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

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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 using 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, drinking water and vitamin samples.

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

INTRODUCTION

In 1988, Bosch Reig and Campins Falco delineated the fundamentals of H-point standard addition method (HPSAM), [1], with which two species with mostly or even totally overlapping spectra can be determined [2-6]. The HPSAM, as a modified version of the standard addition method, allows for the direct correction of both proportional and constant errors produced by the sample matrix. Where only the analyte concentration is required [7], absorbance increments can be used, eliminating blank bias error due to the use of an absorbent blank [8,9]. HPSAM has also been applied to HPLC, spectrofluorimetry, synchronous spectrofluorimetry and to kinetic data analysis with an additional variant, time [10-13]. This method is also of use when unknown interfering substances are involved [14].

Due to their biocidal activity resulting from the ability to precipitate and denature bacterial proteins, zinc compounds have been used in dermatology as antiseptic and disinfectant agents, in ophthalmic solutions, mouthwashes and vitamin-mineral preparations [15]. Insufficient or excessive intake of cobalt, a biologically essential trace element required only in minute amounts, may lead to deficiency or toxicity [16].

Several techniques, such as ion chromatography [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 [24] have been applied for the simultaneous determination of zinc and cobalt ions in different samples. A few UV-Vis spectrophotometric applications have been developed for the simultaneous determination of zinc and cobalt using a color reagent in the sample. However, UV-Vis spectrophotometry, one of the most widely used analytical methods, has been an attractive method for its rapidity, simplicity and broad application.

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

EXPERIMENTAL

Reagents

All chemicals used were of analytical reagent grade from Fluka or Merck chemical companies. Doubly distilled water was used throughout.

A standard Co^{2+} solution (1000 μg ml^{-1}) was prepared by dissolving an appropriate amount of hydrated cobalt(II) chloride salt in a volumetric flask. The solution was standardized as previously published [25]. A stock Zn^{2+} solution (1000 μg ml^{-1}) was prepared from the chloride salt in a volumetric flask, and standardized [25]. An aqueous solution of the sodium salt of MTB (8.0×10^{-4} M) was prepared daily. The pH of the working solution was adjusted using acetic acid, sodium acetate, sodium dihydrogen phosphate and sodium monohydrogen phosphate solution. Foreign ions were prepared using their suitable salts.

Apparatus

A Cary 100 UV-Vis scanning spectrophotometer was used to record the absorbance spectra of Zn-MTB and Co-MTB complexes using a 1.0-cm path length quartz cell equipped with a thermostat to control the cell temperature. A Metrohm 691 pH meter with a combined glass electrode was used for measurement of the pH.

General Procedure

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

Using HPSAM, the simultaneous determination of Zn(II) and Co(II) was performed by measuring the absorbance at 565 and 637 nm with the standard addition of Zn(II) solution and graphed to ascertain the concentration of Co(II). Two straight lines for concentration and absorption were plotted and the values for the absorption and concentration at the intersection of these two lines (H-point), termed C_H and A_H, respectively, were obtained by extrapolation. Zinc(II) and cobalt(II) were determined simultaneously using the concentration ratios of Zn(II) and Co(II) varying from 20:1 to 1:7.5 in mixed samples. Synthetic samples containing different concentration ratios of zinc(II) and cobalt(II) were prepared, and standard additions of Zn(II) up to 1 μg ml^{-1} were made.

The contents of a 2-ml B12 ampoule were decomposed in a 50 ml round-bottom flask by heating with a mixture of concentrated nitric (10 ml) and sulfuric acid (1 ml) on a hot plate until near dryness. The residue, neutralized with a dilute solution of sodium hydroxide, was then diluted in a 25-ml volumetric flask. The cobalt content of an appropriate amount of the solution was analyzed using the recommended procedure [27].

