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Determination Of Cobalt (II) In Various Environmental Samples With 3,5-Dibromosalicylaldehyde Thiosemicarbazone Using Spectrophotometry Coupled With Flow-Injection Technique



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ABSTRACT

The present paper describes a facile, rapid and economical flow-injection method for the determination of cobalt(II) in various environmental samples. The method is based on the formation of green colored complex between cobalt(II) and 3,5-dibromosalicylaldehyde thiosemicarbazone (3,5-DBSTSC) at pH 4.0 ± 0.2 . The absorbance of the complex was measured spectrophotometrically at 431 nm. The influence of various parameters such as pH, flow-rate of samples, volume of the sample and length of reaction coil were investigated. Beer's law was obeyed over the range of $0.6-12 \mu\text{g ml}^{-1}$. The molar absorptivity and Sandell's sensitivity were $1.387 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$ and $0.004125 \mu\text{g cm}^{-2}$ respectively. Detection limits for cobalt(II) was found to be $0.0155 \mu\text{g ml}^{-1}$. The detailed study of various interferences made the method more selective. The method was successfully applied to the determination of cobalt(II) in various environmental samples. The performance of proposed method was evaluated in terms of Student's *t*-test and Variance ratio *f*-test which indicates the significance of proposed method over reported method. Recoveries obtained revealed that the proposed procedure shows good accuracy at 95 % confidence level.

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KEYWORDS

Cobalt(II);
3,5-Dibromosalicylaldehyde
thiosemicarbazone;
Flow-injection
apparatus(FIA);
Spectrophotometry;
Environmental samples.

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INTRODUCTION

Cobalt is an essential element for human and animals because it is associated with the synthesis of vitamin B₁₂. Cobalt is used in the manufacture of alloys, permanent magnets, paint driers and as industrial catalyst in processes such as manufacture of NH₃ and alcohol. It deficiency is exhibited by a retarded growth rate, loss of appetite and pernicious anemia in human beings. Chronic exposure of cobalt may lead to goiter disease. Ingestion of excessive amounts of cobalt causes intercellular hypoxia and polycythemia. At high concentrations, it inhibits heme biosynthesis and enzyme activities. Moreover, the element is toxic, when taken directly.

Several analytical techniques were reported for the determination of cobalt(II) in various environmental samples. Techniques such as AAS, ICP-AES, X-ray fluorescence spectroscopy, spectrofluorimetry and spectrophotometry. Thiosemicarbazone is an important sulphur containing organic reagent. The metal chelate of this reagent find a wide range of application in medicine^[1,2] and agriculture^[3]. The use of thiosemicarbazones in inorganic analysis has been reviewed^[4-6] and the survey of literature reveals, that only a few thiosemicarbazones are employed for the determination of cobalt(II) with spectrophotometer^[7-18]. Above reported methods have certain limitations such as less stability, selectivity, sensitivity, serious interference of foreign species with chelating agent and high reagent consumption.

Hence, we developed a flow-injection analysis, coupled with a spectrophotometric detector, is simple, rapid, economically advantageous (low reagent consumption), provides reproducible results in statistical validation (elimination of subjective analytical errors and foreign species). It is useful tool for quantification of samples in a variety of spiked and natural samples at low detection level for series of analysis of cobalt(II) in various environmental samples.

EXPERIMENTAL

Instrumentation

A high-pressure liquid chromatography (Perkin-

Elmer Series 3) pump furnished with stainless-steel tubing was used to propel the stream by a Rheodyne injection valve provided with a 100 µL loop. In the FI system, the absorbance of the colored complex formed was measured and coupled with a HITACHI U 2001 spectrophotometer equipped with a flow-through microcell (Spectra SYSTEM UV 3000 HR, Thermo Separation Products, USA), and connected to a computer incorporated with a PC 1000 software program.

An Elico model Li-129 meter with combined glass and calomel electrode was employed for all pH measurement.

