SPECTROPHOTOMETRIC DETERMINATION OF BISMUTH IN WATER SAMPLES BY DISPERSIVE LIQUID-LIQUID MICROEXTRACTION AFTER MULTIVARIATE OPTIMIZATION BASED ON BOX-BEHNKEN

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ABSTRACT

A new simple and rapid dispersive liquid-liquid microextraction has been applied to preconcentration trace levels of bismuth as a prior step to its determination by spectrophotometric detection. In the proposed method, dithizone was used as a chelating agent, and tetrachloride carbon and ethanol are selected as extraction and dispersive solvent. The optimization strategy is carried out by using two level full factorial designs. Results of the two level full factorial design (2^4) based on an analysis of variance demonstrated that the pH, concentration of dithizone, amount of dispersive and extraction solvents were statistically significant. Optimal condition for four variables: pH, concentration of dithizone, amount of dispersive and extraction solvents are obtained by using Box-Behnken design. Under the optimum conditions, the calibration graphs were linear in the range of 5-300 ng mL⁻¹ with detection limit of 1.6 ng mL⁻¹ ($3\delta_b/m$) and the enrichment factor of this method for bismuth reached at 150. The relative standard deviation (R.S.D.) is 2.3% ($n = 5$, $c = 50$ ng mL⁻¹). The method is successfully applied to the determination of trace amount of bismuth in water samples.

Keywords: Bismuth, Dispersive liquid-liquid microextraction, Box-Behnken design, Spectrophotometry.

INTRODUCTION

Bismuth is environmentally important¹⁾, and is of biological interest due to their possible binding to thiolate ligands to form metal-thiolate clusters²⁾. The most commonly used methods for determination of various metal ions are electroanalytical procedures³⁾, atomic absorption spectrometry (AAS)⁴⁾, atomic emission spectrometry (AES)⁵⁾ and mass spectrometry (MS)⁶⁾. However, these techniques have some disadvantages, such as complicated operation, high cost of maintenance, expensive apparatus and requiring well-controlled experiment conditions.

Several procedures have been developed for the separation and preconcentration of contaminants from environmental matrices such as: liquid-liquid extraction⁷⁾, co-precipitation⁸⁾, solid phase extraction⁹⁾. However, liquid-liquid extraction (LLE) is time-consuming and requires large amounts of organic solvents that are potentially toxic. Solid phase extraction (SPE) uses much less solvent than LLE but can be relatively expensive. Additionally, evaporation of the final organic extract into a small volume is necessary to achieve high enrichment of the analytes. Batch-to-batch reproducibility continues to be the major concern for analysts in selecting SPE devices. Elution of sorbed solute must be performed after sample loading. Solvent evaporation and redissolution are often required¹⁰⁾. A novel microextraction technique as a high performance and powerful preconcentration method termed dispersive liquid-liquid microextraction (DLLME) was demonstrated by Y. Assadi and coworkers¹¹⁾. In this method, an appropriate mixture of the extraction solvent and the dispersive solvent is injected into aqueous sample by a syringe and forms a cloudy solution. The cloudy state results from the formation of fine droplets of the extraction solvent which disperse in the sample solution. The cloudy solution shall be centrifuged and the fine droplets sediment at the bottom of the conical test tube. Determination of analytes in the remained phase can be performed by instrumental techniques. Recently, several papers have been published about dispersive liquid-liquid microextraction in preconcentration and determination of metals¹²⁻²⁰⁾.

Spectrophotometric methods are the most commonly used techniques and continue to enjoy wide popularity. The common availability of the instrumentation, the simplicity of procedures, speed, precision and accuracy of the technique still make spectrophotometric methods attractive. In the present work, the DLLME was combined with UV-Visible Spectrophotometry by using micro-sample introduction system for the determination of bismuth was proposed. In this method, dithizone, which forms complexes with bismuth, was selected as chelating reagent. Dithizone contains azo and hydrosulfide groups, which are good electron donors. Dithizone is widely used in extraction spectrophotometry because it can form stable complexes with many metal ions under proper conditions. The factors influencing the efficiency of DLLME and determination were systematically studied and optimized by Box-Behnken design²¹⁾. The applicability of this approach was validated for the determination of bismuth in water samples.

