Microchim Acta (2012) 178:399–404
DOI 10.1007/s00604-012-0852-z

ORIGINAL PAPER

A cloud point extraction for spectrophotometric determination of ultra- trace antimony without chelating agent in environmental and biological samples

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Received: 13 February 2012 / Accepted: 13 June 2012 / Published online: 26 June 2012

Abstract We report on a simple, sensitive and reliable method for the cloud point extraction of antimony (Sb) and its subsequent spectrophotometric detection. It is based on the color reaction of Sb (III) with iodide in acidic medium and subsequent micelle-mediated extraction of tetraiodoantimonate using a non-ionic surfactant in the absence of any chelating agent. The effects of reaction and extraction parameters were optimized. The calibration plot is linear in the range of 0.80–95 ng mL^{-1} of antimony in the sample solution, with a regression coefficient (r) of 0.9994 (for n=9). The detection limit (at SNR=3) is 0.23 ng mL^{-1}, and the relative standard deviations at 10 and 70 ng mL^{-1} of antimony are 3.32 and 1.85 % (at n=8), respectively. The method compared favorably to other methods and was applied to determine antimony in seawater, anti-leishmania drug (glucantime), and human serum.

Keywords Cloud point extraction · Antimony · Spectrophotometry · Triton X-114

Introduction

Interest in the determination of antimony at ultra- trace levels has increased in recent surveys because of the health hazard of this toxic metal which exists in two oxidation states, III and V. It was reported that antimony (III) is more toxic and mobile than antimony (V). Sb has been classified as priority pollutant by the Environmental Protection Agency of the United States (USEPA 1979) and the Council of the European Communities (1976) [1, 2] with a maximum allowable contaminant level of 5 ng mL^{-1} in drinking water. On the other hand, antimony is used in certain therapeutic agents against major tropical diseases, such as Leishmania and HIV [3, 4]. A study of subacute toxicity in rats revealed that if the antimony value in the body exceeds the allowable limit, it causes a fall in hemoglobin and blood glucose concentrations and an increase in liver enzymes and serum creatinine concentration [5]. In addition, water soluble antimony compounds in high levels cause anorexia, nausea, vomiting, muscle ache, headache, lethargy and bone and joint pain. Recent studies put more focus on the possible role of antimony in the sudden infant death syndrome [6]. Thus, in order to avoid human health hazards, it is worthwhile to make efforts to develop a simple and inexpensive method for monitoring the administration of Sb in the body through its determination in serum or urine and also in environment.

Chromatography techniques and liquid–liquid extraction [7–11], inductively coupled plasma mass spectrometry (ICP-MS) [12], electrothermal atomic absorption spectrometry [13, 14] and electrochemical techniques [15, 16] have been recommended for this purpose. However, the most of these procedures are laborious and time-consuming. Recently, hydride generation coupled with different techniques has often been used for the determination of Sb in various environmental samples but these techniques are quite expensive and involve various long-drawn-out procedures [17–19]. Therefore, these techniques are hardly suitable to be employment in the routine analyses. In this regard, spectrophotometry methods are by far the most popular and attractive because of their simplicity, low-cost and compactness.

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As a matter of fact, one of the disadvantages of the conventional UV/Vis spectrophotometry is that it is not suitable for the measurement of metals that exist at low level in environmental and biological samples. Ironically, using one step preconcentration prior to the UV/Vis spectrophotometry determination makes it an appropriate technique for the determination of metals at ultra-trace levels [20].

In recent years, a methodology of separation and preconcentration based on cloud point extractions (CPE) are growing to be an important and practical application of surfactants in analytical chemistry, particularly in separation of toxic solutes from several matrices [22–24]. The merits of the cloud point extraction are that it is easy to manipulate, reliable to scale up, and simple and effective to operate. CPE is in agreement with the principles of "Green Chemistry", because limited amounts of low toxicity surfactants replace the usual toxic organic solvents. In addition, surfactants are non-flammable and present low volatility, minimizing risks in the extraction process [21].

