Full Length Research Paper

Spectrophotometric determination of cobalt (ii) in low concentrations using hydroxytriazene as selective chelating agents

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The present work describes a selective, rapid and simple spectrophotometric method for the determination of cobalt (II) at trace levels using some selected hydroxytriazene. The method is based on the reaction of hydroxytriazene at pH 5.5 to 6.2, 6.0 to 6.8, 7.1 to 7.35, 7.3 to 7.9, 6.75 to 7.25 and 7.2 to 7.75 for hydroxytriazenes no (i), (ii), (iii), (iv), (v) and (vi) respectively and ethanol media with cobalt (II) to produce a highly absorbent yellow coloured chelate product with an absorption maximum at 393,397,417,406,410 and 412 nm for hydroxytriazenes no. (i), (ii), (iii), (iv), (v) and (vi), respectively. The reaction is instantaneous and the absorption remains stable for 24 Hrs. The average molar absorption coefficient were 1.1×10^4, 1.2×10^4, 1.8×10^4, 2.5×10^4, 2.1×10^4, 1.9×10^4 mol^{-1} dm^3 cm^{-1} for hydroxytriazene no (i), (ii), (iii), (iv), (v), (vi) respectively and Sandell's sensitivity in ng/cm^2 were 5.4, 4.9, 3.3, 2.4, 2.9 and 3.1 for hydroxytriazene no. (i), (ii), (iii), (iv), (v) and (vi), respectively. The stoichiometric composition of the chelate is 1:3 for hydroxytriazene no (i), (iv), (v), (vi), 1:2 for (ii) and 1:1 for (iii). A large excess of over 30 cations, anions and complexing agent do not interfere in the determination at equivalent amounts. The method was successfully used in *Mussaenda arcu*. The method has high precision and accuracy.

Key words: Spectrophotometry, *Mussaenda arcu,* hydroxytriazene, cobalt, interference.

INTRODUCTION

Essential elements are of important in many biological systems. Metals can be found incorporated or naturally complexed in proteins, acting like the well known metalloenzymes and cofactors. The metal of interest in the study (Co) has a major biochemical role in biological systems especially in vitamin B12, a coenzyme in some biochemical processes (Sharga and Soomro, 2004).

Element complexing is an important chemical process in chemical analysis and separation of compounds, besides other uses. Spectrophotometric techniques offer considerable advantages in trace analysis and therefore the selection of more specific methods for the wide variety of environmental and biological sample analysis is very important especially where simpler procedures, low cost and high quality of analytical techniques is

guaranteed (Pearson and Seim, 1977).

Several spectrophotometric chelating reagents are available for the determination of the cobalt in trace amounts. For example β-nitroso-α-naphthol forms pink cobaltic complex in citrate medium at pH 2.5 to 5, but its disadvantage is having less sensitivity. Sodium1-nitroso-2-hydroxynaphthalene-3, 6-disulphonate which is preferred due to its sensitivity has disadvantages due to interferences from some of the other heavy metals and also its complex is usually formed in hot acetate-acetic acid medium. EDTA is a non selecting chelating reagent and also forms colourless solution which makes its use in spectrophotometric method limited.

Exposure to cobalt can occur through inhalation, oral or dermal (skin) routes (Jefferson et al., 2002). Human are exposed to natural sources of cobalt in their food, water and air (Agnihotri et al., 1999; Amin et al., 2001; Agwara et al., 2010; Birsen et al., 2000). In addition to naturally occurring forms in the environment cobalt substances

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may also be present in certain occupational settings and in some consumer products (Jamaluddin and Jakir, 2008). Cobalt in the form of vitamin B12 (hydroxocyanocobalamin) is essential in humans; vitamin B12 supports important synthetic reactions in metabolic processes and is essential for the production of red blood cells (Linna et al., 2004). In the past, cobalt was recommended for treating anemia, nephriis and infection in addition to the usual hemopoietic agents (Goizman et al., 2005; Jamaluddin and Jakir, 2008).

