S. Afr. J. Chem., 2005, **58**, 53–56,
<http://journals.sabinet.co.za/sajchem/>.

Study on the Solid Phase Extraction and Spectrophotometric Determination of Mercury in Water and Biological Samples with 5-(p-Aminobenzylidene)-thiorhodanine

Qiufen Hu[1,2], Xianhua Wu[1,2], Zhangjie Huang[1], Guangyu Yang[1]* and Jiayuan Yin[1]

[1]*Department of Chemistry, Yunnan University, Kunming, 650091, P.R. China.*

[2]*Department of Chemistry, Yuxi Teacher's College, Yuxi, 653100, P.R. China.*

Received 10 April 2004; revised and accepted 7 March 2005.

ABSTRACT

This paper presents the synthesis and application of 5-(p-aminobenzylidene)-thiorhodanine (ABTR) as a new chromogenic reagent for the determination of mercury. Based on the rapid reaction of mercury(II) with ABTR and the solid phase extraction of the coloured chelate with a C_{18} disk, a highly sensitive, selective and rapid method for the determination of mercury has been developed. In the presence of pH 3.5 sodium acetate-acetic acid buffer solution and emulsifier-OP medium, ABTR reacted with mercury(II) to form a red chelate in a molar ratio 1:2 (mercury to ABTR). This chelate was enriched by solid phase extraction with a C_{18} disk and eluted from the disk with dimethyl formamide (DMF). An enrichment factor of 50 was achieved. The molar absorptivity of the chelate in DMF was found to be 1.21×10^5 L mol^{-1} cm^{-1} at 555 nm. Beer's law was obeyed in the range of 0.01~3 μg mL^{-1}. The relative standard deviation for eleven replicates with a concentration of 0.01 μg mL^{-1} was 1.98%. This method was applied with good results to the determination of mercury in water and biological samples.

KEY WORDS

Mercury, solid phase extraction, spectrophotometry, 5-(p-aminobenzylidene)-thiorhodanine.

1. Introduction

Mercury is a toxic heavy metal. The determination of traces of mercury in water and biological samples is very important. Chinese Quality Standards as defined by the Chinese Environmental Agency require that the concentration of mercury may not exceed 0.2 μg g^{-1} in food and 0.05 μg L^{-1} in drinking water.[1] Analytical methods such as spectrofluorimetry, X-ray fluorescence spectrometry, neutron activation analysis, atomic absorption spectrometry, chemiluminescence, electrochemical analysis, and others have been widely applied for the determination of mercury.[2–9] A wide variety of spectrophotometric methods for the determination of mercury have also been reported[10–22] as they have an advantage over the above methods in being simple and not needing expensive or complicated equipment. Each of the various chromogenic systems that have been studied was found to differ with respect to their sensitivity, selectivity and convenience.

Most routine spectrophotometric methods for the determination of mercury in low concentrations usually require a preconcentration step. Recently, solid phase extraction has become a popular technique for it has a number of notable advantages (such as higher enrichment factor, reduced contamination of the environment and the ability for rapid and simultaneous preparation of large sample quantities) over other extraction methods.[23–28] In this work, we have synthesized a new chromogenic reagent, 5-(p-aminobenzylidene)-thiorhodanine (ABTR) and thoroughly studied its colour reaction with mercury and the solid phase extraction of the coloured chelate by means of a C_{18} disk. Experiments have shown that the molar absorptivity of this method reaches 1.21×10^5 L mol^{-1} cm^{-1} at 555 nm. When masked with pyrophosphoric acid, most common foreign ions do not interfere with the determination. By solid phase extraction of the Hg(II)-ABTR chelate with a C_{18} disk, an enrichment factor of 50 was achieved. Combining the new reagent with solid phase extraction allowed the development of a highly sensitive, selective and rapid method for the determination of mercury in water and biological samples.

