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A Rapid Spectrophotometric Method for the Determination of Cobalt in Industrial, Environmental, Biological, Pharmaceutical and Soil Samples Using Bis(5-bromosalicylaldehyde)orthophenylenediamine

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A very simple, ultra-sensitive and fairly selective spectrophotometric method is presented for the determination of cobalt at trace levels using bis(5-bromosalicylaldehyde)orthophenylenediamine (BBSOPD). The method is based on the reaction of non-absorbent BBSOPD in a slightly acidic (0.001-0.0025 M H_2SO_4 or pH 3.4-4.0) and 50% N,N-dimethylformamide media with cobalt(II) to produce a highly absorbent orange colored chelate-product with an absorption maximum at 473 nm. The reaction is instantaneous and the absorption remains stable for 24 h. The average molar absorption coefficient and Sandell's sensitivity were found to be 5.84×10^4 l mol^{-1} cm^{-1} and 9.0 ng cm^{-2} of cobalt(II), respectively. Linear calibration graphs were obtained for 0.02-4.0 mg l^{-1} of Co(II). The stoichiometric composition of the chelate is 1:1 (Co(II):BBSOPD). A large excess of over 50 cations, anions and complexing agents do not interfere in the determination. The method was successfully used for the determination of cobalt in several standard reference materials (steels and alloys), environmental waters (potable and polluted), biological samples (human blood and urine), pharmaceutical and soil samples and solutions containing both cobalt(II) and cobalt(III) as well as some complex synthetic mixtures. The method has high precision and accuracy.

Keywords: Spectrophotometry, Bis(5-bromosalicylaldehyde)orthophenylenediamine, Cobalt, Environmental, biological and soil samples, Pharmaceutical sample

INTRODUCTION

Cobalt traces are technically important, which are used mainly as binder in the hard metal industry and as constituents of many alloys [1]. Cobalt toxicity causes different diseases including asthma, contact dermatitis, lung cancer and bronchitis [2-5]. Cobalt(II) ions are also genotoxic and carcinogenic [3,5]. Genotoxicity follows tow mechanisms: (I) DNA breakage by cobalt metals especially hard metal particles and (II) inhibition of DNA repair by cobalt(II) ions [5]. Occupational exposure to hard metal dust, consisting of tungsten carbide (WC) and metallic cobalt particles (Co) is associated with increased lung cancer [6]. Although traces of cobalt is necessary for the synthesis of vitamin B-12, excessive administration of this trace element produces goiter and reduce thyroid activity [7]. A dietary intake of about 50 µg cobalt, of which 40 µg in the form of vitamin B-12, maintains cobalt equilibrium in the human [8]. Some death in man resulted from consumption of large amounts of beer containing 1.2-1.5 mg l^{-1} of cobalt that was added to the beer to promote optimal foam stabilization [9]. The normal level in human urine and blood are about 1.0 and 0.18 µg l^{-1}, respectively [9]. Therefore, the accurate determination of cobalt at trace and ultra-trace levels using simple and rapid

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methods is of paramount importance.

Spectrophotometry is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis. The reagent bis(5-bromosalicylaldehyde)orthophenylene-diamine (BBSOPD) has never been used as a spectrophotometric reagent for the determination of cobalt. This paper reports in its use in a very sensitive and highly specific new spectrophotometric method for the trace determination of cobalt. The method is based on the reaction of non-absorbent BBSOPD in a slightly acidic solution, 0.001-0.0025 M H_2SO_4 or pH 3.4-4.0 with cobalt(II) to produce a highly absorbent orange chelate product followed by the direct measurement of absorbance in aqueous solutions. With suitable masking, the reaction can be made highly selective and the reagent blank solutions do not show any appreciable absorbance. The method possesses distinct advantages over existing methods [11-25].

EXPERIMENTAL

Apparatus

A Shimadzu (Kyoto, Japan) (Model-160) double beam UV-Vis recording spectrophotometer and a Jenway (England, UK) (Model-3010) pH-meter with a combination of electrode were used for the measurements of absorbance and pH, respectively. A Hitachi Ltd., Model 180-50, S.N. 5721-2 atomic absorption spectrophotometer with a deuterium lamp background corrector, equipped with graphite furnace GA-3, with cobalt hollow cathode lamps of Hitachi, and a Hitachi Model 056 recorder was used for comparing the results. An FTIR Spectrophotometer (Model-Nicolet 5700; Detector-DTGS KBr; Smart Accessory ID-012-593; Beamsplitter-XT-KBr) was used for the qualitative information on the complex formation.

