

A Rapid Spectrophotometric Method for the Determination of Trace Amount of Zinc (II) Ion in Aqueous Medium using Alizarin Red S as a Chelating Agent*

Md Rafique Ullah and Mohammad Mahbubul Hasan

Department of Chemistry, Bangladesh University of Engineering and Technology (BUET) Dhaka-1000, Bangladesh, D-10 (3rd floor)
South Bonosree, Goran, Dhaka-1219

E-mail: MdMahbubulHasan@yahoo.com; rafique@chem.buet.ac.bd

(Received June 14, 2011)

Abstract: A very simple, sensitive and highly selective non-extractive spectrophotometric method for the determination of trace amount of Zinc has been developed by using 1,2-dihydroxy anthraquinone-3-sulphonic acid sodium salt named Alizarin Red S (ARS) as a spectrophotometric reagent. ARS reacts in slightly acidic solution ($\text{pH } 4.00 \pm 0.02$) with Zinc to give an orange-red chelate that has an absorption maximum at 502.2 nm. The reaction is instantaneous and absorbance remained constant for over 48 hrs. The average molar absorption co-efficient was found to be $2.1 \times 10^2 \text{ Lmole}^{-1}\text{cm}^{-1}$. Linear calibration graphs were obtained for 0.30-100 $\mu\text{g mL}^{-1}$ of Zn. The stoichiometric composition of the chelate has been found 1:2 (Zn:ARS). Large excess of cations, anions and some complexing agents have been studied. The method was successfully used in the determination of Zinc in several standard reference material, alloys & environmental water and made a comparison with those obtained by Atomic Absorption Spectrophotometer.

1. Introduction

Human contains the second largest amount of Zn (2gm) after Fe (4 gm). It is a constituent of over 100 enzyme systems¹. Not only high concentration (50-100 μM) of Zn but also low concentration (<15 μM) of Zn decreases the immune responses² and causes abnormal pain, diarrhea, Cu deficiency, sideroblastic anemia³, low birth weight⁴, etc.

According to Schwartz term "Concentration Window" metals are (a) Essential at definite trace level, (b) Deficient than that level cause metabolic disorder, (c) Higher than that level termed "Toxic level", and causes adverse effects.⁵

*Paper presented in CONIAPS XIII at UPES, Dehradun during June 14-16, 2011.

As Zn is both toxic and essential element, so it is necessary for continuous monitoring and control the trace balanced level of Zn by cheaper, easier and rapid analytical method like UV-Vis spectroscopy rather than AAS or MAS.⁶

Spectrophotometry is essentially a trace-analysis technique and is one of the most powerful tools in chemical analysis. Alizarin Red S (ARS) has been reported to be a spectrophotometric reagent for aluminium⁷, cadmium⁸, molybdenum⁹ and arsenic¹⁰ but has not previously been used for the spectrophotometric determination of zinc. This paper reports on its use in a very sensitive, highly specific spectrophotometric method for the trace determination of zinc. The method possesses distinct advantages over existing methods¹¹⁻¹³ with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH range, thermal stability, accuracy, precision, and ease of operation. It is based on the reaction of ARS in a slightly acidic solution (pH 4.00) with zinc to produce a highly absorbent orange-red chelate product, followed by a 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

2.1 Apparatus

A Shimadzu (Kyoto, Japan) (Model 160) double-beam UV-Visible recording spectrophotometer and a Denver (UB-10) ultra basic pH-meter with a combination of electrodes were used for measurements of the absorbance and pH, respectively. A Shimadzu (Kyoto, Japan) (Model AA6401F) atomic-absorption spectrophotometer equipped with a microcomputer-controlled nitrous oxide-acetylene flame was used to compare the results.

2.2 Reagents and solutions

All chemicals used were of analytical-reagent grade or the highest purity available. Doubly distilled deionized water, which is non-absorbent under ultraviolet radiation, was used throughout. Glass vessels were cleaned by soaking in acidified solutions of $K_2Cr_2O_7$, followed by washing with nitric acid (1 + 1) and rinsed several times with high-purity 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.

2.3 ARS solution 0.5% or 14.605×10^{-3} M

Prepared by dissolving the requisite amount of ARS (1, 2-dihydroxyanthraqui-none-3-sulphonic acid, sodium salt) (BDH chemicals) (DBH chemicals) in a known volume of distilled water. More dilute solutions of the reagent were prepared as required.