In clinical situations where decreased numbers of red blood cells were associated with anaemia, ingestion of inorganic cobalt {Co^{2+} ion in the form of cobalt chloride} stimulated an increase in the production of red blood cells{polycythemia or erythroytosis}(Jefferson et al., 2002). Having high amounts of cobalt for long periods of time could affect the heart and might decrease fertility in men. An excessively high intake of cobalt may damage the heart muscles and may cause a over-production of red blood cells or damage to the thyroid glands (Kaul et al., 2001). A deficiency of cobalt leads to anaemia, a lethal disorder. Increased consumption of vitamin B12 via food /or supplements would alleviate any deficiency symptoms (Jefferson et al., 2002; Linna et al., 2004). Therefore, the accurate determination of cobalt at trace levels using simple, rapid and selective methods is paramount importance.

The synthesized hydroxytriazenes were used to determine the amount of cobalt in *Mussaenda arcu* Poir (Local name: Adieremo), which is a shrub of about 2 to 4.4 m high. It has elliptic leaves which are cuneate to rounded, apex acuminate, 4 to 14 by 2 to 6 cm, glabrous o sparsely pubescent. The shrub has yellow flowers with orange hairy centre and it produces blue fruits which are subglobose or ellipsoid. It is found in wooded bush land forests margins. The reason it was chosen for this study is because it is used for local treatment of anemic patients (Kokwaro and Johns, 1972).

Spectrophotometry is essentially a trace analysis technique and is one of the most powerful tools in chemical analysis. The six reagents have never been used as a spectrophotometric method for the trace determination of cobalt. The objective of this work is therefore to come up with a spectrophotometric method which is selective, sensitive, rapid, involving simple procedures and providing high quality results with less costs for cobalt determination in trace amounts.

MATERIALS AND METHODS

Experimental apparatus

Systronics double beam UV-V is recording spectrophotometer 108 (Brand: Systronics, manufacturer: Systronics (India) Limited) and a Systronics pH meter 324 with a combination of electrode were used for the measurements of absorbance and pH, respectively. Atomic absorption spectrophotometer (model CTA 2000 AAS) was used for Comparing the results. The melting point and composition

verification of hydroxytriazenes were carried out using melting point apparatus {Model Kruess M500} and elemental analyzer {Perkin Elmer 2400 Series II CHN Analyzer}, respectively.

Reagents and solutions

All the chemicals used were of analytical reagent grade. Doubly distilled deionized water, which is non-absorbent under visible radiation, was used throughout. Glass vessels were cleaned by soaking in acidic solutions of K_2Cr_2O7, followed by washing with concentrated HNO_3 and rinsed several times with deionized water. Stock solutions of 1000 ml were kept in polyethylene bottles containing 1 ml of concentrated HNO_3.

Synthesis and characterization of hydroxytriazenes

The six hydroxytriazenes were synthesized according to the recommended method (Purohit et al., 2003; Sharma et al., 2006), by coupling of arylhydroxylamine in sodium acetate medium of pH 5 to 6 and temperature range of 0 to 5°C. The yellow precipitate formed was filtered off on cooling, washed with ethanol and recrystallized from ethanol and dried under vacuum over silica gel. The prepared hydroxytriazenes were characterized by elemental analyses and melting point determination apparatus.

Solutions for spectrophotometric determination

Standard stock solution of cobalt (II)

A 1.0×10^{-2} M stock solution of cobalt (II) was prepared by dissolving required quantity of A.R grade cobalt nitrate hexahydrate in double distilled deionized water. To prevent hydrolysis a few drops of concentrated nitric acid were added to the solution. These solution was then standardized using 1.0×10^{-2} M EDTA solution and xylenol orange as indicator.

Reagent solution

Stock solution of each hydroxytriazene (1.0×10^{-2} M) was prepared by dissolving the required quantity of it in ethanol.

Solutions for pH adjustments

1.0% tris-buffer (1.0 gm of tris-buffer was dissolved in minimum quantity of water and then diluted to 100 ml with alcohol) and 1% perchloric acid solution (1.0 ml of perchloric acid was diluted to 100 ml with alcohol) were used to adjust the desired pH.

Solutions for atomic absorption spectrophotometric determination

Standard solutions

Standard solutions for cobalt were prepared from 1000 ppm spectro econ stock solution by serial dilution to the range of standards as recommended by the manufacturers of AAS CTA 2000 model.

