Spectrophotometric determination of Micro amount of Cobalt (II) in raw Milk by Using (Antipyriyl azo 1-Nitroso-2-naphthol) as New reagent

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Abstract

A new , simple , sensitive and rapid spectrophotometric method is proposed for the determination of trace amount of cobalt (II) . The method is based on the formation of a 2:1 complex with 3-(4′- antipyriyl azo) 1- nitroso-2-naphthol (APAN) as a new reagent is developed . The complex has a maximum absorption at 432 nm and εmax of 3.3 X 104 L. mol-1. cm-1 . A linear correlation (0.1 – 2.5 µg. ml-1) was found between absorbance at λmax and concentration . The accuracy and reproducibility of the determination method for various known amounts of cobalt (II) were tested . The results obtained are both precise (RSD was better than 0.60 %) and accurate (relative error was better than 0.2 %) . The effect of diverse ions on the determination of cobalt (II) to investigate the selectivity of the method were also studied . The stability constant of the product was 1.0 X 108 L. mol-1 . The proposed method was successfully applied to the analysis of synthetic mixtures and raw milk without any preliminary concentration or separation.

Key Word:- Cobalt (II) determination , spectrophotometry , antipyriyl azo 1-nitroso-2-naphthol , milk raw .

Introduction

Azo compounds are the most widely class of industrial synthesized organic dyes due to their versatile application in various fields such as dyeing textile fiber,biological-pharmacological activities and advanced application in organic synthesis[(1)]. azo compounds are know to be involved in a number of biological reaction such as inhibition of DNA,RNA and protein synthesis[(2)].

Heavy metals are grouped within the category of environmental toxins; it is however a fact that many metals to survive. They play significant roles in biological processes, in diseases, as well as in familial amyotrophic lateral sclerosis and others.[3-6] . Cobalt is know to be an essential trace element to man for metabolic processes . It is the core of vitamin B_{12} and possesses anti-anemic properties. However, some cobalt compounds are carcinogenic at higher concentrations. Due to these properties, the determination of cobalt in biological and environmental samples is of great significance from the public health environmental point of view. [7-9] . In chemical analysis , metal chelation followed by solvent extraction and spectrophotometric detection is the preferred mode of analysis for a number of metal ions[10,11] .due to its rapidity , simplicity and wide applications . Pyrazolon azo forms colored water insoluble complexes with a large number of metal ions[12]. At present , several techniques such as ions chromatography(13), liquid-liquid extraction with atomic absorption spectrometry(14), atomic fluorescence spectrometry(15), X-ray fluorescence spectrometry(16,17) and inductively coupled plasma atomic emission spectrometry (18) , show good sensitivity but is limited because of expensive instrumentation and high cost for routine analysis . According to the best of our knowledge , this reagent has not been reported in the literature as being used for any cation determination . In this study , we wish to report this reagent as a selective reagent in spectrophotometric determination of micro amounts of cobalt (II) .