EXPERIMENTAL

Apparatus

A Hewlett-Packard 8453 diode array spectrometer controlled by a Hewlett-Packard computer and equipped with a 1-cm path length quartz cell. A centrifuge (Behdad Universal Centrifuge) was used to accelerate the phase separation process. The pH was determined with a model 780 Metrohm pH-meter with combined glass-calomel electrode.

Reagents and materials

All reagents were of analytical reagent grade. The water utilized in all studies was double-distilled and deionized. Stock solution of bismuth (1000 µg mL⁻¹) was prepared by dissolving appropriate amounts of their corresponding salts in deionized water. Standards of working solution were made by appropriate dilution daily as required. The dithizone and all solvents, such as methanol, ethanol, chloroform, carbon tetrachloride, acetonitrile and acetone were obtained from Merck. Universal buffer solutions were prepared from boric acid, citric acid and phosphoric acid.

Dispersive liquid-liquid microextraction procedures

A 10 mL of sample of standard solution containing 10-300 ng mL⁻¹ of bismuth and potassium nitrate (5%) was poured in a test tube with a conical bottom and 1 mL of dithizone (0.42 mmol L⁻¹) as chelating agent was added into the solution. After a few minutes an orange complex of bismuth was formed and the solution pH was adjusted to 9.0 by universal buffer. Then a binary solution containing 850 mL of ethanol (disperser solvent) and 150 mL of tetrachloride carbon (extraction solvent) was injected rapidly into the sample. Then, Bismuth-Dithizone complex was extracted into fine droplets of carbon tetrachloride. After that the mixture was centrifuged for 10 min at 4000 rpm. After this process the fine droplets of carbon tetrachloride were joined together and sedimented at the bottom of the conical test tube. After removing the whole aqueous solution, and transferred to a 70 mL cell and the absorbance was measured at 485 nm. The volume of the sedimented phase determined to be about 100 µL.

Experimental design

The traditional optimization procedure varying "one variable-at-a-time", is a strategy based on experience that does not guarantee the attainment of a true optimum of the extraction conditions. Conversely, the chemometric approach relies on a rational experimental design, which allows the simultaneous variation of all experimental factors, saving time and materials. Thus in present study, full factorial design was employed to determine the main effects as well the interactive effects of the selected factors. According to the previous experiments, four factors including the pH, concentration of dithizone, volume of disperser solvent and volume of extraction solvent were studied. Therefore, a full factorial design included $2^4 = 16$ experiments used to explore important factors and their interactions. The experiments were run in random manner to

minimize the effect of uncontrolled variables. Then, the analysis of variance (ANOVA) was carried out to calculate the values of effects and interactions of factors²²⁾. The variables that are found significant in this initial screening can be further optimized using response surface methodology such as Box-Behnken design²¹⁾.

Sample preparation before DLLME

Tap water, mineral water and waste water samples were collected from Kermanshah (Kermanshah, Iran). Prior to the preconcentration procedure, all the water samples were filtered through a 0.45 µm pore size membrane filter to remove suspended particulate matter and then were stored at 4 °C in the dark.

RESULTS AND DISCUSSION

In order to select the optimum DLLME conditions for the determination of bismuth, a procedure was required to optimize the different parameters that affect the DLLME extraction process. Some of these parameters are selection of suitable pH, concentration of dithizone, extraction solvent, disperser solvent, volume of extraction solvent, volume of disperser solvent, ionic strength of aqueous phase, and extraction time. It is very important to optimize them in order to obtain good recovery.

Optimization of DLLME procedure

A two level factorial 2⁴ design with two replicates of center point was performed in order to determine the influence of these factors their interactions. The factorial design was evaluated using analytical response (Absorbance in 485 nm) of Bismuth-Dithizone. An analysis of the variance (ANOVA) demonstrated that, within the experimental range, pH, concentration of dithizone, volume of extraction solvent and disperser solvent were statistically significant and Pareto chart (Fig. 1) interpretation it.

Figure 1: Pareto chart of main effects obtained from 2⁴ full factorial designs. The vertical line defines the 90% confidence interval (A = pH, B = Concentration of dithizone, C = Volume of disperser solvent and D = Volume of extraction solvent).

