ULTRA-TRACE MONITORING OF COPPER IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES BY INDUCTIVELY COUPLED PLASMA ATOMIC EMISSION SPECTROMETRY AFTER SEPARATION AND PRECONCENTRATION BY USING OCTADECYL SILICA MEMBRANE DISKS MODIFIED BY A NEW SCHIFF'S BASE

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Ultra-trace amounts of Cu(II) were separated and preconcentrated by solid phase extraction on octadecyl-bonded silica membrane disks modified with a new Schiff's base (Bis- (2-Hydroxyacetophenone) -2,2-dimethyl-1,3-propanediimine) (SBTD) followed by elution and inductively coupled plasma atomic emission spectrometric detection. The method was applied as a separation and detection method for copper(II) in environmental and biological samples. Extraction efficiency and the influence of sample matrix, flow rate, pH, and type and minimum amount of stripping acid were investigated. The concentration factor and detection limit of the proposed method are 500 and 12.5 pg mL^{-1}, respectively.

Keywords: copper; solid phase extraction; Shiff's base.

INTRODUCTION

The role of trace transition and heavy metal ions in animal and plant biological systems is very important, but very complex. A lake of some of these microelements in an organism can cause many diseases and illnesses, however, their sufficient quantities can also induce many harmful consequences. The most frequently present copper ion is introduced into human and animal bodies by food, water, air, etc. On the other hand, rain, snow, fertilizer and water of irrigation are the most common routes for copper introduced into plants[1].

Human can not exist without food and drinking water. Therfore, the development of accurate and rapid determination method for monitoring the level of copper in water and food is necessary and indispensable.

The most common methods for determination of copper ion are flame and graphite furnace atomic absorption spectrometry[2], spectrophotometry[3], constant current stripping analysis[4], differential pulse anoding stripping voltametry[5], potentiometry[6] and inductively coupled plasma atomic emission spectrometry[7]. But, their sensitivity is usually insufficient for very low concentrations in environmental and biological samples. Thus, a preconcentration and matrix elemination step is usually required. The most widely used techniques for the separation and preconcentration of trace amounts of copper ions are liquid-liquid extraction (LLE)[8,9], precipitation and cation exchange resins[10], absorption of their complexes on the micro-crystalline benzophenone[11]and solid phase extraction (SPE)[12].

Solid phase extraction (SPE) is an alternative technique that reduces solvent usage and exposure, disposal cost, and extraction time for sample preparation[13]. Recently, hydrophobic SPE disks have been used extensively for the determination of organic environmental polluents[14,15]. These disks, made of 90% ($^w/_w$) octadecyl silane in an inert polytetrafluoroethylene matrix, have atypical diameter of 47 mm and a thickness of 0.5 mm. Typical capacity of a disk for well–retained compounds ranges from 10 to 20 mg. The proclaimed major

advantages of the SPE disks include (1) shorter sample processing time due to the large cross–sectional area of the disk and decreased pressure drop, which allows the sample processing at higher flow rates, (2) decreased plugging by particles due to large cross–sectional area of the disk and (3) reduced channeling resulting from the use of smaller diameter sorbent and a greater mechanical stability of the sorbent bed[16].

We have recently reported solid phase extraction and atomic absorption determination of some cations including Cu^{2+}, Pb^{2+}, Ag$^+$ and Ce^{3+}[17-22]. In this paper we reported the solid phase extraction and determination of ppt amounts of copper in environmental and biological samples using octadecyl silica membrane disks modified with a new Schiff base (SBTD, Figure 1) and inductively coupld plasma atomic emission spectrometry.
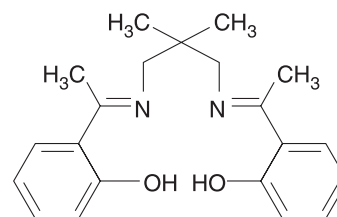

Figure 1. *Structure of SBTD*

EXPERIMENTAL PART

Reagents and apparatus

Hydrochloric acid, hydrobromic acid, acetic acid and nitric acid, high purity methanol, 2-hydroxyacetophenone and 2,2-dimethyl-1,3-propanediamine and ethanol (all from Merck) were used as received. Analytical grade nitrate salts of copper, sodium, potassium, magnesium, calcium, strontium, cobalt, nickel, zinc, chromium, silver, barium, lead and cadmium (all from Merck) were of the highest purity available and were dried in a vacuum over P$_2$O$_5$.

