24. A. Sengupta, V. C. Adya, P. K. Mohapatra, S. V. Godbole and V. K. Manchanda, J. Radioanal. Nucl. Chem. 283, 777 (2010).
25. A. Sengupta, V. C. Adya, M. Kumar, S. K.Thulasidas, S. V.Godbole andV. K. Manchanda, At. Spectrosc. 32(2), 49 (2011).
26. P. K. Mohapatra, A. Sengupta, M. Iqbal, J. Huskens and W. Verboom, Inorg. Chem. 52(5), 2533 (2013).
27. P. K. Mohapatra, A. Sengupta, M. Iqbal, J. Huskens and W. Verboom, Chem. Eur. J. 19(9), 3230 (2013).

Determination and Speciation of Selenium Species in Real Water and Food Samples Using Dispersive Liquid–liquid Microextraction and Electrothermal Atomic Absorption Spectrometry

Özgür Dogan Uluözlü
Tokat Gaziosmanpasa University, Faculty of Science and Arts,
Chemistry Department, 60250 Tokat, Turkey

ABSTRACT

A new method was developed for the determination and speciation of selenium species in real water and food samples using dispersive liquid–liquid microextraction and electrothermal AAS. Sodium dibenzyldithiocarbamate was used as the complexing agent and THF (tetrahydrofuran) and chloroform as a dispersing and extraction solvent. Excellent quantitative recovery for Se(IV) was obtained at pH 2, while the recovery for Se(VI) was below 14% at the pH ranging from 1-9. Determination of total selenium was performed after Se(VI) was reduced to Se(IV). The concentration of Se(VI) was obtained by calculating the difference between total selenium and Se(IV). The validation of this method was verified by using certified reference materials and good results were obtained. The limit of detection and the preconcentration factor of the described method were found to be 0.97 µg L^{-1} and 75, respectively, with the relative standard deviation at <1.4%.

INTRODUCTION

While many metal ions are necessary for humans and other mammals, some can be harmful and toxic. Selenium (Se) is a basic and necessary microelement for the organism and depending on the amount ingested, can have a wide range of pleiotropic effects from antioxidant immune functions to the production of active thyroid hormones (1–4). In general, selenium is found in plants and meats (5-7). It also has an important role in the biological system, while selenium environmental toxicity often depends on the concentration and the chemical forms (8, 9). The inorganic forms of selenium are more toxic than the organic forms (10).

Environmental samples generally contain very low concentrations of selenium species. Therefore, fast, easy, and inexpensive enrichment methods must be developed for the separation and selective speciation of selenium. Recently, several methods have been developed to determine the toxic forms of these metal ions present in very low concentrations. However, the instruments used for these methods are very expensive and, therefore, not commonly available in many academic laboratories. However, an electrothermal atomic absorption spectrometer (ETAAS) for selenium analysis is a very common method, far superior and more economical, sensitive, and achieves very low detection limits (11).

Razaee and co-workers (12) were first in proposing the dispersive liquid–liquid microextraction (DLLME) technique as a liquid phase microextraction technique. DLLME is a mini-sample preparation technique and has attracted a wide interest in analytical chemistry. It is a very simple, low-cost and fast extraction technique (10, 13, 14). DLLME is a miniaturized LLE method in which the volume of the extraction solvent is in the microliter range (15). This technique consists of three steps: (a) a mixture of dispersing and extraction solvent is prepared, (b) this mixture is quickly injected into the aqueous phase, producing a cloudy solution and extracting the target analyte into the organic phase, (c) involves centrifugation and a microliter level extraction solvent to separate the analyte from the water and ETAAS analysis.

The analytical parameters of this proposed ETAAS method were optimized and used for analysis of the selenium species. The results showed that this new method is very useful and successful for the speciation of Se(IV) and Se(VI) in water samples, as well as for the analysis of total selenium and Se(IV) in the CRMs and food samples.

EXPERIMENTAL

Materials and Reagents

All chemicals used were of analytical purity. Deionized water with a resistivity of 18.2 MΩ–cm^{-1} was used for all dilutions. Ethanol (E. Merck, Germany) was used as solvent material for sodium dibenzyldithiocarbamate (Fluka, Switzerland). All stock solutions of 1,000 mg L^{-1} Se(IV) and Se(VI) were prepared by dissolving appropriate amounts of SeO_2 (Aldrich, Germany) and Na_2SeO_4 (Sigma, Germany). Sodium dibenzyldithiocarbamate (Fluka) was used as the complexing agent. A mixture of tetrahydrofuran and chloroform (Merck) solution

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**Corresponding author.*
E-mail: ozgurdogan.uluozlu@gop.edu.tr
E-mail: uluozlu@gmail.com
Tel: +90 356 2521616
Fax: +90 356 2521585

was used as the dispersing and extraction solution, respectively. 10% HNO_3 in ethanol (Merck) was used for dilution and solving of the extraction solvent containing the analyte. Each matrix modifier solution was diluted from 10,000 ppm Pd and $MgNO_3$ original solutions (Inorganic Ventures). The pH of the sample was adjusted to 0.1 mol L^{-1} buffer solution of the desired pH.

Instrumentation

In this study, a model AAnalyst™ 700 graphite furnace atomic absorption spectrometer was used, equipped with a deuterium lamp and background correction (PerkinElmer, Inc., Shelton, CT, USA). High purity argon gas was used at a flow rate of 250 mL per minute. A selenium electrodeless discharge lamp (wavelength 196 nm, slit width 0.7 nm, 196A energy) was used. The ashing and atomization temperatures were optimized at 1100 °C and 2600 °C, respectively. For this study, a 2 µL mixture of 0.015 mg Pd + 0.010 mg $Mg(NO_3)_2$ as the matrix modifier was used. This mixture and a 20 µL sample solution were injected into the furnace of the AS-800 automatic sampler. The pH measurements and adjustment were performed using a P-15 Model (glass electrode) pH meter (Sartorius, Germany). An Ethos D brand microwave system (Milestone, Inc., Europe) was used for dissolving the food samples and the certified reference materials.

Analytical Procedure for Se(IV)

A 0.5 mL (0.1% w/v) sodium dibenzyldithiocarbamate was added to a 15 mL sample solution containing 0.005 µg of Se(IV). The pH of the solution was adjusted to 2 (NaH_2PO_4 and H_3PO_4 buffer solution). Then the mixture of 0.5 mL THF (disperser solvent) and 50 µL chloroform solution (extraction solvent) was rapidly injected into the sample solution and shaken for 5 minutes). The phase separation was achieved by centrifugation for 10 minutes at 5000 rpm. The organic phase was diluted to 0.2 mL by adding 10% HNO_3 in ethanol. Analysis of Se(IV) was performed by ETAAS.

Design of DLLME for Selenium Speciation

The reduction of Se(VI) to Se(IV) was achieved by adding 1.5 mL concentrated HCl to a mixture of Se(IV) and Se(VI) sample solution (16) in a PTFE flask. Then it was placed into the microwave digestion system using the program listed in Table I.

Then the proposed DLLME procedure was applied to this solution. Se(VI) concentration was calculated as the difference between total selenium and Se(IV).

TABLE I
Program of Microwave Digestion System

Time (Minute)	Power (W)
5	250
5	400
10	500
8	0

RESULT AND DISCUSSION

Effect of pH

The pH plays an important role in the formation of the metal complexes for extraction and speciation studies since it provides selectivity for the metals in the different oxidation states. The pH effect on the recovery of Se(IV) and Se(VI) ions were investigated in the pH range of 1-9. Quantitative recovery was obtained for the Se(IV) in the pH range of 1-4, but the highest recovery of 98±3 % was found at pH 2. On the other hand, the recovery result for Se(VI) was 11±2 % at the same pH. Moreover, the recoveries of Se(VI) were below 11±2 % for all pH values. The pH 2 was selected for all further experiments. The recovery results are given in Figure 1.

Effect of Amount of Sodium dibenzyldithiocarbamate

In the extraction studies, the amount of ligand is of great importance for the formation of the complexes. The effect of the amount of sodium dibenzyldithiocarbamate on the recoveries of selenium was investigated in the range of 0-600 µL (0.1%, w/v) at pH 2. The best quantitative recovery was obtained with

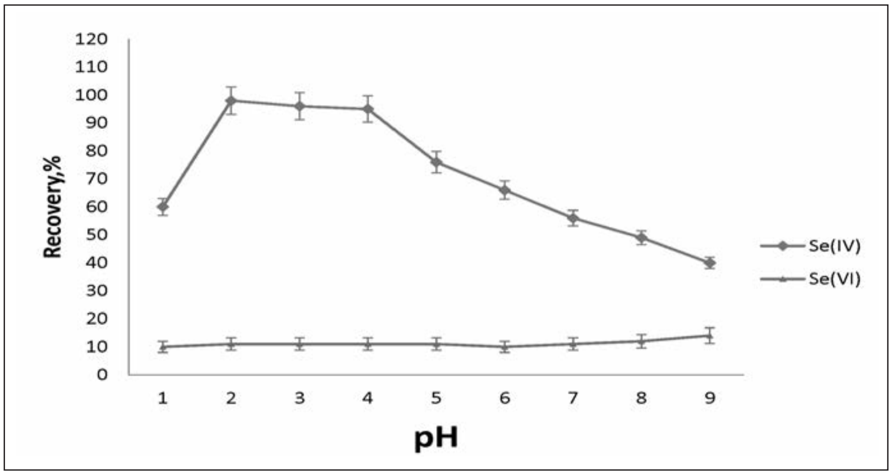

Fig. 1. Effect of different pH on the recovery of selenium species.

200 µL for sodium dibenzyldithiocarbamate (see Figure 2). For all further studies, 200 µL sodium dibenzyldithiocarbamate was used.

Effect of Volume of Extraction Solvent

The properties of the extraction solvent must be of low toxicity, low solubility in water, and have high extraction efficiency in water. In this study, chloroform ($CHCl_3$) was used as the extraction solvent for Se(IV). For the DLLME procedure, the effects of amount of $CHCl_3$ was investigated in the range of 25-200 µL. The recoveries were quantitative in the range of 50-200 µL, but not quantitative under 50 µL as is shown Figure 3. Therefore, 50 µL of $CHCl_3$ was used for further experiments.

