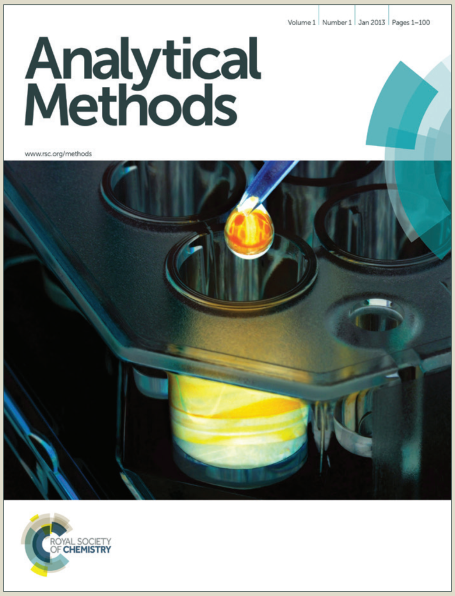
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Statement of Societal Impact

Significance

Bismuth (Bi) is a heavy metal with relatively wide applications in different fields such as cosmetic industry, medicine and preparation of semiconductors. It is used in the cosmetic industry as a pigment in paint for eye shadows, hair sprays and nail polishes. Also, in the field of medicine it is an ingredient in some pharmaceuticals for treatment of antidiarrheal, syphilis and eye infections. As the use of bismuth increases, it has spread in the environment and the chance of exposure of organisms to bismuth has increased. Bismuth is toxic which can affect kidney and liver; therefore, analyzing and monitoring the content of bismuth in environmental samples is of great significance.

Novelty

Spectrophotometric investigation of bismuth thiocyanate complex has been performed by Kingery and Hume. They have reported different positive and negative charged Bi-thiocyanate complexes formed at different concentrations of thiocyanate anion. Based on the reported results, at certain concentration of thiocyanate anion, negative charged Bi-thiocyanate complexes of $Bi(SCN)_6^{3-}$ and $Bi(SCN)_4^-$, are predominant. **Based on our knowledge, there is not any publication to use thiocyanate anion for microextraction of Bismuth (III).** In this paper, we used thiocyanate anion at a critical concentration to produce negative charged Bi-thiocyanate complexes followed by addition of cetylpyridinium chloride (CPC) as a positive counter ion to form hydrophobic ion pair complexes. The resulting ion pair complexes were extracted into the fine droplet of extraction solvent by DLLME and quantitatively determined by electrothermal atomic absorption spectrometry (ETAAS).

Societal impact

Ultra-trace monitoring of heavy metals in environmental samples has great importance. Here, we have used a simple, rapid and efficient ion paired based dispersive liquid liquid microextraction for microextraction and determination of ultra-trace levels of Bi (III). This method is easy to use and can be replaced with some other time consuming methods.

Ion pair based dispersive liquid liquid microextraction for preconcentration of ultra-trace levels of Bismuth (III) and its determination by electrothermal atomic absorption spectroscopy

Samira Vakilzadeh, Mohammad Eftekhari*, Mahmoud Chamsaz, Farzaneh Javedani-Asleh
Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

Abstract

A simple, selective and efficient ion pair based dispersive liquid liquid microextraction method was used for preconcentration of ultra-trace levels of Bi (III) prior to its determination by electrothermal atomic absorption spectrometry. This method is based on the complexation of Bi (III) with thiocyanate anion to form anionic complexes of $Bi(SCN)_6^{3-}$ and $Bi(SCN)_4^-$. A cationic surfactant, cetylpyridinium chloride (CPC) was used as a counter ion. The resulting ion pair complexes were extracted into the fine droplets of carbon tetrachloride by rapid injection of the mixture of acetone (as disperser solvent) and carbon tetrachloride (as extractant solvent) into the sample solution. The important parameters affecting the extraction efficiency including sample pH, thiocyanate and CPC concentrations, type and volume of the extraction and disperser solvents were examined and optimized. Under the optimum conditions, the calibration graph was linear in the range of 0.3 - 8 μg L^{-1} Bi (III) with a correlation coefficient of 0.9979. The relative standard deviation (RSD, %) based on eight replicate analysis of 2 μg L^{-1} Bi (III) was 4.8 % and the detection limit (DL) of Bi (III) was 0.07 μg L^{-1}. The validation of the proposed method was examined by the analysis of a certified reference material and spike method. The obtained results were in very good agreement with the certified values. The proposed method was successfully applied for the determination of ultra-trace levels of Bi (III) in different water and human serum samples.

Keywords: Bismuth, Thiocyanate, Dispersive liquid liquid microextraction, Electrothermal atomic absorption spectrometry

Corresponding to:
Mohammad Eftekhari (meftekhari85@yahoo.com)
Tel: + 98 5138795162
Fax: + 98 5138796416

1. Introduction

In recent years, environmental pollution by heavy metals has received considerable attentions [1,2]. Bismuth (Bi) is a heavy metal with relatively wide applications in different fields such as cosmetic industry,[3] medicine[4] and preparation of semiconductors.[5] It is used in the cosmetic industry as a pigment in paint for eye shadows, hair sprays and nail polishes. Also, in the field of medicine it is an ingredient in some pharmaceuticals for treatment of antidiarrheal, syphilis and eye infections.[3,4] As the use of bismuth increases, it has spread in the environment and the chance of exposure of organisms to bismuth has increased. Bismuth is slightly toxic which can affect kidney and liver; therefore, analyzing and monitoring the content of bismuth in environmental samples is of great significance[6]. A variety of techniques have been developed for the determination of bismuth in different samples. These include UV-Vis spectrophotometry,[7,8] atomic absorption spectrometry,[9,10] inductively coupled plasma mass spectrometry[11] and inductively-coupled plasma optical emission spectrometry.[12] Among the mentioned techniques, atomic absorption spectrometry is the most often used technique because of its availability and simplicity. However, direct determination of trace metals in real samples containing complex matrices or very low concentrations of analytes is difficult. In order to achieve this goal, separation and preconcentration procedures for samples preparation are necessary[13].

