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Development of a new highly sensitive and selective spectrophotometric method for the determination of cobalt at nanotrace levels in various complex matrices using *N,N'*-bis(salicylidene)-ethylenediamine

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RESEARCH ARTICLE

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ABSTRACT

A new spectrophotometric reagent, *N,N'-bis*(salicylidene)-ethylenediamine (Salen), has been synthesized and characterized through novel reaction techniques. A very simple, ultrasensitive, and nonextractive spectrophotometric method has been developed for the determination of the picotrace amount of cobalt (II) using Salen. Salen undergoes a reaction in a slightly acidic solution (0.001-0.003 M H_2SO_4) with cobalt in 20% ethanol to give a light orange chelate, which has an absorption maximum at 459 nm. The reaction is instantaneous, and the absorbance remains stable for over 24 hours. The average molar absorption coefficient and Sandell's sensitivity were found to be 6.04×10^5 L/mol.cm and 5.0 ng/cm^2 of Co, respectively. Linear calibration graphs were obtained for 0.001-40 mg/L of Co with a detection limit of 0.1 μg/L and RSD of 0-2 %. The stoichiometric composition of the chelate is 1:1 (Co:Salen). A large excess of over 60 cations, anions and some common complexing agents such as chloride, azide, tartrate, EDTA, SCN^- etc. do not interfere in the determination. The developed method was successfully used in the determination of cobalt in several Certified Reference Materials (Alloys, steel, bovine liver, human hair, drinking water, sewage sludge, soil, and sediments) as well as in some environmental waters (Potable and polluted), biological fluids (Human blood, urine, and milk), soil samples, food samples (Vegetables, rice, and wheat) and pharmaceutical samples and solutions containing both cobalt (II) and cobalt (III) as well as complex synthetic mixtures. The results of the proposed method for assessing biological, soil, food and vegetable samples were comparable with ICP-OES and AAS were found to be in excellent agreement. The method has high precision and accuracy (s = ±0.01 for 0.5 mg/L).

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1. Introduction

Cobalt is found in relatively low abundance in the Earth's crust [1]. Cobalt traces are technically important metals, used mainly as a binder in the hard metal industry and as a constituent of many alloys [2]. Cobalt is an important element, not only for industry but also for biological systems. It is present in vitamin B_{12}, which is involved in red blood cell production and the prevention of pernicious anemia [3]. Insufficient natural levels of cobalt in feed causes cobalt deficiency diseases characterized by anemia, loss of weight, retard growth [4], and failure in infants [5]. Excess cobalt shows toxicity and is responsible for cardiac failure and polycythemia [6]. It is reported that cobalt toxicity causes different diseases, including asthma, contact dermatitis, lung cancer, bronchitis [7-11], pulmonary disorders, nausea, vomiting, diarrhea, blood pressure, slowed respiration, giddiness, cardiomyopathy, hyperglycemia and so on [3]. Cobalt (II) ions are also genotoxic and carcinogenic [8,10]. Chronic oral administration of Co for the treatment of anemia may result in the production of goiter; epidemiological studies suggest that the incidence of goiter is higher in regions containing increased levels of cobalt in water and soil [12]. All these findings cause great concern regarding public health, demanding accurate determination of this metal ion at trace levels and ultra-trace levels.

Spectrophotometry is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis. The development of the field of bioinorganic chemistry has increased because the interest in Schiff base has been recognized and many of the Schiff base complexes may serve as models for biologically important species. In transition metal chemistry, the most commonly used ligands are those that contain O, N donor sets. Schiff's bases are characterized by the presence of a C=N double bond (imine) which is bound to an aryl group through the carbon or the nitrogen atom to avoid rapid decomposition or polymerization [13]. Salen is a Schiff's base reagent that has been reported to be used in cloud point extraction (CPE) - spectrophotometric method for the determination of Cu, Cr, and Co [14], but has never been used as a selective direct spectrophotometric reagent for the determination of cobalt.

This study demonstrates a simple unswerving spectrophotometric method of ultratrace determination of cobalt.

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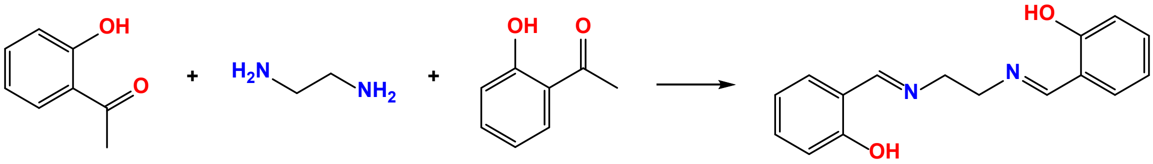

Scheme 1. Synthesis of 2-[2-[(2-hydroxyphenyl)methylideneamino]ethyliminomethyl]phenol.

The method has distinct advantages over existing methods [15-20] with respect to sensitivity, selectivity, range of determination, simplicity, speed, pH/acidity range, thermal stability, accuracy, precision, and ease of operation. From the above-mentioned literature survey (Table 1), it reveals that those methods are lengthy, time-consuming, pH dependent, and in most of the above-mentioned methods, interference was high. The method is based on the formation an absorbance complex of non-absorbent Salen and cobalt in a slightly acidic (0.001-0.003 M H_2SO_4) solution in the presence of ethanol. The complex gives with a highly absorbent light-orange chelate product followed by a direct measurement of the absorbance in an aqueous solution. The reagent blank solutions did not show any significant absorbance. The selectivity of the method was tested with suitable masking agent in some complex mixtures.

2. Experimental

2.1. Instrumentation

A Shimadzu (Kyoto, Japan) (Model-1800) double beam UV/VIS spectrophotometer and Jenway (England, U.K) (Model-3010) pH meter with a combination of electrodes were used for the measurements of absorbance and pH, respectively. A Shimadzu (Model: AA7000) atomic absorption spectrophotometer equipped with microcomputer-controlled air-acetylene flame and A Shimadzu (Japan) (Model: 9800) Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES), (λ = 418 nm, plasma gas flow rate (L/min) = 15, LOD: < 1 µg/L of Co, RF Power (W) = 1400, Nebulizer gas flow rate (L/min) = 1-10) were used for comparison of the results. The elemental Analyzer (Exeter Analytical Inc. Model: CE 440) equipped with a supersensitive thermal conductivity detector was used to simultaneously determine CHN. Infrared spectrum was recorded with a FTIR Spectrophotometer, Shimadzu (Model-IR Prestige 21, Detector-DTGS KBr) in the range 7500-350 cm^{-1} and model: JEOL 500SS, magnetic field strength: 500 MHz, solvent used: DMSO-d_6, standard: TMS, four-channel NMR spectrometer with a signal-to-noise ratio of ~500:1 for proton were used for ligand characterization. A Shimadzu QP-2010S Mass Spectrometer, Model: QP-2010S, AOC-20I Auto Injector - AOC-20S Auto Sampler with suitable software and accessories was used for the measurements of *m/z* (%).

2.2. Synthesis and characterization of the reagent

2.2.1. Synthesis of the reagent

The reagent may be synthesized by the condensation of ethylenediamine and salicylaldehyde [21,22]. The reagent was synthesized in our laboratory according to the method of Gahnn [23], Diehl [24], and Bakir [25]. At first, salicylaldehyde (20 mmol) was dissolved in 12 mL of ethanol in a 25 mL round flask. In a separate 25 mL flask, ethylenediamine (10 mmol) was dissolved in 10 mL ethanol. The ratio of ethylenediamine and salicylaldehyde should be 1:2. The salicylaldehyde solution was added drop wise to the ethylenediamine solution while stirring and refluxed for 2 hours. The precipitation of the product was observed by the formation of yellow precipitate and completed within 30 minutes. Afterwards the reaction flask was placed in an ice water bath for 15 minutes and then reaction mixture is filtrated. The precipitate was washed with ice-cold ethanol and dried off [26] to form yellow flakes and finally in a desiccator under vacuum over silica gel whose melting point 122.5 °C (Lit. 126.8 °C [23]). The yield of the product was 80% (Scheme 1).