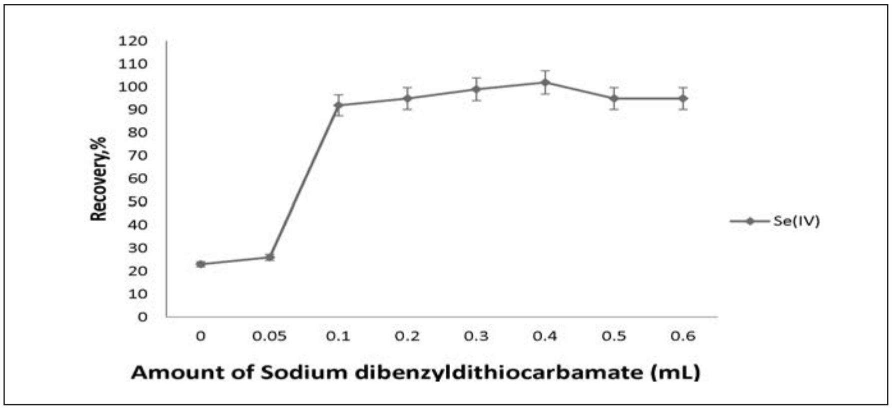

Fig. 2. Effect of sodium dibenzyldithiocarbamate amount on the recovery of Se(IV).

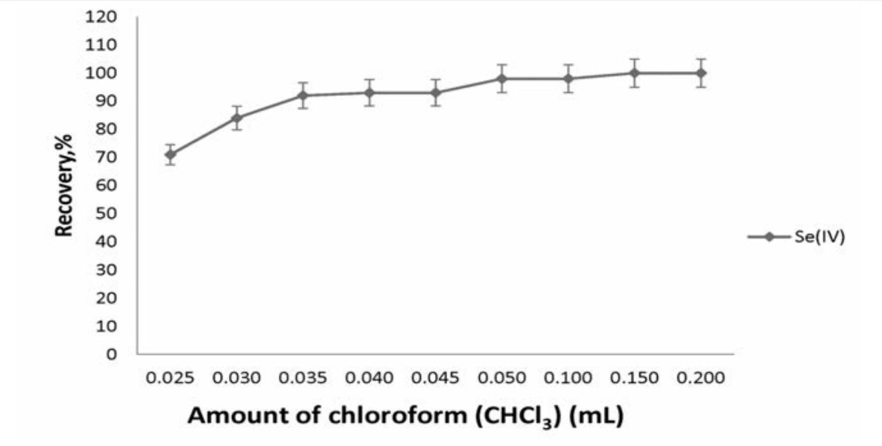

Fig. 3. Effect of extraction solvent volume on the recovery of Se(IV).

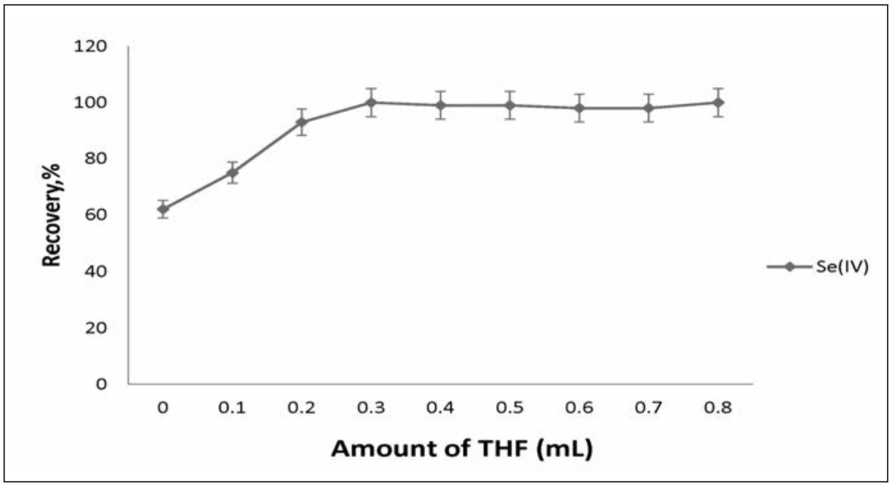

Fig. 4. Effect of disperser solvent volume on the recovery of Se(IV).

Effect of Disperser Solvent Volume

The effect of amount of disperser solvent on the recovery of Se(IV) was investigated. The effect of THF for the proposed DLLM method was investigated in the range of 0-500 µL. According to the experimental results shown in Figure 4, the quantitative signal of Se(IV) increased with an increasing volume of THF. The recovery results remained almost constant at 300 µL and above amounts. Further experimental studies were executed using 300 µL THF.

Effect of Centrifugation Time and Centrifugation Rate

The centrifugal force (rate and time) is a very important factor for fast and excellent phase formation. A short centrifugation time and slow centrifugation rate can diminish the extraction efficiency of the analyte. Therefore, a centrifugation rate of 2500-5000 rpm and a centrifuge time of 2-12 minutes were tested for achieving quantitative results. The results showed that the optimal centrifugation rate and time were 3500 rpm and 8 minutes, respectively, and used for further experimental studies.

Effect of Interference of Foreign Ions

The selectivity of the DLLME procedure was checked by investigating the effect of foreign ions. The maximum concentrations of different matrix ions were chosen to examine the recovery of Se(IV). Therefore, 0.005 µg Se(IV) ions was added to the test solution containing different amounts of foreign ions, and the method was applied as developed previously. The matrix ions (Table II) were unaffected for the applied DLLME, and the method was successfully able to be applied to the speciation of selenium in environmental water samples.

Determination of Total Selenium

The recommended reduction procedure was performed to the different amounts of Se(IV) and Se(VI) solutions, and quantitative results were obtained (see Table III). The quantitative Se(VI) level was found using the difference of total selenium and Se(IV) concentration and the results are listed in Table IV. It can be seen that the proposed method was able to be applied for the speciation of selenium species in water samples.

Analytical Performance

Under the optimum conditions, the analytical performance of the procedure for the selenium species was calculated. The calibration graph was linear for Se(IV) in the range of 10–50 µg L^{-1}. The detection limit (DL) of the proposed procedure was calculated as 0.97 µg L^{-1} by using 10 blank measurements. The preconcentration factor was calculated by the ratio of maximum sample volume (15 mL) to the lowest sample volume (200 µL). The preconcentration factor (PF) of this DLLME method was 75 and the relative standard deviations were < 1.4%.

Application to Water and Food Samples

The validity of the method was checked by using CRMs LGC 6010 Hard Drinking Water (U.K. Laboratory of the Government Chemist, and OLY, UK)), SRM 1577b Bovine Liver and SRM 1570a Spinach Leaves (National Institute of Standards and Technology, Gaithersburg, MD, USA). The obtained values were in good agreement with the certificate values. Also, the DLLME procedure was applied to some real water and food samples. The present method was applied to environmental water samples (drinking water, seawater, river water, hot spring water, mineral water, and tap water) for the speciation of Se(IV) and Se(VI). All water samples were filtered through a 0.45 µm membrane filter. Then the proposed speciation procedure was applied to the water samples and the selenium concentration was determined by ETAAS.

Also, the proposed procedure was applied to some food samples (mushroom, honey, cinnamon, leek, corn flour, kiwi, grape molasses, rice, ground beef, and egg) for the determination of total selenium. The food samples were collected from local supermarkets. The samples were weighed as 1 gram and digested in the mixture of 6 mL of HCl (36%) and 2 mL of HNO_3 (65%) solution with microwave digestion using the following progam: 5 minutes for 250 W, 5 minutes for 400 W, 10 minutes for 500 W, vent: 8 minutes. Finally, the proposed procedure was applied to the digested SRMs and the food samples for the preconcentration of total selenium. Good results were obtained and the results are listed in Table V. Also, the comparison of this study with some other studies are given in Table VI.

TABLE II
Influences of Some Foreign Ions on the Recoveries of Se(IV) (N=3)

Ion	Concentration (mg L^{-1})	Recovery (%)[a]
Na^+	2000	95±2
Mg^{2+}	2000	99±2
K^+	2000	95±2
PO_4^{3-}	1500	95±3
Cl^-	3100	97±2
NO_3^-	10000	99±3
SO_4^{2-}	2500	97±2
Fe^{3+}	100	99±2
Zn^{2+}	100	95±1
Mn^{2+}	100	99±3

[a] Mean ±standard deviation

CONCLUSION

In this study, a new preconcentration method for the determination and speciation of selenium species is proposed. In terms of cost, simplicity, and eco-friendly application, this method is more advantageous than the previously developed method reported in the literature. The validity of the method was performed by the control analysis with standard refer-

TABLE III
Total Se Determinations in Spiked Test Solutions (Volume: 15 mL, N=4)

Added (µg)		Found (µg)			Recovery (%)[a]		
Se(IV)	Se(VI)	Se(IV)	Se(VI)	Total Se	Se(IV)	Se(VI)	Total Se
0	0.500	-	0.480	0.480	-	96±2	96±2
0.200	0.300	0.191	0.295	0.486	96±3	98±2	97±2
0.250	0.250	0.245	0.246	0.491	98±2	98±2	98±2
0.300	0.200	0.294	0.197	0.491	98±2	99±2	98±2
0.500	0	0.496	-	0.496	99±2	-	99±2

[a] Mean ±standard deviation.

TABLE IV
Speciation of Se(IV) and Se(VI) and Total Se in Some Natural Water Samples (sample volume: 15 mL, N=4)

Samples	Added ($\mu g\ L^{-1}$)		Found ($\mu g\ L^{-1}$)			Recovery (%)		
	Se(IV)	Se(VI)	Se(IV)	Se(VI)	Total Se	Se(IV)	Se(VI)	Total Se
Drinking water	-	-	12.8±1	1.3±0.2	14.1±1	-	-	-
	10	20	22.7±1	20.8±1	43.5±2	99±2	98±2	99±2
	20	10	31.5±2	11.2±1	42.7±2	96±2	99±2	97±2
Sea water	-	-	15.3±0.7	1.2±0.2	16.5±1	-	-	-
	10	20	24.3±0.2	20.9±1	45.2±1	96±2	99±2	97±2
	20	10	33.7±0.7	10.8±2	44.5±2	95±2	96±2	96±2
River Water	-	-	10.6±1	1.6±1	12.2±1	-	-	-
	10	20	19.7±1	21.5±1	41.2±2	96±2	99±2	98±2
	20	10	30.5±2	11.3±1	41.8±2	100±2	97±2	99±2
Spring Water	-	-	13.8±1	1.4±0.2	15.2±1	-	-	-
	10	20	23.1±1	20.8±1	43.9±2	97±2	97±2	97±2
	20	10	32.8±2	11.1±1	43.9±2	97±2	97±2	97±2
Mineral Water	-	-	11.2±1	1.1±0.2	12.3±1	-	-	-
	10	20	20.9±1	20.7±1	41.6±2	99±2	98±2	98±2
	20	10	30.3±2	11.1±1	41.4±2	97±2	100±2	98±2
Tap Water	-	-	11.7±1	1.1±0.2	12.8±1	-	-	-
	10	20	20.8±2	21.0±1	41.8±2	96±2	100±2	98±2
	20	10	30.8±2	10.9±1	41.7±2	97±2	98±2	97±2

ence materials. The limit of detection and the preconcentration factor of the described method were found to be 0.97 $\mu g\ L^{-1}$ and 75, respectively, with the relative standard deviation at <1.4%. Finally, this proposed DLLME method has low DL and high PF and can be applied to a successful speciation of selenium in environmental water samples.