The significant variables like pH; concentration of dithizone, volume of extraction solvent and disperser solvent were chosen as the critical variables and designated as pH, C_{Ligand}, V_{disperser} and V_{extraction}, respectively. The low, middle and high levels of each variable were designated as -, 0 and + respectively, are given in Table 1. In a system involving four significant independent variables, the mathematical relationship of the response on these variables can be approximated by the quadratic polynomial equation:

$$Y = b_0 + b_1 \text{pH} + b_2 C_{\text{Ligand}} + b_3 V_{\text{disperser}} + b_4 V_{\text{extraction}} + b_{12} \text{pH} \times C_{\text{Ligand}} + b_{13} \text{pH} \times V_{\text{disperser}} + b_{14} \text{pH} \times V_{\text{extraction}} + b_{23} C_{\text{Ligand}} \times V_{\text{disperser}} + b_{24} C_{\text{Ligand}} \times V_{\text{extraction}} + b_{34} V_{\text{disperser}} \times V_{\text{extraction}} + b_{11} \text{pH}^2 + b_{22} C_{\text{Ligand}}^2 + b_{33} V_{\text{disperser}}^2 + b_{44} V_{\text{extraction}}^2$$

Equation 1

A multiple regression analysis is done to obtain the coefficients and the equation can be used to predict the response. The degree of experiments chosen for this study was Box-Behnken, a fractional factorial design for four independent variables. It is applicable once the critical variables have been identified. In the model given in equation (1), interactions higher than second order have been neglected. A total of 27 experiments were necessary to estimate of the full model. The results from this experimental design provided a

statistical process, which was used to identify high yield trends for the DLLME process. Table 3 shows the matrix with coded and the responses as absorbance of Bismuth-Dithizone complex at 490 nm. The equation below illustrates the relationship of the four variables.

$$A = 0.7151 - 0.0895 (\text{pH}) + 0.1336 (C_{\text{Ligand}}) + 0.0546 (V_{\text{disperser}}) - 0.0898 (V_{\text{extraction}}) - 0.0542 (\text{pH})^2 - 0.0526 (C_{\text{Ligand}})^2 + 0.3152 (\text{pH})(C_{\text{Ligand}}) - 0.0005 (\text{pH})(V_{\text{disperser}}) + 0.0004 (\text{pH})(V_{\text{extraction}}) - 0.004 (C_{\text{Ligand}})(V_{\text{disperser}}) + 0.0002 (C_{\text{Ligand}})(V_{\text{extraction}})$$

Equation 2

The critical point in the surface response are found by solving the derivation of equation 2 for the condition of $d(A)/d(\text{pH}) = 0$, $d(A)/d(C_{\text{Ligand}}) = 0$, $d(A)/d(V_{\text{disperser}}) = 0$ and $d(A)/d(V_{\text{extraction}}) = 0$. The calculated values for the critical point are follows: pH = 9.0, C_{Ligand} = 0.042 mmol L⁻¹, V_{disperser} = 850 mL and V_{extraction} = 150 mL.

Table 1. The levels of variables chosen for the trials.

Variable	Low level (-)	Middle level (0)	High level (+)
pH	8.0	9.0	10.0
C _{Ligand} (mmol L ⁻¹)	0.04	0.045	0.05
V _{disperser} (µL)	800	850	900
V _{extraction} (µL)	100	150	200

Effect of type of the extraction and disperser solvents

Selecting the extraction solvent by paying attention to its characteristic properties is very important. It must have a higher density than water, be capable of extracting the compounds of interest, and have low solubility in water. Chloroform, carbon dichloride and carbon tetrachloride were compared in this extraction and obtained recoveries were higher for carbon tetrachloride. The main criterion for the selection of the disperser solvent is its miscibility in the extraction solvent and aqueous solution. In addition, the type of disperser directly influences the viscosity of the binary solvent. Thus, this solvent can control droplet production and extraction efficiency. To study this effect, four different solvents such as acetone, acetonitrile, ethanol and methanol were tested and obtained recoveries were higher for ethanol.

