

## Quenchofluorimetric Determination of Cobalt (II) At Ultra trace Levels Using Bathophenanthrolinedisulphonate

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**Abstract:** A very simple, rapid, highly selective and ultrasensitive fluorimetric method for the determination of Cobalt (II) has been developed. The method is based on the instantaneous quenching action of Cobalt(II) on the native fluorescence of Bathophenanthrolinedisulphonate (4,7-diphenyl-1,10-phenanthroline disulphonate) having excitation and emission wavelength maxima at 288nm and 444.8nm respectively in the optimum pH range of 4.0-4.7. The fluorescence intensity versus Cobalt (II) concentration calibration graph was rectilinear for a total range of 0.001-1.0ppm Cobalt (II). The standard deviation and relative standard deviation for 11 replicate determinations of 50ppb Cobalt (II) were found to be 0.435 and 0.868% respectively.

**Keywords:** Bathophenanthrolinedisulphonate, Cobalt (II), Environmental samples, Quenchofluorimetry, Toxicity.

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### I. Introduction

Cobalt is relatively rare element, comprising only 0.001% of the earth's crust. Cobalt is necessary for vitamin B<sub>12</sub> or cyanocobalamin which is needed for our bodies to form haemoglobin and deficiencies cause pernicious anaemia. Cobalt is also used as an enzyme activator, e.g. in carbonic anhydrase or carboxypeptidase [1]. Acute toxicity of cobalt results diarrhoea, loss of appetite, paralysis of the hind legs and lowering of body temperature prior to death [2]. It is also reported to be carcinogenic in both plants [3] and animals [4]. The effective toxicity, genotoxicity and micronutrient activity of cobalt are dose dependant which need to be accurately determined with much confidence to properly assess areas of health risk, otherwise prognosis and diagnosis of disease will be erratic that may culminate into wrong treatment and fatal end. Therefore, its trace and ultra trace analysis is of paramount importance. Very few works on fluorimetric determination of cobalt (II) have been reported in the literature. Review on previous works reveals that these are low sensitive, unselective and encountered with high interferences [5-13]. Another quenchofluorimetric method for Co (II) determination was reported from this laboratory by Pal and Banerjee [14] using bathophenanthroline in 50% ethanolic medium. The insolubility of the reagent in water is one of the drawbacks and further applications to alloys and ores are yet to be done. The present method is simple, virtually selective, and sensitive and has successfully applied to complex matrices.

### II. Experimental

#### 2.1 Instrumentation

All fluorescence measurements were performed on a Shimadzu Spectrofluorophotometer (RF 5000) equipped with a 150 W Xenon lamp, 12 inch colour video display, parallel line thermo sensitive printer recorder, 1 x 1cm quartz cells and a Shimadzu ASC-5 auto sample changer. A digital pH meter (Model pH 5651; Electronic Corporation of India) was used to measure pH. A Hanovia fluorescence lamp (Model 11A; Hanovia Limited, England) was employed for visual study of fluorescence intensity changes qualitatively.

#### 2.2 Reagents

##### 2.2.1. Cobalt (II) standard solution

A standard stock solution of Co (II) (1ng/ml) was prepared by dissolving 0.4769 g. of CoSO<sub>4</sub>.7H<sub>2</sub>O (E. Merck, Pro-analysis) in 100ml volumetric flask with deionised water. Suitable aliquots from the stock solution were standardised by EDTA titration method using Xylenol orange as indicator [13].

##### 2.2.2. Bathophenanthrolinedisulphonate solution

A 0.1 % (W/V) corresponding to 1.693 x 10<sup>-3</sup>M bathophenanthrolinedisulphonate (Liba – Chemie Wein – Fischamend) solution was prepared in deionised water. This solution was stable for more than a month if preserved in refrigerator. From this stock solution, more diluted solutions were prepared as and when needed.

### 2.2.3. Buffer solution

A 100ml acetate buffer solution (pH 4.27) was prepared by mixing 70ml of 0.2M acetic acid and 30ml of 0.2M sodium acetate solutions.

### 2.2.4. Other solutions

A large number of solutions of inorganic ions and complexing agents were prepared from their Analar grade or equivalent grade water soluble salts. In case of insoluble salts, special dissolution methods were adopted [15]. Doubly distilled demineralised water was used throughout the study.

