

Selective Cloud Point Extraction and Preconcentration of Copper by the Use of Dithizone as a Complexing Agent

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ABSTRACT: *The aim of this work was to develop a selective cloud point extraction method for the separation and preconcentration of copper(II) prior to spectrophotometric determination. For this purpose dithizone was used as a complexing agent and the experimental solution was acidified with sulfuric acid. Triton X-114 was used as a surfactant and after phase separation, based on the cloud point of the mixture, the rich phase was diluted with tetrahydrofuran (THF) and the enriched analyte determined by spectrophotometric analysis. The chemical and thermodynamic variables affecting the complexation and phase separation were optimized. Calibration plot of absorbance vs. concentration was linear within the range of 15-250 ng ml⁻¹ Cu(II) the limit of detection being 4.6 ng ml⁻¹. The proposed procedure was successfully applied to the determination of copper in liver samples.*

KEY WORDS : *Cloud point extraction, Spectrophotometry, Copper, Dithizone.*

INTRODUCTION

Copper is a nutritionally essential metal and is widely distributed in nature [1-3]. There is a great need to develop a simple, sensitive, selective and inexpensive method for the determination and continuous monitoring of Cu levels in environmental and biological specimens [2, 4, 5].

Electrothermal atomic absorption spectrometry (ETAAS) and inductively coupled plasma mass spectrometry (ICP-MS) are the most commonly used techniques in the determination of trace levels of metals, but the use of more accessible instruments such as spectrophotometer is important for the determination of

trace metals. Its availability, simplicity of use, speed, precision and the accuracy of the technique still make the spectrophotometric methods an attractive alternative [6,7]. Major difficulties involved in determination of trace metals by spectrophotometric analysis are the lack of selectivity and sensitivity, requiring separation and preconcentration of the samples to be analysed. The classical liquid-liquid extraction and separation methods are usually time consuming, labor extensive and require relatively large volumes of high purity solvents. In addition there is concern regarding the disposal of the

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1021-9986/05/4/47

6/\$/2.60

solvents used. Cloud point extraction (CPE) is an attractive technique that reduces the consumption of the solvent, extraction time and the disposal costs [6,8-20]. Cloud point methodology has been used for the extraction and preconcentrations of metal ions after the formation of sparingly water soluble complexes. *Watanabe, et al.* [21] used phenolic derivatives of 2-(2-Pyridylazo) and 2-(2-thiazolylazo) and *Wang, et al.* [22] used dodecylphenyl-methyl- β -diketone (LIX 54) for the cloud point extraction of copper. These ligands are not selective for copper making it necessary to use, masking reagents.

Dithizone has been widely used in the presence of different surfactants, for spectrophotometric determination of some metal ions [23, 24]. It is known that dithizone is highly selective for extraction of copper under acidic conditions [25]. The aim of this study was the use of dithizone and Triton X-114 for selective cloud point extraction and preconcentration of copper prior to its spectrophotometric determination.

EXPERIMENTAL

Apparatus

A Shimadzu (Kyoto, Japan) (Model 265FW) UV-VIS spectrophotometer with 1cm optical path cell was used for the measurements of absorbance. A thermostated bath (Tokyo Rikakikai LTD, Japan) Model UA-1, maintained at the desired temperature, was used for cloud point preconcentration experiments and phase separation was assisted using a centrifuge (Hettich) in 15 ml calibrated centrifuge tubes (Superior, Germany). A Shimadzu (Kyoto, Japan) Model AA-670G atomic absorption spectrometer with deuterium lamp background correction and a copper hollow cathode lamp were used for comparing the results.

Reagents

The nonionic surfactant Triton X-114, was obtained from Sigma (St. Louis, MO, USA) and was used without further purification. A stock standard solution of Cu (II) at a concentration of 1000 $\mu\text{g ml}^{-1}$ was prepared from pure $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (E. Merck, Darmstadt, Germany). Working standard solutions were made by appropriate dilution of the stock standard solution. A solution of $1 \times 10^{-3} \text{ mol l}^{-1}$ dithizone (E. Merck) was prepared by dissolving appropriate amounts of this reagent in tetrahydrofuran (E. Merck). A 4M of sulfuric acid (E. Merck) was prepared in water. All other reagents were of

analytical grade and all solutions were prepared in triply distilled water. The materials and vessels used for trace analysis were kept in sulfochromic acid mixture at least 1h and subsequently washed four times with triply distilled water before use.