Dispersive liquid liquid microextraction (DLLME) [14, 15] is a preconcentration technique which has the advantages of simplicity, rapidity and use very reduced amount of organic solvents. In this method a mixture of disperser and extraction solvent is rapidly injected into the sample solution causing the formation of fine droplets of extraction solvent. Owing to the considerably large surface area between the extracting solvent and the aqueous sample, an equilibrium state is achieved quickly.

Spectrophotometric investigation of bismuth thiocyanate complexes has been performed by Kingery and Hume.[16] They have reported different positive and negative charged Bi-thiocyanate complexes were formed at different concentrations of thiocyanate anion. Based on the reported results, at a certain concentration of thiocyanate anion, negative charged Bi-thiocyanate complexes of $Bi(SCN)_6^{3-}$ and $Bi(SCN)_4^-$, are predominant. In this paper, we have used thiocyanate anion at a critical concentration to produce negative charged Bi-thiocyanate complexes followed by addition of cetylpyridinium chloride (CPC) as a positive counter ion to form hydrophobic ion pair complexes. The resulting ion pair complexes were extracted into the fine droplet of extraction solvent by DLLME and quantitatively determined by electrothermal atomic absorption spectrometry (ETAAS). Different parameters affecting the extraction efficiency were extensively investigated and optimum conditions were selected.

2. Experimental

2.1. Instrumentation

A Nov AA 400, Analytik Jena graphite furnace atomic absorption spectrometer equipped with deuterium lamp as a background correction system was used for determination of Bismuth in the extraction solvent. Pyrolytically coated graphite tubes with L'vov platform were used. A Bismuth hollow cathode lamp at 223.1 nm was employed as radiation source and measurements were carried out in peak area mode, using a spectral bandwidth of 0.5 nm. Argon of 99.99 % purity was used as the inert gas and its flow rate was maintained at 300 mL min^{-1} during all stages, except for the atomization where the flow was stopped. The instrumental parameters are given in Table 1. The pH values in the aqueous phase were measured with Metrohm 827 pH lab (Switzerland) glass-electrode pH meter. Phase separation was assisted using a Centurion Scientific Centrifuge (Model Andreas Hettich D72, Tuttlingen, Germany).

2.2. Reagents and solutions

Bismuth standard solution (1000 mg L^{-1}) was prepared from bismuth (III) nitrate pentahydrate (Sigma Alderich, USA) in deionized water (Barnstead, Nanopure Diamond purification system). The working standard solution was obtained daily by stepwise dilution from the standard stock solution with deionized water. A solution of 2.0 mol L^{-1} thiocyanate anion was prepared from potassium thiocyanate (Merck, Darmstadt, Germany) in deionized water. Carbon tetrachloride, chloroform and dichloromethane as extraction solvents and ethanol, acetonitrile, acetone and methanol as disperser solvents were purchased from Merck (Darmstadt, Germany). A solution of 10^{-2} mol L^{-1} of cetylpyridinium chloride (CPC) (Merck, Darmstadt, Germany) was prepared in deionized water and used as the positive counter ion.

2.3. Preparation of real samples

2.3.1. Water samples

Different water samples including, tap (Obtained from Mashhad, Iran), river (Kashaf Rood, Mashhad, Iran), mineral and spring (Nowchah, Mashhad, Iran) waters were collected from their local sources and filtered through a No. 42 Whatman® paper to remove any suspended particles and then acidified with dilute nitric acid and stored in glass bottles at 5 ºC. For the analysis of Bi content in real samples, six milliliter of each sample solution was transferred into the sample vial, and analyzed according to the microextraction procedure.

2.3.2. Serum samples

The human serum samples (Sample No 1, 2 and 3) were collected from the Iranian Blood Transfusion Organization (Mashhad, Iran). Each sample (2.0 mL) was acidified with 30% (w/v) trichloroacetic acid solution to precipitate the proteins [17]. The mixture was then shaken in a vortex for 5min and centrifuged for 15 min. The supernatant was decanted and filtered through 0.22 μm membrane and diluted to 10 mL with deionized water. For the analysis of Bi (III) content in serum samples, 2.5 mL of each sample was analyzed according to the section 2.4.

2.4. Microextraction Procedure

10 mL of sample solution containing 2 μg L^{-1} Bi (III), 0.80 mol L^{-1} SCN^- and 1.5×10^{-4} mol L^{-1} CPC was adjusted at pH 2 with diluted nitric acid and transferred into conical bottom glass centrifuged tube. 750 μL of acetone containing 60 μL of carbon tetrachloride was rapidly injected into the sample solution. A cloudy solution (water, acetone and carbon tetrachloride) was formed in the test tube. In this step, hydrophobic ion pair based complexes of bismuth were extracted into the fine droplets of carbon tetrachloride. The mixture was then centrifuged for 7 min at 3,000 rpm, by this way; the dispersed fine droplets of carbon tetrachloride were sedimented at the bottom of the test tube. The volume of the sedimented phase was determined using a 100 μL micro-syringe which was about 40.0 μL. 25 μL of the sedimented phase was removed by the syringe and diluted to 50 μL with ethanol. Finally, 20 μL of this solution was injected into the electrothermal atomizer by an auto-sampler to obtain the analytical signal.