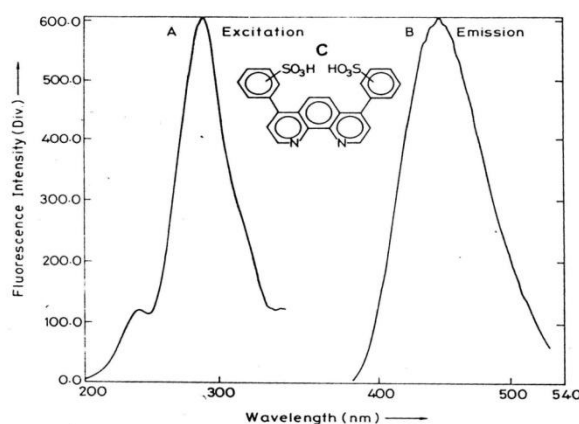
### 2.3. Procedure

To 1ml sample solution containing 0.02-0.2 $\mu$ g or 0.2-2.0 $\mu$ g or 2.0-10 $\mu$ g of cobalt (II) in a 10ml volumetric flask, were respectively added 0.1ml of 10<sup>-4</sup>M or 0.3ml of 10<sup>-4</sup>M or 1ml of 10<sup>-3</sup>M bathophenanthrolinedisulphonate solution and 2ml of acetate buffer (pH 4.27). The volume was then made up to the mark with deionised water. Fluorescence intensity of the complex was then measured against a corresponding reagent blank at 444.8nm, keeping the excitation wavelength maximum at 288nm. The concentration of cobalt (II) in the unknown sample was determined with the help of a concurrently prepared calibration graph from standard cobalt (II) solution.

## III. Results And Discussion

### 3.1. Spectral characteristics

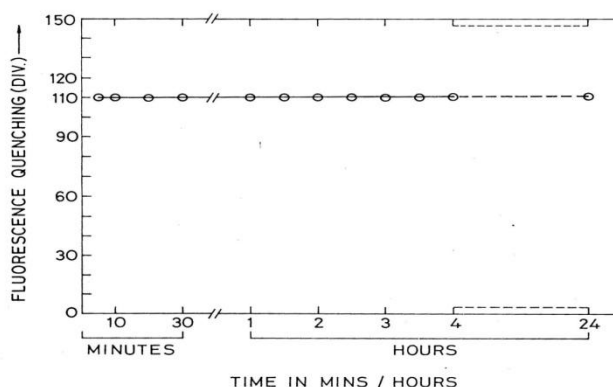
The uncorrected excitation and emission spectra of the bathophenanthrolinedisulphonate solution are shown in Fig.1. The wavelength maxima of excitation and emission were found to occur at 288nm and 444.8nm, respectively.



**Fig.1.** Excitation (A) and emission (B) spectra of Bathophenanthrolinedisulphonate (4, 7-diphenyl -1, 10-phenanthrolinedisulphonate), (C) Structure of Bathophenanthrolinedisulphonate.

### 3.2. Effect of time

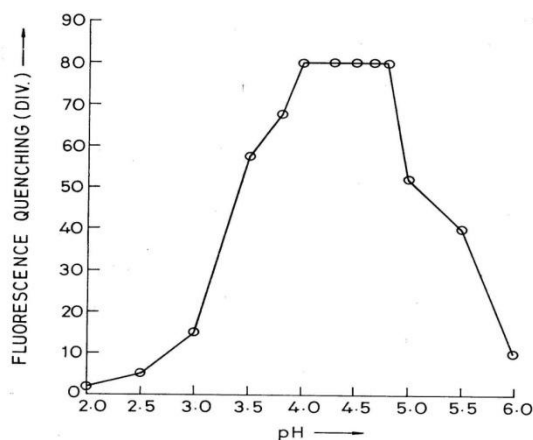
The extent of fluorescence quenching intensity remained constant from 5min of volume make-up to 24hrs. Longer than this time was not studied. Fig.2 shows effect of time.



**Fig.2.** Effect of time on the fluorescence quenching intensity of Co(II) – Bathophenanthrolinedisulphonate system.

### 3.3. Effect of pH

The study of the effect of pH while other conditions in the procedure remained the same showed that the maximum and constant fluorescence quenching intensity occurred in the pH range 4.0 – 4.7. Fig.3 shows effect of pH.