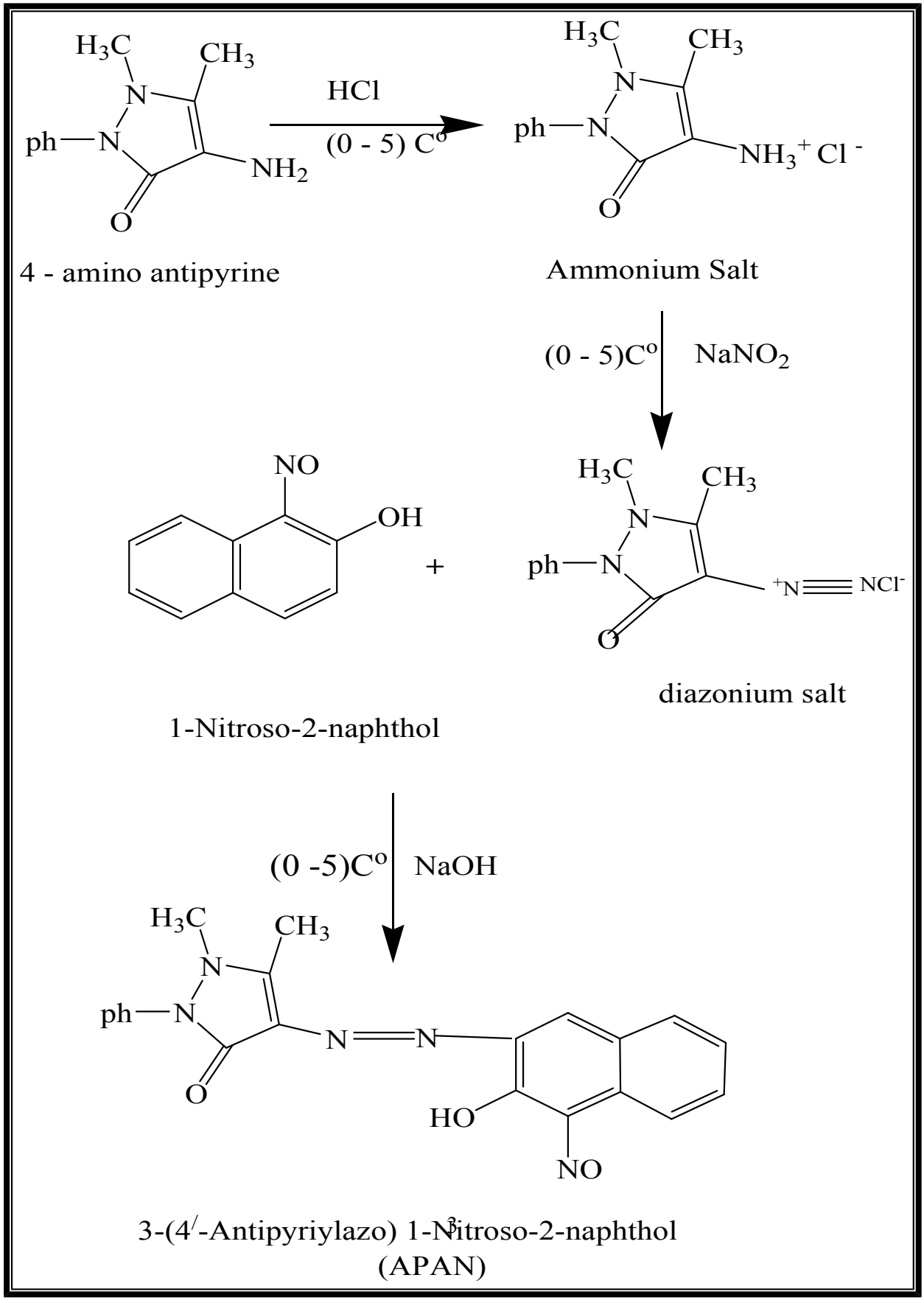
Experimental

I/ Preparation of the reagent (APAN)

The reagent was prepared by coupling 1-nitroso-2-naphthol with diazotate 4-amino antipyrine in alkaline alcoholic solution. A diazonium solution was prepared by taking

1 g 4-amino antipyrine in 25 mL of ethanol and concentrated hydrochloric acid with 5 mL of distilled water and adding sodium nitrite solution drop wise at (0-5 C^0). 1-nitroso-2-naphthol 1.2 g was dissolved in 25 mL of ethanol and 30 mL of 0.1 M from sodium hydroxide were added at (0-5 C^0) .The mixture was left to stand over night. The precipitate was filtered off and recrystallized from

ethanol[19].Schem1.



Scheme.1 preparation of reagent (APAN)

II/Preparation of Cobalt(II)complex

The complex was prepared by stoichiometric amount from ligand in 100 mL of ethanol then
added drop wise with stirring to a stoichiometric amount 2:1 for cobalt salt in 50 mL hot
distilled water. The solid product thus formed off, washed with ethanol and dried.

Apparatus

Spectrophotometric measurement were made with **Shimadzu UV – visible – 1700 double beam spectrophotometer** using 1.00 cm glass cells . Vibrational spectra were recorded on **Test scan Shimadzu FT.IR 8000 series** . Measurements of pH were made using an **Hanna, HI9811-5 pH – meter** equipped with a glass – saturated calomel combined electrode Melting points of both ligand and complex were obtained with an electrothermal melting point apparatus. Conductivity was measured in DMSO (10^{-3}) solution with an Alpha digital conductivity model -800. Elemantal analysis (C.H.N) were carried out with a **EuroEA Elemental Analyser.**

Reagents

All chemicals used were of analytical grades

Cobalt (II) stock solution (100 µg . ml^{-1})

prepared by dissolving 0.0807 g of cobalt chloride in 200 ml of distilled water , working standard of Co (II) solutions were prepared by simple dilution of the appropriate volume of the standard Co (II) solution (100 µg . ml^{-1}) with distilled water .