Reagents

All reagent used were of analytical reagent grade. Deionised doubly distilled water was used throughout the experiment. A stock solution of cobalt(II) (0.01 M) was prepared by dissolving 2.380 g of CoCl₂·6H₂O (E-Merck, Germany) in deionised doubly distilled water in 1000 ml standard flask. Cobalt(II) working standard stock solutions were prepared freshly by appropriate dilution of the standard stock solution with deionised doubly distilled water. 0.05M 3,5-dibromosalicylaldehyde thiosemicarbazone (3,5-DBSTSC) was prepared by dissolving 1.765 g of 3,5-DBSTSC in 100 ml of 40% aqueous dimethyl formamide.

Solution of 1M sodium acetate and 1M acetic acid were prepared in deionised doubly distilled water. Suitable portions of the solutions were mixed to get acetate buffer solution of pH 4.0 ± 0.2.

Finally, 0.1 M HNO₃, 25% KI, 2 M HCl and isobutylmethylketone (E-Merck, Germany) were used in the present investigation.

Synthesis of 3,5-dibromosalicylaldehyde thiosemicarbazone(3,5-DBSTSC)

3,5-DBSTSC is not commercially available and was synthesized in laboratory. 50 ml of methanolic 3,5-dibromosalicylaldehyde (28 g) and 50 ml of aqueous thiosemicarbazide (9.10 g) solution, 3 ml of concentrated hydrochloric acid is added. The reaction mixture is kept on a hot water bath for about 30 min. A white colored product is obtained; separated by filtration and dried at room temperature. The prod-

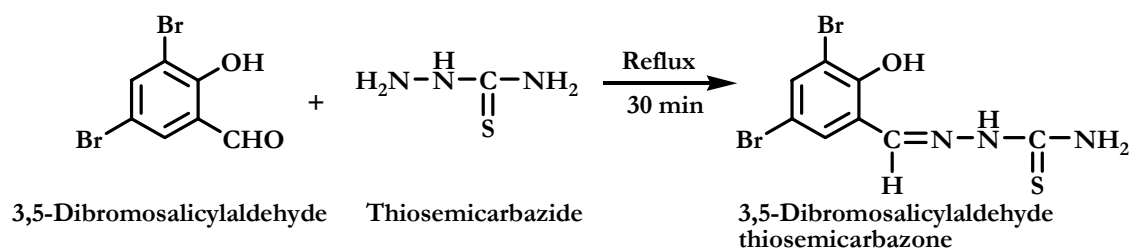


Figure 1: Synthesis of 3,5-Dibromosalicylaldehyde thiosemicarbazone

uct is recrystallized from ethanol. The yield was 89% and melting point of the product is 230-234°C. The structure of color forming reagent is shown in figure 1. IR data with tentative assignment of various stretching and bending frequencies of 3,5-DBSTSC are listed. The band at 1137.01 cm^{-1} is due to the –OH group and absorption bands at 1613.34 cm^{-1} , 1535.18 cm^{-1} and 1448.83 cm^{-1} are due to the C=N group of reagent. The absorption band at 595.27 cm^{-1} is due to C-Br on benzene ring. Absorption bands appear at 1264.14 cm^{-1} and 1237.21 cm^{-1} are due to N-CS-N group.

Procedure

The FI system for the determination of cobalt(II) in various environmental samples was presented in the figure 2. The method was tested with model solution before its application to natural samples. A 200 μL volume of sample solution was injected into the reagent carrier solution (acetate buffer). The sample solution along with carrier combines with

reagent (3,5-DBSTSC) in the FI system and mixed solution was sent to a reaction coil, the colored solution was carried to the flow-injection spectrophotometric detector coupled to PC, the absorbance of the complex was selectively measured at 431 nm. In the optimized studies, the parameters studied were pH, flow-rate, reagent concentration, sample volume, length of reaction coil and method evaluation.