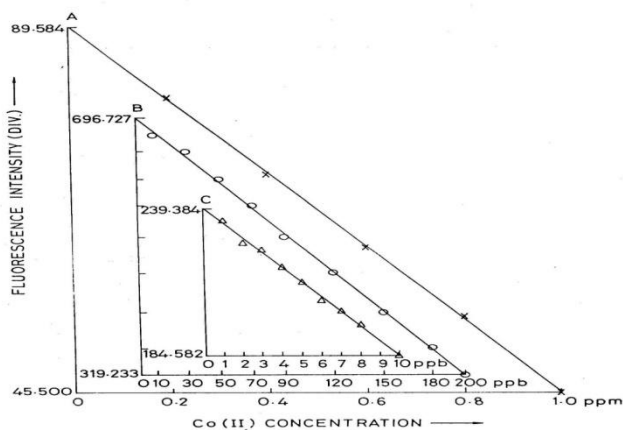
**Fig.3.** Effect of pH on the fluorescence quenching intensity of Co(II) – Bathophenanthrolinedisulphonate system.

### 3.4. Effect of buffer solution

Study of the effect of buffer solution showed that the presence of 1 – 5 ml sodium acetate – acetic acid buffer of pH 4.27 added to the total 10ml volume of metal – reagent system had no effect on the fluorescence quenching intensity. Consequently, 2ml of this buffer was used throughout the study.

### 3.5. Effect of metal concentration (calibration graph)

The fluorescence intensity versus cobalt (II) concentration calibration graph was rectilinear for a total range of 0.001 – 1.0 ppm cobalt (II). For convenience of measurements, it was distributed into 3 sets; 1.0 – 10.0 ppb, 10.0 – 200.0 ppb and 0.2 – 1.0 ppm of Co (II). Typical calibration graphs as displayed in the video screen of the Spectrofluorophotometer are reproduced in Fig.4.



**Fig.4.** Calibration graphs; (A) 0.2 – 1.0 ppm, Co(II); (B) 10.0 – 200.0 ppb, Co(II); (C) 1.0 – 10.0 ppb, Co (II).

### 3.6. Effect of foreign ions

The individual effects of over 60 anions, cations and some common complexing agents on the determination of 50ppb Co (II) were studied separately by the procedure described earlier. Less than 3% error by (W/W) ratio of foreign ion to Co (II) added was the criterion of non-interference. 1000 – fold excess of Li(I), Na(I), K(I), Ba(II), Ca(II), Sr(II), Mg(II), Ag(I), As(III), La(III), Th(IV), U(VI), Cl<sup>-</sup>, I<sup>-</sup>, Br<sup>-</sup>, NO<sub>3</sub><sup>-</sup>, HSO<sub>4</sub><sup>-</sup>, HSO<sub>3</sub><sup>-</sup>, HPO<sub>4</sub><sup>2-</sup>, SO<sub>3</sub><sup>2-</sup>, SO<sub>4</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup>, ClO<sub>4</sub><sup>-</sup>, SCN<sup>-</sup>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup>, H<sub>2</sub>O<sub>2</sub>, H<sub>2</sub>C<sub>4</sub>H<sub>4</sub>O<sub>6</sub>(tartaric acid), SC(NH<sub>2</sub>)<sub>2</sub>(thiourea) and CH<sub>3</sub>COO<sup>-</sup>, EDTA do not interfere. This is not the maximum tolerance limit of these foreign ions but the actual amounts studied. The maximum tolerance limits of the following ions are; 500 – fold excess: H<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub> (citric acid), Sn(II), Sn(IV), F<sup>-</sup>; 100 – fold excess: H<sub>2</sub>C<sub>6</sub>O<sub>4</sub> (oxalic acid), C<sub>6</sub>H<sub>8</sub>O<sub>6</sub>(ascorbic acid), Pb(II), Al(III), Tl(III); 50 – fold excess: Zn(II), Mn(II), Se(IV), Bi(III) (in presence of thiourea); 10 - fold excess: Cd(II), Cr(III),

Sb(III), Fe(III), Sb(III), Cr(VI), Zr(VI), Hg(II) (in presence of thiosulphate), V(V) (in presence of tartarate), W(VI) (in presence of F<sup>-</sup>), Mo(VI) (in presence of citrate). Cu(II), Ni(II) and Fe(II) interfere in the determination procedure. However, these interferences could be removed by a quick ion – exchange separation process using Amberlite IRA – 400 (Chloride form) [15].

