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Atomic Absorption Spectrometric Determination of Trace Amounts of Copper in Natural Waters and Standard Biological Samples after Separation and Preconcentration onto Amberlite XAD-2 Loaded with 2-(5-Bromo-2-Pyridylazo)-5-Diethylaminophenol

MOHAMMAD ALI TAHER*, ALI MOSTAFAVI and TAHEREH ROHANI
Department of Chemistry
Shahid Bahonar University of Kerman, Kerman, Iran
E-mail: taher@arg3.uk.ac.ir; /ma_taher@yahoo.com

A procedure for separation and preconcentration of trace amounts of copper has been proposed. It is based on the adsorption of copper(II) ions solution onto a column of amberlite XAD-2 resin loaded with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP). Copper is quantitatively retained on the column in the pH range 6.5–9.5 at a flow rate of 2 mL/min. The copper complex dissolved out from the column with 5 mL of dimethylformamide (DMF) and copper was measured by atomic absorption spectrometry at 324.8 nm. In this case, 0.15 μg of copper can be concentrated in the column from 600 mL of aqueous sample, where its concentration is as low as 0.25 ppb. Eight replicate determinations of 2.0 ppm of copper gave a mean absorbance of 0.210 in final DMF solution with a relative standard deviation of 1.7%. The sensitivity for 1% absorption was 42 ppb. The interference of a large number of anions and cations has been studied and the optimized conditions developed were utilized for the trace determination of copper in natural waters and various standard samples.

Key Words: Atomic absorption spectrometry, Amberlite XAD-2, Separation and preconcentration of copper, 2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol, Standard biological samples.

INTRODUCTION

Copper is an essential element not only for life in mammals but also for plants and lower forms of organisms. It has varied and many biological effects as an essential element as well as a toxic one depending on the concentration. It is usually used as algicide and herbicide[1, 2]. In natural water and biological samples its level is low, and previous steps of separation and enrichment are usually required. This way many preconcentration procedures for copper determination[3–14] have been developed and they involve different analytical techniques and several materials. Liquid-liquid extraction by using dithiocarbamate[3] and trioctylmethylammonium chloride[4] as complexing reagents, coprecipitation with magnesium hydroxide as collector[5], precipitation as rubeanic acid complex and filtration by using membrane filter[6], and also several systems of adsorption that use sorbents such as polyurethane foam loaded with diethyldithiocarbamate[7], activated carbon[8, 9], amberlite XAD resins[10, 11], naphthalene[12, 13] and silica gel loaded with diethyldithiocarbamate[14]. Solid phase extraction process[6–14] has received more acceptance due to a number of possible advantages including availability and easy recovery of the solid phase, reach of high preconcentration factors and easiness of separation and enrichment using continuous flow systems. This paper proposes an analytical procedure for determination of trace copper by FAAS, using a column packed with amberlite XAD-2 resin loaded with 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) for preconcentration and separation. Amberlite XAD-2 (polystyrene-divinylbenzene polymer) is a frequently used resin in preconcentration procedures, due to its good physical and chemical properties such as porosity, high surface area, durability and purity[10]. The metal complex can easily be dissolved with a suitable organic solvent such as dimethylformamide (DMF) and the copper is then determined by atomic absorption spectrometry. The interference of a number of metal ions and anions on the estimation of copper has been studied in detail. The developed method is found to be highly sensitive and selective and has been employed for the estimation of copper in complex materials.

EXPERIMENTAL

A Shimadzu AA 670 flame atomic absorption spectrophotometer was used in the following conditions: wavelength: 324.8 nm, lamp current: 3.0 mA, slit width: 0.5 mm, burner height: 6.0 mm, acetylene flow: 1.8 L min^{-1}, air flow: 8.0 L min^{-1}. A Beckman pH-meter was employed for pH measurements. A funnel-tipped glass tube (60 × 6 mm) was used as a column for preconcentration. All glassware and column were washed with mixture of concentrated sulfuric acid and concentrated nitric acid (1 : 1) before use. All reagents were of analytical reagent grade. A standard copper solution was prepared from copper(II) nitrate in distilled water and standardized by known methods[15]. A 0.02% solution of 5-Br-PADAP in ethanol was prepared. Buffer solutions of pH 3–6, 6–8 and 8–11 were prepared by mixing appropriate ratios of a 0.5 M acetic acid and 0.5 M sodium acetate solution, 0.1 M sodium dihydrogen phosphate solution and 0.1 M disodium hydrogen phosphate solution and 0.5 M ammonia solution and 0.5 M ammonium acetate solution, respectively. Solutions of alkali metal salts (1%) and various metal salts (0.1%) were used for studying the interference of anions and cations, respectively.

