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Metal substitution method for the spectrophotometric determination of zinc(II) in real samples

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Abstract : A spectrophotometric procedure for the determination of zinc(II) by its quantitative displacement with copper(II) from diethyldithiocarbamate complex, $Cu(DDTC)_2$ is described. Subsequently, Cu(II) complex was extracted with $CHCl_3/CCl_4$ and the absorbance was measured at 435 nm. The molar absorptivity (ε) and Sandall's sensitivity at this wavelength are 2.86×10^5 mole^{-1} L cm^{-1} and 3.076 ng cm^{-2}, respectively. Reproducibility within 4% detection limit was 0.029 μg mL^{-1} and linear calibration range was 0.2-14 μg mL^{-1} with the regression coefficient (r^2) 0.998 for zinc determination. In the presence of a suitable masking agent (ascorbic acid and sodium thiosulfate) very good selectivity was achieved. The method was extended to the determination of zinc in a number of environmental water and soil samples, biological, pharmaceutical, fertilizer and food samples.

Keywords : Spectrophotometric procedure; Diethyldithiocarbamate; Zinc(II); Quantitative displacement; Real samples.

INTRODUCTION

Zinc is most commonly used as an anti-corrosion agent, galvanization as an anode material for batteries[1,2] as a substitute for the traditional lead/tin alloy in pipes, in the automotive, electrical, and hardware industries[3]. Zinc oxide has antibacterial properties, for which it is extensively used to treat a number of skin conditions, sunscreen lotion. Zinc compounds have been employed in dermatology, mouthwashes and ophthalmic solutions as antiseptic and disinfectant agents[4]. Zinc oxide is added in dietary or vitamin supplements and some food products, as a source of zinc. Zinc sulfate works well to treat zinc deficiencies. Crystals of ZnS are used in lasers that operate in the mid-infrared part of the spectrum[5]. Zinc ensures a healthy immune system and takes part in DNA synthesis as well[6]. Zinc is an essential micronutrient needed not only by people but also by crops. Zinc dithiocarbamate complexes are used as agricultural fungicides[7]. Zinc sulfide (ZnS) is used in luminescent pigments such as on the hands of clocks, X-ray and television screens, and luminous paints[8]. Due to the tremendous applications their exposure to environment and thereby in food cycle is obvious. Therefore, trace determination of zinc is of great importance, as some of them have nutritional significance, whilst others are toxic.

Determination of zinc was carried out by differential pulse anodic stripping voltammetry[9], ICP-AES[10], ICP-MS[11], FAAS[12]. Spectrophotometric methods for the determination of zinc have been reported a few that use thiosemicarbazones[13,14], dithiozone[15] or hydrazones[16,17] as a sensitive colourful reagent. Diethyldithiocarbamate (DDTC) is commonly used for spectrophotometric determination of Cu(II), Ni(II), Mn(II), and V(V)[18]. The complexes are insoluble in water and are extracted for measurement. On the other hand, several dithiocarbamate reagents have been used as coprecipitating agents together with a carrier element such as Cd(II)[19], Cu(II)[18] and Bi(III)[20] prior to the FAAS determination to preconcentrate the trace metal ions.

Zn(II) gives white insoluble complexes which is less stable than the yellow Cu(II) complex thus zinc can be indirectly determined by a displacement reaction after extraction with CCl_4. After displacement of zinc the absorbance of Cu(II) complex was measured. However, the use of DDTC as spectrophotometric reagent is the most interesting one for the determination of nontransition metal like zinc though they form colourless complexes. Finally, the aim of this study was to develop a rapid spectrophotometric method for the determination of zinc with sodium diethyldithiocarbamate (Na-DDTC) which has not previously been used for the determination of zinc by a spectrophotometric method. The present method was successfully applied to the determination of zinc in series of synthetic mixtures of various compositions and also in a number of real samples. The method was also extended to the determination of zinc in a number of environmental water and soil samples, biological, pharmaceutical, fertilizer and food samples.

EXPERIMENTAL

Chemicals and reagents

High-purity carbontetrachloride, various acids, salts and reagent grade Na-DDTC (Merck) were used. The standard stock solutions (1000 $\mu g\ mL^{-1}$) were prepared by dissolving appropriate amount of each salt in water. Solutions of a large number of inorganic ions and complexing agents were prepared from their analytical grade or equivalent grade, water soluble salts. Doubly distilled deionized water, which is non-absorbent under ultraviolet radiation, was used throughout. Suitable portions of these solutions were mixed to get the desired pH. Stock solutions and environmental water samples (1000-mL each) were kept in polypropylene bottles containing 1-mL of concentrated HNO_3. Both $[Zn(NH_3)_6][HgI_4]$ and $[Zn(en)_3][HgI_4]$ complex were analyzed by proposed method. Experimental zinc content was compared to theoretical values. $[Zn(NH_3)_6][HgI_4]$ and $[Zn(en)_3][HgI_4]$ complex were synthesized as following procedure published by Uddin *et al.*[21].

Instrumentation

A Shimadzu UV Visible UV-1800 spectrophotometer model with suitable settings equipped with 1-cm quartz cells was used for absorbance. The spectral band length was 1 nm, the wavelength accuracy was 0.5 nm with automatic wavelength correction, and the recorder was a computer-controlled in the wavelength range 350–600 nm. A Jenway (England, U.K) (Model-30100) pH meter were used for the measurements of pH. A Varian (Australia) ICP-MS spectrophotometer was used for comparing the results.