Determination of cobalt(II) in natural water samples

Different river lake and spring water samples were collected from various places in and around Tirupati, A.P., India. The samples (150 ml) were stored at 0-5°C in metal free polyethylene bottles. Water samples were filtered through Whatman filter paper no.41 and clean solution is collected into 250 ml beaker. The contents are diluted up to the mark with deionised doubly distilled water. 15 ml of this solution is further diluted to get working solution for determination of cobalt(II) as described in above

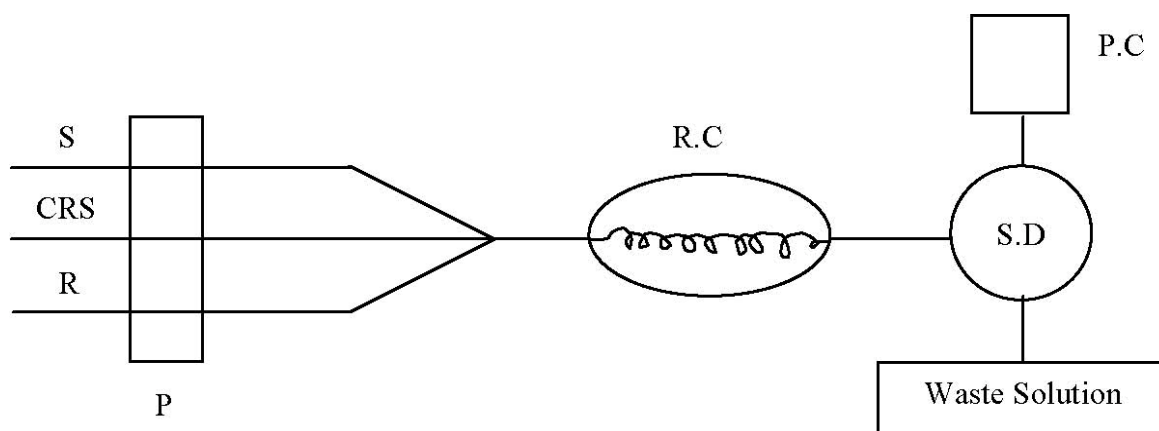


Figure 2: Flow-injection apparatus for the determination of cobalt(II) in various environmental samples. S=Sample; CRS=Carrier reagent solution; R=Reagent; P=Peristaltic Pump; RC=Reaction Coil; P.C=Personal Computer; S.D.= Spectrophotometric detector

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TABLE 1: Determination of cobalt(II) in various environmental samples

Samples	Proposed Method					Reported Method ^[18]	
	Cobalt(II) added ($\mu\text{g ml}^{-1}$)	Cobalt(II) found ($\mu\text{g ml}^{-1}$)	Mean recovery (%) ^a	t-test	f-test	Cobalt(II) found ($\mu\text{g ml}^{-1}$)	Mean recovery (%) ^a
Spiked Water-I	1.0	0.98	98.0 $\pm$ 0.36	1.07	0.042	0.97	97.0 $\pm$ 0.45
Spiked Water-II	3.0	2.98	99.33 $\pm$ 0.10	0.53	0.015	2.92	97.33 $\pm$ 0.07
River Water ^b	-	9.42	-	-	-	9.40	-
	5.0	14.27	97.0 $\pm$ 0.45	0.56	0.22	14.20	96.0 $\pm$ 0.18
Lake Water ^c	-	5.06	-	-	-	-	-
	7.0	13.89	98.42 $\pm$ 0.08				
Spring Water ^d	-	2.91	-	-	-	-	-
	9.0	11.7	97.65 $\pm$ 0.12				
Soil Sample ^e	-	10.85	-			11.40	-
	11.0	21.75	99.0 $\pm$ 0.16	0.47	0.60	22.14	97.63
Tomato ^f	-	1.41	-	-	-	-	-
	13.0	14.26	98.84 $\pm$ 0.18				
Carrots ^g	-	1.16	-	-	-	-	-
	15.0	15.97	98.73 $\pm$ 0.05				

^aMean $\pm$ Standard deviation(n=4), ^bCollected from Chandragiri area, ^cCollected from Narsingapuram area, ^eCollected from Rangampet area,

^fCollected from local market

procedure and compared with the reported method. The results were summarized in TABLE 1.