2-[2-[(2-Hydroxyphenyl) methylideneamino] ethylimino methyl]phenol (*N,N'-bis*(salicylidene)-ethylenediamine): Color: Yellow. Yield: 80%. M.p.: 124.5-125.5 °C. FT-IR (KBr, ν, cm^{-1}): 3300-3600 (OH), 1636 (C=N), 1283 (C-O), 742 (benzene ring). ^1H NMR (500 MHz, DMSO-d_6, δ, ppm): 8.47 (s, 2H, CH=N), 7.33-7.29 (m, 4H, Ar-H), 6.83-6.87 (m, 4H, Ar-H), 3.97 (s, 4H, CH_2). MS (EI, *m/z* (%)): 268.32 (268), 268.12 (147), 268.08 (107). HRMS (EI, *m/z*) calcd. for 268.35; found 268.31. Anal. calcd. for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.56; H, 5.96; N, 10.43%. HPLC (Isocratic: CH_3CH_2OH: H_2O = 80:20, 100 min; 0.1%): R_t = 7.8 min, purity = 99.5%. Λ_m(S.m^2.mol^{-1}): 23. n_D^{25}: 1.252. $[\alpha]_D^{25}$: -68.7 (*c* 0.5, CH_3CH_2 OH). UV/Vis (C_2H_5OH, λ_{max}, nm, (1.00)): 314.5 [22].

2.3. Live subject statement

We were not aiming to carry out detailed human studies, but some samples from individuals were used in our study, and as such we abided by all necessary procedures and regulations, and our university gave consent. The University of Chittagong, Bangladesh, is committed to the protection and safety of human subjects involved in research.

2.4. Reagents and solutions

Analytical grade reagents were used throughout the whole experiment. High-purity absolute ethanol and high-purity deionized water were used throughout. More rigorous contamination control was used when the cobalt levels in the samples were low.

2.4.1. Salen solution

Salen solution (7.45×10^{-3} M) was prepared by dissolving the requisite amount of Salen in a known volume solution of distilled absolute ethanol. A more dilute solution of the reagent was prepared as required.

2.4.2. Cobalt (II) standard solution

A 100 mL amount of stock solution (1.70×10^{-2} M) (1 mg/mL) of divalent cobalt was prepared by dissolving 403.76 mg of cobalt chloride ($CoCl_2.6H_2O$), (Merck proanalysis grade, purity 99.5%) in doubly distilled de-ionized water. Aliquots of this solution were standardized by titrimetric analysis with EDTA [27]. More dilute standard solutions were prepared by appropriate dilution of aliquots from the stock solution with deionized water as and when required. A freshly standardized solution was always used [28].

2.4.3. Cobalt (III) standard solution

A 100 mL amount of stock solution (1.70×10^{-2} M) (1 mg/mL) was prepared by treating a 10 mL aliquot of the above stock solution with a few mL of hydrogen peroxide in dilute

Table 1. Summary of reviews on existing spectrophotometric methods for the determination of cobalt.

Reagent	Pyridoxal-4-phenyl-3-thiosemicarbozone (PPT) [15]				
Solvent	**Medium**	**Analytical parameters**		**Interference**	**Remarks**
n-Butanol	Aqueous	pH	5.0	Many	i) pH dependent. ii) Less sensitive. iii) Less selective due to much interference. iv) Application in limited samples.
		λ_{max} (nm)	450		
		Beer's law (mg/L)	0.5-6.0		
		Molar absorption co-efficient (L/ mol.cm)	1.4×10^4		
		Detection limit (ng/cm^2)	55		
		RSD (%)	2.0		
Reagent	1-(2-Pyridylazo)-2-naphthol (PAN) [16]				
Solvent	**Medium**	**Analytical parameters**		**Interference**	**Remarks**
Ethanol	Triton X-100 micellar medium	pH	8.0	Many	i) pH dependent. ii) Less sensitive. iii) Less selective due to much interference. iv) Less accuracy and precision.
		λ_{max} (nm)	540-700		
		Beer's law (mg/L)	0.2-4.0		
		Molar absorption co-efficient (L/ mol.cm)	4×10^3		
		Detection limit (ng/cm^2)	50		
		RSD (%)	5.0		
Reagent	Cyanex-272 [17]				
Solvent	**Medium**	**Analytical parameters**		**Interference**	**Remarks**
Organic solvent	Extraction into organic solvent	pH	4.0	Many	i) pH dependent. ii) Less sensitive. iii) Less selective due to much interference. iv) Limited application. v) Solvent extractive hence lengthy and time consuming.
		λ_{max} (nm)	635		
		Beer's law (mg/L)	0.295-2.36		
		Molar absorption co-efficient (L/ mol.cm)	3.07×10^3		
		Detection limit (ng/cm^2)	70		
		RSD (%)	5.0		
Reagent	2,6-Dithiolphenol (DTP) [18]				
Solvent	**Medium**	**Analytical parameters**		**Interference**	**Remarks**
Chloroform	Hydrophobic amines	pH	4.1-5.6	Many	i) Less sensitive. ii) pH dependent. iii) Less selective due to much interference. iv) Solvent extractive, hence lengthy and time consuming.
		λ_{max} (nm)	540		
		Beer's law (mg/L)	0.05-3.2		
		Molar absorption co-efficient (L/ mol.cm)	$(2.56\text{-}3.15)\times10^4$		
		Detection limit (ng/cm^2)	12-15		
		RSD (%)	6.0		
Reagent	2-Hydroxy-1-naphthaldehyde-p-hydroxybenzoichydrazone (HNAHBH) [19]				
Solvent	**Medium**	**Analytical parameters**		**Interference**	**Remarks**
DMF	Aqueous	pH	5.0	Many	i) Less sensitive. ii) Less selective due to much interference. iii) pH and temperature dependent. iv) Less accuracy and precision.
		λ_{max} (nm)	425		
		Beer's law (mg/L)	0.12-3.53		
		Molar absorption co-efficient (L/ mol.cm)	2.3×10^4		
		Detection limit (ng/cm^2)	40		
		RSD (%)	1.37		
Reagent	5-(2-Benzothiazolylazo)-8-hydroxyquinolene (BTAHQ) [20]				
Solvent	**Medium**	**Analytical parameters**		**Interference**	**Remarks**
Absolute ethanol	Cetyl pyridenium chloride (CPC)	pH	6.4	Many	i) Less sensitive. ii) Less selective due to much interference. iii) pH dependent. iv) Less accuracy and precision.
		λ_{max} (nm)	658		
		Beer's law (mg/L)	0.01-0.38		
		Molar absorption co-efficient (L/ mol.cm)	2.42×10^5		
		Detection limit (ng/cm^2)	3.1		
		RSD (%)	1.37		
Reagent	*N,N'-Bis*(salicylidene)-ethylenediamine (Salen) [Proposed method]				
Solvent	**Medium**	**Analytical parameters**		**Interference**	**Remarks**
20% Absolute ethanol	Aqueous	pH	2.8-3.8	Using suitable masking agents, the reaction can be made highly selective.	i) Highly selective. ii) Ultra-sensitive. iii) Simple and rapid and non-extractive. iv) Aqueous reaction medium. v) Complex color stable more than 24 hours at room temperature. vi) Application in various complex Matrices.
		λ_{max} (nm)	459		
		Beer's law (mg/L)	0.001- 40		
		Molar absorption co-efficient (L/ mol.cm)	6.04×10^5		
		Detection limit (ng/cm^2)	0.1		
		RSD (%)	0-2		

sulfuric acid, followed by complete removal of the peroxide by boiling and the volume was made up to the mark with water. The working standard of Co(III) was prepared by appropriate dilution of this solution. To check the accuracy of the prepared solution, the aliquots of this solution were standardized by titrimetric analysis with EDTA [28].

