ISSN-1996-918X

Pak. J. Anal. Environ. Chem. Vol. 14, No. 1 (2013) 01 – 15

A Simple Spectrophotometric Method for the Trace Determination of Zinc in Some Real, Environmental, Biological, Pharmaceutical, Milk and Soil Samples Using 5,7- Dibromo-8-hydroxyquinoline

M. Tazul Islam and M. Jamaluddin Ahmed*

Laboratory of Analytical Chemistry, Department of Chemistry, University of Chittagong, Chittagong-4331, Bangladesh.

Received 26 March 2013, Revised 04 June 2013, Accepted 05 June 2013

Abstract

A very simple, ultra-sensitive and highly selective non-extractive spectrophotometric method for the determination of trace amount of zinc using 5,7-dibromo-8-hydroxyquinoline (DBHQ) has been developed. DBHQ reacts in a slightly acidic solution (0.000001-0.000007 M H_2S0_4) with zinc to give a pale-yellow chelate, which has an absorption maximum at 391 nm. The reaction is instantaneous and absorbance remains stable for over 24 hrs. The average molar absorption co-efficient and Sandell's sensitivity were found to be 1.62 x 10^5 L mol^{-1} cm^{-1} and 10 ng cm^{-2} of Zn, respectively. Linear calibration graphs were obtained for 0.02-4 mg L^{-1} of Zn having detection limit of 5 µg L^{-1} and RSD 0 - 2%. The stoichiometric composition of the chelate is 1:2 (Zn : DBHQ). A large excess of over 50 cations, anions and some common complexing agents (such as chloride, azide, tartrate, EDTA, oxalate, SCN^- etc) do not interfere in the determination. The method was successfully used in the determination of zinc in several Standard Reference Materials (alloys and steels) as well as in some environmental waters (potable and polluted), biological samples (human blood and urine), soil samples, milk samples, pharmaceutical samples and complex synthetic mixtures.The results of the proposed method for biological samples were comparable with AAS and were found to be in excellent agreement.The method has high precision and accuracy (s = ± 0.01 for 0.5 mg L^{-1}).

***Keywords*:** Spectrometry; Zinc determination; 5,7–dibromo-8-hydroxyquinoline; Alloy; Steel; Environmental; Biological; Milk; Pharmaceutical samples; Soil samples.

Introduction

Zinc in trace amounts is important industrially, as a: biological nutrient, epidemiological preventive, toxicant, environmental pollutant and occupational hazard [1-6]. Therefore, its accurate determination at trace levels using simple and rapid methods is of paramount importance.

Zinc is an essential element for all animals including human beings. It plays an important physiological role in human blood distributed 75-85% in erythrocytes (mostly as carbonic anhydrase), 12 to 22% in plasma and 3% in leukocytes. One third of zinc in plasma is loosely bound to serum albumins, the reminder being more firmly attached to α-globulins, with minor fractions complexed in histidine and cysteine [7-10]. Zinc is associated with many enzyme systems, both as metallo-enzyme and enzyme activator, as well as filling a structural role. In addition, it plays a number of important biological roles such as with

*Corresponding Author Email: pmjahmed55@gmail.com

Table 1. **Summary of review on the existing spectrophotometric methods for thedetermination of zinc**