The purpose of this study is to introduce a new method for the spectrophotometric determination of total antimony after preconcentration in a very simple cloud point extraction process. The method is based on the color reaction of Sb (III) with iodide in acidic medium and in the presence of ascorbic acid as a reducing agent; subsequently the product was extracted in micellar medium. This method was applied to the analysis of several real and spiked samples. To the best of our knowledge, this is the first report on the preconcentration of antimony by CPE method which require no chelating agent, centrifugation and cooling after centrifugation.

Experimental

Apparatus

A Perkin-Elmer UV-visible spectrophotometer (Lambda 25) was used for recording absorption spectra and absorbance measurements using quartz Suprasil microcell (0.7 mL). A Colora thermostat bath was applied to maintain the desired temperature within ±1.0 °C.

Reagents

All reagents were of analytical-reagent grade and used without further purification. A stock standard solution (100 mg L^{-1}) of antimony (III) was prepared by dissolving 0.019 g of antimony trichloride (Merck, Darmstadt, Germany, www.merck.de) in 10 mL of concentrated H_2SO_4 and diluting to 100 mL in a volumetric flask. Working standard solutions of Sb (III) were prepared daily by dilution of the standard stock solution. The non-ionic surfactant solution was prepared by dissolving 2 g of Triton X-114 (Fluka, Sigma-Aldrich Co., Germany, www.sigmaaldrich.com) in deionized water and diluting to 100 mL in a volumetric flask. The fresh solution of 5 mol L^{-1} of KI (Merck, Darmstadt, Germany, www.merck.de) was prepared by the addition of 41.5 g of KI to water and diluting to 50 mL. A 2 mol L^{-1} solution of ascorbic acid (Sigma, Sigma-Aldrich Co., USA, www.sigmaaldrich.com) was prepared by dissolving 8.8 g of this reagent in water and diluting to 25 mL in a volumetric flask. This solution was also prepared daily just before use. A solution of sulfuric acid (10 mol L^{-1}) was prepared by dissolving 27.2 mL of concentrated acid (Merck, d=1.84 gmL^{-1} and 98 %) in water and diluting to 50 mL.

Procedure

An aliquot of the solution containing 0.80–95 ng $mL^{-1}$of Sb (III) ion was transferred in to a 100 mL volumetric flask containing 10 mL of 2 mol L^{-1} ascorbic acid solution, 11 mL of 5 mol L^{-1} potassium iodide solution and 8 mL of sulfuric acid solution. The solution was shaken and allowed to stand still for 3 min at room temperature. Then, 20 mL of 2 % (w/v) Triton X-114 solution was added and made up to the mark with water. Resultant solution was transferred to a 100 mL container with long thin neck and was placed in a thermostat bath at 60 °C for 35 min. Since the density of surfactant rich phase is lower than that of the aqueous one, the organic phase remains in upper layer. By means of a syringe-pipette, the organic phase was transferred to a quartz cell. To reduce viscosity, 0.10 mL of methanol was added to the surfactant-rich phase, and the absorbance of the solution was measured at the wavelength of 330 nm. A blank solution was also run using the entire mentioned components except antimony.

Result and discussion

In this work, Sb^{3+} complexed with I^- in the presence of an excess amount of iodide to initially form SbI_4^- complex which has a maximum absorption at 330 nm. SbI_4^- reacts with H^+ cation to form the yellowish hydrophobic ion-associated complex [H^+, SbI^{4-}] that could be extracted into surfactant-rich phase of Triton X-114. After the cloud point extraction with Triton X-114, the absorption spectra of surfactant rich phase also showed a maximum band at 330 nm (Fig. 1). Hence, all the measurements were carried out at this wavelength. The effects of various parameters on the performance of the method were investigated in order to achieve the highest sensitivity.