Preparation of standards

Stock solutions of Na-DDTC, copper, zinc were prepared by dissolving an appropriate amount of each compound in water and were stored at 4°C, protected from light and used within 3 months. A 0.1 % (4.4×10^{-3} mol L^{-1}) Sodium Diethyldithioarbamate, DDTC stock solution was prepared by dissolving 0.1 g sodium diethyldithiocarbamate reagent in approximately 80 mL heated (60 oC) water. Afterwards, the volume was made up to 100 mL in a volumetric flask and filtered. The filtrate solution was taken in a 100 mL volumetric flask and made up to the mark by adding distilled water. This solution was stable for two weeks, at least. A stock solution (13.06 g L^{-1}) of zinc (2×10^{-3} M) was prepared by dissolving 5.7508 g of zinc sulphate heptahydrate (Merck, Germany) in 100 mL of doubly distilled deionized water. The working standard of zinc solution was prepared by suitable dilutions of this stock solution. A stock solution (12.70 g L^{-1}) of copper (2×10^{-3}M) was prepared by dissolving 4.992 g of copper sulphate pentahydrate (Merck, Germany) in 100 mL of doubly distilled deionized water in a mixture of 15 mL of concentrated HNO_3. The working standard of copper solution was prepared by suitable dilutions of this stock solution. The buffer solutions were prepared by mixing 1M HCl and 1M sodium acetate (pH 1–3), 0.2M acetic acid and 0.2M sodium acetate (pH 3.2–6.0), 1M sodium acetate and 0.2M acetic acid (pH 7.0), and 2 M ammonium hydoxide and 2 M ammonium chloride (pH 8.0–12.0). The pH of these solutions as prepared in doubly distilled water. Suitable portions of these solutions were mixed to get the desired pH. A 100-mL stock solution of tartrate (0.01%) was prepared by dissolving 10 g of A.C.S. grade (99%) potassium sodium tartrate tetrahydrate in (100-mL) deionized water. 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. A 100-mL stock solution of EDTA (4000 mg L^{-1}) was prepared by dissolving 0.4 gm of A.C.S. grade (≥90%) ethylenediaminetetraacetic acid, dissodium salt dehydrate in (100-mL) deionized water.

General procedure

To determine zinc, a solution of (1 -15 mL) 2×10^4 M Zn(II) was placed in a 25-ml separating flask along with 0.5 mL 0.05 M H_2SO_4, and 1 mL of the reagent 6×10^{-4} M DDTC solution was mixed. The mixture was stirred at 10 min. The solid product, $Zn(DDTC)_2$ complexes so formed, was extracted carefully with the addition of 10 mL CCl_4 in two phases congaing 5 mL each. Its pH value was adjusted to 5 using acetate buffer. 1 mL of 2×10^{-4} M Cu(II) solution was added when immediate yellow colour was formed, and organic phase was further separated after vigorous stirring for 10 min. The absorbance was measured by a spectrophotometer at 435 nm against a blank. Optimum experimental conditions for acid and reagent concentration, stability, aqueous phase volume and extraction period was studied. To determine the one effect other parameters were kept constant and the general procedure given above was applied.

Method validation

Method was validated in terms of ICH[22] analytical performance parameters; precision, accuracy, specificity, limit of detection, limit of quantitation, linearity and range, suitability and robustness.

RESULTS AND DISCUSSION

Method optimization

(a) Absorption spectra

The absorption spectra of the reagent and the complex are recorded in the wavelength range 350–600 nm at pH 5.0 against CCl_4 (standard) or reagent blank (real samples). The typical superimposed UV-vis spectra of $Cu(DDTC)_2$ or $Zn(DDTC)_2$ in CCl_4 and reagent blank are presented in Figure 1. The spectra show that the Cu(II) complex solution has an absorption maximum at 435 nm, whereas $Zn(DDTC)_2$ or the reagent does not show appreciable absorbance at this wavelength. It was found that the molar absorptivity (ε) and Sandell's sensitivity (for the absorbance of 0.001) at this wavelength are 2.86×10^5 $mole^{-1}$ L cm^{-1} and 3.076 ng cm^{-2}, respectively. Therefore, UV-vis spectrophotometric measurements were carried out at a wavelength of 435 nm for subsequent studies. Its determination in the different matrices based on the direct measurement of its absorption for ultraviolet light is not susceptible to potential interferences from the matrix excipients.

(b) Effect of acid

Acid effect was primarily tested for HCl, HNO_3, H_2SO_4 while last one was supposed to be suitable for Zinc-complexation. The influence of acid concentration on the reaction was investigated by carrying out the reaction in varying acid values. Test at different acid concentration ($x\times10^{-2}$ M, H_2S0_4) for a constant concentration (1.0 μg mL^{-1}) of Zn(II) produced a constant absorbance for $(0.1\text{-}0.8)\times10^{-2}$ M, H_2S0_4. Therefore, 0.5×10^{-2} M, H_2S0_4 was selected as optimized concentration and all measurements have been peformed at this concentration level.