2.4.4. Other solutions

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); those of niobium, tantalum, titanium, zirconium and hafnium were specially prepared from their corresponding oxides (Specpure, Johnson Matthey) according to the Mukherji recommended procedures [29]. In the case of insoluble substances, special dissolution methods were adopted [30,31].

2.5. General procedure

A volume of 0.1-1.0 mL of neutral aqueous solution containing 0.01-400-µg of cobalt in a 10 mL volumetric flask was mixed with a 1:15 to 1:120 fold molar excess (preferably 2 mL of 7.45×10^{-3} M) of Salen reagent solution followed by the addition of 0.5-2.5 mL (preferably 1 mL) of 0.002 M sulfuric acid. The solution was mixed well. After 1 minute, 3.0 mL of ethanol was added. The mixture was diluted to the mark with deionized water. The absorbance was measured at 459 nm against a corresponding blank of reagent. The cobalt content in an unknown sample was determined using a concurrently prepared calibration graph.

2.6. Sample collection and preservation

2.6.1. Environmental samples

Water and soil samples were collected in polythene bottles from different places of Bangladesh. After collection, HNO_3 (1 mL/L) was added as a preservative.

2.6.2. Blood, urine, and milk

Blood and urine samples were collected in polypropylene bottles from effected persons of Chittagong Medical College Hospital, Bangladesh. Milk sample was collected from a Bangladeshi lactating mother. Immediately after collection, they were stored in a salt-ice mixture and later, in the laboratory, were at 20 °C.

2.6.3. Soil samples

Soil samples were collected from different locations in Bangladesh. The samples were dried in air and homogenized with a mortar.

2.6.4. Food samples

Food samples (Rice, wheat, fruits, and vegetables) were collected from local market of Chittagong. After collection the samples (Fruits and vegetables) were stored in refrigerator for preservation. Samples (Rice and wheat) were used as dry conditions and homogenized with a mortar.

2.6.5. Pharmaceutical samples

Pharmaceutical samples (Tablet and syrup) from different companies were collected from the Chittagong local pharmacy. The samples (tablets) were homogenized with a mortar.

3. Results and discussion

3.1. Characterization of the reagent

The reagent was characterized by taking melting point, elemental analysis, FTIR, and ^{1}H NMR spectrums. The melting point of the reagent was 124.5 °C (Lit. 126.8 °C) [23]. The elemental analysis was performed by the National Center of Excellence in Analytical Chemistry, University of Sindh, Pakistan. The results of elemental analysis of the reagent was in good coincidence with the calculated values. FT-IR spectrum was recorded with FT-IR spectrophotometer, a Shimadzu (IR-Prestige 21, Detector DTGS, KBr) in the range 7500-350 cm^{-1} in our Laboratory, Department of Chemistry, University of Chittagong. The presence of FT-IR peak at 1636 cm^{-1} was due to the characteristic C=N double bond ($\nu_{C=N}$, 1612–1635 cm^{-1}) of Salen [25]. The C-O frequency value appeared at 1283 cm^{-1}. The appearance of broad band (3300-3600 cm^{-1}) refer to the frequency of OH (Lit. 3433-3468 cm^{-1}) [25]. A large broad band appearance at 742 cm^{-1} refers to the frequency of the benzene ring (Lit. 748 cm^{-1}) [25]. The ^{1}H NMR spectrum was recorded from Jahangirnagar University, Savar, Dhaka. The presence of ^{1}H NMR peaks at δ 6.85, 7.52, and 8.47 ppm due to the characteristic of the reagent is consistent with the values of the literature [26]. Both the FTIR and ^{1}H NMR data and the elemental analysis data indicated the formation of the reagent. The steadiness of the thermogravimetric curve obtained for approximately 1 g of the reagent at 80-90 °C indicated that the reagent did not contain any moisture.

3.2. Factors affecting the absorbance

3.2.1. Absorption spectra

The absorption spectrum of a cobalt-Salen system in aqueous medium in presence of 1 mL 0.002 M sulfuric acid solution, was recorded using the spectrophotometer. The absorption spectrum of cobalt-Salen is an asymmetric curve with maximum absorbance at 459 nm and an average molar absorptivity of 6.04×10^5 L/mol.cm (Figure 1). The reagent blank exhibited negligible absorbance in the range of determination. In all instance's measurements were made at 459 nm against a reagent blank. The reaction mechanism of the present method is as previously reported [31].

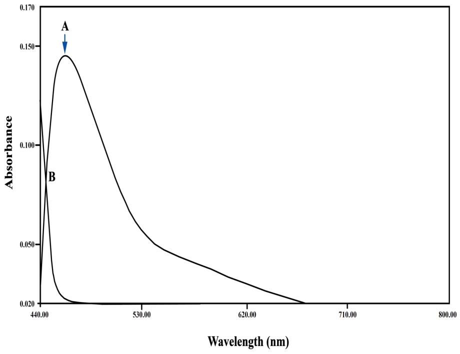

Figure 1. Spectra A and B are the absorbance spectra of cobalt (II)-Salen and the reagent blank (λ_{max} = 459 nm) in aqueous solutions, respectively. (1.0 mg/L, acidity: 0.001-0.003 M H_2SO_4; ethanol = 3 mL, Salen = 1:20 molar ratio; temperature: 25±5 °C).

3.2.2. Optimization of some parameters on the absorbance

3.2.2.1. Effect of solvent

Because Salen is partially soluble in water, an organic solvent was used for the system, consideration of cost, availability, toxicity, volatility of the solvent, *etc*. Among the various solvents (Acetone, benzene, carbon tetrachloride, chloroform, ethanol, 1-butanol, isobutyl methyl ketone, *N*,*N*-dimethylformamide, methanol, and 1,4-dioxane) studied, ethanol was found to be the best solvent for the system. Different volumes (0-8 mL) of ethanol were added to fixed metal ion concentration and the absorbance was measured according to the general procedure. Maximum absorbance was observed in 30±2% (v/v) ethanol/water medium, hence, a 30% ethanol solution was used in the determination procedure. It was observed that 10-60% (1-6 mL) ethanol produced a constant absorbance of the Co-chelate. For all subsequent measurements, 30% (3.0 mL) of ethanol was added.

3.2.2.2. Effect of acidity

Among the various acids (Nitric, sulfuric, hydrochloric, and phosphoric acids) studied, sulfuric acid was found to be the best acid for the system. The variation of the absorbance was noted after the addition of 0.2-4.0 mL of 0.002 M sulfuric acid to every 10 mL of test solution. The maximum and constant absorbance was obtained in the presence of 1.0-2.8 mL of 0.002 M sulfuric acid at room temperature 25±5 °C. For all subsequent measurements, 1.0 mL of 0.002 M sulfuric acid was added. This range of acidity (0.001-0.003 M H_2SO_4) also measured the corresponding pH was 3.3-3.1. This type of conversion has also been previously reported [32].

Table 2. Summary of selected analytical parameters obtained with optimization experiments.

Parameters	Studied value	Selected value
Wavelength, λ_{max} (nm)	200-800	459
Solvent (mL)	0-8	1-8 (preferably 3.0)
H_2SO_4 (M)	0.0001-0.01	0.001-0.003 (preferably 0.002)
pH	2.1-5.5	2.8-3.8 (preferably 3.5)
Time	0 -24 h	1 min - 24 h (preferably 2 min)
Temperature (°C)	10-80	25±5
Reagent (Fold molar excess, M:R)	1:1 -1:120	1:15-1:120 (preferably 1:20)
Linear range (mg/L)	-	0.001-40
Molar absorptivity (L/mol.cm)	-	6.04×10^5
Limit of quantification (µg/L)	-	1.0
Detection limit (µg/L)	-	0.1
Sandell's sensitivity (ng/cm^2)	-	5.0
Reproducibility (% RSD)	-	0-2
Regression coefficient, R^2	0.9740-0.9994	0.9993

Table 3. Effect of interfering radicals.