Reagent	$\lambda_{max/nm}$	€(L mol^{-1} cm^{-1})	Beer's Law mg L^{-1}	Interference	Remarks	Ref.
Pyridoxal-4-phenyl-3-thio-semicarbazone	430	1.60×10^3	1.0- 18.0	Many	i. Less sensitive ii. Less selective due to much interference iii. Solvent extractive	14
7-(4-nitrophenylazo)-8-hydroxyquinoline-5-sulphonic acid	520	3.75×10^3	0.05-1.0	Cu^{+2},Ni^{+2} ,Co^{+2} , Cd^{+2},Fe^{+3} ,Fe^{+2}	i. Less sensitive ii. Less selective due to much interference iii. pH dependent	15
Benzildithiosemicarbazone	395	0.42×10^3	1.0- 18	Cu^{+2},Ni^{+2} ,Co^{+2},Pb^{+2},Mn^{+2},Ag^{+}	i. Less sensitive ii. Less selective due to much interference iii. Solvent extractive	16
1,3-Cyclohexandionedithiosemi carbazone	570	1.42×10^3	0.1-20	Many	i. Less sensitive ii. Less selective due to much interference iii. Time dependent	17
1,2-Cyclohexandionedithiosemi carbazone	415	0.73×10^3	1-100	Cu^{+2}, Cd^{+2} , Fe^{+2} Ni^{+2}, Co^{+2}, Hg^{+2}	i. Less sensitive ii. Less selective due to much interference iii. Solvent extractive.	18
Glyoxaldithiosemicarbazone	433	1.3×10^3	0.1-50	Cd^{+2} , Fe^{+2} Ni^{+2}, Co^{+2}, Hg^{+}	i. Less sensitive ii. Less selective due to much interference iii. pH dependent	19
Methylglyoxal bis (4-phenyl-3-thiosemicarbazone)	445	0.21×10^3	0.1-50	Many	i. Less sensitive ii. Less selective due to much interference iii. Solvent extractive.	20
Bis-[2,6-(2-hydroxy-4-sulpho-1-napthylazo)]pyridine	565	4.3×10^3	0.1-0.8	Ni^{+2},Co^{+2},Pb^{+2},Mn^{+2} ,Ag^{+}	i. Less sensitive ii. Less selective due to much interference iii. Solvent extractive and lengthy.	21
8-hydroxyquinoline derivative,7,(4nitrophenylazo)-8-hydroxyquinoline-5-sulfonicacid(pNIAZOXS).	408	3.75×10^3	0.05-1.0	Many	i) pH dependent. ii) Less sensitive. iii) Less selective due to much interference. iv) Solvent extractive hence, lengthy and time consuming.	22
2-benzoylpyridine thiosemicarbazone (BPT).	430	1.8×10^4	0.26-2.61	Many	i) Less sensitive. ii) Less selective due to much interference. iii) pH dependent. iv) Solvent extractive hence.	23
Zincon(2-carboxy-20-hydroxy-50 sulfoformazylbenzene)	560	2.8×10^3	0.1-2.5	Many	i) pH dependent. ii) Less sensitive. iii) Less selective due to much interference. Solvent extractive hence, lengthy and time consuming.	24
2-(2-quinolylazo)-5-dimthylaminophenol (QADMAP)	590	1.22×10^4	0-1.0	Many	i) Less sensitive. ii) Less selective due to much interference. iii) pH- dependent. iv) Limited application.	25
Di-2-pyridylketone benzoylhydrazone (DPKBH)	405	3.64×10^4	0.02-1.87	Many	i) Less sensitive. ii) Less selective due to much Interference. iii) pH dependent iv) Limited application.	26
5,7-dibromo-8-hydroxyquinoline	391	6.2×10^5	0.02-4.0	Using suitable masking agents, the reaction can be made highly selective	i) Highly selective. ii) Ultra sensitive. iii) Aqueous reaction medium. iv) Simple and rapid. v) Color stable more than 24 h at room temperature (25 ± 5^0C.) vi) Non -extractive. vii) Application in various real, environmental, biological, soil, milk and pharmaceutical samples.	*(Present Method)*

the synthesis of deoxyribonucleic acid (DNA) and ribosomal ribonucleic acid (RRNA). Zinc has been extensively studied in recent years, as it is essential in the human diet. Zinc is released into the environment by chemical weathering of zinc minerals. However, its mobility is restricted by adsorption onto clays and secondary oxides. Several compounds of zinc are commonly used in the preparation of ophthalmic solutions, insulin's, mouthwashes, and mineral–vitamin preparations. In pharmaceutical analysis, the ease with which zinc compounds can be analyzed by spectrophotometry does not seem to be complicated. In copper alloys, there are several elements that are added to provide specific attributes for the material. Zinc is seldom present in copper as an impurity, but it is intentionally added to form a series of industrial alloys. When it is added in concentrations of 5% or less, zinc acts as a deoxidizer. Industrially, Zinc increases the density, melting point, electrical and thermal conductivity of the copper alloy, decreasing the elasticity, but the strength hardness and the coefficient of expansion are increased. The effect of zinc content on the color of the alloy is also commercially important, because many brasses are used for decorative purposes.

On the other hand toxic role of the metal ion is also well recognized [5]. Although a little zinc is vital to health, too much is harmful; a single 220 mg zinc sulphate capsule can cause nausea and vomiting. The strong toxicity is due to the swelling of too large quantities of zinc resins, accidentally or intentionally. At high concentrations in water systems, however, zinc is accepted as a hazardous contaminant [5]. Toxic effects may include abdominal pain, fever and also severe anemia resulting from eating acidic foods or drinks that have been stored in galvanized containers. All these findings cause great concern regarding public health, demanding accurate determination of this metal ion at trace and ultra trace levels.

Spectrophotometry is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis. 5,7-Dibromo-8-hydroxyquinoline (DBHQ) has been reported as a spectrophotometric reagent for vanadium, molybdenum and cadmium, but has not previously been used for spectrophotometric determination of zinc [11-13]. This paper reports its use in a very sensitive, highly specific spectrophotometric method of trace determination of zinc. The method possesses distinct advantages over existing methods with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH /acidity range, thermal stability, accuracy, precision and ease of operation. From above mentioned literature survey (Table-1) it reveals that those methods are lengthy, time-consuming, pH dependent and in most of above mentioned methods, interference was high and applied on limited samples [14-26]. It is needless to emphasize further that the direct spectrophotometric method in non-extractive way is more useful if it offers high sensitivity and selectivity. Search should be directed a new in order to develop simpler spectrophotometric method for non-extractive estimation of zinc in very selective and sensitive ways. The method is based on the reaction of non-absorbent DBHQ in a slightly acidic solution with zinc to produce a highly absorbent yellow chelate product followed by a direct measurement of the absorbance in an aqueous solution with suitable masking, the reaction can be made highly selective and the reagent blank solution do not show any absorbance.