Cloud point preconcentration procedure

For the cloud point extraction, to the aliquots of the standard or sample solution, 0.8 ml Triton X-114 (1% V/V), 0.1 ml dithizone ($1 \times 10^{-3} \text{ mol l}^{-1}$) and 0.5 ml of 4 mol l^{-1} sulfuric acid were added and the volumes were made up to 10 ml. The mixtures were kept for 10 min in the thermostated bath at 40 °C. Separation of the two phases was accomplished by centrifugation for 10 min at 3500 rpm. On cooling in an ice acetone mixture (5 min), the surfactant rich phase became viscous. The aqueous phase was then separated completely by a 5 ml syringe centered in the tube. The surfactant phase in the tube was dissolved by adding 1 ml of THF. The absorbance of the resultant solution was measured in a micro cuvette against a blank at 552 nm.

Real samples

Appropriate amounts of wet liver of cow were dried at 100 °C. After cooling, the sample was powdered. Two hundred milligrams of the powdered sample was placed in a 50 ml beaker, and 8 ml of concentrated HNO_3 and HClO_4 mixture (2:1) was added. The beaker was covered with a watch glass and allowed to stand overnight. The contents in the beaker were heated on a hot plate (100°C, 15 min, 150°C, 10 min). The watch glass was removed and the acid evaporated to dryness at 150°C. To the obtained white residue, 8 ml of HClO_4 was added, and the mixture was heated at 160°C to dryness. All of the heating processes were carried out under a hood with necessary precautions. Ten milliliters of 1 M HCl were added, and the contents were heated at 150°C for 1 min. The resulted clear solution was neutralized by a 3 M NaOH solution and the volume was made up to 50 ml in a volumetric flask. Aliquots (6 ml) of these solutions were subjected to cloud point separation according to the mentioned procedure.

RESULTS AND DISCUSSION

Absorption spectrum of copper complex

The absorption spectrum of copper(II) dithizonate in the final extracted and diluted phase against THF as a

blank at the maximum absorbance for the complex (552 nm) is shown in Fig. 1.

Stability of dithizone

Dithizone has a poor stability in most solvents and thus the solution should be prepared freshly before each analysis and this may be considered as a disadvantage for the method. We used THF as solvent for preparing solution of dithizone. The changes in the absorption spectrum of a 5×10^{-6} M solution of dithizone in THF with time is shown in Fig. 2. The solution seems to be reasonably stable over a period of 60 min in the condition of laboratory. We also investigated the stability of dithizone in the condition that the solutions were kept in refrigerator. The investigation showed that in this condition dithizone is stable for at least 4 weeks and there is no need to prepare it freshly.

Effect of sulfuric acid concentration on extraction recovery of Cu(II)

Cloud point extraction of copper(II) dithizonate was performed in acidic condition such as H_2SO_4 and HNO_3 . Results showed dithizone is stable in the presence of H_2SO_4 while the presence of strong oxidizing acids such as HNO_3 could decompose dithizone. Cloud point extraction of copper was studied in different concentrations, from 0.001 to 1.5 mol l^{-1} of sulfuric acid. Results (Fig. 3) indicate that for sulfuric acid concentrations below 1 mol l^{-1} , recovery of copper is close to 100%. However, with sulfuric acid concentrations over 1 mol l^{-1} , the surfactant rich phase can not be separated from the aqueous solution. Hence a concentration of 0.2 mol l^{-1} H_2SO_4 was chosen for subsequent experiments.

Effect of dithizone concentration on extraction of Cu(II)

The extraction recovery as a function of the dithizone concentration is shown in Fig. 4. For this study, 10 ml of a solution containing 200 ng ml^{-1} Cu(II) in 0.08% (V/V) Triton X-114 and 0.2 mol l^{-1} sulfuric acid plus various amounts of dithizone were subjected to the cloud point preconcentration process. For this copper concentration, $\sim 100\%$ extraction of Cu(II) is achieved above a dithizone concentration of $8 \times 10^{-6} \text{ mol l}^{-1}$. A concentration of $1 \times 10^{-5} \text{ mol l}^{-1}$ dithizone was chosen for subsequent experiments.