* To whom correspondence should be addressed: Fax: +86-871-8316812p;
E-mail: huqiufena@163.com

2. Experimental

2.1. Apparatus

A UV-160A spectrophotometer (Shimidzu, Japan), equipped with a 1 cm microcell (0.5 mL) was used for all absorbance measurements. The pH measurements were made with a Beckman Φ-200 pH meter. The extraction was carried out with a Waters Solid Phase Extraction (SPE) device that can handle twenty samples simultaneously. A Zorbax C_{18} membrane disk [47 mm (diameter) × 0.5 mm (thickness), 8 μm, 50 mg] (Agilent Technologies, USA) was used.

2.2. Reagents

ABTR was synthesized by the following procedure: 40 mL of acetic acid were added to 1.5 g of thiorhodanine and 1.2 g of p-aminobenzaldehyde, and the mixture was heated gently to completely dissolve the thiorhodanine and p-aminobenzaldehyde. The solution was refluxed for about 1.5 h. During the course of refluxing, 1 mL of concentrated sulfuric acid was added dropwise. After the colour of the solution had turned red, the refluxing was stopped and the sample was poured into 200 mL of distilled water. A small amount of aqueous ammonia

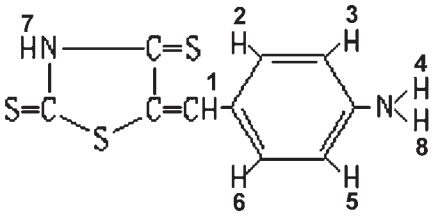

Figure 1 The structure of ABTR.

was added to this solution. Thereafter, the precipitate was separated by filtration and recrystallized twice from absolute alcohol. The yield was 55% (m. p. 282~285°C). The composition of ABTR as shown in Fig. 1 was verified by elemental analysis, IR, ^{1}HNMR and MS. Elemental analysis: calculated (found), 47.59 (47.23)% C, 3.19 (3.28)% H, 11.10 (11.02)% N, 38.12 (37.53)%. IR (KBr) (cm^{-1}): 3470, 3450, 3355 (ν_{N-H}); 3060, 3020 ($\nu_{-C=C-H}$); 1628 (δ_{N-H}); 1566, 1548, 1515, 1450 ($\nu_{C=C}$); 1292 (ν_{C-N}); 1171 ($\nu_{C=S}$); 825 (δ_{Ar-H}); 806 ($\delta_{C=C-H}$). ^{1}HNMR (solvent: DMSO-d$_6$) (δ, ppm): 7.46 (1H, s, H-1); 7.26, 7.35 (2H, d, J DWL 9Hz, H-2 and H-6); 6.62, 6.72 (2H, d, J DWL 9Hz, H-3 and H-5); 2.21 (1H, s, H-7); 3.36 (2H, w, H-4 and H-8). MS (EI) (m/z): 252 (M$^+$).

All solutions were prepared with ultra-pure water obtained from a Milli-Q50 SP Reagent Water System (Millipore Corporation). High purity dimethyl formamide (DMF) (Fisher Corporation, USA) was used. A 3.0×10^{-4} mol L^{-1} of ABTR solution was prepared by dissolving ABTR in DMF. A stock solution of mercury standard (1.0 mg mL^{-1}) was obtained from the Chinese Standard Material Center, and a stock solution of 0.5 μg mL^{-1} was prepared by diluting this solution. 0.5 mol L^{-1} of pH 3.5 sodium acetate-acetic acid buffer solution (containing 0.2 mol L^{-1} of pyrophosphoric acid) was used. Emulsifier-OP solution (2.0 % (v/v)) was prepared by dissolving emulsifier-OP with water. All chemicals used were of analytical grade unless otherwise stated.

2.3. General Procedure

To a standard or sample solution containing no more than 3.0 μg of Hg(II) in a 50 mL calibrated flask, 5 mL of 0.5 mol L^{-1} sodium acetate-acetic acid (containing 0.2 mol L^{-1} pyrophosphoric acid) of pH 3.5, 3.0 mL of 3.0×10^{-4} mol L^{-1} ABTR solution and 2.0 mL of 2.0 % emulsifier-OP solution were added. The mixture was diluted to volume of 50 mL and mixed well. After 10 min, the solution was passed through the C_{18} disk at a flow rate of 50 mL min^{-1}. The coloured chelate was retained on the disk. After the enrichment, the chelate was eluted from the disk at a flow rate of 5 mL min^{-1} with 1.0 mL of DMF in reverse direction. The eluent was adjusted to the accurate volume of 1.0 mL in a 1.0 mL calibrated flask by adding small quantities of DMF with a 200 μL syringe. The absorbance of this solution was measured at 555 nm in a 1cm microcell (0.5 mL) against a reagent blank prepared in a similar way.