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Digestion of tea and pepper samples

The procedure used for the extraction of these ions from tea sample was similar to that reported in the literature[28]. 10 mg of the dry tea sample (dried at 110 °C) was placed in a 50-mL beaker, followed by the addition of 7 mL of concentrated nitric acid, and the beaker was covered with a glass watch. The beaker was allowed to stand over night, and the contents were heated on a hot plate (150 °C for 15 min). Then the sample was cooled, 8 mL of perchloric acid was added, and the mixture was heated again at 200 °C until the solution became clear (about 1 h). The glass watch was removed and the acid evaporated to dryness at 150 °C. The residue was completely dissolved in 5 mL of 1 mol L^{-1} nitric acid and the solution was transferred to a 100 mL calibrated flask. Then, the solution was neutralized with a proper NaOH solution and diluted to the mark, and the recommended procedure was followed.

Digestion of standard biological samples

10 mg sample was taken in a beaker and dissolved in 5 mL of concentrated nitric acid with heating. The solution was cooled, diluted and filtered. The filtrate was neutralized with NaOH diluted to 100 mL with water in a calibrated flask. An aliquot of the sample solution was taken individually and Cu^{2+}ions were determined by the general procedure.

Synthesis of SBTD

2-hydroxyacetophenone (0.02 mL, 2.72 g) was mixed with 150 mL of distilled ethanol in a 500 mL round bottom flask, which was stirred using a magnetic stirrer. 2,2-dimethyl-1,3-propanediamine (0.015 mol, or 1.02 g) dissolved in 25 mL of distilled ethanol was added drop by drop, using a dropping funnel to the above solution. The contents were refluxed for 2 h to get a yellow solid precipitate of Schiff's base ligand, which was cooled, filtered, washed with Petroleum ether and dried. The ligand was then re-crystallized from ethanol.

Analytical calculation of SBTD with formula $C_{12}H_{26}N_2O_2$ is shown C, 74.52; H, 7.74; N, 8.28%. Exprimental analysis of SBTD was shown C, 73.94; H, 7.68; N, 8.41%.

The copper, sodium, potassium, magnesium, calcium, strontium, cobalt, nickel, zinc, chromium and silver determination were carried out on a Varian Liberty 150 AX Turbo.

Extraction were performed with 47 × 0.5 mm (diameter × thickness) Empore membrane disks containing octadecyl-bonded silica (8-µm particles 60-Å pore size, 3M Co.,Paul, MN). The disk was used in conjunction with standard Millipore 47-mm filtration apparatus.

Preparation of the SPE membrane disks

To remove potential interferences and to ensure optimal extraction of the analyte of interest, the disk cleaning and conditioning should be done before its use. Thus, after placing the membrane disk in the filtration apparatus, 10 mL of methanol was poured onto the disk immediately drawn through the disk by applying a slight vacuum. Afterall of the solvent has passed through the disk, it was dried by passing air through it for few minutes. The disk conditioning was then begun by pouring 10 mL of methanol onto the disk and a low vacuum was applied and the solvent was drawn through the disk until the solvent surface almost reached the surface of the disk. The disk should not be allowed to soak without vacuum, and air should not be allowed to wake contact with surface of the disk. It is preferably

extra methanol above the disk rather than to allow any air to make contact with the surface of the disk. Immediately, 10 mL of water was introduced onto the disk and was drawn through the disk. The disk was then dried under vacuum for 5 min, this is especially important for the disks that are used for the first time. Finally, a solution of 5 mg of SBTD dissolved in 4 mL of methanol was introduced onto the disk so that the solution was spread on the whole disk surface. The solution was allowed to penetrate inside the membrane completely without applying any vacuum. After about 1min, the filtration funnel containing the modified disk was transferred into an oven and the solvent was completely evaporated at 60 ºC. Then the sample solution containing Cu^{2+} (with pH about 5) was passed through the membrane. After the extraction, the disk was stripped from the membrane disk using minimum 5 mL of a 3 mol L^{-1} nitric acid solution. Then the copper concentration was determined by inductively coupld plasma atomic emission spectrometry (off-line analysis).

RESULTS AND DISCUSSION

Our recently reserches showed that, the Schiff's base compounds can form a fairly stable and selective complex with transition and heavy metal ions, specially copper[23]. To the best of our knowledge, there is no report on the stability of SBTD complexes with metal ions. Thus, in preliminary experiments, the complexation of SBTD with a number of alkali, alkaline earth, transition and heavy metal ions was studied conductometrically in acetonitrile solution at 25±0.05 °C[24,25], in order to obtain information about the stability and selectivity of the resulting complexes. The complex formation constant, K_f, of the resulting 1:1 complexes were evaluated by computer fitting of the molar conductance-mole ratio data to appropriate equations[24,25], and the results are summarized in Table 1. As can be seen, the SBTD could act as a suitable complexing agent for pre-concentration and separation of copper ions via solid extraction, by using octadecyl bonded silica membrane disks.

