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A Highly Selective and Sensitive Spectrophotometric Method for the Determination of Lead at Ultra-trace Levels in Some Real, Environmental, Biological, Food and Soil Samples Using 5,7-Dibromo-8-Hydroxyquinoline

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Authors' contributions

This work was carried out in collaboration among all authors. Author MJA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MTI and SA managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

A very simple, ultra-sensitive and highly selective non-extractive spectrophotometric method for the determination of trace amounts of lead using 5,7-dibromo-8-hydroxyquinoline (DBHQ) has been developed. DBHQ reacts in a slightly acidic (0.0006-0.0025 M HCl) aqueous solution with lead (II) in 30% ethanolic media to produce highly absorbent a greenish-yellow chelate which has an absorption maximum at 390 nm. The reaction is instantaneous and the absorbance remains stable for over 24 h. The average molar absorption co-efficient and Sandal's sensitivity were found to be 6.16×10^5 L $mol^{-1}cm^{-1}$ and 5 ng cm^{-2} of lead (II), respectively. Linear calibration graphs were obtained for 0.01- 60.0 mg L^{-1} of lead (II) having detection limit of 1.0 µg L^{-1} and RSD 0-2%. The

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stoichiometric composition of the chelate is 1:2 (Pb: DBHQ). A large excess of over 60 cations, anions and complexing agents (like, chloride, phosphate, azide, tartrate, oxalate, SCN^- etc.) do not interfere in the determination. The developed method was successfully used in the determination of lead levels in several Standard Reference Materials (alloys, steels, natural water, bovine liver, human urine and hair) as well as in some environmental waters (potable and polluted), biological samples (human blood, urine and hair), soil samples, food samples (vegetables, rice, wheat) solutions containing both lead (II) and lead (IV) and complex synthetic mixtures. The results of biological and food analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS.

Keywords: Spectrophotometry; lead determination; 5, 7-dibromo-8-hydroxyquinoline; alloys; steels; environmental; biological; soil; food samples.

1. INTRODUCTION

Lead in trace amounts is important industrially [1], as a: toxicant [2], biological nutrient [3], environmental pollutant [4] and occupational hazards [5]. Lead [6] is a cumulative body poison [7] that enters the body from lead water pipes, lead-based paints and leaded petrol. Presence of even traces of Pb(II) in environmental samples leads to environmental pollution and many fatal diseases including dysfunction of renal blood and neurological systems. Pb(II) easily deposits in blood, kidney, reproductive system, nervous system and brain, and acute lead poisoning can result in colic shock, severe anemia and irreversible brain damage. Lead compounds is used as anti-knocking agents in automobile fuels cause air pollution. The toxicity [8] of lead has been studied extensively [9]. Inorganic lead (Pb^{2+}) binds itself with the -SH group in enzymes or proteins and acts as an enzyme inhibitor. Lead interferes with the calcium metabolism and gets deposited in the bones. Organic lead compounds, such as tetramethyl lead, are highly poisonous because they are absorbed readily by the body through skin and mucus membranes. Acute lead poisoning in humans causes severe damage in the kidneys, liver, brain, reproductive system, and central nervous system, and sometimes causes death. Mild lead poisoning causes anemia, headache, and sore muscles and the victim may feel fatigued and irritable. All these findings cause great concern regarding public health, demanding accurate determination of this metal ion at trace and ultra-trace levels.

Lead [10] in environmental and biological samples has been determined by NAA [11], inductively coupled plasma – atomic [12] emission spectrometry(ICP-AES) [13], inductively coupled plasma - mass spectrometry(ICP-MS) [14], anodic stripping voltammetry [15] and reversed – phase high performance liquid chromatography coupled with UV-Vis or fluorescence detection [16], AAS [17] and spectrophotometry [18-65]. The first four methods have disadvantages [66] in terms of cost and instrument used in routine analysis. AAS is often lacking in sensitivity and affected by matrix condition of samples such as salinity. Spectrophotometry [67] is a relatively sensitive method for lead (II) as it is of low cost, simple and within the reach of even ordinary laboratories, which is based on reaction between lead and chromogenic reagents. For this reason there is an ongoing search for new chromogenic reagents for direct and rapid spectrophotometric estimation lead at trace levels, especially in aqueous solution. Spectrophotometry [68] is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis.

The aim [69] of this study is to develop a simpler direct spectrophotometric method for ultra-trace determination of lead. 5, 7-dibromo-8-hydroxyquinoline (DBHQ) has been reported as a spectrophotometric reagents and forms colored water – soluble complexes with vanadium [70] and cadmium [71] and has not previously been used for spectrophotometric determination of lead. This paper reports its use in a very sensitive, highly specific spectrophotometric method for the ultra-trace determination of lead. The method possesses distinct advantages over existing methods [18–65] with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH/acidity range, thermal stability, accuracy, precision, and ease of operation. The method is based on the reaction of non-absorbent DBHQ in slightly acidic solution (0.0006-0.0025 M HCl) with lead(II) to produce a highly absorbent greenish-yellow chelate product, followed by direct measurement of the absorbance in aqueous solution. With suitable masking, the reaction can be made highly selective and the reagent blank solutions do not show any absorbance.

2. EXPERIMENTAL SECTION

2.1 Apparatus

A Shimadzu (Kyoto, Japan) (Model-1800) double-beam UV/VIS spectrophotometer and a Jenway (England, UK) (Model-3010) pH meter with combination of electrodes were used [72] for measurements of the absorbance and pH, respectively. The calibration and linearity of the instrument were frequently checked with standard quinine sulphate (100-μgL^{-1}). A Thermo Fisher [73] Scientific (Model-iCE 3000, origin USA) atomic absorption spectrophotometer equipped with a microcomputer-controlled nitrous oxide-acetylene flame was used to compare of the results [74]. Infrared spectrum was recorded with FTIR Spectrophotometer, Shimadzu (Model-IR Prestige 21, Detector-DTGS KBr) in the range 7500-350 cm^{-1}.

2.2 Reagent and Solutions

All of the chemicals used were of analytical reagent grade or the highest purity available. Doubly distilled de-ionized water, HPLC-grade ethanol which is non-absorbent under ultraviolet radiation, were used throughout. High- purity water was obtained by passing tap water through cellulose absorbent and to mixed bed ion exchange columns, followed by distillation in a corning AG-II unite. Glass vessels were cleaned by soaking in acidified solution of $KMnO_4$ or $K_2Cr_2O_7$ followed by washing with concentrated HNO_3 and rinsed several times with deionized water. Stock solutions and environmental water samples (1000-mL each) were kept in polypropylene bottles containing 1-mL of concentrated HNO_3. More rigorous contamination control was used when the lead levels in the specimens were low.

2.3 DBHQ Solution (3.3×10^{-3} M)

A 25-mL amount of stock solution was prepared by dissolving the requisite amount (0.025g) of DBHQ (Merck, Darmstadt, Germany, proanalysis grade 99%) in a known volume solution of distilled ethanol. More dilute solutions of the reagent were prepared as requited as when required. A freshly prepared reagent [75] (DBHQ) solution (10^{-4}M) was used whenever as required.