Reagents and Solutions

All the chemicals used were of analytical reagent grade or the highest purity available. Doubly distilled deionized water, which is non-absorbent under visible radiation, was used throughout. Glass vessels were cleaned by socking in acidic solutions of $KMnO_4$ or $K_2Cr_2O_7$, followed by washing with concentrated HNO_3 and rinsed several times with deionized water. Stock solutions and environmental water sample (1000 ml each) were kept in a polypropylene bottles containing 1 ml of concentrated HNO_3. More rigorously contamination control was applied when the cobalt level in the specimen is low.

Synthesis and Characterization of BBSOPD

The reagent BBSOPD was synthesized according to the recommended method [26] by refluxing a mixture of 5-bromosalicylaldehyde (100 mmol) and *ortho*-phenylene-diamine (50 mmol) at 60 ºC for 1 h. The yellow precipitate formed was filtered off on cooling, washed with ethanol and recrystallized from ethanol and dried under vacuum over silica gel. Yield 80% and m.p.: 220-222 ºC. The prepared BBSOPD was characterized by IR spectra (νC=N at 1590-1660 cm^{-1}) and elemental analyses.

Stock Solutions

A 5.27×10^{-3} M solution of BBSOPD was prepared by dissolving the requisite amount of the reagent in a known volume of N,N-dimethylformamide (DMF). More dilute solutions of the reagent were prepared as required.

A 100 ml amount of stock solution (1 mg ml^{-1}, 1.70×10^{-2} M) of cobalt was prepared by dissolving 403.6 mg of cobalt chloride ($CoCl_2.6H_2O$) in doubly distilled deionized water. Aliquots of this solution were standardized by titrimetric analysis with EDTA [27]. More dilute standard solutions were prepared by appropriate dilution of the stock solution.

Solutions of a large number of inorganic ions and complexing agents were prepared from their Analar grade or equivalent grade water soluble salts (or the oxides and carbonates in hydrochloric acid). The stock solutions of niobium, tantalum, titanium, zirconium and hafnium were specially prepared from their corresponding oxides (Specpure, Matthey) according to the recommended procedure [28]. In the case of insoluble substances, special dissolution methods were adopted [29,30].

Sample Collection and Preservation

Water samples were collected in polythene bottles from shallow tube-wells, river, sea and drain of different places of Bangladesh. After collection, 1 ml l^{-1} nitric acid was added as preservative.

Soil (surface) samples were collected from different locations of Bangladesh. Samples were dried in air and

homogenized with mortar.

Procedure

A volume of 0.1-1.0 ml of a neutral aqueous solution containing 0.2-40.0 μg of cobalt(II) in a 10-ml calibrated flask was mixed with a 1:10-1:30 fold molar excess of BBSOPD solution (preferably 1 ml of 5.27×10^{-3} M) followed by the addition of 0.5-2.5 ml (preferably 1.0 ml) of 0.01 M sulfuric acid and 3.0-6.0 ml (preferably 5.0 ml) of DMF. The mixture was diluted to the mark with deionized water. The absorbance was measured at 473 nm against a corresponding reagent blank. The cobalt content in an unknown sample was determined using a concurrently prepared calibration graph.

Determination of Cobalt in Alloy and Steel (Certified Reference Materials)

A 0.1 g amount of alloy or steel sample containing (0.02-1.45)% of cobalt was accurately weighed and placed in a 50-ml erlenmeyer flask following a recommended method [31]. To it 10 ml of concentrated HNO_3 and 2 ml concentrated H_2SO_4 were carefully added and then covered with a watch-glass until the brisk reaction subsided. The solution was heated and simmered gently after the addition of another 5 ml of concentrated HNO_3 until all carbides were decomposed. The solution was carefully evaporated to dense white fumes to drive off the oxides of nitrogen and then cooled down to the room temperature (25 ± 5 ºC). After suitable dilution with deionized water, the content of erlenmeyer flask was warmed to dissolve the soluble salts. The solution was cooled and neutralized with dilute NH_4OH solution in the presence of a 0.1% (w/v) EDTA solution. The resulting solution was filtered, if necessary, through Whatman No. 40 filter paper into a 25-ml calibrated flask. The residue was washed with a small volume (5 ml) of hot (1:99) H_2SO_4 followed by water; the volume was made up to the mark with deionized water. A suitable aliquot (1-2 ml) of the above mentioned solution was taken into a 10-ml calibrated flask and the cobalt content was determined as described under procedure using EDTA as masking agent.