TABLE V
Level of Total Se in Certified Reference Materials and Food Samples (N=4)

Samples	Certified Value	Value Present Work	Relative Error (%)
NIST-SRM 1570a Spinach leaves ($\mu g\ g^{-1}$)	0.117	0.126±0.05	7.7
NIST-SRM 1577b Bovine liver ($\mu g\ kg^{-1}$)	0.73	0.72±0.53	-1,4
LGC 6010 Hard drinking water ($\mu g\ L^{-1}$)	9.3	9.7±0.82	4.3
Edible mushroom ($\mu g\ g^{-1}$)	-	0.29±0.01	-
Honey ($\mu g\ g^{-1}$)	-	0.32±0.03	-
Cinnamon ($\mu g\ g^{-1}$)	-	0.38±0.04	-
Leek ($\mu g\ g^{-1}$)	-	0.35±0.03	-
Cornflour ($\mu g\ g^{-1}$)	-	0.29±0.02	-
Kiwi ($\mu g\ g^{-1}$)	-	0.30±0.01	-
Grape molasses ($\mu g\ g^{-1}$)	-	0.35±0.02	-
Rice ($\mu g\ g^{-1}$)	-	0.23±0.01	-
Ground beef ($\mu g\ g^{-1}$)	-	0.32±0.02	-
Egg ($\mu g\ g^{-1}$)	-	0.29±0.01	-

Mean expressed as 95% tolerance limit.

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The author is also thankful to the Unit of the Scientific Research Projects of Tokat Gaziosmanpasa University for the financial support.

COMPLIANCE WITH ETHICAL STANDARDS

This paper does not contain any studies with human participants or animals performed by the author.

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TABLE VI
Comparison of Proposed Work With Recent Studies

Analyte	Detection	Media	Technique	PF	DL ($\mu g\ L^{-1}$)	RSD (%)	References
Se(IV), Se(VI)	ETV-ICP-MS	5-mercapto-3-phenyl-1,3,4-thiadiazole-2-thione potassium	DLLME	64.8	0.047	7.2	(10)
Se(IV), Se(VI)	ETAAS	APDC	LLE	-	1	3.5	(11)
Se(IV), Se(VI)	GFAAS	APDC	DLLME	70	0.050	4.5	(17)
Se(IV), Se(VI)	ETV-ICP-MS	Diethyldithiocarbamate (DDTC)	CPE	-	0.05	3.5	(18)
Se(IV), Se(VI)	HPLC–ICPMS	–	HF-LPME	49	0.23	<15	(19)
Se(IV), Se(VI)	ETAAS	3,3- Diaminobenzidine (DAB)	UALPME-DES	50	0,005	4.1	(20)
Se(IV), Se(VI)	ETAAS	Sodium dibenzyldithiocarbamate	DLLME	75	0.97	1.4	Present work

REFERENCES

1. M.P. Rayman, Lancet 379, 1256 (2012).
2. Y. Shizhong, H. Zhang, H. Zhen, Z. Lin, Y. Qiao, Microchem. J. 145, 1 (2019).
3. MB. Arain, TG. Kazi, MK. Jamali, N. Jalbani, HI. Afridi, GA. Kandhro, R. Ansari, RA. Sarfraz, J. of Hazardous Materials 55, 216 (2008).
4. Naeemullah, TG. Kazi, M. Tuzen, Food Chem. 172, 161 (2015).
5. Q. Zhou, M. Lei, J. Li, M. Wang, D. Zhao, A. Xing, K. Zhao, J. of Separation Science 38, 1577 (2015).
6. WHO, Cambridge University Press (1987).
7. C. Reilly, Elsevier Science (1991).
8. M.P. Rayman, Lancet 356, 233 (2000).
9. Z. Pedrero, Y. Madrid, Anal. Chim. Acta 634, 135 (2009).
10. Y. Zhang, J. Duan, M. He, B. Chen, B. Hu, Talanta 115, 730 (2013).
11. N.M. Najafi, S. Seidi, R. Alizadeh, H. Tavakoli, Spectrochim. Acta Part B 65, 334 (2010).
12. M. Rezaee, Y. Assadi, S.M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, J. of Chromatography A 1116, 1 (2006).
13. E.M. Martinis, P. Berton, R.P. Monasterio, R.G. Wuilloud, TrAC – Trends in Anal. Chem. 29, 1184 (2010).
14. L.B. Escudero, E.M. Martinis, R.A. Olsina, R.G. Wuilloud Food Chem. 138, 484 (2013).
15. M. Saraji, M.K. Boroujeni, Anal Bioanal Chem. 406, 2027 (2014).
16. T. Ferri, S. Rossi, P. Sangiorgio, Anal. Chim. Acta 361, 113 (1998).
17. A. Bidari, E.Z. Jahromi, Y. Assadi, Microchem. J. 87, 6 (2007).
18. Y. Li, B. Hu, M. He, G. Xiang, Water Research 42(4-5), 1195 (2008).
19. F. Morenoa, T. García-Barreraa, J.L. Gómez-Arizaa, J. of Chromatography A 1300, 43 (2013).
20. A.H. Panhwar, M. Tuzen, T.G. Kazi, Talanta 175, 352 (2017).

Sensitive and Accurate Determination of Copper in Blood and Urine Samples by Vortex-assisted Dispersive Liquid-liquid Microextraction-slotted Quartz Tube - Flame Atomic Absorption Spectrometry After Complexation with a Novel Schiff-base Ligand

Emine Gülhan Bakirdere[a,*] and Esra Maltepe[b]
[a] Yildiz Technical University, Faculty of Education, Department of Science Education, 34210 Istanbul, Turkey
[b] Yildiz Technical University, Faculty of Art and Science, Chemistry Department, 34210 Istanbul, Turkey

ABSTRACT

Due to the low sensitivity of flame atomic absorption spectrometry for trace copper determination, dispersive liquid-liquid microextraction and slotted quartz tube were used to increase the instrument's detection power by about 102-fold. This high detection improvement was achieved by performing a comprehensive optimization study on the microextraction method and slotted quartz tube parameters.

A novel ligand was used to form the copper complex prior to the extraction process. The limits of detection and quantification obtained under optimum experimental conditions were 0.52 and 1.7 ng/mL, respectively. The calibration plot was linear over a wide concentration range, and the percent relative standard deviation of the lowest concentration in the linear calibration plot was less than 5.0%, indicating high precision for the extraction procedure and instrumental measurements.

In order to determine the validity and applicability of the developed method, recovery studies were carried out on blood and urine samples at different spiked concentrations. The recovery results recorded for both samples ranged between 85% and 113% which confirmed that the method can be applied to these matrices with high accuracy and precision.

INTRODUCTION

Copper plays an important role in regulating the DNA/RNA transcription processes, the metabolism of nitrogen-containing compounds and plant photosynthesis, thus, it is referred to as a biogenic element which is essential for both plants and animals (1-3). Copper is widely used in products such as fertilizers, wood preservatives, pesticides and water distribution systems (pipes) (4). Activities such as mining and industrial waste disposal, combustion of unrefined or contaminated gasoline could lead to the release of this element into the environment (5, 6). It was reported that copper enters and accumulates in aquatic plants, fish and other environmental organisms and since it moves up the food chain, it becomes biomagnified, and the organisms at the higher trophic levels become exposed to higher concentrations (7). The common routes for Cu exposure are through drinking water, eating food, inhaling air, and having direct skin contact with the metal (8). At high exposure levels, copper causes health disorders such as diarrhea, nausea, kidney damage, liver damage, even death under extreme conditions (8). Lethal doses of copper in adults range from 4.0 - 400 mg per kilogram of body weight as stated by the World Health Organization for suicidal and accidental ingestion (9). Due to the low levels of copper in the environment, sensitive analytical techniques are required for its accurate and precise determination.

The complex nature of environmental and biological sample matrices makes direct instrumental analysis difficult as the matrix components could affect the results or cause harm to the instrument. Sample preparation is, therefore, used to obtain the analyte in a clean and compatible form for instrumental reading, with extraction being a commonly used method. Recently developed microextraction methods are simple, fast, require low amounts of toxic solvent, result in low analyte loss, high enrichment factors, and high analytical recovery (10). Methods such as single-drop microextraction (SDME), solidified floating organic microextraction (SFODME), solid phase microextraction (SPME), and hollow fiber liquid phase microextraction (HF-LPME) have been used to preconcentrate copper and other metals for trace determination (11-14). Dispersive liquid-liquid microextraction (DLLME) is an important method that was introduced by Rezaee et al. in 2006 (15), and it has since become a widely used method for both organic and inorganic analyses. DLLME employs dispersive solvents that are miscible with both an aqueous sample and

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** Corresponding author.*
E-mail: gulhan063@gmail.com
Tel: +902123834896

an extractant, and this miscibility ensures a fine distribution of extraction solvent and increases extraction surface area. Extraction is completed in a few seconds, and the high surface area results in high extraction output (16). The extract after DLLME is compatible with several instrumental techniques used for qualitative and quantitative determination of the analytes.