2.4 Zinc standard solution, 0.1529×10^{-3} M

A 100-mL amount of stock solution (10 mg L^{-1}) of divalent zinc was prepared by dissolving 4.40 mg of A. R. crystallized zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) (Merck) in doubly distilled de-ionized water. Aliquots of this solution were standardized by EDTA titration using xylenol orange as indicator. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with deionized water, as and when required.

2.5 Procedure

A volume of 0.1-1.0 mL of neutral aqueous solution containing 1-300 μg of zinc in a 10-mL calibrated flask was mixed with a 1:2 to 1:9 fold molar excess of the ARS solution, followed by the addition of required amount of HClO_4 acid to maintain the pH at 4.00 and the mixture diluted up to the mark with water of pH 4.00 (D.D. water with HClO_4 acid). The absorbance was measured at 502.2nm against corresponding zinc blank. The zinc content of an unknown sample was determined using a concurrently prepared calibration graph.

3. Results and Discussion

Factors affecting the absorbance

3.1 Absorption spectra

The absorption spectra of the Zinc-ARS system in a pH 4.00 (using HClO_4 and NaOH) was recorded using a spectrophotometer. The absorption spectra of Zinc- ARS is a symmetric curve with the maximum absorbance at 502.2 nm is shown in Fig-1. In all instances measurements were made at 502.2 nm against zinc blank.

3.2. Effect of pH

The effects of pH have been studied over the range of pH 3.85 to 4.10, the pH 4.00 was found to be the best for the system. Below the pH 4.00 the complex formation is not completed and over the pH 4.00 there may form metal hydroxide as a result the absorbance decreases in both cases. These effects are shown in Fig-2.

3.3 Effect of Acid

Of the various acids (Nitric, Sulfuric, Hydrochloric, Per-chloric, Phosphoric, Acetic acid, Oxalic acid and Citric Acid) studied, per-chloric acid was found to be the best acid for the system. The absorbance was at a maximum at per-chloric acid medium. The organic acids may form co-ordination with the zinc and the other inorganic acid have low dissociation constant as a result they give the lower absorbance comparing to per-chloric acid. These effects are shown in Fig-3.

3.4 Effect of time

The reaction is instantaneous. Constant maximum absorbance was obtained just after diluting to volume, and remained strictly unaltered for 48 hours.

3.5 Effect of temperature

The zinc-ARS system attained the maximum and constant absorbance at room temperature (25 ± 5)°C. Outside of this range of temperature, the absorbance decreased.

3.6 Effect of the reagent concentration

Different molar excesses of ARS were added to a fixed metal ion concentration, and the absorbances were measured according to the standard procedure. It was observed that at 50 mg L⁻¹ zinc metal, the reagent molar ratios of 1:2 and 1:9 produced a constant absorbance of the Zn-chelate (Fig-4). Greater excesses of the reagent were not studied.

3.7 Calibration graph (Beer's law and sensitivity)

A well-known equation for spectrophotometric analysis in a very dilute solution was derived from Beer's law. The effect of the metal concentration was studied over 0.1-100 µg mL⁻¹. The absorbance was linear for 0.3 - 30 µg mL⁻¹ of zinc at 502.2 nm (Fig-5). The molar absorption co-efficient was found to be 2.10×10^2 L mol⁻¹ cm⁻¹.

3.8 Effect of foreign ions

The effect of over 30 ions and complexing agents on the determination of only $1 \mu\text{g mL}^{-1}$ of zinc was studied. The criterion for interference¹⁴ was the absorbance value varying by more than $\pm 5\%$ from the expected value for zinc alone. As can be seen, a large number of ions had no significant effect on the determination of Zinc. The most serious interference was from Cu(II), Pb(II) and Fe(III) ions. Interference from these ions was probably due to complex formation with ARS. A greater tolerance limits for these ions can be achieved by using several masking methods like potassium thiocyanate. During interference studies, if a precipitate was formed, it was removed by centrifugation.

3.9 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 ($n = 11$) was 2.0% for $100 \mu\text{g mL}^{-1}$ of zinc, indicating that this method is highly precise and reproducible. The results are given in Table 1.