Determination of Cobalt in Environmental Water Samples

Each filtered samples (1000 ml) was evaporated nearly to dryness with a mixture of 5 ml of concentrated H_2SO_4 and 10 ml of concentrated HNO_3 in a fume cupboard, following a method recommended by Greenberg and then cooled down to room greenberg [32]. The residue was then heated with 10 ml of deionized water in order to dissolve the salts. The solution was then cooled and neutralized with dilute NH_4OH in the presence of a 1-2 ml of 0.1% (w/v) EDTA solution. The resulting solution was then filtered and quantitatively transferred into a 25-ml calibrated flask and made up to the mark with deionized water. An aliquot (1-2 ml) of this preconcentrated water sample was pipetted into a 10-ml calibrated flask and then cobalt content was determined as described under the procedure using EDTA as a masking agent.

Determination of Cobalt in Biological Samples

Human blood (2-4 ml) or urine (5-7 ml) sample was taken into a 100-ml micro-Kjeldahl flask. A glass bead and 10 ml of concentrated nitric acid were added, and the flask was placed on a digester under gentle heating. When the initial brisk reaction was completed, the solution was removed and cooled, and digested following a recommended method [33]. A 1 ml of volume of concentrated sulfuric acid was carefully added followed by the addition of 1 ml of 70% perchloric acid, and heating was continued to dense white fumes, while repeating nitric acid addition if necessary. Heating was continued for at least half an hour and then cooling was applied. The content of the flask was filtered and neutralized with NH_4OH in the presence of 1-2 ml of a 0.01% (w/v) EDTA solution. The resultant solution was then filtered and transferred quantitatively into a 10-ml calibrated flask and made up to the mark with deionized water. A suitable aliquot (1-2 ml) of the final solution was pipetted out into a 10-ml calibrated flask and the cobalt content was determined as described under procedure using EDTA as masking agent.

Determination of Cobalt in Soil Samples

An air-dried homogenized soil sample (100 g) was accurately weighed and placed in a 500-ml beaker. The sample was digested in the presence of an oxidizing agent following a recommended method by Jackson [34]. The content of the beaker was filtered through Whatman No. 40 filter paper into a 25-ml calibrated flasx and neutralized with dilute ammonia

solution in the presence of 1-2 ml of a 0.01% (w/v) EDTA solution. It was then diluted up to the mark with deionized water. A suitable aliquot (1-2 ml) of the final solution was pipetted out into a 10-ml calibrated flask and a calculated amount of 0.01 M H_2SO_4 to give the final acidity 0.001-0.0025 M or pH 3.4-4.0 was added followed by 1-2 ml of a 0.01% fluoride or thiocyanate solution as a masking agent. The cobalt content was determined by the recommended procedure and quantified from a calibration graph prepared concurrently.

Determination of Cobalt in Some Pharmaceutical Samples

Finished pharmaceutical samples (cobalt containing tablets) were quantitatively taken in a beaker. Added 10 ml of conc. nitric acid and heated to dryness and then added 10 ml of 20% (v/v) of sulfuric acid and 1-2 drops of perchloric acid. The volume was reduced to 2-5 ml and then cooled to room temperature. The solution was then neutralized with dilute NH_4OH in the presence of a 1-2 ml of 0.1% (w/v) EDTA solution. The resulting solution was then filtered and quantitatively transferred into a 25-ml calibrated flask and made up to the mark with deionized water. An aliquot (1-2 ml) of this preconcentrated sample was pipetted into a 10-ml calibrated flask and then cobalt content was determined as described under the procedure using EDTA as a masking agent.

Determination of Cobalt(II) and Cobalt(III) Speciation in Mixture

A few drops of 1 M sulfuric acid and 1-2 ml of 1% (w/v) potassium permanganate solution were added to oxidize a suitable concentration of divalent cobalt solution. Now suitable aliquots (1-2 ml) of cobalt(II+III) mixtures (preferably, 1:1, 1:2, 1:3) were taken in a 25-ml conical flask. Then, 1 ml of freshly prepared sodium azide solution (2.5%, w/v) were added to the mixtures and heated on a steam bath for 20-30 min with occasional gentle shaking in addition with of 2-3 ml of water, if necessary. Excess azide was driven off on heating and then cooled to room temperature. The reaction mixture was made slightly acidic in a 10 ml volumetric flask. Then total cobalt(II+III) content was determined according to the general procedure with the help of calibration graph. An equal aliquot of the above cobalt(II+III) mixtures (preferably, 1:1, 1:2, 1:3) were taken in a 25-ml beaker and 1 ml of 0.1% (w/v) EDTA solution was added to mask cobalt(III) and then neutralized with dilute ammonia. After that, the content of the beaker was transferred into a 10-ml volumetric flask and its cobalt(II) was determined according to the general procedure. This value is subtracted from that of the total cobalt(II+III) to calculate the cobalt(III) in the mixtures.