Preparation of Amberlite XAD-2 column loaded with 5-Br-PADAP

Amberlite XAD-2 was treated with an ethanol : hydrochloric acid : water (2 : 1 : 1) solution overnight. Then resin was rinsed with deionized water until supernatant water pH got neutral. The packing of the column must be done using ethanol as eluent since water makes resin beads float. The resin was saturated with the reagent by passing 3.0 mL of a 0.02% 5-Br-PADAP solution in ethanol at a flow rate of 1 mL min^{-1}. Afterwards it was washed with water until reagent excess was eliminated from the resin. All experiments were done in a funnel-tipped glass tube (60 × 6 mm) which was used as a column for preconcentration. It was plugged with polypropylene fibre and then filled with the XAD-2 to a height of 1.0–1.2 cm (0.2 g). Before sample loading the column must be preconditioned by passing a buffer solution. Then the column could be used repeatedly for ten times at least.

Procedure for the sorption of copper on the column

25 to 600 mL of sample solution of copper ions in the range of 0.35 to 50 μg was transferred into a beaker and 2 mL of buffer solution (pH 7.0 was added to it). This solution must be passed through the column at a flow rate of 2.0 mL min^{-1}. After this solution had been passed the column was washed with 10 mL of deionized water. The adsorbed copper chelate on the column was then eluted with 5.0 mL dimethylformamide (DMF) at a flow rate of 1.0 mL min^{-1}. The eluent was collected in a 5 mL volumetric flask and copper was determined by flame atomic absorption spectrometry.

RESULTS AND DISCUSSION

In order to determine the optimum conditions for quantitative extraction of copper by using amberlite XAD-2 resin loaded with 5-Br-PADAP several parameters were assessed.

Reaction conditions

These were established with the use of 3.5 μg of copper. The sorption of copper on the column was found to be a maximum in the pH range 6.5– 9.5. In subsequent studies, the pH was maintained at approximately 7. Addition of 0.5–10 mL of the buffer did not affect the retention of copper and the use of 2 mL is recommended.

The flow rate was varied fiom 0.2 to 5 mL/min. It was found that a flow rate of 0.2–4.0 mL min^{-1} did not affect adsorption. A flow rate of 2 mL/min was recommended in all experiments.

The volume of the aqueous phase was varied in the range of 10–900 mL under the optimum conditions, keeping the other variables constant. It was observed that the signal height was almost constant up to 600 mL (preconcentration factor of 120). However, for convenience, all the experiments were carried out with 40 mL of the aqueous phase.

Sorption capacity

The sorption capacity of the amberlite XAD-2 resin loaded with 5-Br-PADAP for copper was also evaluated. The resin had a sorption capacity of 1.5 mg of copper per gram of XAD-2 resin.

Choice of solvent

A number of solvents were tried, to dissolve the metal complex from the XAD-2 resin. Since the metal complex is dissolved in a small volume (3–5 mL) of solvent, it is essential to select a solvent in which the chelate is highly soluble and one which is also sensitive for atomic absorption spectrometric measurements. Dimethyl formamide (DMF) was preferred because of the high solubility and stability of the metal complex. It was found that 2–5 mL of this solvent was sufficient to dissolve the entire mixture, thus further enhancing the sensitivity of the method.

Calibration and sensitivity

Since it is possible to retain 0.15 μg of copper from 600 mL of solution passing through the column, the dissolution with 5.0 mL DMF gives a detection limit of 0.25 ppb for copper at the minimum instrumental settings. The linearity was maintained in the concentration range of 0.58 ppb to 5 ppm copper in aqueous solution or 0.07 to 10 ppm copper in final DMF solution with a correlation factor of 0.9997. Eight replicate determinations of 10 μg of copper in 5 ml DMF solution gave a mean absorbance of 0.210 with a relative standard deviation of 1.7%. The sensitivity for 1% absorption was 42 ppb.

Effect of Diverse Ions

Various salts and metal ions were added individually to a solution containing 10 μg of copper and the general procedure was applied. The tolerance limit was set as the concentration of the diverse ions required to cause ±3% error in the determination of copper. The results obtained are given in Table-1. Among the salts examined, most could be tolerated up to gram or milligram levels except EDTA. Citrate or tartrate enhanced the tolerance limit of coexisting metal ions. Thus the proposed method is relatively selective and can be used for the determination of copper in alloys and biological samples without any prior separation.