Table-1: Standard deviation and relative standard deviation of Zn(II)-ARS system

Sam ple No.	Zn(II) taken mg L^{-1}	Zn(II) Found $X_1 \text{ mg L}^{-1}$	Mean $\bar{X}$ mg L^{-1}	$X_1 - \bar{X}$	$(X_1 - \bar{X})$	Standard deviation ($\pm s$)	Relative standard deviation (s_r)%
1	100.0	99.0	100.3 2	1.32	1.74	± 1.102	1.098
2	100.0	99.5		0.82	0.67		
3	100.0	101.0		0.68	0.46		
4	100.0	101.5		1.18	1.40		
5	100.0	99.0		1.32	1.74		
6	100.0	101.5		1.18	1.40		
7	100.0	101.0		0.68	0.46		
8	100.0	99.5		0.82	0.67		
9.	100.0	99.0		1.32	1.74		
10	100.0	101.5		1.18	1.40		
11	100.0	101.0		0.68	0.46		
N = 11		ΣX_1 = 1103.5		$\Sigma X_1 - \bar{X}$ = 11.181	$\left(\Sigma (X_1 - \bar{X}) \right)^2$ = 12.1364		

$$\text{Mean, } \bar{X} = \frac{\sum X_1}{N} = \frac{1103 \cdot 5}{11} = 100 \cdot 32$$

$$\text{Standard deviation, } S = \sqrt{\frac{\sum (X_1 - \bar{X})^2}{N - 1}} = \sqrt{\frac{12 \cdot 136}{11 - 1}} = \sqrt{1 \cdot 2136} = \pm 1 \cdot 102$$

$$\text{Relative standard deviation (S}_r\text{) \%} = \frac{S}{\bar{X}} \times 100 \%$$

$$= \frac{1 \cdot 102 \times 100}{100 \cdot 32} \% = 1 \cdot 098 \%$$

3.10 Composition of the absorbent complex.

Job's method¹⁵ of continuous variation and the molar-ratio¹⁶ method were applied to ascertain the stoichiometric composition of the complex. A zinc-ARS (1:2) complex was indicated by both methods.

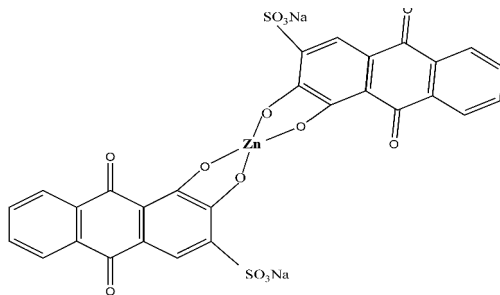


Fig.1: Probable structure of Zinc-Alizarin Red S (1:2) complex

4. Applications

4.1 Determination of zinc in alloy

A 0.1 g amount of a brass alloy sample was accurately weighed into a 50 mL Erlenmeyer flask following a method recommended by Parker *et al.*¹⁷ To it, 10 mL of nitric acid was added, carefully covering with a watch-glass until the brisk reaction subsided. The solution was heated and simmered gently after the addition of 5 mL of concentrated HNO₃ until all carbides were decomposed. Then 2 mL of con HClO₄ was added and the solution was carefully evaporated to dense white fumes to drive off the oxides of nitrogen, and then cooled to room temperature (25 ± 5)°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 dilute NaOH in the presence of few drops of dilute potassium thiocyanate solution. The resulting solution was filtered, if necessary, through Whatman No. 40 filter paper into a 50 mL calibrated flask. The residue was washed with a small volume of hot water and the volume was made up with deionized water. A suitable aliquot (0.1 - 1.0 mL) of the above solution was taken into a 10 mL calibrated flask and the zinc content was determined as described under Procedure using potassium thiocyanate as a masking agent. The results are given in Table 2.

Table 2 Determination of zinc in a standard brass sample solution

Sample Source	% of Zn W/W	
	Proposed method	AAS method
Brass	32.027 %	32.000 %

4.2 Determination of zinc in environmental waters

Each filtered (with Whatman No. 40) environmental water sample (1000 mL) was evaporated nearly to dryness with a mixture of 5 mL of concentrated HClO₄ and 10 mL of concentrated HNO₃ in a fume cupboard following a method recommended by Greenberg *et al.*,¹⁸ and was then cooled to room temperature. The residue was then heated with 10 mL of deionized water in order to dissolve the salts. The solution was cooled and neutralized with dilute NaOH in the presence of 1- 2 mL of 0.01% (w/v) potassium thiocyanate solution. The resulting solution was filtered and quantitatively transferred into a 25 mL calibrated flask and made up to the mark with deionized water.