2.4 Purity Test

The purity of DBHQ was tested by taking [76] the melting point, an FTIR spectrum and thermogravimetric analysis. The melting of the reagent (DBHQ) was (200± 2)ºC (Lit. 198º-200ºC) [77]. The FTIR spectrum of the reagent (DBHQ) shows a peak at 1581.70 cm^{-1} due the characteristic C=N double bond peak (C=N,1580-1660 cm^{-1}) [78], the peak at 1607.74 cm^{-1} due to the characteristics C=O double bond peak (C=O, 1600-1735 cm^{-1}), the presence of a peak at 1568.19 cm^{-1} due to the characteristic C==C double bond peak (C=C,1560-1620) [78] of DBHQ.

Both melting point and FTIR spectral analysis [79] data indicated the purity of the reagent DBHQ. The steadiness of the thermogravimetric curve obtained for about 1g of the reagent at 80-90ºC, which indicates that the reagent did not contain any moisture.

2.5 Lead (II) Standard[8] Solution (4.83×10^{-3} M)

A 100-mL amount of stock solution (1mg mL^{-1}) of divalent lead was prepared by dissolving 159.9 mg of lead nitrate {$Pb(NO_3)_2$}(Merck, proanalysis grade, 99.8%) in doubly distilled de-ionized water. One ml of dilute nitric acid was added to the stock solution to prevent hydrolysis. Aliquots of this solution were standardized with EDTA titration using xylenol orange as indicator [80]. Working standard solution was prepared by suitable dilutions of the stock solution as when required.

2.6 Lead (IV) Standard Solution (4.83×10^{-3} M)

A 100-mL volume of lead(IV) stock solution (1mg mL^{-1}) was prepared by dissolving 110.3 mg of purified grade lead(II) oxide (The British Drag House Ltd. England) in de-ionized water containing 1-2-mL of hydrochloric acid (1+1). The working standard was prepared by approximate dilution of this stock solution.

2.7 EDTA Solution

A 100-mL stock solution of EDTA (0.01%) was prepared by dissolving 10 mg of A.C.S. grade (≥90%) ethylenediaminetetraacetic acid, disodium salt dehydrate in (100-mL) de-ionized water.

2.8 Tartrate Solution

A 100-mL stock solution of tartrate (0.01%) was prepared by dissolving 10 mg of A.C.S. grade

(99%) potassium sodium tartrate tetrahydrate in (100-mL) deionized water.

2.9 Dilute Ammonium Hydroxide Solution

A 100-mL solution of dilute ammonium hydroxide was prepared by diluting 10-mL concentrated NH_4OH (28-30% A.C.S. grade) to 100-mL with de-ionized water. The solution was stored in a polypropylene bottle.

2.10 Other Solutions

Solutions of a large number of inorganic ions and complexing agents were prepared from their Analytical grade or equivalent grade water soluble salts (or the oxides and carbonates in hydrochloric acid); those of niobium, tantalum, titanium, zirconium and hafnium were specially prepared from their corresponding oxides (Specupure, Johnson Matthey) according to the recommended procedures of Mukharji [81]. In the case of insoluble substances, special dissolution methods were adopted [82].

2.11 General Procedure

A volume of 0.1-1.0-mL of neutral aqueous solution containing 0.1-600 µg of lead in a 10-mL volumetric flask was mixed with a 1:50 to 1:200 fold molar excess (preferably 1-mL of 3.3 $x10^{-3}$ M) of 5,7-dibromo-8-hydroxyquinoline (DBHQ) reagent solution followed by the addition of 0.05 – 0.70-mL (preferably 0.5-mL) of 0.001M hydrochloric acid. The solution was mixed well. After 1 minute 3-mL of ethanol was added. The mixture was diluted up to the mark with de-ionized water. The absorbance was measured at 390 nm against a corresponding reagent blank. The lead content in an unknown sample was determined using a concurrently prepared calibration graph.

2.12 Sample Collection and Preservation

2.12.1 Water

Water samples were collected in polythene bottles from shallow tube-wells, tap-wells, river, sea and drain of different places of Chittagong region, Bangladesh. After collection, HNO_3 (1 mL L^{-1}) was added as preservative.

2.12.2 Blood and Urine

Blood and urine samples were collected in polypropylene bottles from effected persons of CSCR Hospital & Chittagong Medical College Hospital, Bangladesh. Immediately after collection they were stored in a salt-ice mixture and latter, at the laboratory, were kept at-20ºC.

2.12.3 Soil

Soil [73] (surface) samples were collected from different locations in Chittagong region, Bangladesh. Samples were dried in air and homogenized with a mortar.

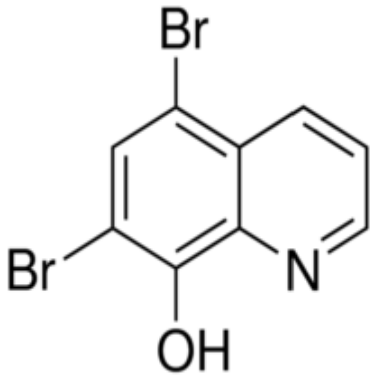
2.12.4 Food samples

Food samples (rice, wheat, and vegetables) were collected from local market of Chittagong. After collection the samples (vegetables) were stored in refrigerator for preservation. Samples (rice, wheat,) were used as dry condition and homogenized with a mortar.

3. RESULTS AND DISCUSSION

3.1 Factors Affecting the Absorbance

Absorption spectra: The absorption spectra of a lead-DBHQ system in aqueous medium in presence of 1-mL 0.001M hydrochloric acid [74] solution, was recorded using the spectrophotometer. The absorption spectra of the lead- DBHQ is a asymmetric curve with maximum absorbance at 390 nm and an average molar absorptivity of 6.16 x 10^5 L $mol^{-1}cm^{-1}$ (Fig. 2). The reagent blank exhibited negligible absorbance despite having wavelength at 390 nm. The reaction mechanism of the present method is as reported earlier [83]. The structure of the reagent (DBHQ) is shown Scheme 1.



Scheme 1. The structure of 5, 7-Dibromo-8-Hydroxyquinoline (DBHQ)