Effect of extraction time and centrifuging rate

In DLLME, extraction time is defined as the time between the injection of the binary solvent and starting to centrifuge. The effect of extraction time was examined in the range of 1-10 min with constant experimental conditions. The surface area between extraction solvent and aqueous phase is infinitely large. Thereby, transfer of complex from aqueous phase to extraction solvent is fast. Subsequently, equilibrium state is achieved quickly; therefore, the extraction time is very short, which is the advantage of DLLME technique. In this method, the most time-consuming step is the centrifuging of sample solution, which is about 1 min. A series of same solutions were tested at various rates of centrifugation. The rate of centrifugation was adjusted between 1000 to 6000 rpm for 5 minutes. The rate of 4000 rpm was selected as the optimum rate for centrifuging.

Effect of salt

For studying the influence of ionic strength on the performance pf DLLME, we investigated KNO₃ concentration in the range of 0-10 % (w/v) while other experimental conditions were kept constant. By increasing KNO₃ concentration extraction efficiency slowly increase due to salting-out effect and then approximately stays constant. These studies showed the possibility of DLLME for separation of bismuth from saline solution to 10 % (w/v).

Interferences study

The potential interference in the present method was investigated. The interference was due to the competition of other heavy metal ions for the chelating agent and their subsequent co-extraction with bismuth. In these experiments, solutions containing 50 ng mL⁻¹ of bismuth and the interfering ions were treated according to the recommended procedure. The tolerance limits of the co-existing ions, defined as the largest amount making the recovery of bismuth less than 95% (Table 2).

Table 2. Tolerance limits of co-existing ions in DLLME of bismuth.

Co-existing ions	Tolerance limits (ng mL ⁻¹)
Ca ²⁺ , Mg ²⁺ , Ba ²⁺ , Sr ²⁺ , Mn ²⁺ , Li ⁺ , Rb ⁺ , K ⁺ , Na ⁺ , NH ₄ ⁺ , Cr ³⁺	1000
CO ₃ ²⁻ , NO ₃ ⁻ , Br ⁻ , S ₂ O ₃ ²⁻ , SO ₃ ²⁻ , I ⁻ , CH ₃ COO ⁻ , Cl ⁻ , SCN ⁻ , BrO ₃ ⁻ , C ₂ O ₄ ²⁻ , PO ₄ ³⁻	1000
Cs ⁺ , Cd ²⁺ , Hg ²⁺ , As ³⁺ , In ³⁺ , Tl ⁺	500
Zr ⁴⁺ , Ga ²⁺ , Pd ²⁺ , Al ³⁺	400
Ni ²⁺ , Co ²⁺ , Cu ²⁺	100

Analytical performance

Table 3 summarizes the analytical characteristics of the optimized method, including linear range, limit of detection, reproducibility and enhancement factor. The calibration graph was linear in the range of 5-300 ng mL⁻¹ of bismuth. The limit of detection, defined as $C_{DL} = 3 S_B/m$ (where C_{DL} , S_B and m are the limit of detection, standard deviation of the blank and slope of the calibration graph, respectively), was 1.6 ng mL⁻¹. The relative standard deviation (R.S.D.) for five replicate measurements of 50 ng mL⁻¹ bismuth was 2.3%. The enhancement factor was obtained from the slope ratio of the calibration graph after and before extraction, which was about 150. The equations of calibration graphs after and before extraction summarized in Table 3.

Table 3. Analytical characteristics of DLLME for determination of bismuth.

Parameter	Analytical feature
Calibration curve before extraction	$A = 2.638C_{Bi} + 0.0536$ ($R^2 = 0.9903$)
Calibration curve after extraction	$A = 0.0176C_{Bi} + 0.1264$ ($R^2 = 0.9924$)
Linear range (ng mL ⁻¹)	5 - 300
Limit of detection (ng mL ⁻¹)	1.6
Repeatability (R.S.D., %) (n = 10)	2.3
Enhancement factor (mL)	150

Analytical applications

The proposed DLLME method was validated by extraction and determination of bismuth from spiked tap, mineral and waste water and the results are summarized in Table 5. For this purpose, 10 mL of the water samples were spiked with 50 and 150 ng mL⁻¹ bismuth standard solution and the recommended procedure was followed to assess the matrix effects.

Table 4. Determination of bismuth in different samples.