(c) Effect of reagent concentration

Studying the effect of DDTC concentration on its reaction with zinc revealed that the reaction was dependent on the DDTC concentration as the readings increased with the increase in the reagent concentration (Figure 3). The absorbance of the complex solution at

435 nm is measured according to the standard procedure at different molar excesses (1:1-1:30) of sodium diethyldithiocarbamate keeping Zn(II) concentration (1.0 µg mL^{-1}) constant at optimized acid concentration.

It was revealed that zinc metal and (optical path length 1 cm), the reagent molar ratios of 1:10 to 1:40 produced a constant absorbance of $Zn(DDTC)_2$. A greater excess of the reagent was not studied. Therefore, a 15

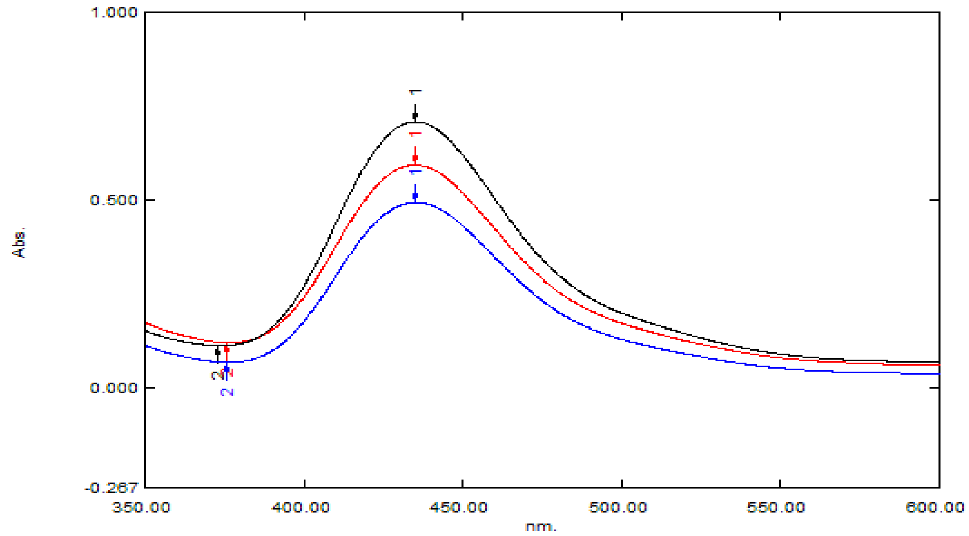

Figure 1 : The typical superimposed UV-vis spectra of (A) $Cu(DDTC)_2$

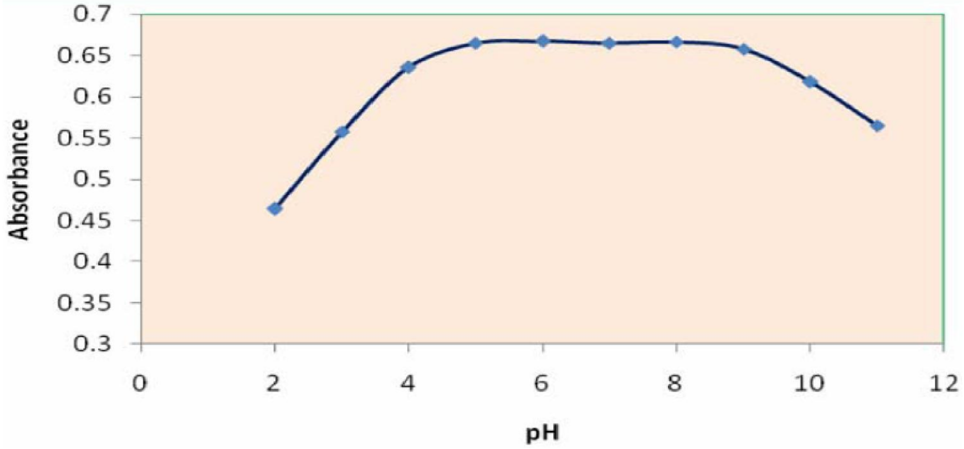

Figure 2 : Effect of pH on the extraction of the Cu(II)-DDTC complex in organic phase.

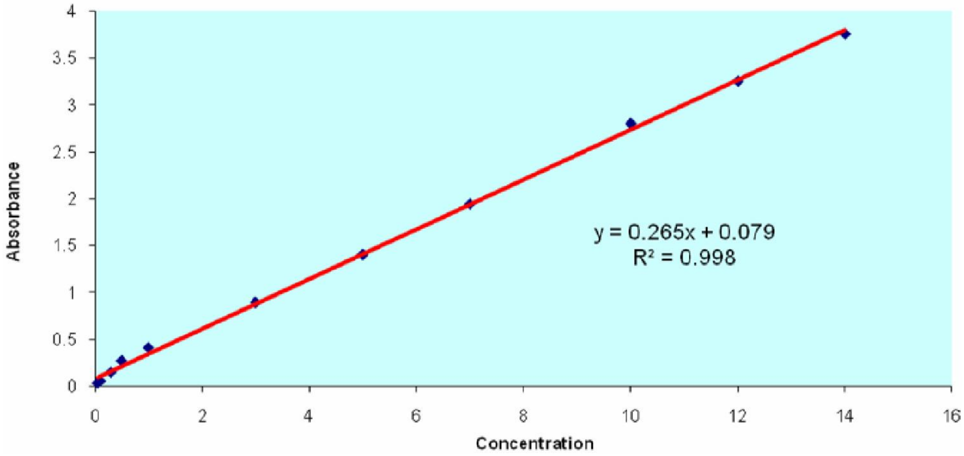

Figure 3 : Calibration curve constructed by plotting absorbance against corresponding concentrations (µg mL^{-1}) of zinc.

fold molar excess of DDTC was optimized for constant color development and was used in all the subsequent experiments. Excess of the reagent has no effect on the absorbance of the complex.