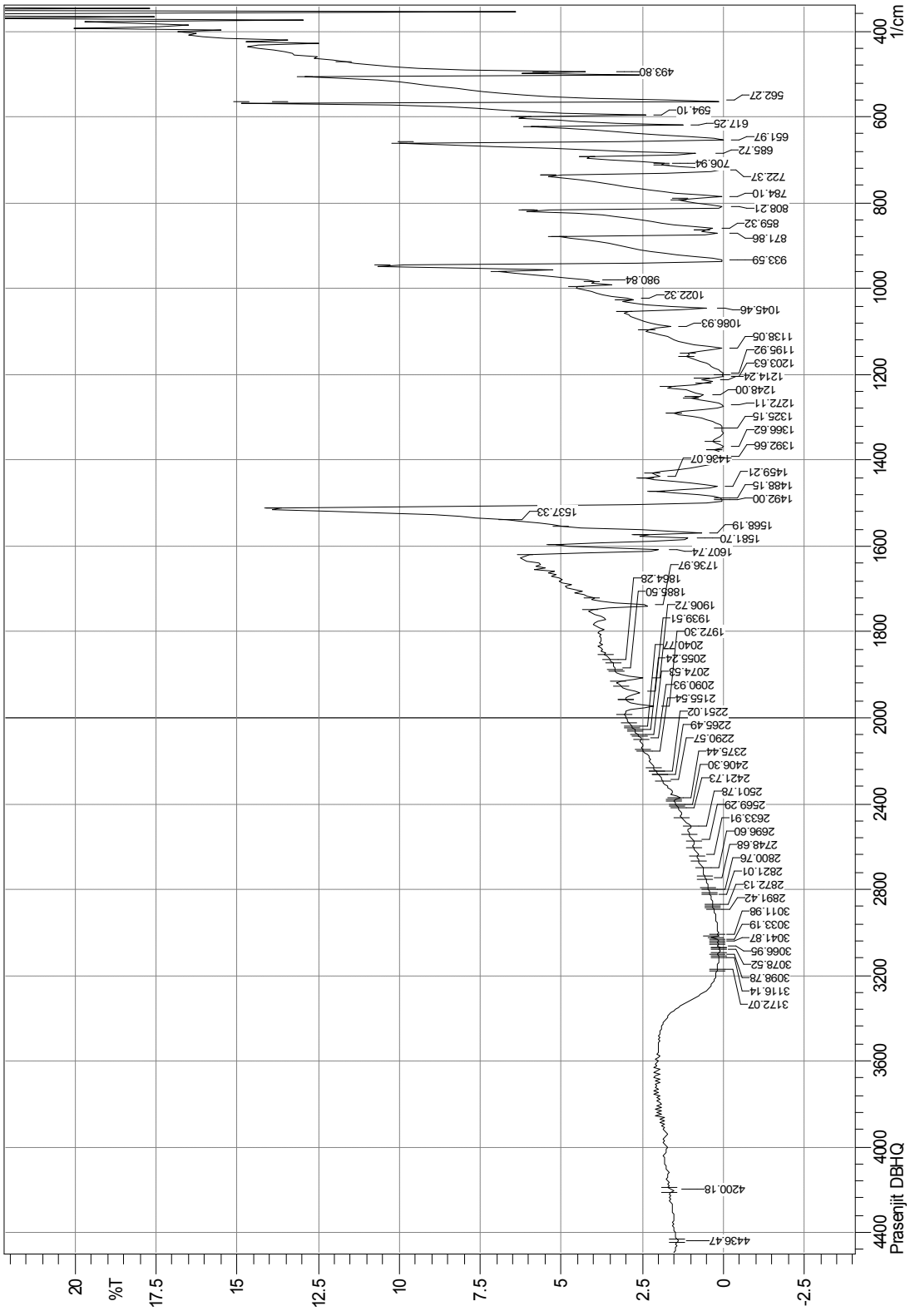


Fig. 1. FTIR spectrum of 5,7-dibromo-8-hydroxyquinoline (DBHQ)

3.2 Live Subject Statement

We were not aiming to carry out detailed human studies but some samples from individuals were used in our study and as such we abided by all the necessary procedures and regulations and our University gave consent. University of Chittagong, Bangladesh is committed to the protection and safety of human subjects involved in research.

3.3 Optimization of Some Parameters on the Absorbance

3.3.1 Effect of solvent

Because DBHQ is partially soluble in water, an organic solvent was used for the system, consideration of cost, availability, toxicity and volatility of the solvent etc. Of the various solvents (acetone, benzene, carbon tetrachloride, chloroform, ethanol, 1-butanol, isobutyl methyl ketone, N.N-dimethyl formamide (DMF), methanol [74] and 1,4-dioxane) studied, ethanol was found to be the best solvent for the system. Different volumes (0-7mL) of ethanol were added to fixed metal ion concentration and the absorbance were measured according to the general procedure. Maximum absorbance was observed in (30 ± 2%) (v/v) ethanol/water medium, hence, a 30% ethanol solution was used in the determination procedure. It was observed that 20-70% (2-7mL) ethanol produced a constant absorbance of the Pb-chelate (Fig. 3). For all subsequent measurements, 30% (3-mL) of ethanol was added.

3.3.2 Effect of acidity

Of the various acids [74] (nitric, sulfuric, hydrochloric, percloric and phosphoric) studied, hydrochloric acid was found to be the best acid for the system. The variation of the absorbance was noted after the addition of 0.05-2.0-mL of 0.001 M hydrochloric acid to every 10-mL of test solution. The maximum and constant absorbance was obtained in the presence of 0.1-1.0-mL of 0.001M sulfuric acid at room temperature (25±5)ºC. Outside this range of acidity, the absorbance decreased (Fig. 4). For all subsequent measure - ements 0.5-mL of 0.001 M hydrochloric acid was added.

3.3.3 Effect of time

The reaction [73] is instantaneous. The lead (II)-DBHQ system attained maximum and constant absorbance immediately (within 1 min) just after dilution of the solution to final volume and remained strictly constant for over 24 h.

3.3.4 Effect of temperature

The Pb - DBHQ system attained maximum and constant absorbance at room temperature (25±5)ºC. Outside this range of temperature, the absorbance decreased. All subsequent measurements were done at room temperature (25±5)ºC.

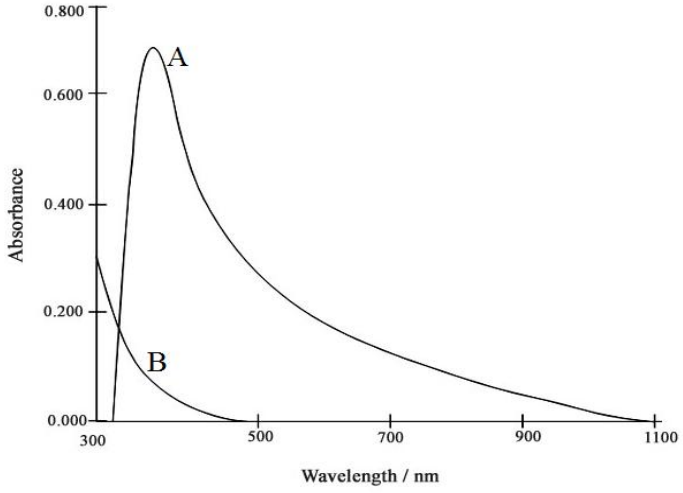


Fig. 2. A and B absorption spectra of Pb^{II}-DBHQ system and the reagent blank (λ_{max} =390 nm) in aqueous solutions, respectively