Modern analytical instruments have been conveniently used over the classical gravimetric and titrimetric methods due to a rapid, accurate, and precise analysis. Inductively coupled plasma (ICP) techniques, equipped with mass spectrometry (ICP-MS), and optical emission spectrometry (ICP-OES) detection systems offer multi-elemental analysis and very low detection limits for several metals (17, 18). However, these plasma techniques tend to be relatively expensive in terms of instrument and operational cost. Atomic absorption spectrometry (AAS) techniques, such as graphite furnace (GFAAS) and hydride generation (HG-AAS), have good sensitivities and are comparable to the plasma techniques, but they can only be used to determine one analyte at a time, and HG-AAS is only applicable to hydride-forming elements such as Te, Pb, Sb, Se, and As (19). Flame atomic absorption spectrometry (FAAS) is a very common metal detetection technique due to its robustness, ease of operation, and relatively low instrument cost (10). The nebulizer unit of the FAAS is considered its weakest point because only a fraction (<10%) of aspirated sample reaches the flame for atomization, and this results in low sensitivity for most metals (16). A basic external component attached to the flame unit to improve absorbance measurements of the FAAS is a slotted quartz tube (SQT). The SQT works on two principles: increasing residence time of the atoms in the flame, leading to a 2–5-fold enhancement in absorbance (Figure 1), and trapping atoms within the inner walls of the SQT for the subsequent sudden release to obtain a sharp analytical signal (20).

The main purpose of this study was to develop an analytical method for the determination of copper at trace levels in blood and urine samples by slotted quartz tube-flame atomic absorption spectrometry after dispersed liquid-liquid microextraction. The complexation of copper for extraction was achieved using a ligand synthesized in our laboratory (21) and all variables were systematically optimized to improve the analytical signal of copper.

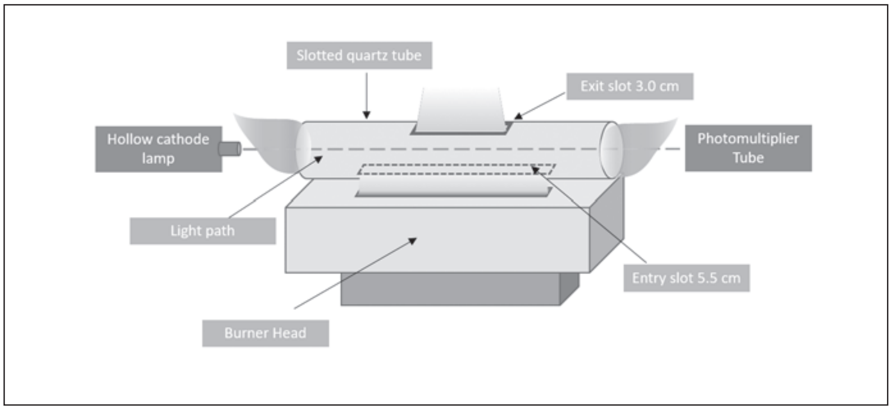


Fig. 1. Schematic diagram of FAAS burner head with an attached SQT (16).

EXPERIMENTAL

Instrumentation

All absorbance measurements were performed with a NovAA (Analytik Jena AG, Germany) FAAS system. The flame was generated with a mixture of air and acetylene, and the flow rate of the acetylene was optimized to enhance the atomization efficiency. The 324.8 nm analytical line of Cu was selected from a multi-element hollow cathode lamp (Fe, Co, Ni, Mn, Cu, Cr) operating at 6.0 mA current and 0.50 nm spectral bandpass. A deuterium lamp (D_2) was used as background correction for all measurements. A 140 mm long quartz tube was used in this study which was cut at two different sides (entry and exit slots) at an angle of 180°. The lengths of the entry and exit slots were 55 mm and 30 mm, respectively. The SQT-FAAS system is represented in Figure 1 (16). Other instruments used in the study were EBA20 Andreas Hettich centrifuge, M-100 model HAPA ultrasonicator, Kermanlar mechanical shaker, and a HI2020 Hanna Instruments pH meter.

Reagents and Standard Solutions

Appropriate aliquots from a 1000 mg/L copper stock standard solution (purchased from High Purity Standards) were diluted to prepare working standards and calibration standard solutions. Ultrapure deionized water (resistivity 18.2 MΩ·cm) was used for dilution of the standards and for general cleaning purposes. All chemicals and reagents used in this study were of analytical grade. Optimization of the extraction method was carried out with methanol, ethanol, chloroform, 2-propanol, carbon tetrachloride, dichloromethane, 1,2-dichloroethane, sodium chloride, potassium chloride, sodium sulfate and potassium nitrate, all purchased from Merck (Darmstadt, Germany). Also used were ligand chemicals.

Ligand Synthesis

In the synthesis of the ligand, the procedure listed in the literature was slightly modified (21). 3-methoxy-5-bromosalicylaldehyde (10 mmol 2.31 g) and *p*-toluenesulfonic acid (0.01 g) were dissolved in 25 mL of ethyl alcohol. 1,4-phenylenediamine (20 mmol, 2.16 g) dissolved in 25 mL of ethyl alcohol was slowly added dropwise under reflux for 1.0 hour at 60 °C. The aldehyde carbonyl group was followed by IR. The dark orange product obtained as a result of the reaction was allowed to stand overnight and then filtered off from the solvent. The product was washed several times with hot distilled water, ethyl alcohol, and diethylether. The resulting product was then dried under vacuum and stored in a desiccator. Characterization of the synthesized ligand ($C_{14}H_{13}O_2N_2Br$) was performed.

Procedure of DLLME

1.0 mL of pH 8 buffer solution and 0.50 mL of ligand solution (0.02% w/v) were consecutively added to 8.0 mL of standard/sample solution. Mixing by hand-shaking for 15 seconds was done to ensure uniform distribution of the ligand throughout the aqueous solution. A mixture of 2-propanol (3.0 mL) and chloroform (300 µL) was then injected into the complex solution, resulting in a cloudy solution. After 15 seconds of vortexing, the solution was centrifuged at 6000 rpm for 2.0 minutes. Finally, the extracted phase was taken into a separate tube for complete evaporation of the chloroform. The residue was dissolved with 200 µL concentrated HNO_3 and sent to the FAAS for measurements. An amount of 0.2 mL is enough to get a sharp analytical signal in FAAS.

RESULTS AND DISCUSSIONS

Step-by-step optimizations were carried out for the complex formation and DLLME to enhance the sensitivity for the Cu determination. In the optimization of one parameter, the other parameters were held at constant values, and the highest mean absorbance of triplicate measurements was used to determine the optimum values. Optimization studies were performed with appropriate aqueous standards with absorbance values within the linear calibration range.

Complex Formation Optimization

Parameters varying in this optimization step were pH and amount of buffer solution, ligand concentration and ligand amount. Buffer solutions are added to sample solutions in order to provide a favorable medium for complexation and maintain the stability of the complex. For this reason, buffer solutions with pH values ranging from 5 to 11 were tested for their effects on the Cu complex formation. The absorbance values recorded increased linearly from pH 5 up to 8, after which it decreased marginally. A pH 8 buffer solution was favorable for the complex formation because an acidic medium presents free hydroxonium ions which compete with complexation, and a highly basic medium could lead to deprotonation of the ligand's hydroxyl groups (22). The volume of buffer solution used is also important because an excess amount could result in analyte dilution. The optimum amount of pH 8 buffer solution was determined by testing 0.50, 1.0, 1.5, and 2.0 mL volumes. The absorbance values recorded were not different from each other, thus, 0.50 mL was selected in order to lower the use of chemicals.

The concentration of ligand solution is another important factor for complexation, and this was optimized by testing 0.02, 0.065, and 0.10% (w/v) ligand concentrations. The absorbance signals recorded were not significantly different from each other, but 0.065% was selected for being slightly higher than the other two concentrations. The optimum amount of the ligand required for efficient complexation of 8.0 mL sample/standard solution was determined by testing 0.25, 0.50, 1.0, 1.5, and 2.0 mL volumes. The absorbance values increased steadily from 0.25 mL up to 1.5 mL, after which it plateaued as is shown in Figure 2. For an even distribution

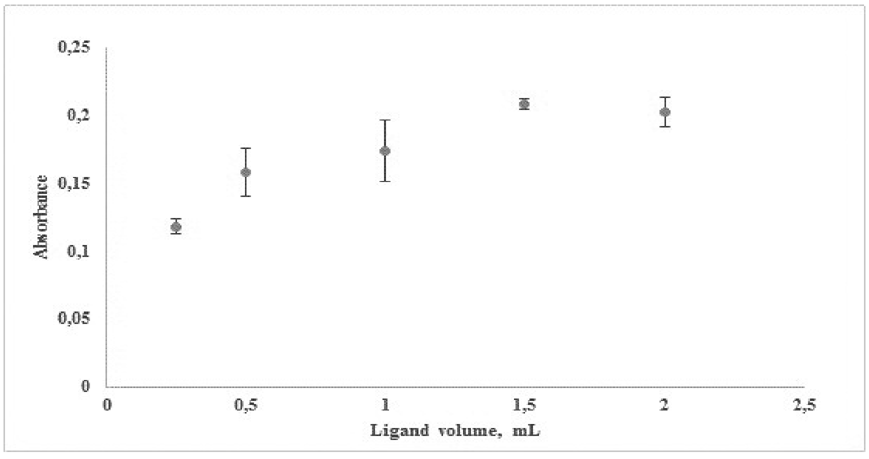


Fig. 2. The graphic of ligand volume optimization.

of ligand throughout the aqueous solution, 15 seconds of vortexing was employed in this study. Some studies in the literature reported that excess mixing periods lower the absorbance signals (10, 22) and for this reason, mixing during complexation was not optimized.

Optimization of Extraction Solvent Type and Volume

An ideal extraction solvent should be immiscible with water and should extract an appreciable amount of analyte(s). The centrifuge tubes (15 mL) used in the study had a tapered bottom which was convenient for collecting the microliter volume of extraction solvents used. For this purpose, only high density solvents (p> 1.0) including carbon tetrachloride, 1,2-dichloroethane, chloroform, and dichloromethane were tested for their extraction efficiencies on the copper complex. 500 µL of each solvent was mixed with 3.0 mL of 2-propanol and injected in separate extractions. Chloroform recorded the highest average absorbance as well as the least relative standard deviation value. A very low amount of extraction solvent might not be sufficient to extract appreciable amounts of analyte and an excess amount could make processing of the extract for instrumental read-out difficult. The optimum amount of chloroform was determined by testing 200, 250, 300, 400, and 500 µL volumes of chloroform under similar conditions. Absorbance signals increased marginally from 200 to 300 µL, and then decreased marginally again at the higher volumes. The optimum chloroform volume for further optimizations was therefore set at 300 µL.