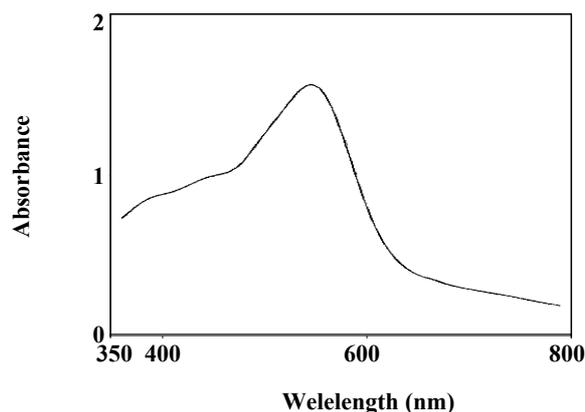


Fig. 1: The absorption spectrum of copper (II) dithizonate in the final extracted and diluted phase.

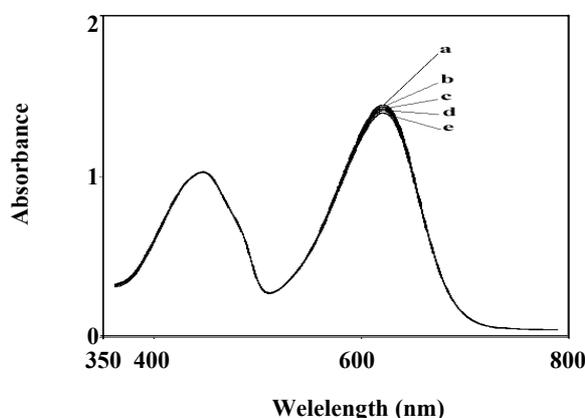


Fig. 2: Change in the absorption spectrum of $5 \times 10^{-6} \text{ mol l}^{-1}$ solution of dithizone in THF with time. a = 0, b = 15, c = 30, d = 45, and e = 60 min.

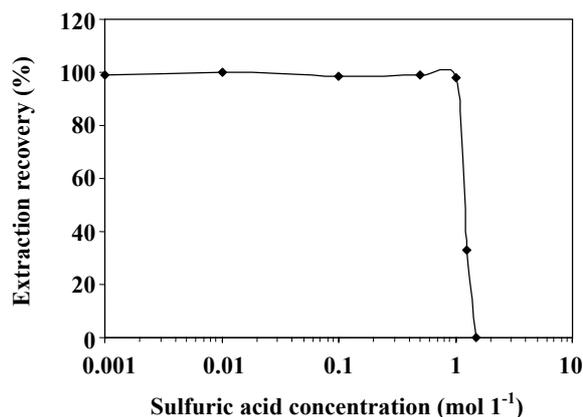


Fig. 3: Effect of sulfuric acid concentration on the extraction recovery of copper(II). Conditions: 200 ng ml^{-1} Cu(II), 0.08% (V/V) Triton X-114, $1 \times 10^{-5} \text{ mol l}^{-1}$ dithizone.

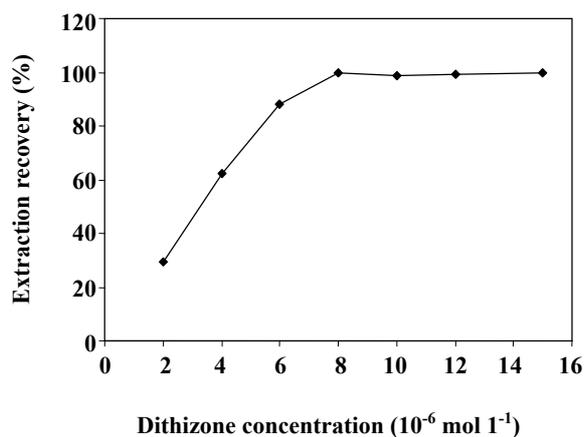


Fig. 4: Effect of Dithizone concentration on the extraction recovery of copper(II). Conditions: $200 \text{ ng ml}^{-1} \text{ Cu(II)}$, 0.08 % (V/V) Triton X-114, 0.2 M sulfuric acid.