**3.7. Separation of Interfering Ions on Anion Exchanger ion exchanger [15]**

About 2 g. of anion exchange resin, Amberlite IRA – 400 (Chloride form) was taken in a beaker and repeatedly washed with deionised water to remove the fine particles. The resin was then transferred in a ion exchange column of specific i.d. (1cm) containing a plug of glass wool until a column of well-packed resin about 5 cm long was obtained. The resin in the column was washed twice with deionised water. 20ml of 9M HCl was then passed through the column and drained to almost bed level. A solution containing known amount of Co (II) and interfering ions was mixed with conc. HCl (11.3M) so that the final molarity of the solution was 9M. It was then passed through the resin at the rate of 2 ml/min. Another 20ml of 9M HCl was again passed in order to elute the interfering ions completely. Co (II) was finally eluted with 50ml of deionised water at the rate of 3 ml/min. The effluent was collected in a beaker and concentrated to a small volume inside fume cupboard in order to remove the excess of acid. After cooling, it was neutralised with dil. NaOH solution and finally diluted to 50ml with deionised water. From suitable aliquots, cobalt content was then fluorimetrically determined as described earlier with the help of concurrently prepared calibration graph from standard Co(II) solution, also absorbed in the similarly conditioned resin bed and eluted identically.

**IV. Applications**

The proposed method was successfully applied to the determination of Cobalt (II) in a series of synthetic mixtures of various compositions, certified reference materials and environmental water samples. The results of analyses were in excellent agreement with their certified values. In case of environmental water samples of unknown composition, the analyses were done in both Cobalt (II) - spiked and – unspiked conditions. These results were in agreement with those reported by AAS method. These results are shown in Table 1, 2 and 3.

**TABLE 1. Determination of Cobalt (II) in Synthetic Mixtures.**

Composition of the mixture <sup>a</sup>	Co (II) present (%)	Co (II) found <sup>b</sup> (%)	Recovery (%)
A. Fe <sup>3+</sup> (2), Cu <sup>2+</sup> (1), Ni <sup>2+</sup> (1), Co <sup>2+</sup> (0.1).	2.43	2.68 <sup>c</sup> ± 0.0008	110
B. Mg <sup>2+</sup> (50), Pb <sup>2+</sup> (1), As <sup>3+</sup> (10), Th <sup>4+</sup> (50), La <sup>3+</sup> (50), Co <sup>2+</sup> (0.1).	0.062	0.064 ± 0.0007	103
C. As in B, Citrate (50), Mo <sup>6+</sup> , Bi <sup>3+</sup> (1), Thio-urea (100).	0.031	0.032 ± 0.0020	103
D. As in C, Cd <sup>2+</sup> (1), S <sub>2</sub> O <sub>3</sub> <sup>2-</sup> (100), Hg <sup>2+</sup> (1).	0.024	0.026 ± 0.0010	108
E. As in D, Tartarate (100), Zn <sup>3+</sup> (5), Fe <sup>3+</sup> (1).	0.019	0.019 ± 0.0010	108
F. As in E, Cr <sup>6+</sup> (1), Al <sup>3+</sup> (100), Zr <sup>6+</sup> (10), Mn <sup>2+</sup> (2).	0.015	0.015 ± 0.0007	100

a – Composition in ppm. b – Mean + Standard Deviation (n = 5). c – Using ion-exchange column.