Optimization of Dispersive Solvent Type and Volume

Low carbon chain alcohols have mid polarity index values which makes them miscible with most polar and apolar organic solvents. This miscibility makes them ideal for the role of dispersive solvents in DLLME (23, 24). Ethanol, methanol, and 2-propanol are all miscible with chloroform, and 3.0 mL of each was mixed with 300 µL of chloroform, then tested for their dispersive efficiencies. 2-propanol recorded the highest average absorbance for triplicate measurements and was approximately 2–3 times higher than methanol and ethanol, respectively (Figure 3). The degree of extractant dispersion depends on the volume of the dispersive solvent used, and the optimum volume of 2-propanol was determined by testing 1.5, 2.0, 2.5, and 3.0 mL. The absorbance value increased gradually from 1.5 mL up to 3.0 mL as shown in Figure 4. The 2-pro-panol volumes higher than 3.0 mL were not tested because their sum total with the volumes of sample, ligand, and buffer solution would have exceeded the volume of the tube used. A significant increase would not be expected for higher volumes as the results can be seen to have plateaued. 3.0 mL was therefore selected as optimum volume of 2-propanol.

Salt Effect

In liquid-liquid extraction methods, salting out of analyte(s) is achieved by adding highly soluble inorganic salts to aqueous solutions. The salts displace analytes in the aqueous medium, thereby enhancing their mass transfer into the extractant. However, an appropriate amount of the salt needs to be added in order to avoid saturation which could hinder the extraction of the analytes. For extraction solvents, which have

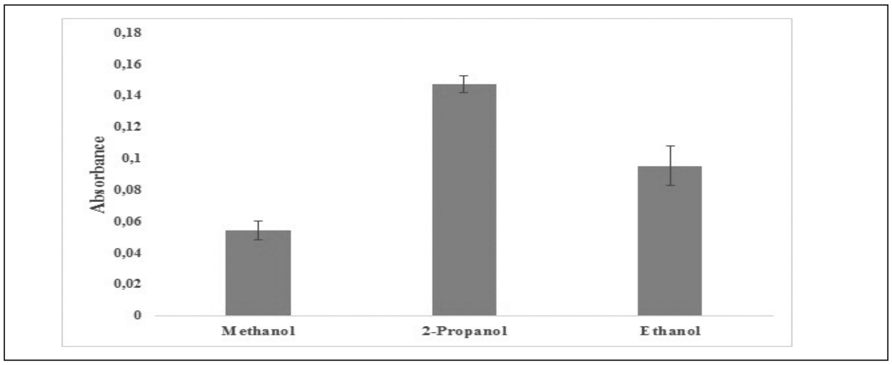
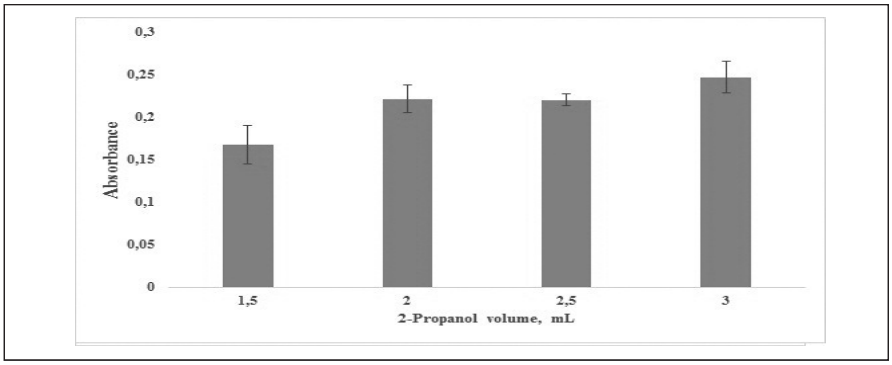


Fig. 3. A chart of dispersive solvents against average absorbance values (n=3 error bars).



Fi. 4. A chart of 2-propanol volumes against average absorbance values (n=3 error bars).

densities very similar to the sample solution, addition of a salt prior to extraction could alter the density of the solution and result in a better phase separation (10). For these reasons, 1.0 g each of NaCl, KNO_3, Na_2SO_4, and KCl were totally dissolved in equivalent aqueous standards, and their extraction results were compared to salt-free extraction. Although the highest yields were obtained with KCl, it was not significantly higher than the other salt-added extractions. The KCl-added extraction was about 1.1 times higher than the salt-free extraction, and this enhancement was not deemed significant to continue further extractions with KCl.

Analytical Performance

Table I is a summary of the optimal experimental parameters, and using these parameters, the analytical performance of all systems were determined using calibration plots. Table II summarizes the figures of merit calculated for FAAS, SQT-FAAS, DLLME-FAAS, and DLLME-SQT-FAAS. A comparison of these systems to other literature studies is also presented in Table II. The calibration plots were linear over wide concentration ranges, and the regression coefficients (R^2) were greater than 0.9993. The limits of detection (LOD) and quantification (LOQ) were determined using the following equations:

$$LOD = 3Stdev/m \qquad (Eq. 1)$$

$$LOQ= 10Stdev/m \qquad (Eq. 2)$$

The standard deviation (Stdev) was calculated from six replicate absorbances of the lowest concentrations of each calibration plot, and the slope (m) was taken from the linear regression equation.

According to the LOD values, increases in detection power with respect to FAAS were 2.3-, 38-, and 102-fold for SQT-FAAS, DLLME-FAAS, and DLLME-SQT-FAAS, respectively.
The LOD and LOQ values for the optimum system (DLLME-SQT-FAAS) were found to be 0.52 and 1.7 ng/mL, respectively. The precision of the extraction procedure and the instrumental measurement was appreciably high as all measurements recorded percent relative standard deviations lower than 10%.

TABLE I
Optimized Parameters of DLLME-SQT-FAAS

Parameters	Value
pH of buffer solution (amount)	pH 8.0 (1.0 mL)
Ligand concentration (amount)	0.02%, w/v (0.50 mL)
Complexing period	15 s (Vortex)
Extraction solvent (amount)	Chloroform (300 µL)
Dispersive solvent (amount)	2-Propanol (3.0 mL)
Sample flow rate*	6.44 mL/min
Acetylene flow rate*	40 L/hr
SQT height*	0.0 mm

* Parameters taken from Özzeybek et al. (10).

TABLE II
Analytical Figures of Merit for Different FAAS Systems Under Study

Analytical Method	LOD (ng/m	LOQ, (ng/mL)	Range (ng/mL)	RSD (%)^a	
FAAS	53	177	200 – 10,000	5.5	
SQT-FAAS	23	75	50 - 1000	8.4	
DLLME-FAAS	1.4	4.6	5.0 - 500	8.9	
DLLME-SQT-FAAS	0.52	1.7	2.0 - 250	3.6	
DLLME-SQT-FAAS	0.7	2.2	2.0 - 100	4.7	(10)
DLLME-FAAS	1.9	_	8.0 - 400	3.8	(25)

*For FAAS and SQT-FAAS systems, the values of LOD and LOQ taken from Özzeybek et al. (10).

Recovery

A recovery study was conducted to test the accuracy and applicability of this method to biological samples. It is a fact that heavy metals are not easily removed from the human body. For this reason, it is very important to be able to perform heavy metal determination at trace levels in biological samples such as urine and blood. These biological samples have very complex matrices which affects the accurate quantification of the analytes. The acid digestion procedure reported by Erarpat et al. (16) was therefore used to decompose the organic matrix, leaving the metal (inorganic) component for analysis. The solution after digestion was diluted with deionized water after evaporating to near dryness which was repeated several times. Then, 1.0 mL of pH 8 buffer solution was added to the solution to adjust the pH of the solution to 8.0. The sample analysis was then performed, and the absence of copper signals suggested that the analyte was not present in the samples according to the limit of detection. The digested blood and urine samples were selectively spiked to final concentrations of 25 and 50 ng/mL and analyzed under optimum experimental conditions. The reason for the selection of the stated concentrations was that they were within the linear calibration range, and gave distinct signals. The recovery results obtained using aqueous calibration standards ranged between 85 and 113% for both samples as given in Table III. The precision of the method for the biological matrices was also confirmed by low %RSD values (<5.0%). These results were satisfactory and established that the method can be used for an accurate and precise quantification of copper in blood and urine samples.

TABLE III
Percent Recovery Results for Blood and Urine

Sample	25 ng/mL	50 ng/mL
Blood	85.1 ± 3.4	112.9 ± 4.2
Urine	95.4 ± 3.3	110.6 ±3.1

CONCLUSION

In this study, a simple, accurate, and precise analytical method was developed for the determination of copper at trace level by FAAS. Dispersive liquid-liquid microextraction was comprehensively optimized to obtain a high preconcentration factor and slotted quartz tube was used to further enhance the detection power of the FAAS instrument. Under the optimum extraction and system conditions, the method was found to have an approximately 102-fold enhancement in detection power with a detection limit of 0.52 ng/mL. Other figures of merit were very satisfactory, and applicability of the method was then tested for the urine and blood samples. Recovery results from the spiked tests were obtained in the range of 85– 113% and confirmed that the method is appropriate for testing biological matrices. The developed method is economical, easy, rapid, and agrees with green chemistry which is aimed at reducing toxic chemical usage.

Declaration of interest: None.

