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A rapid spectrophotometric method for the determination of mercury in environmental, biological, soil and plant samples using diphenylthiocarbazone

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Abstract. A simple, sensitive and highly selective direct spectrophotometric method for the determination of trace levels of mercury(II) in various samples is described. Diphenylthiocarbazone (dithizone) reacts in slightly acidic 50% aqueous 1,4-dioxane media (0.18–1.80 M sulphuric acid) with mercury(II) to give an orange chelate which has an absorption maximum at 488 nm. The average molar absorption co-efficient and Sandell's sensitivity were found to be $2.5 \times 10^4\,\mathrm{l\,mol^{-1}\,cm^{-1}}$ and 0.015 μg of Hg(II) $\mathrm{cm^{-2}}$, respectively. The reaction is immediate and absorbance remains stable for over 24 h. Beer's law is obeyed for concentration range of mercury(II) between 0.1 $\mu\mathrm{g\,ml^{-1}}$ and 25 $\mu\mathrm{g\,ml^{-1}}$; the stoichiometric composition of the chelate is 1 : 2 (mercury : dithizone). The various analytical parameters, such as effect of time, acidity, reagent concentration and foreign species, were studied. The method was applied successfully to a number of environmental waters (portable and polluted), biological samples (human blood, urine and fish), soils, plant samples (potato, cabbage, lettuce, carrot and tomato), solutions containing both mercury(I) and mercury(II) and complex synthetic mixtures. The method is very simple and requires no solvent extraction or pre-concentration steps.

Keywords: Non-extractive spectrophotometry, mercury(II) : dithizone, environmental, biological and plant samples

1. Introduction

Mercury is an extremely toxic metal [1] and symptoms of mercury (methyl mercury) poisoning include instantaneous neurological damages [1] particularly irritability, paralysis, insanity, blindness, chromosome damage and birth defects [2]. One example of acute mercury poisoning is the "Minemata disease" which causes mental disturbance, loss of balance, speech, sight and hearing difficulty in swallowing and degeneration of brain [2]. The toxicity of mercury depends on its chemical state [3]. Some forms of mercury are relatively non-toxic and have been used as medicines, e.g., for the treatment of syphilis [4]. Speciation of mercury at trace and ultra-trace levels is a matter of current interest [4].

Mercury enters the environment mainly through human activities [5]. The chief sources of mercury pollution are chlor-alkali plants, paper, pulp, cellulose and plastic industries, electrical, paint, pharmaceutical industries, etc. Uses of mercury as fungicides, pesticides, etc., also add mercury to the environment [5]. Mercury is also believed to be the most dangerous of all the metal contaminants which may

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be present in our daily foods [5]. Compounds of mercury consumed in fish, cereals and other food stuffs have resulted in numerous poisoning [6].

At present few analytical techniques with sufficient sensitivity and selectivity are available for the determination and speciation of trace and ultra-trace levels of mercury in environmental and biological samples. Some form of preliminary separation, solvent extraction and pre-concentration is required to determine the low levels of individual mercury species by sensitive techniques such as cold vapour atomic absorption spectrphotometry [7] and neutron activation analysis [8]. Although these sophisticated techniques are available for the determination of mercury at trace levels in numerous complex materials, factors such as the low cost of instrument, easy handling, lack of requirement for consumables and almost no maintenance have caused spectrophotometry to remain a popular technique, particularly in laboratories of developing countries with limited budgets. The colorimetric solvent extractive method [9–11] employing orange complex of dithizone is still a valuable method for analyzing mercury(II). However, this method [9–11] is lengthy and time consuming, require large amounts of reagents, is pH dependent and it lacks selectivity due to much interferences [12,13] (e.g., Pt(IV), Pd(II), Au(III), Ag(I), Cu(II), Pb(II), Cd(II), etc.). The method described here records for the first time the non-extractive direct spectrophotometric determination of mercury(II) in aqueous media without recourse of any "clean-up" step. This method is far more selective (virtually specific) sensitive, non-extractive simple and rapid than all the existing spectrophotometric [10,11,14] methods.