Species X	Tolerance ratio X/Co (w/w)	Species X	Tolerance ratio X/Co (w/w)
Aluminum	100	Lead(II)	100
Arsenic(III)	100	Magnesium	100
Arsenic(V)	100	Mercury(II)	100
Antimony	100	Molybdenum(VI)	50
Azide	50	Manganese(II)	20 [a]
Ammonium	50	Manganese(VII)	100
Bismuth	100	Nickel(II)	50
Bromide	100	Oxalate	100
Barium	50	Phosphate	100
Cadmium	50	Potassium	100
Calcium	100	Selenium(IV)	50
Carbonate	100	Selenium(VI)	100
Citrate	50	Silver	100
Chromium(III)	100 [a]	Strontium	50
Chromium(VI)	20 [a]	Sulfate	100
Cesium	100	Sodium	100
Copper(II)	100	Tartrate	100
Cerium(III & IV)	20 [b]	Tin(II)	50
Chloride	100	Tellurium(IV)	100
Dimethylglyoxime	100	Titanium(IV)	100
EDTA	100	Thallium(I)	100
Fluoride	100	Thiocyanate	100
Iron(II)	50 [a]	Tungsten(VI)	50
Iron(III)	20 [a]	Thiosulphate	100
Iodide	100	Uranium	20 [a]
Lithium	100	Vanadium (V)	100
Cobalt (III)	50 [a]	Zinc	50

The tolerance limit was defined as the ratio that causes less than ±5 percent interference.

[a] with 10 mg/L EDTA.

[b] with 10 mg/L tartrate.

3.2.2.3. Effect of time

The reaction is very fast. A constant maximum absorbance was obtained just after dilution within a few seconds to volume and remained strictly constant for over 24 h; a longer period of time was not studied.

3.2.2.4. Effect of temperature

The influence of temperature was studied between 10-80 °C. From the temperature studies, it can be observed that the temperature effect is not pronounced between 20-80 °C and so room temperature (25±5°C) is recommended for all subsequent measurements.

3.2.2.5. Effect of reagent concentration

Different molar excesses of Salen were added to a fixed metal ion concentration and the absorbance was measured according to the general procedure. It was observed that cobalt metal, the reagent molar ratio of 1:15 to 1:120 produced a constant. For different (0.5 and 1 mg/L) cobalt concentrations an identical effect of varying the reagent concentration was noticed. Further concentration of the reagent was not studied. For all subsequent measurements, 2 mL of 7.45×10^{-3} M Salen reagent was added.

3.2.3. Calibration graph (Beer's law and sensitivity)

The well-known equation for a spectrophotometric analysis in a very dilute solution was derived from Beer's law. The effect of the metal concentration was studied over 0.001-100 mg/L distributed in five different sets (0.001-0.01, 0.01-0.1, 0.1-1.0, 1.0-10.0 and 10.0-100.0 mg/L) for convenience of measurement. The absorbance was linear for 0.001-40 mg/L at 459 nm. Of the five calibration graphs, one showing the limit of the linearity is given in Figure 2. The other four were straight-line graphs passing through the origin (R^2 = 0.9993). The molar absorption co-efficient and the Sandell's sensitivity [33] were found to be 6.04×10^5 L/mol.cm and 5.0 ng/cm^2 of cobalt, respectively. The selected analytical parameters obtained from the optimization experiments are summarized in Table 2.

3.2.4. Effect of foreign ions

The effect of more than 60 anions, cations, and complexing agents was studied on the determination of only 10 mg/L of cobalt. The criterion for an interference [34] was an absorbance value varying by more than 5% from the expected value for cobalt alone. The results are summarized in Table 3. As can be seen, a large number of ions have no significant effect on the determination of cobalt.

Table 4. Determination of cobalt levels in a variety of synthetic mixtures.

Sample	Composition of mixtures (mg/L)	Co(II) (mg/L)		
		Added	Found (n=5) [a]	Recovery ± SD [b] (%)
A	Co^{II}	0.50	0.50	100±0.0
		1.00	0.99	99±0.5
B	As in A + Te^{4+} (25) + Pb^{2+} (25) + Zn (25) + Ba (25) + Cu^{2+} (25) + EDTA (50)	0.50	0.49	98±1.0
		1.00	1.02	102±1.4
C	As in B + K (25) + V^{5+} (25) + Na (25) + Ni^{2+} (25) + Mn^{2+} (25)	0.50	0.48	96±1.2
		1.00	0.98	98±1.5
D	As in C + As^{3+} (25) + As^{5+} (25) + Li (25) + W^{6+} (25) + Se^{4+} (25) + Tl^{+} (25)	0.50	0.52	104±1.6
		1.00	1.04	104±1.5
E	As in D + Hg^{2+} (25) + Fe^{3+} (25) + Sr (25) + Ag (25) + Cr^{6+} (25) + Tartrate (50)	0.50	0.53	106±1.7
		1.00	1.06	106±1.8
F	As in E + Ce^{3+} (25) + Mo^{6+} (25) + Sn^{2+} (25) + Mn^{7+} (25) + Bi^{3+} (25) + U^{6+} (25)	0.50	0.54	108±1.9
		1.00	1.09	109±2.0

[a] Average of five analysis of each sample.

[b] The measure of precision is the standard deviation (SD).

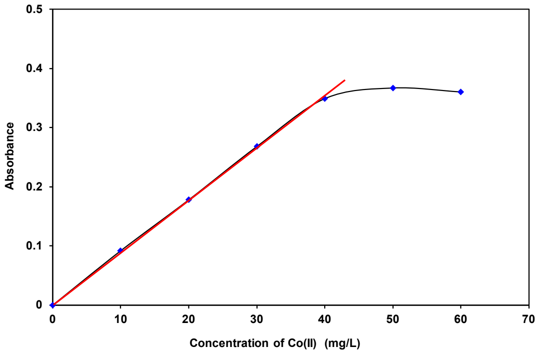

Figure 2. Calibration graph: 10-40 mg/L of cobalt (II). (Acidity: 0.001-0.003 M H_2SO_4; Ethanol = 3 mL, Salen = 1:20 molar ratio; Temperature: 25±5 °C).

The most serious interference was from Fe(III) and Mn(II) ions. Interference from these ions is probably due to complex formation with Salen. Greater tolerance limits for these ions can be achieved by using several masking methods. In order to eliminate interference of Cr(III), Cr(VI), Fe(II), Fe(III), Co(III), U(VI), and Mn(II), 10 mg/L EDTA is used for Cr(III) and Cr(VI), 10 mg/L tartrate is used as masking agent for rest of the ions, respectively. During interference studies, if a precipitate was formed, it was removed by centrifugation and filtered [35]. The amount mentioned is not the tolerance limit but the actual amount studied. However, for those ions whose tolerance limits have been studied, their tolerance ratios are mentioned in Table 3.

3.3. Precision and accuracy

The precision of the present method was evaluated by determining different concentrations of cobalt (each analysed at least five times). The relative standard deviation (n = 5) was 0-2.0 % for 0.01-400 µg of cobalt in 10 mL, indicating that this method is highly precise and reproducible. The detection limit (3s/S of the blank) and Sandell's sensitivity (concentration for 0.001 absorbance unit) for cobalt were found to be 0.1 µg/L and 5.0 ng/cm^2, respectively. The method was also tested by analysing several synthetic mixtures containing cobalt and various ions (Table 4). The results for total cobalt were in good agreement with certified values (Table 5). The reliability of our Co-chelate procedure was tested by recovery studies. The average percentage recovery obtained by addition of cobalt spike to some environmental water samples was quantitative as shown in Table 6. The results of biological analyses by the spectrophotometric method were in excellent agreement with those obtained by AAS and ICP-OES (Table 7). The results of soil analyse using the spectrophotometric method were in excellent agreement with those obtained by AAS (Table 8). The results of food and vegetable analyses by the spectrophotometric method were also found to be in excellent agreement with those obtained by AAS and ICP-OES (Table 9). The results of pharmaceutical samples obtained by the spectrophotometric method were in excellent agreement with those obtained by the claimed values and by ICP-OES (Table 10). The results of speciation of cobalt(II) and cobalt(III) in the mixtures were highly reproducible (Table 11). Hence, the precision and accuracy of the method were excellent. With the appropriate masking, the reaction can be made highly selective.