Sample collection and treatment

Preparation of sample solutions

The samples were collected from different villages of Mfangano Island in Nyanza province Kenya. Samples were dried in air,

grounded into fine powder and mixed by coning and quartering to obtain an analytical sample. 100.0 gms sample was taken into a 500 ml Kjeldahl flask.10 ml of 60% $HClO_4$ was then added .This was followed by swirling gently and digesting slowly at moderate heat. The heating was increased later. Digestion continued until white fumes appeared. The solution was then cooled and filtered into 50 ml volumetric flask through Whatmann Filter paper No.44.The solution was thereafter made to the mark using doubled distilled water. The blank determination was also prepared in similar manner (Stewart, 2003).

Determination of working wavelength

1 ml of 5 x 10^{-4} M Co^{2+} was taken into a 10 ml volumetric flask. Then 5 ml of 5x 10^{-4} M hydroxytriazenes was added. The pH of this solution was adjusted to pH of 6.15 to 6.5 using tris-buffer and perchloric acid .The solution was topped to the mark using double distilled deionized water. The absorbance was then determined in entire visible region against reagent blank. The spectrum of reagent was also measured in the same wavelength region against ethanol. The plot of absorbance against wavelength was then done on the same graph. The working wavelength was chosen where there was maximum difference between the absorbance due to complex and the reagent.

Determination of pH range

A set of solutions having concentrations of Co^{2+} and hydroxytriazenes were prepared in such a manner that, the ratio of [M]:[R] was 1:5 .The pH of these solutions was adjusted in such a manner that, each solution was having the pH different from others. Then the absorbance of each solution was measured against a reagent blank. The absorbance obtained was then plotted against the corresponding pH.

Molar composition of Cobalt (II) complex with each of the hydroxytriazenes

Three different methods, namely Job's method, mole ratio method and slope ratio method of Harves and Manning were used for the determination of molar composition of metal: reagent.

Validity of Lambert-beer's law

Under corresponding optimum conditions of pH, solvent and cobalt (II) to reagent ratio, the validity of Beer –Lambert's Law was studied. The results of absorbance obtained were plotted against the corresponding concentration of cobalt (II). The calibration curve obtained was used to determine the concentration of cobalt in sample solution.

Precision studies

This was carried out by measuring the absorbance of ten solutions. Each solution contained the same concentration of cobalt (II) and the reagent. The absorbance was measured against reagent blank under optimum conditions of pH, solvent and cobalt (II) to reagent ratio. On the basis of their absorbance values, standard deviation was determined.

Interference studies

Cobalt (II) was determined with each reagent under its optimum conditions of pH, solvent and cobalt (II) to reagent ratio, in the presence of equivalent amounts, five and ten fold excess of 35 diverse ions. From group VIII elements only Ni^{2+} was checked for interference, this was due to time and financial limitations.

Procedure for atomic absorption spectrophotometric analysis

The instrument was put on and left to warm up for about 0.5 h. The optimum operating conditions were set before any measurement as recommended by the manufacturer. Thereafter, aspiration of the sample, series of standard containing the sample having the same concentration of cobalt (II) were aspirated hence standard addition method was used.

Hydroxytriazene used as a complexing reagent in spectrophotometric determination of Cobalt (II)

(i). 3-hydroxy-3-0-tolyl-1-p-sulphonato (sodium salt) phenyltriazene
(ii). 3-hydroxy-3-0-tolyl-1-o-carboxyphenyltriazene
(iii). 3-hydroxyl-3-m-tolyl-1-o-carboxyphentyltriazxene
(iv). 3-hydroxy-3-m-tolyl-1-p-methoxyphenyltriazene
(v). 3-hydroxy-3-p-tolyl-1-m-hydroxyphenyltriazene
(vi). 3-hydroxy-3-m-tolyl-1-m-hydroxyphenyltriazene.

Quality control

The quality of the method was compared with the known reagent for cobalt spectrophotometric analysis, nitroso-R-salt [sodium-1-nitroso-2-hydroxyl naphthalene-3, 6-disulphonate). It was found out that the hydroxytriazenes synthesized were better reagents, with higher sensitivity and selectivity and also time saving. The purity of standard hydroxytriazenes and other reagents were checked for the presence of cobalt (II) using AAS.