Materials and Methods

Apparatus

A Shimadzu (Kyoto, Japan) (Model-160) double beam UV/VIS spectrophotometer and Jenway (England, U.K) (Model-3010) pH meter with a combination of electrodes were used for the measurements of absorbance and pH, respectively. A Thermo Fisher Scientific (Model: iCE 3000 series, origin USA) atomic absorption spectrometer was used for comparison of the results. Infrared spectrum was recorded with FTIR Spectrophotometer, Shimadzu (Model-IR Prestige 21, Detector-DTGS KBr) in the range 7500-350 cm^{-1}.

Reagent and solutions

All of the chemicals used were of analytical reagent grade or the highest purity available. Doubly distilled deionized water and HPLC-grade ethanol, which is non-absorbent

under ultraviolet radiation, was used throughout. 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 zinc levels in the specimens were low.

DBHQ solution, 3.3×10^{-3} M

Prepared by dissolving the requisite amount of DBHQ (Merck, Darmstadt, Germany) in a known volume solution of distilled ethanol. More dilute solutions of the reagent were prepared as requited.

Zinc standard solution 1.53×10^{-2} M

A 100-mL amount of stock solution (1mg mL^{-1}) of Zn was prepared by dissolving 440.0 mg of zinc sulfate heptahydrate ($ZnSO_4$. 7 H_2O) in doubly distilled deionized water. Aliquots of this solution were standardized with EDTA titration using Eriochrome Black T as indicator [27].Working standard solution was prepared by suitable dilutions of the stock solution

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, dissodium salt dehydrate in (100-mL) deionized water.

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.

Dilute ammonium hydroxide solution

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

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 Mat they) according to the recommended procedures of Mukharji [28]. In the case of insoluble substances, special dissolution methods were adopted [29].

General Procedure

A volume of 0.01-1.0-mL of neutral aqueous solution containing 0.2-40 µg of zinc in a 10 mL volumetric flask was mixed with a 1:270 to 1:700 fold molar excess (preferably 1 mL of 3.30 $x10^{-3}$ 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 0.0001 M sulfuric acid. The solution was mixed well. After 1 minute 5-mL of ethanol was added. The mixture was diluted up to the mark with deionized water. The absorbance was measured at 391 nm against a corresponding reagent blank. The zinc content in an unknown sample was determined using a concurrently prepared calibration graph.

Sample collection and preservation

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.

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-20^0C.

Soil: Soil (surface) samples were collected from different locations in Bangladesh. Samples were dried in air and homogenized with a mortar.

Milk samples: Milk samples were collected from local market of Chittagong. Human milk were collected from Chittagong Medical College Hospital. After collection the samples were stored in refrigerator for preservation.

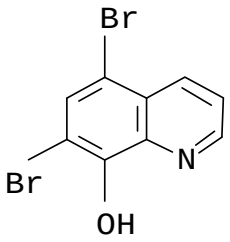
Pharmaceutical samples: Pharmaceutical samples (tablet and syrup) of different companies were collected from local Pharmacy of Chittagong. Samples (tablet) were homogenized with a mortar.

Results and Discussion

Factors Affecting the Absorbance

Absorption spectra

The absorption spectra of a zinc-DBHQ system in aqueous medium in presence of 1 mL 0.0001 M sulfuric acid solution, was recorded using the spectrophotometer. The absorption spectra of the zinc - DBHQ is a asymmetric curve with maximum absorbance at 391 nm and an average molar absorptivity of 1.62 x 10^5 L mol^{-1} cm^{-1} (Fig. 1). The reagent blank exhibited negligible absorbance despite having wavelength at 391 nm. The reaction mechanism of the present method is as reported earlier [30].