(d) Effect of temperature and time

The effect of temperature on the reaction was not studied due to the lake of instrumental facilities. Therefore, further experiments were carried out at room temperature (25 ± 2°C). In order to determine the optimum time that is required for completion the reaction, it was allowed to proceed at room temperature for varying periods of time. It was found that the reaction goes to almost completion within 1 min, however for higher precision readings, the reaction was allowed to proceed for quite longer time; reactions in all the subsequent experiments were carried out for 10 min.

(e) Stability of the Cu(II)-DDTC complex

The effect of time on the stability of the Cu(II)-DDTC complex was studied by following the absorption intensity of the reaction solution (after dilution) at different time intervals. It was found that the absorbance of the complex remains stable for at least 72 hrs. This allowed the processing of large batches of samples, and their comfortable measurements with convenience. This gives the high throughput property to the proposed method when applied for analysis of large number of samples in quality control/analytical laboratories.

(f) Effect of pH on the extraction of the complex

The pH is one of the most important parameters, and first of all pH must be determined. The influence of pH on the exchange reaction, replacement of Zinc by copper and subsequent extraction in organic phase, was investigated by carrying out the reaction in buffer solution of varying pH values. The other parameters being constant, the general procedure was applied and the concentrations of metals were measured by UV-VIS spectrophotometer. The effect of pH on the color intensity is studied in the pH range 2–11. The stability constant of $Cu(DDTC)_2$ is greater than that of $Zn(DDTC)_2$. Experimental results presented in Figure 4 shows that as the pH increased, the readings increased rapidly. The optimum pH value for the reaction of Cu(II) and $Zn(DDTC)_2$ is attained at pH 5 and remains constant up to 9 (Figure 2). At higher pH, sharp decrease in the readings occurred. The pH value should not be lower than 4 due to the fast decomposition of dithiocarbamates at these experimental conditions. On the other hand, pH value over 9 would accelerate the $Cu(OH)_2$ precipitation. Hence, pH 5.0 is chosen for further studies and as convenient. Acetic acid-acetate buffer solution (pH = 5.0) was chosen for the subsequent studies. In order to investigate the effect of buffer components on the reaction, different buffer solutions of pH 5 were tested and buffer was used as mentioned in experimental section. As the highest absorbance was obtained it was used in all the subsequent experiments.

(g) Effect of the aqueous phase volume

The volume of the aqueous phase is an important factor for the extraction of metal ions as Cu(II)-DDTC is sparingly soluble in aqueous phase[23]. The effect of volume of the aqueous phase was also studied. The solutions containing 1 μg mL^{-1} of Cu(II) were diluted in the volume range from 5 cm^3 to 30 cm^3 with deionized

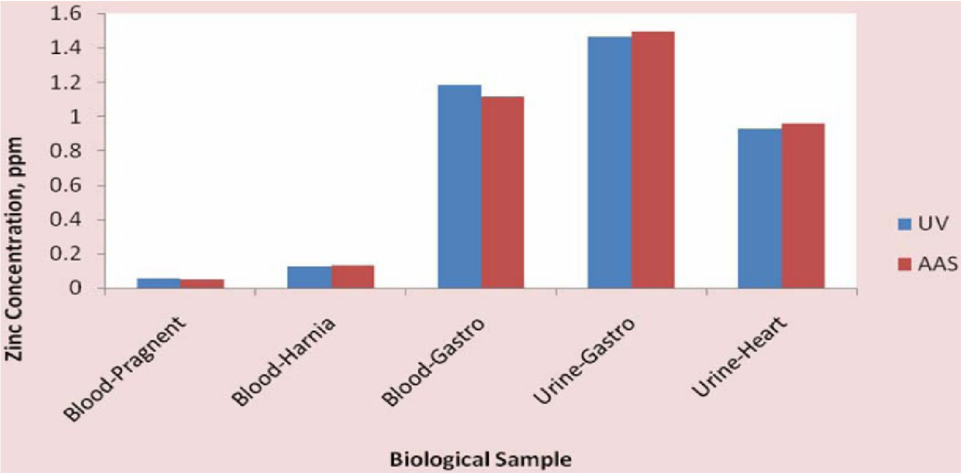

Figure 4 : Comparative presentation of concentration obtained by proposed method and ICP-MS.

water. The general procedure given above was applied and the recovery percentages were calculated. The recovery of Cu(II) from aqueous phase was plotted against the aqueous phase volume. The recovery of Cu(II) from aqueous phase decreases when the aqueous phase volume increases. It is indicated that the exchange reaction of metals is rapid, and the efficiency is high. It is revealed that aqueous volume beyond 15 mL rapid decrease in absorbance was occurred hence total aqueous volume was always confined to maximum volume 15 mL throughout all experiments for convenient of operation with confidence.