3. Results and Discussion

3.1. Optimization of graphite furnace conditions

In order to obtain high extraction efficiency for ETAAS measurement of Bi in the extraction solvent, optimization of important parameters, including pyrolysis and atomization temperature in the graphite furnace, were studied. As the matrix of extraction solvent is carbon tetrachloride and ethanol, it can be easily removed at 130 °C. The influence of pyrolysis temperature on Bi absorbance was studied in the range of 400–800 °C. Optimal pyrolysis temperature was observed at 550- 650°C, with a hold time of 20 s. Therefore, 600 °C was selected as the optimum pyrolysis temperature. The effect of atomization temperature on Bi absorbance was studied within the interval of 1600–2100°C. The maximum signal was observed at 2000 °C, with a hold time of 3s.

Finally, a temperature of 2400 °C and a hold time of 3 s were chosen for the cleaning step. No matrix modifier was found to be useful for enhancement of the sensitivity of Bi. Therefore, all analysis were performed without addition of matrix modifier.

3.2. Effect of pH

The effect of pH on the absorbance of Bi (III) was studied in the range of 1 – 8. The results are presented in Fig. 1, and show that the absorbance reached to its maximum value at pH 2 and then decreased gradually at higher pH values. Based on the results, bismuth ion paired complex is stable in acidic conditions and its stability reduced at higher pH values. Therefore, pH 2 was selected as the optimum value.

3.3. Effect of thiocyanate anion concentration

Thiocyanate anion has an important effect on the formation of negative charged $Bi(SCN)_6^{3-}$ and $Bi(SCN)_4^-$ complexes.[16] To study the effect of thiocyanate concentration on the extraction efficiency of Bi (III), its concentration was studied in the range of 0.2 – 1.4 mol L^{-1}. The results are shown in Fig. 2. Based on the results, the absorbance increased gradually by increasing the thiocyanate concentration upto 0.75 mol L^{-1} and it remained constant afterwards. Therefore, a concentration of 0.80 mol L^{-1} thiocyanate anion was selected as the optimum value.

3.4. Effect of CPC concentration

A cationic surfactant cetyl pyridinium chloride (CPC) was used as a counter ion due to its ability to form ion pairs with $Bi(SCN)_6^{3-}$ and $Bi(SCN)_4^-$ complexes. Different concentrations of CPC were used in the concentration range of $5.0 \times 10^{-5} – 2.0 \times 10^{-4}$ mol L^{-1}. As it can be seen in Fig. 3, an increase in the extraction recovery of the ion pair occurred with increasing the CPC concentration up to 1.30×10^{-4} mol L^{-1} and the absorbance remains constant at higher CPC concentrations. Therefore, a concentration of 1.50×10^{-4} mol L^{-1} CPC was selected as the optimum value.

3.5. Effect of type of extraction solvent

In order to obtain high extraction efficiency, the selection of type of extraction solvent and disperser solvent must be optimized in DLLME system. The effect of different extraction solvents including: carbon tetrachloride, chloroform and dichloromethane were investigated. 750 µL of acetone containing different volumes of carbon tetrachloride, dichloromethane and chloroform were injected into the sample solution to obtain 40 µL of sedimented extraction solvents. As shown in Fig. 4, carbon tetrachloride has the highest extraction efficiency and therefore, it was selected as the optimum extraction solvent.

3.6. Effect of volume of extraction solvent

To study the effect of extraction solvent volume on the absorbance of Bi (III), different volumes of carbon tetrachloride in the range of 50 – 150 μL were examined. The results show that (data not shown) 60 μL of carbon tetrachloride provides maximum extraction efficiency for determination of Bi (III). Therefore, 60 μL of carbon tetrachloride was selected as the optimum value.

3.7. Effect of type of disperser solvent

The selection of a dispersive solvent is limited to the solvents such as methanol, acetonitrile, ethanol and acetone, which are miscible with both water and extraction solvents. To study the effect of type of disperser solvent on the absorbance of Bi (III), 750 μL of different disperser solvents containing 60 μL of carbon tetrachloride for acetone and acetonitrile and 70 μL of carbon tetrachloride for ethanol and methanol, to obtain 40 μL of sedimented extraction solvent, was injected into the sample solution. The results in Fig. 5 show that, acetone has maximum extraction efficiency for microextraction of Bi (III) and therefore, it was selected as the optimum disperser solvent.

3.8. Effect of volume of disperser solvent

To study the effect of volume of disperser solvent on the absorbance of Bi (III), different volumes of disperser solvent in the range of 0.5 – 2 mL containing 60 μL of carbon tetrachloride were examined. The results are shown in Fig. 6. According to the results, the absorbance is increased by increasing of disperser solvent upto 750 μL and decreases by further increase of acetone volume. It seems that at low volumes of acetone, the cloudy state is not formed well; thereby, the absorbance decreases and at high volumes of acetone, the solubility of Bi (III) ion pair complex in water increases and hence, the extraction efficiency decreases. Therefore, 750 μL of acetone was selected as the optimum value.

3.9. Effect of centrifuge conditions

The effect of centrifugation rate on the absorbance of Bi (III) was studied in the range of 2000-3500 rpm with a constant centrifuge time of 10 min. The results show that 3000 rpm centrifuge rate is an optimum value for separation of two immiscible phases. Also, the effect of centrifugation time on the absorbance of Bi (III) was studied in the range of 1-10 min with a constant centrifuge rate of 3000 rpm. The results show that (data not shown), 7 min centrifugation time is an optimum centrifuge time. Therefore, 7 min centrifugation time at 3000 rpm was selected as the optimum value.

3.10. Effect of interfering ions

The effect of different cations and anions on the recovery of Bi (III) was investigated to check the selectivity of the proposed method. An ion was considered as interfering when it caused a variation in the absorbance of the analyte greater than ± 5%. The recoveries of Bi (III) determined in various amounts of matrix ions are given in Table 2. As the results show, there is no interference from the coexisting ions in different real samples for the determination of Bi (III).