3. Results and Discussion

3.1. Absorption Spectra

The absorption spectra of ABTR and its Hg(II) chelate are shown in Fig. 2. The absorption peaks of ABTR and its complex in DMF medium were located at 430 nm and 555 nm.

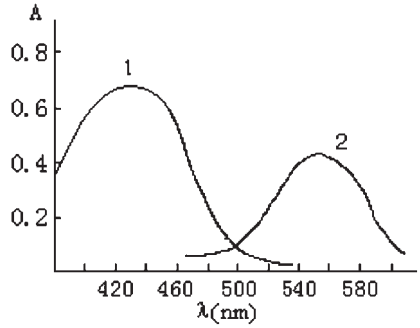

Figure 2 Absorption spectra of ABTR and its Hg(II) complex: 1 ABR-emulsifier-OP blank against water; 2 ABR-emulsifier-OP-Hg(II) chelate against reagent blank.

3.2. Effect of Acidity

The results showed that the optimal pH for the reaction of Hg(II) with ABTR was 1.2~4.2. A sodium acetate-acetic acid buffer solution of pH 3.5 is recommended in order to control the pH. The use of 4~6 mL of the buffer solution (pH 3.5) per 50 mL of final solution was found to give the best results in terms of absorbance reproducibility. The experiments showed further that a buffer solution containing 0.15~0.25 mol L^{-1} of pyrophosphoric acid greatly increases the selectivity without affecting the sensitivity. The use of 5 mL of a 0.2 mol L^{-1} pyrophosphoric acid in buffer was in summary found to give the best results.

3.3. Effect of Surfactants

The effects of surfactants on the Hg(II)-ABTR system were studied. The results (Table 1) showed that in the absence of surfactants or in the presence of anionic or cationic surfactants, the Hg(II)-ABTR chromogenic system gave a low absorption, whereas in the presence of nonionic surfactants medium, the absorption of the chromogenic system increased markedly. Various nonionic surfactants enhanced the absorbance in the following sequence: Emulsifier-OP > Tween-80 > Tween-20 > Tween-60. Accordingly, emulsifier-OP was found to be the best additive, and the use of 0.5~3 mL of emulsifier-OP solution was found to give the best results in terms of absorbance reproducibility. Consequently, the use of 2.0 mL was recommended.

3.4. Effect of ABTR Concentration

For up to 2.0 μg of Hg(II), the use of 3 mL of 3.0×10^{-4} mol L^{-1} of ABTR solution was found to be sufficient for a complete reaction. Accordingly, 3.0 mL of ABTR solution were added in all further measurements.

3.5. Stability of the Chromogenic System

After mixing the components, the absorbance reaches its maximum within 5 min at room temperature and remains stable for at least 8 h. After having been extracted into the DMF medium, the chelate was stable for at least 12 h.

3.6. Solid Phase Extraction

Both the enrichment and the elution were carried out on a

Table 1 The effect of surfactants on Hg(II)-ABTR chromogenic system.

Surfactant	Absence	Emulsifier-OP	Tween-80	Tween-20	Tween-60	SDS	CTMAB	CPB
λmax (nm)	530	555	540	545	550	520	525	525
ε ($\times 10^4$) L mol^{-1} cm^{-1}	6.19	12.1	8.96	9.12	8.23	6.25	6.12	5.85

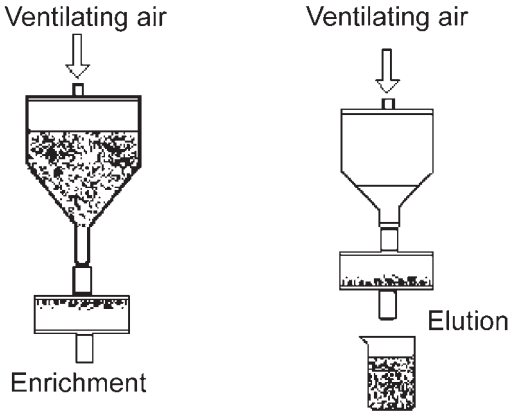

Figure 3 Enrichment and the elution in the course of solid phase extraction.