**TABLE 2. Determination of Cobalt (II) in Certified Reference Materials (CRM).**

Composition of CRM (%)	Co (II) spiked (ng/ml)	Co (II) found <sup>a</sup> (ng/ml)	Recovery (%)
BAS (20b) Al – alloy	0.00	0.00 ± 0.063	100.0
Cu=4.1, Fe=0.43, Mn=0.19, Mg=1.61, Si=0.24, Al=90.50, Ni=1.93	20.0 40.0	20.7 ± 0.418 40.5 ± 0.360	103.5 101.2
BAS (32a) Al – Bronze	0.00	0.00 ± 0.0085	100.0
Al=8.8, Cu=85.9, Mn=0.27, Ni=1.16, Zn=0.94, Fe=2.69	20.0 40.0	21.1 ± 0.519 40.7 ± 0.396	105.5 101.7

a – Mean ± Standard Deviation (n=5)

**TABLE 3. Determination of Cobalt (II) in Environmental Water Samples.**

Sample	Composition by AAS ( $\mu\text{g/ml}$ )	Amount of Co (II) ( $\mu\text{g/ml}$ )		Recovery (%)	
		Added	Found <sup>a</sup>		
Tap water	---	0.00	0.00	100	
		0.02	$0.02 \pm 0.0017$	100	
		0.05	$0.052 \pm 0.002$	104	
Deep tube well water	---	0.00	0.00	100	
		0.02	$0.02 \pm 0.001$	100	
		0.05	$0.053 \pm 0.002$	106	
Factory drain Waters	A. As <sup>3+</sup> <0.01, Hg <sup>2+</sup> <0.01, Pb <sup>2+</sup> <0.01, Cr <sup>3+</sup> =0.09, Ni <sup>2+</sup> =0.20, Ag <sup>+</sup> =0.02, Zn <sup>2+</sup> =0.19, Na <sup>+</sup> =56, Cu <sup>2+</sup> =0.03, Cl <sup>-</sup> =108, SO <sub>4</sub> <sup>2-</sup> =25	0.00	$0.023 \pm 0.0017$	100 98	
		0.02	$0.043 \pm 0.0021$		
		0.05	$0.072 \pm 0.0022$		
		0.00	$0.017 \pm 0.001$		
		0.02	$0.04 \pm 0.001$		
		0.05	$0.068 \pm 0.001$		
	B.	As <sup>3+</sup> =0.01, Hg <sup>2+</sup> =0.01, Pb <sup>2+</sup> =0.01, Cr <sup>3+</sup> =0.04, Ni <sup>2+</sup> =0.18, Ag <sup>+</sup> =0.008, Zn <sup>2+</sup> =0.19, Na <sup>+</sup> =52, Cu <sup>2+</sup> =0.02, Cl <sup>-</sup> =105, SO <sub>4</sub> <sup>2-</sup> =23	0.00	$0.017 \pm 0.001$	115 102
			0.02	$0.04 \pm 0.001$	
			0.05	$0.068 \pm 0.001$	
	C.	As <sup>3+</sup> =0.01, Hg <sup>2+</sup> =0.01, Pb <sup>2+</sup> =0.01, Cr <sup>3+</sup> =0.04, Ni <sup>2+</sup> =0.15, Ag <sup>+</sup> =0.005, Zn <sup>2+</sup> =0.32, Na <sup>+</sup> =54, Cu <sup>2+</sup> =0.01, Cl <sup>-</sup> =108, SO <sub>4</sub> <sup>2-</sup> =24	0.00	$0.011 \pm 0.002$	105 104
			0.02	$0.032 \pm 0.002$	
			0.05	$0.063 \pm 0.002$	
D.	As <sup>3+</sup> =0.01, Hg <sup>2+</sup> =0.01, Pb <sup>2+</sup> =0.01, Cr <sup>3+</sup> =0.004, Ni <sup>2+</sup> =0.1, Ag <sup>+</sup> =0.03, Zn <sup>2+</sup> =0.12, Na <sup>+</sup> =53, Cu <sup>2+</sup> =0.01, Cl <sup>-</sup> =105, SO <sub>4</sub> <sup>2-</sup> =23	0.00	$0.017 \pm 0.0017$	105 102	
		0.02	$0.038 \pm 0.0026$		
		0.05	$0.068 \pm 0.002$		

a – Mean  $\pm$  Standard Deviation (n=5)

## V. Conclusion

The fairly solubility of the reagent, bathophenanthrolinedisulphonate, in water and non-requirement of any other solvents like alcohol are added advantages over previous methods. The method could be improved further if suitable complexing agents are available for masking the interfering ions instead of applying the ion-exchange process as suggested. Though the present method requires a quick ion-exchange separation process for removing interfering ions, it is far superior to others in terms of simplicity, sensitivity, wider determination range, prolong stability of fluorescence, non-requirement of any other solvent and applicability to complex matrices.

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