Determination of cobalt(II) in soil samples

Soil samples were collected from Rangampet area, A.P., India. The sample was homogenized in the laboratory, using a pestle and motor and air-dried for 24 h approximately, before analysis. An aliquot of 500 mg of finely pulverized soil is digested with 5 ml of nitric acid (65%) in metal free teflon vessel. The sample is digested for about 1 h in a microwave oven and again redigested at 160°C for 15 min. After treating with double distilled water, the supernatant liquid was made up to the mark in a 25 ml standard flask. The aliquot was analyzed for cobalt(II) using 3,5-DBSTSC, following the recommended procedure and results were tabulated in TABLE 1.

Determination of cobalt(II) in vegetable samples

The vegetable matter was dried at 105°C ground in agate, and homogenized. Samples (10-15 g) were weighed into evaporated dishes and ashed at 300-400°C in a microwave oven for 10 min. Then, the ash was dissolved in 15 ml of 0.1 M HNO₃ and the solution was transferred into a 100 ml separating

funnel, 3 ml of 25 % (w/w) KI (To mask interference of Ca²⁺ and Ba²⁺ ions during extraction) and 5 ml of 2 M HCl were added and the solution was diluted with deionised doubly distilled water.

The solution was extracted with 15 ml of isobutylmethylketone, and the aqueous solution was transferred to another 100 ml separating funnel. The aqueous solution was re-extracted with 15 ml of isobutylketone, the aqueous layer was discarded and the organic phase was washed with 20 ml of deionised doubly distilled water. The organic phase was merged, then back-extracted with 1 ml of 2 M NaOH and 9 ml of deionised doubly distilled water and determined the cobalt(II) content by general procedure. The results were presented in TABLE 1.

RESULTS AND DISCUSSION

Under the experimental condition, the absorption spectra of (3,5- DBSTSC) and [Cobalt(II)-3,5-DBSTSC] complexes were scanned. The absorption maximum of 3,5-DBSTSC vs reagent blank was measured at 60 nm, where as [Cobalt(II)-3,5-DBSTSC] complex gave an absorption peak at 431 nm. The contrast (bath chromic shift) of the two peaks was

