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Short Report

Solid Phase Extraction and Spectrophotometric Determination of Mercury in Tobacco and Tobacco Additives with 5-(*p*-Aminobenzylidene)-Thiothiorhodanine

Weizhu Yang,[a,b,c] *Qun Hu,*[b,c] *Jing Ma,*[b] *Liming Wang,*[b] *Guangyu Yang**,[a] *and Gang Xie*[b,c]

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

[b]*School of Materials & Metallurgy, Kunming Science and Technology University, Kunming, 650031, P.R. China*

[c]*Yunnan Academy of Tobacco Science, Kunming 650106, P. R. China*

Foi desenvolvido um método altamente sensível, seletivo e rápido para a determinação de mercúrio, a partir da reação rápida de mercúrio(II) com 5-(*p*-aminobenzilideno)-tiorodanina (ABTR) e posterior extração em fase sólida do quelato colorido, utilizando discos C_{18}. Em pH 3,5 e na presença do emulsificante-OP, ABTR reage com mercúrio(II) para formar um quelato vermelho na razão molar 1:2 (mercúrio:ABTR). O quelato foi enriquecido pela extração em fase sólida com discos C_{18} e o quelato retido, eluído com dimethyl formamida (DMF). Um fator de enriquecimento na ordem de 50 foi obtido. Em DMF, a absortividade molar do quelato é $1{,}21\times10^5$ L mol^{-1} cm^{-1} a 545 nm, e a lei de Beer é obedecida no intervalo 0,01~3 μg mL^{-1} na solução medida. O desvio padrão relativo para onze replicatas a 0,01 μg mL^{-1} é 1,7%. Este método foi aplicado para a determinação de mercúrio em tabaco e aditivos de tabaco. Bom coeficiente de preconcentração foi encontrado, comparando-se o método proposto com outros similares.

A highly sensitive, selective and rapid method for the determination of mercury based on the rapid reaction of mercury(II) with 5-(*p*-aminobenzylidene)-thiorhodanine (ABTR) and the solid phase extraction of the colored chelate with C_{18} disks has been developed. At pH 3.5 and in the presence of emulsifier-OP medium, ABTR reacts with mercury(II) to form a red chelate of a 1:2 (mercury to ABTR) molar ratio. This chelate was enriched by solid phase extraction with C_{18} disks and the retained chelate eluted form the disks with dimethyl formamide (DMF). An enrichment factor of 50 was achieved. In the DMF medium, the molar absorptivity of the chelate is 1.21×10^5 L mol^{-1} cm^{-1} at 545 nm, and Beer's law is obeyed in the 0.01~3 μg mL^{-1} range in the measured solution. The relative standard deviation for eleven sample replicate measurements at the 0.01 μg mL^{-1} level is 1.7%. This method was applied to the determination of mercury in tobacco and tobacco additives and good preconcentration was found between proposed and comparative methods results.

Keywords: mercury, solid phase extraction, spectrophotometry, 5-(*p*-aminobenzylidene)-thiothiorhodanine

Introduction

Mercury is a toxic heavy metal. Thus, trace mercury determination in tobacco and tobacco additives is a very important issue. The Quality Standards of Tobacco in Chinese Tobacco Company says that the concentration of mercury should not exceed 0.2 μg g^{-1} in tobacco and tobacco additives.[1] Many sensitive instrumental techniques, such as spectrofluorimetry, X-ray fluorescence spectrometry, neutron activation analysis, atomic absorption spectrometry, chemiluminescence, electrochemical analysis, and other have been widely applied to the determination of mercury.[2-8] However, the spectrophotometric method has still the advantage of its simplicity and accessibility, not needing expensive or complicated equipments. For this reason, a wide variety of spectrophotometric methods for the determination of mercury have been reported.[9-19] Each chromogenic system has its advantages and disadvantages with respect to sensitivity, selectivity and analysis speed.

Nevertheless, for the routine spectrophotometric determination of mercury trace, a preconcentration step is usually required. Solid phase extraction is attractive technique because of its notable advantages, namely simplicity and high pre-concentration factors.[20-24] In the

*e-mail: ygy1110@163.com

present work, a new chromogenic reagent, 5-(*p*-aminobenzylidene)-thiothiorhodanine (ABTR) was synthesized, and its reaction with mercuric ions and solid phase extraction of the formed chelate with C_{18} disks were studied. Based on this, a highly sensitive, selective and rapid method for the determination of total mercury in tobacco and tobacco additives was developed.