2. Experimental section

2.1. Apparatus

A Shimadzu (Model-160) double beam UV-visible recording spectrophotometer and Philips PW 9418 pH-meter with a combination electrodes were used for measurements of absorbance and pH, respectively. A Perkin-Elmer (Model-560) atomic absorption spectrometer equipped with a cold vapour technique (whereby free mercury was produced from an aqueous solution by reducing with Sn(II) or hydroxylamine) was used for comparison of the results.

2.2. Reagents and solutions

All the chemicals used were of analytical-reagent grade or the highest purity available (e.g., Aldrich ACS or Merck pro-analysis grade). Triply distilled 1,4-dioxane and de-ionized water were used throughout.

Diphenylthiocarbazone (dithizone) 1.56×10^{-3} *M:* This solution was prepared by dissolving the requisite amount of diphenylthiocarbazone (Aldrich Chemical Co. Ltd., Proanalysis) in a known volume of triply distilled 1,4-dioxane. More dilute solutions of the reagent were prepared as required.

Mercury(II) standard solutions: A 100 ml stock solution (4.99×10^{-3} M) of divalent mercury was prepared by dissolving 135 mg of mercuric chloride (E. Merck. Proanalysis) in de-ionized water. Aliquots of this solution were standardized with EDTA using xylenol orange as indicator. Diluted standard solutions were prepared from this stock solution as and when required.

Mercury(I) stock solution: 100 ml of mercury(I) stock solution (10^{-4} M) was prepared by treating a 10 ml aliquot of stock solution of mercury(II) with a few crystals of hydroxylamine hydrochloride in dilute sulphuric acid (10 ml), followed by complete removal of the hydrochloride by boiling and diluting with de-ionized water to 100 ml.

Potassium permanganate solution: A 1% potassium permanganate solution (Merck) solution was prepared by dissolving requisite amount in de-ionized water. Sodium azide solution (2.5% w/v) (Fluka purity 99%) was also used.

Tartrate solution: A 100 ml stock solution of tartrate (0.01% w/v) was prepared by dissolving 10 mg of ACS grade (99%) potassium sodium tartrate tetrahydrate in (100 ml) de-ionized water.

EDTA solution: A 100 ml stock solution of EDTA (0.01% w/v) was prepared by dissolving 10 mg of A. C. S. grade ($\geqslant$99%) ethylenediaminetetraacetic acid, disodium salt dihydrate in (100 ml) de-ionized water.

Other solutions: Solutions of a large number of inorganic ions (such as Cu(II), Cd, Ni(II), etc.) and complexing agents (EDTA, tartrate, SCN^-) were prepared from their water soluble salts (or oxides and carbonates in hydrochloric acid); those of niobium, tantalum, titanium, zirconium and hafnium were prepared from their corresponding oxides (Specpure, Johnson Matthey) according to the recommended procedures of Mukherji [15].

2.3. Procedure

A series of standard solution of a neutral aqueous solution containing 1–250 μg of mercury(II) in a 10-ml volumetric flask was mixed with 0.8 ml of 1.45×10^{-3} M of the diphenylthiocarbazone reagent solution followed by the addition 0.1 ml of 4.5 M sulphuric acid (0.45 M). After 1 min, 5 ml of 1,4-dioxane was added and the mixture was diluted to the mark with de-ionized water. The absorbance was measured at 488 nm against a corresponding reagent blank. The mercury content in an unknown sample was determined using a concurrently prepared calibration graph.

3. Results and discussion

3.1. Factors affecting the absorbance

Absorption spectra: The absorption spectra of Hg(II)–dithizone system in 4.5 M H_2SO_4 medium was recorded using the spectrophotometer. The absorption spectra of the Hg(II)-dithizone as a symmetric curve with maximum absorbance at 488 nm and average molar absorption co-efficient of $2.5 \times 10^4\,\mathrm{l\,mol^{-1}\,cm^{-1}}$. The reagent blank exhibited negligible absorbance despite having wavelength in the same region. The reaction mechanism of the present method is as reported earlier [1].

Effect of solvent: Chloroform, benzene, carbon tetrachloride, isobutanol, ethanol and 1,4-dioxane were tested as solvents for the system. No absorbance was observed in the organic phase with the exception of chloroform and carbon tetrachloride. In $50 \pm 2\%$ (v/v) 1,4-dioxane medium, however, maximum absorbance was observed; hence a 50% 1,4-dioxane solution was used in the determination procedure.