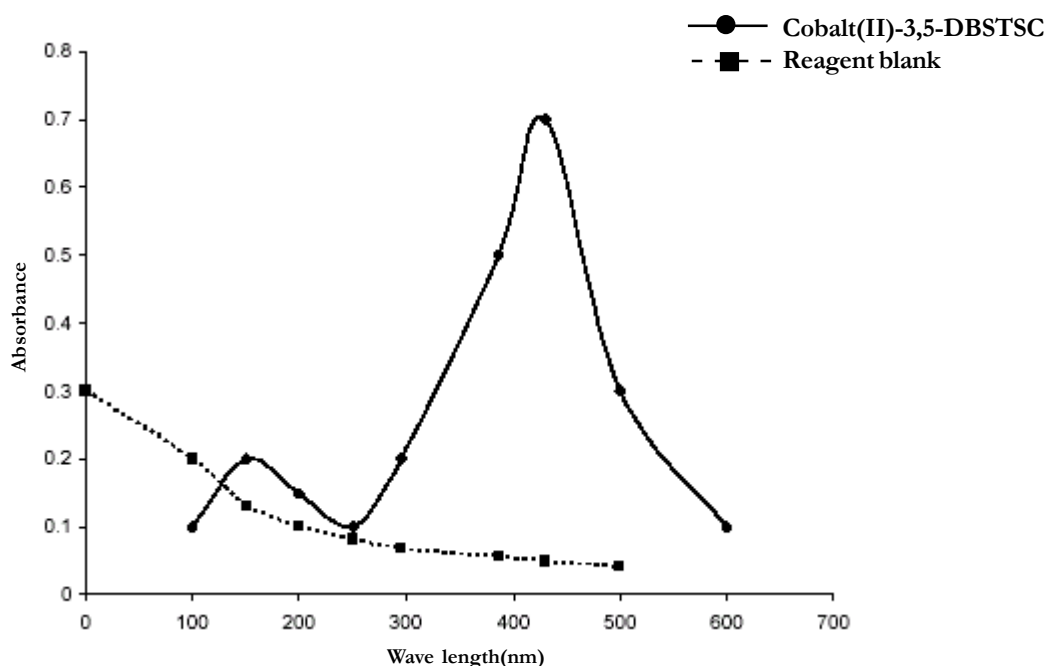


Figure 3: Absorption spectrum of cobalt(II) against 3,5-DBSTSC

371 nm, and could be obviously distinguished. Thus the absorption peak at 431 nm was chosen as the determination of $\lambda_{\max}$ for [Cobalt(II) – 3,5-DBSTSC] complex (Figure 3). The colour formation was instantaneous and stable for more than 10 days.

Analytical parameters

Beer's law was obeyed in the concentration range $0.9\text{--}19\text{ }\mu\text{g ml}^{-1}$ of cobalt(II). The molar absorptivity of complex at 431 nm and at pH 4.0 ± 0.2 was calculated as $0.3932 \times 10^4\text{ l mol}^{-1}\text{ cm}^{-1}$. Sandell's sensitivity of the method was found to be $0.001255\text{ }\mu\text{g cm}^{-2}$. The other characteristics are shown in TABLE 2.

Composition of cobalt(II)-3,5-DBSTSC complex

Spectrophotometric investigation of the metal complex was made to obtain the composition of the complex. The composition of the complex was established by molar ratio and job's continuous variation methods and it indicates that the composition of the M:L was 1:2.

Optimization of flow-injection system

Effect of pH

The effect of pH on the peak height of cobalt(II)

TABLE 2: Optical characteristics of Co(II)-3,5-DBSTSC and precision data.

Optical characteristics of present method	
Color	Green
$\lambda_{\max}$ [nm]	431
Stability	More than 10 days
Beer's law range [$\mu\text{g ml}^{-1}$]	0.6 – 12
Molar absorptivity [$\text{l mol}^{-1}\text{ cm}^{-1}$]	1.387×10^4
Sandell's sensitivity [$\mu\text{g cm}^{-2}$]	0.004125
Regression equation (Y) ^b	
Slope ^a	0.16318
Intercept ^b	0.0182
Correlation coefficient ^c	0.9991
Standard deviation [%] ^c	0.05
Range of error (95% confidence level)	± 1.50
% error	0.04150

^aExperiments performed under optimized conditions (see text) with 5 ml of 0.01 M $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ solution. ^b $Y = ax + b$, where x is the concentration of Cobalt(II) in $\mu\text{g ml}^{-1}$, ^c $n = 4$

at different concentrations was investigated with a fixed 0.05 M 3,5-DBSTSC concentration in the reagent carrier solution. The pH of acetate buffer in the reagent carrier solution was changed over a range of 2.0–6.0 and the peak height were measured for each concentration level of cobalt(II). At all con-

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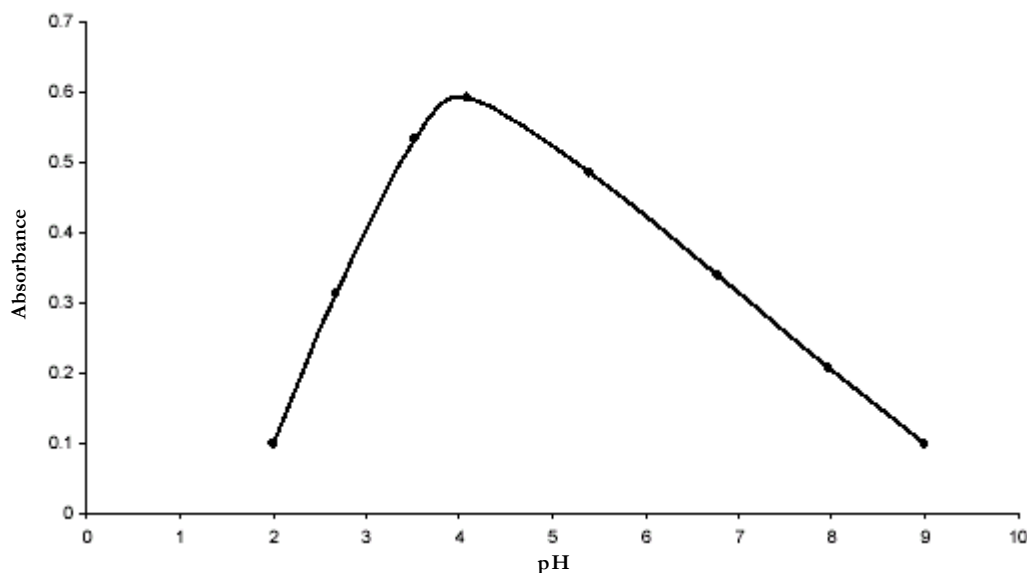