3.11. Analytical figures of merit

Under the optimum conditions, the calibration curve was linear in the range of 0.3 – 8 µg L^{-1} Bi (III) with a correlation coefficient of 0.9979. The calibration equation after preconcentration step was A= 0.0246 + 0.0678 C, where A is the analytical signal measured as absorbance and C is the concentration of Bi (III) in µg L^{-1}. Also the equation of the calibration curve without the preconcentration step was A= 0.012 + 0.0005 C, within a dynamic range from 50 to 200 µg L^{-1} Bi (III). The relative standard deviation (RSD, %) based on the eight replicate analysis of 2 µg L^{-1} Bi (III) was 4.8 %. The limit of detection (LOD) based on $3S_b/m$ (where S_b is the standard deviation for six replicate analysis of the blank signals and m is the slope of calibration curve after extraction) was calculated to be 0.07 µg L^{-1}. The preconcentration factor (PF), calculated as the ratio between the volumes of the aqueous phase and the final volume of the extraction phase, was 125. The enhancement factor was calculated by the slope ratio of the calibration curves for Bi determination with and without ion paired based-DLLME was 136.

3.12. Analysis of real samples

In order to assess the applicability of the proposed method, different water and human serum samples were analyzed according to the microextraction procedure. The results are shown in Table 3. In order to check the validity of the proposed method, spike methods were also examined on the samples. As shown in Table 3, the recovery values were in the range of 96 - 107 %. The results show that the procedure is reliable for determination of ultra-trace levels of Bi (III) in water and human serum samples. The accuracy of the method was validated by the analysis of CRM-TMDW (drinking water) certified reference material (http://www.highpuritystandards.com/store/home.php?cat=44). The observed value of the analysis of CRM was 9.73 ± 0.58 µg L^{-1} (mean ± standard deviation based on three replicate analysis) which is in very good agreement with the certified value of 10 µg L^{-1} Bi (III).

3.13. Comparison to other method

A comparison of the proposed method with other reported preconcentration methods for determination of Bi (III) is given in Table 4. As can be seen, the linear range, LOD and preconcentration factor (PF) of the proposed method is better or comparable to other reported methods. The simplicity of operation, rapidity, high sensitivity, low cost and high preconcentration factor are some advantages of the proposed method.

4. Conclusions

An efficient, simple and sensitive ion paired based dispersive liquid liquid microextraction was used for preconcentration of ultra-trace levels of Bi (III) followed by its determination by ETAAS. This method is based on the complexation of Bi (III) with thiocyanate anion (SCN^-) to form a negatively charged $Bi(SCN)_6^{3-}$ and $Bi(SCN)_4^-$. Cetyl pyridinium chloride (CPC) was used as a counter ion to form hydrophobic ion pair complexes which extractable into the fine droplet of carbon tetrachloride by DLLME. The proposed method was successfully applied for determination of trace levels of Bi (III) in different water and human serum samples.

References

1. A. H. Panhwar, T. G. Kazi, H. I. Afridi, S. A. Arain, M. S. Arain, K. D. Brahaman, Naeemullah, S. S. Arain, *Environ. Geochem. Health*, 2015, in press, DOI: 10.1007/s10653-015-9715-y.

2. A. H. Panhwar, T. G. Kazi, H. I. Afridi, S. A. Arain, M. S. Arain, K. D. Brahaman, N. Ullah, J. Ali, S. S. Arain, *Environ. Monit. Assess.*, 2015, **187**, 37.

3. F. J. Maile, G. Pfaff, P. Reynders, *Prog. Org. Coat.*, 2005, **54 (3)**, 150.

4. T. E. Sox, C. A. Olson, *Antimicrob. Agents Chemother*., 1989, **33(12)**, 2075.

5. N. Pistofidis, G. Vourlias, S.Konidaris, El. Pavlidou, A.Stergiou, G. Stergioudis, *Mater. Lett.*, 2007, **61 (4-5)**, 994.

6. R. Pamphlett, M. Stoltenberg, J. Rungby, G. Danscher, *Neurotoxicol. Teratol*., 2000, **22**, 559.

7. S. Rastegarzadeh, N. Pourreza, A. Larki, *Anal. Meth.,* 2014, **6**,3500.

8. P. D. Tzanavaras, D. G. Themelis and A. Economou, *Anal. Chim. Acta*, 2004, **505**, 167.

9. M. Fayazi, D. Afzali, A. Mostafavi, *J. Anal. At. Spectrom*., 2011, **26**, 2064.

10. X. Wen, Y. Zhao, Q. Deng, S. Ji, X. Zhao and J. Guo, *Spectrochim. Acta Part A*, 2012, **89**, 1.

11. X. Jia, Y. Han, X. Liu, T. Duan, H. Chen, *Microchim. Acta*, 2010, **171**, 49.

12. H. Sereshti, Y. Entezari Heravi, S. Samadi, *Talanta*, 2012, **97**, 235.

13. A. H. Panhwar, T. G. Kazi, H. I. Afridi, S. A. Arain, Naeemullah, K. D. Brahman, M. S. Arain, *Spectrochim. Acta Part A*, 2015, **138**, 296.

14. M. Rezaee, Y. Assadi, M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, *J. Chromatogr. A,* 2006, **1116**, 1.

15. M. Chamsaz, M. Eftekhari, S. Tafreshi, A. Yekkebashi, A. Eftekhari, *Inter. J. Environ. Anal. Chem.,* 2014, **94**, 348.

16. Wm. D. Kingery, D. N. Hume, *J. Am. Chem. Soc.*, 1949, **71 (7)**, 2393.

17. J. M. Padro, M. E. Marson, G. E. Mastrantonio, J. Altcheh, F. Garcia-Bournissen, M. Reta, *Talanta*, 2013, **107**, 95.