(h) Optimum extraction period

The efficiency of Cu(II) extraction in organic phase depends on the extraction period. Optimum extraction period was determined by extracting copper from the solutions containing 1 μg mL^{-1} of Cu(II) for various periods of time according to the procedure described previously. Then, the amount of Cu(II) dissolved in CCl_4 was determined on the basis measurement of the absorbance using a UV-VIS spectrophotometer. After 10 min constant absorbance was obtained up to studied period of 30 mins. Hence, reaction mixture was extracted at least10 min for getting maximum recovery.

Composition of the complex

Under the optimum conditions (TABLE 1), the stoichiometry of the reaction between Cu(II) and DDTC was investigated by Job's[14] and mole ratio methods. Experimental data has been found and the stoichiometry was found to be 1:2 (Copper: Ligand).

Validation of the proposed method

(a) Preparation of calibration curves

The calibration curves were made as described in the experimental procedure and good correlation coefficients were found. It was constructed by plotting absorbance against corresponding concentrations for ten standard solutions containing 0.01-16.0 μg mL^{-1} of zinc or copper according to the general procedure. The linearity range, regression equation and correlation coefficient were obtained by the method of least squares. The liner plot between the absorbance and the amount of zinc(II) ion corresponded to the Cu(II) and Cu(II) alone is drawn and the straight line obeyed the equation $y=0.256x+0.079$ and $y=0.208x+0.075$ for zinc and copper, respectively having correlation coefficient of (r^2) 0.998 each (Figure 3). Linearity range for zinc according to the Beer's law corresponded to the Cu(II) concentrations of 0.2-14 μg mL^{-1} and Cu(II) alone was obtained of 0.2-12 μg mL^{-1}.

The analytical sensitivity, the calibration sensitivity, which is the slope of the analytical curve, the limit of detection, and the limit of quantitation as well as other analytical characteristics of the procedure are calculated and summarized in TABLE 1.

(b) Accuracy

The accuracy of the proposed method was evaluated by the recovery studies for three different concentrations (1, 5, 8 μg mL^{-1}) of standards by the analysis of spiked deep well water. The recovery values were 94.77 – 105.00%, indicating the accuracy of the proposed method. The recoveries in both 'spiked' and the 'unspiked' samples are in good agreement. Higher recovery in spiked well water is due to the average presence of 0.041 μg mL^{-1}. For an accuracy check of the method, certified reference substances (alloy, amalgam, synthetic compound) and zinc spiked potable water sample were used. An aliquot of the solution of the respective certified substances was taken and the metal contents were determined by the proposed method. The recovery values calculated for the standard additions were between 101.16-105.57% and for the certified reference material were 94.20 – 97.57%. The results given in TABLE 3 indicate that the certified and found values are very concordant.

(c) Precision

The intra-assay precision of the proposed method was determined on sample solutions at varying concentration levels by analyzing 3 replicates of each sample. The inter assay precision was determined by analyzing the same samples as duplicates (n=3) in three consecutive days. The relative standard deviations (RSD) did not exceed 4.43% proving the high precision of the proposed method for the routine application in the analysis of zinc.

(d) Specificity/Selectivity

Application of the proposed method to analysis of zinc in pharmaceutical formulations it is evident that the proposed method gave satisfactory results. Specificity or selectivity was demonstrated showing that analytes were free of interference from ingredients in pharmaceutical formulations or excipients in biological samples by the analysis of five blank matrices ensuring that peak response in the wavelength is due to examined components only.

(e) Sensitivity

The analytical sensitivity, the calibration sensitivity, which is the slope of the analytical curve, the limit of detection, and the limit of quantitation as well as other analytical characteristics are calculated from the data obtained for constructing calibration curve. The calculation method is based on the standard deviation of the response (S_{xy}) and the slope of the calibration curve (a). The limit of detection were calculated from calibration graph by the formula; $LOD=3 \cdot S_{xy}/a$, and the limit of quantification; $LOQ=10 \cdot S_{xy}/a$. The results are presented in TABLE 1. The standard deviation of the method for ten determinations is found to be not more than 0.066. The lower detection limit and quantification limit of Zn(II) were found to be 0.029 μg mL^{-1} and 0.098 μg mL^{-1}, respectively.

(f) Robustness

Robustness was examined by evaluating the influence of small variation in the method variables (DDTC) concentration, buffer pH, reaction and extraction period) on its analytical performance. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. It was found that small variation in the method variables did not significantly affect the procedures; recovery values were 98.87 - 101.41 ± 0.26 - 0.86%. This indicated the reliability of the proposed method during its routine application for the analysis of zinc.

(g) Ruggedness

Ruggedness was tested by applying the proposed methods to the assay of zinc using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day-to-day variations were reproducible, as the relative standard deviations (RSD) did not exceed 2%. A pre-validated ICP-MS method was used as a reference method for determination of zinc in whole blood and urine samples. The result obtained in proposed method for the determination of zinc in whole blood and urine samples was compared with that obtained from the reference method by statistical analysis with respect to the accuracy (by t-test) and precision (by F-test). No significant differences were found between the calculated and theoretical values of t- and F-tests at 95% confidence level proving similar accuracy and precision in the determination of zinc by both methods.