RESULTS AND DISCUSSION

Table 1 consists of the results of the physical characteristics and elemental analysis. The hydroxytriazenes prepared were either light yellow coloured shining needles or orange–yellow coloured needles. The melting point of these compounds ranged from 112 to 178°C. Ethanol was used to crystallize these compounds. The results of C, H, and N indicate that the theoretical values and experimental values are identical.

The spectrum of the complex formed with each reagent ([M]:[R]=1:5) was obtained in wavelength region 375 to 500 nm against a reagent blank. Further, spectrum of reagent was also measured in the same wavelength region against ethanol. The working wavelength was chosen such that there was maximum difference between the absorbance due to complex and the reagent. The result in Table 2 indicates that, the working wavelengths are very close to λ max. This ensures that sensitivity is high, molar absorptivity remains constant and Lambert-beers law is obeyed.

The absorbance of a series of solutions containing cobalt (II) and reagent in the molar ratio 1:5 ([M]:[R]) was measured against reagent blank at corresponding working wavelength at various pH values to determine

Table 1. Physical characteristic and elemental analysis of hydroxytriazenes.

Reagent No.	Synthesized hydroxytriazene	Physical characteristics		M.P	Elemental analysis						Molecular formula
		Colour and shape of crystals	Crystallized from		C (%)		H (%)		N (%)		
					Th	Exp	Th	Exp	Th	Exp	
i	3-Hydroxy-3-o-tolyl-1-p-sulphanato (sodium salt)phenyltriazene)	Light yellow shining needles	ethanol	128	47.39	47.26	3.67	3.65	12.76	12.37	$C_{13}H_{12}N_3O_4SNa$
ii	3-Hydroxy-3-o-tolyl-1-o-carboxyphenyltriazene	light yellow shining needles	ethanol	138	61.96	60.48	4.83	4.63	15.49	14.69	$C_{14}H_{13}N_3O_3$
iii	3-Hydroxy-3-m-tolyl-1-o-carboxyphenyltriazene	Light yellow shining needles	ethanol	178	61.96	61.44	4.83	4.69	15.49	14.66	$C_{14}H_{13}N_3O_3$
iv	3-Hydroxy-3-m-tolyl-1-p-methoxyphenyltriazene	Light yellow shining needles	ethanol	112	65.33	64.56	5.88	5.77	16.34	16.87	$C_{14}H_{15}N_3O_2$
v	3-Hydroxy-3-p-tolyl-1-m-hydroxyphenyltriazene	Orange- yellow needles	ethanol	124	64.16	63.40	5.38	5.03	17.28	17.89	$C_{13}H_{13}N_3O_2$
vi	3-Hydroxy-3-m-tolyl-1-m-hydroxyphenyltriazene	Orange- yellow needles	ethanol	122	64.16	63.21	5.38	4.87	17.28	17.63	$C_{13}H_{13}N_3O_2$

Table 2. Spectrophotometric determination of cobalt (ii) with hydroxytriazenes.

Reagent No.	Composition of the complex Co:R	Colour of the complex	λmax in nm	Working wavelength nm	Optimum pH range	Beer's law rangex 10^{-5}M	Molar absorptivity in $mol^{-1}dm^3cm^{-1}$	Sandell's sensitivity ng/cm2	Stability constant for complex	Free energy of formation of the complex at 25°C at Kcal/mol	Standard deviation in ppm
(i)	1:3	Yellow	387	393	5.5-6.20	0.4-2.4	11,000	5.357	5.24×10^{14}	-19.383	0.021
(ii)	1:2	Yellow	392	397	6.0-6.8	0.3-1.8	11,944	4.934	3.100×10^{11}	-15.4012	0.012
(iii)	1:1	Yellow	409	417	7.1-7.35	0.2-1.2	18,000	3.274	1.609×10^7	-9.336	0.010
(iv)	1:3	Yellow	399	406	7.3-7.9	0.15-0.9	24,556	2.400	5.519×10^{15}	-21.733	0.007
(v)	1:3	Yellow	403	410	6.75-7.25	0.2-1.2	20,167	2.922	1.733×10^{16}	-22.012	0.009
(vi)	1:3	Yellow	406	412	7.2-7.75	0.2-1.2	19,333	3.048	1.719×10^{15}	-20.433	0.009

pH range of constant and maximum absorbance. The pH of the solution was adjusted using tris-buffer and perchloric acid solutions. The colour developed in pH range of constant absorbance was yellow for each reagent. The colour development in each case was

Table 3. Determination of cobalt (II) in prescence of interfering species at equivalent amount.