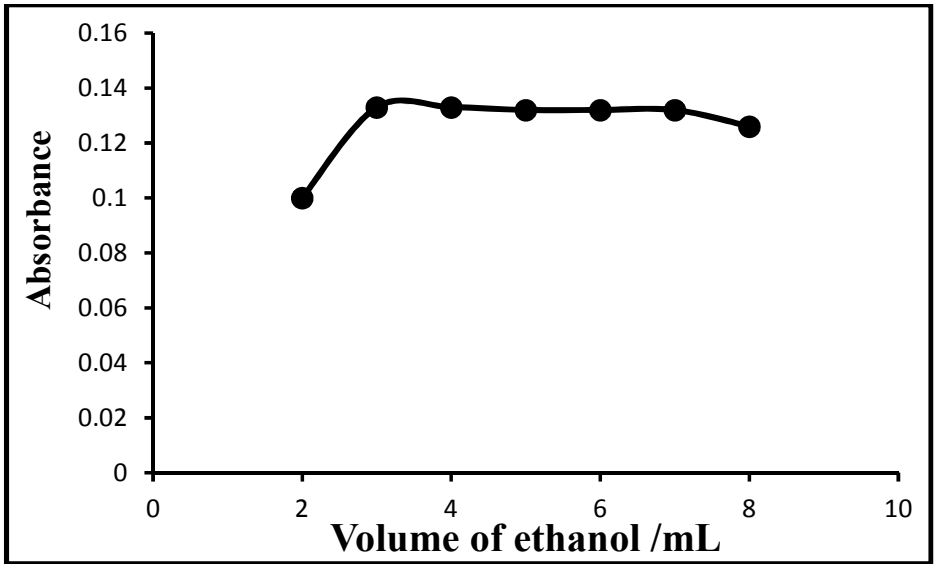


Fig. 3. Effect of solvent (ethanol) on the absorbance of Pb^{II}-DBHQ system

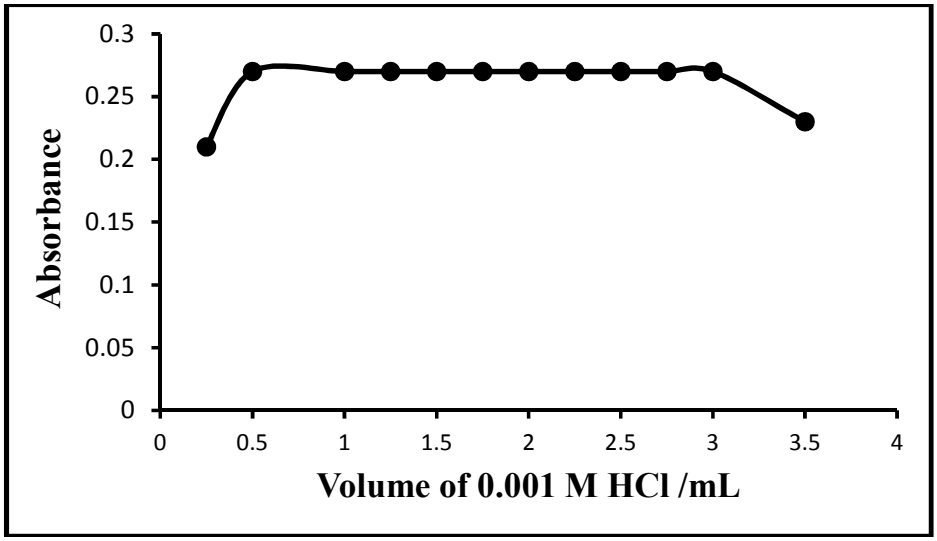


Fig. 4. Effect of acidity on the absorbance of Pb^{II}-DBHQ system

3.3.5 Effect of reagent concentration

Different molar excesses of DBHQ were added to a fixed metal ion concentration and the absorbance was measured according to the general procedure. It was observed that 1.0-mgL^{-1} lead metal, the reagent molar ratios of 1:50 to 1:200 produced a constant absorbance of Pb-chelate (Fig. 5). For different (0.5 and 10.0 mgL^{-1}) lead concentrations an identical effect of varying the reagent concentration was noticed. A greater excess were not studied. For all subsequent measurements, 1 mL of 3.30×10^{-3} M DBHQ reagent was added.

3.3.6 Calibration graph (Beer's law and sensitivity)

The well known equation for a spectrophotometric analysis in a very dilute solution was derived from Beer's law. The effect of the metal concentration was studied over 0.01-100 mg L^{-1} distributed in four different sets (0.01 -0.1, 0.1-1.0, 1.0-10 and 10.0-100.0 mg L^{-1}) for convenience of the measurement. The absorbance was linear for 0.01-60.0 mg L^{-1} at 390 nm. Of four calibration graphs, the one showing the limit of the linearity range Fig. 6; the next three were straight-line graphs passing

through the origin (R^2=0.9986). The molar absorption co-efficient and the Sandal's sensitivity [84] were found to be 6.16 x 10^5 L mol^{-1} cm^{-1} and 5 ng cm^{-2} of lead, respectively. The selected analytical parameters obtained with the optimization experiments are summarized in Table 1.

3.3.7 Effect of foreign ions

The effect of over 60 anions, cations and complexing agents on the determination of only 1 mg L^{-1} of lead was studied. The criterion for interference [86] was an absorbance value varying by more than 5% from the expected value for lead alone. The results are summarized in Table 2. As can be seen, a large number of ions have no significant effect on the determination of lead. The interference were from V (V), Mo (VI) and Cd(II) ions. Interference from these ions is probably due to complex formation with DBHQ. The greater tolerance limits for these ions can be achieved by using several masking methods. In order to eliminate interference of V (V), Mo (VI) and Cd (II); EDTA and tartarate used as masking agent, respectively. During the interference studies, if a precipitate was formed, it was removed by centrifugation. The strong reducing agents such as tin (II), chloride, iron (II), sulfate, hydroxylamine hydrochloride and sodium azide, which would otherwise reduce lead (IV) had no reducing effect on lead (II). The amount mentioned is not the tolerance limit butthe actual amount studied. However, for those ions whose tolerance limit has been studied, their tolerance ratios are mentioned in Table 2.

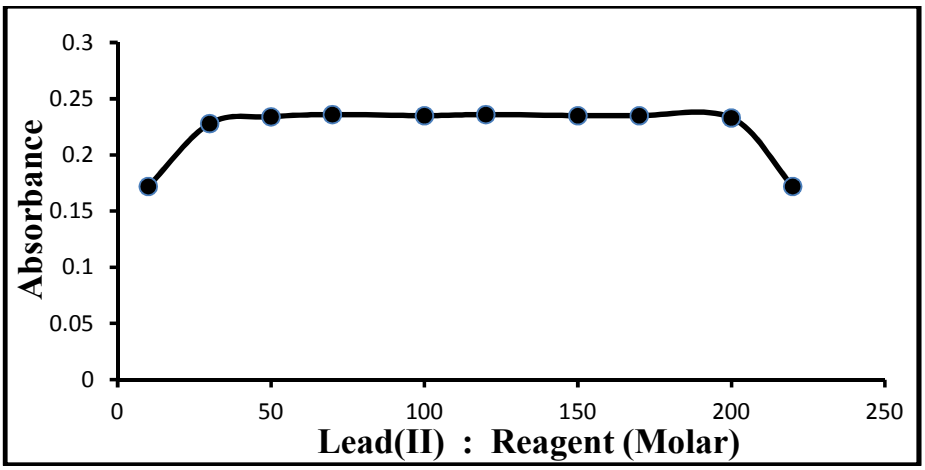


Fig. 5. Effect of reagent on the absorbance of Pb^{II}-DBHQ system

Table 1. Selected analytical parameters obtained with the optimization experiments [85]

Parameters	Studied range	Selected value
Wavelength / λ_{max} (nm)	200-800	390
Solvent / % (Ethanol)	10-80	20-70 (Preferably 30)
Acidity / M HCl	0.0001-0.05	0.0006-0.0025 (Preferably 0.001)
pH	4-1.30	3.10-2.48 (Preferably 3)
Time / h	0-72	1min-24 h (Preferably 1 min)
Temperature / °C	10-80	20-70 (Preferably 25±5)
Reagent (fold molar excess, M:R)	1:1 - 1:500	1:50 - 1:200 (Preferably 1: 100)
Linear range/mg L^{-1}	0.001-100	0.01-60.0
Molar absorption coefficient / L mol^{-1} cm^{-1}	5.0 x 10^5 –7.32 x 10^5	6.16 x 10^5
Sandell's sensitivity/ng cm^{-2}	1-100	5.0
Detection limit/ µg L^{-1}	0.01-10	1.0
Reproducibility (% RSD)	0-10	0-2
Regression Co-efficient (R^2)	0.9989-0.9998	0.9995
Molar Ratio (Pb : DBHQ)	1 : 9 – 9 : 1	1 : 2