RESULTS AND DISCUSSION

Absorption Spectra

The absorption spectra of the cobalt(II)-BBSOPD system and reagent blank in 0.01 M sulfuric acid are shown in Fig. 1. It is obvious, the spectrum of cobalt(II)-BBSOPD is a symmetric curve with maximum absorbance at 473 nm and an average molar absorption coefficient of 5.8×10^4 l mol^{-1} cm^{-1}. The reagent blank did not show any absorbance in the range of determination. In all instances measurements were made at 473 nm against a reagent blank.

Effect of Solvent

Because BBSOPD is insoluble in water, an organic solvent should be selected for the system. The criteria for choice of the solvent are persistence of strong colored complex in true solution, absorbance maximization with minimum use of solvent, obtaining sharp and well defined absorption spectrum of complex in visible region, consideration of cost, availability, toxicity and volatility of solvent *etc*. Among

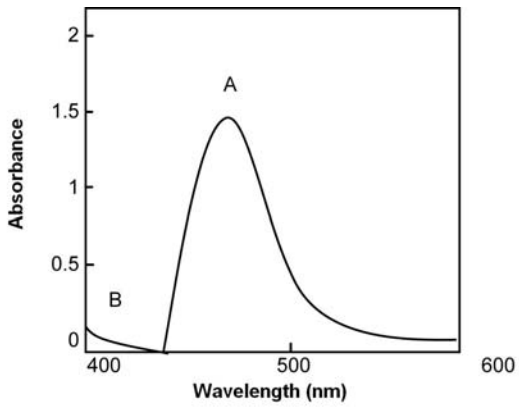

Fig. 1. A and B absorption spectra of cobalt-BBSOPD and the reagent blank (λ_{max} = 473 nm) in aqueous solutions.

different solvents including benzene, chloroform, acetone, carbon tetrachloride, nitrobenzene, isobutyl alcohol, 1-butanol, isobutyl methyl ketone, ethanol, 1,4-dioxan and DMF, DMF was found to be the best solvent for the system. Maximum absorbance was observed in (50 ± 2)% (v/v) DMF/water medium; hence, a 50% DMF solution was used in the determination procedure. It was observed that at 1 mg l^{-1} of Co-BBSOPD, 30-80% of DMF solution produced a constant absorbance of the Co-chelate.

Effect of Acidity/pH

Of the various acids (nitric acid, sulfuric acid, hydrochloric acid and phosphoric acid) studied, sulfuric acid was found to be the best acid for the system. The variation of absorbance was noted after the addition of 0.5-3.0 ml of 0.01 M sulfuric acid to every 10 ml of test solution (Fig. 2). As it is seen from Fig. 2, a maximum and constant absorbance was obtained in the presence of 1.0-2.5 ml of 0.01 M sulfuric acid (or pH 3.4-40) at room temperature. This is corresponding to 0.001-0.0025 M acidity range or pH 3.4-4.0 in the final dilution. Thus, for all subsequent measurements, 1 ml of 0.01 M sulfuric acid (or pH 3.88) was added.

Effect of Time

The reaction is very fast. A constant maximum absorbance was obtained just after dilution to volume and remained strictly unaltered for 24 h.

Effect of Reagent Concentration

Different molar excess of BBSOPD were added to a fixed metal ion concentration and the absorbance were measured according to the standard procedure and the results are shown in Fig. 3. It was observed that at 1 mg l^{-1} of cobalt metal, the reagent molar ratios of 1:10-1:30 produce a constant absorbance of the Co-BBSOPD. For two different cobalt-concentrations of 0.5 and 1.0 mg l^{-1} an identical effect of varying the reagent concentration was noticed. A greater excess of reagent were not studied. Thus, for all subsequent measurements, 1 ml of 5.27×10^{-3} M BBSOPD reagent was added.

Composition of the Absorbent Complex

Job's method [35] of continuous variation and the molar-ratio method [36] were applied to ascertain the stoichiometric composition of the complex. A Co:BBSOPD (1:1) complex was indicated by both methods.