(h) System suitability

For standard at five different concentration levels within 1.0-10.0 μg mL^{-1} maximum wavelength (435.52±0.33) of absorption was checked and their relative standard deviations were calculated was to be 0.077%. For each concentration molar absorptivity was calculated and plotted against respective concentration levels (1, 5, 8 μg mL^{-1}) and good linearity was found. Both indicate the excellent system suitability for the proposed method.

Effect of diverse ions

The tolerance limit of various anions and cations on the determination of Zn(II) under optimal conditions in the present method. The tolerance limit of a foreign ion is taken as the amount that caused an error in the absorbance value of ≤10%. Large amounts of commonly associated cations and anions do not interfere in the present method. Among the various ions studied, all the anions and the cations Pb(II), Te(IV), U(VI), Na(I), K(I), Li(I), Th(IV), W(VI), Ce(VI), Ti(IV), Al(III) do not interfere even when present in more than 100 fold excess. Copper(II), nickel(II), cobalt(II), mercury(II), cadmium(II), iron (III) and iron(II) ions interfered seriously at all proportions. Interference from Co, Ni, Cr, Mn, Bi, Pb, Cd can be eliminated using EDTA as masking agent. Cyanide/thiocyanate is a stronger complexation agent for Zn(II) ions than DDTC ligand, therefore Zn(II) ion forms a stable complex with the cyanide ligand, thus decreasing dramatically the absorption. However, selective extraction of zinc from thiocyanate solution in HCl 0.5 mol L^{-1} medium followed by its back extraction with an ammoniacal solution solves this problem. Iron and copper were the main interferences for the analysis of Zn by the proposed procedure. This effect was suitably avoided by prior addition of ascorbic acid and sodium thiosulfate to the samples. If iron is present in the sample, it is necessary to add 1300 μg mL^{-1} NaF/NaI before the zinc extraction. The addition of the acid phosphate reagent inhibits the reaction of iron is due to the formation of the insoluble iron phosphate in slightly acid solution. In presence of 950 μg mL^{-1} of phosphate, V(V) is also tolerable up to 100 fold excess. Ni(II) (up to 4 μg) can be masked by 180 μg of citrate/1860 μg mL^{-1} of EDTA. However in the presence of 1860 μg mL^{-1} of EDTA, Cu(II) and Ni(II) do not interfere even in 100-fold excess.

APPLICATIONS

The present method was successfully applied to the determination of zinc in series of synthetic mixtures of various compositions and also in a number of real samples. The method was also extended to the determination of zinc in a number of environmental water and soil samples, biological, pharmaceutical, fertilizer, food samples. The results of biological analyses by spectrophotometric method were found to be in excellent agreement with those obtained by ICP-MS. The results of pharmaceutical, fertilizer, food and soil samples analyses by the spectrophotometric method shown in tables gave the satisfactory RSD in analytical agreement.

Determination of zinc in synthetic mixtures

Several synthetic mixtures of varying compositions containing zinc (3 μg mL^{-1}) and diverse ions of known concentrations were analyzed for zinc content and the results were found to be highly reproducible as shown in TABLE 2. The accurate recoveries were achieved in all solutions with maximum RSD value of 1.36 for triplicate measurements. It is indicated that zinc can be measured eliminating possible interferences from diverse ions.

Determination of zinc in alloys (Certified Reference Substances)

A 0.1g amount of Certified Reference Materials containing different composition of metals was accurately weighed and placed in a 50-mL Erlenmeyer flask. 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

TABLE 1 : Summary for the optimization and validation of variables of the proposed spectrophotometric method

Optimization parameters			Validation parameters	
Variables	**Studied range**	**Optimum**	**Parameter**	**Values**
Acid concentration/M	(0.2-1.2) × 10^{-2}	0.5 × 10^{-2}	Linear range (μg mL^{-1})	0.20-14
Reagent Molar fold excess (M:L)	1:1-1:30	15	Intercept Slope	0.079 0.256
Temperature	Ambient	Ambient	Standard deviation of the intercept	0.025
Time/min	1-20	10	Standard deviation of the slope	0.004
Stability	1 min-72 hrs		Correlation coefficient (r)	0.9993
Wavelength (nm)	300-650	435	Relative standard deviation (%)	4.43
Extraction variables			Limit of detection, LOD (μg mL^{-1})	0.029
Extraction period/min	1-30	10	Limit of quantification, LOQ (μg mL^{-1})	0.090
aqueous phase volume/mL	5-30	15	Molar absorptivity, ε (L mol^{-1} cm^{-1})	2.86 × 10^5
pH	2–11	5	Sandall's sensitivity (ng cm^{-2})	3.076

TABLE 2 : Determination of zinc in synthetic mixture by the proposed spectrophotometric method

