MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Environmental Science and Toxicology (ISSN: 2350-2266) Vol. 2(3) pp. 044-052, June, 2014
Available online http://www.meritresearchjournals.org/est/index.htm

Original Research Article

Spectrophotometric determination of trace amount of mercury (II) in dental-unit wastewater and fertilizer samples using the novel reagent 6-{4-(2,4-dihydroxyphenyl)diazenyl)phenyl}-2-oxo-4-phenyl-1,2-dihydropyridine-3-carbonitrile

Alaa S. Amin[*1], Ahmed H. Moustafa[2] and Asmaa El-Haggar[2]

[1]Chemistry Department, Faculty of Science, Benha University, Benha, Egypt

[2] Chemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt

***Corresponding Author's E-mail: asamin2005@hotmail.com
Tel.: +20 552350996; Fax: +20 133222578;**

Abstract

A simple and low cost method was developed and validated for the determination of trace mercury(II) ions in dental-unit wastewater and fertilizer samples. The method was based upon the reaction of mercury(II) ions with the novel reagent 6-{4-(2,4-dihydroxyphenyl)diazenyl)phenyl}-2-oxo-4-phenyl-1,2-dihydropyridine-3-carbonitrile (DDPODC), the formed complex shows an absorption maximum at 444 nm (λ_{max}) in borate buffer of pH 9.0 and SDS. Beer's–Lambert law and Ringbom's plots of the colored Hg–reagent complex were obeyed in the concentration range of 0.1–5.0 and 0.4–4.70 µg mL^{-1} mercury (II) ions, respectively with a relative standard deviation in the range of 1.8 ± 0.7%. The limits of detection (LOD) and quantification (LOQ) of the procedure were 0.3 and 0.98 µg mL^{-1} Hg^{2+}, respectively. The proposed method was applied for the analysis of mercury (II) in dental-unit wastewater and fertilizer samples. The validation of the method was tested by comparison with the data obtained by the inductively coupled plasma-mass spectrometry (ICP-MS). The statistical treatment of data in terms of Student's t-tests and variance ratio F-tests has revealed no significance differences.

Keywords: Mercury (II) determination, Spectrophotometry, Water and Fertilizer analysis, ICP-MS.

INTRODUCTION

Among the heavy metal ions found in environmental waters mercury (II) is one of the most hazardous. It can be taken up by plankton, which is then consumed by fish and finally reach humans, the last destination in the food chain, or be ingested through polluted drinking water. The permissible mercury (II) level in the drinking water set by the WHO is 1.0 µg L^{-1} (Ebdon, et al., 2002). Mercury (II) is essentially nephrotoxic (Peixoto and Pereira, 2007). It has been claimed to inhibit or deactivate the biological functions of several enzymes by binding to the sulfhydryl group of the enzyme (Peixoto *et al.,* 2007; Franciscato *et al.,* 2011). Due to the toxicological effects and potential accumulation of mercury onto human bodies and aquatic organisms, the determination of mercury(II) or organo mercury (II) has seen an upsurge of interest in the last few years (Stone *et al.,* 2003).

The determination of low concentrations of mercury is a vital task. Therefore, considerable efforts and progress have been carried out to develop accurate, low cost and reliable methods for mercury determination in contaminated samples without any complicated processing steps (Hussein and Moghaddam, 2005). The most common techniques in natural samples are ICP-MS (Schmit *et al.,* 1991; Nixon et al., 1999; Iwashita et al., 2007); atomic fluorescence (Vijayakumar et al., 1980; Saouter and Blattmann, 1994; Cava-Montesinos *et al.,* 2004); cold vapour atomic absorption (Navarro and Lopez, 1992; Martine et al., 2009; Manzoori et al., 1998; Zhang and Adeloju, 2008); GC (Nevado *et al.,* 2005); stripping voltammetry (Zhang et al., 1996; Faller et al., 1999); X-ray fluorescence spectrometry (Bennun and Gomez, 1997; Alcalde-Molina *et al.,* 2009); neutron activation analysis (Devi *et al.,* 1991) and atomic fluorescence spectrometry (Shafawi et al., 1999). The determination and chemical speciation of mercury(II) and/or methyl mercury in a series of complicated matrices, e.g. Mushroom from Tokat-Turkey, water and fish have been reported by Tuzen et al. (Tuzen and Soylak, 2006; Tuzen$_a$ *et al.,* 2009). Moreover, the use of Lichen (Xanthoparmelia conspersa) biomass and Streptococcus pyogenes loaded Dowex optipore SD-2 has been reported as efficient materials for the removal of mercury(II) and methylmercury from aqueous media (Tuzen$_b$ *et al.,* 2009; Tuzen$_c$ et al., 2009). Among these techniques, visible absorption spectrophotometry represents the most convenient technique because of the availability of the instrumentation, simplicity, speed, precision, accuracy and low cost. A series of chromogenic reagents has been reported for mercury (II) determination in different samples (Suresha *et al.,* 2002; El-Sayed 1998; Sandell 1959; He *et al.,* 2007; Chatterjee et al., 2002). Most of these methods are suffered from the lack of sensitivity due to the significant interference of the excess of chromogenic reagent with the analyte at λ_{max}. This problem was solved by employing the β-correction spectrophotometric method to calculate the real absorbance of the complex (Abbaspour and Baramakeh, 2002; Cao and Zhang, 1994)