18. X. Wen, S. Yang, H. Zhang, J. Wang, *Anal. Methods*, 2014, **6**, 8773.

Figure Captions:

Fig. 1. Effect of pH on the absorbance of Bi (III).
Conditions: 2 μg L^{-1} Bi (III), 0.80 mol L^{-1} [SCN^-], 1.50×10^{-4} mol L^{-1} CPC and 750 μL of acetone containing 60 μL CCl_4.

Fig. 2. Effect of Thiocyanate concentration on the absorbance of Bi (III).
Conditions: 2 μg L^{-1} Bi (III), pH 2, 1.50×10^{-4} mol L^{-1} CPC and 750 μL of acetone containing 60 μL CCl_4.

Fig. 3. Effect of CPC concentration on the absorbance of Bi (III).
Conditions: 2 μg L^{-1} Bi (III), pH 2, 0.80 mol L^{-1} [SCN^-] and 750 μL of acetone containing 60 μL CCl_4

Fig. 4. Effect of type of extraction solvent on the absorbance of Bi (III).
Conditions: 2 μg L^{-1} Bi (III), pH 2, 0.80 mol L^{-1} [SCN^-], 1.50×10^{-4} mol L^{-1} CPC.

Fig. 5. Effect of type of disperser solvent on the absorbance of Bi (III).
Conditions: 2 μg L^{-1} Bi (III), pH 2, 0.80 mol L^{-1} [SCN^-], 1.50×10^{-4} mol L^{-1} CPC.

Fig. 6. Effect of volume of disperser solvent on the absorbance of Bi (III).
Conditions: 2 μg L^{-1} Bi (III), pH 2, 0.80 mol L^{-1} [SCN^-], 1.50×10^{-4} mol L^{-1} CPC.

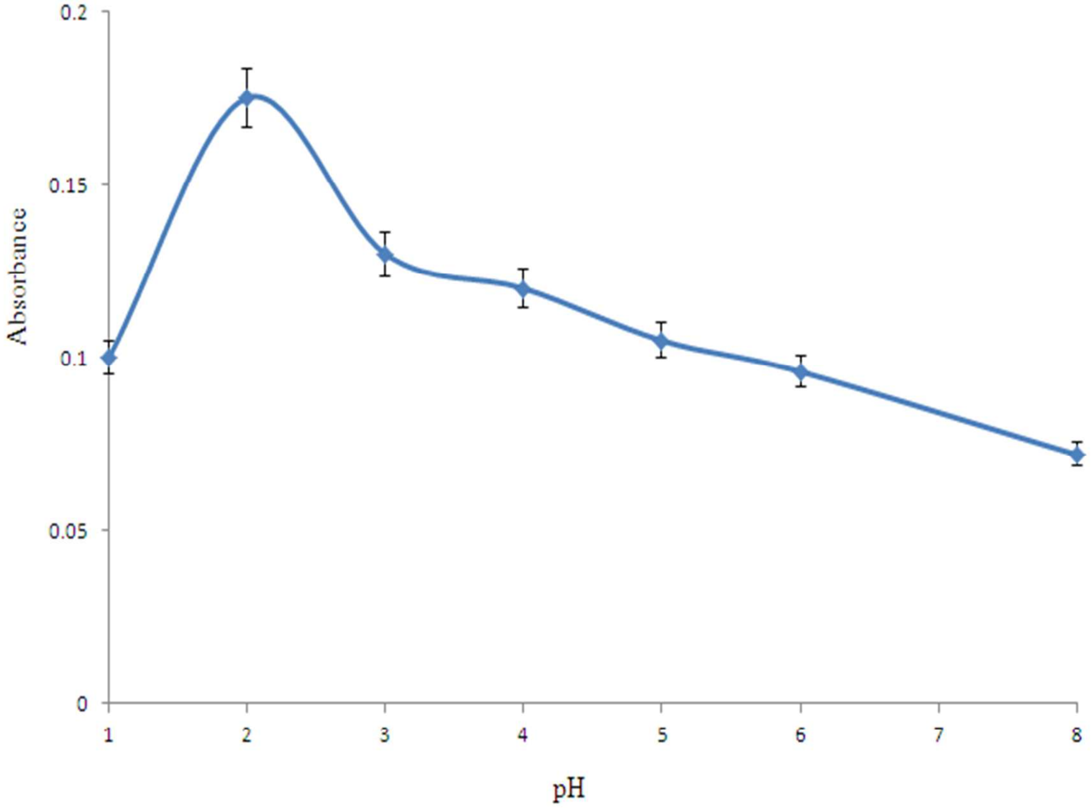


Fig. 1.