RESULTS AND DISCUSSION

As shown in Fig. 1, the absorption spectra of the colored

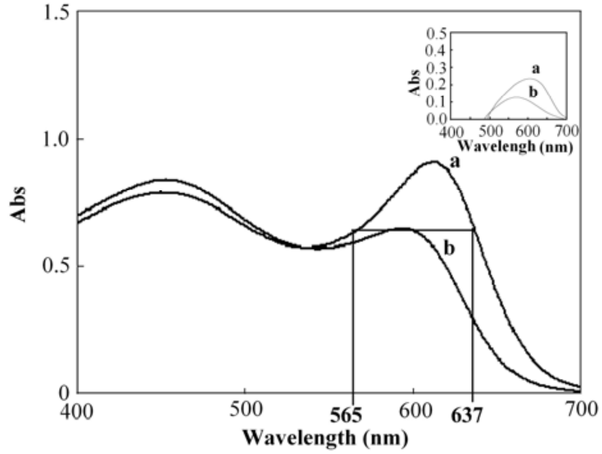

Fig. 1. Absorption spectra of Co-MTB (a) and Zn-MTB (b) complexes at 2.00 μg ml^{-1} of Co^{2+} and Zn^{2+}, $C_{MTB} = 1.0 \times 10^{-4}$ M, pH = 6 at 25 °C. The inset of the figure shows the absorption spectra of Co-MTB (a) and Zn-MTB (b) complexes against a reagent blank.

Zn^{2+}-MTB and Co^{2+}-MTB complexes overlap with each other, which interferes with the spectrophotometric determination of the ions. However, after application of the optimal conditions and HPSAM, the system allows for the accurate and simple simultaneous determination of Zn(II) and Co(II).

Determination of Optimal Conditions

The effect of the solution pH was studied in the range of pH 3-7, using buffers containing acetic acid-sodium acetate and potassium dihydrogen phosphate-potassium monohydrogen phosphate. As shown in Fig. 2, the absorbance of both MTB-Zn and MTB-Co increases up to pH 6, after which the absorbance decreases. Therefore, phosphate buffered solution at pH 6 was selected as the optimum solution.

The effect of the MTB concentration on the sensitivity of the system was investigated in the range of 1.0×10^{-5}-1.0×10^{-4} M. The results given in Fig. 3 show that the absorbance increases with increasing [MTB] up to 8.0×10^{-5} M, remaining constant thereafter. Thus, MTB was used at a concentration of 8.0×10^{-5} M.

The influence of the temperature on the reaction was studied in the range of 15-35 ºC. The results show that up to 25 ºC the absorbance of both Zn-MTB and Co-MTB increases, remaining nearly constant or decreasing slightly at higher temperatures. Therefore, 25 ºC was selected as the optimum temperature. The decrease in absorbance may be due to the lower stability of these complexes at higher temperatures.

Applying HPSAM

In the proposed system, Zn(II) and Co(II) ions are the analyte and interfering ions, respectively. As seen in Fig. 1, at the selected wavelengths of $\lambda_1 = 565$ and $\lambda_2 = 637$ nm, the Zn-MTB complex signal increases linearly with the increase in concentration of zinc ions, whereas the Co-MTB complex signal does not change with the increase in analyte concentration.

The concentration of Zn(II) ion is determined by HPSAM using two wavelengths, $\lambda_1 = 565$ and $\lambda_2 = 637$ nm, at which the interfering species, Co(II) ion, should have the same absorbance. Known amounts of Zn^{2+} ion are then consecutively added to the mixture. After each addition the absorbance (A) is measured at the two wavelengths, and expressed by the following equations.

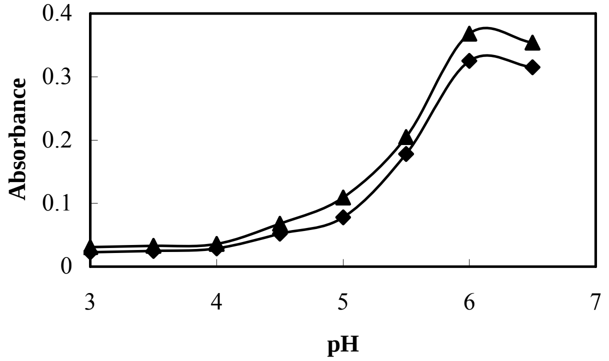

Fig. 2. Effect of pH on the absorbance of Co-MTB (▲) and Zn-MTB (♦) complexes.