Figure 4: Effect of pH on cobalt(II)-3,5-DBSTSC complex

centration levels of cobalt(II), maximum peak heights were found between pH 3.0 and 5.0. Therefore, the pH 4.0 ± 0.2 for the acetate buffer system was chosen throughout in the study as shown in figure 4.

Effect of flow-rate

The effect of the flow-rate on the peak height was studied in the range of $0.5\text{--}3.0\text{ ml min}^{-1}$. The peak height decreased with the increasing of the flow-rate. Taking into consideration of the stability of the pump, peak shape and sampling time, the flow-rate of the reagent carrier solution was adjusted to 2.0 ml min^{-1} .

Sample volume

The influence of the sample volume on the absorbance was investigated by injecting different volumes ($150\text{--}250\text{ }\mu\text{L}$) of cobalt(II) standard solutions into the system at the 2.0 ml min^{-1} flow-rate. The injection volume has a significant effect, yielding increased peak height and reproducibility with increase in the injection volume. However, increasing the injection volume widens the sample zone and lowers the sampling rate, so that $200\text{ }\mu\text{L}$ was taken for compromise.

Effect of 3,5-DBSTSC concentration

The effect of concentration of 3,5-DBSTSC, in

the reagent carrier solution, on the peak height was investigated at $\text{pH } 4.0 \pm 0.2$ and a flow-rate of 2.0 ml min^{-1} , by using 4.2 and $6.8\text{ }\mu\text{L}^{-1}$ cobalt(II) solutions. The concentrations of 3,5-DBSTSC was varied over in the range of $0.01\text{--}0.1\text{ M}$. Maximum peak height was obtained at a concentration of 0.05 M 3,5-DBSTSC as color developing reagent in the carrier solution for lower concentration level of cobalt (II) in the sample solution.

Effect of length of reaction coil

The effect of length of reaction coil on the flow-injection spectrophotometric determination of cobalt(II) in various environmental samples was studied by varying the length of reaction coil in the range of $50\text{--}120\text{ mm}$ with internal diameter 3 mm . It can be observed that the absorbance of [Cobalt(II)-3,5-DBSTSC] increases with increase in length of reaction coil up to 90 mm . After increasing the length of reaction coil, the absorbance remains constant. Thus, 90 mm of reaction coil was chosen for maximum absorbance of [Cobalt(II)-3,5-DBSTSC] complex.

Ringbom's plot for cobalt(II)- 3,5-DBSTSC

Ringbom's plot is the established standard adopted to know the optimum range of concentration for a system that obeys Beer's law. The plot is drawn between $\log C$ of cobalt(II) and $(1-T)$ (where