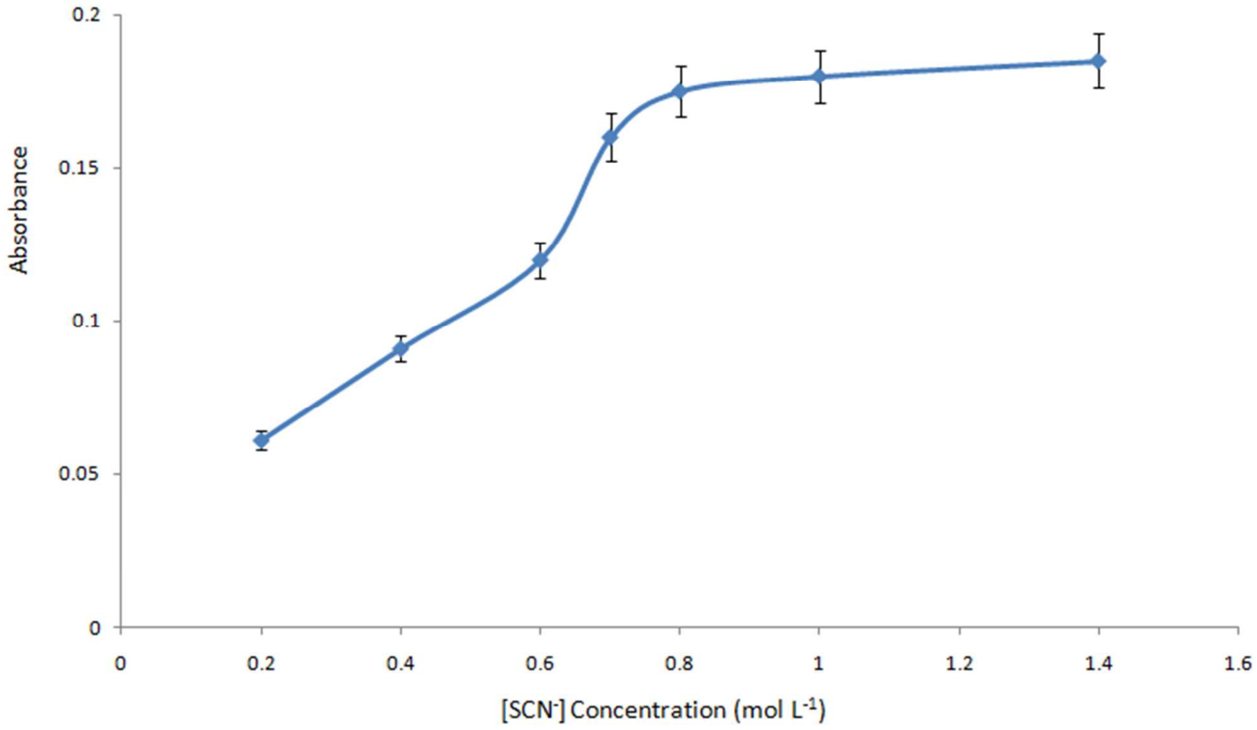
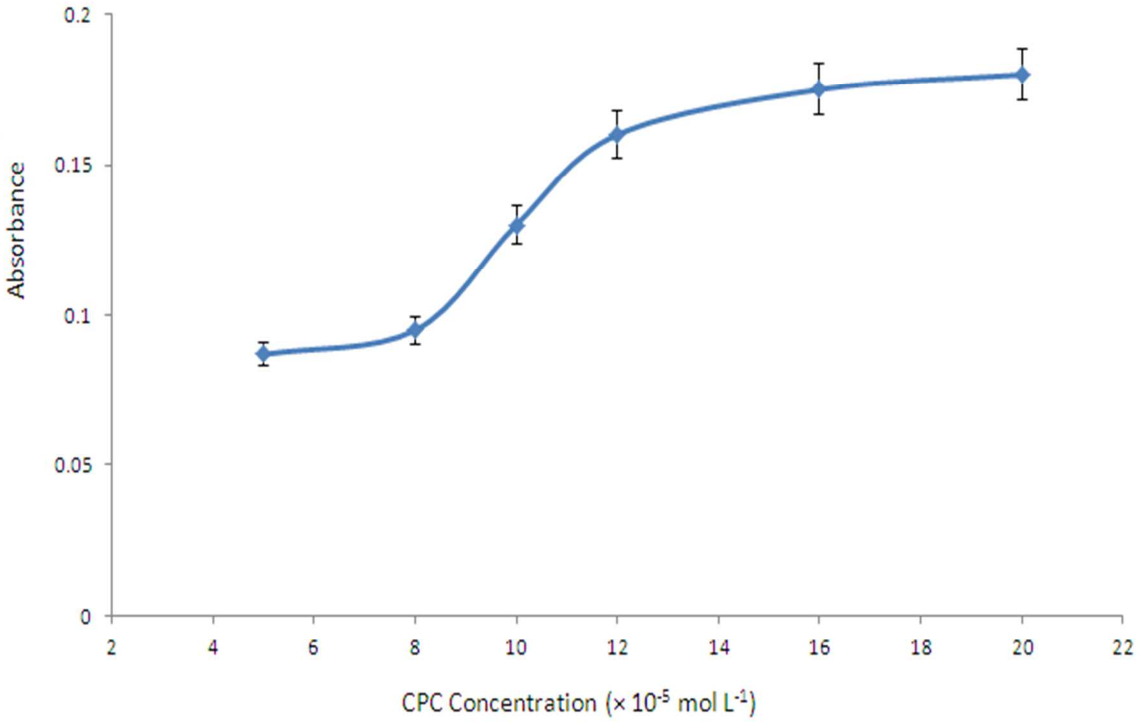

Absorbance
0
0.05
0.1
0.15
0.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
[SCN$^-$] Concentration (mol L^{-1})

Fig. 2.



Absorbance
0.2
0.15
0.1
0.05
0
2
4
6
8
10
12
14
16
18
20
22
CPC Concentration (× 10⁻⁵ mol L⁻¹)

Fig. 3.