A recent literature on the analytical applications of the entitled reagent 6-{4-(2,4-dihydroxyphenyl)diazenyl) phenyl}-2-oxo-4-phenyl-1,2-dihydropyri-dine-3-carbonitrile (DDPODC) (Fig. 1) has revealed no study on the use of the reagent for mercury(II) determination and/or other trace metal ions. Therefore, the goals of the present manuscript are focused on the synthesis and spectroscopic characterization (UV–Vis, IR and ^{1}H–NMR) of the DDPODC reagent. Moreover, the stiochiometry of the formed mercury (II)- DDPODC chelate was elucidated in an attempt to develop an accurate method for the analysis of mercury(II) in different water and fertilizer samples. The effect of different parameters that control the absorbance of the formed complex was determined.

Experimental

Reagents and materials

Unless otherwise stated, all chemicals and solvents used were of analytical reagent grade and were used without further purification. A stock solution of mercury (1000 µg mL^{-1}) was prepared from mercury(II) chloride (BDH, Poole, England). More diluted standard (0.05–20 µg mL^{-1}) solutions were then prepared by dilution and were stored in low density polyethylene (LDPE) bottles. An appropriated weight of the pure reagent DDPODC (Fig. 1) was dissolved in lest amount of ethanol followed by dilution with absolute ethanol (100 mL). A series of borate buffer (pH 6.5–10.5) were prepared as reported earlier (Vogel, 1966). A 500 mL Sodium dodecyl sulphate (SDS) 0.6 M was prepared by dissolving 86.4 g of pure SDS (Merck, Darmstadt, Germany) in 250-300 mL bidistilled water, sonicated for 15 min and diluted with deionized water when it became transparent.

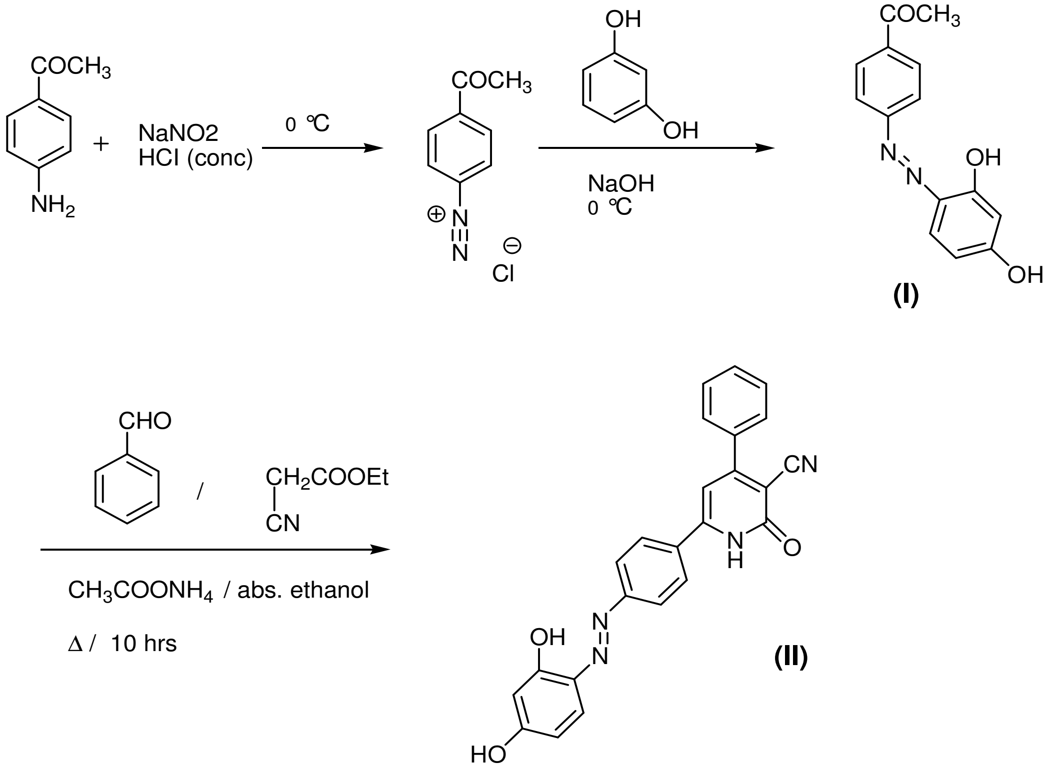
Apparatus

The UV–Vis (190–1100 nm) and IR (200–4000cm−1) spectra were recorded on a Perkin Elmer (Lambda 25, Shelton, CT, USA) and a Perkin Mattson 5000 FTIR spectrophotometers, respectively. The absorbance measurements of the reagent and its mercury(II) complex were also measured with a Perkin Elmer (Lambda 25, USA) spectrophotometer (190–1100 nm) with 10 mm(path width) quartz cell. A Bruker NMR (model VanceDPX400 MHz) was used for recording the ^{1}H–NMR spectra of the reagent in deuterated DMSO solution using TMS as internal standard.