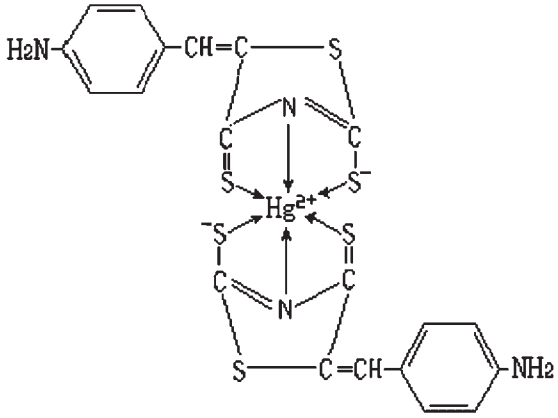

Figure 4 The structure of Hg(II)-ABTR chelate.

Waters SPE device, which can handle twenty samples simultaneously. The flow rate was set to 50 mL min^{-1} for enrichment and 5 mL min^{-1} for elution.

Some experiments were carried out in order to investigate the retention of ABTR and its Hg(II) chelate on the disk. It was found that the ABTR and its Hg(II) chelate were quantitively retained on the disk when the separation on the disk was carried out in an aqueous solution. The capacity of the disk was 32 mg 50 mL^{-1} and 28 mg 50 mL^{-1} for ABTR or the Hg(II) chelate, respectively. Therefore, the disk had an adequate capacity for the enrichment of the Hg(II)-ABTR chelate.

In order to choose the most suitable eluant for the elution of ABTR and its Hg(II) chelate, various organic solvents were studied and the following sequence (in order of decreasing eluation power) was obtained: DMF > acetonitrile > acetone > ethanol > methanol. DMF was therefore selected as the eluant of choice. The experiment showed that it was easier to elute the retained ABTR and its Hg(II) chelate in reverse direction than in forward direction. Therefore, it was necessary to elute the retained chelate in reverse direction (Fig. 3). 1.0 mL of eluant was sufficient to elute the ABTR and its Hg(II) chelate from disk at a flow rate of 5 mL min^{-1}. A volume of 1.0 mL eluant was selected.

3.7. Calibration Curve and Sensitivity

The calibration curve showed that Beer's law was obeyed over the concentration range of 0.01~3 μg Hg(II) per mL solution. Linear regression analysis resulted in the best fit for the equation $A = 0.548\,C\,(\mu g\,mL^{-1}) + 0.0164$ ($r = 0.9991$). The molar absorptivity was calculated to be 1.21×10^5 L mol^{-1} cm^{-1} at 555 nm. The relative standard deviation at a concentration level of 0.01 μg mL^{-1} of Hg(II) (11 repeat determination) was 1.98%.

3.8. Composition of the Complex

The composition of the complex was determined by the method of continuous variations and the mole ratio method. Both showed that the molar ratio of Hg(II) to ABTR is 1:2. The probable structure of the chelate is shown in Fig. 4.

Table 2 Tolerance limits for the determination of 1.0 μg of Hg(II) with ABTR (relative error ±5%).