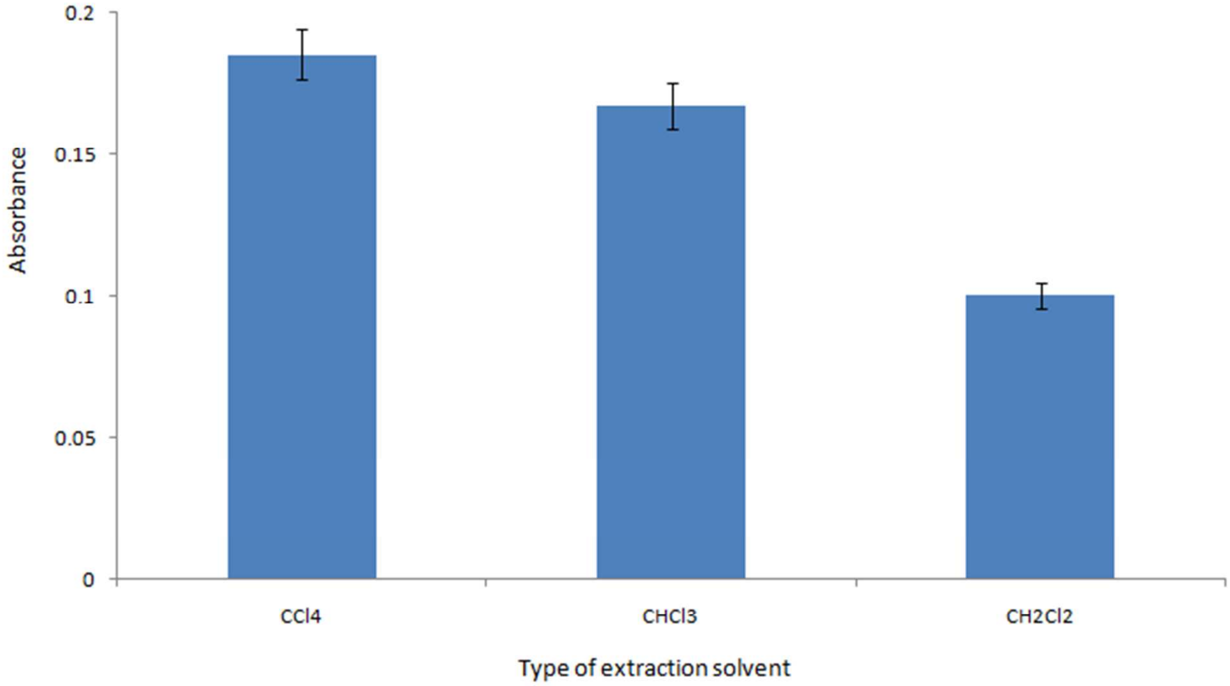

0.2
0.15
0.1
0.05
0
Absorbance
CCl4
CHCl3
CH2Cl2
Type of extraction solvent

Fig. 4.

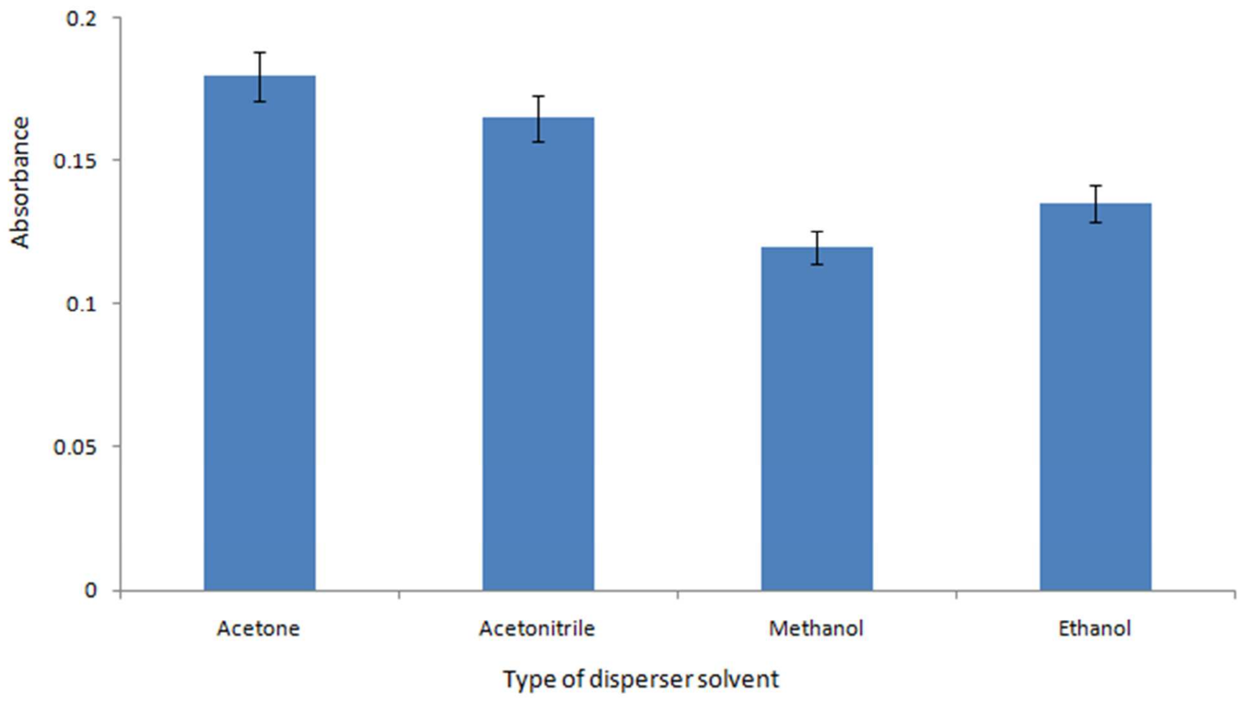

0.2
0.15
0.1
0.05
0
Absorbance
Acetone
Acetonitrile
Methanol
Ethanol
Type of disperser solvent

Fig. 5.