Effect of Triton X-114 concentration on extraction recovery of Cu(II)

Triton X-114 was chosen for the formation of the surfactant rich phase. The low cloud point temperature and high density of the surfactant rich phase, facilitates phase separation by centrifugation. Fig. 5 highlights the effect of the surfactant concentration in the range of 0.02 - 0.1% (V/V) on the extraction recovery. Triton X-114 was found to quantitatively extract the Cu-dithizone complex from aqueous sample at surfactant concentrations above 0.04%, using a single step extraction procedure. A concentration of 0.08% Triton X-114 was chosen for subsequent experiments.

Selection of the dilution agent for the surfactant rich phase

Different solvents such as methanol, ethanol and THF were tried to select among them the one that can completely dissolve the surfactant rich phase and the extracted materials (complex of copper and excess of dithizone) and give the best sensitivity. The best results were obtained with the use of THF, therefore, it was chosen as the diluent for further experiments.

Effect of equilibration temperature and time

It is desirable to have the shortest incubation time and the lowest possible equilibration temperature, which compromise completion of the reaction and efficient separation

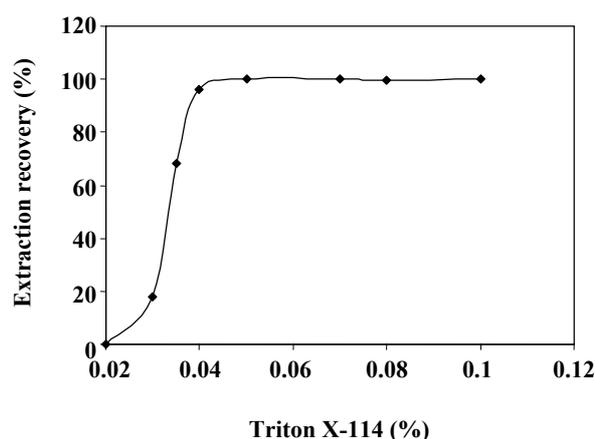


Fig. 5: Effect of Triton X-114 concentration on the extraction recovery of copper (II). Conditions: $200 \text{ ng ml}^{-1} \text{ Cu(II)}$, $1 \times 10^{-5} \text{ mol l}^{-1}$ dithizone, 0.2 M sulfuric acid.

of the phases. The variation of the extraction recovery with the temperature was studied in the range of 25 – 90 °C. The results showed excellent recovery for equilibration temperature from 40 to 70 °C. The dependence of extraction efficiency upon incubation time was also studied in the range of 5-20 min. An incubation time of 10 min was optimal for quantitative extraction of copper(II).

Selectivity of the method for copper

Dithizone is known to be fairly selective for copper at $\text{pH} < 1$, and only palladium, platinum, gold, mercury and silver can be extracted with copper in acidic solution [23]. The concentration of palladium, platinum, gold, mercury and silver is usually very low in most biological samples and thus they have no interference in the extraction and determination of copper.

Characteristics of the method

Table 1 shows the analytical characteristics of the method. In the optimum experimental conditions, the calibration curve for copper was linear from 15 to 250 ng ml^{-1} and the limit of detection was sufficiently low. Further improvement is also feasible, either by pre-concentrating larger amounts of the sample solution or diluting the surfactant rich phase to a smaller volume of the THF solution.

APPLICATION

In order to confirm the usefulness of this proposed

Table 1: Analytical characteristics of the method.

Concentration range (ng ml ⁻¹)	15-250
Slope	4.012×10 ⁻³
Intercept	0.057
Correlation coefficient(r)	0.998
RSD(%) (n=5)	2.9(100) ^a , 3.4(25) ^a
LOD (ng ml ⁻¹) ^b	4.6

a) Values in parentheses are the copper concentrations (ng ml⁻¹) for which the RSD was obtained.

b) Limit of detection. Calculated as three times the standard deviation (3σ) of the blank signal.

Table 2: Determination of copper in some cow liver samples.

Sample	Cu (μg g ⁻¹) ^b	
	Proposed method ^a	AAS ^a
A	52.3±2.5	53.8±0.8
B	80.5±3.4	79.1±1.1
C	65.0±2.8	63.5±0.9

a) Mean of three experiment ± standard deviation.

b) Concentration in dried liver sample.

method, it was applied for the determination of copper in some liver samples. The results in Table 2 show that the proposed method can be successfully applied to the determination of copper in biological samples. The results of analyses by the developed method were found to be in good agreement with those obtained by AAS.