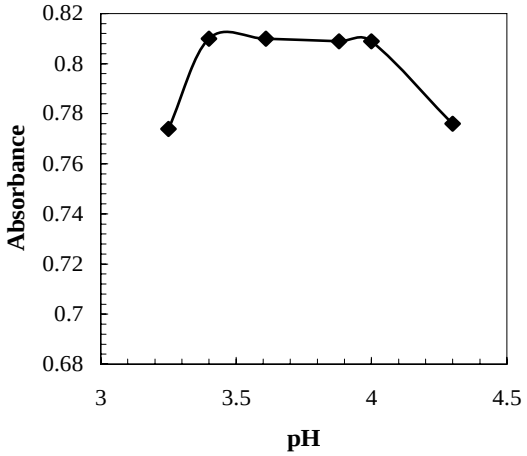

Fig. 2. Effect of pH on the absorbance of the Co(II)-BBSOPD system.

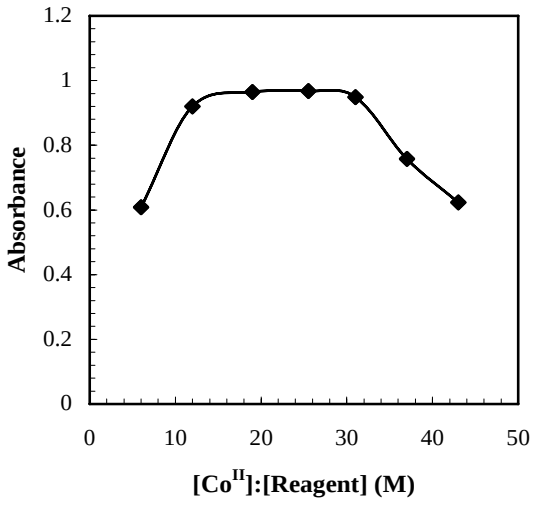

Fig. 3. Effect of the reagent (BBSOPD:Co(II) M concentration ratio) on the absorbance of the Co(II)-BBSOPD system.

Calibration Graph

The well-known equation for spectrophotometric analysis in a very dilute solution was derived from Beer's law. The

effect of metal concentration was studied over 0.01-8 mg l^{-1} distributed in two different sets (0.01-0.1 and 0.1-8 mg l^{-1}) for convenience of the measurements. The resulting calibration graph obtained in the range of 0.1-8 mg l^{-1} is shown in Fig. 4. The other graph was straight line passing through the origin. The absorbance was linear for 0.02-4.0 mg l^{-1} of cobalt at 473 nm. The molar absorption coefficient and the Sandell's sensitivity were found to be 5.8×10^4 l mol^{-1} cm^{-1} and 9.0 ng cm^{-2} of cobalt(II), respectively [37].

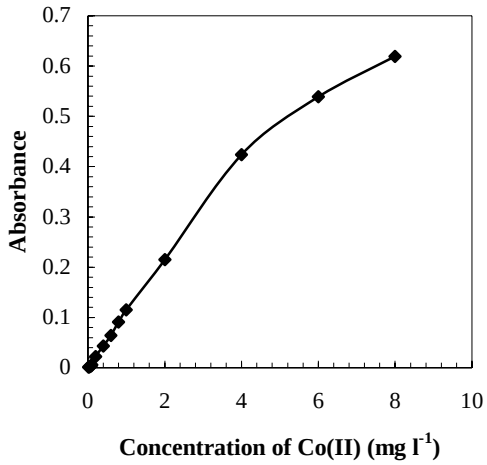

Fig. 4. Calibration graph-B, 0.2-4.0 mg l^{-1} of cobalt(II).

Effect of Foreign Ions

The effect of over 50 cations, anions and complexing agents on the determination of 1 mg l^{-1} of cobalt(II) was studied (Table 1). The criterion for interference was

Table 1. Table of Tolerance Limit of Foreign ions[a]

Species x	Tolerance ratio [Species (x)/Co(II) (w/w)]	Species x	Tolerance ratio [Species (x/Co(II) (w/w)]
Zinc(II)	100	Magnesium(II)	100
Arsenic(III)	100	Beryllium(II)	100
Arsenic(V)	100	Manganese(II)	100
Azide	100	Mercury(II)	100[c]
Barium(II)	100	Iron(II)	100[b]
Potassium(I)	100	Iron(III)	50[b]
$Chloride^-$	200	Silver(I)	100
$Citrate^{2-}$	100	Copper(II)	50[d]
$Tartrate^{2-}$	1000	$Phosphate^{3-}$	100
$EDTA^{4-}$	500	$Thiocyanate^-$	100
$Bromide^-$	500	Sodium(I)	100
$Fluoride^-$	500	Strontium(II)	100
$Oxalate^{2-}$	200	Molybdenum(V)	30[b]
$Iodide^-$	100	Cerium(III)	20[b]
Aluminum(III)	200	Vanadium(V)	50[b]
Calcium (II)	200	Tin(IV)	40[c]
Cadmium(II)	200	Ammonium(I)	100
$Acetate^-$	1000	Selenium(IV)	100
$Nitrate^-$	1000	Selenium(VI)	100
Chromium(III)	500	Nickel(II)	50
$Cyanide^-$	100	Tungsten(VI)	100
Chromium(VI)	50[b]	Lead(II)	50[c]