3.4. Composition of the absorbent complex

The Job method [36] of the continuous variation method was applied to determine the stoichiometric composition of the complex under the optimal conditions (Table 2). This method indicated a Co-Salen (1: 1) complex. The molar ratio method [37] was also applied to ascertain the stoichiometric composition of the complex. A Co-Salen complex was indicated by both methods, and the stoichiometry was also found to be 1:1 (Metal:Ligand). The similar square planar structure of cobalt (II) with Schiff base ligands have been reported by Tsumaki [21].

3.5. Applications of the proposed method

To verify the validity of the proposed method was successfully applied to the determination of cobalt in a number of environmental, biological, soil, vegetable, food and pharmaceutical samples, e.g., speciation of cobalt (II) and cobalt (III) in mixtures.

3.5.1. Determination of cobalt in some synthetic mixtures

Several synthetic mixtures of varying compositions containing cobalt and diverse ions of known concentrations were determined by the present method using tartrate or EDTA as masking agent, and the results were found to be highly reproducible. The results are shown in Table 4.

Table 5. Determination of cobalt in some certified reference materials.

Sample	Certified reference materials (Composition, %)	Cobalt (%)		
		Certified value	Found (n=5) [a]	RSD [b]
1	BCS-261 Straight Nb 18/12: Stainless steel [c] (C = 0.083, Si = 0.39, Cr = 17.20, Ni = 13.08, Mn = 0.66, Nb & Ta = 0.71, Co = 1.0)	1.00	1.015	1.3
2	YSBC11515-93(K3): Dies Steel [c] (Cr = 10.93, C = 0.15, Si = 0.06. Zr = 0.096, Fe = 0.20, Mo = 3.98, Ce = 0.15, Ti = 2.72, W = 4.91, Co = 5.41, Al = 5.53)	5.41	5.395	1.5
3	YSBC11515-93(264): Unalloyed Steel [c] (Si = 0.08, Mn = 0.35, Cr = 0.21, Ni = 28.91, Co = 17.17)	17.17	17.158	2.0
4	YSBC1013-1-95- 9Cr$_{17}$MoVCo: High tensile steel [c] (Cr = 16.3, C = 90, Si = 0.44, Mn = 0.81, Mo = 0.52, Co = 1.45, V = 0.24)	1.45	1.435	1.8
5	CRM-TMDW-A-100: Trace Metals in Drinking Water [d] (Ag = 2, Tl = 10, Se = 11, Be & Li = 15, Cr, Cu & Pb = 20, Co = 25, V = 35, Mn = 40, As & Sb = 55, Ni = 60, Zn = 75	25.00 [f]	25.15	2.0
6	CRM®-BCR®-142: Sandy Soil [e]	10.3±0.4 [g]	10.25±0.8	1.8
7	CRM®-BCR®-143: Sewage Sludge [e]	11.8±1.2 [g]	11.75±1.5	2.0
8	CRM®-BCR®-277: Estuarine Sediment [e]	15.6±1.0 [g]	15.6±1.0	2.0
9	CRM®-185®: Bovine Liver	1.0±0.1 [g]	0.99±0.5	1.0
10	NIES®-CRM®: Human hair No.5 [e]	0.9±0.2 [g]	0.88±0.5	1.0

[a] Average of five analysis of each samples.

[b] The measure of precision is the relative standard deviation (RSD).

[c] The CRMs were obtained from the NCS Analytical Instruments Co. Ltd. Beijing, China.

[d] CRM was obtained from High Purity Standards (HPS), Amazon, North Charleston, USA.

[e] These CRMs were obtained from the National Research Council, Govt. of Canada.

[f] Values in µg/L.

[g] Values in mg/kg.

Accurate recoveries were achieved in all solutions in the range 98±1.0 to 109±2.0%. The reliability of our cobalt-Salen procedure was approved by quantitative recovery of cobalt (II) spiked in several synthetic mixtures containing cobalt (II) and diverse ions. The method has high precision and accuracy (s = ±0.01 for 0.5 µg/L).

3.5.2. Determination of cobalt in some certified reference materials

A 0.1 g amount of an alloy or steel sample containing 1.0-25.0 % of cobalt was weighed accurately and placed in a 50 mL Erlenmeyer flask in the presence of excess reducing agent to reduce cobalt (III) to cobalt (II) following a method recommended by Mitra [38]. To it, 10 mL of 20% (w/v) sulfuric acid was added and while carefully covered with a watch glass until the brisk reaction subsided. The solution was heated and simmered gently after the addition of 10 mL of concentrated HNO_3 until all residual carbides were decomposed. Then a further 2 mL of 1+1 H_2SO_4 and 2 mL 2.5% (w/v) freshly prepared sodium azide solution were added and the solution was evaporated carefully to dense white fumes to remove excess azide, then cooled to room temperature (25±5 °C). After suitable dilution with deionized water, the contents of the Erlenmeyer flask were warmed so as to dissolve the soluble salts. The solution was then cooled and neutralized with dilute NH_4OH solution in the presence of 1-2 mL of 0.01% (w/v) EDTA solution. The resulting solution was filtered if necessary, through a Whatman No. 40 filter paper into a 100 mL calibrated flask. The residue (Silica and tungstenic acid) was washed with a small volume of hot 1+99 H_2SO_4, followed by water; the volume was made up to the mark with de-ionized water.

A suitable aliquot (1-2 mL) of the above-mentioned solution was taken into a 10 mL calibrated flask and the cobalt (II) content was determined; as described under the general procedure using EDTA or tartrate as masking agent. The proposed procedure for the spectrophotometric determination of cobalt was applied to the analysis of single element CRM of Co, estuarine sediment (CRM BCR-277), sewage sludge (CRM BCR-143), sandy soil (CRM-BCR-142), bovine liver (CRM^{R}-185R), human hair (NIES CRM), these CRMs obtained from the National Research Council of Canada, using tartrate or EDTA as masking agents, following a method recommended by Sun *et al.* [39]. Based on five replicate analyses, the average cobalt concentration determined by the spectrophotometric method was in an excellent agreement with the certified values. The results are given in Table 5.

3.5.3. Determination of cobalt in environmental water samples

Each filtered (with Whatman No. 40) environmental sample (25 mL) contained in a 50 mL Pyrex beaker were added 1 mL of concentrated H_2SO_4 and 2 mL of concentrated HNO_3 in the presence of freshly prepared excess sodium azide solution in a fume cupboard to reduce cobalt (III) to cobalt (II) and the mixture was heated on a hot plate until white fumes of azide to remove completely, following a method recommended by Greenberg *et al.* [40]. The solution was cooled and neutralized with a dilute NH_4OH solution in the presence of 1-2 mL of 0.01% (w/v) EDTA solution. The resulting solution was then filtered through a Whatman No. 40 filter paper and quantitatively transferred to a 25 mL calibrated flask and made up to the mark with deionized water.

An aliquot (1-2 mL) of this water sample was pipetted into a 10 mL calibrated flask and the cobalt content was determined as described under the general procedure using tartrate or EDTA as masking agent. To test the validity of our method, we have analysed different types of potable and polluted waters in spike and un-spike conditions. The reliability of our spectrophotometric method was tested by recovery studies. The average percentage recovery obtained by the addition of a cobalt (II) spike to some environmental water samples was quantitative. The results of the analyses of environmental water samples from various sources of cobalt are shown in Table 6.

Most spectrophotometric methods for determination of cobalt in natural and sea-water require preconcentration or standard addition of cobalt [41]. The concentration of cobalt in surface water is 0.01-0.04 mg/L for Halda and Karnaphuli River, respectively, and very low levels of cobalt levels to 0.009 mg/L for greater Chittagong region [42]. The average geological cobalt distribution in the Chittagong Division is 0.007 mg/L and the average concentration for household drinking water is 0.006 mg/L [43]. Therefore, preconcentration or standard addition must be required [42] for accurate measurements.

