J. Iran. Chem. Soc., Vol. 5, No. 4, December 2008, pp. 546-552.

JOURNAL OF THE
Iranian
Chemical Society

H-point Standard Addition Method for Simultaneous Determination of Cobalt(II) and Zinc(II) Ions

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(Received 5 February 2006, Accepted 26 April 2007)

The H-point standard addition method (HPSAM) was applied to the simultaneous determination of zinc(II) and cobalt(II). This method is based on the difference in the absorbance of methylthymol blue complexes of Zn(II) and Co(II) at pH 6 buffered solution in different wavelength pairs. The results showed that Zn(II) and Co(II) can be determined simultaneously with concentration ratios of 20:1 and 1:7.5. Under working conditions, the proposed method was successfully applied to the simultaneous determination of zinc and cobalt in synthetic and real samples.

Keywords: H-point standard addition, Zinc, Cobalt, Methylthymol blue

INTRODUCTION

H-point standard addition method (HPSAM), which is a modification of the standard addition method, permits both proportional and constant errors produced by the matrix of the sample to be corrected directly.

The fundamentals of HPSAM were outlined by Bosch Reig and Campins Falco in 1988 [1]. It permits the determination of two species with extensively or even completely overlapping spectra [2-6]. Absorbance increments were used where only the analyte concentration was required [7]. This variant allows the elimination of the blank bias error due to the use of absorbent blank [8,9]. In addition, it has been applied to HPLC analysis [10,11] and to the analysis of kinetic data [12], with an additional variant, time. The basis of the method has also been used in spectroflourimetry and synchronous spectroflourimetry [13]. The method is also of use when the interferents are not known [14].

Zinc compounds have biocidal activity because they precipitate and denature the bacterial proteins. For this reason it has been used in dermatology as an antiseptic and disinfectant agent in ophthalmic and mouthwash solutions and mineral-vitamin preparations [15]. Cobalt is a biologically essential trace element. Although cobalt is required only in minute amounts for maintaining normal health, a lower or higher concentration of it may lead to deficiency or toxicity states, respectively, resulting in impairment or abnormalities of health [16].

Several techniques such as ion chromatogeraphy [17], liquid-liquid extraction with atomic absorption spectrometry [18], atomic fluorescence spectrometry [19], X-ray fluorescence spectrometry [20,21] graphite furnace atomic absorption spectrometry [22], inductively coupled plasma atomic emission spectrometry [23] and chemometrics method [24] have been applied for the simultaneous determination of zinc and cobalt ions in different samples. The use of UV-Vis spectrophotometry for simultaneous determination of zinc and cobalt using a color reagent in a real sample is rare. However, among the most widely used analytical methods, the UV-Vis

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spectrophotometry has been an attractive method for the analysis of samples due to its experimental rapidity, simplicity and wide applications.

In this work, a selective H-point standard addition method has been developed for simultaneous determination of zinc and cobalt using methylthymol blue (MTB) as a chromogenic complexing agent in a buffered solution at pH 6.0. The obtained selectivity was due to the selected pH and the type buffer as well as the use of a mixture of suitable masking agents.

EXPERIMENTAL

Reagents

All chemicals used were of analytical reagent grade. Doubly distilled water was used throughout.

A stock Co^{2+} solution (1000 μg ml^{-1}) was prepared by dissolving appropriate amounts of hydrated Co(II) chloride salt in a proper volumetric flask. Standardization of the solution was performed by a reported procedure [25]. A stock solution of Zn^{2+} (1000 μg ml^{-1}) was prepared by dissolving appropriate amounts of zinc chloride in a proper volumetric flask and standardized [25]. A 50 ml of 8.0×10^{-4} M aqueous solution of the sodium salt of methylthymol blue (MTB) was prepared and used daily. Adjusting the pH values of the working solution was carried out using acetic acid-sodium acetate and sodium dihydrogen phosphate-sodium monohydrogen phosphate solutions.

Apparatus

A carry 100 UV-Vis scanning spectrophotometer was used for recording the absorbance spectra and absorbance measurements in selected wavelengths against a reagent blank of Zn-MTB and Co-MTB complexes using a 1.0-cm path length quartz cell. The cell temperature was controlled by circulating water around the thermostated cell. A Metrohm 691 pH meter using a combined glass electrode was used for pH measurements.