A digital micro-pipette (Volac), an Orion pH-meter (model EA 940) and the scientific melting point SMP1 (UK) were employed for the preparation of the standard and test solutions, pH measurements and melting point, respectively. De-ionized water was obtained from Milli-Q Plus system (Millipore, Bedford, MA, USA) and was used for the preparation of all solutions. Carbon, hydrogen, nitrogen and sulfur content were determined by a Perkin Elmer 2400 C series elemental analyzer, USA. A Perkin Elmer ICP-MS spectrometer (model Elan DRC II, USA) was used under the optimum experimental conditions.

Synthesis of the chromogenic reagent, DDPODC

Firstly compound I was prepared from diazotization of p-aminoacetophenone and coupling with resorcinol at – 5.0 ºC. The reagent DDPODC was prepared as literature procedure by the reaction of diazoaceto-phenone (I)b with benzaldhyde, ethyl cyanoacetate in the presence of ammonium acetate and absolute ethanol on refluxing for10 hrs. The reaction mixture was then cooled, poured



(I)

(II)

Figure 1 and 2. Compounds

onto an ice bath and filtered off. The solid was separated out, washed with ether and acetone, recrystallized from ethanol and finally characterized. (Figure 1, 2)

Recommended procedure

In a series of measuring flasks (10 mL), an appropriate concentration (0.1–5.0 µg mL^{-1}) of mercury (II) solution was added to the reagent solution (2.0 mL, 10^{-3} M). To the test solution, an approximate volume (4.0 mL) of borate buffer of pH 9.0 and 1.0 ml of 0.6 M SDS was added and finally the solution was made up to the mark with distilled water. The solution mixtures were allowed to stand at room temperature for 2.0 min before measuring the absorbance at 444 nm.

Analytical application

Determination of mercury (II) in tap and mineral water

Tap water collected from the laboratories of Chemistry Department, Benha University, Benha city, Egypt, and mineral water, commercially available in Egyptian market, were filtered through 0.45 µm cellulose membrane filter prior to analysis and stored in LDPE sample bottles (250 mL). The recommended general spectrophotometric procedure used to prepare the standard curve was followed.

Analysis of mercury in dental-unit (DU) wastewater

DU wastewater samples were collected from dental chair, Benha Hospital, Zagazig city, Egypt, at the end of working day. An accurate volume of sample was digested by UV-digester in the presence of suitable volumes of both concentrated HNO_3 and H_2O_2 (30%) for one hr. the obtained solution was neutralized by NaOH (5.0 M) and 10 mL of this solution was treated under the conditions of recommended procedure.

Determination of mercury (II) in fertilizer

In a 50 mL beaker, an accurate weight (4.50–5.70 g) of the local fertilizer (Broxals) was dissolved in de-ionized water after constant stirring for few minutes. The aqueous solution was then completed to 250 mL with double distilled water. An accurate volume of the test solution (5.0 mL) was then adjusted to pH 9.0 with borate buffer, transferred into volumetric flask (25 mL) in the presence of the reagent (2.0 mL, 10^{-3} M) and various concentrations (0.2 – 5.0 µg mL^{-1}) of mercury (II) were

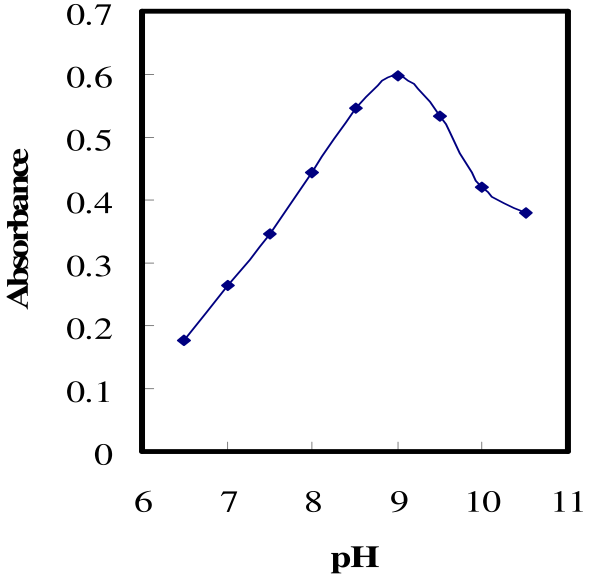


Figure 3. Effect of borate buffer solution on the complexation of 3.0 µg mL^{-1} Hg (II) with DDPODC

added separately. The solutions were then completed to the mark with doubly de-ionized water and the absorbance of the test solutions was measured under the conditions of recommended procedure.