[a]Tolerance limit defined as ratio that causes less than 5% interference. [b]With 10 μg ml^{-1} EDTA. [c]With 10 μg ml^{-1} tartrate. [d]With 10 μg ml^{-1} hydrazine hydrate/Thiocyanate.

absorbance value varying ±5% from the expected value for cobalt alone [38]. The results are summarized in Table 1. As can be seen, a large number of ions have no significant effect on the determination of cobalt. The most serious interference were from Cu(II) and Fe(III) ions. Interference from these ions is probably due to complex formation with BBSOPD. The greater tolerance limits for these ions can be achieved by using different masking methods. In order to eliminate the interference of Cu(II), 10 mg l^{-1} aqueous hydrazine or thiocyanate was used. For Cr(VI), Fe(II), Fe(III), Mo(V), Ce(III) and V(V) 10 mg l^{-1} EDTA and for Sn(IV), Hg(II) 10 mg l^{-1} tartrate were added and the precipitate formed in any case was filtered off (Table 1) [39]. Thus, the selectivity of the proposed method is greatly improved and practically is increased and the cobalt amounts in complex samples may be determined by using the proposed method. Moreover, the tolerance limits of NO_3^-, ClO_4^-, SO_4^{2-} are especially high which is advantageous with respect to the digestion of the samples.

Analytical Parameters and Applications

The precision of the present method was evaluated by determining different concentrations of cobalt (each analyzed at least five times). The relative standard deviation (n = 5) was 2-0% for 0.2-40.0 µg of cobalt in 10 ml solutions, indicating that this method is highly precise and reproducible. The detection limit (3 s of the blank) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for cobalt(II) were found to be 1.0 ng ml^{-1} and 9.0 ng cm^{-2}, respectively.

The analytical results must be evaluated with regard to the validity of the analytical method. Poor analytical quality may lead to false conclusions [40]. Keeping this in our mind the validity of our method was tested by recovery studies (Tables 2 and 3), analyzing several standard reference materials (Table 4) and also comparing the results with conventional analysis (AAS) (Table 5).

The reliability of our cobalt-chelate procedure was approved by quantitative recovery of cobalt(II) spiked to several synthetic mixtures containing cobalt(II) and divers ions (Table 2) and environmental water samples (Table 3). As it is obvious from Table 4, there are satisfactory agreements between the certified values and those obtained by the proposed method in several certified samples. The results of biological analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS (Table 5). The results of soil samples analyses by the spectrophotometric method are shown in (Table 6). The results of some

Table 2. Determination of Cobalt in Some Synthetic Mixtures

Sample	Composition of mixtures (mg l^{-1})	Cobalt(II) (mg l^{-1})		
		Added	Found[a]	Recovery ± s (%)
A	Co^{2+}	0.50	0.50	100 ± 0.0
		1.00	0.99	99 ± 0.5
B	As in A + Mn^{2+} (25) + Zn^{2+} (25)	0.50	0.49	98 ± 1.0
		1.00	1.02	102 ± 1.3
C	As in B + Cr^{2+} (25) + Na^{+} (25)	0.50	0.48	96 ± 1.2
		1.00	0.98	98 ± 1.6
D	As in C + Ni^{2+} (25) + Ca^{2+} (25)	0.50	0.52	104 ± 1.5
		1.00	1.03	103 ± 1.3
E	As in D + Al^{3+} (25) + Mg^{2+} (25)	0.50	0.54	108 ± 1.6
		1.00	1.09	109 ± 1.8

[a]Average of five replicate determinations.