3 –(4'- antipyriyl azo)1-nitroso-2-naphthol (1 mM)

0.097 g of reagent was dissolved in 250 ml of ethanol .

Foreign ion solutions (10 µg . ml^{-1})

These solutions were prepared by dissolved an amount of the compound in distilled
water completing the volume in a volumetric flask .

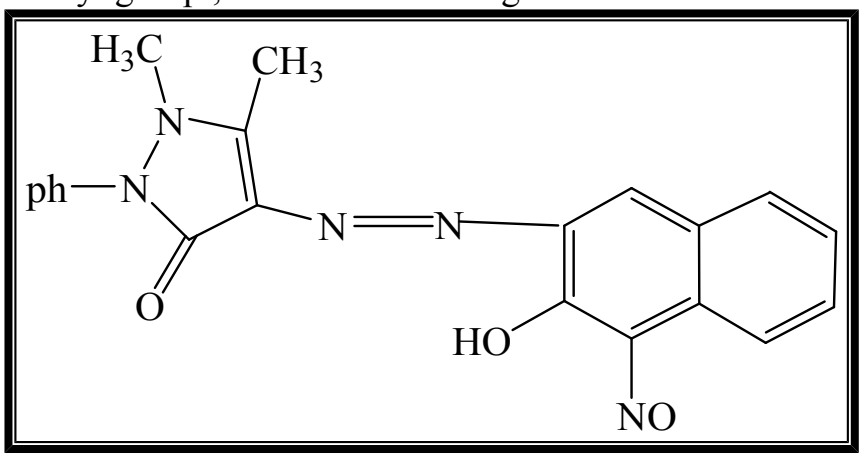
General Procedure

In to a series of 10 ml calibrated flask, transfer increasing volumes of Co(II) working solution 10 ppm to cover the range of calibration curve, add 2 ml 0f 1Mm of (APAN) solution and pH was adjusted to 8.5. The complexes formed were solubilized in water and diluted up to 10 ml in a standard flask . The absorbance of the resulting solution was measured at the respective absorption maxima against a reagent blank prepared under similar condition .

Results and Discussion

Properties of (APAN) and its metal chelate

APAN is a tridentate with coordination of azo group nitrogen , hydroxyl group and carbonyl group ; it has the following structure :



structure of APAN

Owing to the large conjugated system , the compound showed excellent chelating ability to form inner metal chelates . APAN and its metal chelates can be easily solubilized in an aqueous solution .Elemental analyses were carried out on the resulting compound (C% calc. 65.11 ; found 64.46, H% calc.4.42 ; found 4.15, N% calc.18.08 ; found 17.99). (m.p (201 C^0

Spectra

The result of this work indicated that the reaction of Co (II) with APAN at pH yield highly soluble product which can be utilized as a suitable assay procedure for Co (II) . This product has a maximum absorption at 432 nm at which the blank at this wavelength shows zero absorbance Fig. 1 was adopted in all subsequent experiments .