3.5.4. Determination of cobalt in some biological samples

The biological samples were digested accordingly following the method reported by Khayatian *et al.* [44]. The samples were initially dried in an oven at 120 °C for 24 h. Blood serum samples were further dried in an oven at 20 °C for an additional 24 h. Then, the biological samples were dry-ashed in a muffle furnace at 300 °C for 24 h, then at 450 °C for 4 h.

Table 6. Determination of cobalt in some environmental water samples.

Samples		**Cobalt (µg/L)**		**Recovery ± s (%)**	**s_r [b] (%)**
		Added	**Found [a]**		
Tap water		0.0	6.0	-	-
		100.0	106.5	100.5±0.5	0.37
		500.0	505.95	99.9±0.3	035
Mineral Water (commercial bottle)		0.0	11.0	-	-
		100.0	111.5	100.5±0.5	0.29
		500.0	511.0	100.0±0.0	0.00
Rainwater		0.0	3.0	-	-
		100.0	103.0	100.0±0.0	0.00
		500.0	504.0	100.1±.1.0	0.29
Well water		0.0	12.5	-	-
		100.0	113.0	100.4±0.5	0.32
		500.0	515.0	100.5±1.0	0.35
River water	Kanaphuli (upper)	0.0	8.5	-	-
		100.0	110.0	100.4±0.5	0.32
		500.0	512.0	100.5±1.0	0.35
	Karnaphuli (lower)	0.0	10.0	-	-
		100.0	113.0	100.4±0.5	0.32
		500.0	515.0	100.5±1.0	0.35
	Halda (upper)	0.0	7.8	-	-
		100.0	107.8	100.0±0.0	0.00
		500.0	510.0	100.5±0.5	0.29
	Halda (lower)	0.0	9.5	-	-
		100.0	110.0	100.5±1.0	0.35
		500.0	512.0	100.5±0.8	0.21
Lake water	Science Faculty, Chittagong University	0.0	35.8	-	-
		100.0	136.0	100.1±0.5	0.45
		500.0	535.8	100.0±0.0	0.00
Seawater	Bay of Bengal (upper) (Patenga Beach)	0.0	3.5	-	-
		100.0	102.5	99.0±0.6	0.23
		500.0	503.5	100.0±0.0	0.00
	Bay of Bengal (lower) (Patenga Beach)	0.0	6.5	-	-
		100.0	136.0	99,6±0.8	0.35
		500.0	536.5	100.0±0.0	0.00
	Kolatoli Beach, Cox's Bazaar (lower)	0.0	7.5	-	-
		100.0	108.0	100.4±0.8	0.45
		500.0	507.5	100.0±0.0	0.00
Drain Water	KAFCO [c]	0.0	15.5	-	-
		100.0	116.0	100.8±0.8	0.25
		500.0	517.5	100.4±1.0	0.21
	Eastern Refinery [d]	0.0	165.0	-	-
		100.0	267.5	100.9±0.8	0.45
		500.0	670.0	99.9±1.0	0.35
	Elite Paint [e]	0.0	45.0	-	-
		100.0	148.0	102.4±1.0	0.28
		500.0	545.0	100.0±0.0	0.00
	PHP Steels [f]	0.0	85.0	-	-
		100.0	186.0	100.5±1.0	0.55
		500.0	585.0	100.0±0.0	0.00

[a] Average of five replicate determinations of each sample.
[b] The measure precision is the relative standard deviation (s_r).
[c] Eastern Cables Ltd., North Patenga, Chittagong.
[d] Eastern Refinery Ltd., North Patenga, Chittagong-4204.
[e] Elite Paint and Chemical Industries Ltd., Bayazid Bostami Road, Chittagong.
[f] PHP Steels, Agrabad, Chittagong-4100.

After dry-ashing, samples were wet-ashed with 5 mL concentrated nitric acid and 2 mL of freshly prepared 2.5% sodium azide solution. The mixture was heated to just below boiling until complete reduction of cobalt (III) to cobalt (II). The samples were cooled and wet-ashed three more times in the same manner. At completion, the white residue was dissolved with 10 mL of 1 M HNO_3 by heating of an excess reducing agent according to the method recommended by Stahr [45] and diluted to 20.0 mL for analysis. After neutralizing pH by addition of dilute NH_4OH in the presence of 1-2 mL of 0.01% (w/v) tartrate or EDTA solution. The resultant solution was then filtered and transferred quantitatively into a 25 mL calibrated flask and made up to the mark with deionized water.

A suitable aliquot (1-2 mL) of the final solution was pipetted into a 10 mL calibrated flask and the cobalt content was determined as described under the procedure using tartrate or EDTA as masking agent. The results of biological analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by AAS and ICP-OES. The results are shown in Table 7.

Healthy people who take cobalt as a dietary supplement may consume ≤ 1000 µg/daily, and expert governmental advisory groups have concluded that supplementation ≤ 1400 µg/daily (which results in whole blood levels of ≤ 18 µg/L) produces no adverse health effects [46]. Cobalt can cause a distinctive, rapidly progressive, and reversible depression of cardiac systolic function, which is readily distinguished from other causes of cardiomyopathy [47] and excessive administration of this trace element produces goiter and reduced thyroid activity [48].

3.5.5. Determination of cobalt in some surface soil samples

An air-dried homogenized soil sample (10 g) was accurately weighed and placed in a 100 mL micro-Kjeldahl flask. The sample was digested in the presence of an excess reducing agent (2 mL of 2.5% freshly prepared sodium azide solution) to reduce cobalt(III) to cobalt(II) following method recommended by Jackson [49].

Table 7. Determination of cobalt in some human fluids.

No	Sample	Cobalt (µg/L)						Sample source [a]
		AAS (n=5)		Proposed method (n=5)		ICP-OES (n=5)		
		Found [b]	RSD (%)	Found [b]	RSD (%)	Found [b]	RSD (%)	
1	Blood	17.8	1.5	18.5	1.5	18.8	1.6	Normal adult (M)
	Urine	6.15	1.0	6.8	1.2	7.2	1.3	
2	Blood	35.0	1.8	36.0	1.8	35.8	2.0	Skin disease patient (F)
	Urine	9.3	1.3	9.5	1.5	9.8	1.5	
3	Blood	45.5	1.5	46.2	1.8	47.0	2.1	Anemia patient (M)
	Urine	16.6	1.3	16.8	1.5	17.0	1.8	
4	Blood	105.8	2.5	107.6	2.5	108.0	2.8	Mild Cognitive Impairment (MCI) patient (M)
	Urine	28.8	1.8	30.0	1.8	30.5	1.9	
5	Blood	485.0	2.5	486.8	2.5	487.0	3.0	Pneumonia patients (F)
	Urine	107.3	1.8	108.0	1.8	108.5	2.0	
6	Blood	710.0	3.0	709.8	3.0	711.0	4.0	Paralysis patient (M)
	Urine	180.0	2.5	181.5	2.8	182.0	3.0	
7	Blood	115.0	3.0	116.8	2.8	117.8	3.0	Thyroid patient (F)
	Urine	29.8	2.0	30.5	1.8	31.0	1.5	
8	Blood	135.8	3.0	136.5	2.5	137.8	2.8	Heart disease patient (F)
	Urine	34.8	1.9	35.5	1.8	36.2	2.0	
9	Blood	176.0	3.0	178.0	2.8	177.5	3.0	Kidney Dialysis patient (M)
	Urine	44.8	1.7	45.5	1.8	46.0	2.0	
10	Blood	84.8	2.2	85.5	2.0	86.0	2.5	Hyperplasia patient (M)
	Urine	27.0	1.6	28.5	1.8	29.0	1.8	
11	Human milk	8.5	1.0	8.45	1.0	8.98	1.5	Lactating Mother

[a] Samples were collected from Chittagong Medical College Hospital, Chittagong.
[b] The measure of precision is the relative standard deviation (RSD).