Experimental

Apparatus

An UV-2401 spectrophotometer (Shimadzu, Japan), equipped with 1 cm microcells (0.5 mL) was used in all absorbance measurements. The pH measurements were made with a Beckman Φ-200 pH meter (Beckman Instruments, USA). The extraction was performed with a Waters Solid Phase Extraction (SPE) Device (Water Corporation, USA), and Zorbax C_{18} membrane disks (47 mm (diameter) × 0.5 mm (thickness), 8 μm, 50 mg) (Agilent Technologies, USA) were also used. A microwave oven (Model WL 5001, 1000W, Fei Yue Analytical Instrument Factory, Shanghai, China) equipped with PTFE high-pressure microwave acid-digestion vessels (Fei Yue, Analytical Instrument Factory, Shanghai, China) was used.

Reagents

The ABTR was synthesized by the following procedure: 40 mL of acetic acid was added to 1.5 g of thiorhodanine and 1.2 g of p-aminobenzaldehyde, and the mixture was gently heated until total dissolution. The solution was refluxed for about 1.5 h, and 1 mL of concentrated sulfuric acid was added dropwise. After the solution has turned red, the reflux was stopped and the sample was poured into 200 mL of distilled water. After the precipitation of a red solid took place, a small amount of aqueous ammonia was added. The solid was separated by filtration. They were recrystallized twice from absolute alcohol and the yield was 36% (mp 282 ~ 285 °C). The structure (Figure 1) has been characterized by elemental analysis, IR, ^{1}H NMR and MS. Elemental analysis, found: C, 47.23; H, 3.28; N, 11.02; S, 37.53. Calc.for $C_{10}H_8S_3N_2$: C, 47.59; H, 3.19; N, 11.10, S, 38.12%. IR (KBr) ν_{max}/cm^{-1}: 3470, 3450, 3355 (N-H); 3060, 3020 (C-H); 1628 (N-H); 1566, 1548, 1515, 1450 (C=C); 1292 (C-N); 1171 (C=S); 825 (Ar-H); 806 (C=C-H). ^{1}H NMR (DMSO-d_6) δ 7.46 (s, 1H, C=C-H); 7.26, 7.35 (d, 2H, *J* 9Hz, H-2 and H-6); 6.62, 6.72 (d, 2H, *J* 9Hz, H-3 and H-5); 3.36 (w, 2H, -NH_2). EIMS *m/z* 252 [M^+].

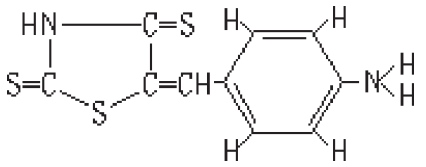

Figure 1. The structure of ABTR.

All of the solutions were prepared with ultra-pure water obtained from a Milli-Q50 SP Reagent Water System (Millipore Corporation, USA). High purity dimethyl formamide (DMF) (Fisher Corporation, USA) was used. A 3.0×10^{-4} mol L^{-1} ABTR solution was prepared by dissolving ABTR in ethanol. A stock standard solution of mercury (1.0 mg mL^{-1}) was obtained from the Chinese Standard Material Center, and a working solution (0.5 μg mL^{-1}) was prepared by adequate dilutions of this solution. Sodium acetate-acetic acid buffer solution (0.5 mol L^{-1}, pH 3.5, containing 0.2 mol L^{-1} of pyrophosphoric acid) was prepared by dissolving the 40.8 g sodium acetate, 180 mL acetic acid and and 44.2 g of sodium pyrophosphate in water and diluted to 1 L. Emulsifier-OP solution (2.0%, v/v) was prepared by dissolving the emulsifier-OP in water. All chemical used were of analytical grade unless otherwise stated.

General procedure

To a proper volume (up to 40 mL) of standard or sample solution containing no more than 3.0 μg of Hg(II), 5 mL of pH 3.5 buffer solution, 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 made up to 50 mL and well mixed. After 10 min, the solution was passed through the C_{18} disks at a flow rate of 50 mL min^{-1}. The elution was then performed at a flow rate of 5 mL min^{-1} in the reverse direction with 1.0 mL of DMF, and the final volume accurately made up to 1.0 mL in a 1.0 mL calibrated flask by adding microamounts of DMF with a 200 μL syringes. The absorbance of this solution was measured at 545 nm in a 1 cm microcell (0.5 mL) against a reagent blank prepared in a similar way.