TABLE-1
EFFECT OF DIVERSE SALTS AND METAL IONS

Salt or ion	Tolerance limit (mg)
$CH_3COONa{\cdot}3H_2O$, KNO_3	1000
K_2SO_4	185
KI	85
NH_4Cl, $Na_3PO_4{\cdot}12H_2O$, K_2CO_3	45
NaF	100
Sodium potassium tartrate	18
Thiourea, KSCN, $NaClO_4$	14
Sodium oxalate, $Na_2S_2O_3$	32
Trisodium citrate	10
Na_2 EDTA	80
Mg(II)	140
Ca(II)	85
Cd(II), Sb(II), Pb(II)	36
Ti(IV), Fe(III)	23
Zn(II), Ag(I), Al(III)	13
Cr(VI), Cr(III)	9
Se(VI), Ga(III)	8
Mo(VI), U(VI), Mn(II)	7.5
Pd(II), Te(IV)	6
Os(VIII), Be(III)	6
Rh(III), V(V)	5.5
Ru(III), Hg(II), Co(II)	4

Analysis of Copper in Standard Alloys and Steel Samples

The proposed method was applied to the determination of copper in Nippo Keikinzoku Kogyo (NNK) CRM 916, No. 920 aluminum alloy, NKK No. 1021, Al, Si, Cu, Zn alloy, Japanese standards of iron and steel (JSS) CRM 651–7 and 653–7 stainless steel. A 0.1 g sample of the standard aluminum alloy or steel was completely dissolved in 6–14 mL of hydrochloric acid (1 + 1) by heating on a water-bath and then 1 mL of 30% (v/v) hydrogen peroxide was added to it. The excess of peroxide was decomposed by heating the sample on a water-bath. The solution was cooled, filtered if needed and diluted to 100 mL with distilled water in a standard flask. An aliquot (1–2 mL) of this sample was taken in a 20 mL beaker and the general procedure was applied. The results obtained are given in Table-2. These results are in agreement with the certified values.

TABLE-2
ANALYSIS OF COPPER IN STANDARD ALLOYS

Sample	Composition (%)	Found*
JSS 651-7 Stainless steel	C, 0.047; Si, 0.072; N, 0.0312; P, 0.028; Cr, 18.60; Al, 0.002; S, 0.0063; Mo, 0.84; Co, 0.22; Mn, 1.72; Ni, 9.20; Cu, 0.082	0.080 ± 0.004
JSS 653-7 Stainless steel	C, 0.068; Si, 0.63; N, 0.0276; Cr, 22.53; Co, 0.35; Mn, 1.72; Ni, 13.91; Cu, 0.030	0.032 ± 0.002
NKK No. 916 Aluminum alloy	Si, 0.41; C, 0.41; Fe, 0.54; Mg, 0.10; Cr, 0.05; Sn, 0.05; Zn, 0.30; Ti, 0.10; Pb, 0.04; Sb, 0.01; B, 0.0006; Zr, 0.05; Co, 0.03; Mn, 0.11; Bi, 0.03; Ni, 0.06; V, 0.02; Cu, 0.27	0.26 ± 0.03
NKK No. 1021 Al, Si, Cu, Zn alloy	Pb, 0.18; Zn, 1.76; Si, 5,56; Sn, 0.10; Mg, 0.29; Ti, 0.04; Cr, 0.03; Fe, 0.99; Bi, 0.01; Zr, 0.01; Sb, 0.01; Ca, 0.004; Mn, 0.20; Ni, 0.14; V, 0.007; Cu, 2.72	2.69 ± 0.06
NKK No. 920 Aluminum alloy	Cr, 0.27; Si, 0.78; Bi, 0.06; Ti, 0.15, Sn, 0.20; Ga, 0.05; Pb, 0.10; Fe, 0.72; Zn, 0.80; Ca, 0.03; Mg, 0.46; Sb, 0.10; Co, 0.10; Mn, 0.20; Ni, 0.29; V, 0.15; Cu, 0.71	0.74 ± 0.03

*Average of five determinations, ± standard deviation.

Analysis of Copper in Biological Samples

The accuracy and applicability of the proposed method has been applied to the determination of copper in National Institute for Environmental Studies (NIES) No. 1 pepperbush; NIES, No. 3 chlorella; NIES, No. 5 human hair and

NIES, No.7 tea leaves. A 0.1 g sample was taken in a beaker and dissolved in concentrated nitric acid (*ca.* 5 mL) with heating. The solution was cooled, diluted and filtered. The filtrate was made to 100 mL with water in a calibrated flask. An aliquot (10–50 mL) of the sample solution was taken individually and copper was determined by the general procedure. The results are given in Table-3 which are in good agreement with the certified values.