Table 8. Determination of cobalt in some surface soil samples.

Serial	Cobalt (mg/kg)				Sample source [c]
	Proposed Method (n=5)		AAS (n=5)		
	Found [a] (n=5)	RSD [b] (%)	Found [a] (n=5)	RSD [b] (%)	
S_1	65.6	1.5	66.8	1.5	Roadside soil (Dhaka-Chittagong)
S_2	16.8	1.2	17.0	1.3	Agricultural soil (Chittagong University Campus)
S_3	155.0	2.0	158.5	2.2	Industrial soil (Eastern Cables Ltd., Chittagong)
S_4	125.5	1.8	124.8	2.0	Industrial soil (Eastern Refinery Ltd., Chittagong)
S_5	163.8	2.5	165.0	2.8	Industrial soil (BSRM Ltd., Bayazid, Chittagong)
S_6	115.5	2.2	116.8	2.5	Madina Tannery soil (Jalalabad, Chittagong)
S_7	170.8	2.8	172.0	3.0	Paint soil (Elite Paint & Chemical Industries Ltd., Bayazid, Chittagong)
S_8	36.5	1.5	36.8	1.5	Karnaphuli River Bank soil (Chittagong)
S_9	45.8	1.8	47.0	2.0	T.S.P complex soil (Patenga, Chittagong)
S_{10}	88.9	2.0	90.6	2.3	Estuarine soil (Karnaphuli River)

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

As the heating process continued, 1 mL of H_2SO_4 is added and heated for about 5 minutes to dense white fumes to remove excess azide. The solution was then cooled at room temperature and neutralized with dilute NH_4OH solution in the presence of 1-2 mL of 0.01% (w/v) EDTA solution. The content of the flask was then filtered through a What-man No. 40 filter paper and quantitatively transferred into a 25 mL calibrated flask and made up to the mark with de-ionized 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 the general procedure using tartrate or EDTA as masking agent. The cobalt content was then determined by the above procedure and quantified from a calibration graph prepared concurrently. The results of soil analyses by spectrophotometric method were also found to be in excellent agreement with those obtained by AAS. The average value of cobalt in the Chittagong region surface soil was found to be 99.53 mg/kg. The results are shown in Table 8.

3.5.6. Determination of cobalt in vegetable, food and fruit samples

The vegetable and fruit samples collected prior to determination were pre-treated in the following way: The edible portion of the samples was first washed clean with tap water followed by rewashing with deionized water. After removing de-ionized water from the surface of vegetables and fruits, the samples were cut into small pieces and dried at 65 °C in oven. Air dried vegetable and fruit samples (10 g) were ground in a mortar and taken in a 100 mL micro-Kjeldahl flask in the presence of excess reducing agent and digested following a method recommended by Stahr [45] and 10 ml of concentrated nitric acid were added and the flask was placed on the digester under gentle heating. When the initial brisk reaction was over, the solution was removed and cooled at room temperature. 1 mL volume of concentrated sulfuric acid was added carefully, followed by the addition of 1 mL of concentrated HF, and the heating was continued for at least ½ hr and then cooled.

Table 9. Determination of cobalt in some samples of food, fruits, and vegetables.

No	Sample	Cobalt (µg/kg)						Sample source
		Proposed method (n=5)		AAS (n=5)		ICP-OES (n=5)		
		Found [a]	RSD [b] (%)	Found [a]	RSD [b] (%)	Found [a]	RSD [b] (%)	
1	Chicken meat *(Gallus cibum)*	4.9	1.0	5.1	1.1	5.0	1.2	Local Market, Chittagong
2	Chicken liver *(Gallus jecur)*	5.8	1.3	6.2	1.5	6.0	1.5	Local Market, Chittagong
3	Egg white *(Albumen)*	5.2	1.2	5.5	1.5	5.8	1.5	Local Market, Chittagong
4	Egg yolk *(Vitellus)*	6.8	1.5	7.0	1.8	7.3	1.8	Local Market, Chittagong
5	Carrot *(Daucus carota)*	6.5	1.6	6.8	1.8	7.0	1.8	Local Market, Chittagong
6	Tomato (*Lycopersicon esculentum)*	8.9	1.8	9.0	2.0	9.5	2.0	Local Market, Chittagong
7	Radish *(Raphanus sativus)*	7.5	1.6	7.8	1.8	8.2	2.0	Local Market, Chittagong
8	Tea *(Camellia sinensis)*	12.6	2.0	12.5	2.5	12.8	2.5	Local Market, Chittagong
9	Spinach *(Spinacia oleracea)*	10.8	2.1	10.5	2.5	11.0	2.3	Local Market, Chittagong
10	Black pepper *(Piper nigrum)*	8.5	2.0	8.8	2.3	8.6	2.2	Local Market, Chittagong
11	Cashew nut *(Anacardium occidentale)*	13.8	2.0	14.5	2.5	14.0	2.8	Local Market, Chittagong
12	Wheat (*Triticum aestivum*)	2.8	1.5	3.0	1.5	2.6	1.5	Local Market, Chittagong
13	Rice *(Oryza sativa)*	2.5	1.2	2.8	1.5	2.6	1.8	Local Market, Chittagong
14	Tobacco *(Nicotana tabacum)*	29.5	2.5	30.3	3.0	30.5	3.0	Local Market, Chittagong
15	DiaryMilk(powder) (*Bos taurous* milk)	4.5	1.5	4.8	1.8	4.6	1.8	Local Market, Chittagong

[a] Samples were collected from local market, Chittagong.

[b] The measure of precision is the relative standard deviation (RSD).

In the resulting solution 2 mL of 2.5% (w:v) of freshly prepared sodium azide solution was added. The mixture of each foodstuff was heated below the boiling point for 5-10 min to reduce cobalt (III) to cobalt (II). The excess azide was removed by further heating. The solutions were then cooled and neutralized with dilute NH_4OH in the presence of 1-2 mL of 0.01% (w/v) EDTA solution. The resulting solution was filtered through a Whatman No. 40 filter paper and quantitatively transferred into a 25 mL calibrated flask and mixed well and made up to the mark with deionized water.

The food samples used were rice, wheat and corn and these were used under dry conditions. Each sample was first ground in a mortar. Corn and fruit samples (2 g) or rice and wheat samples (1 g) were accurately weighed and placed in a porcelain crucible and charred in an electric furnace; the sample was ashen at 555 °C in a muffle furnace in the presence of excess oxidizing agent following a method recommended by Mitra [38]. To it, 2.0 mL of HCl and 10 mL of water were added to the ash. The mixture of each foodstuff was heated with 2 mL of 2.5% (w:v) freshly prepared sodium azide was added below the boiling point for 5-10 min to complete the reduction from Co(III) to Co(II). Then the solution was heated for another 5 min to remove excess azide. The solutions were cooled and neutralized with dilute NH_4OH in the presence of 1-2 mL of 0.01% (w/v) EDTA solution and filtered. The resulting solution was quantitatively transferred into a 25 mL calibrated flask and mixed well and made up to the mark with deionized water.

A suitable aliquot (1-2 mL) of the final digested solution was pipetted into a 10 mL calibrated flask and the cobalt content was determined as described under the general procedure using tartrate as masking agent. The results of food and vegetable analyses by the spectrophotometric method were also found to be in excellent agreement with those obtained by AAS and ICP-OES. The results are shown in Table 9.

3.5.7. Determination of cobalt in pharmaceutical samples

Finished pharmaceutical samples (each Co containing tablets or 10 mL insulin or required weight) were quantitatively taken in a beaker and digested following a method recommended by Abrarin and Ahmed [50]. 10 mL of concentrated nitric acid was added and heated to dryness and then added 10 mL of 20% (v/v) of H_2SO_4. The mixture was heated with 2 mL of 2.5% (w/v) freshly prepared sodium azide solution was added below the boiling point for 5-10 min to complete reduction of Co(III) to Co(II). The excess azide was removed by heating and 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 1-2 mL of 0.01% (w/v) EDTA or tartrate solution. The resulting solution was then filtrated and quantitatively transferred to a 25 mL calibrated flask and made up to the mark with deionized water.