Scheme 1. **Structure of 5,7-Dibromo-8-hydroxyquinoline (DBHQ)**

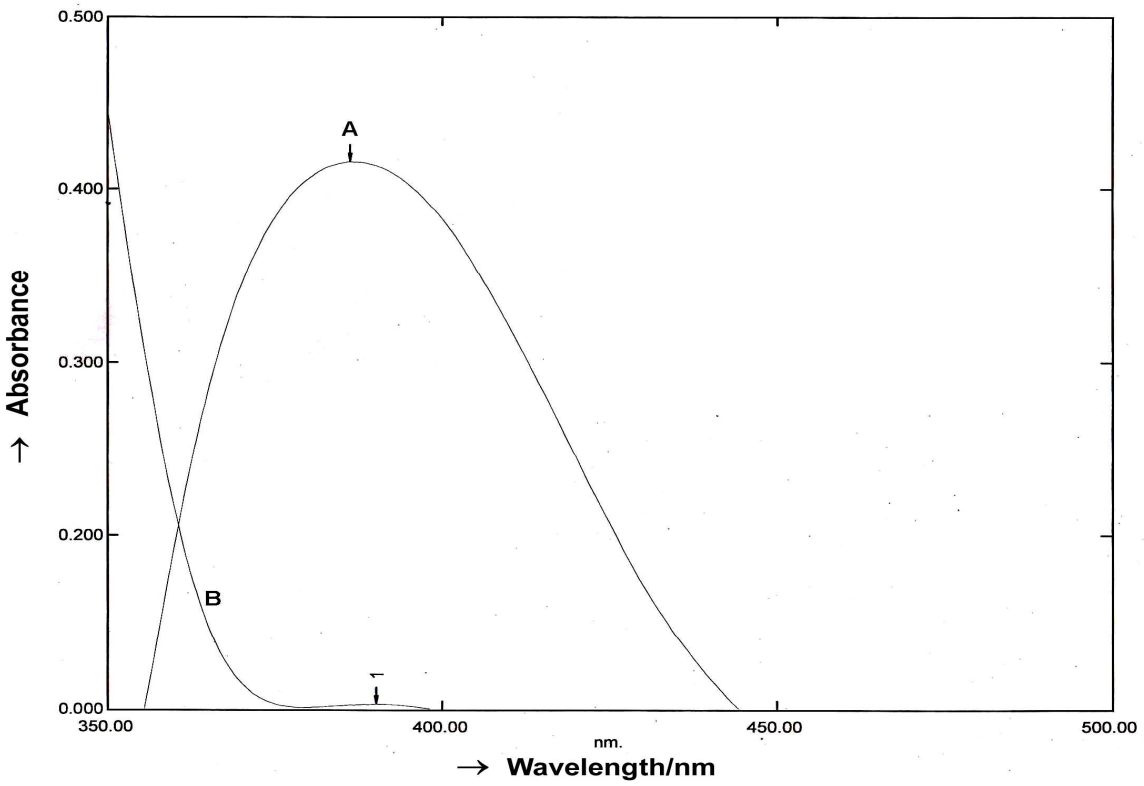


Figure 1. **A and B absorbance spectra of Zinc-DBHQ and the reagent blank (λ_{max} = 391 nm) in aqueous solutions.**

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, dimethylformamide, methanol and 1,4-dioxane) studied, ethanol was found to be the best solvent for the system. Different volumes (0-7-mL) of ethanol was added to fixed metal ion concentration and the absorbance were measured according to the general procedure. Maximum absorbance was observed in (50 ± 2%) (v/v) ethanol/water medium, hence, a 50% ethanol solution was used in the determination procedure. It was observed that 50-70% (5-7 mL) ethanol produced a constant absorbance of the Zn-chelate (Fig. 2). For all subsequent measurements, 50% (5 mL) of ethanol was added.

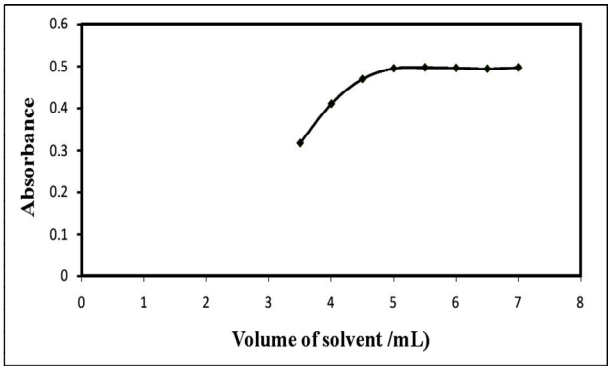


Figure 2. **Effect of solvent on the absorbance of Zn-DBHQ system.**

Effect of acidity

Of the various acids (nitric, sulfuric, hydrochloric and phosphoric) studied, sulfuric 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 0.0001 M sulfuric acid to every 10 mL of test solution. The maximum and constant absorbance was obtained in the presence of 0.1-0.7 mL of 0.0001M sulfuric acid at room temperature $(25\pm5)^0$C. Outside this range of acidity, the absorbance decreased (Fig. 3). For all subsequent measurements 0.5 mL of 0.0001 M sulfuric acid was added.

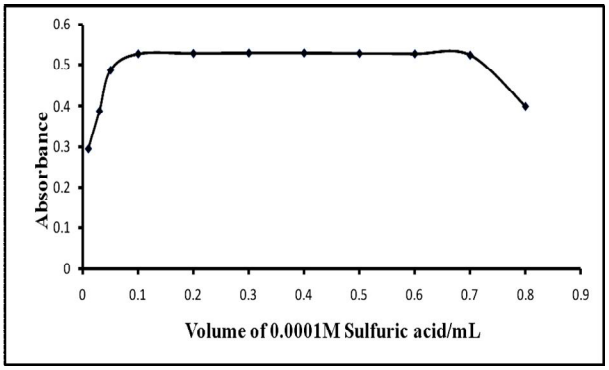


Figure 3. **Effect of acidity on the absorbance of Zn-DBHQ system.**

Effect of time

The reaction is very fast. A constant maximum absorbance was obtained just after dilution within few seconds to volume and remained strictly constant for over 24 h; a longer period of time was not studied.

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 zinc metal, the reagent molar ratio of 1:270 to 1:700 produced a constant absorbance of Zn - chelate (Fig. 4). For different (0.5 and 1.0 mgL^{-1}) zinc 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 $\times10^{-3}$ M DBHQ reagent was added.