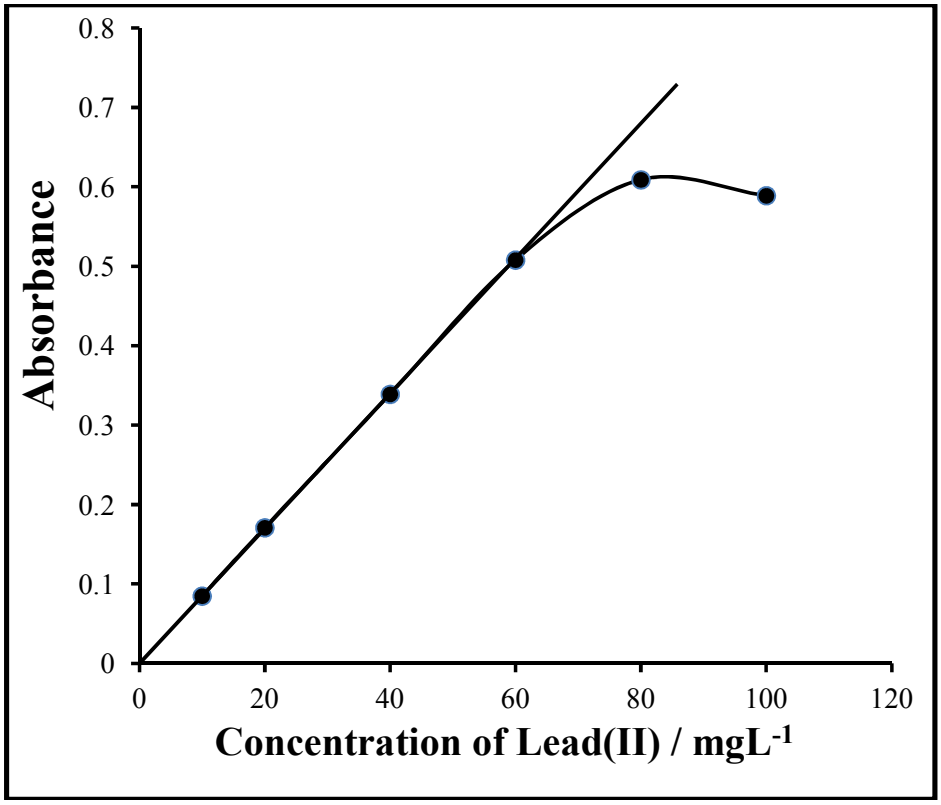


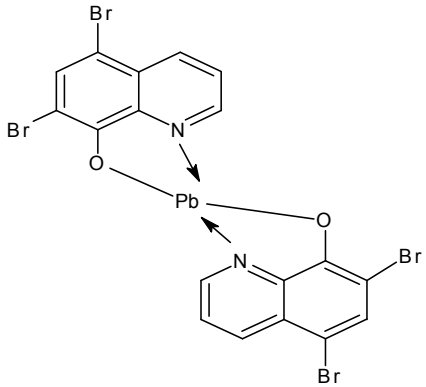
Fig. 6. Calibration graph: 10 – 60 mgL^{-1} of lead (II)

3.4 Composition of the Absorbent Complex

Job's method [87] of continuous variation method was applied to ascertain the stoichiometric composition of the complex under the optimum conditions (Table 1). A Pb - DBHQ (1:2) complex was indicated by this method. The molar- ratio method [88] was also applied to ascertain the stoichiometric composition of the complex. A Pb - DBHQ complex was indicated by both methods and the stoichiometry was also found to be 1:2 (Metal: Ligand).

3.5 Precision and Accuracy

The precision of the present method was evaluated by determining different concentrations of lead (each analyzed at least five times). The relative standard deviation (n = 5) was 2-0% for 0.1-60 µg of lead in10-mL, indicating that this method is highly precise and reproducible. The detection limit (The detection limit (3s/S 's' is the standard deviation of the blank & 'S' is slope)) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for lead were found to be 1 µg L^{-1} and 5 ng cm^{-2}, respectively. The method was also tested by analyzing several synthetic mixtures containing lead and diverse ions (Table 3).The results for total lead were in good agreement with certified values (Table 4). The reliability of our Pb-chelate procedure was testedby recovery studies. The average percentage recovery obtained for addition of lead spike to some environmental water samples was quantitative as shown in (Table 5.) The results of



Scheme 2. Structure of [Pb(DBHQ)$_2$] complex

biological and food analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS (Tables 6 and 8). The results of speciation of lead (II) and lead (IV) in mixtures are shown in Table 9. Hence, the precision and accuracy of the method were excellent. With suitable masking, the reaction can be made highly selective.

3.6 Applications

The proposed method [89] was successfully applied to the determination of lead in a series of synthetic mixtures of various compositions (Table 3) and also in a number of real samples e.g. several Certified Reference Materials (CRMs) (Table 4). The method was also extended to the determination of lead in a number of environmental, biological, food, vegetable and soil samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such samples were analyzed for lead content; the recoveries in both the "spiked" (added to the samples before the mineralization or dissolution) and the "unspiked" samples are in good agreement (Table 5). The results of biological analyses by spectrophotometric method were found to be in excellent agreement with those obtained by AAS (Table 6). The results of soil samples analyzed by the spectrophotometric method are shown in Table 7. The results of vegetable and food analyses by the spectrophotometric method were [90] found to be in excellent agreement with those obtained by AAS (Table 8). The results of speciation of lead (II) and lead (IV) in mixtures are shown in Table 9. The precision and accuracy of the method were excellent.

Table 2. Table of tolerance limits of foreign ions[a], tolerance ratio [species(x) / Pb(II)(w/w)]

Species x	Tolerance ratio x/Pb^{II}(w/w)	Species x	Tolerance ratio x/Pb (w/w)
Ammonium (I)	50	Lithium	50[b]
Arsenic (III)	50	Lead (II)	100
Arsenic (V)	50	Magnesium	100
Aluminium	100	Manganese (II)	100
Azide	100	Manganese (VII)	100
Ascorbic acid	100	Mercury (II)	100
Antimony	100	Molybdenum (VI)	50[b]
Barium	100	Nitrate	100
Bromide	100	Nickel	50
Bismuth (III)	100	Oxalate	1000
Beryllium (II)	100	Potassium	50
Calcium	50	Phosphate	100
Chloride	100	Selenium (VI)	50
Cobalt (II)	100	Selenium (IV)	50
Cobalt (III)	100	Silver	100
Chromium (III)	100	Sodium	100
Chromium (VI)	50	Strontium	50
Cadmium	50[c]	Sulfate	100
Carbonate	1000	Titanium (IV)	50
Cerium(III)	100	Tellurium (IV)	100
Cerium(IV)	100	Tellurium (VI)	100
Cesium	50	Tartrate	1000
Citrate	100	Thiocyanate	1000
Copper (II)	50	Thiourea	1000
Cyanide	100	Tungsten (VI)	100
EDTA	1000	Tin (II)	100
Fluoride	100	Tin (IV)	100
Iodide	100	Vanadium (V)	50[c]
Iron (II)	100	Zinc	50[c]
Iron (III)	100		

[a] Tolerance limit was defined as ratio that causes less than ± 5 percent interference.
[b] with 10 mg L^{-1} tartrate.
[c] with 10 mg L^{-1} EDTA

3.7 Determination of Lead in Synthetic Mixtures

To test the validity of the proposed method several synthetic mixtures [91] of varying compositions containing lead and diverse ions of known concentrations were determined by the present method using tartrate or EDTA as masking agent and the results were found to be highly reproducible. The results are shown in Table 3. Accurate recoveries were achieved in all solutions.