General Procedure

Appropriate volumes of Zn^{2+} and Co^{2+} standard solutions, 1 ml of 8.0×10^{-4} M MTB, 5 ml of buffered solution at pH 6 were added into a 10 ml volumetric flask. The solution was diluted to the mark and allowed to stand for 5 min at 25 ºC. Then, a portion of the solution was transferred into a 1-cm quartz cell to measure its absorbances against a reagent blank at appropriate wavelengths.

The simultaneous determination of Zn(II) and Co(II) with HPSAM was performed by measuring the absorbances at 565 and 637 nm with standard addition of Zn(II) solution for obtaining graphs. The C_H and A_H values in HPSAM were obtained from the intersection point of the two derived straight. The amounts of Zin(II) and Co(II) can be determined simultaneously at Zn(II) to Co(II) concentration ratios varying from 20:1 to 1:7.5 in mixed samples.

RESULTS AND DISCUSSION

Methylthymol blue forms colored complexes with Zn^{2+} and Co^{2+} ions. Figure 1 shows the absorption spectra of MTB complex with Zn^{2+} and Co^{2+} ions. As can be seen, the spectra of the complexes overlap with each other and, therefore, each compound interferes with the spectrophotometric determination of the other ions. However, the system is suitable for the simultaneous determination of Zn(II) and Co(II) using H-point standard addition method (HPSAM).

Effect of Variables

The effect of the pH of solution was studied in the pH range of 3-7 buffered with acetic acid-sodium acetate and potassium dihydrogen phosphate-potassium monohydrogen phosphate. The results are shown in Fig. 2. The absorbance both complexes increased by increasing pH of the solution up to a pH of 6 and then decreased at higher pH value. Therefore, a pH 6 phosphate buffered solution was selected as the optimum condition.

The effect of MTB concentration on the sensitivity of the systems was investigated in the range of 1.0×10^{-5}-1.0×10^{-4} M. The results are shown in Fig. 3. As it is seen form Fig. 3, the absorbance of complexes increased with increasing C_{MTB} from 1.0×10^{-5} M up to 8.0×10^{-5} M and remained constant at higher concentration. Thus, a 8.0×10^{-5} M concentration of MTB was used as the optimum concentration.

The influence of temperature on the reaction was studied in the range of 15-35 ºC. The results show that, the absorbance of both Zn-MTB and Co-MTB complexes increased by

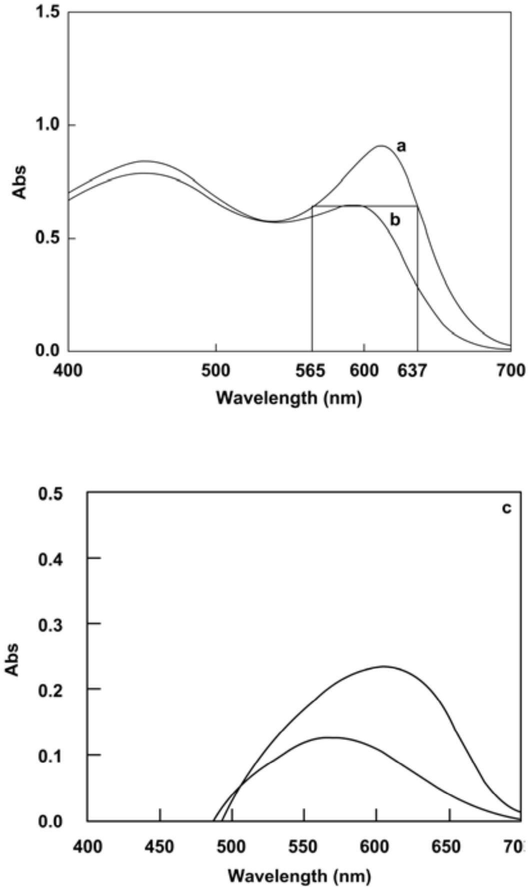

Fig. 1. Absorption spectra of a) Co-MTB; b) Zn-MTB complexes at conditions: 2.00 μg ml^{-1} of Co^{2+} and Zn^{2+}, C_{MTB} =1.0 × 10^{-4} M, pH = 6 and 25 ºC; c) the absorption spectra against a reagent blank.