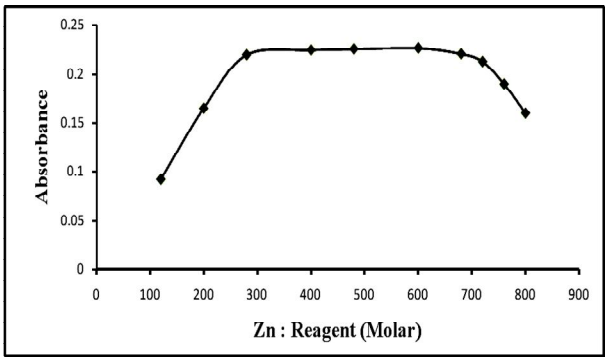


Figure 4. **Effect of reagent on the absorbance of Zn –DBHQ System.**

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, 10.0-100.0 mgL^{-1}) for convenience of the measurement. The absorbance was linear for 0.02-4.0 mg L^{-1} at 391 nm. Of the three calibration graphs one showing the limit of the linearity is given in (Fig. 5). The next two are straight-line graphs passing through the origin (R^2 = 0.9998).The molar absorption co-efficient and the Sandell's sensitivity [31] were found to be 1.62 x 10^5 L mol^{-1} cm^{-1} and 10 ng cm^{-2} of zinc, respectively. The selected analytical parameters obtained with the optimization experiments are summarized in (Table 2).

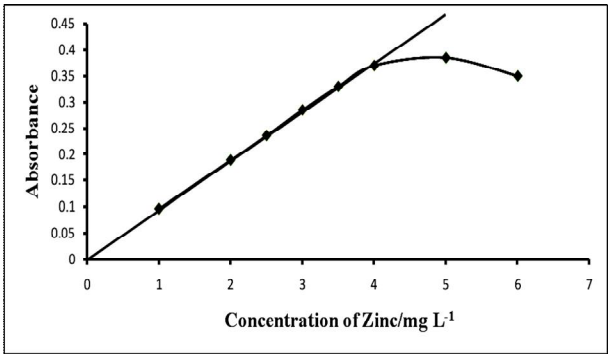


Figure 5. **Calibration graph C : 1 – 4 mg L^{-1} of zinc.**

Precision and accuracy

The precision of the present method was evaluated by determining different concentrations of zinc (each analyzed at least five times). The relative standard deviation (n = 5) was 2-0% for 0.2-40 µg of zinc in10-mL, indicating that this method is highly precise and reproducible. The detection limit (3s of the blank) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for zinc were found to be 5 mg L^{-1} and 10 ng cm^{-2}, respectively. The method was also tested by analyzing several synthetic mixtures containing zinc and diverse ions (Table 4). The results for total zinc were in good agreement with certified values (Table 5). The reliability of our Zn-chelate procedure was tested by recovery studies. The average percentage recovery obtained for addition of zinc spike to some environmental water samples was quantitative as shown in (Table 6.) The results of biological analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS (Table 7). Hence, the precision and accuracy of the method were excellent. With suitable masking, the reaction can be made highly selective.

Effect of foreign ions

The effect of over 50 anions, cations and complexing agents on the determination of only 1 mg L^{-1} of zinc was studied. The criterion for an interference[32] was an absorbance value varying by more than 5% from the expected value for zinc alone. The results are summarized in (Table 3). As can be seen, a large number of ions have no significant effect on the determination of zinc. The interference were from V(V), Mo(VI) and Cd(II) ions. Interference from these ions are 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 tartrate used as masking agent, respectively. During the interference studies, if a precipitate was formed, it was removed by centrifugation. The amount mentioned is not the tolerance limit but the actual amount studied. However, for those ions whose tolerance limit has been studied, their tolerance ratios are mentioned in (Table 3).

Composition of the absorbent complex

Job's method[33] of continuous variation and the molar ratio method[34] were applied to ascertain the stoichiometric composition of the complex. A Zn : DBHQ(1 : 2) complex was indicated by both methods.

Applications

The proposed method was successfully applied to the determination of zinc in a series of synthetic mixtures of various compositions (Table 4) and also in a number of real samples e.g. several Certified Reference Materials (CRMs) (Table 5). The method was also extended to the determination of zinc in a number of

Table 2. **Summary of Selected analytical parameters obtained with optimization experiments**

Parameters	Studied value	Selected value
Wavelength/ λ_{max} (nm)	200-800	391
Acidity/ M H_2SO_4	0.0000001-0.00002	0.000001-0.000007 (preferably 0.000005)
pH	4.83 – 3.46	4.39 -4.01 (preferably 4.04)
Time/h	0-24h	1min-24h (preferably 2 min)
Temperature/ ^oC	10-95	25±5
Reagent (fold molar excess,M:R)	1:1-1:800	1:270-1:700 (preferably 1mL)
Linear range/mgL^{-1}	0.01-100	0.02-4.0
Molar absorptivity/$Lmol^{-1}cm^{-1}$	1.00×10^5 - 2.35×10^5	1.62×10^5
Detection limit/μgL^{-1}	0-100	5
Sandell's sensitivity/$ngcm^{-2}$	0-100	10
Reproducibility(%RSD)	0-10	0-2
Regression coefficient,R^2	0.9987-0.9998	0.9998

Table 3. **Tolerance limits of foreign ions, tolerance ratio [Species(x)]/Zn (w/w)**