Effect of acidity: As H_2SO_4 was found previously to be the most suitable acid [10,14] for determination of mercury, the proposed procedure for the spectrophotometric determination of mercury in aqueous solution with dithizone included H_2SO_4. However, the effects of H_3PO_4, H_2SO_4 and HNO_3 on the absorbance of the Hg(II)-dithizone system were studied separately after the addition of 0.05–4.0 ml of 6.66 M H_3PO_4, 0.01–0.9 ml of 4.5 M H_2SO_4, 0.02–3.0 ml of 7 M HNO_3 to each 10 ml of the test solution. Maximum and constant absorbance was obtained in the presence of 0.1–2.0 ml of 6.66 M H_3PO_4, 0.04–0.4 ml of 4.5 M H_2SO_4 or 0.2–2.5 ml of 7 M HNO_3. The optimum acidity range in final dilution is therefore 0.18 M H_2SO_4. For all subsequent measurements 0.1 ml of 4.5 M sulphuric acid was used.

Table 1
Selected analytical parameters obtained with optimization experiments

Parameter	Studied range	Selected value
Wavelength, λ_{max} (nm)	200–800	488
Acidity, H_2SO_4 (M)	0.01–3.0	0.18–1.8
Time (h)	0–48	24
Temperature (°C)	1–50	25 ± 5
Reagent [fold molar excesses (M : R)]	1 : 6−1 : 50	1 : 12−1 : 36
Linear range (μg ml^{-1})	0.01–100	0.1–25
Detection limit (ng ml^{-1})	1–100	20
Reproducibility (% RSD)	0–10	0–2.5

Effect of time: The reaction is very fast and constant absorbance is achieved within 1 min that remains unaltered for 24 h.

Effect of reagent: The excess of reagent is not critical, in tests with μg ml^{-1} Hg(II), the mercury/reagent mole ratio was varied from 1 : 6 to 1 : 48. Constant maximum absorbance was obtained for mole ratios between 1 : 12 and 1 : 36 within the prescribed acidity.

Effect of metal concentration (calibration graph and Beer's law): The well known equation [19] for spectrophotometric analysis in very dilute solution, derived from Beer's law, Plots of absorbance against mercury(II) concentration were linear and passed through the origin for a wide range (0.1 μg ml^{-1} to 25 μg ml^{-1}) of mercury concentrations, when three different scale expansions were used. The average molar absorption co-efficient and the Sandell's sensitivity [20] were found to be 2.5×10^4 l mol^{-1} cm^{-1} and 0.015 μg of Hg(II) cm^{-2}, respectively. The selected analytical parameters obtained with the optimization experiments are summarized in Table 1.

Precision and accuracy: The precision of the present method was evaluated by determining different concentrations of mercury (each analyzed at least 5 times). The relative standard deviation ($n = 5$) was 0.0–2.5 for 1–250 μg of mercury(II) in 10.0 ml, indicating that this method is highly precise and reproducible. The detection limit (3 s of the blank) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for mercury(II) were found to be 20 ng ml^{-1} and 15 ng cm^{-2}, respectively.

Effect of foreign ions: The effect of over forty ions and complexing agents on the determination of only 1 μg ml^{-1} mercury(II) was studied. The criterion for an interference [21] was an absorbance value varying by more than 5% from the expected value for mercury alone. There was no interference from the following: a 1000-fold amounts of tartrate, citrate, oxalate, EDTA, nitrate, sulphate, fluoride, bromide, iodide, alkali metals or acetate, a 100-fold amount of alkaline earth metals, persulphate, perchlorate, phosphate, nickel(II), manganese(II), copper(II), cerium(IV), chromium(III), cadmium, zinc, molybdenum(VI), tungsten(VI) or selenium(VI). In the presence of EDTA or sodium tartrate, a 100-fold amount of iron(III), chromium(VI), selenium(IV), beryllium, arsenic(III), antimony(V), mercury(I), palladium(II), thorium, zirconium, chloride, tin(IV) or cobalt(III). A 20-fold excess of cyanide, thiocyanide, vanadium(V) and thiourea have been tolerated. A 10-fold excess of cobalt(II) has been tolerated with 0.1% w/v of sodium tartrate. Interference from permanganate was removed simply by using hydrogen peroxide and boiling the solution.