RESULTS AND DISCUSSION

The characteristics IR vibrations of the solid reagent in KBr disk are observed at 3525-3340 cm^{-1} (2 O-H); 2219 cm^{-1} (C=N); 1658 cm^{-1} (C=O); and 1597 cm^{-1} (N=N). ^{1}H–NMR spectrum of the reagent in d6-DMSO show signals at δ = 6.67 (s, 1H, pyridine ring); 6.99 – 8.08 (m, 12H, Ar-H), and 12.05 (t, 1H, NH). Elemental analysis of the reagent after solvent evaporation and crystallization from ethanol for the structure [$C_{24}H_{16}N_4O_3$] (408.41); required: C, 70.58; H, 3.95; N, 13.72; whereas found is C, 70.59; H, 3.92; and N, 13.70.

Absorption spectra of the reagent and its mercury(II) complex

Preliminary screening investigation on the interaction of the title reagent DDPODC (Figure 1) with mercury (II) ions in the aqueous media and shaking has revealed the formation of a red colored complex. The absorption electronic spectra of the reagent and its mercury (II) complex are shown in Figure 2. The spectrum of the reagent versus water, showed one well defined peak at 392 nm (λ1), while in the spectrum of its mercury(II) complex against the reagent blank at pH 9.0 a well defined absorption peak (λ2) at 444 nm.

Optimization of the system

To take full advantage of the procedure, the reagent concentrations and reaction conditions must be optimized. Various experimental parameters were studied in order to obtain optimum conditions. These parameters were optimized by setting all parameters to be constant and optimizing one each time.

The effect of pH on the absorbance at a constant concentration of complex was investigated in the range of 2.56–12.41. The absorbance of the Hg (II) – DDPODC at 444 nm was studied against the reagent blank. The absorbance was nearly constant in the pH range of 8.5–9.5. Therefore, pH 9.0 was selected as optimal (Figure 3). Moreover the amount of pH 9.0 was studied to select the optimum volume. A 4.0 mL of pH 9.0 gave the highest absorbance value, which selected for all further studies.

Effect of DDPODC concentration on determination of copper was investigated in the range of 1.0–5.0 × 10^{-4} M. The sensitivity of the method increased by increasing DDPODC concentration up to 2.0 × 10^{-4} M (Figure 4) and decreased at higher concentrations. It was expected that increasing DDPODC causes an increase in the absorbance of complex, because increasing in DDPODC concentration caused an increase in concentration of the complex. At concentrations higher than 2.5 × 10^{-4} M, the concentration of uncomplexed DDPODC increases significantly. Therefore, much probably decrease of absorbance change at concentrations higher than 2.5 × 10^{-4} M is due to this fact that the free DDPODC competes with the complexes. A concentration of 2.0 × 10^{-4} M of DDPODC was selected as the optimum.

Examining different types of nonionic, cationic and anionic surfactants reveals that anionic ones increase the

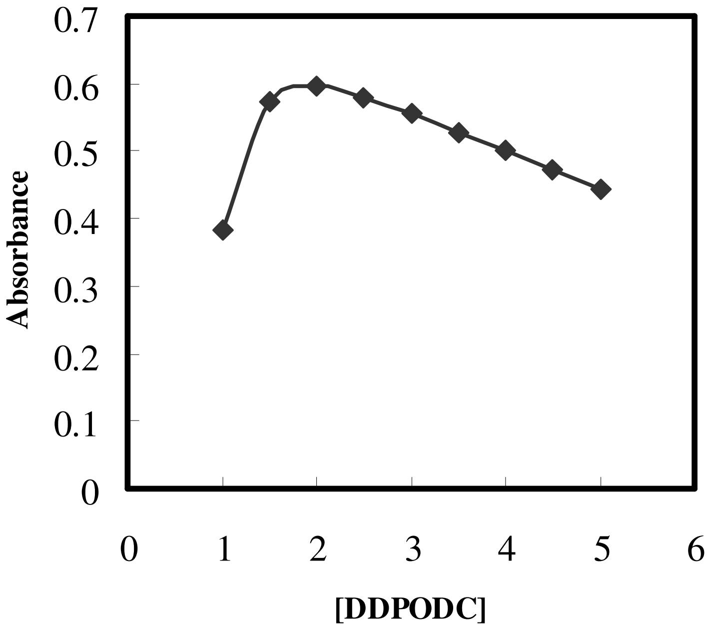


Figure 4. Effect of (DDPODC) on the complex formation of 3.0 µg mL^{-1} Hg (II) at the optimum conditions