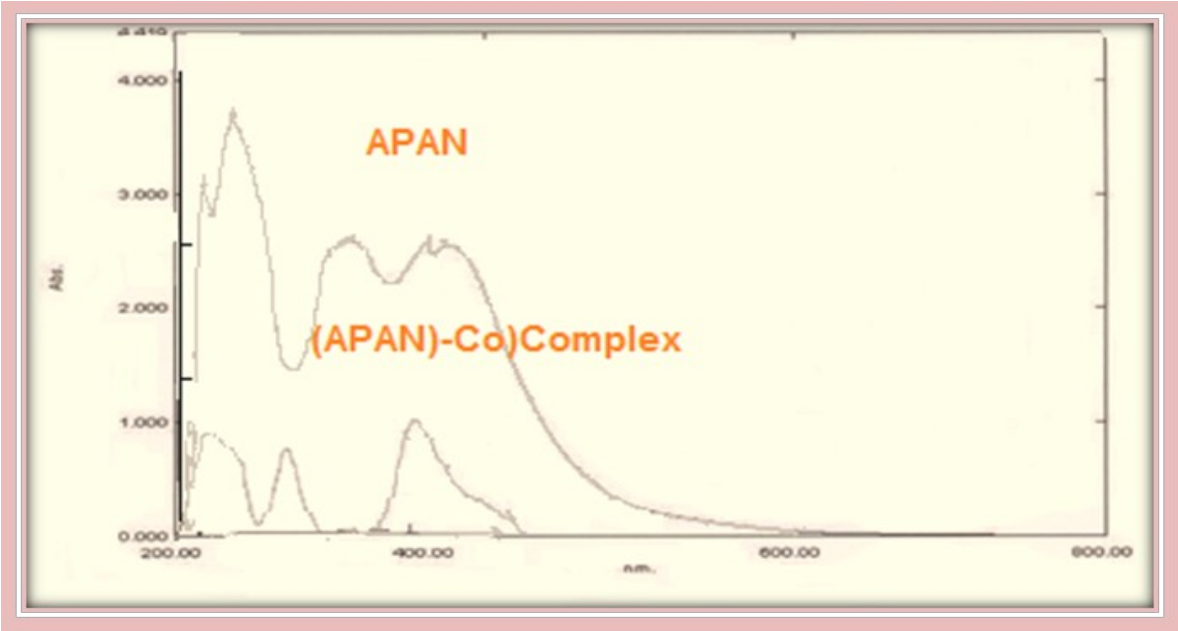


Fig.1 :- Absorption spectra of [Co (II) + APAN] treated as described under procedure and against a reagent blank and reagent blank against ethanol .

The effect of various parameters on the absorbance intensity of the formed products were studied and the reaction conditions were optimized .

Effect of pH

The electronic absorption of APAN and its complex in ethanol have been recorded in the wavelength range (200 – 800) nm Fig. 1 .
The electronic absorption of complex showed a red shift for ($\pi - \pi^*$) electronic transition band. [Co (L)] shows one broad in visible region at (16000-21000) cm^{-1} refer $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)[\nu_2]$) that is in accordance with octahedral geometry) ,)[ν_1] $^4T_{2g}$ $^4T_{1g}(F) \rightarrow$)to
of Cobalt metal ion[20,21] The pH of metal complex solutions was adjusted using dilute solutions (0.05M) NaOH and (0.05 M) HCl , and the effect on absorbance was studied Fig. 2 The absorbance of the complex was maximum and constant in the pH range given in Table. 1