Table 1. Stability constants (K_f) of SBTD-cation complexes in acetonitrile solution

Cations	Log K_f
Na$^+$	2.72±0.03
K$^+$	2.20±0.04
Ca^{2+}	2.95±0.05
Ba^{2+}	2.79±0.06
Cu^{2+}	5.86±0.07
Co^{2+}	3.95±0.03
Zn^{2+}	2.98±0.05
Ni^{2+}	4.01±0.02
Pb^{2+}	3.75±0.04
Cd^{2+}	3.72±0.02

Effect of eluent on the stripping of copper

Some preliminary experiments were carried out in order to choose a proper eluent for the retained Cu^{2+} ions after extraction of 2 µg copper in 100 mL solution by the modified disks. The copper was stripped with varying volumes (5-20 mL) of different acids (HNO$_3$, CH$_3$COOH, HCl and HBr). Results showed that among four different acid solutions used, 5 mL of 3.0 mol L^{-1} nitric acid can accomplish the quantitative elution of copper from the membrane disk, while other acids (5 mL) used are ineffective for the complete elution of these ions. Nitric acid can penetrate into the modified disk and react

with the SBTD (by protonation of nitrogen atoms and reducing its complex stability) and formation of free copper ion that can easily pass through the membrane.

Influence of flow rate on the separation of copper

The influence of flow rates of the sample (2 µg of copper in 100 mL water) and stripping solution from the modified membrane disk on the retention and recovery of Cu^{2+} ions was investigated. The results showed that in the range of 1-15 mL min^{-1}, the retention of copper by the membrane disk is not affected by the sample solution flow rate. At higher flow rate than 15 mL min^{-1}, the retention and recovery of the copper reduced considerably.

Optimization of amount of BSTD for modification of disk

In order to investigate the optimum amount of SBTD on the quantitative extraction by membrane disk, copper ion extraction was conducted by varying the amount of ligand from 0 to10 mg (Table 2). As seen, the extraction of the copper ions is quantitative using above 5 mg of SBTD. Hence, subsequent extraction experiments were carried out with 5 mg of SBTD.

Table 2. Effect of amount of SBTD on Cu^{2+} extraction

Amount of SBTD (mg)	%Recovery [a] (Cu^{2+} ions)
0	0.8 (1.1)[b]
2	55.3 (1.4)
4	91.2 (1.0)
5	100.1 (1.0)
7	100.2 (1.1)
10	99.8 (1.2)

[a] Initial samples contained 1 µg of Cu^{2+} ions in 100 mL water; [b] values in parentheses are RSDs based on three replicate analysis

Effect of pH of sample solution on the extraction of copper

The pH influence on the recovery of 2 µg Cu^{2+} from 100 mL solutions was studied in range 2.0 to 8.0. The pH was adjusted by using 0.1 mol L^{-1} of either nitric acid or sodium hydroxide solutions and the results are shown in Figure 2. The results obtained indicated that the modified membrane disk through the pH range of 4.0-8.0 could retain the Cu^{2+} ions quantitatively. Higher pH values (>8.0) were not tested because due to the possibility of octadecyl silica hydrolysis of on the disk. At lower pH than 4.0, the extraction efficiency was reduced. This is most probably due to the protonation of nitrogen donor atoms of SBTD and reduction of its complex formation ability.
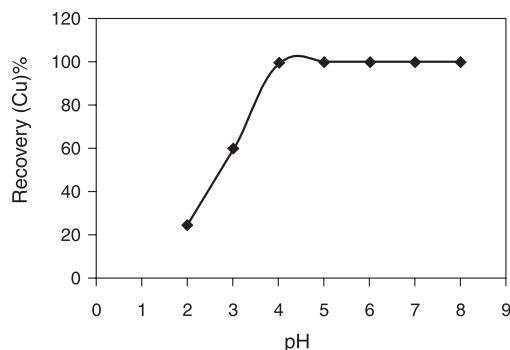
Figure 2. *Effect of pH on the extraction of Cu^{2+}*

Determination of break-through volume

The break-through volume of sample solution by dissolving 2 µg of copper in 25, 50, 100, 250, 500, 750, 1000, 1250, 1500, 1750, 2000, 2250, 2500, 3000 and 3500 mL of water, was tested and the recommended procedure was followed and the results showed that the extraction by modified membrane disk was found to be quantitative, up to 2500 mL. Thus, the break-through volume for the method is more than 2500 mL.

Detection limit of the proposed method

The limit of detection (LOD) of the proposed method for the determination of copper was studied under the optimal experimental conditions. The LOD obtained from $C_{LOD}=K_b S_b m^{-1}$ [26,27] for a numerical factor K_b=3 and concentration factor 500, is 12.5 pg mL^{-1}.

Retention capacity of the modified disk

The maximum capacity of the membrane disk modified by 5 mg of SBTD was determined by passing 100 mL portions of an aqueous solution containing 2000 µg copper through the disk, followed by determination of the retained metal ions using ICP-AES. The maximum capacity was found 202±2 µg of copper.