An aliquot (1-2 mL) of this digested sample was pipetted into a 10 mL calibrated flask and then cobalt content was determined as described under the general procedure using tartrate as a masking agent. The results of some pharmaceutical analyses by the spectrophotometric method were found to be in excellent agreement with those obtained by the reported values and the ICP-OES. The analyses of pharmaceutical samples from several Pharmaceutical Companies for cobalt are given in Table 10.

3.5.8. Determination of Cobalt (II) and Cobalt (III) speciation in mixtures

Suitable aliquots (1-2 mL) of cobalt (II+III) mixtures (preferably 1:1, 1:5, 1:10) were taken in a 250 mL Pyrex conical flask. A few drops (3-5 drops) of 4 M H_2SO_4, and 5-10 mL of 2.5% (w:v) freshly prepared sodium azide were added to reduce Co(III) to Co(II) and the mixture was heated gently with further

Table 10. Determination of cobalt in some pharmaceutical samples.

No	Sample type [a]	Brand name	Trade name	Cobalt (mg/kg or mg/L)				
				Reported value / Claimed value	Proposed method (n=5)		ICP-OES (n=5)	
					Found (n = 5)	RSD [b] (%)	Found (n = 5)	RSD [b] (%)
1	Tablet	ESKAYEF	Solbion	0.2	0.19	1.0	0.185	1.2
2	Tablet	Square	Methicol	0.5	049	1.5	0.485	1.5
3	Tablet	Chemico Lab. Ltd.	Kavit-M (Cyanocobalamin)	702.5	709.5	3.5	710.8	4.0
4	Tablet	Aristopharma	Neobion (Cyanocobalamin)	13.75	13.58	2.5	13.69	2.8
5	Tablet	Incepta Pharma Ltd.	Mecolagin (Mecobalamin)	307.25	308.98	3.0	309.18	3.5
6	Tablet	Renata	Anorexon DS (vet)	100.0	99.5	2.0	100.15	2.5
7	Tablet	Opsonin	Zovia Gold	6.0	5.985	2.0	5.99	2.5
8	Tablet	Incepta	Proviten A-Z	6.0	5.99	2.5	5.989	2.5
9	Syrup	ACME	Nutrum Junior	3.0[c]	2.98 [c]	2.5	2.99	2.0
10	Tablet	Square	Multivit Plus	6.0	5.989	2.5	5.99	2.5
11	Tablet	Square	Bicozinc	1.5	1.49	1.0	1.489	1.5
12	Tablet	Drug International Ltd.	Supravit-G	6.0	5.99	2.0	5.989	2.5
13	Tablet	Beximco	Bextram Gold	6.0	5.999	2.5	5.99	2.5

[a] Samples were collected from local market, Chittagong.

[b] The measure of precision is the relative standard deviation (RSD).

[c] Values in mg/dL.

Table 11. Determination of cobalt (II) and cobalt (III) speciation in mixtures.

No	Co(II):Co(III)	Co, taken (mg/L)		Co, found (mg/L)		Error (mg/L)	
		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	1:5	1.00	5.00	0.98	4.98	0.02	0.02
2	1:5	1.00	5.00	0.99	4.99	0.01	0.01
3	1:5	1.00	5.00	0.98	4.98	0.02	0.02
Mean error: Co(II) = ±0.016 Co(III) = ±0.016 Standard deviation: Co(II) = ±0.0058 Co(III) = ±0.0058							
1	1:10	1.00	10.00	0.99	9.99	0.01	0.01
2	1:10	1.00	10.00	0.98	9.98	0.02	0.02
3	1:10	1.00	10.00	0.98	9.98	0.02	0.02
Mean error: Co(II) = ±0.016 Co(III) = ±0.016 Standard deviation: Co(II) = ±0.015 Co(III) = ±0.015							

addition of 10 mL water, if necessary, for 5 minutes to drive off the excess azide, then the mixture was cooled to room temperature (25±5 °C) following the methods recommended by Ahmed *et al.* [50-53]. The reaction mixture was then cooled and neutralized with dilute NH_4OH in presence of 1-2 mL of 0.01% (w:v) EDTA solution. The solution was transferred quantitatively into a 25 mL volumetric flask and 2.5 mL of 7.45×10^{-3} M Salen reagent solution was added followed by the addition of 1 mL of 0.002 M H_2SO_4 and 3 mL of ethanol. It was made up to the mark with de-ionized water. The absorbance was measured then being cooled at room temperature (25±5 °C) at 459 nm against a reagent blank. The total cobalt content was calculated with the help of a calibration graph prepared concurrently.

An equal aliquot (1-2 mL) of the above cobalt (II + III) mixture was taken into a 250 mL Pyrex conical flask. The solution was neutralized with dilute NH_4OH in presence of 1-2 mL of 0.01% (w:v) EDTA solution. After, the content of the beaker was transferred quantitatively into a 25 mL volumetric flask, 2.5 mL of 7.45×10^{-3} M Salen reagent solution was added, followed by the addition of 1 mL of 0.002 M H_2SO_4. Then added 3 mL of ethanol to it. It was made up to the mark with de-ionized water. After 5 min the absorbance was measured following the general procedure at 459 nm against a reagent blank, as before. The cobalt concentration was calculated in mg/L or µg/L with the aid of a calibration graph. This gives a measure of cobalt (II) originally present in the mixture. This value was subtracted from that of the total cobalt to determine the cobalt (III) present in the mixture. The results of the assessment of speciation of Co(II) and Co(III) were found to be highly reproducible. The occurrence of such reproducible results is also reported for different oxidation states of cobalt [28]. The results of a set of determination are given in Table 11.

4. Conclusions

A new simple, sensitive and inexpensive method with the cobalt-Salen complex was developed for the determination of cobalt in some real, environmental, biological, soil, food and pharmaceutical samples, for continuous monitoring to establish the trace levels of cobalt in different samples matrices. Compared with other methods [15-20] in the literature Table 1, the proposed method has several remarkable analytical characteristics: (*i*) The proposed method is highly sensitive with molar absorptivity of the complex of 6.04×10^5 L/mol.cm. Thus, amount of ng/g of cobalt can be determined without preconcentration. (*ii*) The proposed method is very simple, rapid and stable. The reaction of cobalt (II) with Salen is completed rapidly in 1 min at room temperature so it does not involve any stringent reaction conditions and offer the advantage of high complex stability (24 h). (*iii*) The method has added the advantage of determining individual amounts of Co(II) and Co(III). With suitable masking agents, the reaction can be made highly selective.

The proposed method using Salen in aqueous solutions not only is one of the most sensitive methods for determination of cobalt but also is excellent in terms of selectivity and simplicity. Therefore, this method will be successfully applied to the routine monitoring of trace and ultra-trace amounts of cobalt in real, environmental, biological, soil, food and pharmaceutical samples. It is a new method needs neither heating nor extraction to organic phase, works satisfactorily and could be an alternative method for the rapid determination of cobalt in a wide variety of sample solutions and found superior to spectrophotometric methods described in different literature [15-20].

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CRediT authorship contribution statement CR

Conceptualization: Muhammad Jamaluddin Ahmed; Methodology: Tahmina Happy; Software: Tahmina Happy; Validation: Muhammad Jamaluddin Ahmed; Formal Analysis: Tahmina Happy; Investigation: Tahmina Happy; Resources: Muhammad Jamaluddin Ahmed; Data Curation: Tahmina Happy; Writing - Original Draft: Muhammad Jamaluddin Ahmed; Writing - Review and Editing: Muhammad Jamaluddin Ahmed; Visualization: Tahmina Happy; Supervision: Muhammad Jamaluddin Ahmed; Project Administration: Muhammad Jamaluddin Ahmed.

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