Table 3. Determination of Cobalt in some Environmental Water Samples

Sample		Cobalt ($\mu g\ l^{-1}$)		Recovery ± s (%)	s_r (%)[b]
		Added	Found[a]		
Tap water		0	5.0	-	-
		100	104.0	99 ± 0.3	0.45
		500	505.0	100 ± 0.0	0.00
Well water		0	12.5	-	-
		100	113.0	100.4 ± 0.5	0.32
		500	515.0	100.5± 0.2	0.35
River water	Karnaphuly (upper)	0	8.5	-	-
		100	110.0	101.4 ± 0.3	0.19
		500	512.0	100.8 ± 0.2	0.25
	Karnaphuly (lower)	0	10.0	-	-
		100	109.5	99.5 ± 0.5	0.30
		500	510.0	100.0 ± 0.0	0.00
	Padma (upper)	0	7.8	-	-
		100	107.8	99 ± 0.5	0.45
		500	510.0	100.4 ± 0.3	0.29
	Padma (lower)	0	9.5	-	-
		100	110.0	100.5 ± 1.0	0.35
		500	512.0	100.5 ± 0.8	0.21
Sea water	Bay of Bengal (upper)	0	3.5	-	-
		100	102.5	99 ± 0.6	0.21
		500	503.5	100 ± 0.0	0.00
	Bay of Bengal (lower)	0	6.5	-	-
		100	105.0	99 ± 0.5	0.35
		500	508.0	100.3 ± 0.3	0.23
Drain water	Chemico Lab. Ltd.[c]	0	25.0	-	-
		100	125.0	100.0 ± 0.0	0.00
		500	526.0	100.2 ± 0.4	0.18
	Elite Paint Drain[d]	0	45.0	-	-
		100	148.0	102 ± 1.0	0.28
		500	545.0	100 ± 0.0	0.00
	Eastern Refinery[e]	0	165.0	-	-
		100	167.5	101 ± 0.8	0.49
		500	670.0	100.7 ± 0.3	0.35
	KPM[f]	0	88.5	-	-
		100	190.0	100.8 ± 0.9	0.15
		500	592.0	100.6 ± 0.5	0.26

[a]Average of five replicate determinations. [b]The measure of precision is the relative standard deviation (s_r). [c]Chemico Laboratories Ltd., Rajshahi, Bangladesh. [d]Elite Paint, Chittagong, Bangladesh. [e]Eastern Refinery, Chittagong, Bangladesh. [f]Karnaphuly Paper Mill, Chandraghona, Chittagong,

Table 4. Determination of Cobalt in Certified Reference Materials

Certified reference materials (Composition, %)	Cobalt		
	Added/Certified Value[a]	Found (n = 5)	RSD (%)
BCS-261 Straight Nb 18/12 Stainless steel (C = 0.083, Si = 0.39, Cr = 17.20, Ni = 13.08, Mn = 0.66, Nb + Ta = 0.71)	1.0[b]	1.015[b]	1.3
BAS-5g, Brass (Cu = 67.4, Sn = 1.09, Pb = 2.23, Zn = 28.6, Ni = 0.33, P = 0.01)	1.0[b]	1.02[b]	2.0
BAS-10g, High tensile brass (Cu = 60.8, Fe = 1.56, Pb = 0.23, Ni = 0.16, Sn = 0.21, Al = 3.34, Zn = 32.0, Mn = 0.12)	1.0[b]	1.03[b]	3.0
GSBH 40101-96, Cr_{12} MoV-Dies Steel (Cr = 11.63,, Ni = 0.095, Cu = 0.082, Mo = 0.986, V = 0.411, Co = 0.02)	0.02[c]	0.021[c]	2.5
YSBC1013-1-95, $9Cr_{17}$ MoVCo High tensile steel (C = 90, Si = 0.44, Mn = 0.81, Cr = 16.3, Mo = 0.52, V = 0.24, Co = 1.45)	1.45[c]	1.43[c]	1.3

[a]These CRMs were obtained from Beijing NCS Analytical Instruments Co. Ltd., China. [b]Values in mg l^{-1}. [c]Values in percentage (%).

Table 5. Concentration of Cobalt in Blood and Urine Samples

Sl. No.	Samples	Cobalt (µg l^{-1})				
		AAS (n = 5)		Proposed method (n = 5)		Sample source[a]
		Found	RSD (%)	Found	RSD (%)	
1	Blood	17.8	1.2	18.5	1.3	Normal adult
	Urine	6.75	0.8	7.2	1.0	(male)
2	Blood	45.6	1.5	44.8	1.8	Anaemia patient (male)
	Urine	16.8	1.3	16.5	1.5	
3	Blood	550.7	1.6	558.5	1.0	Paralysis patient
	Urine	137.6	1.8	140.7	1.6	(male)
4	Blood	152.5	1.7	158.6	1.4	Pulmonary patient
	Urine	45.8	1.4	48.2	1.2	(male)

[a]Samples were collected from LUMHS Hospital, Hyderabad, Pakistan.

pharmaceuticals tablets are also in good agreement with the desired specifications (Table 7). Hence the precision and accuracy of the method were found to be excellent.