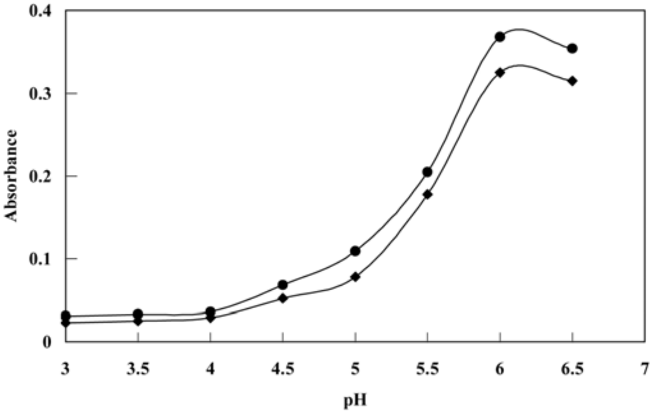

Fig. 2. Effect of pH on the absorbance of (●) Co-MTB and (♦) Zn-MTB complexes.

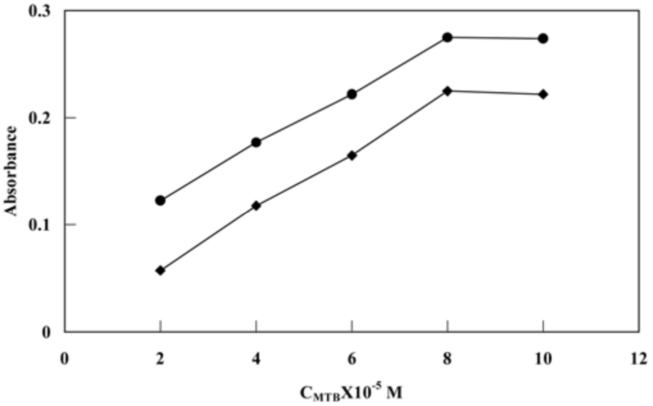

Fig. 3. Effect of methylthymole blue (MTB) concentration on the absorbance of (●) Co-MTB and (♦) Zn-MTB complexes.

increasing temperature up to 25 ºC, and then remained nearly constant at higher temperatures. Therefore, 25 ºC was selected as the optimum temperature.

Applying HPSAM

For the selection of appropriate wavelengths for applying HPSAM, the following principles should be followed. At the two selected wavelengths, the analyte signal should be linear with its concentration, while the interferent signals should remain equal, even if the analytical concentrations are changed. The analytical signals of the mixture composed of the analyte and interferent should be equal to the sum of the individual signals of the two compounds. In addition, the slope difference of the two straight lines obtained at λ_1 and λ_2 should be as large as possible, in order to obtain a good accuracy.

In the proposed system, Zn(II) and Co(II) ions could be considered as the analyte and interfering ions, respectively. At selected wavelengths of λ_1 = 565 and λ_2 = 637 nm (Fig. 1), the absorbances of Zn-MTB complex found to increase linearly with the concentration of zinc ions, whereas, the absorbances of Co-MTB complex remained equal, even with increasing analyte concentration.

After appropriate selection of the two wavelengths, known amounts of Zn^{2+} ion are successively added to the mixture, and the resulting absorbances are measured at the two wavelengths and expressed by the following equations:

$$A_{565} = M_{565}C_{Zn} + b_o + b \quad (1)$$

$$A_{637} = M_{637}C_{Zn} + A_o + A' \quad (2)$$

where A_{565} and A_{637} are the analytical signals measured at wavelengths of 565 and 637 nm, respectively, b_o and A_o ($b_o \neq A_o$) are the original analytical signals of Zn(II) ion at 565 and 637 nm, respectively, b and A' are the analytical signals of Co(II) ion at 565 and 637 nm, respectively, M_{565} and M_{637} are the slopes of the standard addition calibration lines at 565 and 637 nm, respectively and C_{Zn} is the added Zn(II) ion concentration. The two straight lines thus obtained intersect at the so-called H point ($-C_{Zn}$, A_{Co}) (Fig. 4).