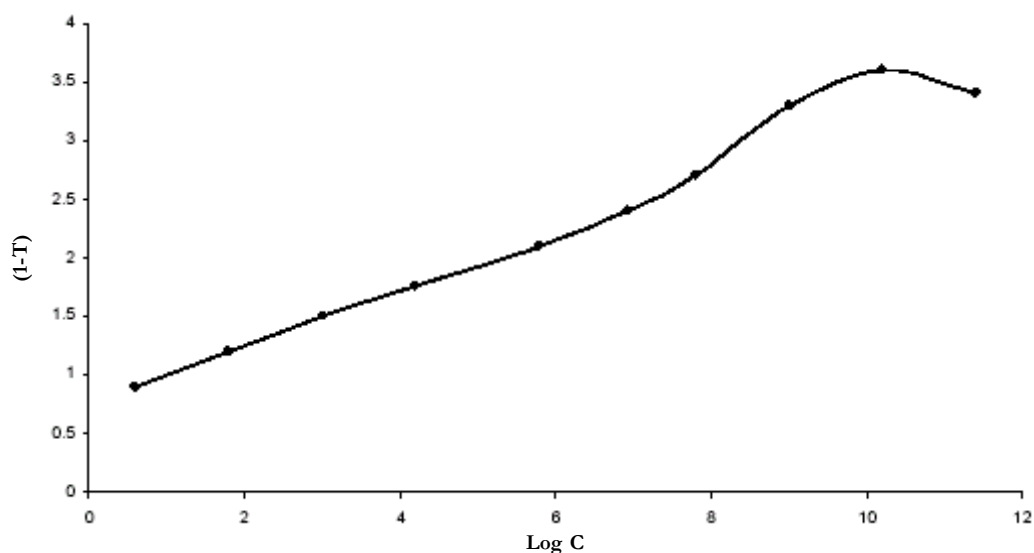


Figure 5: Ringbom's plot for cobalt(II)-3,5-DBSTSC complex

T is the transmittance). The plot has a sigmoid shape with a linear segment at intermediate absorbance values 1.2-3.3) and concentration values ($1.8-9.0 \mu\text{g ml}^{-1}$). The slope of Ringbom plot from figure 5 is 0.025. Hence, the ratio between the relative error in concentration and photometric error is 1.8, for a concentration of 1.2, for 0.6 percent photometric error.

Physico chemical properties of 3,5-DBSTSC

3,5-DBSTSC, being an colorless solid, easily soluble in 40% DMF and slightly soluble in methanol. The spectra of 3,5-DBSTSC in different pH are shown in figure 6. It can be that 3,5-DBSTSC in acidic medium ($\text{pH} < 2$) in phosphate buffer with an absorption peak at 400 nm, corresponding to less

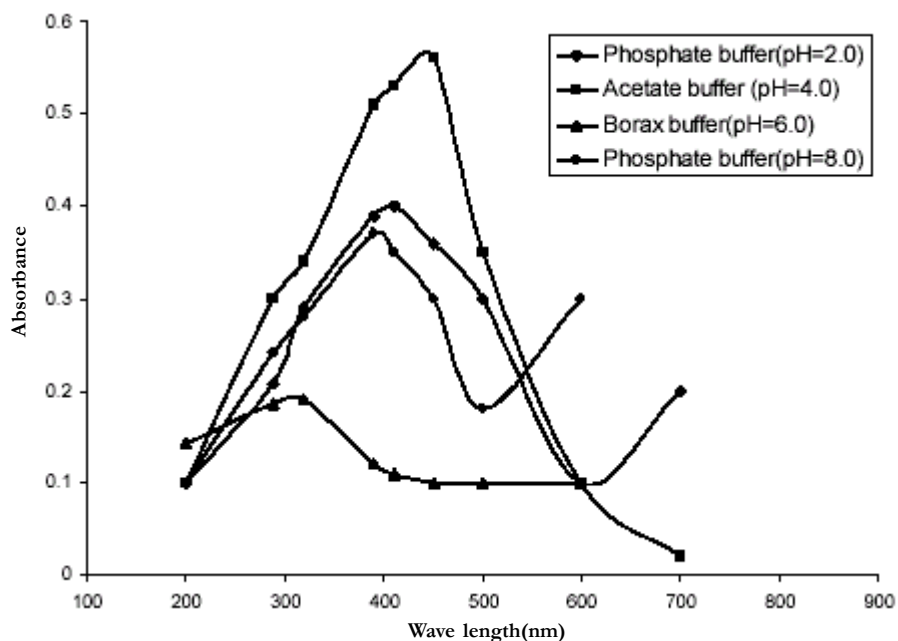


Figure 6: Spectra of 3,5-DBSTSC against water at different pH values

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protonized ligand possessing yellowish green. It changes to intense green in weak acidic up to pH 5 in acetate buffer with absorption peak at 431 nm, corresponding to strong protonized form(H_2L^+). In borax buffer pH 6.0, it appears light yellow at absorption peak of 330 nm corresponding to feasible protonization of the ligand. At phosphate buffer, dark yellow, instability and maximum absorption at 390 nm, corresponding to the protonation appears and disappears. According to the absorbance of the reagent at 431 nm. The dissociation constant of 3,5-DBSTSC was measured to be 1.03×10^{-12} by potentiometric titration.