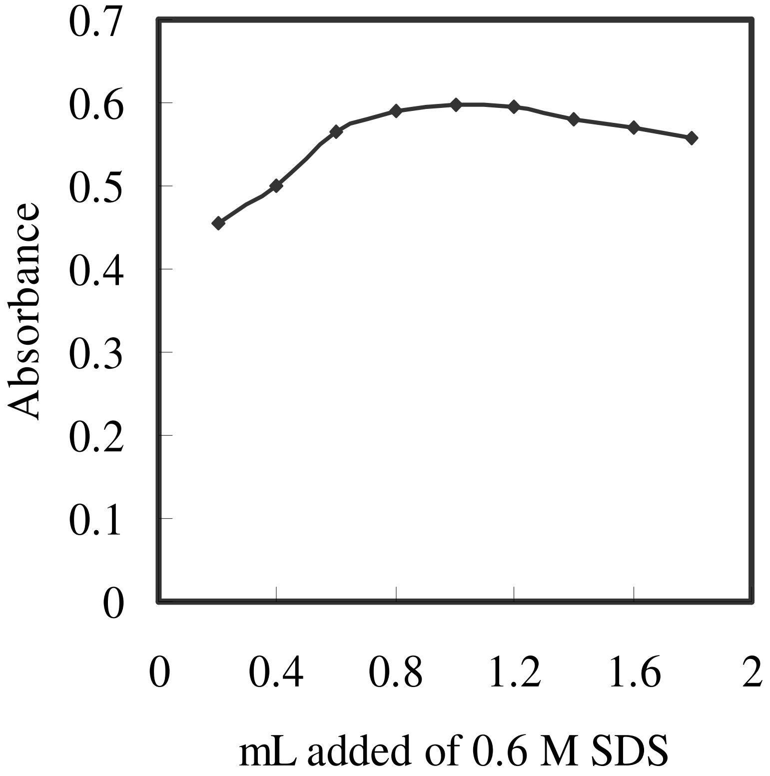


Figure 5. Effect of (SDS) on complexation of 3.0 µg mL^{-1} Hg (II) at the optimum conditions

absorbance and solubility of the formed complex. SDS was found the best anionic surfactant to be used. In 0.6 M SDS maximum absorbance is obtained. Moreover a different volume of 0.6 M SDS was examined to a fixed metal ion concentration and the absorbance was measured according to the recommended procedure. A

Table 1. Analytical features of the proposed method

Parameters	Proposed method
Amount of buffer	4.0 mL
pH	9.0
Optimum [BTAHQ]	2.0 × 10^{-4} M
0.6 M SDS	1.0 mL
Reaction time (min)	2.0
Beer's range (µg mL$^{-1)}$	0.1 – 5.0
Ringbom range (µg L$^{-1)}$	0.4 – 4.70
Molar absorptivity (L mol^{-1} cm^{-1})	2.01 × 10^{4}
Sandell sensitivity (ng cm^{-2})	1.02
Regression equation[a]	
Intercept	- 0.005
Slope	0.12
intercept	- 0.004
Correlation coefficient (*r*)	0.9992
RSD [a] (%)	1.75
Detection limits (µg mL^{-1})	0.30
Quantification limits (µg mL^{-1})	0.98

[a] A = a + bC, where C is the concentration in µg mL^{-1} and A is the absorbance units.

1.0 ml of 0.6 M SDS gave the highest absorbance. For all subsequent measurements 1.0 mL 0.6 M SDS was used (Figure 5).

Effect of time on the reaction procedure was investigated. The results showed that complexation reaction was completed in 2.0 min. Raising the temperature upto 60 ºC has no effect on the complex formation.

Stiochiometry of the mercury(II) complex

The chemical structure of the produced mercury (II) complex species was determined by the method of continuous variations at various concentrations of the mercury (II) ions and reagent (Sawyer, et al., 1984). A plot of the absorbance of the produced colored solution at 444 nm versus the mole fraction of the reagent revealed a graph that indicated the formation of complex having mercury (II) to a reagent molar ratio of 1:1. These data confirmed that, the colored species is most likely fit with the molecular formula of mercury (II)–reagent.

The stability of the complexes was evaluated. The formation of the complexes was rapid and the orange color was stable at least for 15 hrs without any change in color intensity and with the maximum absorbance at room temperature. The conditional stability constant of the complex was calculated from the continuous variation data using the Harvey equation (ICH Harmonized Tripartite Guideline, 2005). The conditional stability constant was found to be 8.84.

Interference study

The determination of mercury(II) ions at concentration 3.0 µg mL^{-1} in the presence of a relatively high excess (0.05–1.0 mg) of some diverse ions relevant to water, e.g. alkali and alkaline earth metals, Al^{3+}, Ag^{+}, Au^{3+}, Ni^{2+}, Co^{2+}, Cd^{2+}, Fe^{3+}, VO_3^{-}, AsO_2^{-}, SO_4^{2-}, and PO_4^{3-} ions was critically investigated by the developed procedure. The tolerance limit (w/w) was defined as the concentration of the divers ions added causing a relative error within ± 5.0 % in the true absorbance of mercury(II)– DDPODC complex. The results revealed that the presence of large amounts of the following foreign ions: Ag^{+}, Ca^{2+}, NH_4^{+}, Li^{+} and Mg^{2+} and the anions PO_4^{-3}, BO_4^{-3} CH_3COO^{-} and NO_3^{-} did not cause any significant change in the corrected absorbance of the Hg complex even at 1:2500 tolerable concentrations of Hg(II) to the foreign ions, respectively. The ions Co^{2+}, Ni^{2+}, Cd^{2+}, Au^{3+}, F^{-} and Cl^{-} at 500-fold excess to the mercury(II) ions also did not interfere. The ions Pb^{2+} and Fe^{3+} at concentrations 150 times higher than those of the analyte interfered seriously. Addition of few drops of NaCl (0.1%, w/v) and NaF (1.0%, w/v) to the aqueous solution eliminates the positive interferences

Analytical characteristics

Table 1 summarizes the analytical characteristics of the optimized method, including regression equation, linear range and limit of detection, and reproducibility. The limit of detection, defined as $C_L = 3S_B/m$ (where C_L, S_B, and m are the limit of detection, standard deviation of the blank

Table 2. Analysis of mercury(II) ions in tap, mineral, DU wastewater and fertilizer samples by the developed (A) and the ICP-MS (B) methods [a].