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REFERENCES

1. I. Yruela, Metallomics 5(9), 1090 (2013).
2. J. Lakshmipraba et al., Spectrochim. Acta Part A: Molecular and Biomol. Spectrosc. 109, 23 (2013).
3. G. Güleryüz, Chemistry and Ecology 31(6), 494 (2015).
4. C. Mauceri and S. Mishra, Copper (Cu), in Health Effects of Metals and Related Substances in Drinking Water, M. Ferrante, O.C. Gea, and Z. Rasic-Milutinovic, Editors, IWA Publishing: London, UK (2013).
5. A. Tobiasz, Talanta 96, 82 (2012).
6. S.N. Do Carmo et al., Microchem. Journal 110, 320 (2013).
7. G.S. Rocha et al., Ecotoxicol. and Environmental Safety 133, 235 (2016).
8. V.A. Lemos et al., Environmental Monitoring and Assessment 148(1), 245 (2009).
9. WHO, Copper in drinking-water: Background document for development of WHO Guidelines for Drinking-Water Quality. World Health Organization, 2004 (WHO/SDE/WSH/03.04/88).
10. G. Özzeybek et al., Microchem. Journal 132, 406 (2017).
11. X. Wen, Q. Deng, and J. Guo, Spectrochim. Acta Part A: Molecular and Biomolecular Spectrosc. 79(5), 1941 (2011).
12. Ç.A. Sahin and İ. Tokgöz, Anal. Chim. Acta 667(1), 83 (2010).
13. S.A. Arain et al., J. of Anal. At. Spectrom. 29(12), 2362 (2014).
14. Z. Es'haghi and R. Azmoodeh, Arabian Journal of Chem. 3(1), 21 (2016).
15. M. Rezaee et al., J. of Chromatography A 1116(1), 1 (2006).
16. S. Erarpat et al., Chemosphere 189, 180 (2017).
17. M.C. Bruzzoniti et al., Talanta 99, 703 (2012).
18. J. Moreda-Piñeiro et al., Microchem. Journal 137, 402 (2018).
19. N.A. Kasa,et al., Microchem. J. 133, 144 (2017).
20. I. Demirtas, S. Bakirdere, and O.Y. Ataman, Talanta 138, 218 (2015).
21. E.G. Bakirdere, Synthesis and characterization of complexes produced from N_2O_2 type Schiff bases with Co(II), Ni(II), Cu(II) and Zn(II) metals in chemistry. Fırat University, Turkey (2008).
22. M.S. Findikoglu et al., Water, Air, & Soil Pollution 229(2), 37 (2018).
23. M. Firat et al., Spectrochim. Acta Part B: At. Spectrosc. 129, 37 (2017).
24. E. Öztürk Er, et al., J. of Trace Elements in Medicine and Biology 49, 13 (2018).
25. M. Rajabi et al., J. of Molecular Liquids 194, 166 (2014).

Speciation of Fe(II) and Fe(III) in Geological Samples by Solvent Extraction and Flame Atomic Absorption Spectrometry (FAAS)

Susanta Kumar Pradhan[a,*], Balram Ambade[b], and Pranab Kumar Tarafder[a]
[a] Chemical Laboratory, Atomic Minerals Directorate for Exploration and Research, Department of Atomic Energy, Eastern Region, Khasmahal, Jamshedpur 831002, India
[b] Department of Chemistry, National Institute of Technology, Jamshedpur 831014, India

INTRODUCTION

Iron (Fe) is one of the most common metallic elements found in the earth's crust. Determination of the Fe(II) and Fe(III) species is of great importance in geological, biological and environmental materials due to the specific role of their oxidation states. The analysis of ferrous oxides in rocks and minerals is of great interest to petrologists and mineralogists (1). The ratio of ferrous to ferric oxide plays an important role not only in interpreting geological data, but also in determining the redox condition of iron in natural water. It is an essential nutritional element for all life forms, i.e., a cofactor in many enzymes and essential for oxygen transport and electron transfer (2). Hence, it is very important from an analytical point of view to develop a sensitive, selective, and rapid method for the quantitative determination of ferrous and ferric iron present in various materials.

There are several individual methods for the determination of ferrous oxide (3-7) as well as ferric oxide (8). However, very few methods are available for the simultaneous or sequential determination of both oxides in geological samples such as rocks, soils, cores, stream sediments, minerals, etc. In general, Pratt's titrimetric method using dichromate as a titrant has been used for the determination of ferrous oxides in geological samples. However, this particular method has its limitations when applied to samples containing ferrous oxide at the parts per million (ppm) levels. A variety of colorimetric methods have been employed for the determination of ferrous and ferric ions in sample solution. The reagent 1,10-phenanthrolene has been extensively used in the colorimetric determination of ferrous iron and total iron after the reduction of ferric iron by chemical means or by photochemical reduction. Several authors applied 1,10-phenanthrolene to determine ferrous and total iron. However, the major concern of the method is the instability of the color. Stuky and Anderson (9) identified several sources of error in the 1,10-phenanthrolene method. One of the errors is the falsely high ferrous value due to photo-reduction of ferric to ferrous ions in the presence of 1,10-phenanthrolene with ordinary fluorescence light. They corrected this problem in the ferrous analysis by conducting the digestion and dilution steps under red light (typical dark room) condition. They obtained reliable results for ferrous, even in the presence of ferric ion, after conducting the experiment in typical dark room conditions. Many other workers have tried to improve the spectrophotometric method for ferrous and ferric ion determination by carrying out the reaction in dark room conditions. Recently, Tarafder and Thakur (10) reported that the error in the determination of ferrous ion in the presence of ferric ion is not due to the reduction of ferric ion into ferrous iron by any fluorescence light. Instead, the reason was assigned to the presence of organic matter in the sample solution which is responsible for the reduction of ferric to ferrous ion over time. Most of the methods used for the sequential determination of ferrous and ferric ion with 1,10-phenanthrolene are not able to yield accurate results.

In view of the above, an atomic absorption spectrometry (AAS) method has been devised to

ABSTRACT

A procedure has been devised for the selective determination of ferrous and ferric oxides in geological samples such as rocks, soils, cores, stream sediments, etc. This is based on the selective dissolution of both oxides with an ammonium bi-fluoride and sulfuric acid mixture, followed by solvent extraction separation of Fe(III) into ethyl acetate using thiocyanate. The extracted Fe(III) was stripped off into dilute hydrochloric acid solution and determined by flame atomic absorption spectrometry (FAAS). Fe(II) in the aqueous solution was determined by FAAS separately. The method was applied to a number of geological samples including certified reference materials (CRMs) and synthetic mixtures. The results obtained by the proposed method for the CRMs were found to be in excellent agreement with the reported values. Similarly, the results of a set of geological samples were comparable with those obtained by the colorimetric method involving 1,10-orthophenanthrolene and the standard titrimetric (Pratt's) method.

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*Corresponding author.
E-mail: skpradhan.amd@gov.in*

accurately estimate the ferrous and ferric ions sequentially; thus eliminating all of the limitations associated with the earlier reported methods. This novel scheme is based on the ammonium bi-fluoride dissolution of the sample in the presence of sulfuric acid, followed by the selective extraction of the ferric ions into ethyl acetate as thiocyanate complexes. The Fe(III) ions were then stripped off into diluted HCl solution and determined by flame AAS. Glokas et al. (11) have worked on the speciation of ferrous and ferric ions in water samples by flame AAS after cloud point microextraction. Their method involves cloud point extraction (CPE) of both species with ammonium pyrrolidinecarbodithioate (APDC) under standard conditions, which facilitates the *in situ* complexation and extraction of both species. They used mathematical equations to overcome the interference of Fe(III) in the spectrometric determination of Fe(II) present in the same sample solution. However, they used ferrozine which has the disadvantages of reducing Fe(III) to Fe(II), unless Fe(III) is sequestered with fluoride or EDTA. Therefore, the presence of fluoride is advisable in the ferrozine method. Speciation of ferrous and ferric ions in combination with solvent extraction/solid phase extraction and FAAS in water, fruit juice, cola, tea infusion, etc., has also been reported in the literature (12-13).

In the proposed method, the ferrous and ferric ions are leached out into the solution with an ammonium bi-fluoride and sulfuric acid treatment. Fe(III) in solution forms a strong fluorosulfate complex, and extraction of the ferric ion bound in this complex is not possible unless Fe(III) is freed by breaking the complex. However, when boric acid is added, the complex is broken due to the formation of a strong H[BF_4] complex, and immediately Fe(III) is released in the solution and can be extracted as thiocyanate complex into the organic solvent, such as ethyl acetate, leaving the Fe(II) ions in solution, which does not form a similar fluorosulfate complex as formed by Fe(III). This way the ferric ion is separated from the ferrous ion. Fe(III) is stripped off from the organic solvent into the diluted HCl medium and determined by FAAS. Fe(II) in aqueous solution can be directly estimated by aspirating into the FAAS. This way, both Fe(II) and Fe(III) in the aqueous solution can be determined selectively and sequentially in the geological samples. It is important to mention that the ferric fluorosulfate or trifluorosulfato ferrate(III) $[Fe^{III}(SO_4)F_3]^{2-}$ complex had been synthesized earlier by Chaudhury and Islam (14).

The proposed method is a novel approach for the speciation of ferrous and ferric oxides in geological samples such as rocks, soils, cores, and stream sediments, and to the best of the authors' knowledge, speciation of iron by this proposed technique has not been reported in the literature and is based on the following reaction scheme:

$$NH_4HF_2 = NH_4F + HF \qquad \textbf{(i)}$$

$$Fe_2O_3 + 6HF = 2FeF_3 + 3H_2O \qquad \textbf{(ii)}$$

$$FeF_3 + H_2SO_4 + 2NH_4F = (NH_4)_2[Fe^{III}(SO_4)F_3] + 2HF \qquad \textbf{(iii)}$$

$$4(NH_4)_2[Fe^{III}(SO_4)F_3] + 3H_3BO_3 + 6H_2SO_4 = 4(NH_4)_2SO_4 + 2Fe_2(SO_4)_3 + 3H[BF_4] + 9H_2O \qquad \textbf{(iv)}$$

$$Fe^{3+} + 6SCN^- = [Fe(SCN)_6]^{3-} \qquad \textbf{(v)}$$

Using this approach, most of the earlier drawbacks found by using photochemical reduction or reduction due to organic matter can be completely eliminated.

EXPERIMENTAL

Instrumentation

For this study, a ZEEnit 700 flame atomic absorption spectrometer (Analytik Jena, Germany) was used for measurement of the absorbance of iron. An iron hollow cathode lamp (HCL) was used for the light source. Details of the optimum instrumental operating conditions are listed in Table I.

A Specord 250+ double-beam UV-visible spectrophotometer (Analytik Jena, Germany) with a 1 cm path length quartz cell was used for the absorbance measurements of the iron-phenanthrolene color complex at $\lambda = 560$ nm.

An Ultima-2 inductively coupled plasma optical emission spectrometer (Horiba Scientific, Yobin Yvon Technology, France) was also used for the determination of total iron at the wavelength of 259.940 nm.