Table 3 Determination of zinc in some environmental water samples

Sample	Zinc / mgL ⁻¹		Recovery ±S ^b (%)
	Added	Found ^a	
Tap water	0	3.5	
	100	105.0	101 ± 0.5
	500	504.0	100 ± 0.5
Drain water (Olympic Battery).	0	120	
	100	223	101 ± 0.4
	500	630	101 ± 0.6

a. Values given represent the average of triplicate determinations.

b. The measure of precision is the standard deviation(s)

An aliquot (1-2 mL) of this pre-concentrated water sample was pipetted into a 10 mL calibrated flask and the zinc content was determined as

described under procedure. The analyses of environmental water samples from various sources for zinc are given in Table 3.

5. Conclusions

In this paper, a new simple, sensitive, selective and inexpensive method with a Zn-ARS complex was developed for the determination of zinc in industrial, environmental, samples, for continuous monitoring to establish trace levels of zinc in different samples 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 a requirement for consumables, and almost no maintenance have caused spectrophotometry to remain a popular technique, particularly in laboratories of developing countries with limited budgets.

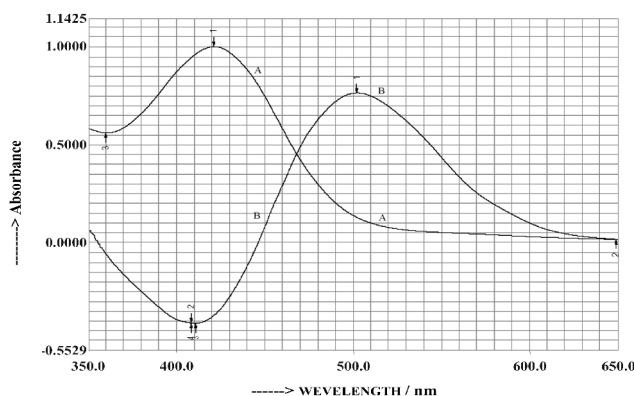


Fig.1 A and B absorbance spectra of ARS with respect to water and the Zn-ARS with respect to ARS in aqueous solution. ($\lambda_{\text{max}} = 502.2\text{nm}$)

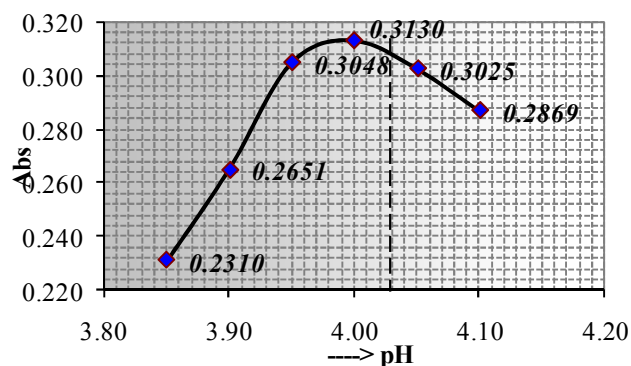


Fig 2 Effect of pH on the absorbance of Zn-ARS complex system.

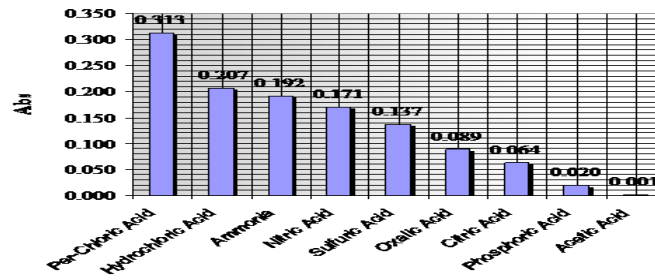


Fig.3. Effect of Acids and Base on the absorbance of Zn-ARS complex at 502.2nm.
Absorbance Vs Acid or Base used.

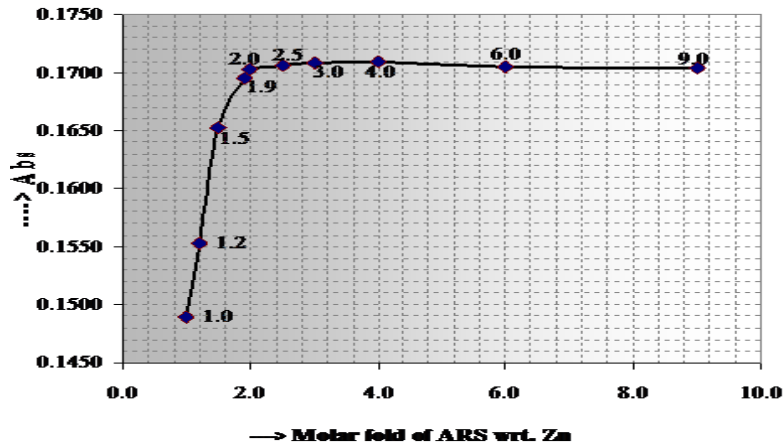


Fig-4 Effect of ligand's (ARS) molar concentration on the absorbance of Zn-ARS complex