Water sample	Hg(II) added (µg mL^{-1})	Hg(II) found (µg mL^{-1})[a]		Recovery (%)[a]		t-test[b]	F-value[b]
		A	B	A	B		
Tap water	–	ND	ND	–	–	–	–
	1.5	1.51 ± 0.14	1.57 ± 0.45	100.67	104.67	1.32	2.97
	3.0	3.05 ± 0.21	2.94 ± 0.63	101.67	98.00	1.07	2.74
	4.5	4.44 ± 0.27	4.60 ± 0.32	98.67	102.22	1.36	3.11
Mineral water	0.0	ND	ND	–	–		
	1.2	1.21 ± 0.32	1.18 ± 0.41	100.83	98.33	1.22	2.87
	2.4	2.38 ± 0.18	2.44 ± 0.63	99.17	101.67	0.98	2.37
	3.6	3.65 ± 0.40	3.50 ± 0.57	101.39	97.22	1.19	2.67
DU waste water	0.0	3.83 ± 0.27	3.76 ± 0.56	–	–		
	0.4	4.25 ± 0.34	4.15 ± 0.72	100.47	99.76	1.43	3.38
	0.8	4.60 ± 0.17	4.70 ± 0.47	99.35	103.07	1.51	3.52
	1.2	5.00 ± 0.25	5.15 ± 0.59	99.40	103.83	1.37	3.16
Broxal	0.0	ND	ND	–	–		
	1.6	1.65 ± 0.32	1.54 ± 0.73	103.13	96.25	1.06	2.66
	3.2	3.26 ± 0.15	3.30 ± 0.64	101.88	103.13	1.34	3.04
	4.8	4.88 ±0.38	4.70 ± 0.56	101.67	97.92	1.27	2.71

[a] Average of six measurements ± standard deviation.
[b] The theoretical values of t- and F- at P = 0.05 are 2.57 and 5.05, respectively.

and slope of the calibration graph, respectively), was 0.3 µg mL^{-1}. The relative standard deviation (RSD) and relative error for six replicate measurements of 2.0 µg mL^{-1} of copper was 1.76 and 1.34 and for 4.0 ng mL^{-1} of Hg (II) was 1.67 and 1.32%, respectively.

Validation and analytical applications of the developed method

The validity of the proposed method was tested by the analysis of mercury(II) in tap, mineral and DU wastewater samples. For this purpose, different concentrations of mercury(II) ions at concentration range 0.1–5.0 µg mL^{-1} were spiked onto the tested water samples. The mercury content in each sample was then determined via the developed method and the results are summarized in Table 2. The obtained results were compared with the standard

ICP-MS method in terms of Student's t-test and F-test (Miller and Miller, 2005). The results summarized in Table 2 revealed that, the percentage recoveries of both methods were in good agreement and always higher than 95% confirming the accuracy of developed procedure and its independence from matrix. Moreover, the validity of the proposed method was also tested by the analysis of mercury(II) on the Broxal fertilizer under the conditions described in Section 2.5.2. The spiked mercury (II) concentration was determined via the standard addition curve and the results were successfully compared with the value of mercury (II) determined by ICP-MS (Table 2).

CONCLUSIONS

The method described provides a simple and reliable means of determination of trace amounts of mercury(II) ions in aqueous media by spectrophotometry. The method is sensitive ($\varepsilon = 2.01 \times 10^4$ L mol^{-1} cm^{-1}), inexpensive and less toxic than most of the reported extractive spectrophotometric methods (Mudakavi, 1984; Murti and Khopkar, 1977; Tsubouchi, 1970; Saad and Sultan, 1994; Ahmed and Alam, 2003). Moreover, the method also has the advantage of virtual freedom from interference from extraneous ions. Thus, it can act as an alternative approach to the widely used flameless AAS and ICP-OES in rapid and precise determination of trace amounts of mercury in natural water and industrial effluent samples. The method requires no complex pretreatment of chromatographic separations and/or preconcentration of the analyte.

REFERENCES

Abbaspour A, L Baramakeh (2002). "Dual-wavelength β-correction spectro-photometry for selective determination of Zr," Talanta, vol. 57, pp. 807–812.

Ahmed MJ, MS Alam (2003). "A rapid spectrophotometric method for the determination of mercury in environmental, biological, soil and plant samples using diphenylthiocarbazone," Spectroscopy, vol. 17, pp. 45–52.