Sample preparation

The samples (0.50 g) were accurately weighted into the PTFE high-pressure microwave acid-digestion vessels, and 3.0 mL of concentrated nitric acid plus 5.0 mL of 30% hydrogen peroxide were added. The vessels were sealed tightly and then positioned in the carousel of the microwave oven. The system was operated at full power

for 8.0 min. The digest was evaporated to near dryness. The residue was dissolved with 5 mL 5% (m/v) nitric acid, and quantitatively transferred to a 50 mL volumetric flask for further analysis.

Results and Discussion

Absorption spectra

The absorption spectra of ABTR in water and its Hg(II) chelate in DMF are shown in Figure 2. The absorption peak maxima of ABTR in water and its complex in DMF medium are located at 420 nm and 545 nm, respectively.

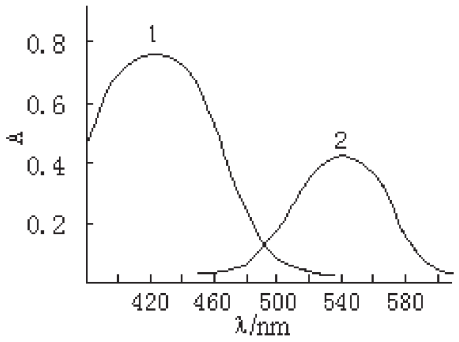

Figure 2. Absorption spectra of ABTR and its Hg(II) complex: 1) ABTR-Emulsifier-OP in water; 2) ABTR-Emulsifier-OP-Hg(II) chelate in DMF.

Effect of acidity and masking

Results showed that the optimal pH for the complexation of Hg(II) by ABTR is at the range 1.2~4.2. The use of the sodium acetate-acetic acid buffer solution (pH 3.5) is recommended, and the addition of 4~6 mL of this solution to a final volume of 50 mL was found to give a maximum and constant absorbance. A volume of 5 mL of the buffer solution was then recommended. The addition to the buffer solution of 0.15~0.25 mol L^{-1} pyrophosphoric acid do not affect the sensitivity and can greatly increase the selectivity. For instance, the tolerance limits of foreign ions such as Cu(II), Ag(I), Pd(II) and Pb(II), increased from 0.005, 0.005, 0.002 and 0.002 to 0.5, 0.2, 0.1 and 0.1 mg *per* 50 mL, respectively if pyrophosphoric acid is present. Thus, the presence of 0.2 mol L^{-1} of pyrophosphoric acid in the buffer solution is recommended.

Effect of surfactants

The influence of surfactants on the Hg(II)-ABTR chromogenic system was studied. The results (Table 1) showed that in the absence of surfactants, or in the presence both anionic or cationic surfactants, lower molar absorptivity (ε) values were found, whereas in the presence of nonionic surfactants, the chromogenic system ε values increases markedly. Considering the various nonionic surfactants invatigated the absorbance enhancement follows the sequence: Emulsifier-OP >Tween-80 > Tween-20 > Tween-60. Accordingly, the use of 0.5~3 mL of the Emulsifier-OP solution give a constant and maximum absorbance. The use of 2.0 mL of this surfactant was then recommended.

Effect of ABTR concentration

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

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 extracted into the DMF medium, the chelate was stable for at least 12 h.

Solid phase extraction

Both the enrichment and elution were carried out on a Waters SPE device (able to prepare twenty samples simultaneously). The flow rate was set to 50 mL min^{-1} during enrichment and 5 mL min^{-1} during elution.

Some experiments were carried out in order to investigate the retention of ABTR and its Hg(II) chelate on the disks. It was found that the ABTR and its Hg(II) chelate could be quantitatively retained on the disks as they pass through the disks in aqueous solution. The capacity of the disks was determined as 30 mg for ABTR and 25 mg for its Hg(II) chelate both in 50 mL solution. This experiment has shown that the disks have adequate capacity to enrich the Hg(II)-ABTR chelate to adequate levels.