TABLE I
Optimum FAAS Instrumental Operating Conditions

Light source	Iron Hollow Cathode Lamp (HCL)
Wave Length	248.3 nm
Slit width	0.5 nm
Lamp current	5.0 mA
Atomizer	100 mm slot burner
Back ground correction	Deuterium lamp background
Fuel	Acetylene
Support	Air
Flame stoichiometry	Oxidizing
Calibration curve	Five point calibration curves (1-10 µg mL^{-1})
No. of replicates for measurement	4

A laboratory stirrer with a glass ceramics heater and temperature sensor was used for the leaching studies.

Standard Solution and Reagents

Specpure® standard solution of iron (1000 µg mL-1) (Alfa Aesar, USA) in 5% HNO_3 was used as a standard stock solution. The working standard solutions (1–10 µg mL^{-1}) were prepared by diluting the standard stock solution with distilled water.

Unless otherwise stated, all reagents used were of analytical/ guaranteed grade.

Recommended Procedure

Preparation of Sample Solution

A finely crushed sample of 0.2 g (–200#) was transferred to a clean Teflon® beaker (125 mL capacity), and 1.0 g solid NH_4HF_2 and 10 mL of 1:1 H_2SO_4 added. The beaker was covered with a Teflon lid and heated to about 70–80 ºC on a hotplate for 15–20 minutes. Then the beaker was removed and cooled to room temperature. The content of the Teflon beaker was transferred into a 50 mL volumetric (plastic) flask and made up to volume with distilled water.

Extraction of Fe(III) as Fe(III)-Thiocyanate Complex

An aliquot (1 to 5 mL) of sample solution depending on the iron concentration was taken into a 100 mL separating funnel. Approximately 1.0 g boric acid powder was added and mixed thoroughly for 2 minutes. Then 5 mL of 10% KCNS (w/v) solution and 10 mL ethyl acetate were added, and shaken for 2–3 minutes. The resulting color complex of Fe(III) thiocyanate was extracted into the organic layer. The aqueous layer was separated out into a 100 mL beaker. Fe(III) in the organic layer was stripped off using 10 mL 5% HCl (v/v) solution, then placed into a 50 mL volumetric flask, and made up to the mark with distilled water.

Determination of Fe(II) and Fe(III) by Flame AAS

Fe(II) in the aqueous layer (which was kept in a 100 mL beaker) was transferred into a 50 mL volumetric flask and made up to volume with distilled water. Direct measurement of Fe(II) was carried out by aspirating the solution into the FAAS. Similarly, the extracted Fe(III) in the separated solution was also determined by aspiration into the FAAS. In both cases, the absorbances of iron were measured using a calibration curve prepared by absorbance against the standard iron solutions (1 to 10 µg mL^{-1}). A blank was also run in a similar manner.

RESULTS AND DISCUSSION

As has already been discussed above, the titrimetric determination of ferrous oxide based on using the Fe(II)/Fe(III) dichromate/permanganate redox system for geological samples (rocks, soils, and stream sediments) is very difficult, particularly at trace levels, i.e., the ppm levels. Similarly, determination of ferrous oxide in rock samples by UV-visible spectrometry using the reagent orthophenanthrolene and ferrozine is also not free from errors due to several reasons (9-11). In order to eliminate the above-mentioned drawbacks, the authors devised a system for the accurate and sequential determination of ferrous and ferric oxides in all types of geological samples, particularly rocks, soils, and stream sediments, by FAAS.

The main advantages of FAAS for the determination of metal ions are speed and high selectivity. It is also a moderately sensitive technique for some transition elements, including iron. However, determination of ferrous ions in geological samples, particularly rocks, soils, and stream sediments by FAAS, to the best of the authors' knowledge have so far not been reported in the literature. In this paper, a robust and reliable method for the determination of both ferrous and ferric oxide in most geological samples is described. The method is based on the rapid leaching of ferrous and ferric ions using a combination of H_2SO_4 and NH_4HF_2 at a moderate temperature (70–80 ºC) as per the procedure described in the literature (10). Of the ferrous and ferric ions leached into the solution after H_2SO_4/NH_4HF_2 treatment, Fe(III) rapidly forms a strong fluorosulfate complex (14) leaving ferrous ions free in the solution. The ferric ion strongly bound in the mixed ligand ternary fluorosulfate complex is released into the solution on the breach of the said ternary complex by the addition of boric acid, which has stronger affinity towards fluoride ions bound in the complex. The released ferric ion reacts with potassium thiocyanate forming the ferric thiocyanate complex, which is easily extracted into ethyl acetate leaving the ferrous ions in the leached solution. The ferric ion bound in the extracted thiocyanate complex was stripped off into the diluted HCl solution and measured by FAAS. The ferrous ion present in the aqueous solution after separation of the ferric ions by solvent extraction was directly measured by FAAS. In this way, both ferrous and ferric ions are determined by FAAS.

A mixture of 10 mL of (1:1) H_2SO_4 and 1 g NH_4HF_2 at 70–80 ºC for about 15–20 minutes are the optimum conditions for leaching the ferrous and ferric ions for 0.2 g geological samples. The details of effect of such variables as the effects of acid and salt (H_2SO_4 and NH_4HF_2), time and temperature on the leaching of both oxides are discussed in the literature (10). However, the effects of other variables such as the effect of boric acid, thiocyanate concentration, choice of solvent, effects of stripping agents, etc., are discussed below.

Effects of Boric Acid

Boric acid is used to release Fe(III) from a strong fluorosulfate complex of Fe(III) at the prevailing reaction condition, i.e., in a strong H_2SO_4 medium. The ferric ions strongly bound in the mixed ligand ternary fluorosulfate complex are released into the solution on the breach of the said ternary complex by the addition of boric acid, which has a stronger affinity towards fluoride ions forming the $H[BF_4]$ complex. This aspect was also ascertained by the addition of thiocyanate solution without addition of boric acid. Formation of no red color solution of ferric thiocyanate indicates that the leached Fe(III) ions are not available in the solution to form a complex with thiocyanate ions; instead, they formed the ternary fluorosulfate complex. The effect of boric acid on the release of Fe(III) ions from the ternary fluorosulfate complex was studied for boric acid concentrations ranging from 0.1 to 5 g for 1 mL leached solution of certified reference material (SY-3) from the Canadian Certified Reference Materials Project (CCRMP). An amount of 0.5 g boric acid is sufficient to mask fluoride ions present in the solution. However, in order to allow for impurities, present in the reagent as well as to allow for the extra consumption of the reagent by the matrix elements present in the sample solution, a 1 g boric acid was found to be optimum for 99% recovery of ferric ions. This is illustrated in Figure 1.

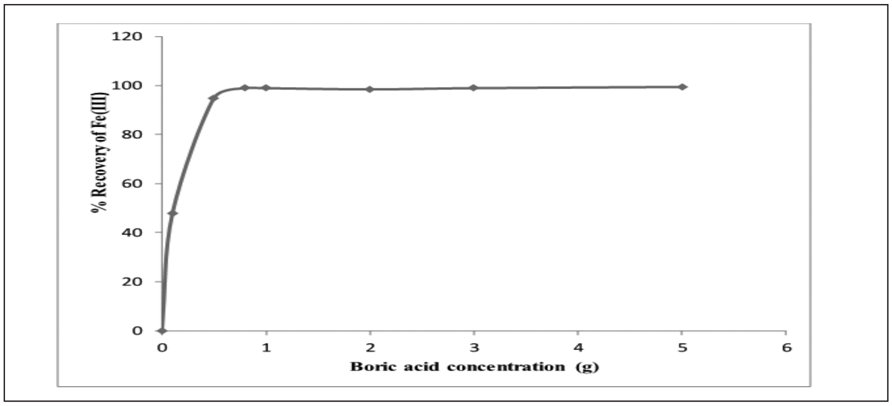

Fig. 1 Effect of boric acid concentration (g) on the release of Fe(III) from its fluorosulfate complex.

TABLE II
Recovery of Fe(II) and Fe(III) in Synthetic Mixtures

Fe(II)[a] Taken (µg)	Fe(II) Found (µg)	Recovery (%)	Fe(III)[b] Taken (µg)	Fe(III) Found (µg)	Recovery (%)
50	47	94	200	214	107
100	95	95	150	162	108
150	190	95	100	110	110
200	142	95	50	55	110

[a] For Fe(II), salt used ammonium ferrous sulfate hexahydrate (GR, Merck).
[b] For Fe(III), salt used ammonium ferric sulfate dodecahydrate (GR, Merck).

Effects of Thiocyanate Concentration on the Extraction of Ferric Ions

Iron(III) reacts with thiocyanate and results in a series of intensely red colored complexes, which remain in solution. Iron(II) does not react. Depending on the thiocyanate concentration, a series of complexes can be obtained, which are red in color and can be formulated as $[Fe(SCN)n]^{3-n}$, where n=1,…, 6. At low thiocyanate concentration, the predominant colored species is $[Fe(SCN)]^{2+}$, whereas at 0.1 M thiocyanate concentration it is largely $[Fe(SCN)_2]^{+}$, while at very high thiocyanate concentration it is $[Fe(SCN)_6]^{3-}$. In the colorimetric determination, a large amount of thiocyanate is used since it increases the intensity and stability of the color. Thiocyanate colorimetric determination of Fe(III) is a well-established method (15-16). A 5 mL of 2 M thiocyanate solution (15) was used to form the Fe(III) thiocyanate complex for 1 mL leached solution of the synthetic mixtures under study. It was observed that the extraction of this ferric thiocyanate complex into ethyl acetate is quantitative (more than 99% recovery) which is shown in Table II.

Effects of Diverse Ions on the Extraction of the Fe(III)-Thiocyanate Complex

The thiocyanate spectrometry method used for the determination of Fe(III) is reported in the literature (15-16). Hence, further studies on the effects of diverse ions for the formation of the ferric thiocyanate complex are redundant here. However, the effects of borate, fluoride, and sulfate ions were examined on the extraction of the ferric thiocyanate complex into ethyl acetate. Though sulfate ions have a tendency to form a complex with

Fe(III), it has been observed that up to a 1000-fold excess concentration of sulfate ions had no effect on the extraction of the Fe(III) thiocyanate complex from the leach solution. Similarly, the fluoride ions, which have a tendency to form a complex with Fe(III), have been removed by masking these by the addition of excess boric acid. No adverse effect from the borate ions on the extraction of the Fe(III) thiocyanate complex in the leached solution was observed.