Sample ^a	Bismuth spiked (ng mL ⁻¹)	Bismuth found ^b (ng mL ⁻¹)	Recovery (%)
Tap water	0	N.D. ^c	-
	50	48.6 ± 0.5	97.2
	150	145.1 ± 0.7	96.7
Mineral water	0	N.D.	-
	50	49.7 ± 0.5	99.4
	150	143.3 ± 0.5	95.5
Waste water	0	46.3 ± 0.7	-
	50	97.3 ± 0.6	102.0
	150	198.4 ± 0.8	101.4

^a From water systems of Arak, Iran. ^b Mean ± S.D., n = 3. ^c Not detected.

As it is obvious from Table 3, in all cases, the extraction efficiency of bismuth was excellent, and showed no serious matrix effects.

CONCLUSION

A new method of DLLME combined with spectrophotometric has been proposed for the determination of bismuth in tap, mineral and waste water

samples. The proposed DLLME method takes advantages from low cost, use of minimized toxic organic solvents, simplicity of operation, rapidity, high enhancement factor and high sensitivity and selectivity. The limit of detection of proposed method (1.6 ng mL⁻¹) is significantly improved over that of the best of previously reported spectrophotometric methods for bismuth determination. Also, the application of a Box-Behnken matrix became possible, rapid, economical and efficient way of an optimization strategy of the proposed procedure.

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REFERENCES

1. M. Nimmo and G. Fones, *Anal. Chim. Acta*, 291, 321 (1997).
2. M. J. Stillman, *Coord. Chem. Rev.*, 144, 461 (1995).
3. A. Koper, M. Grabarczyk, *J. Electroanal. Chem.*, 663, 67 (2011).
4. H. Matusiewicz and M. Krawczyk, *Chemia Analityczna*, 52, 565 (2007).
5. M. Grotti, C. Lagomarsino and R. Frache, *J. Anal. Atomic Spec.*, 20, 1365 (2005).
6. F. Shemirani, M. Baghdadi, M. Ramezani and M. R. Jamali, *Anal. Chim. Acta*, 534, 163 (2005).
7. A.N. Anthemidis, D.G. Themelis, J.A. Stratis, *Talanta* 54, 37 (2001).
8. G. Doner, A. Ege, *Anal. Chim. Acta* 547, 14 (2005).
9. A.M. Starvin, T. Prasada Rao, *J. Hazard. Mat.* B113, 75 (2004).
10. D. Nagaraju, Sh. Da Huang, *J. Chromatogr. A* 1161, 89 (2007).
11. M. Rezaee, Y. Assadi, S.M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, *J. Chromatogr. A* 1101, 1 (2006).
12. A. Bidari, E. Zeini Jahromi, Y. Assadi, M.R. Milani Hosseini, *Microchem. J.* 87, 6 (2007).
13. E. Zeini Jahromi, A. Bidari, Y. Assadi, M.R. Milani Hosseini, M.R. Jamali, *Anal. Chim. Acta* 585, 304 (2007).
14. P. Liang, H. Sang, *Anal. Biochem.* 380, 21 (2008).
15. M.T. Naseri, P. Hemmatkaki, M.R. Milani Hosseini, Y. Assadi, *Anal. Chim. Acta* 610, 135 (2008).
16. M. Gharehbaghi, F. Shemirani, M. Baghdadi, *Intern. J. Environ. Anal. Chem.* 88, 513 (2008).
17. R.E. Rivas, L. Lopez-Garcia, M. Hernandez-Cordoba, *Spectrochim. Acta Part B* 64, 329 (2009).
18. P. Liang, E. Zhao, F. Li, *Talanta* 77, 1854 (2009).
19. P. Liang, L. Peng, P. Yan, *Microchim. Acta* 166, 47 (2009).
20. Z. Xiao-Huan, W. Qiu-Hua, Z. Mei-Yue, X. Guo-Hong, W. Zhi, *Chin. J. Anal. Chem.* 37, 161 (2009).
21. M. Khajeh, *J. Hazard. Mat.* 177, 385 (2009).
22. S.H. Chang, T.T. Teng, N. Ismail, A.F.M. Alkarkhi, *J. Hazard. Mater.* 190, 197 (2011).