Species x	Tolerance ratio x/Zn (w/w)	Species x	Tolerance ratio x/Zn (w/w)
Aluminium	500	Lithium	20
Arsenic(III)	30[a]	Lead(II)	50
Arsenic(V)	50	Magnesium	100
Ammonium(I)	50	Mercury(II)	100
Antimony	100	Molybdenum(V)	50
Azide	100	Molybdenum(VI)	20[b]
Bismath(III)	20	Manganese(II)	100
Bromide	100	Nickel(II)	50
Barium	50	Nitrate	200
Cadmium	20[a]	Oxalate	20
Cobalt(II)	10	Phosfate	100
Cobalt(III)	100	Potassium	100
Calcium	100	Selenium(IV)	20
Chloride	100	Selenium(VI)	50
Citrate	10	Strontium	100
Chromium(VI)	30[a]	Sulfate	100
Chromium(III)	50	Sodium	200
Caesium	20	Tartarate	1000
Copper(II)	20[b]	Tin(II)	50
Cerium	20[a]	Tin(IV)	50
EDTA	100	Titanium(IV)	20
Fluoride	100	Tellurium(V)	20
Iron(II)	100	Thiocyanate	50
Iron(III)	20	Tungsten(VI)	50
Iodide	100	Vanadium(V)	10[a]

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

Table 4. **Determination of zinc in some synthetic mixtures**

Sample	Composition of mixtures (mgL^{-1})	Zn / mgL^{-1}		
		Added	Found[a]	Recovery ± s[b] (%)
A	Zn^{2+}	0.50 1.00	0.49 1.00	98 ± 0.5 100 ± 0.0
B	As in A + Fe^{2+}(50) + Mn^{2+}(50) + EDTA(10)	0.50 1.00	0.50 1.02	100 ± 0.0 102 ± 0.8
C	As in B + Mo^{VI}(20) + K(50) + EDTA(10)	0.50 1.00	0.49 0.99	98 ± 0.6 99 ± 0.7
D	As in C + Sr(50) + Sb^{III}(50) + EDTA(10)	0.50 1.00	0.53 1.05	106 ± 1.3 105 ± 1.0
E	As in D + Hg^{2+}(50) + Ni^{2+}(50) +Tartrate(50)	0.50 1.00	0.54 1.08	108 ± 2.0 108 ± 1.8

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

Table 5. **Determination of zinc in certified reference materials.**

Certified Reference Materials (Composition, %)	Zn, %		
	Certified value	Found* (n=5)	R.S.D. %
BAS-CRM-10g, High tensile brass (Cu=60.8, Fe=1.56, Pb=0.23, Ni=0.16, Sn=0.21, Al=3.34, Zn=32.0 and Mn=0.12)	32.0	31.89	1.2
BAS-CRM-5g; brass (Cu=67.4, Sn=1.09, Pb=2.23, Zn=28.6 and Ni=0.33	28.6	28.38	1.5
Brass-CRM-5f, Cu=70.8, Zn=24.2, Sn=1.84, Fe=0.31, Ni=0.17 and Mn=0.12	24.2	24.05	1.8
ZLD104*, Si=6.84, Fe=0.82, Cu=0.77, Mg=0.64, Zn=0.64, Mn=0.08, Ti=0.17 and Pb=0.10	0.64	0.66	2.0
ZLD108*,Si=14.02,Fe=0.69, Cu=3.37, Mg=0.68, Mn=0.61, Zn=0.55, Ni=0.20 and Pb=0.045	0.55	0.54	2.5

*These CRMs obtained from Beijing NCS Analytical Instrument Co. Ltd., China.

Table 6. **Determination of zinc in some environmental water samples**

Sample		Zinc / µg L^{-1} Added	Zinc / µg L^{-1} Found [a]	Recovery ± s (%)	s_r [b] (%)
Tap water		0	250.0		
		100	355.0	98.5 ± 0.5	0.31
		500	760.0	98.6 ± 0.3	0.35
Well Water		0	125.0		
		100	230.0	98 ± 0.4	0.35
		500	625.0	100 ± 0.0	0.00
Rain water		0	30.0	100 ± 0.0	0.00
		100	130.0	99 ± 0.3	0.29
		500	535.0		
River water	Karnaphuly (upper)	0	50.0		
		100	150.0	100 ± 0.0	0.00
		500	560.0	98 ± 0.2	0.24
	Karnaphuly (lower)	0	55.0		
		100	160.0	103 ± 0.1	0.24
		500	565.0	98 ± 0.2	0.27
	Halda (upper)	0	40.0		
		100	140.0	100 ± 0.0	0.00
		500	545.0	99 ± 0.3	0.09
	Halda (lower)	0	45.0		
		100	145.0	102 ± 0.8	0.01
		500	550.0	99 ± 0.5	0.08
Sea water	Bay of Bengal (upper)	0	10.0		
		100	110.0	100 ± 0.0	0.00
		500	515.0	99 ± 0.6	0.21
	Bay of Bengal (lower)	0	12.0		
		100	110.0	98 ± 0.7	0.45
		500	512.0	100 ± 0.0	0.00
Drain water	PHP Steels Ltd[b].	0	500.0		
		100	600.0	100.0 ± 0.0	0.00
		500	1010.0	99 ± 0.6	0.34
	Eastern Refinery[c]	0	260.0		
		100	360.0	100 ± 0.0	0.00
		500	770.0	99 ± 0.8	0.30
	K.P.M.[d]	0	550.0		
		100	660.0	98 ± 0.4	0.29
		500	1060.0	99 ± 0.5	0.47