The proposed method was also applied to cobalt speciation and determination of Co(II) and Co(III) in their different binary mixtures. The results of a set of determination are given

Table 6. Determination of Cobalt in Some Surface Soil[a,b]

Sl. No.	Cobalt (μg g^{-1})	Sample source
S_1[c]	18.5 ±1.2	Agricultural soil (Tikapara, Rajshahi, Bangladesh)
S_2	16.8 ± 1.0	Marine soil (Bay of Bengal, Chittagong, Bangladesh)
S_3	25.3 ± 1.5	River soil (River Padma, Rajshahi, Bangladesh)
S_4	45.8 ± 1.3	Eustrain soil (Junction of Bay of Bengal and River Karnaphuly, Chittagong, Bangladesh)
S_5	125.6 ± 1.5	Industrial soil (Karnaphuly Paper Mill, Chandraghona, Chittagong, Bangladesh)

[a]Average of five analyses of each sample. [b]The measure of precision is the standard deviation. [c]Composition of the soil samples: C, N, PO_3^{3-}, K^+, Na^+, Ca^{2+}, Mg^{2+}, Cu^{2+}, Fe^{2+}, Pb^{2+}, NO_3^-, NO_2^-, Zn^{2+}, SO_4^{2-}, Mn^{2+}, Mo^{VI}, Co^{2+}, *etc.*

Table 7. Determination of Cobalt in Some Pharmaceutical Samples

Sl. No.	Composition of tablet	Trade name	Cobalt (μg g^{-1})		RSD (%)
			Reported	Found	
1	Cyanocobalamin (0.2 mg)	Neobeon (Aristopharma Ltd.)	13.78	14.39	4.0
2	Cyanocobalamin (6.0 mg)	Kvit-M (Chemico Lab. Ltd.)	702.05	711.76	1.4
3	Mecobalamin (0.5 mg)	Mecolagin (Incepta Pharma Ltd.)	307.26	295.68	3.5

in Table 8. As it can be seen from Table 8, the results are very accurate and highly reproducible.

CONCLUSIONS

In this paper, a new simple, sensitive, selective and inexpensive method with the Co(II)-BBSOPD complex developed for the determination of cobalt in industrial, environmental, biological and soil samples, for continuous monitoring to establish the trace levels of cobalt in difficult samples matrices. It offers also a very efficient procedure for speciation analysis. Although many sophisticated techniques such as pulse polarography, HPLC, AAS, ICP-AES and ICP-MS, are available for the determination of cobalt at trace levels in numerous complex materials, factors such as low cost of the instrument, easy handling, lack of requirement for consumables and almost no maintenance have caused spectrophotometry to remain a popular technique, particularly in laboratories in developing countries with limited budgets. The method possesses distinct advantages over existing methods [11-25] with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH/acidity range, thermal

Table 8. Determination of Cobalt(II) and Cobalt(III) in Mixtures

Sl. No.	Co(II):Co(III)	Co, taken ($mg\ l^{-1}$)		Co, found ($mg\ l^{-1}$)		Error ($mg\ l^{-1}$)	
		Co(II)	Co(III)	Co(II)	Co(III)	Co(II)	Co(III)
1	1:1	1.00	1.00	0.98	0.99	0.02	0.01
2	1:1	1.00	1.00	1.00	1.02	0.00	0.02
3	1:1	1.00	1.00	0.97	0.99	0.03	0.01
Mean error: Co(II) = ±0.016; Co(III) = ±0.013; Standard deviation: Co(II) = ±0.015; Co(III) = ±0.011							
1	1:2	1.00	2.00	0.99	1.98	0.02	0.02
2	1:2	1.00	2.00	0.98	1.99	0.01	0.01
3	1:2	1.00	2.00	0.98	1.98	0.02	0.02
Mean error: Co(II) = ±0.016; Co(III) = ±0.016; Standard deviation: Co(II) = ±0.006; Co(III) = ±0.0058							
1	1:3	1.00	3.00	0.99	2.99	0.01	0.01
2	1:3	1.00	3.00	0.98	2.98	0.02	0.02
3	1:3	1.00	3.00	0.98	2.98	0.02	0.02
Mean error: Co(II) = ±0.016; Co(III) = ±0.016; Standard deviation: Co(II) = ±0.015; Co(III) = ±0.015							

stability, accuracy, precision and ease of operation. The sensitivity in terms of molar absorptivity and precision in terms of relative standard deviation of the present method are very reliable for the determination of cobalt in real samples down to ng g^{-1} levels in aqueous medium at room temperature.

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