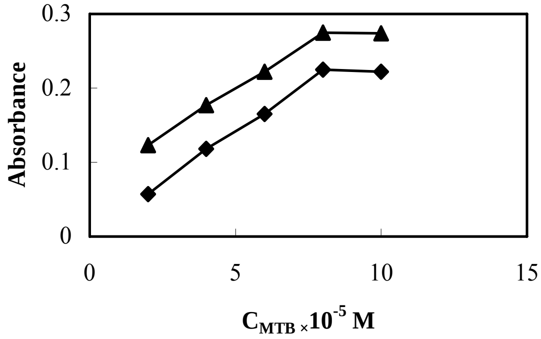

Fig. 3. Effect of MTB concentration on the absorbance of Co-MTB (▲) and Zn-MTB (♦) complexes.

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

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

The two straight lines obtained intersect at the H-point ($-C_{Zn}$, A_{Co}) (Fig. 4).

To achieve the 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. Results of the analysis of the different mixtures in the proposed system revealed a dynamic range of 0.2-2.0 and 0.1-1.5 μg ml^{-1} for Zn^{2+} and Co^{2+} ions, respectively.

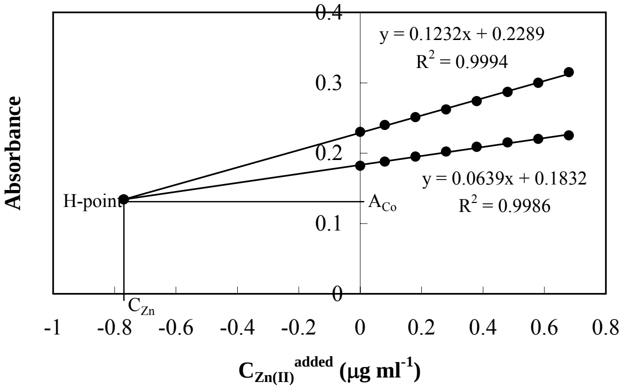

Fig. 4. H-point standard addition plot for the simultaneous determination of zinc and cobalt. Conditions: 1.00 μg ml^{-1} of Co^{2+}, 0.76 μg ml^{-1} of Zn^{2+}, $C_{MTB} = 8.0 \times 10^{-5}$ M, pH = 6 at 25 °C.

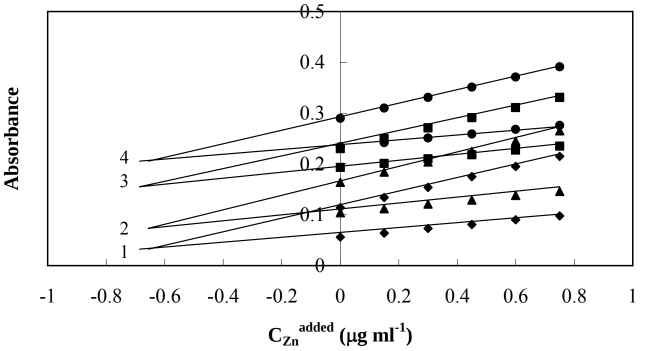

Fig. 5. H-point standard addition plot for the simultaneous determination of zinc and cobalt under optimized conditions with a constant concentration of zinc (0.70 μg ml^{-1}) and cobalt concentrations of: (1) 0.10, (2) 0.50, (3) 1.00 and (4) 1.50 μg ml^{-1}.

Reproducibility and Accuracy of the Method

Five replicate experiments on binary samples of Zn(II) and Co(II) ions were performed to check the reproducibility of the proposed method (Table 1). A recovery of 96-105%, with a standard deviation of ≤0.03, was obtained for the Zn(II) and Co(II) ions in the samples.

In addition, several experiments were carried out on a series of samples containing a fixed amount of zinc(II) with varying amounts of cobalt(II) (Fig. 5), and a fixed amount of cobalt(II) with varying amounts of zinc(II) (Fig. 6), accomplished by the addition of standard solutions of the varied ion. Figures 5 and 6 show the determination of Zn^{2+} and Co^{2+} ions in the samples at concentrations of 0.2-2.0 and 0.1-1.5 μg ml^{-1}, respectively, to be independent of each other and accurate, thereby confirming the applicability of the proposed procedure.

Limit of Detection

The detection limit of this system is calculated by the

Table 1. Determination of Zinc and Cobalt in 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	101.0
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).