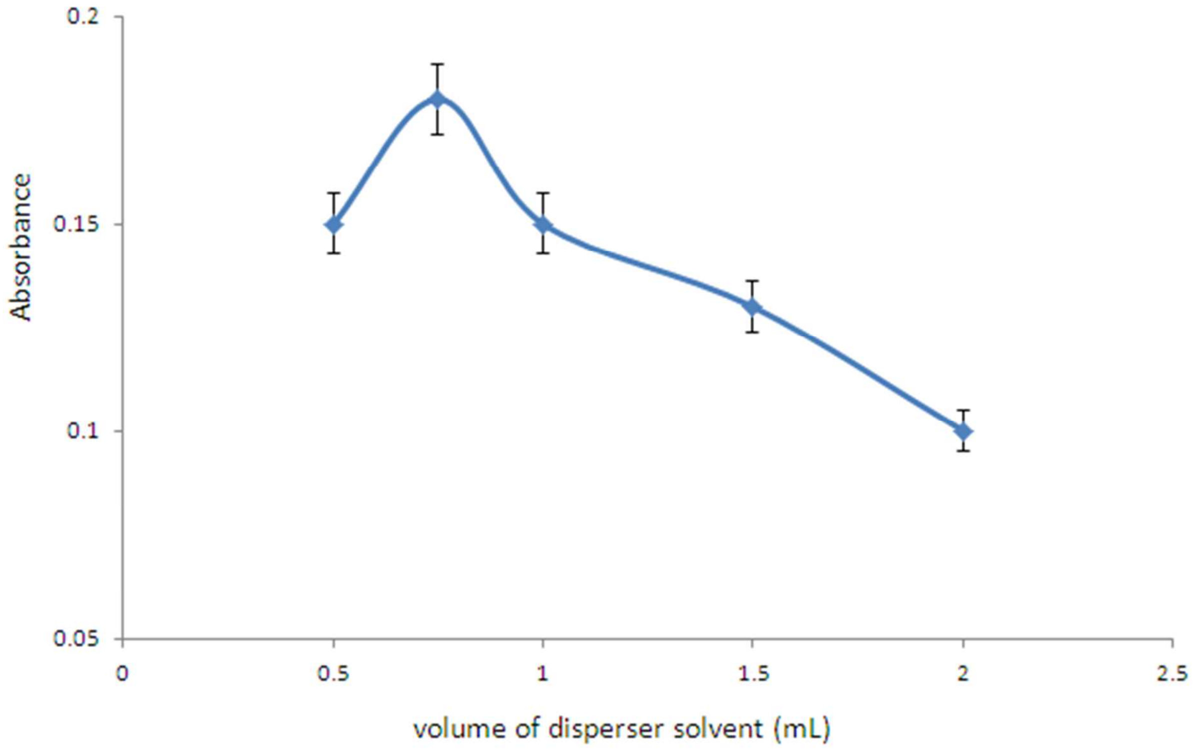

Absorbance
0.2
0.15
0.1
0.05
0
0.5
1
1.5
2
2.5
volume of disperser solvent (mL)

Fig. 6.

Table 1. Instrumental parameters and temperature program for Bismuth analysis.

Step	Temp. (°C)	Ramp time (sec)	Hold time (sec)	Argon flow rate (mL min^{-1})
Drying 1	90	5	10	300
Drying 2	130	3	15	300
Ashing	600	15	20	300
Atomization	2000	0	3	0
Cleaning	2400	2	3	300

Table 2. The effect of interfering ions on the absorbance of 2 µg L^{-1}Bi (III).

Interfering ions	Added as	Concentration (µg L^{-1})	Recovery, %
Na^+	$NaNO_3$	200000	98.1
Zn^{2+}	$Zn(NO_3)_2$	500	96.9
Cu^{2+}	$Cu(NO_3)_2.5H_2O$	500	96.7
Mn^{2+}	$Mn(NO_3)_2.H_2O$	500	98.6
Ni^{2+}	$Ni(NO_3)_2$	500	99.0
Pb^{2+}	$Pb(NO_3)_2$	500	98.9
Cd^{2+}	$Cd(NO_3)_2.H_2O$	500	98.9
Fe^{3+}	$Fe(SO_4)_2.NH_4.12H_2O$	400	96.5
Al^{3+}	$Al(NO_3)_3.9H_2O$	400	96.7
Co^{2+}	$Co(NO_3)_2.6H_2O$	200	97.1
SO_4^{2-}	K_2SO_4	100000	98.5
Cl^-	KCl	100000	100

Table 3. Analysis of real samples for determination of Bi (III). Results (mean±standard deviation based on three replicate analyses)

Sample	Added (μg L^{-1})	Founded (μg L^{-1})	Recovery (%)
Tap Water [a]	-	0.69 ± 0.03	-
	0.80	1.50 ± 0.08	100.7
	2.00	2.67 ± 0.11	99.2
Spring Water [b]	-	1.1 ± 0.06	-
	0.80	2.01 ± 0.10	105.8
	2.00	3.25 ± 0.15	104.8
Mineral Water	-	ND [d]	-
	0.80	0.86 ± 0.04	107.5
	2.00	2.11 ± 0.14	105.5
River Water [c]	-	2.80 ± 0.15	-
	1.00	3.75 ± 0.17	98.7
	2.00	4.65 ± 0.20	96.9
Sample No. 1	-	ND	-
	4.00	4.18 ± 0.17	104.5
Sample No. 2	-	ND	-
	4.00	4.06 ± 0.16	101.5
Sample No. 3	-	7.00 ± 0.41	-
	6.00	12.9 ± 0.55	99.2

[a] : Obtained From Mashhad, Iran

[b]: NowChah, Mashhad, Iran

[c]: Kashaf Rood, Mashhad, Iran

[d]: Not detected

Table 4. Comparison of the ion paired based DLLME with other reported methods for determination of Bi (III).

Method	Linear Range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	RSD (%)	PF	Ref.
DLLME-UV-Vis	5 - 400	1.6	1.14-2.66	62.5	7
CPE-FAAS	-	4.0	4.2	-	10
UA-DLLME-W-coil ET-AAS	2 - 30	0.2	4.7	-	18
DLLME- flow injection inductively coupled plasma mass spectrometry	0.01-1	0.0047	2.6 – 6.7	-	11
DLLME-FAAS	30 - 1700	3.0	1.5	28.6	9
Ion paired DLLME-ETAAS	0.3 - 8	0.07	4.8	125	Present work