3.8 Determination of Lead in Some Certified Reference Materials

Certified Reference Materials, alloys, steels, brass and some synthetic compounds were analyzed to evaluate the validation of the method. A 0.1g amount of an alloy or steel or brass containing 0.23 –2.25% of lead was accurately weighed and placed in a 50-mL Erlenmeyer flask in the presence excess reducing agent to reduce Pb(IV) to Pb(II), following a method recommended by Mitra [92]. To it, 10-mL of concentrated HNO_3 and 2-mL of concentrated H_2SO_4 were carefully added. The solution was heated and simmered gently after the addition of another 10-mL of concentrated HNO_3 until all carbides were decomposed. The solution was carefully evaporated to dense white fumes to drive off the oxides of nitrogen and then cooled to room temperature $(25\pm5)^0$C. After suitable dilution with de-ionized water, the contents of the Erlenmeyer flask were warmed to dissolve the soluble salts. The solution was then cooled and neutralized with a dilute NH_4OH solution in the presence of 1-2-mL of 0.01 %(w/v) tartrate solution. The resulting solution filtered, if necessary, through Whatman no. 40 filter paper into a 100-mL calibrated flask. The residue (silica and tungstic acid) was washed with a small volume of hot (1+99) H_2SO_4, followed by water; the volume was made up to the mark with de-ionized water.

A suitable aliquot (1-2 mL) of the above solution was taken into a 10-mL calibrated flask and the lead content was determined as described under general Procedure using EDTA or tartrate as masking agent. The proposed method for the spectrophotometric determination of lead was applied to the analysis of natural water(NIST-SRM-1640a), bovine liver(NIST-SRM-1677c), human urine(NIST- SRM-2670a) and human hair(CRM-397) obtained from National Research Council of Canada using EDTA or tartrate as a masking agent, following a method recommended by Sun et al. [93]. Based on five replicate analyses, the average lead concentrations determined by spectrophotometric method were found to be in good agreement with the certified values. The results are shown in Table 4.

3.9 Determination of Lead in Some Environmental Water Samples

Each filtered (with Whatman No. 40) environmental water sample (1000 mL) was evaporated nearly to dryness with a mixture of 3-mL concentrated H_2SO_4 and 10-mL of concentrated HNO_3 in a fume cupboard in presence fresh excess sodium azide solution(2.5%), following a method recommended by Greenberg et al. [94] and was cooled to room temperature. The residue was heated with 10-mL of de-ionized water in order to

Table 3. Determination of lead in some synthetic mixtures

Sample	Composition of mixtures (mg L^{-1})	Lead (II) / mg L^{-1}		
		Added	Found[a] (n=5)	Recovery ± SD[b] (%)
A	Pb^{II}	0.50	0.49	98 ± 0.3
		1.00	1.00	100 ± 0.0
B	As in A + Ag (25) + Cd (25) + K(25) + Co^{2+} (25)	0.50	0.50	100 ± 0.0
		1.00	1.02	102 ± 0.5
C	As in B + Na (25) + Te^{IV} (25) + Sn^{2+}(25) + Mg(25)	0.50	0.49	98 ± 0.6
		1.00	0.99	99 ± 0.2
D	As in C + Ca (25) + Cr^{III} (25) + Ba(25) + Hg^{2+} (25) + EDTA(50)	0.50	0.52	104 ± 1.3
		1.00	1.04	104 ± 1.5
E	As in D + Sr (25)+ Ce^{IV} (25) + Mn^{2+} (25) + Sb(25)	0.50	0.54	108 ± 1.6
		1.00	1.07	107 ± 1.8

[a] *Average of five analyses of each sample.*
[b] *The measure of precision is the standard deviation (s)*

Table 4. Determination of lead in some certified reference materials

Sample	Certified reference materials (Composition, %)	Lead (%)		
		In C.R.M sample	Found (n=5)	RSD[a] (%)
1	BAS-CRM -5g, Brass (Cu=67.4, Sn=1.09, Pb=2.23, Zn=28.6, Ni=0.33, P=0.01)	2.23	2.25	1.5
2	BAS-CRM-10g, High-tensile Brass: (Cu=60.8, Fe=1.56, Pb=0.23, Ni=0.16, Sn=0.21, Zn=32.0,Al=3.34 Mn=0.12,)	0.23	0.22	1.0
3	BAS-Brass-5f: (Cu=70.8, Fe=0.31, Pb=2.52, Ni=0.17, Sn=1.84, Zn=24.2, Mn=0.12, P=0.06)	2.25	2.30	1.8
4	NIST-SRM-1640_a :Natural water[b]	12.101±0.05	12.05±0.06	2.0
5	NIST-SRM^R-1677_c: Bovine liver[b]	62.8±1.0	62.5±1.2	2.5
6	NIST-SRM^R-2670_a :Urine(Freez-dried)[c]	125.0±4.3	123.0±3.5	2.8
7	CRM-397 :Human hair[d]	33.0±1.2	32.8±1.5	2.6

[a] *The measure of precision is the relative standard deviation (RSD).*
[b] *Values in μg kg^{-1}*
[c] *Values in μg L^{-1}*
[d] *Values in μg g^{-1}*

dissolves the salts. The solution was then cooled and neutralized with dilute NH_4OH solution in the presence of a 1–2-mL of 0.01 % (w/v) tartrate or EDTA solution. The resulting solution was then filtered (if necessary) and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with de-ionized water.

An aliquot (1-2-mL) of this preconcentrated water sample was pipetted into a 10-mL calibrated flask and the lead content was determined as described under the Procedure, using tartrate or EDTA as a masking agent. The analyses of environmental water samples for lead from various sources are shown in Table 5.

Most spectrophotometric methods for the determination of lead in natural and sea-water require preconcentration of lead [94]. The concentration of lead in natural and sea-water is a few μgL^{-1} in Japan[6]. The mean concentration of lead in US drinking water is $5\mu gL^{-1}$ [94].

3.10 Determination of Lead in Some Biological Samples

Human blood (2-5-mL) or urine (20-50-mL) was collected in polyethane bottles from the affected persons. Immediately after collection, they were stored in a salt-ice mixture and later, at the laboratory, were kept at -20°C. The samples were taken into a 100-mL micro-Kjeldahl flask. A glass bead and 10-mL of concentrated nitric acid were added, and the flask was placed on the digester under gentle heating. The sample was digested in presence of excess reducing agent according to the method recommended by Stahr [95]. When the initial brisk reaction was over, the solution was removed and cooled at room temperature. 2-mL volume of concentrated sulfuric acid was added carefully, followed by the addition of 2-mL of concentrated HF and heating was continued to dense white fumes, repeating nitric acid addition if necessary. Heating was continued for at least ½ hr and then cooled. The content of the flask was filtered then neutralized with dilute NH_4OH solution in the presence of 1-2-mL of a 0.01 % (w/v) tartrate or EDTA solution. The resultant solution was then transferred quantitatively into a 10-mL calibrated flask and made up to the mark with de-ionized water.

A suitable aliquot (1-2-mL) of the final solution was pipetted into a 10-mL calibrated flask and the lead content was determined as described under the Procedure using tartrate or EDTA as masking agent. The results of biological analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS. The results are shown in Table 6.