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
λ_{max} / (nm)	520	545	540	540	535	530	530	530
ε / (10^4 L mol^{-1} cm^{-1})	6.67	12.1	11.2	9.86	8.76	5.92	6.33	6 .76

In order to choose a proper eluent for the retained ABTR and its Hg(II) chelate, various organic solvents were studied. Their effectivences was evaluated and the following sequence was obtained: DMF > acetonitrile > acetone > ethanol > methanol. So DMF was selected as eluent. Experiments have shown that it is easier to elute the retained ABTR and its Hg(II) chelate in the reverse direction in comparison to the forward direction. Thus, it is also recommended to invert the disks before elution. Only 1.0 mL of eluent was sufficient to elute all the ABTR and its Hg(II) chelate retained at the disks at a flow rate of 5 mL min^{-1}.

Figures and merit

The calibration curve showed that the Beer's law is obeyed in the 0.01 to 3 μg Hg(II) *per* mL concentration range. The linear regression equation obtained was A =0.581 C (μg mL^{-1}) + 0.0126 (r=0.9994). The molar absorptivity was calculated to be 1.21×10^5 L mol^{-1} cm^{-1} at 545 nm. The relative standard deviation (n=11) at a 0.01 μg mL^{-1} concentration level was 1.7%. The detection limit, based on the three times of standard deviation is 0.02 μg L^{-1}, in the original sample solutions.

Composition of the chelate

The composition of the complex was determined by the continuous variation (Figure 3) and molar ratio (Figure 4) methods. Both showed a 1:2 Hg(II) to ABTR molar ratio. A possible chelate structure is suggested in Figure 5.

Interferences

The selectivity of the proposed method was investigated by analysis of 20 μg L^{-1} Hg(II) solutions containing various potentially interfering ions. A relative deviation of ±5% was tolerated and the results are given in Table 2. The results showed that the ions commonly present in Hg determinations do not interfere at a preoccupting concentration range.

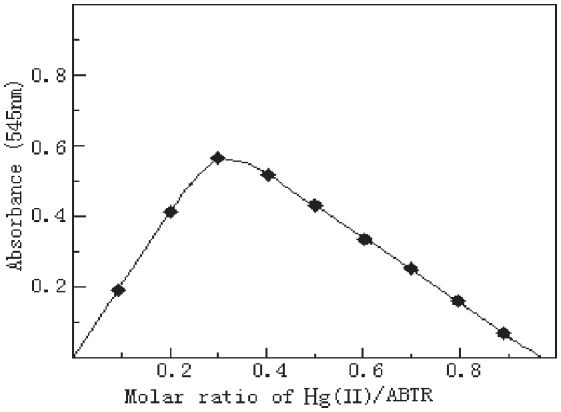

Figure 3. Composition of Hg(II)-ABTR complex by continuous variation method. Hg(II)+ABTR concentration: 1.2×10^5 mol L^{-1}; other conditions as in standard procedure.

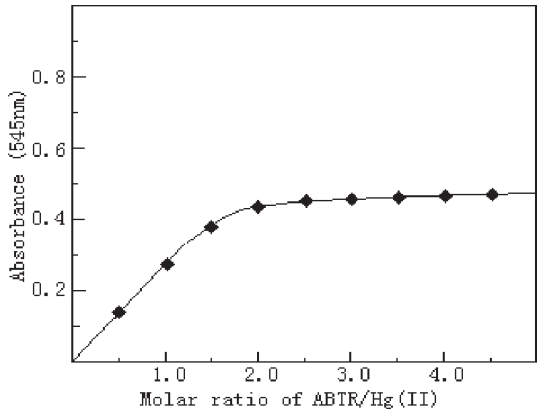

Figure 4. Composition of Hg(I1)-ABTR complex by molar ratio method. Hg(II) concentration: 0.35×10^5 mol L^{-1}; other conditions as in standard procedure.