Reagent no.	Working wavelength (nm)	pH range	$[Co^{2+}] \times 10^{-5}$ M	Absorbance in absence of interfering species	$[Co^{2+}]:[R]$	Absorbance when interfering species was present						
						Cl^-	CH_3COO^-	CO_3^{2-}	Br^-	PO_4^{3-}	SO_4^{2-}	$C_2O_4^{2-}$
(i)	393	5.5-6.2	3	0.327	1:6	0.329	0.321	0.326	0.322	0.219	0.325	0.310
(ii)	397	6.0-6.8	2	0.242	1:6	0.241	0.240	0.240	0.237	0.209	0.242	0.230
(iii)	417	7.1-7.35	1.5	0.270	1:6	0.270	0.261	0.267	0.269	0.160	0.268	0.181
(iv)	406	7.3-7.9	1.5	0.374	1:6	0.367	0.367	0.376	0.377	0.344	0.371	0.365
(v)	410	6.75-7.25	2	0.412	1:6	0.422	0.406	0.406	0.402	0.216	0.402	0.380
(vi)	412	6.75-7.25	1.5	0.290	1:6	0.290	0.294	0.297	0.285	0.123	0.290	0.278

Reagent no.	Absorbance when interfering anions were present									
	I^-	$S_2O_3^{2-}$	NO^{2-}	SO_3^{2-}	F^-	S^{2-}	HPO_4^{2-}	NO_3^-	$Mo_7O_{24}^{6-}$	WO_4^{2-}
(i)	0.327	0.320	0.328	0.327	0.327	0.329	0.301	0.325	0.321	0.326
(ii)	0.242	0.242	0.238	0.240	0.244	0.245	0.210	0.242	0.240	0.239
(iii)	0.276	0.270	0.265	0.268	0.270	0.267	0.193	0.269	0.262	0.188
(iv)	0.369	0.367	0.376	0.365	0.368	0.377	0.297	0.374	0.370	0.370
(v)	0.403	0.416	0.412	0.418	0.403	0.422	0.320	0.410	0.412	0.416
(vi)	0.286	0.289	0.286	0.297	0.293	0.293	0.167	0.290	0.290	0.283

Reagent no.	Absorbance when interfering cations were present									
	K^+	UO^{2+}	Mn^{2+}	Ba^{2+}	Pb^{2+}	Hg^{2+}	Sn^{2+}	Th^{4+}	Cd^{2+}	Mg^{2+}
(i)	0.320	0.334	0.330	0.323	0.327	0.321	0.253	0.321	0.327	0.324
(ii)	0.242	0.242	0.237	0.242	0.265	0.244	0.240	0.242	0.236	0.236
(iii)	0.268	0.275	0.272	0.271	0.270	0.269	0.273	0.267	0.270	0.263
(iv)	0.368	0.366	0.367	0.371	0.369	0.374	0.375	0.369	0.374	0.369
(v)	0.408	0.420	0.408	0.414	0.420	0.420	0.280	0.404	0.410	0.410
(vi)	0.296	0.288	0.287	0.290	0.291	0.293	0.108	0.290	0.289	0.289

Reagent no.	Absorbance when interfering cations were present						
	Ca^{2+}	NH_4^+	ZrO^{2+}	Na^+	Ni^{2+}	Cu^{2+}	Zn^{2+}
(i)	0.326	0.320	0.323	0.329	0.293	0.320	0.325
(ii)	0.239	0.241	0.240	0.239	0.557	0.323	0.350
(iii)	0.273	0.271	0.274	0.270	0.534	0.320	0.359
(iv)	0.369	0.371	0.369	0.369	0.538	0.453	0.484
(v)	0.420	0.410	0.402	0.408	0.572	0.416	0.416
(vi)	0.285	0.291	0.284	0.291	0.406	0.290	0.297

Table 4. Determination of cobalt (II) in prescence of interference species at five fold excess.