3.2. Composition of the absorbent complex

Job's method [22] of continuous variation and the molar-ratio method [23] were applied to ascertain the stoichiometric composition of the complex. A 1 : 2 (Hg : dithizone) complex was indicated by both methods.

3.3. Applications

(A) *Determination of mercury in synthetic mixtures*: Few synthetic mixtures of varying compositions containing mercury(II) and diverse ions of known concentrations were determined by the present method using EDTA as a masking agent and the results were found to be highly reproducible.

(B) *Determination of mercury in environmental water samples*: Each filtered environmental water sample (100 ml) was mixed with 2 ml of 4.5 M sulphuric acid in a 500-ml distillation flask. The sample was digested in presence of excess potassium permanganate solution according to the method recommended by De [3]. The digest was transferred into a 50-ml calibrated flask and diluted up to the mark with de-ionized water.

Table 2
Determination of mercury in some environmental water samples

Environmental water sample	Mercury(II) "spiked"		Mercury(II) "unspiked"	
	Added (μg ml^{-1})	Found (μg ml^{-1})	Recovery (%)	Found (μg ml^{-1})
Tap water	0.10	0.099[a]	99±0.2[b]	0.00
Well water	0.10	0.098	98±0.5	0.00
Pond water	0.10	0.103	100.9±0.8	0.002
River water				
(i) Halda (upper stream)	0.10	0.118	101.7±1.0	0.016
(ii) Halda (lower stream)	0.10	0.124	99±1.2	0.025
(i) Karnaphuli (upper)	0.10	0.117	100.8±1.5	1.016
(ii) Karnaphuli (lower)	0.10	0.118	99±1.0	0.019
Sea water				
(i) Bay of Bengal (upper)	0.10	0.134	99.3±0.6	0.035
(ii) Bay of Bengal (lower)	0.10	0.138	98.6±0.8	0.040
Drain water				
(i) T.S.P. Complex[c]	0.10	0.158	99.4±1.2	0.059
(ii) Chemical Complex[d]	0.10	0.149	98.7±1.5	0.051
(iii) Ctg. Urea Fertilizer[e]	0.10	0.165	100.6±1.8	0.064
(iv) Eastern Refinary[f]	0.10	0.163	98.8±1.4	0.065
(v) Karnaphuli Paper Mill[g]	0.10	0.217	99±1.5	0.119

[a]Values given represent the average of five analysis of each sample.
[b]Standard deviation is a measure of precision.
[c]T.S.P. Complex, North Patenga, Chittagong.
[d]Chittagong Chemical Complex, Barakunda, Chittagong.
[e]Chittagong Urea Fertilizer Limited, Rangadia, Chittagong.
[f]Eastern Refinary, North Patenga, Chittagong.
[g]Karnaphuli Paper Mill, Chandraghona, Chittagong.

An aliquot (1–2ml) of this solution was pipetted into a 10-ml calibrated flask and the mercury content was determined as described under procedure using tartrate as a masking agent. The analysis of environmental water from various sources for mercury is shown in Table 2.

(C) *Determination of mercury in food or other biological samples*: Human blood (10–20 ml) or urine (10–25 ml) or 10–20 g of fish sample was taken in a 100 ml micro-Kjeldahl flask with a B24 socket attached to a standard double surface reflux condenser. The sample was digested according to the method recommended by the Analytical Methods Committee [16]. The digest was filtered (if necessary) and neutralized with dilute ammonia in the presence of 1–2 ml of 0.01% (w/v) EDTA solution. The solution was transferred quantitatively into a 25-ml volumetric flask and diluted up to the mark with de-ionized water.

An aliquot (1–2 ml) of the final solution was pipetted into a 10-ml calibrated flask and mercury content was determined as described under procedure using tartrate as a masking agent. The results of biological analysis by spectrophotometric method were found to be in excellent agreement with those obtained by atomic absorption spectrophotometric method (cold vapour technique). The results are shown in Table 3.