CONCLUSIONS

The combined advantages of the cloud point methodology and the selectivity of the dithizone as a ligand for Cu(II) in acidic conditions were utilized for spectrophotometric determination of copper in liver samples. The method is simple, clean, safe, inexpensive, selective and sensitive. It can be used as an alternative to other analytical methods for the determination of copper in the biological samples. The method gives a good limit of detection and solvent-free extraction of the element from its initial matrix following a single step extraction procedure without any interferences.

Received : 17th February 2004 ; Accepted : 26th April 2005

REFERENCES

- [1] Ahmed, M.J., Jahan I., Banoo, S., *Anal. Sci.*, **18**, 805 (2002).
- [2] Richter, P., Toral, M.I., Tapia, A.E., Fuenzalida, E., *Analyst*, **122**, 1045 (1997).
- [3] Toral, M.I., Richter, P., Rodriguez, C., *Talanta* **45**, 147 (1997).
- [4] Yamini, Y., Tamaddon, A., *Talanta*, **49**, 119 (1999).
- [5] Shamsipur, M., Avanes, A., Rofouei, M.K., Sharghi, H., Aghapour, G., *Talanta*, **54**, 863 (2001).
- [6] Sombra, L., Luconi, M., Fernanda Silva, M., Olsina, R.A., Fernandez, L., *Analyst*, **126**, 1172 (2001).
- [7] Dalmon, O., Tufekci, M., Nohut, S., Guner, S., Karabocek, S., *J. Pharm. Biomed. Anal.*, **27**, 183 (2002).
- [8] Manzoori, J. L., Karim-Nezhad, G., *Anal. Sci.*, **19**, 579 (2003).
- [9] Manzoori, J.L., Karim-Nezhad, G., *Anal. Chim. Acta*, **484**, 155 (2003).
- [10] Manzoori, J.L., Bavili-Tabrizi, A., *Anal. Chim. Acta*, **470**, 215 (2002).
- [11] Manzoori, J.L., Bavili-Tabrizi, A., *Microchim. Acta*, **141**, 201 (2003).
- [12] Silva, M.F., Fernandez, L., Olsina, R.A., Stacchiola, D., *Anal. Chim. Acta.*, **342**, 229 (1997).
- [13] Silva, M.F., Fernandez, L.P., Olsina, R.A., *Analyst*, **123**, 1803 (1998).
- [14] Fernandez, A. E., Ferrera, Z. S., Rodrinuez, J. J. S., *Analyst*, **124**, 487 (1999).
- [15] Carabias - Martinez, R., Rodriguez - Gonzalo, E., Dominguez - Alvarez, J., Hernandez - Mendez, J., *Anal. Chem.*, **71**, 2468 (1999).
- [16] Laespada, M. E. F., Pavon, J. L. P., Cordero, B. M., *Analyst*, **118**, 209 (1993).
- [17] Igarashi, S., Endo, K., *Anal. Chim. Acta*, **320**, 133 (1996).
- [18] Oliveros, M. C. C., Jimenez de Blas, O., Pavon, J.L.P., Cordero, B.M., *J. Anal. At. Spectrom.*, **13**, 547 (1998).
- [19] Mesquita da Silva, M.A., Frescura, V.L.A., Nome Aguilera, F.J., Curtius, A.J., *J. Anal. At. Spectrom.*, **13**, 1369 (1998).
- [20] Paleologos, E.K., Giokas, D.L., Tzouwara-Karayanni, S.M., Karayannis, M.I., *Anal. Chim. Acta*, **458**, 241 (2002).

- [21] Watanabe, H., Saitoh, T., Kamidate, T., Haraguchi, H., *Mikrochim. Acta*, **106**, 83 (1992).
- [22] Wang, C.H., Martin, D.F., Martin, B.B., *J. Environ. Sci. Health A*, **31**,1101 (1996).
- [23] Fiedler, H. D., Westrup, J.L., Souza, A.J., Pavei, A.D., Chagas, C.U., Nome, F., *Talanta*, **64**, 190 (2004).
- [24] Singh, H.B., Kumar, B., Sharma, R.L., *Analyst*, **114**, 853 (1989).
- [25] Lurie, J., "Handbook of Analytical Chemistry", Mir Publishers, Moscow, pp.336-339 (1975).