Reagent no.	Working wavelength (nm)	pH range	$[Co^{2+}] \times 10^{-5}$ M	Absorbance in absence of interfering species	$[Co^{2+}]:[R]$	Absorbance when interfering species was present						
						Cl^-	CH_3COO^-	CO_3^{2-}	Br^-	PO_4^{3-}	SO_4^{2-}	$C_2O_4^{2-}$
(i)	393	5.5-6.2	3	0.327	1:6	0.329	0.327	0.329	0.320	-	0.326	-
(ii)	397	6.0-6.8	2	0.242	1:6	0.242	0.240	0.245	0.238	-	0.237	0.173
(iii)	417	7.1-7.35	1.5	0.270	1:6	0.274	0.265	0.265	0.267	-	0.261	-
(iv)	406	7.3-7.9	1.5	0.374	1:6	0.367	0.372	0.377	0.376	-	0.365	0.218
(v)	410	6.75-7.25	2	0.412	1:6	0.416	0.402	0.410	0.414	-	0.402	-
(vi)	412	6.75-7.25	1.5	0.290	1:6	0.294	0.290	0.294	0.287	-	0.287	-

Reagent no.	Absorbance when interfering anions were present									
	I^-	$S_2O_3^{2-}$	NO_2^-	SO_3^{2-}	F^-	S^{2-}	HPO_4^{2-}	NO_3^-	$Mo_7O_{24}^{6-}$	WO_4^{2-}
(i)	0.321	0.294	0.299	0.364	0.320	0.323	-	0.328	0.319	0.319
(ii)	0.237	0.244	0.242	0.312	0.234	0.242	-	0.238	0.214	0.224
(iii)	0.264	0.189	0.263	0.153	0.263	0.270	-	0.271	0.263	-
(iv)	0.367	0.365	0.369	0.357	0.354	0.376	-	0.379	0.369	0.379
(v)	0.402	0.409	0.405	0.416	0.406	0.405	-	0.402	0.380	0.407
(vi)	0.283	0.283	0.282	0.298	0.285	0.296	-	0.287	0.277	0.281

Reagent no.	Absorbance when interfering cations were present									
	K^+	UO^{2+}	Mn^{2+}	Ba^{2+}	Pb^{2+}	Hg^{2+}	Sn^{2+}	Th^{4+}	Cd^{2+}	Mg^{2+}
(i)	0.319	0.326	0.314	0.327	0.319	0.327	-	0.299	0.325	0.321
(ii)	0.245	0.249	0.243	0.243	-	0.249	0.229	0.273	0.229	0.239
(iii)	0.265	0.280	0.263	0.274	0.263	0.263	0.143	0.349	0.173	0.265
(iv)	0.367	0.368	0.380	0.373	0.390	0.383	0.312	0.328	0.365	0.367
(v)	0.410	0.416	0.418	0.408	0.402	0.406	-	0.220	0.407	0.421
(vi)	0.293	0.284	0.291	0.283	0.286	0.283	-	0.200	0.281	0.293

Reagent no.	Absorbance when interfering cations were present						
	Ca^{2+}	NH_4^+	ZrO^{2+}	Na^+	Ni^{2+}	Cu^{2+}	Zn^{2+}
(i)	0.326	0.325	0.319	0.325	-	0.267	0.267
(ii)	0.238	0.248	0.246	0.238	-	-	-
(iii)	0.277	0.269	0.279	0.270	-	-	-
(iv)	0.365	0.369	0.364	0.370	-	-	-
(v)	0.417	0.408	0.412	0.402	-	0.544	0.404
(vi)	0.295	0.290	0.289	0.289	-	0.297	0.395

- Indicates that interfered at lower concentration and hence interference studies at next concentration.

Table 5. Determination of cobalt (Ii) In prescence OF interference species at ten fold excess.