Since, at the H-point, $A_{565} = A_{637}$ and $C_{Zn} = -C_{Zn}$, the following equations can be driven from Eqs. (1) and (2):

$$b_o + b + M_{565}(-C_{Zn}) = A_o + A' + M_{637}(-C_{Zn}) \quad (3)$$

$$-C_{Zn} = [(A_o - b_o) + (A' - b)]/(M_{565} - M_{637}) \quad (4)$$

From Eq. (4), the following conclusions can be made. (i) If the Co(II) component is the known interferent and the analytical signals corresponding to Co(II) ion, *i.e.*, b (at 565) and A' (at 637), do not change with the addition of Zn(II) ion (b = A' = constant), we can write:

$$C_{Zn} = (A_o - b_o)/(M_{565} - M_{637}) = b_o/M_{565} = A_o/M_{637} \quad (5)$$

where $C_{Zn} = -C_{Zn}$ corresponds to the analyte concentration in the mixture. This is because $-C_{Zn}$ depends only on variables related to the analyte [26]. This is equivalent to

$$-C_{Zn} = (A_o - b_o)/(M_{565} - M_{637}) = b_o/M_{565} = A_o/M_{637} \quad (6)$$

If the value of $-C_{Zn}$ is included in Eq. (1), the A_{Co} ordinate value of the intersection point is described as follows:

$$A_{Co} = b_o + b + M_{565}(-C_{Zn}),\ \text{as}\ b_o = -M_{565}C_{Zn} \quad (7)$$

Then A_{Co}= b and similarly A_{Co}= A'.

Hence, the A_{Co} value is only related to the signal of the interferent, Co(II) ion, at the two selected wavelengths. To evaluate the interferent concentration from the ordinate value of the H-point (A_{Co}), (i) a calibration graph or the absorbance value of an interferent Co(II) ion standard is needed. (ii) If component Co(II) ion is the unknown interferent, Eq. (4) is tenable as long as the Co(II) ion analytical signals (b at 565 and A' at 637) remain equal with the addition of analyte Zn(II) ion. According to the above discussion at the H-point, because C_{Zn} is independent of the concentration of the interferent, A_{Co} is also independent the analyte concentration.

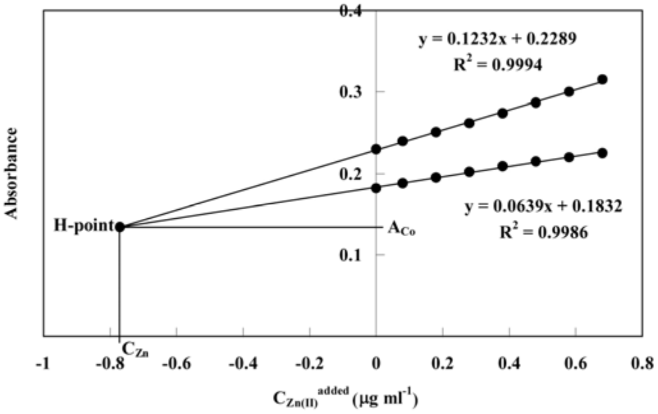

Fig. 4. H-Point standard addition plot for the simultaneous determination of zinc and cobalt. Conditions: 1.00 μg ml^{-1} of Co^{2+}, 0.76 μg ml^{-1} of Zn^{2+}, $C_{MTB} = 8.0 \times 10^{-5}$ M, pH = 6 and 25 ºC.

Under the optimum conditions, simultaneous determination of Co^{2+} and Zn^{2+} was carried out using HPSAM. The concentration of the Co^{2+} ions was calculated in each test solution by the calibration method with a single standard and the ordinate value of the H-point (A_{Co}). For simultaneous determination of Co^{2+} and Zn^{2+} in a sample, several synthetic mixtures with different concentration ratios of Zn^{2+} and Co^{2+} were analyzed using HPSAM. With analysis of different mixtures, the results revealed that the proposed system can be applied in the dynamic ranges of 0.2-2.0 and 0.1-1.5 μg ml^{-1} for Zn^{2+} and Co^{2+} ions, respectively.