[a] average of the five replicate determinations - [b]PHP Steel Mill, Bara Kumira, Chittagong - [c]Eastern Refinery, North Patenga, Chittagong
[d]Karnaphuli Paper Mill, Chandraghona, Chittagong

Table 7. **Determination of zinc in human fluids**

Serial No.	Sample	Zinc / mgL^{-1} AAS (n = 5) *Found*	AAS (n = 5) *RSD,%*	Proposed method n = 5 *Found*[b]	Proposed method n = 5 *RSD,%*	Sample source[a]
1	Blood	1.15	1.0	1.21	1.0	Normal adult (Male)
	Urine	0.32	1.2	0.34	1.5	
2	Blood	1.74	0.3	1.81	1.0	Harnia (Male)
	Urine	0.59	0.7	0.62	1.4	
3	Blood	2.66	0.4	2.72	0.8	Liver cirrhosis patient (Male)
	Urine	0.76	0.8	0.78	1.2	
4	Blood	1.35	0.5	1.42	0.7	Pregnant Woman
	Urine	035.	0.8	0.40	1.6	
5	Blood	1.36	0.4	1.45	1.2	Kidney diseases patient (Male)
	Urine	0.44	0.8	0.46	1.8	
6	Blood	1.38	0.7	1.40	0.9	Diabetic patient(Male)
	Urine	0.45	1.2	0.47	2.0	

[a] Samples were collected from Chittagong Medical College Hospital and C.S.C.R. Hospital Chittagong.
[b] Average of the five replicate determinations

Table 8. **Determination of zinc in some surface soil[a, b]**

Serial No.	Zinc (mg kg^{-1}) (n=5)[a]	Sample source
S_1[b]	10 ± 0.8	Marine soil (Bay of Bengal, Chittagong, Bangladesh)
S_2	70 ± 1.5	Industrial Soil (Welding Industry, Chittagong, Bangladesh.)
S_3	20.0 ± 1.2	Esturine Soil (Karnaphuly River, Chittagong, Bangladesh),
S_4	45 ± 2.0	Roadside soil, (Chittagong-Dhaka Highway)
S_5	15 ± 1.0	Agricultural Soil (Chittagong University Campus)
S_6	55 ± 1.8	Industrial soil (PHP Steels Ltd. Bara Kumira, Chittagong, Bangladesh)

[a]Average of five analysis of each sample
[b]Measure of precision is the standard deviation
[c]Composition of the soil samples: C, N, P, K, Na, Ca, Mg, Fe, Pb, Cu, Zn, Mn, Mo, Co, NO_3, NO_2, SO_4, etc.

Table 9. **Determination of zinc in some pharmaceutical samples**

Composition of Pharmaceutical samples	Brand name	Claimed value $mgkg^{-1}$	Expt. Value $mgkg^{-1}$	Recovery (%)	RSD (%)
Each tablet contains 20 mg of zinc	BIOPHARMA	1.00	0.98	98 ±1.0	1.9
	ORION PHARMA	1.00	0.99	99 ±0.8	1.8
5 mL syrup contains 10 mg of zinc	SQUARE PHARMA	1.0	1.05	105±1.2	1.5
	APEX PHARMA	1.0	0.95	95 ±1.5	2.5

Table 10. **Determination of zinc in some milk samples**

Milk Samples*	Zinc			
	Claimed Value	*Found*	*Recovery (%)*	*RSD (%)*
Marks Milk[a]	0.79	0.84	106 ± 0.8	1.2
Arong Milk[a]	0.81	0.86	106 ± 1.0	1.8
Dano Milk[a]	0.80	0.78	98 ± 1.2	2.0
Cow Milk[b]	0.95	1.00	105 ± 1.0	1.5
Goat Milk[b]	0.73	0.78	106 ± 0.8	1.8
Human Milk[b]	0.11	0.12	106 ± 1.0	2.5

*Samples were collected from local Market and Hospital of Chittagong.
[a]Values in mg kg^{-1} - [b]Values in mg L^{-1}

environmental, biological, pharmaceutical, milk and soil samples. In view of the unknown composition of environmental water samples, the same equivalent portions of each such samples were analyzed for zinc 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 6). The results of biological analyses by spectrophotometric method were found to be in excellent agreement with those obtained by AAS (Table 7). The results of soil samples analyzed by the spectrophotometric method are shown in Table 8. The results of pharmaceutical sample by the spectrophotometric method are shown in Table 9. The results of milk sample by the spectrophotometric method are shown in Table 10. The precision and accuracy of the method were excellent.

Determination of zinc in synthetic mixtures

Several synthetic mixtures of varying compositions containing zinc 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 4. Accurate recoveries were achieved in all solutions.

Determination of zinc in brass, alloys and steels (Certified reference materials)

Certified Reference Materials, alloys, 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 0.55 – 32% of zinc was accurately weighed and placed in a 50 mL Erlenmeyer flask following a method recommended by Parker[35]. 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 deionized 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 deionized water.