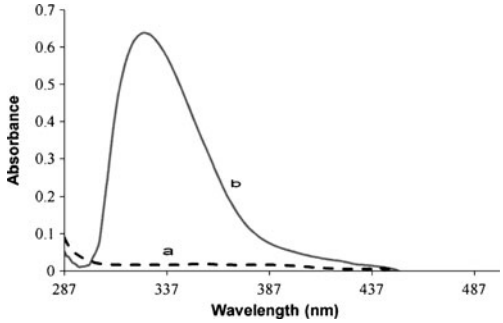

Fig. 1 Absorption spectra of (**a**) blank solution (**b**) antimony after CPE. Conditions: 0.4 % (w/v) Triton X-114, 0.55 mol L^{-1} KI, 0.2 mol L^{-1} ascorbic acid, 0.8 mol L^{-1} H_2SO_4, equilibrium temperature 60 °C and incubation time 35 min

Effect of potassium iodide concentration

The effect of potassium iodide on the extraction of the metal with surfactant was examined. The results revealed that the recorded absorbance intensity enhanced with increasing iodide concentration. The optimum concentration of KI for maximum extraction of the metal was found to be in the range of 0.50–0.60 mol L^{-1}, as shown in the Fig. 2. Thus, a potassium iodide concentration of 0.55 mol L^{-1} in the final solution was chosen as the optimum concentration for subsequent experiments.

Effect of ascorbic acid concentration

The main purpose of using ascorbic acid is to reduce Sb (V) to Sb (III) and also prevent the liberation of iodine by atmospheric oxygen and any other oxidizing species present in the analyte. Due to ease of oxidation of ascorbic acid, it can reduce the produced iodine in solution to iodide and avoid decreasing its concentration; it also removes the color interference of the iodine. Therefore, the effect of concentration of ascorbic acid on the performance of the extraction efficiency of Sb was evaluated. The maximum value in the antimony absorbance was obtained at 0.20 mol L^{-1} concentration of ascorbic acid and the signal remained constant at higher concentration, as it is represented in Fig. 3.

Effect of sulfuric acid concentration

The efficiency of the preconcentration process depends on the acid concentration. Since the formation of both complexes of SbI_4^- and $[H^+, SbI^{4-}]$, ion-associated, depends on the acid concentration, the effect of sulfuric acid concentration on the performance of the extraction efficiency of Sb was investigated in the range of 0.00–1.5 mol L^{-1}. At zero point concentration of H_2SO_4, the absorbance of the surfactant rich phase was zero, then increased by increasing the sulfuric acid concentration up to 0.8 mol L^{-1} and decreased at higher concentrations. A decrease in absorbance at higher concentrations is due to the prevention of cloud formation in the solution. Therefore, 0.80 mol L^{-1} sulfuric acid concentration was selected as optimum.

Effect of Triton X-114 concentration

A successful CPE would be the one which maximizes the extraction efficiency through minimizing the phase volume ratio, and thus maximizing its enrichment factor. Hence, the effect of Triton X-114 concentration on the absorbance of the extracted phase was investigated in the range of 0.10 to 0.70 % (w/v). As demonstrated in Fig. 4, the highest absorbance was obtained with 0.40 % (w/v) Triton X-114. The signal was enhanced by increasing the surfactant concentration to 0.4 % (w/v) while in higher concentration a slight decrease was observed in the recorded signal. The results might be duo to the presence of the high amount of surfactant, leading to an increase in the volume of the surfactant-rich phase. In addition, the viscosity of the surfactant-rich phase increases, causing to poor sensitivity [25, 26].

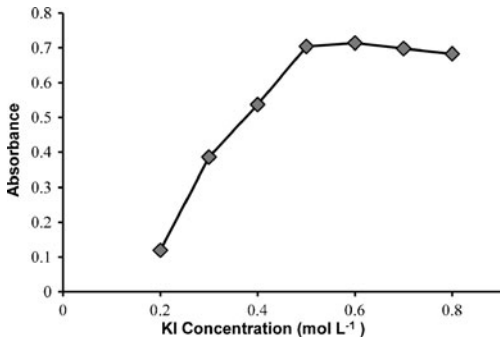

Fig. 2 The effect of KI concentration on the absorption of 70 ng mL^{-1} of antimony. CPE conditions: 0.4 %(w/v) Triton X-114,0.25 mol L^{-1} ascorbic acid, 0.8 mol L^{-1} H_2SO_4, equilibrium temperature 60 °C and incubation time 40 min