Method evaluation

The proposed flow injection method was critically evaluated with regard to reproducibility, accuracy, detection limit and sample frequency.

1. Reproducibility

To test the reproducibility of the FIA, four repetitive analysis cycles of each sample were run. A S.D. (%) in the range 0.05-0.45 was obtained as shown in TABLE 1.

2. Accuracy

The accuracy of the proposed flow injection method was evaluated by comparing the results with reported method. The results shown in Table 1 reveals that the good correlation between the two methods indicative of present method and reported method^[18].

3. Sampling frequency

The experimental period for one complete analytical cycle was 180 s long giving an overall sampling rate of 20 samples h^{-1} .

Detection limit

Under optimized conditions the detection limit for determination of cobalt(II) by flow injection method (signal to noise ratio=2) obtained was $0.0155 \mu g ml^{-1}$ for a 200 μl injection volume Cu(II) ion solution.

Effect of foreign species

In order to analysis the recoveries and sensitivity of the present method, the effect of the some foreign ions were also investigated, by carrying out

TABLE 3: Effect of foreign species for the determination of cobalt(II) with FIA system

Ion	Tolerance limit $\mu g ml^{-1}$
As(III), Mg(II), Mn(II), Zr(IV), Ba(II), Ti(I), As(V)	4500 ^c
Hg(II), Th(IV), Al(III), V(V), Be(II), U(VI)	3000 ^b
Cu(II), Ni(II), Fe(II), Cd(II), Fe(III), Pd(II)	5000 ^a
F ⁻ , SCN ⁻ , thiosulfate	4000
Chlorides, tartrate, thiourea and bromide	2000

^aMasking with 20 $\mu g ml^{-1}$ of 0.5% EDTA, ^bMasking with 20 $\mu g ml^{-1}$ 1% flourides. ^cMasking with 200 $\mu g ml^{-1}$ 1 % citrate.

determination of 50 $\mu g l^{-1}$ of cobalt(II) with a known amount of the ions using the recommended procedure. The criterion for interference is a variation of more than ± 1.5 % in absorbance from the expected value for cobalt(II) alone. The results are shown in TABLE 3.

Analytical applications

To evaluate the applicability of the proposed flow injection method, it was applied to the determination of cobalt(II) in various environmental samples. The samples collected from different sources were filtered and then directly injected into the FIA. The obtained results were compared with reported method^[18]. The analytical data summarized in TABLE 1 suggest that the percentage of recovery of cobalt(II) from various water and vegetable samples ranges from 97.0 to 98.66 % which is more reliable and sensitive than the methods reported in literature.

CONCLUSION

The proposed method is simple, highly sensitive and rapid for the flow-injection spectrophotometric determination of cobalt(II) in various environmental samples. The limit of detection of the proposed method is superior to the other methods available in the literature^[18]. The method has additional advantages over owing to it's:

- Simplicity in synthesis of color forming reagent at ordinary laboratory conditions.
- Synthesized organic reagent is distinct in terms of sensitivity and selectivity.
- Proposed method is sensitive due to the stabili-

zation of complex [Cobalt(II)- 3,5-DBSTSC] for more than 10 days.

- (iv) Less interference, more accuracy, non- extractive procedure and statistical validation which made present method to be placed among the more sensitive ones.
- (v) Ligand is not only acts as anti microbial agent but also complexing agent useful in the quantitative analysis of metal ions at low concentrations in various environmental matrices.
- (vii) Complex formation is in one step, made additional advantage for monitoring the cobalt(II) in various environmental matrices.

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