A suitable aliquot (1-2 mL) of the above solution was taken into a 10-mL calibrated flask and the zinc content was determined as described under general Procedure using EDTA or tartrate as masking agent. Based on five replicate analyses, the average zinc concentrations determined by spectrophotometric method were found to be in good agreement with the certified values. The results are shown in Table 5.

Determination of zinc in 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, following a method recommended by Greenberg *et al.*[36] and was cooled to room temperature. The residue was heated with 10 mL of deionized water in order to 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 deionized water.

An aliquot (1-2 mL) of this preconcentrated water sample was pipetted into a 10-mL calibrated flask and the zinc content was determined as described under the Procedure, using tartrate or EDTA as a masking agent. The analyses of environmental water samples for zinc from various sources is shown in Table 6. Most spectrophotometric methods for the determination of zinc in natural and sea-water require preconcentration of zinc[36]. The concentration of zinc in natural and sea-water is a few μgL^{-1} in India [14]. The mean concentration of zinc found in US drinking waters is 1.33 mgL^{-1} [36].

Determination of zinc in 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. When the initial brisk reaction was over, the solution was removed and cooled following a method recommended by Stahr [37]. 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 deionized water. A suitable aliquot (1-2-mL) of the final solution was pipetted into a 10-mL calibrated flask and the zinc 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 7.

The abnormally high value for the liver cirrhosis patient is probably due to the involvement of high zinc concentrations with As and Pb. Occurrence of such high zinc contents are also reported in liver cirrhosis patient from some developed countries[2]. The low value for the pregnant woman is probably due to a low zinc concentration in the environment.

Determination of zinc in 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, following the method recommended by Hesse.[38] The content 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 deionized water.

Suitable aliquots (1-2 mL) were transferred into a 10-mL calibrated flask and a calculated amount of 0.0001 M H_2SO_4 needed to give a final acidity of 0.000001-0.000007 M H_2SO_4 was added followed by 1-2 mL of 0.01% (w/v) tartrate or EDTA solution as masking agent. The zinc content was then determined by the above Procedure and quantified from a calibration graph prepared concurrently. The results are shown in Table 8. The average value of zinc in Chittagong region surface soil was found to be 35.83 $mgkg^{-1}$.

Determination of zinc in pharmaceutical samples

Finished pharmaceutical samples (each Zn containing 1mg tablet or 5 mL syrup or required weight) were quantitatively taken in a beaker and digested following a method recommended by Ahmed *et al*[29]. 10-mL of concentrated nitric acid was added and heated to dryness and then added 10-mL of 20% (v/v) of H_2SO_4. The volume was reduced to 2.5 mL and then cooled to room temperature. The solution was than neutralized with dilute NH_4OH in the presence of a 1-2mL of 0.01% (w/v) EDTA or tartrate solution. The resulting solution was then filtrated and quantitatively transferred to a 25-mL calibrated flask and made up to the mark with deionized water.

An aliquot (1-2 mL) of this digested sample was pipetted into a 10-mL calibrated flask and then zinc content was determined as described under the general Procedure using tartrate as a masking agent. The results of some pharmaceutical analyses are in excellent agreement with the reported values. The analyses of pharmaceutical samples from several Pharmaceutical Companies for zinc are given in Table 9.

Determination of zinc in milk samples

Each 10g amount of milk powder (Dano,Denmark;Marks,Australia) or liquid milk sample (100 mL) containing different composition

metals was accurately taken and evaporated nearly to dryness with a mixture of 3 mL concentrated H_2SO_4 and 10 mL of concentrated HNO_3 to sulfur trioxide fumes in a fume cupboard, following a method recommended by Stahr.[37] After cooling the residue was heated with 10 mL of deionized water in order to 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 deionized water.

An aliquot (1-2 mL) of this preconcentrated water sample was pipetted into a 10-mL calibrated flask and the zinc content was determined as described under the procedure, using mixture of tartrate and EDTA as masking agent. The results of mild analyses are in excellent agreement with the claimed values.The analyses of milk samples for zinc from various sources is shown in Table 10.

Conclusions

In this paper, a new, simple, sensitive, selective and inexpensive method with the Zn-DBHQ complex was developed for the determination of zinc in some industrial, environmental, biological, pharmaceutical, milk and soil samples, for continuous monitoring to establish the trace levels of zinc in different sample matrices. Although many sophisticated techniques such as pulse polarography, HPLC, AAS, ICP-AES and ICP-MS are available for the determination of zinc at trace levels in numerous complex materials, factors such as the low cost of the instrument, easy handling, lack of requirement for consumables and almost no maintenance have caused spectrophtometry to remain a popular technique, particularly in laboratories of developing countries with limited budget. The sensitivity in terms of molar absorptivity and precision in terms of relative standard deviation of the present method are very reliable for the determination of zinc in real samples down to ng g^{-1} levels in aqueous medium at room temperature (25 ± 5°C).

Acknowledgements

The authors thank to the authorities of Faculty of Biological Science, University of Chittagong for analyzing the biological samples by AAS. We are especially indebted to the authorities of Chittagong Medical College Hospital and CSCR Hospital for their generous help in supplying biological samples.

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