Ion added	Tolerance (mg)
NO_3^-, K^+, borate, Na^+	50
Li^+, Al^{3+}, PO_4^{3-}, NO_2^-, SO_4^{2-}, ClO_4^-	20
Ca^{2+}, Mg^{2+}, SO_3^{2-}, Sr^{2+}, Ba^{2+}, IO_3^-, BrO_3^-, ClO_3^-	10
Mn^{2+}, Ce(IV), W(VI), Mo(VI), U(IV), Fe^{3+}	4
Ti(IV), Bi(III), V(V), Cr(VI), Zr(IV), F^-, Fe^{2+}, Cl^-	1
Cd^{2+}, Cr^{3+}, La^{3+}, Sn(IV), Zn^{2+}, Zr(IV) , Co^{2+}, Ni^{2+}	0.5
Ru(III), Bi(III), Pb^{2+}, Sb^{3+}, Th(IV), Br^-, Os(VIII) , I^-, Cu^{2+}	0.2
Se(IV), Te(IV), $S_2O_3^{2-}$, Ag^+	0.1
Ir(IV) , Rh(III), Ru(III)	0.05
Pt(IV), Au^{3+}	0.01
CN^-, SCN^-	0.005

3.9. Interference

The selectivity of the proposed method was investigated by measuring the concentration of Hg(II) (0.5 μg in 50 mL^{-1}) in the presence of various ions. From the results (relative error ±5%) in Table 2 it can be concluded that most common ions do not interfere with the determination. The described method is therefore highly selective.

3.10. Application

The proposed method was successfully applied for the determination of mercury in aqueous and biological samples.

For biological samples, 0.50 g of sample was weighed accurately into a teflon high-pressure microwave acid-digestion bomb (Fei Yue Analytical Instrument Factory, Shanghai, China). 2.5 mL of concentrated nitric acid and 2.5 mL of 30% hydrogen peroxide were added. The bombs were tightly sealed and then positioned in the carousel of the microwave oven (Model WL 5001, 1000 W, Fei Yue Analytical Instrument Factory, Shanghai, China). The system was operated at full power for 6.0 min. The digested sample was neutralized, and the mercury contents were analysed according to the general procedure. The results are

Table 3 Determination of mercury in the certified standard biological samples.

Samples	Standard value ($\mu g\,g^{-1}$)	By this method ($\mu g\,g^{-1}$)	RSD% ($n = 5$)
Human hair (GBW07601)	As(0.28), B(1.3), Bi(0.34), Ca(2900), Cd(0.11), Ce(1.2), Co(0.71), Cr(0.37), Cu(10.2), Fe(54), Hg(0.36), Mg(360), Mn(6.3), Mo(0.073), Ni(0.83), Pb(8.8)	0.325	2.4
Tea leaf (GBW08505)	As(0.191), Ba(15.7), Ca(2840), Cd(0.032), Co(0.2), Cr(0.8), Cu(16.2), Fe(373), Hg(0.014), Mg(2240), Mn(766), Ni(7.61), Pb(1.06), Se(0.041), Zn(38.7),	0.0128	2.6

Table 4 Determination of mercury in the water sample.

Samples	Reference method (μg L^{-1})	Found (μg L^{-1})	RSD% (n = 5)	Recovery (%) (n = 5) (add 1.0 mg mercury)
River water	18.2	17.8	2.3	95
Lake water	12.7	13.2	2.5	103
Tap water	0.00	15.6	2.4	94

shown in Table 3.

The water sample was acidified with nitric acid and filtered with a 0.45 μm filter. The concentration of mercury was determined according to the general procedure. The results are shown in Table 4, together with the results of a recovery test. A standard method using ICP-MS has also been used as reference method. The results are also shown in Table 4.

4. Conclusion

This method is highly selective and highly sensitive. ABTR is a sensitive and selective spectrophotometric reagent for mercury. The molar absorptivity of the chelate reaches 1.21×10^5 L mol^{-1} cm^{-1}. Most foreign ions do not interfere with the determination when masked with pyrophosphoric acid. Enrichment by solid phase extraction of the ABTR-Hg(II) chelate with a C_{18} disk increased the sensitivity of the method by a factor of 50. The consumption of organic solvents in this method is much lower than in the liquid–liquid extraction method. Using a Waters SPE device allows the rapid and simultaneous preparation of large quantities of a given sample.

References

1 Edited by Chinese Environment Protection Agency, *Analysis Method of Water and Waste Water,* 3th edn., Chinese Environmental Science Press, Beijing, P.R. China, 1989, pp. 578–560.