Solution	Ions	Conc. (μg mL^{-1})	Recovery (μg mL^{-1})	% Er	RSD (%) n=3
A	Zn^{2+}+Ni^{2+}+SO_4^{2-}+ EDTA	3.00	3.13±0.02	2.96	0.65
B	Zn^{2+}+Ni^{2+}+ Mg^{2+}+ SO_4^{2-}EDTA		2.87±0.03	-5.83	1.06
C	Zn^{2+}+Ni^{2+}+ Mg^{2+}+Al^{3+}+ Cl^- + SO_4^{2-} EDTA		3.17±0.02	4.22	0.63
D	Zn^{2+}+Ni^{2+}+ Mg^{2+}+Al^{3+} +K^++ Cl^-+ SO_4^{2-} +EDTA		3.21±0.03	5.47	0.93
E	Zn^{2+}+Ni^{2+}+ Mg^{2+}+Al^{3+}+K^+ +Ca^{2+}+Cl^- + SO_4^{2-} +EDTA		2.95±0.04	-3.31	1.36

nitrogen and then cooled to room temperature (25±5)^{0}C. After suitable dilution with deionized water, the contents of the Erlenmeyer flask were warmed to dissolve the soluble salts. Based on five replicate analyses, the average zinc concentration determined in certified reference materials, alloy, amalgam, synthetic compounds by spectrophotometric method was in good agreement with the certified values. The average percentage recovery of zinc obtained to some certified reference substances was quantitative as shown in TABLE 3 previously mentioned. RSD value obtained indicates reasonable precision of the measurements.

TABLE 3 : Certified substances analysis for the determination of zinc by proposed method

Certified Reference substance		Zinc			
		Certified values μg mL^{-1}	Expt. values μg mL^{-1}	RSD	Recovery (%)
Alloy		0.50	0.48±0.01	5.34	95.43
Amalgam		3.00	2.93±0.05	6.28	97.57
Synthetic compounds	$[Zn(en)_3][HgI_4]$	1.37	1.29±0.04	3.09	94.20
	$[Zn(NH_3)_6][HgI_4]$	1.49	1.42±0.02	1.41	95.03

Determination of zinc in biological samples

Human blood 2-mL or urine 25 mL was taken into beaker. 5 mL of concentrated nitric acid and 1 mL H_2SO_4 were added and the beaker was placed on the digester under gentle heating. The solution was heated to dryness. After cooling 1 mL of concentrated nitric acid and 10 mL distilled water were added. Heating was continued to reduce volume 3-4 mL. The content of the beaker was filtered and neutralized with dilute ammonia. The resultant solution was then transferred quantitatively into a 20-mL calibrated flask and made up to the mark with de-ionized water. An aliquot (1-mL) of this digested biological sample was pipetted into a calibrated flask and the zinc content was determined under the general procedure as described using ascorbic acid and sodium thiosulfate. The results of the biological sample analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by ICP-MS. The results are given in table indicating the method reproducible. Figure 4 indicates the comparative concentration obtained by proposed and ICP-MS method and inter method variation of measurements were calculated and presented in TABLE 4 which was obtained in the range -4.00-7.00%.

Determination of zinc in environmental water samples

250-mL environmental water samples (KY steel, PHP steel water, Berger paint, ship yard) was mixed with 5-mL of concentrated HNO_3, 2-mL of concentrated H_2SO_4 and 2 drops $HClO_4$ acid in a distillation flask. The sample was digested until a paste was formed. The resulting solution was then filtered and quantitatively transferred into a 25-mL calibrated flask and made up to the mark with deionized water. Ammonia buffer solution was added (pH < 7) to precipitate excess iron as hydroxide and the precipitate was filtered. This step is not required for other potable water samples (tap water, Deep Tube wall, Wasa) was taken and pre-concentrated by the simple evaporation. An aliquot (1-mL) of pre-concentrated environmental water was analyzed by proposed method and the zinc content was determined. The results of analyses of environmental water samples from various sources for zinc are given in TABLE 5. High concentration of zinc was obtained in Chittagong WASA water while remaining potable water samples contain reasonable level. All industrial water samples higher zinc level as expected except that from KY steel. Triplicate measurements possess good precision (RSD not greater than 7%).

Determination of zinc in soil samples (Acid Digestion)

Soil samples were dried well in sunlight. 1 g of each soil sample was placed into a 250 mL flask. 0.2 mL of sulfuric acid, 1 mL of nitric acid and 1 mL of perchloric acid were added. The soil and acid mixture was heated to 180°C for 3 h on a hotplate to dryness. After cooling, 1 g of ammonium chloride and 20 mL of 0.5 N HCl were added. Samples were reheated to 180°C for one hour and evaporated to approximately 10 mL. After cooling, the extracts were filtered into 100 mL plastic bottles through an ashless 5B filter paper (Advantec, Tokyo, Japan). Ammonia buffer solution was added to this solution to precipitate excess iron as hydroxide. After filtration 5 mL of this solution was taken to determine the concentrations of Zn by using UV/Visible spectrophotometer followed by general procedure using ascorbic acid and sodium thiosulfate as a masking agent. Results of triplicate measurements of soil samples collected from industrial area to determine the zinc concentrations by using proposed method are given in TABLE 5 that indicate RSD well.