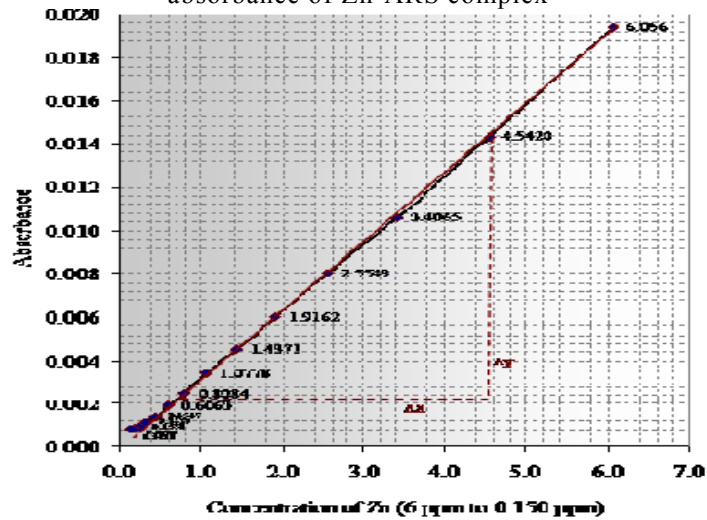


Fig-5: Observation of Beer Lambert laws for Zinc-ARS complex at 502.2 nm.

References

1. J. D. Lee, Concise Inorganic Chemistry, *Blackwell Science Ltd*, **5**(1998) 851.
2. B. Bao, A. Prasad, F. W. J. Beck, A. Suneja and F. Sarkar, *Toxicology Letters*, **25** (2006) 222-228.
3. Martindale, The complete Drug Reference, *Pharmaceutical Press*, **32** (1999) 1373.
4. The Merck Manual, **18** (2006) 55.
5. K. Schwartz, Clinical Chemistry and Toxicology of Metals, *Elsevier*, (1977) 3.
6. G. D. Christian, Analytical Chemistry, *John Wiley and sons, New York*, **5** (1994) 398.
7. S. Hernández-Méndez, R. Carabias-Martínez, B. Moreno-Cordero and L. Gutiérrez-Dávila, *Analytica Chimica Acta* **149** (1983) 379-383.
8. M. R. Ullah and M. E. Haque, (M.Phil Thesis), Bangladesh University of Engineering and Technology, Dhaka. Bangladesh, Spectrophotometric Determination of Toxic Elements in Aqueous Media, 2007.
9. Mahir Alkan, Myroslava Kharun and Fedor Chmilenko, Spectrophotometric determination of molybdenum with Alizarin Red S in the presence of poly sulfonypiperidinylmethylene hydroxide, *Dniepropetrovsk, Ukraine. Talanta*, **59** (2003) 605-611.
10. M. J. Ahmed and J. Hasan, Non-Extractive Spectrophotometric Method for the Determination of Arsenic in Environmental, Industrial, Biological and Soil Samples using Alizarin-Red S. *Research journal of Chemistry and environment*, **3** (1999) 9.
11. Ashok K.. Sharma and Ishwar Singh. *Springer-New York*. **2** (2009) 311-316.
12. Stanislaw Zarba, *Pharmaceutica Acta Helveticae*, **70** (1995) 195-198.
13. M. Blanco, J. Coello, H. Iturriaga, S. Maspocho and E. Bertran, *Microchimica Acta*, **108** 53-59.
14. C. Bosch Ojeda, A. Garcia de Torres, F. Sanchez Rojas, and J.M. Cano Pavon, *Analyst*, (1987) 112-1499.
15. P. Job, *Ann.Chim. (Paris)* **9** (1928),113.
16. J. A. Yoe and A. L. Jones, *Ind. Eng. Chem. Anal. Ed.*, (1944) 16, 11.
17. G. A. Parker, Analytical Chemistry of Molybdenum, *Spring-Verlag Berlin*, (1983).
18. E. A. Greenberg, S. L. Clesceri and D. A. Eaton (ed.), Standard Methods for the Examination of Water and Wastewater, *American Public Health Association, Washington, D. C.*, **18** (1992) 3-82.