The abnormally high values for the lung cancer and convulsion patients are probably due to the involvement of high lead concentration with As and Zn. The occurrence of such high lead content are also reported in lung cancer and convulsion patients from developed countries

[96]. The abnormally high value for the lung cancer patient is also probably due to high lead concentrations in air due to use of tetramethyl lead in gasoline [97].

Table 5. Determination of lead in some environmental water samples

Sample		Lead (II) / µg L^{-1}		Recovery ± s (%)	s_r [b] (%)
		Added	Found [a] (n=5)		
Tap water		0	25.0		
		100	119.0	99±0.5	0.35
		500	525.0	100±0.0	0.00
Well water		0	16.0		
		100	115.0	99.5±0.4	0.27
		500	517.0	100.0±0.3	0.17
Rain water		0	10.0		
		100	108.0	98.18±1.0	0.24
		500	510.0	100.0±0.0	0.00
River water	Karnaphully (upper)	0	25.0		
		100	123.0	98.4±1.0	0.25
		500	524.0	99.8±0.5	0.23
	Karnaphully (lower)	0	30.0		
		100	131.0	100.0±0.03	0.15
		500	530.0	100.0±0.0	0.00
	Halda (upper)	0	20.0		
		100	121.0	100.8±0.5	0.26
		500	519.0	99.8±0.7	0.27
	Halda (lower)	0	22.0		
		100	120.0	98.0±1.0	0.25
		500	524.0	104.0±0.6	0.23
Sea water	Bay of Bengal (upper)	0	15.0		
		100	116.0	100.8±1.0	0.42
		500	514.0	99.8±0.7	0.26
	Bay of Bengal (lower)	0	13.0		
		100	114.0	100.8±0.8	0.09
		500	512.0	99.8±0.5	0.10
Drain water	T. S. P. Complex [c]	0	65.0		
		100	165.0	100.0±0.0	0.00
		500	568.0	99.5±0.7	0.21
	PHP[d]	0	125.0		
		100	230.0	102.0±0.8	0.28
		500	635.0	101.6±0.9	0.26
	BSRM [e]	0	95.0		
		100	198.0	98.4±0.5	0.21
		500	590.0	101.0±0.4	0.29
	K.P.M Water[f]	0	120.0		
		100	225.0	97.8±0.9	0.37
		500	620.0	100.0±0.0	0.00
	Eastern Refinery [g]	0	150.0		
		100	255.0	98.0±0.5	0.35
		500	660.0	98.5±0.8	0.48

[a] Average of five replicate determinations of each sample .
[b] The measure precision is the relative standard deviation(s_r).
[c] T. S. P. Complex Ltd., Patenga, Chittagong.
[d] PHP glass factory, Kumira, Chittagong.
[e] Bangladesh Steel Re-rolling Mills Ltd.(BSRM) , Baizid Bosthami, Chittagong.
[f] Karnaphuly Paper Mills, Chandraghona, Chittagong.
[g] Eastern Refinery, North Patenga, Chittagong

3.11 Determination of Lead in Some Surface Soil Samples

An air dried homogenized soil sample (100 g) was weighed accurately and placed in a 100-mL micro-Kjeldahl flask. The sample was digested in presence of excess reducing agent (1-mL of 2.5%(w/v) freshly [98] prepared sodium azide solution), following the method recommended by Jackson [99]. The contents of the flask was filtered through a Whatman No. 40 filter paper into a 25-mL calibrated flask and neutralized with dilute NH_4OH solution in the presence of 1-2-mL of a 0.01% (w/v) tartrate or EDTA solution. Then the solution of the flask was made up to the mark with de-ionized water.

Suitable aliquots (1-2-mL) were transferred into a 10-mL calibrated flask and a calculated amount of 0.001 M HCl needed to give a final acidity of 0.0006-0.0025 M HCl was added followed by 1-2-mL of 0.01% (w/v) tartrate or EDTA solution as masking agent. The lead content was then determined by the above Procedure and quantified from a calibration graph prepared concurrently. The results are shown in Table 7. The average value of different surface soil samples of Bangladesh containing 78.47 mg kg^{-1} of Pb.

3.12 Determination of Lead in Some Vegetable and Food Samples

The vegetable and food samples collected prior to the determination were pretreated in the following way. Edible portion of samples was first washed clean with tap water followed by rewashing with de - ionized water. After removing de-ionized water from the surface of vegetables and fruits, the samples were cut into small pieces and dried at 65ºC in oven. An air dried vegetables and fruits samples (10 gm) were taken in a 100-mL micro-Kjeldahl flask in presence of reducing agent and digested following a method recommended by Stahr [95]. A glass bead and 10-mL of concentrated nitric acid were added and the flask was placed on the digester under gentle heating. When the initial brisk reaction was over, the solution was removed and cooled at room temperature. 1-mL volume of concentrated sulfuric acid was added carefully, followed by the addition of 2-mL of concentrated HF, and heating was continued for at least ½ hr and then cooled. The content of the flask was reduced from lead (IV) to lead(II) by using freshly prepared sodium azide solution (2.5% w/v) and excess of azide was removed by boiling and then filtered. The solution of flask then neutralized with dilute ammonia in the presence of 1-2-mL of a 0.01% (w/v) tartrate or EDTA solution. The resultant solution was then transferred quantitatively into a 25-mL calibrated flask and made up to the mark with de-ionized water.

A suitable aliquot (1-2-mL) of the final solution was pipetted into a 10-mL calibrated flask and the lead content was determined as described under the Procedure using tartrate as masking agent. High value of lead for *Brassica oleracea cupitata* (White Cabbage) is probably due to the involvement of high lead concentration in the soil [100]. The results are shown in Table 8.

Table 6. Determination results of lead for human fluids and hair samples

Serial no.	Sample	Lead / µg L^{-1}				Sample Source[a]
		AAS (n=5)		Proposed method (n = 5)		
		Found	RSD[b] (%)	Found	RSD[b] (%)	
1	Blood	252.0	2.0	258.0	2.0	Convulsion patient(Male)
	Urine	72.8	1.2	75.5	1.1	
2	Blood	210.5	2.3	215.8	1.8	Hemolytic anemia
	Urine	55.6	1.3	58.5	1.2	patient(Female)
3	Blood	373.5	2.2	381.6	2.5	Lung cancer
	Urine	94.8	1.0	95.7	1.3	patient (Male)
4	Blood	85.0	1.5	91.0	1.7	Asthma patient (Male)
	Urine	21.5	0.8	23.8	1.2	
5	Blood	33.5	1.8	35.8	1.9	Normal Adult(Female)
	Urine	8.6	0.8	8.8	1.0	
6	Hair[b]	45.5	1.3	48.6	1.5	Normal human hair (Female)

[a]Samples were from Chittagong Medical College Hospital; [b]Values in µg g^{-1}

Table 7. Determination of lead in some surface soil samples

Serial No.	Lead (mg kg^{-1}) [a] (n=5)	RSD (%)	Sample Source[b]
S_1[b]	10.5	1.0	Agriculture soil (Chittagong University Campus)
S_2	6.55	1.5	Marine soil (Bay of Bengal)
S_3	175.0	1.3	Traffic soil (Kadamtali Bus Terminal)
S_4	76.5	2.0	Industrial soil (Estern Cables)
S_5	85.6	2.1	Industrial soil (T.S.P. Complex, Chittagong)
S_6	104.5	2.5	Industrial soil (Bangladesh Steel Re-rolling Mills Ltd., Chittagong, Bangladesh)
S_7	78.6	2.0	Road side soil (Dhaka-Chittagong Highway)
S_8	125.0	2.3	Paint soil (Elite Paint, Chittagong)
S_9	56.0	1.6	River soil (River Halda, Chittagong)
S_{10}	67.0	1.8	River soil (River Karnaphully, Chittagong)