TABLE-3
ANALYSIS OF COPPER IN BIOLOGICAL SAMPLES

Sample	Composition	Found*†
NIES, No. 1 Pepperbush	K, 1.51 ± 0.06; Mn, 0.203 ± 0.107; Mg, 0.408 ± 0.020; Ca, 1.38 ± 0.07 Cd, 6.7 ± 0.5; Ni, 8.7 ± 0.6; Fe, 205 ± 17; Co, 23 ± 3; Pb, 5.5 ± 0.8; Cu, 12 ± 1; Zn,340 ± 20; Ba,165 ± 10; Na, 106 ± 13; Rb, 75 ± 4; Sr, 36 ± 4; As, 2.3 ± 0.3; P, (1100); Cr (1.3); Cs, (1.2); Tl, (0.13); Hg, (0.056) μg/g	11 ± 1
NIES, No. 3 Chlorella	K, 1.24 ± 0.06; Mg, 0.33 ± 0.02; Ca, 0.46 ± 0.03; Fe, 0.185 ± 0.010; P (1.7)%; Zn, 20.5 ± 1.0; Sr, 40 ± 3; Co, 0.87 ± 0.05; Cd, (0.026); Mn, 69 ± 5; Pb, (0.60); Cu, 3.5 ± 0.3; Sc, (0.013) μg/g	3.4 ± 0.2
NIES No. 5 Human hair	Pb, 6.0; Cd, 0.20; K, 34; Rb, 0.19; Sb, 0.07; Zn, 169; Al, 240; Fe, 225; Mg, 208; Hg, 4.4; Sc, 0.05; Se, 1.4; Na, 26; Sr, 2.3; Ti, 3.2; Ca, 728; Cr, 1.4; Ba, 2.2; Co, 0.10; Mn, 5.2; Cu, 16.3; Ni, 1.8 μg/g	16.24 ± 0.09
NIES, No. 7 Tea leaves	Pb, 0.80; Cd, 0.030; Sb, 0.014; Zn, 33; Cr, 0.15; Al, 775; Mg, 1530; Ba, 5.7; K, 18600; Sc, 0.011; Na, 15.5; Sr, 3.7; Ca, 3200; Cs, 0.22; Co, 0.12; Mn, 7.00; Ni, 6.5; Cu, 7.0 μg/g	7.1 ± 0.1

*Average of five determinations, ± standard deviation

†Standard addition method was applied.

NIES: National Institute of Environmental Studies reference materials.

NIES, No. 1 & No. 3 (Values in parentheses were approximate and not certified).

Analysis of copper in natural waters and hair

The method has been employed for the determination of copper in hot spring, coastal, river, ground and lake water samples at levels as low as ng/mL. A 500 mL water sample was adjusted to pH 1.5 with nitric acid, filtered to remove suspended material and then analyzed by the general procedure. The results given in Table-4 are comparable to those of the standard DDTC-MIBK method[16].

The method was also applied to the determination of copper in human hair; a 10 g sample was decomposed by heating with 30 mL of concentrated nitric acid and 3 mL of 60% perchloric acid in a Kjeldahl flask. The solution was cooled, filtered and diluted to 200 mL with water in a calibrated flask. An aliquot of this solution was taken through the general procedure. The results obtained for these samples are also shown in Table-4.

TABLE-4

ANALYSIS OF COPPER IN NATURAL WATERS AND HAIR

Sample	Copper found[a] (ng/mL)	
	Present method	Alternative method[b]
Coastal water	1.1 ± 0.2	1.2 ± 0.2
River water, A	1.5 ± 0.3	1.4 ± 0.3
River water, B	1.8 ± 0.2	1.9 ± 0.3
River water, C	5.1 ± 0.4	5.4 ± 0.5
River water, D	1.3 ± 0.2	1.2 ± 0.2
Hot spring water	12 ± 0.6	13 ± 0.8
Lake water	1.0 ± 0.5	11 ± 0.6
Human hair (male)[c]	14.3 ± 0.7	14.5 ± 0.9
Human hair (female)[c]	25.7 ± 0.8	25.4 ± 1.0

[a] Mean of five determinations, ± standard deviation

[b] DDTC-MIBK method after vacuum evaporator concentration.

[c] Results are given in $\mu g\ g^{-1}$.

Conclusion

The main advantages of this procedure are: (i) the preparation of the extractor system is simple and fast; (ii) during copper desorption the 5-Br-PADAP reagent remains in the resin, which allows using the column several times; (iii) a good enrichment factor (120x) can be achieved. The achieved recovery measured by standard addition technique showed that the proposed procedure had good accuracy. The proposed procedure has been applied for copper determination in various samples. This reagent is fairly sensitive and selective for copper but, with the preconcentration step and the use of atomic absorption spectrometry, its sensitivity and selectivity have been further improved. It is not possible to develop selective methods for metal ions using this adsorbent in spectrophotometry, since many metal-5-Br-PADAP complexes absorb at close wavelengths. However, with the use of AAS this problem can be easily solved. The proposed procedure has been applied for copper determination in various samples.

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