Reagent no.	Working wavelength (nm)	pH range	$[Co^{2+}]$ x 10^{-5} M	Absorbance in absence of interfering species	$[Co^{2+}]$:$[R]$	Absorbance when interfering species was present						
						Cl^-	CH_3COO^-	CO_3^{2-}	Br^-	PO_4^{3-}	SO_4^{2-}	$C_2O_4^{2-}$
(i)	393	5.5-6.2	3	0.327	1:6	0.329	0.320	0.323	0.328	-	0.318	-
(ii)	397	6.0-6.8	2	0.242	1:6	0.242	0.240	0.244	0.236	-	0.225	-
(iii)	417	7.1-7.35	1.5	0.270	1:6	0.271	0.269	0.263	0.264	-	0.177	-
(iv)	406	7.3-7.9	1.5	0.374	1:6	0.367	0.370	0.373	0.375	-	0.341	-
(v)	410	6.75-7.25	2	0.412	1:6	0.418	0.407	0.402	0.418	-	0.260	-
(vi)	412	6.75-7.25	1.5	0.290	1:6	0.293	0.293	0.295	0.289	-	0.122	-

Reagent no.	Absorbance when interfering anions were present									
	I^-	$S_2O_3^{2-}$	NO^{2-}	SO_3^{2-}	F^-	S^{2-}	HPO_4^{2-}	NO_3^-	$Mo_7O_{24}^{6-}$	WO_4^{2-}
(i)	0.278	-	-	-	0.263	0.284	-	0.320	0.250	0.289
(ii)	0.230	0.222	0.220	-	0.214	0.246	-	0.241	-	-
(iii)	0.174	-	0.197	-	0.179	0.263	-	0.267	0.188	-
(iv)	0.341	0.370	0.328	-	0.347	0.374	-	0.368	0.371	0.354
(v)	0.234	0.254	0.399	0.262	0.254	0.388	-	0.406	-	0.254
(vi)	0.111	0.240	0.131	0.133	0.151	0.281	-	0.293	-	0.259

Reagent no.	Absorbance when interfering cations were present									
	K^+	UO^{2+}	Mn^{2+}	Ba^{2+}	Pb^{2+}	Hg^{2+}	Sn^{2+}	Th^{4+}	Cd^{2+}	Mg^{2+}
(i)	0.325	0.229	-	0.316	0.300	0.319	-	-	0.258	0.319
(ii)	0.241	0.253	0.249	0.245	-	0.242	-	-	-	0.242
(iii)	0.271	0.287	0.282	0.279	0.290	0.261	-	-	-	0.269
(iv)	0.371	0.369	0.387	0.375	-	0.395	-	-	0.339	0.365
(v)	0.414	0.404	0.406	0.388	0.398	0.326	-	-	0.246	0.422
(vi)	0.295	0.257	0.297	0.289	0.306	0.278	-	-	0.191	0.297

Reagent no.	Absorbance when interfering cations were present						
	Ca^{2+}	NH_4^+	ZrO^{2+}	Na^+	Ni^{2+}	Cu^{2+}	Zn^{2+}
(i)	0.328	0.327	0.228	0.325	-	-	-
(ii)	0.239	0.239	0.223	0.240	-	-	-
(iii)	0.280	0.263	0.285	0.270	-	-	-
(iv)	0.366	0.373	0.302	0.374	-	-	-
(v)	0.426	0.410	0.421	0.412	-	-	0.382
(vi)	0.299	0.290	0.247	0.289	-	0.210	-

- Indicates that interfered at lower concentration and hence interference studies at next concentration not done.

Table 6. Determination of cobalt in BAS-5g Brass, {Cu=67.4%, Sn=1.09%, Pb=2.23%, Zn=28.6%, Ni=0.33%, P=0.01%) a certified reference material.

Reagent no.	Cobalt		
	Certified value in mg/L	Found (n=5) in mg/l	RSD (%)
(i)	1.0	1.053	1.69
(ii)	1.0	1.048	1.48
(iii)	1.0	1.041	1.56
(iv)	1.0	1.017	1.32
(v)	1.0	1.028	1.45
(vi)	1.0	1.031	1.37

Table 7. Estimation of cobalt (II) in *Mussaenda arcu* using uv-visible spectrophotometric and atomic absorption spectrophotometric methods.