Application and validation

The proposed method has been successfully applied to the determination of mercury in tobacco and tobacco additives. The digested sample was quantitatively

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

Ion added	Tolerance limit / (mg *per* 50 mL)
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+}, Cu^{2+}	0.5
Ru(III), Bi(III), Pb^{2+}, Sb^{3+}, Th(IV), Br^-, Os(VIII) , I^-	0.2
Se(IV), Te(IV), $S_2O_3^{2-}$, Pd^+, Ag^+	0.1
Ir(IV) , Rh(III), Ru(III)	0.05
Pt(IV), Au^{3+}	0.01
CN^-, SCN^-	0.005

Table 3. Determination of mercury in real samples

Samples	Mercury found / (μg g^{-1})		RSD % / (n=5)	Recovery Range % / (n=5)
	Reference Method	This Method		
Tobacco leaf (SA1)	0.152	0.165	2.4	93 - 98
Tobacco leaf (SA2)	0.186	0.197	2.1	98 - 106
Glycerol (AL)	0.212	0.228	2.3	97 - 105
Tobacco sauce (AM)	0.162	0.157	2.2	101 - 107
Cigarette (SF)	0.217	0.232	2.1	98 - 104
Tobacco essence (AG)	0.176	0.185	2.5	94 - 102

Table 4. The comparison reagents for the spectrophotometric determination of mercury

Reagents	λ_{max} / (nm)	ε / (10^4)	Linear range / (up to μg mL^{-1})	Detect limit / (ng mL^{-1})	Conditions	Interfering ions	Reference
H_2Dz	485	7.1	2	1	pH 2~5, extraction $CHCl_3$	Cu, Zn, Cd, Pb	1
CPDAAB	540	22.2	0.8	20	alkaline medium, pH 10	Co,Zn, Cd, Ni	11
DAA	514	22	0.8	2	pH 9.0, Triton X-100	Cd, Ag, Pb, Ni	13
BG	625	59.6	00.5	-	pH 5, flotation of $Hg\text{-}I^-$-BG with cyclohexane	Ni	14
CMPQ	512	8.0	2	40	pH 4~4.8, Triton X-100	Co, Cu, Pb, Zn, Ni	15
$TPPS_4$	413	27.6	0.6	0.5	pH 8.0, heated in 100 °C water bath for 30nin	Mn, Cu, Ag, Pb, Fe, Co, Ni, Zn, Cd, Mg	16
PAR	500	6.8	2	10	pH 10	Cu, Ni, Co, Fe, Zn, Ag, Cr, V, W, Mo, Mn	17
NBS	430	-	10	0.2	nitric acid medium	Cu, Zn, Cu	18
ABTR	545	12.1	3	0.02	pH 3.5, Emulsifier-OP	CN^-, SCN^-	This Work

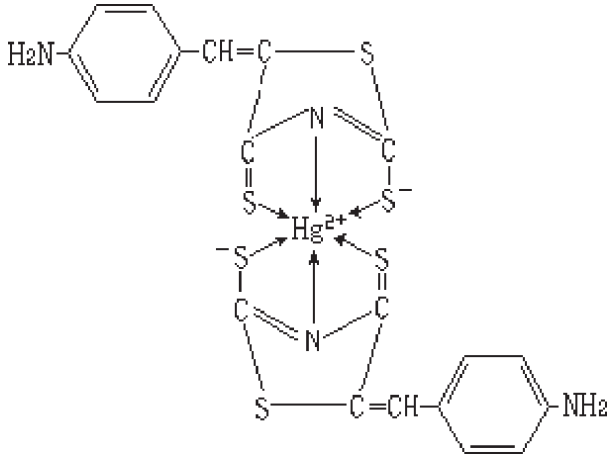

Figure 5. Proposed structure for the Hg(II)-ABTR chelate.

transferred to a 50 mL of volumetric flask and analyzed by the proposed procedure. Recovery tests of 0.2 μg mercury were carried out, as well as the parallel sample analysis by cold vapor atomic absorption spectrometry.[1] The results are shown in Table 3.

Conclusions

A comparison of the proposed the ABTR method with other molecular absorptiometric methods (Table 4) shows that ABTR is a competitive spectrophotometric reagent for mercury determination. The molar absorptivity of the chelate reaches 12.1×10^4 L mol^{-1} cm^{-1}, and no other system achieves this figure at the same low pH range. When masked with pyrophosphoric acid, most of the routine foreign ions do not interfere with the determination in the expected concentration range. By solid phase extraction of the ABTR-Hg(II) chelate with C_{18} disks, an enrichment factor of 50 as well as a detection limit is 0.02 μg L^{-1} in the sample solution were achieved. This is equivalent to 2×10^{-3} μg g^{-1} in the original samples. The consum of organic solvents in this method is much lower than that observed in liquid-liquid extraction methods. Twenty samples can be prepared simultaneously. This is more rapid than that of routine method.

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