Separation of Cu^{2+} ions from synthetic samples

In order to investigate the selective separation and determination of Cu^{2+} ion from their binary mixtures with diverse metal ions, an aliquot of aqueous solution (100 mL) containing 2 µg Cu^{2+} ions and various amounts of Na^+, K^+, Cr^{3+} (100 mg), Mg^{2+}, Ca^{2+}, Sr^{2+} and Zn^{2+} (50 mg) and Co^{2+}, Ni^{2+} and Ag^+ (30mg) was taken and the recommended procedure was followed and the results show that the copper ions in the binary mixtures are retained almost completely by the modified membrane disk . Meanwhile, retention of other cations by the disk is relatively low.

In order to assess the applicability of the method to real samples, with different matrices containing varying amounts of a variety of ions, it was applied to the determination of copper in spring water and waste water samples, and the results are summarized in Table 3, respectively. As seen, the results of three analyses of each sample show that, in all cases, the recovery of the copper ions is almost quantitative.

Table 3. Determination of copper in spring water and wastewater samples

Sample	Cu µg mL^{-1} (SPE-ICP-AES)	Cu µg mL^{-1} (ICP-AES)
Spring water 1	1.5 (0.3)[b]	1.4 (1.5) [a]
Spring water 2	1.2 (1.1)	1.3 (1.0) [a]
Spring water 3	1.4 (1.3)	1.4 (1.0) [a]
Wastewater 1	10.1 (0.7)	10.2 (0.2)
Wastewater 2	10.5 (0.5)	10.7 (0.2)

[a]Measurements were done after ten-fold concentration by evaporation; [b]values in parentheses are RSDs based on three replicate analysis

Determination of copper ions in black tea, hot and black pepper samples

The proposed method was also applied to determination of copper ions in two black tea, hot pepper and black pepper samples and the

results are given in Table 4. As can be seen, the amount of copper in black tea, hot and black pepper samples can be accurately determined with the proposed method.

Table 4. Determination of copper in black tea samples

Sample	(SPE with ICP-AES)	(ICP-AES)[a]
Iranian black tea (Lahijan)	45.50 (1.3)[b]	46.30 (1.2)
Indian black tea (Seylon)	37.92 (1.3)	38.82 (1.2)
Hot pepper	30.4 (1.3)	30.2 (1.6)
Black pepper	29.6 (1.6)	29.7 (1.4)

[a]Measurements were done after four times concentration by evaporation; [b]values in parentheses are RSDs based on three replicate analysis

Analysis of copper ions in standard 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 Peppcrbush; No. 3 Chlorella; No. 5 Human Hair and No. 7 Tea Leaves and the results are given in Table 5, which are in good agreement with the certified values.

Table 5. Analysis of copper in biological samples

Sample	Composition ($\mu g\ g^{-1}$)	Found ($\mu g\ g^{-1}$)
NIES[a], 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)*	Cu, 11.8 ± 1.1[b]
NIES, No.3[c] Chlorella	K, 1.24 ± 0.02; Mg, 0.33 ± 0.02; Ca, 0.46 ± 0.03; Fe, 0.185 ± 0.010 P (1.7)%; Zn,205 ± 10; Sr, 40 ± 3; Co, 0.87 ± 0.05; Cd, (0.026); Mn, 69 ± 5; Cu, 34.*5* ± 0.3 Sc, (0.013)	Cu, 34.5 ± 0.5
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	Cu, 16.2 ± 0.5
N1ES, 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	Cu, 6.9 ± 0.4

[a] NIES: National Institute of Environmental Studies reference materials; [b]average of five determinations ± standard deviation and [c] 3 (Values in parentheses were approximate and not certified).

Comparison of concentration factor of this work and other reports

The concentration factor of the proposed method and previously reported are compared in Table 6. As it is obvious, the concentration

factor of the method by using BSTD is superior to those previously reported[17,21,22,29-33].

Table 6. Comparison of concentration factor of this work and other reports

Metal ions	Concentration factor	Ref.
Cu^{2+}	400	29
Ag^+	400	21
Ce^{3+}	200	22
$Cu^{2+}, Zn^{2+}, Mn^{2+}$	25	30
Co^{2+}, Cu^{2+}	100	31
UO_2^{2+}	100	32
Pb^{2+}	>100	33
Pb^{2+}	100	17
Cu^{2+}	500	This work

CONCLUSIONS

The proposed method has the following advantageous: The method is relatively rapid, the time taken for the separation, and analysis of copper in a 500 mL-water sample is at the most 35 min per sample. It can selectively separate Cu^{2+} ions from other metal ions associated, even much higher concentrations. The method can be successfully applied to separation and determination of ultra trace (12.5 ng L^{-1}) amounts of copper in environmental and biological samples.

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