Reproducibility and Accuracy of the Method

Five replicate experiments on the binary samples of Zn(II) and Co(II) ions were done to check the reproducibility of the proposed method. The results are given in Table 1. A good recovery of 96-105% and standard deviation ≤0.03 were

Table 1. Determination of Zinc and Cobalt in Different Binary Mixtures

Sample (μg ml^{-1})		Found (μg ml^{-1})[a]		Recovery (%)	
Zinc(II)	Cobalt(II)	Zinc(II)	Cobalt(II)	Zinc(II)	Cobalt(II)
0.70	1.00	0.71 ± 0.01	0.99 ± 0.01	101.4	99.0
1.10	0.50	1.13 ± 0.03	0.48 ± 0.02	102.7	96.0
0.70	0.70	0.69 ± 0.02	0.70 ± 0.01	98.6	100.0
0.35	0.25	0.34 ± 0.03	0.24 ± 0.02	97.0	96.0
1.50	0.50	1.52 ± 0.02	0.49 ± 0.01	101.3	98.0
0.70	1.50	0.69 ± 0.01	1.52 ± 0.02	98.6	101.3
0.20	0.50	0.21 ± 0.01	0.49 ± 0.02	105.0	98.0
1.10	0.50	1.10 ± 0.03	0.49 ± 0.01	100.0	98.0
2.00	1.00	1.98 ± 0.02	1.01 ± 0.02	99.0	101.0

[a]Mean ± S.D. (n = 5).

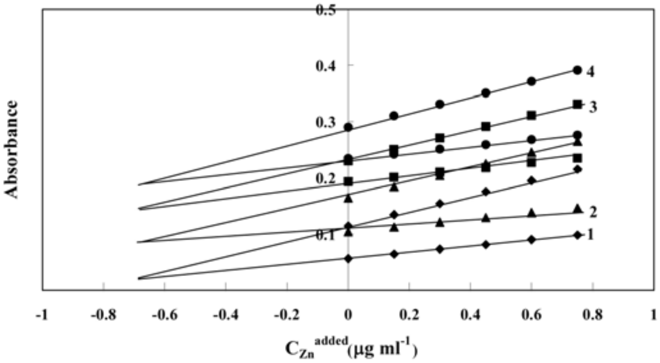

Fig. 5. H-point standard addition plot for the simultaneous determination of zinc and cobalt with constant concentration of zinc (0.70 μg ml^{-1}) and different concentration of cobalt, (1) 0.10, (2) 0.50, (3) 1.00 and (4) 1.50 μg ml^{-1} at optimum conditions.

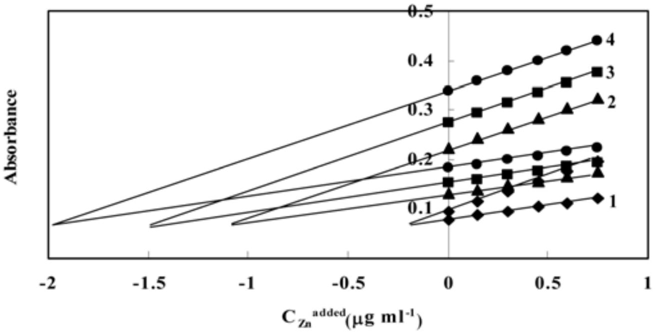

Fig. 6. H-Point standard addition plot for the simultaneous determination of zinc and cobalt with constant concentration of cobalt (0.50 μg ml^{-1}) and different concentration of zinc, (1) 0.20, (2) 1.10, (3) 1.50 and (4) 2.00 μg ml^{-1} at optimum conditions.

obtained for the Zn(II) and Co(II) ions in the samples.

Also, several experiments were carried on a series of samples containing a fixed amount of zinc(II) in the presence of various amounts of cobalt(II) (Fig. 5) or a fixed amount of cobalt(II) in the presence of various amounts of zinc(II) (Fig. 6) by the addition of zinc standard solutions. Figures 5 and 6 show that determination of Zn^{2+} and Co^{2+} ions in concentration ranges of 0.2-2.0 and 0.1-1.5 μg ml^{-1}, respectively, are independent of each other in the samples and the cobalt and zinc contents in the samples can be determined accurately. Therefore, the applicability of the proposed procedure to determine cobalt and zinc has been clarified.