[a]*Average of five analyses of each sample*
[b]*The measure precision is the relative standard deviation(RSD).*
[c]*Composition of the soil samples: C,N, P, K, Na, Ca, Mg, Cu, Mo, Fe, Pb,Zn,V,Mn,Co,NO_3,SO_4*

Table 8. Determination of lead in some food and vegetable samples

Serial no.	Sample	Lead / mg kg^{-1} Found[a] ± s (n=5)				Sample source
		AAS (n=5)		Proposed method (n = 5)		
		Found	RSD[b] (%)	Found	RSD[b] (%)	
1	White Cabbage *(Brassica oleracea cupitata)*	48.0±1.0	1.8	50.0±1.5	2.0	Local Market, Chittagong
2	Spinach *(Spinacia oleracea)*	15.0±0.8	1.5	`17.0±1.0	2.3	Local Market, Chittagong
3	Tomato Leaves *(Lycopersicon esculentum)*	21.0±1.0	2.0	20.0±0.9	1.8	Local Market, Chittagong
4	Lettuce Leaves *(Lactuca sativa)*	35.0±1.2	2.1	36.5±1.3	2.5	Local Market, Chittagong
5	Cauliflower Leaves *(Brassica oleracea)*	34.0±1.0	1.8	35.5±0.8	2.0	Local Market, Chittagong
6	Almonds *(Prunus dulcis)*	22.0±0.6	1.5	25.0±1.0	2.2	Local Market, Chittagong
7	Rice *(Oryza sativa)*	0.20±0.5	1.8	0.30±0.08	1.6	Local Market, Chittagong
8	Wheat powder *(Tritricum aestivum)*	0.50±1.0	1.6	0.6±0.08	1.8	Local Market, Chittagong
9	*Cacao (Theobroma cacao)*	15.0±0.5	1.8	16.0±0.8	1.7	Local Market, Chittagong
10	Cashews *(Anacardium occidentale)*	17.0±1.0	1.8	16.0±1.5	2.0	Local Market, Chittagong
11	Peanuts *(Arachis hypogaea)*	21.0±1.5	2.0	23.0±1.6	2.1	Local Market, Chittagong

[a] *Average of five replicate analyses of each sample.*
[b]*The measure precision is the relative standard deviation(RSD)*

3.13 Determination of Lead (II) and Lead (IV) Speciation in Mixtures

Suitable aliquots (1-2 mL) of lead (II + IV) mixtures (preferably 1: 1, 1: 5, 1:10) were taken in a 25-mL conical flask. A few drops (2-3 drops) of 4 M H2SO4, 3-4 mL of a freshly prepared sodium azide solution (2.5% w/v) was added to reduce the tetravalent lead to divalent lead and heated gently with the further addition of 5 mL of water, if necessary, for 5 minutes to drive off the excess azide cooled to room temperature. The

Table 9. Determination of Pb (II) and Pb (IV) speciation in mixtures

Serial no.	Pb(II) : Pb(VII)	Pb, taken (mg L^{-1})		Pb, found (mg L^{-1})		Error (mg L^{-1})	
		Pb(II)	Pb(IV)	Pb(II)	Pb(IV)	Pb(II)	Pb(IV)
1	1: 1	1.00	1.00	0.99	0.98	0.01	0.02
1	1: 1	1.00	1.00	1.00	1.00	0.00	0.00
1	1: 1	1.00	1.00	0.98	0.99	0.02	0.01
Mean error : Pb(II) = ± 0.017; Pb(IV) = ± 0.017 Standard deviation : Pb(II) = ± 0.05; Pb(IV) = ± 0.006							
1	1: 5	1.00	5.00	0.98	4.99	0.01	0.01
1	1: 5	1.00	5.00	0.98	4.98	0.02	0.02
1	1: 5	1.00	5.00	0.99	4.98	0.01	0.02
Mean error : Pb(II) = ± 0.013; Pb(IV) = ± 0.016 Standard deviation : Pb(II) = ± 0.0058; Pb(IV) = ± 0.006							
1	1:10	1.00	10.00	0.98	9.98	0.02	0.02
1	1:10	1.00	10.00	0.98	9.99	0.02	0.01
1	1:10	1.00	10.00	0.99	9.98	0.01	0.02
Mean error : Pb(II)= ± 0.016; Pb(IV) = ± 0.017 Standard deviation : Pb(II) = ± 0.0058; Pb(IV) = ± 0.006							

reaction mixtures was neutralized with dilute NH4OH and transferred quantitatively into a 10-mL volumetric flask. 1-mL of 3.3 × 10–3M DBHQ reagent solution was added followed by the addition of 0.5-mL of 0.001 M HCl and 3-mL ethanol. It was made up to the mark with de-ionized water. The absorbance was measured after 1 min at 390 nm against a reagent blank. The total lead content was calculated with the help of a calibration graph prepared concurrently.

An equal aliquot (1-2-mL) of the above lead (II+ IV) mixture was taken into a 25-mL beaker. Neutralize the solution with dilute NH_4OH in presence of 1-2 mL of 0.01% (w/v) tartrate solution. After, the content of the beaker was transferred quantitatively into a 10-mL volumetric flask; 1-mL of 3.3×10^{-3} M DBHQ reagent solution was added, followed by the addition of 0.5-mL of 0.001 M HCl and 3-mL ethanol. It was made up to the mark with de-ionized water. After 1 min the absorbance was measured at 390 nm against a reagent blank, as before. The lead concentration was calculated in mg L^{-1} or µg L^{-1} with the aid of a calibration graph. This gives a measure of lead (II) originally present in the mixture. This value was subtracted from that of the total lead to get the lead (IV) present in the mixture. The results were found to be highly reproducible. The occurrence of such reproducible results is also reported for different oxidation states of lead [101]. The results of a set of determination are given in Table 9.

4. CONCLUSION

A new simple, sensitive, and inexpensive method with the lead(II)-DBHQ complex was developed for the determination of lead in some real, environmental, biological, soil, food, and vegetable samples, for continuous monitoring to establish the trace levels of lead in several difficult samples matrices. Compared with other methods, the proposed method has several remarkable analytical characteristics. Firstly, the proposed method is highly sensitive with molar absorptivity of the complex of 6.16×10^5 L mol^{-1} cm^{-1}. Thus, amount of ng g^{-1} of lead can be determined without preconcentration. Secondly, the proposed method is very simple, rapid, and stable. The reaction of lead(II) with DBHQ is completed rapidly in 1 min at room temperature(25±5°C) so it does not involve any stringent reaction conditions and offer the advantages of high complex stability (24h). Thirdly, the method has added the advantage of determining individual amounts of Pb(II) and Pb(IV). With suitable masking agents, the reaction can be made highly selective. The proposed method using DBHQ in aqueous solutions not only is one of the most sensitive methods for the determination of lead but also is excellent in terms of selectivity and simplicity. Therefore, this method will be successfully applied to the monitoring of trace amounts of lead in real, environmental, biological, soil, food and vegetable samples.

CONSENT

We were not aiming to carry out detailed human studies but some samples from individuals were used in our study and as such we abided by all the necessary procedures and regulations and our University gave consent. University of

Chittagong, Bangladesh is committed to the protection and safety of human subjects involved in research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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