Alcalde-Molina M, J Ruiz-Jimenez, MD Luque de Castro (2009). Automated determination of mercury and arsenic in extracts from ancient papers by integration of solid-phase extraction and energy dispersive X-ray fluorescence detection using a lab-on-valve system," Anal. Chim. Acta, vol. 652, pp. 148–153.

Bennun L, J Gomez (1997). "Determination of mercury by total reflection X-ray fluorescence using amalgamation with gold," Spectrochim. Acta, Vol. 52B, pp. 1195–1200.

Cao H, PF Zhang (1994). "Determination of antimony in waste water with chromazurol S by beta-correction spectrophotometry," Analyst, vol. 119, pp. 2109–2111.

Cava-Montesinos P, E Rodenas-Torralba, A Morales-Rubio, ML Cervera, M de la Guardia (2004). "Cold vapour atomic fluorescence determination of mercury in milk by slurry sampling using multicommutation," Anal. Chim. Acta, vol. 506, pp. 145–153.

Chatterjee S, A Pillai, VK Gupta (2002). "Spectrophotometric determination of mercury in environmental samples and fungicides based on its complex with *O*-carboxy phenyl diazoamino *P*-azobenzene," Talanta, vol. 57, pp. 461–465.

Devi PR, T Gangaiah, GRK Naidu (1991). "Determination of trace metals in water by neutron activation analysis after preconcentration on poly(acrylamidoxime) resin," Anal. Chim. Acta, vol. 12, pp. 533–537.

Ebdon L, ME Foulkes, SL Roux, R Munoz-Olivas (2002). "Cold vapour atomic fluorescence spectrometry and gas chromatography-pyrolysis-atomic fluorescence spectrometry for routine determination of total and organometallic mercury in food samples," Analyst, vol. 127, pp. 1108–1114.

El-Sayed AY (1998). "Zero-crossing derivative spectrophotometric determina-tion of mercury(II) and palladium(II) with 5-(3,4-methoxyhydroxyphenyl-methylene)-2-thioxo-1,3-thiazolidine and cetylmethylammonium bromide," Anal. Lett., Vol. 31, pp. 1905–1916.

Faller C, NY Stojke, G Henze, KZ Brainina (1999). "Stripping voltammetric determination of mercury at modified solid electrode: determination of mercury traces using PDC/Au(III) modified electrode," Anal. Chim. Acta, vol. 396, pp. 195–202.

Franciscato, C, L Moraes-Silva, FA Duarte, CS Oliveira, RP Ineu, EMM Flores, VL Dressler, NC Peixoto, ME Pereira (2001). "Delayed biochemical changes induced by mercury intoxication are prevented by zinc pre-exposure," Ecotoxicol. Environ. Saf., vol. 74, pp. 480–486.

He X-L, Y-Q Wang, KQ Ling (2007). "Synthesis of a novel bistriazene reagent 4,4-bis [3-(4-phenylthiazol-2-yl)triazenyl] biphenyl and its highly sensitive color reaction with mercury (II)," Talanta, vol. 72, pp. 747–754.

Hussein M, H Moghaddam (2005). "Sensitized extraction spectrophotometric determination of Hg(II) with dithizone after its flotation as ion-associate using iodide and ferrioin," Talanta, vol. 67, pp. 555–559.

International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2(R1), (2005). Complementary Guideline on Methodology dated 06 November 1996, ICH, London, UK.

Iwashita A, T Nakajima, H Takanashi, A Ohki, Y Fujita, T Yamashita (2007). "Determination of trace elements in coal and coal fly ash by joint-use of ICP-AES and atomic absorption spectrometry," Talanta, vol. 71, pp. 251–257.

Manzoori JL, MH Sorouraddin, AMH Shabani (1998). "Determination of mercury by cold vapor atomic absorption spectrometry after preconcentration with dithizone immobilized on surfactant-coated alumina," J. Anal. Atom. Spectrom., vol. 13, pp. 305–308.

Martine EM, P Berton, RA Olsina, JC Altamirano, RG Wuilloud (2009). "Trace mercury determination in drinking and natural water samples by room temperature ionic liquid based-preconcentration and flow injection-cold vapor atomic absorption spectrometry," J. Hazard. Mater., vol. 167, pp. 475–481.

Miller JN, JC Miller (2005). *"Statistics and Chemometrics for Analytical Chemistry* 5th edn, Chapman & Hall/CRC, London, UK.

Mudakavi JR (1984). "Spectrophotometric determination of trace amounts of mercury with phenanthroline and eosin," Analyst, vol. 109, pp. 1577–1579.

Murti MVR, SM Khopkar (1977). "Extractive spectrophotometric determination of mercury with thiobenzoylacetone: analysis of waste water," Bull. Chem. Soc. Jpn., vol. 50, pp. 738–741.

Nakamoto K (1978). "Infrared and Raman Spectra of Inorganic and Coordination Compounds," 3rd edn., Wiley, New York, p. 305.