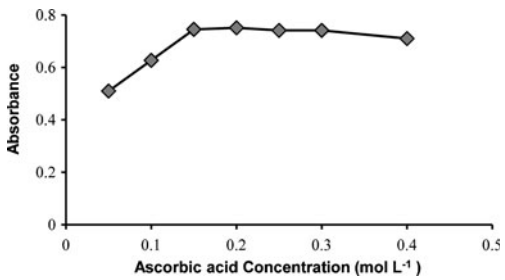

Fig. 3 The effect of ascorbic acid concentration on the absorption of 70 ng mL^{-1} of antimony. Other conditions: 0.4 % (w/v) Triton X-114,0.55 mol L^{-1} KI, 0.8 mol L^{-1} H_2SO_4, equilibrium temperature 60 °C and incubation time 40 min

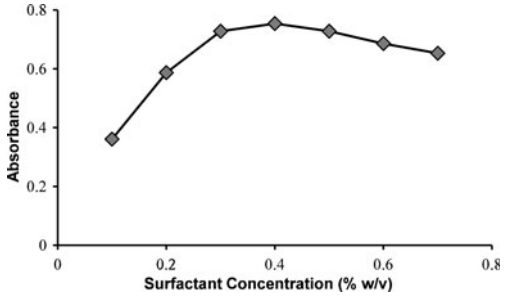

Fig. 4 The influence of Triton X-114 concentration on the absorption of 70 ng mL^{-1} of antimony. Other conditions: 0.55 mol L^{-1} KI, 0.2 mol L^{-1} ascorbic acid, 0.8 mol L^{-1} H_2SO_4, equilibrium temperature 60 °C and incubation time 40 min

Influence of equilibrium time and temperature

Two important and highly effective parameters in cloud point extraction are incubation time and equilibration temperature. As a compromise between completion of extraction and efficient separation of the phases, it is desirable to employ the shortest equilibration time and the lowest possible equilibration temperature. Consequently, the effect of equilibration temperature in the range of 35–70 °C was studied. It was found that temperature of 60 °C is appropriate for the analysis. The dependence of extraction efficiency upon equilibration time was also studied for a time interval of 20–45 min. An equilibration time of 35 min was chosen as the optimum value.

Interference studies

The influence of many cations and anions on the determination of antimony was studied and an error of ±5 % in the absorbance was considered tolerable. Sample solutions containing 70 ng mL^{-1} of antimony and different concentrations of other ions or compounds were prepared. The developed procedure was applied and the results are shown in Table 1. Most of the common cations and anions have no obvious influence on the determination of trace quantities of antimony except bismuth. The interference was due to an increase in absorbance. Although Bi and Hg interfere with Sb, but using a wet chemical method makes this separation possible.

Table 1 The effect of other species on the determination of 70 ng mL^{-1} of antimony

Coexisting ions	Tolerance limits, ng mL^{-1}
Ca^{2+}, Na^{+}, Mg^{2+}, Cu^{2+}, Pb^{2+}, Ag^{+}, Mn^{2+}, Ni^{2+}, Co^{2+}, Al^{3+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Zn^{2+}, $H_2PO_4^{-}$, NO_3^{-}, Cl^{-}, NH_4^{+}, SO_4^{-}, Br^{-}, SCN^{-}, CO_3^{2-}, Acetate, EDTA, Oxalate, Citrate, ClO_4^{-}	1000
Cd^{2+}	400
Hg^{2+}	80
Bi^{2+}	1

Table 2 Determination of total antimony in seawater and human serum samples

Sample	Antimony concentration(ng mL^{-})		
	Added	Found[a]	Recovery (%)
Water	0	1.73±0.14	–
	10	11.56±0.80	98.3
	20	21.84±1.00	100.55
Serum	0	ND[b]	–
	10	10.16±0.73	101.6
	20	19.56±0.90	97.8

[a] $\bar{x} \pm ts/\sqrt{n}$

[b] Not Detected

These cations precipitate as the sulfides (Sb_2S_3, Bi_2S_3 and HgS) in the presence of H_2S (pH=0.5). By adding ammonium sulfide, Bi_2S_3 and HgS remain insoluble while Sb_2S_3 dissolves. |The introduced procedure can be performed after decantation and acidification of the solution [27].