2 G.Y. Yang, X.C. Dong, Y. Dai, Q.F. Hu and J.Y. Yin, *J. Liq. Chromatogr. R. T.*, 2004, **27(3)**, 451.

3 E. Wieteska and A. Ziolek, *Chem. Anal.*, 2000, **45**, 325.

4 Y.H. Zhang, G.Y. Yang, Q.F. Hu, J.Y. Yin and Z. Li, *Microchim. Acta.*, 2004, **146**, 297.

5 M. Morita, J.S. Edmonds and J. Yoshinaga, *Pure. Appl. Chem.*, 1998, **70**, 1585.

6 M. Rose, L. Owen, M. Baxter and M.A. Knaggs, *J. Anal. Atom. Spectrom.*, 2001, **16**, 1101.

7 G.Y. Yang, C.M. Zhang, Q.F. Hu and J.Y. Yin, *J. Chromatogr. Sci.,* 2003, **41**(4), 195.

8 A.T. Ellis, P. Kregsamer, P.J. Potts, C. Streli, M. West, P. Wobrauschek, and M. Holmes, *J. Anal. Atom. Spectrom.*, 1998, **13**(11), 209R.

9 A.Z. Abu Zuhri and W. Voelter, *Fresenius J. Anal. Chem.,* 1998, **360**, 1.

10 G.Y. Yang, Z.Y. Xia, Y.P. Wu and J.Y. Yin, *Bull. Korean. Chem. Soc.*, 2004, **25**(4), 594.

11 A.Y. El-Sayed, *Anal. Lett.*, 1998, **31**, 1905.

12 M.P. Sang and C. Hee-Seon, *Anal. Chim. Acta.*, 2002, **459**, 75.

13 S. Chatterjee, A. Pillai and V.K. Gupta, *Talanta,* 2002, **57**, 461.

14 H.W. Gao, *Asian. J. Chem,* 2000, **12**, 78.

15 Q.F. Hu, G.Y. Yang, Y.Y. Zhao and J.Y. Yin, *Anal. Bioanal. Chem.*, 2003, **375**(6), 831.

16 L. Mathew, M.L. Reddy, P. Prasada-Roa, T.R.R.T. Roa and C.S.P. Iyer, *Microchim. Acta.*, 1997, **127**, 125.

17 A.M. Kiwan, M.S. EI-Shahawi, S.M. Aldhaeri and M.H. Saleh, *Talanta,* 1997, **45**, 203.

18 Z.J. Huang, G.Y. Yang, Q.F. Hu and J.Y. Yin, *Anal. Sci.,* 2003, **19**, 255.

19 Y.L. Feng, H. Narasaki, L.C. Tian, S.M. Wu and H.Y. Chen, *Anal. Sci.*, 1999, **15**, 915.

20 Q.F. Hu, Z.J. Huang, G.Y. Yang and J.Y. Yin, *Chinese. J. Anal. Chem.*, 2004, **32**(4), 551.

21 G.G. Talanova, N.S.A. Elkarim, V.S. Talanov and R.A. Bartsch, *Anal. Chem.,* 1999, **71**, 3106.

22 A.V. Yallouz, R. Calixto-De-Campos and S. Paciornik, *Fresenius' J. Anal. Chem.,* 2002, **366**(5), 461.

23 B.S. Garg, R.K. Sharma, N. Bhojak and S. Mittal, *Microchem. J.*, 1999, 61, 94.

24 G.Y. Yang, X.C. Dong, Q.F. Hu and J.Y. Yin, *Anal. Lett.*, 2002, **35**, 1735.

25 K. Pyrzynska and M. Trojanowicz, *Crit. Rev. Anal. Chem.*, 1999, **29**, 313.

26 Q.F. Hu, G.Y. Yang, J.Y. Yin and Y. Yao, *Talanta,* 2002, **57**(4), 751.

27 P.R. Haddad, P. Doble and M. Macka, *J. Chromatogr. A.*, 1999, **856**, 145.

28 Y.H. Zhang, Z. Li, S.T. Zhang, G.Y. Yang and J.Y. Yin, *S. Afr. J. Chem.*, 2004, **57**, 28.