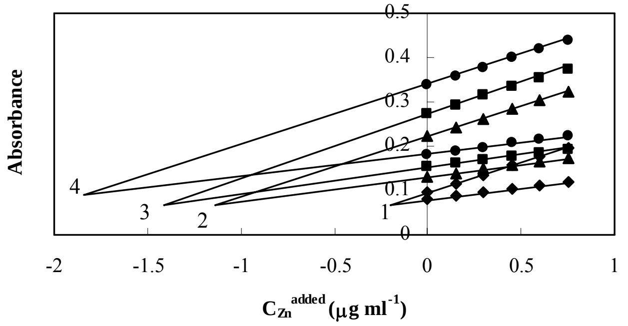

Fig. 6. H-point standard addition plot for the simultaneous determination of zinc and cobalt under optimized conditions, a constant concentration of cobalt (0.50 μg ml^{-1}) and zinc concentrations of: (1) 0.20, (2) 1.10, (3) 1.50 and (4) 2.00 μg ml^{-1}.

equation:

$$DL = B + KS_S$$

where B is the average of the blank solution signal for five replicate analyses, S_S is the standard deviation of the results, K = 3, and DL is the signal corresponding to the detection limit at a confidence interval of 95%. With HPSAM, we used the addition of the analyte ion (Zn^{2+} ion) for blank solutions, combined with the H point values (C_H and A_H) and the above equation, to obtain the theoretical detection limits for Zn^{2+} and Co^{2+} ions of 0.15 and 0.08 μg ml^{-1}, respectively.

Effect of Foreign Ions

We investigated the influence of the presence of several cations and anions on the determination of Zn^{2+} and Co^{2+} ions 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 showed no significant interference at weight ratios greater than 1000. The interference of Pb^{2+} and Cd^{2+} was resolved by precipitating these ions with sulfate and filtering the precipitate. After addition of 0.1 M NaF to the sample solution, the interference of Fe^{3+} and Al^{3+} ions was eliminated using the fluoride ion as a masking agent. The greatest interference is caused by the presence of Cu^{2+} and Ni^{2+} in the sample solution.

Application of the Method

The proposed method was successfully applied to the determination of cobalt and zinc in spiked tap and spring water samples (Table 3), cobalt in vitamin B12 ampoules and zinc in a Megabit tablet (Table 4).

The good agreement between the obtained results and the known values in water samples indicate the applicability of the HPSAM for the simultaneous determination of zinc and cobalt in complex samples. Furthermore, the results of the analyses of the B12 ampoule and Megabit tablet display the applicability of the proposed method for the determination of cobalt and zinc in real samples.

The previously reported spectrophotometric methods using other reagents such as dithizone and DPPH have been applied to the determination of cobalt and/or zinc alone. The proposed

Table 2. Effect of Foreign Ions on the Determination of Zn^{2+} and Co^{2+}

Foreign ion	Tolerance limit: Weight ratio[b]
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+}, [a]Cd^{2+}, [a]Pb^{2+}, [a]Fe^{3+}, [a]Al^{3+}	100
Cu^{2+}, Ni^{2+}	1

[a] After removal of interfering ions using a masking agent or by separation of precipitate.

[b] Foreign ion: 1.5 μg ml^{-1} Zn(II) or Co(II).

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

Sample	Spiked (μg ml^{-1})[a]		Found (μg ml^{-1})[b]	
	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

[a]Zinc and cobalt were not detected prior to spiking samples. [b]Mean ± S.D. (n = 3).

Table 4. Determination of Zinc and Cobalt in the Megabit Tablet and B12 Ampoule

Sample	Claimed		Found[a]	
	Zinc(II)	Cobalt(II)	Zinc(II)	Cobalt(II)
B12 ampoule	-	45.25 μg ml^{-1}	-	46.25 ± 1.40 μg ml^{-1}
Megabit tablet	10.0 mg/tablet	-	10.20 ± 0.20 mg/tablet	-

[a]Mean ± S.D. (n = 3).

method is comparable to HPSAM using the PAN reagent [28] and PLS method using the zincone reagent [24] for determination of cobalt and zinc in a sample.

CONCLUSIONS

An H-point standard addition method has been used in simultaneous determination of cobalt(II) and zinc(II) ions using methylthymol blue as a metallochromic reagent at pH 6.0. The high degree of reproducibility and valuable dynamic range as well as simplicity are the advantages of the proposed method.

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