Reagent no.	Sampling points/concentrations (Mg/Kg)						Mean	% Error	F-test at the 95% confidencE level
	SENA	NYAKWERI	UOZI	UNGINA	SOKLO	GULWE			
(iv)	0.551	0.530	0.562	0.522	0.489	0.511	0.527	+4.98	1.998
AAS	0.526	0.510	0.512	0.500	0.471	0.495	0.502	-	-

instantaneous, stable for about 24 h. The data for pH range and type of colour developed have been incorporated in Table 2. The molar composition of cobalt (II) complex with each of the hydroxytriazenes has been found using three different methods, namely: Job's method, mole ratio method and slope ratio method of Harvey and Manning, under corresponding optimum conditions of pH, solvent and cobalt (II) to reagent ratio. The results have been included in Table 2. The ratio of [M]:[R], which was employed during verification of Labert-beer's law was obtained from mole ratio curve and was such that, absorbance remained constant as the concentration of reagent was being increased and this contributed to Lambert-beer's law being obeyed.

The stability constants of cobalt (II) complexes were determined using Harvey and Manning method. The results from Table 2 reveals that, the complex formed by reagent (iv) and (v) are more stable while that formed by reagent (iii) is the least stable. The free energy of formation were calculated and the results indicates that during the formation of the complex between Co^{2+} and reagent (iv) and (v) high amount of energy is given out while using reagent (iii) the energy produced was least.

Sandell's spectrophotometric sensitivity was calculated from the values of molar absorptivity. This value indicates that reagent (iv) is the most sensitive while reagent (i) is the least sensitive.

The precision studies were carried out in order to check the reliability of the results of cobalt (II) determination with each of the hydroxytriazenes and the results have been given in Table 2. A close examination of Table 2 shows a fair replication of the results with the reagent studied.

Cobalt (II) has been determined spectrophotometrically in the presence of 35 diverse ions at equivalent amounts; five fold excess with each of the hydroxytriazenes under their respective optimum conditions. The results obtained from these studies have been given in Tables 3 to 5. From these results Cl^-,CH_3COO-,CO_3^{2-},Br^-,SO_4^{2-},I^-,NO_3^-, K^+,Ba^{2+},Hg^{2+},Mg^{2+},Ca^{2+},NH_4^+,Na^+, do not interfere with the determination of cobalt (II) even if these are present in ten fold excess with all hydroxytriazenes used. PO_4^{3-}, $C_2O_4^{2-}$,HPO_4^{2-}·Sn^{2+},Ni^{2+},Cu^{2+},Zn^{2+} interfered with cobalt (II) determination with all hydroxytriazenes even at equivalent amounts. These tables of interferences reveals that, reagent nos (vi), (v) and (iv) are the most selective reagents while reagent nos (i), (ii) and (iii) are the least selective reagent.

The validity of the method was tested by analyzing a standard reference material Table 6 and also comparing the results with convectional analysis (AAS) (Table 7).

The estimation of cobalt (II) in *M. arcu* samples were done spectrophotometrically using the most sensitive, selective hydroxytriazenes and the results obtained were compared with those of atomic absorption spectrometer. The results obtained have been summarized in Table 7. The concentration of cobalt in the sample was ranging from 0.489 to 0.562 mg/kg, 0.47 to 0.526 mg/kg for reagent no (iv) and AAS, respectively. The percentage error of reagent no (iv) in comparison with AAS was +4.98%. The slight differences in values using hydroxytriazenes and AAS might have been due to the presence of foreign species which interfered with the determination of cobalt (II).

The tabulated F-value for V1=5 and V2=5 is 5.05.Since the calculated value is less than this, hence there is no significant difference in the precision of the two methods. The detection limit (3S of the blank) was found to be 2.0 ng/ml which is comparable with that of AAS used (1.0

ng/ml). Thus this method is suitable for the determination of cobalt in a wide range of application in environmental and biological samples.

Conclusion

Six hydroxytriazenes are introduced as new reagents for spectrophotometric determination of cobalt (II). One of these is very selective, sensitive and can be used for the determination of cobalt in the presence of a large number of cations and anions and the results are comparable with those of atomic absorption spectrophotometer.

This hydroxytriazenes can be used to determine cobalt content in *M. arcu* samples. However, the differences obtained in the present method and AAS might be due to the following cations and anions which might have interfered with the determination: PO_4^{3-}, HPO_4^{2-}, and Zn^{2+}.

The ease of preparation over many reported reagents, wide pH range, easy methods (direct method) for spectrophotometric determination of cobalt (II), better yield are further advantages of hydroxytriazenes.

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