Navarro M, MC Lopez (1992). "Microwave dissolution for the determination of mercury in fish by cold vapor atomic absorption spectrometry," Anal. Chim. Acta, vol. 257, pp. 155–162.

Nevado J, RC Marti-Domeadios, FJ Bernado, MJ Moreno (2005). "Determination of mercury species in fish reference material by gas chromatography-atomic fluorescence detection after closed-vessel microwave-assisted extraction," J. Chromatogr. A, vol. 1093, pp. 21–28.

Nixon DE, MF Burrit, TP Moyer (1999). "The determination of mercury in whole blood and urine by inductively coupled plasma mass spectrometry," Spectrochim. Acta: Atom. Spectrosc., vol. 54, pp. 1141–1153.

Peixoto NC, ME Pereira (2007). "Effectiveness of $ZnCl_2$ in protecting against nephrotoxicity induced by $HgCl_2$ in newborn rats," Ecotoxicol. Environ. Saf. Vol. 66, pp. 441–446.

Peixoto, NC, CP Kratz, T Roza, VM Morsch, ME Pereira, (2007). "Effects of $HgCl_2$ on porphobilinogen-synthase (E.C.4.2.1.24) activity and on mercury levels in rats exposed during different precocious periods of postnatal life," Cell Biol. Int., vol. 31, pp. 1057–1062.

Saad B, SM Sultan (1994). "Extraction spectrophotometric determination of mercury (II) using thiacrown ethers and Bromocresol Green," Talanta, vol. 42, pp. 1349–1354.

Sandell EB (1959). Colorimetric Method of Analysis, Wiley Interscience, New York, pp. 445.

Saouter E, B Blattmann (1994). "Analyses of organic and inorganic mercury by atomic fluorescence spectrometry using a semiautomatic analytical system," Anal. Chem., vol. 66, pp. 2031–2037.

Sawyer DT, WR Heinemann, JM Beebe (1984). Chemistry Experiments for Instrumental Methods," John Wiley & Sons,; Ringbom A (1939). Fresen. Z. Anal. Chem., vol. 155, pp. 332.

Schmit J, M Youla, Y Gelinas (1991). Multi-element analysis of biological tissues by inductively coupled plasma mass spectrometry," Anal. Chim. Acta, vol. 249, pp. 495–501.

Shafawi A, L Ebdon, M Foulkes, P Stockwell, W Corns (1999) "determination of total mercury in hydrocarbons and natural gas condensate by atomic fluorescence spectrometry," Analyst, vol. 124, pp. 185–189.

Stone ME, ME Cohen, L Liang, P Pang (2003). "Determination of methyl mercury in dental-unit wastewater," Dental Materials, vol. 19, pp. 675–679.

Suresha S, MF Silwadi, AA Syed, (2002). "Sensitive and selective spectrophotometric determination of Hg(II), Ni(II), Cu(II), and Co(II) using iminodibenzyl and 3-chloroiminodibenzyl as new reagent and their application to industrial effluents and soil samples," Int. J. Environ. Anal. Chem., vol. 82, pp. 275–289.

Tsubouchi M (1970). "Spectrophotometric determination of mercury (II) by solvent extraction with variamine blue B," Bull. Chem. Soc. Jpn., vol. 43, pp. 2812–2817.

Tuzen M, M Soylak (2005). "Mercury contamination in Mushroom samples from Tokat-Turkey," Bull. Environ. Contam. Toxicol., vol. 74, pp. 968–972.

$Tuzen_a$ M, I Karaman, D Citak, M Soylak (2009). "Mercury(II) and methylmercury determination in water and fish samples by using solid phase extraction and cold vapour atomic absorption spectrometry combination," Food Chem. Toxicol., vol. 47, pp. 1648–1652.

$Tuzen_b$ M, A Sari, . Mendil, M soylak (2009). "Biosorptive removal of mercury from aqueous solution using Lichen (Xanthoparmelia Conspersa) biomass, kinetic and equilibrium studies," J. Hazard. Mater., vol. 169, pp. 263–270.

$Tuzen_c$ M, I Karaman, D Citak, M soylak (2009). "Mercury and methylmercury speciation on Streptococcus pyogenes loaded Dowex optipore SD-2," J. Hazard. Mater., vol. 169, pp. 345–350.

Vijayakumar M, TV Ramakrishna, G Aravamudan (1980). "Fluorimetric determination of trace quantities of mercury as an ion-association complex with rhodamine 6G in the presence of iodide," Talanta, vol. 27, pp. 911–913.

Vogel AI (1966). "*Quantitative inorganic analysis*," 3rd edn., Longmans Group Ltd, England.

Zhang Y, SB Adeloju (2008). "A novel sequential injection-cold vapour atomic absorption spectrometric system for rapid and reliable determination of mercury," Talanta, vol. 74, pp. 951–957.

Zhang Z, H Liu, H Zhang, Y Li (1996). "Simultaneous cathodic stripping voltammetric determination of mercury, cobalt, nickel, and palladium by mixed binder carbon paste electrode containing dimethylglyoxime," Anal. Chim. Acta, vol. 333, pp. 119–124.