Analytical performance of the method

A linear calibration graph in the range of 0.8–95 ng mL^{-1} of antimony in the initial solution was obtained using the optimized conditions. The equation for the line was $A = 0.012C + 0.026$ with an r value of 0.9994 (n=9) where A is the absorbance and C is the concentration of antimony in ng mL^{-1}. Detection limit (defined as SNR=3) was 0.23 ng mL^{-1} (n=10) and the relative standard deviations (R.S.D.) for 10 and 70 ng mL^{-1} of antimony were 3.32 and 1.85 % (n=8), respectively. Because the amount of antimony in 100 mL of sample solution is measured after preconcentration by CPE in a final volume of 0.50 mL (0.40 mL

Table 3 Comparsion of some reported procedures

Enrichment method	Detection method	Linear range (μg L^{-1})	Detection limit (μg L^{-1})	Reference
LLE[a]	UV–vis spect.	10–1500	5	[10]
LLE	FA-LED	(8.52–87.66)×10^3	3.53×10^3	[7]
SPE[b]	FI-ETAAS	5–60	2	[30]
SPE	GF-AAS	1–4	0.18	[31]
SPE	MP-AES	10–1000	0.38	[33]
CPE[c]	FAAS	Not reported	2.08	[32]
CPE	UV–vis spect.	0.80–95	0.23	This work

[a] liquid-liquid extraction

[b] solid phase extraction

[c] cloud point extraction

surfactant-rich phase +0.10 mL methanol), the solution is concentrated by a factor of 200.

Application to real samples

Determination of antimony in Antileishmanial drug

The validity of the introduced method was determined in Glucantime sample containing Sb (V). The meglumine antimoniate (Glucantime) which is used in Iran for the treatment of leishmaniasis was analyzed. The product is commercialized in 5 mL ampoules and according to the manufacturer; each ampoule contains 1.5 g of meglumine antimoniate, which corresponds to 0.405 g of antimony. A good agreement was obtained between the value found (0.413±0.03 g/ampoule) and that of reported by the producer (n=3, α= 0.05). These results were also checked using ICP-OES as a reference technique obtaining 0.408±0.025 g/ampoule (n= 3, α=0.05) for total antimony concentration.

Determination of antimony in serum and seawater samples

The introduced procedure in this report was successfully applied to the determination of antimony in spiked seawater and human serum samples. The water sample was collected from the Caspian Sea coast (Babolsar) in summer and was filtered immediately on return to the laboratory using 0.45 μm cellulose acetate filters and developed method was successfully applied to the determination of Sb concentration. In order to validate the analytical method, recovery experiments were also carried out by spiking the samples with different amounts of Sb (III) before treatment, and the obtained results were listed in Table 2. The recoveries for the spiked samples were in the acceptable range (97.8–101.6 %) what indicates the capability of the system in the determination of antimony in real samples.

Conclusion

This paper reports a new method for the preconcentration and determination of ultra-trace amounts of antimony using CPE combined with UV/Vis spectrophotometry. The method can be used for the determination of Sb in the range of 0.8–95 ng mL^{-1} with a detection limit of 0.23 ng ml^{-1} and a preconcentration factor of 200. The significant advantages of the presented procedure is that it does not need any chelating agent, centrifugation and cooling after centrifugation. The results of this study clearly show the potential and versatility of this method, which could be applied to antimony monitoring in various samples. It was demonstrated that the suggested procedure is comparable to or even better than some preconcentration procedures that are available for monitoring of antimony. Moreover, the method offers low limit of detection in comparison to the other methods which used conventional UV–Vis spectrophotometer as the detection technique [18, 28, 29]. A comparison of the main features of the present procedure with others reported in the literature is given in Table 3.

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