Limit of Detection

The limit of detection was calculated by $DL = S_b + KS_S$ equation, where S_b is the signal average of the blank solution for five replicate analyses and S_S is the standard deviation of the results. At a confidence interval of 95%, K = 3. By using HPSAM with addition of Zn^{2+} analyte ion for blank solutions and obtaining C_H and A_H, and also by using the above

Table 2. Effect of Foreign Ions on the Determination of 1.5 μg ml^{-1} of Zn^{2+} and Co^{2+} Ions

Foreign ion	Weight tolerance limit
Na^+, Li^+, K^+, Cs^+, Ag^+, NH_4^+, Ba^{2+}, Sr^{2+}, Ca^{2+}, Mg^{2+} NO_2^-, SO_4^{2-}, I^-, Br^-, Cl^-, F^-, ClO_3^-, ClO_4^-, SCN^-	1000
Ti^{4+}, Zr^{4+}, Hg^{2+}, $S_2O_3^{2-}$, IO_3^-, CrO_4^{2-}, MnO_4^- MoO_4^{2-}, WO_3^-	500
Th^{4+}, Sn^{2+}, Cr^{3+}, Mn^{2+}, V^{3+}, Cd^{2+a}, Pb^{2+a}, Fe^{3+a}, Al^{3+a}	100
Cu^{2+}, Ni^{2+}	1

[a]After removal of interfere ion with masking agent or separation of precipitate.

Table 3. Determination of Zinc and Cobalt in Different Real Samples

Sample	Spiked (μg ml^{-1})[a]		Found (μg ml^{-1})[a]	
	Zinc(II)	Cobalt(II)	Zinc(II)	Cobalt(II)
Spring water	1.00	1.00	0.98 ± 0.05	0.92 ± 0.07
Tap water	1.50	0.50	1.47 ± 0.08	0.48 ± 0.05
River water	0.50	1.00	0.54 ± 0.06	0.97 ± 0.05
B12 Ampoule	-	45.25 μg ml^{-1}	-	46.25 ± 1.40
Megabit tablet	10.0 mg/tablet	-	10.20 ± 0.20	-

[a]Mean ± S.D. (n = 3).

equation, the theoretical detection limits of Zn^{2+} and Co^{2+} ions found to be 0.15 and 0.08 μg ml^{-1}, respectively.

Effect of Foreign Ions

The influence of the presence of several cations and anions on the determination of Zn^{2+} and Co^{2+} ions have been investigated by HPSAM under optimum conditions. The tolerance limit was defined as the concentration of the added ion causing less than a ±3% relative error. The results for both ions (Table 2) indicate that most of the cations and anions tested do not show any significant interference at weight ratio greater than 1000. The interference of Pb^{2+} and Cd^{2+} was removed by precipitation with sulfate ion and filtering the precipate. With addition of 0.1 M NaF to sample solution, the interference of Fe^{3+} and Al^{3+} ions was also eliminated. The most interfering effect was observed in the presence of Cu^{2+} and Ni^{2+} ions in sample solution.

Application of the Method

The proposed method was successfully applied to the determination of cobalt in B-complex ampoule, zinc in megabit tablet and cobalt and zinc in real water spiked samples (Table 3).

The content of 2-ml of B-complex ampoule was decomposed in a 50 ml round-bottom flask by heating with 11 ml of a 10+1 mixture of concentrated nitric and sulfuric acids on a hot plate until near dryness. The residue, was neutralized with a dilute sodium hydroxide solution, and then diluted in a 25-ml volumetric flask. The cobalt content was analyzed by the recommended procedure.

The good agreement between obtained results and the known values in water samples indicated the applicability of the HPSAM to the simultaneous determination of zinc and cobalt in complex samples. Also, the results of analysis of B-complex ampoule and megabit tablet were shown applicability of proposed method to the determination of cobalt and zinc in real samples.

CONCLUSIONS

An H-point standard addition method has been used for simultaneous determination of cobalt(II) and zinc(II) ions using methylthymol blue as a metalochromic reagent at buffered solution pH 6.0. The proposed procedure shows a dynamic range of 0.1-1.5 and 0.2-2.0 μg ml^{-1} for Co^{2+} and Zn^{2+} ions, respectively. The proposed method show an excellent reproducibility in determination of zinc and cobalt in different binary mixtures with fixed amount of zinc and various concentrations of cobalt, and vice versa. The proposed HPSAM method was applied successfully to assays of zinc and cobalt in synthetic binary mixtures and real samples.

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