TABLE 4 : Biological sample analysis for the determination of zinc by proposed method

Sample	Proposed Method			ICP-MS (μg mL^{-1})	Inter Method Variation (%)
	Patients	Conc. of Zinc (μg mL^{-1})	RSD (%)		
Blood	Pragnent	0.054±0.002	2.81	0.05	7.41
	Harnia	0.12±0.09	7.55	0.13	-4.321
	Gestroistestinal	1.18±0.03	7.15	1.11	5.841
Urine	Gestroistestinal	1.46±0.002	2.74	1.49	-2.171
	Heart	0.93±0.003	6.59	0.96	-3.671

TABLE 5 : Determination of zinc in some environmental samples

Sample	Source		Zinc	
			Concentration (μg mL^{-1})	RSD (%)
Water	Industrial	Barzer Paint	24.91±0.01	4.08
		PHP Steel	337.16±0.05	2.21
		KY Steel	10.43±0.01	7.21
	Ship breaking yard	Water 1	57.54±0.02	3.31
		Water 2	9.55±0.01	7.10
	Potable Water	Deep Well	0.01±0.01	7.13
		Shallow well	0.03±0.01	7.71
		WASA, Ctg	5.07±0.01	1.04
Soil	Industrial Soil	PHP Steel	9.31±0.01	6.25
		KY Steel	10.24±0.01	2.97
		Berzer Paint	7.38±0.01	4.03
		Elite Paint	10.24±0.01	2.98
	Ship Yard	Soil 1	123.93±0.3	2.80
		Soil 2	6.88±0.01	5.71

Determination of zinc in pharmaceutical samples

Each zinc tablet (20 mg of zinc) or 5 mL syrup (10 mg zinc) or required weight (0.252 g itch guard, 0.191g softi) ointment was dissolved in water as to prepare 200 μg mL^{-1} solution. Necessary dilution was made with deionized water as required. An aliquot amount was analyzed according to the general procedure. Accuracy of the method in terms of percentage recovery compared to levelled value of zinc. Results of pharmaceutical samples analyzed according to the general procedure are summarized in TABLE 6. Accuracy of the method in terms of percentage recovery compared to levelled value of zinc was found to be excellent.

TABLE 6 : Pharmaceutical, fertilizer and food sample analysis for the determination of zinc by proposed method

Pharmaceutical samples	Brand name	Claimed value (μg mL^{-1})	Expt. Value (μg mL^{-1})	Recovery (%)	RSD (%)
Tablet	ACME	10.00	9.56±0.05	95.62	1.93
	SK+F	10.00	9.99±0.14	99.88	5.21
Syrup	ACME	0.10	0.098±0.005	98.14	4.76
	SK+F	0.10	0.10±0.004	101.78	3.83
Ointment	Softi	0.10	0.098±0.003	97.69	2.57
	Itech Guard	0.10	0.099±0.005	99.40	4.99
Fertilizer samples	Mak Gold	1.00	0.93±0.02	93.1	2.15
	Padma	1.00	0.93±0.04	93.4	4.28
Food samples	Boost	1.38	1.31±0.02	94.89	4.46
	Horlicks	0.42	0.40±0.007	95.68	4.03
	Dano Milk	0.802	0.064±0.01	69.40	4.07

Determination of zinc in fertilizer

0.028 g fertilizer containing 36% of zinc was dissolved in water taking in a 100 mL volumetric flask to make 100 μg mL^{-1} solutions. The solution was filtered to remove suspended substances. From the filtrate solution 1 mL was taken in 10 mL volumetric flask and made up to the mark to make 10 μg mL^{-1} solutions. From this solution 1 mL was taken to determine Zn followed by general procedure using ascorbic acid and sodium thiosulfate as a masking agent and it was analyzed for zinc content according to general procedure of the proposed method. Results presented in TABLE 6 shows lower recovery as levelled values.

Zinc in food nutrients

A 1g amount of food nutrients (Boost, Horlicks, Milk) containing different composition of metals was accurately weighed and acid decomposition was done as previous experiment. A suitable aliquot (1mL) of the above solution was taken into a calibrated flask and the zinc content was determined as described under general procedure using ascorbic acid and sodium thiosulfate as masking agent. Based on five replicate analyses of food nutrients (Boost, Horlicks, Milk), the average zinc concentration determined by spectrophotometric method was in good agreement with the leveled values as presented in TABLE 6. In case of milk sample zinc content was obtained about 70% for Dano.

The results of the estimation of Zn content in real and environmental samples presented show good accuracy and precision. For comparison, the Zn(II) content in the biological samples was determined by ICP-MS showing a good agreement with the new analysis method. To assess the usefulness of this method, its accuracy was checked using certified reference materials. The results given in TABLE 2 indicate that the certified and the found values were in good agreement.

CONCLUSION

Among the techniques suitable for the quantification of metal ions, ICP-MS, ICP-AES and atomic absorption or emission spectroscopies are likely to be the most widely employed. However, although these techniques are reliable and sensitive, they suffer from the limitation of being rather costly (considering instrument acquisition and maintenance), time-consuming (with respect to sample preparation), and not always readily available. Thus, simple spectrophotometric (or spectrofluorometric) techniques, which tend to be less costly and labor-intensive, are viable alternatives to those methods requiring more sophisticated instrumentation. The method described herein has many advantages: it is simple and rapid, it has high accuracy and sensitivity, use of inexpensive reagents available in any analytical laboratory. Therefore, the method is practical and valuable for its wide application. The results show a good agreement with certified values in alloys and pharmaceutical preparations and with the results obtained by ICP-AES methods for biological samples. $Cu(DDTC)_2$ is more stable in acidic aqueous media than precipitated $Zn(DDTC)_2$. Dithiocarbamates are more suitable than other complexing reagents because of their selectivity towards Zn(II) and Cu(II).

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