Choice of Solvents

Various solvents were tried for liquid-liquid extraction of the ferric thiocyanate complex. Mention is made of butanol, n-butylacetate, ethylacetate, di-ethylether, MIBK, carbon tetrachloride, hexane, benzene, toluene, etc. However, the extraction was found to be optimum with ethyl acetate. Similarly, several mineral acids were tried for stripping the ferric ions into the aqueous phase. A 10 mL of 5% HCl (v/v) solution was found to be optimum as the stripping solution.

Validation, Accuracy and Precision

In order to validate the method developed, and in order to attest the efficacy of the leaching and extractive system, the proposed method was applied to a set of synthetic mixtures and CRMs of rocks, soils, and stream sediment samples. To ascertain the accuracy and precision of the developed method, a set of synthetic mixtures having variable concentrations of Fe(II) and Fe(III) were prepared and analyzed. The recovery of Fe(II) and Fe(III) was more than 95% and 100%, respectively (see Table II). Similarly, the CRM geo-standards of SY-3, SY-4, and MRG-1 from the Canadian Certified Reference Materials Project (CCRMP), DNC-1a, NKT-1 and sdAR-1 from the U. S. Geological Survey (USGS), and SARM-6 and SARM-48 from Mintek, South African Reference Material, were analyzed for ferrous and ferric oxides by the proposed method. The results for both oxides were in excellent agreement with the reported values and are shown in Table III. The nature of all of the above CRMs used are also listed in Table III. It is pertinent to mention that the reference materials NKT-1 and sdAR-1 were analyzed in this laboratory as a part of the 29th and 31st round of Geo-PT programmes which are an International Association of Geo-analysts' Proficiency Testing Programme for Analytical Geochemistry Laboratories. There is only reported value for total Fe as Fe_2O_3 (T) for both the CRMs of NKT-1 and sdAR-1 instead of ferrous and ferric oxides separately. Hence, to calculate the total Fe as Fe_2O_3 (T) by the proposed method, the following equation has been adopted:

$$\% \; Fe_2O_3 \; (T) = \% \; FeO \times 1.111 + \% \; Fe_2O_3$$

where 1.111 is the conversion factor from FeO to Fe_2O_3. Based on the equation, the total Fe as % Fe_2O_3 (T) by the proposed method for NKT-1 and sdAR-1 are 13.38% and 4.59 % and are comparable with the reported value of 13.29% and 4.63%, respectively. The proposed method yields fairly accurate results under the optimized conditions. The accuracy was found within 5% and 8% for ferrous and ferric oxides, respectively, and was calculated on the basis of reported values for the reference materials under study. The precision (% RSD) of the method was found within 4% and 7% for the ferrous and ferric oxides, respectively, for four replicate determinations (n=4). From the results listed in Tables II and III, it can be observed that there is a slightly higher value of ferric oxide, which may be due to the partial oxidation of ferrous oxide by air or any oxidizing agent present in the sample solution.

TABLE III
Results of FeO and Fe_2O_3 by the Proposed FAAS Method in CRMs

CRMs	Nature of Samples	FeO (%)		Fe_2O_3 (%)		Total Fe as Fe_2O_3(T) %
		Proposed Method[a]	Reported Value	Proposed Method[a]	Reported Value	
SY/3	Synite	3.54±0.15	3.58	2.97±0.10	2.84	-
SY/4	Diorite	2.84±0.16	2.86	3.21±0.21	3.03	-
MRG-1	Gabbro	8.18±0.30	8.63	8.64±0.27	8.48	-
DNC-1a	Dolerite	7.02±0.28	7.32	1.92±0.10	1.79	-
NKT-1	Nephelinite	8.10±0.42	-	4.38±0.22	-	13.29
sdAR-1	River sediment	0.85±0.06	-	3.65±0.20	-	4.63
SARM-6	Soil	14.02±0.35	14.63	0.76±0.10	0.71	-
SARM-48	Soil	0.20±0.01	0.21	0.63±0.06	0.58	-

[a] Mean of four replicate determinations with 95% confidence level.

Analytical Applications

The proposed method was thoroughly applied to a set of rock, soil, and core samples received from the different parts of eastern India. The results of a few of these samples by the proposed extractive method are comparable to those obtained by other standard techniques, such as the colorimetric method involving 1,10-phenanthrolene as the complexing agent as per the procedure devised by Tarafder and Thakur (10) and the standard titrimetric method involving $K_2Cr_2O_7$ as titrant (Pratt's method). One ICP-OES technique was also used for the determination of total iron at the wavelength of 259.940 nm, and the results are listed in Table IV. The results shown in Tables II to IV imply that the method developed is quite effective in the leaching of Fe(II) and Fe(III), selective extraction of Fe(III) into organic solvent and accurate determination of both species by FAAS.

CONCLUSION

The proposed method of ferrous and ferric oxides determination in geological samples offers several advantages such as ability to determine FeO and Fe_2O_3 in ppm to percentage levels, sequentially determine both FeO and Fe_2O_3 from the same sample solution, the results are reproducible and reliable, cost-effective and eco-friendly, and less prone to interference. The proposed ammonium bi-fluoride and sulfuric acid mixture offers an effective method for leaching of the ferrous and ferric ions in geological samples of rocks, soils, cores, sediments, etc. This mixture sequestered ferric ion by formation of its fluorosulfate complex, where reduction of ferric to ferrous is prevented and enables the separation of ferric ions as thiocyanate complex from ferrous ions by solvent extraction. The method is cost-effective in terms of sample throughput because one bottle (500 g) of ammonium bi-fluoride is used for about 250 sample digestions, whereas only 50 samples are digested with the help of one bottle (500 mL) HF, which is almost three times more costly than solid ammonium bi-fluoride; it is also eco-friendly because solid ammonium bi-fluoride is less hazardous than HF which is a liquid; and it completely eliminates most of the earlier drawbacks in other spectrometric methods such as photochemical reduction or reduction due to organic matter of ferric to ferrous iron.

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TABLE IV
Comparison of Results of FeO and Fe_2O_3 in Rock, Soil, and Core Samples With the Proposed FAAS and Other Standard Methods

Sample	Nature of Sample	FeO (%)			Fe_2O_3 (%)		
		Proposed FAAS Method[a]	1,10 Phenanthrolene Method[c]	Standard Titrimetric Method[d]	Proposed FAAS Method[a]	1,10 Phenan throlene Method[c]	ICP-OES Method[b]
JCL-1	Rock	0.20±0.01	0.18	0.26	5.15±0.22	5.27	5.30
JCL-2	Rock	1.25±0.08	1.30	1.40	3.64±0.14	3.57	3.72
JCL-3	Rock	2.15±0.20	2.12	2.00	8.75±0.40	8.80	8.50
JCL-4	Rock	0.78±0.06	0.72	0.70	1.72±0.08	1.78	1.65
JCL-5	Soil	7.58±0.28	7.42	7.85	3.11±0.12	3.25	3.18
JCL-6	Soil	10.15±0.46	10.02	10.50	5.52±0.20	5.40	5.65
JCL-7	Soil	5.90±0.24	5.82	6.20	4.27±0.16	4.50	4.32
JCL-8	Core	4.90±0.20	4.76	5.15	7.17±0.24	7.02	7.28
JCL-9	Core	1.78±0.10	1.73	1.65	0.78±0.04	0.70	0.65
JCL-10	Core	1.10±0.06	1.15	1.25	1.30±0.06	1.22	1.40.

[a] Mean of four replicate determinations with 95% confidence level.

[b] % Fe_2O_3 = [% Fe(Total) – % Fe(II)] x 1.429 (conversion factor from FeIII to Fe_2O_3).

[c] Optimized ortho-phenanthrolene method (10).

[d] $K_2Cr_2O_7$ as titrant (Pratt's method).

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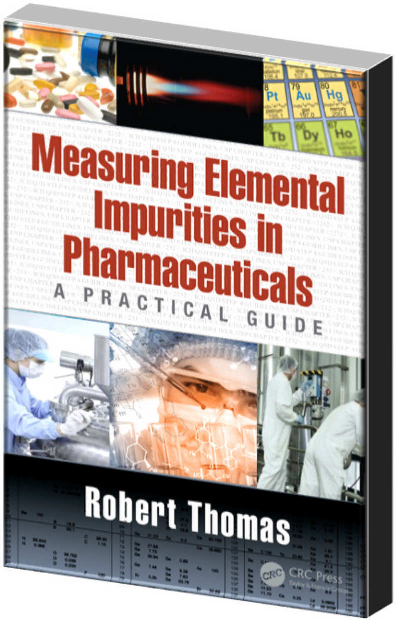
REFERENCES

1. P. Komadel and J. W. Stucki, Clays and Clay Minerals 36(4), 379 (1988).
2. S. Pehkonen, The Analyst 120(11), 2655 (1995).
3. C. B. Brae and N. W. Bower, Geostandards and Geoanalytical Research 11(1), 41 (1987).
4. H. Hong and D. R. Kester, Limnology and Oceanography 31(3), 512 (1986).
5. G. Zhuang, Z. Yi, R. A. Duce and P. R. Brown, Nature 355(6360), 537 (1992).
6. J. E. Amonette and J. Matyas, Anal. Chim. Acta 910, 25(2015).
7. D. Xue, H. Wang, Y. Liu, L. Xie and P. Shen, Geostandards and Geoanalytical Research 43(3), 411 (2017).
8. J. E. Amonette and J. C. Templeton, Clays and Clay Minerals 46(1), 51 (1998).
9. J. W. Stucki, and W. L. Anderson, Soil Science Society of America Journal 45(3), 633 (1981).
10. P. K. Tarafder and R. Thakur, Geostandards and Geoanalytical Research 37(2), 155 (2013).
11. D. Giokas, E. K. Paleologos and M. Karayannis, Analytical and Bioanalytical Chemistry 373(4-5), 237 (2002).
12. E. Pehlivan and D. Kara, Microchim. Acta 158(1-2), 137 (2007).
13. M. Yaman and G. Kaya, Anal. Chim. Acta 540, 77 (2005).
14. M. K. Chaudhuri and N. S. Islam, Inorganic Chem. 25(21), 3749 (1986).
15. G. H. Jeffery, J. Basset, J. Mendhan, and R. C. Denney, Vogel's Textbook of Quantitative Chemical Analysis, 5th edn. ELBS, Longman Group, UK (1989).
16. H. Onishi, Photometric determination of trace metals, part IIB, Wiley, New York, USA (1989).



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