(D) *Determination of mercury in plant samples*: The air dried sample, e.g., 10–25 g of plant tissue or vegetable was taken to a round-bottomed Pyrex flask of 1 litre capacity fitted with a "cold finger" condenser. This sample was digested according to the method recommended by Allport and Brocksopp [17]. The digest was filtered and neutralized with dilute ammonia in presence of 1–2 ml of 0.01% (w/v) tartrate solution; It was then transferred into a 25-ml calibrated flask and diluted upto the mark with deionized water.

A 1–2 ml aliquot of the final solution was pipetted into a 10-ml volumetric flask and mercury content was determined as described under procedure. The results of determinations are given in Table 4.

(E) *Determination of mercury in soil samples*: A 10–20 g amount of air dried soil sample was weighed accurately and placed in a 100 ml micro-Kjeldahl flask. The sample was digested in the presence of

Table 3
Concentration levels ($\mu g\,l^{-1}$ or $\mu g\,g^{-1}$) of mercury in biological samples

Sl. no.	Name of sample	Concentration of mercury (Mean value* ± SD)		Sample source (blood and urine samples in pathological conditions)
		AAS method	proposed method	
1. a.	Blood ($\mu g\,l^{-1}$)	9.50	9.57±1.0	Jaundice patient (male)
b.	Urine ($\mu g\,l^{-1}$)	4.65	4.59±1.2	Nuclear Medical Center, C.M.C.H. Chittagong
2. a.	Blood ($\mu g\,l^{-1}$)	12.60	12.65±0.8	Nurotic patient (female)
b.	Urine ($\mu g\,l^{-1}$)	7.25	7.32±1.3	Nuclear Medical Center, C.M.C.H. Chittagong
3. a.	Blood ($\mu g\,l^{-1}$)	7.80	7.88±0.7	Normal adult (male)
b.	Urine ($\mu g\,l^{-1}$)	3.75	3.79±1.0	Nuclear Medical Center, C.M.C.H. Chittagong
4. a.	Fish (tissue, $\mu g\,g^{-1}$)	0.072	0.0735±1.4	Hilsa (*Clupea alosa*)
b.	Fish (liver, $\mu g\,g^{-1}$)	0.112	0.099±0.8	Padma river, Chandpur
5. a.	Fish (tissue, $\mu g\,g^{-1}$)	0.190	0.185±0.5	Rohue (*Cyprinus denticulatus*)
b.	Fish (liver, $\mu g\,g^{-1}$)	0.230	0.245±1.2	Kaptai lake, Chittagong

*Average of five determinations.

Table 4
Concentration levels of mercury in plant samples

Sl. no.	Plant type	Concentration of mercury $\mu g\,g^{-1}$ (ppm) mean value* ± SD	Sample source
1	Cereal		
	(i) Wheat (grain)	0.01±0.5	Debidwar, Comilla
2	Vegetable		
	(i) Cabbage (leaves)	0.065±1.0	Chandraghona, Chittagong
	(ii) Carrot (roots)	0.057±0.6	Chandina, Comilla
	(iii) Lettuce (leaves)	0.083±0.8	Nasirabad Industrial Area, Ctg.
	(iv) Potato (tuber)	0.095±1.0	Moradnagar, Comilla
	(v) Tomato (fruit)	0.12±0.8	Tongi Industrial Area, Dhaka

*Average of 5 determinations.

excess oxidizing agent according to the method recommended by Jackson [18]. The content of the flask was filtered through Whatman No. 40 filter paper into a 25-ml calibrated flask and neutralized with dilute ammonia. It was then diluted up to the mark with de-ionized water.

Suitable aliquots (1–2 ml) were transferred to a 10-ml volumetric flask and calculated amount of 4.5 M sulphuric acid needed to give a final acidity of 0.18–1.8 M was added followed by 1–2 ml of 0.01% (w/v) tartrate solution as a masking agent. Mercury content was then determined by the above procedure and quantified from a calibration graph prepared concurrently. The average value of mercury in Bangladeshi surface soils was found to be 0.085 $\mu g\,g^{-1}$.

(F) *Determination of mercury (I+II) speciation in mixtures*: The method was also extended to the determination of speciation of mercury(I) and mercury(II) in their admixed solution using EDTA as masking agents. The results were found to be highly reproducible.

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