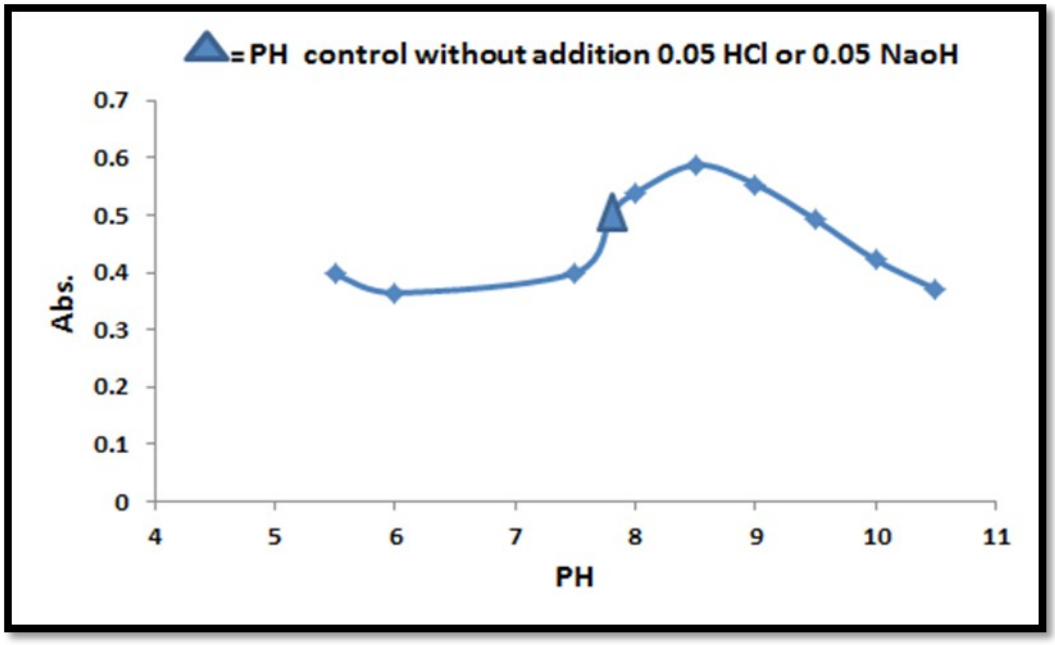


Fig.2 :- Effect of PH

Table.1 :- Analytical characteristics of Co (II) – complex.

Characteristic	Co (II) – complex
Absorption maximum (nm)	432
Beer's law range (ppm)	(0.1– 2.5)
pH range	(7.5 – 8.5)
Sandell's sensitivity μg . cm^{-2}	0.0018
Molar absorptivity (L. mol^{-1}. cm^{-1})	3.3 X 10^4
Stability constant (L. mol^{-1})	1 X 10^8
Melting point for reagent	(199 – 201) C^0
Melting point for Co (II) –	(242 - 244) C^0

complex	

Effect of (APAN) concentration

Various concentration of 3 –(4'- antipyriyl azo) 1-nitroso-2-naphthol was added to fixed concentration of Co (II) 2 ml of 1 mM (APAN) solution was sufficient and gave
minimium blank value was increased causing a decrease in the absorbance of the sample.
Therefore 2 ml of 1 mM of APAN was used in all subsequent experiment Fig. 3 .

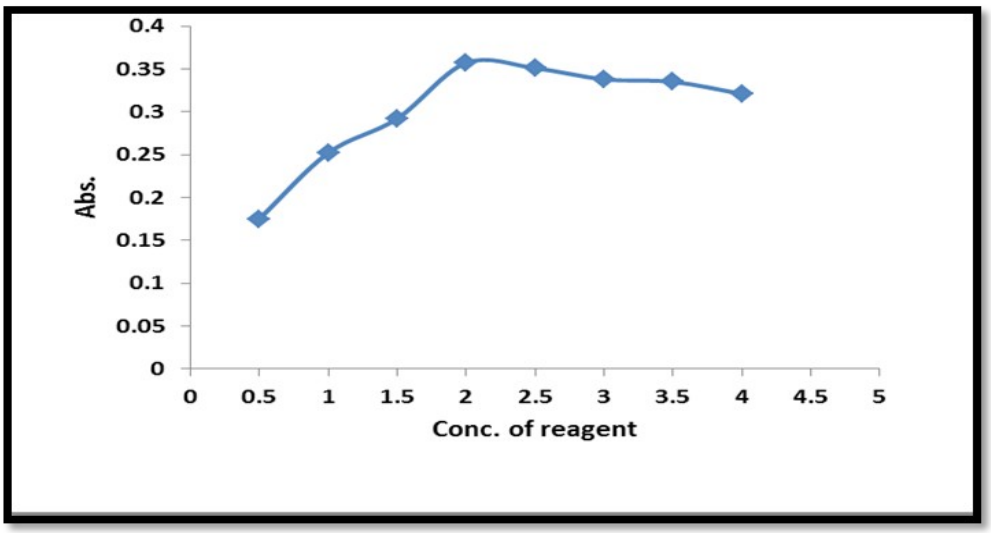


Fig.3 :- Effect of (APAN) concentration

Effect of reaction time

The colour intensity reached a maximum after the Co (II) has been reached immediately with APAN and became stable after one minute , therefore one minute
development time was selected as optimum in the general procedure . The colour obtained was stable for a least 24 hours Fig 4 .

Fig.4:-Effect of time on complex of Co[APAN]$_2$

Effect of temperature

The effect of temperature on the colour intensity of the product was studied .

In practice , the same absorbance was obtained when the colour was developed at room temperature (20 – 40) C°, but when the volumetric flask were placed in a water – bath at (50 – 70) C° a loss in colour intensity and stability were observed , therefore it is recommended that the colour reaction should be carried out at room temperature for complex Fig 5 .

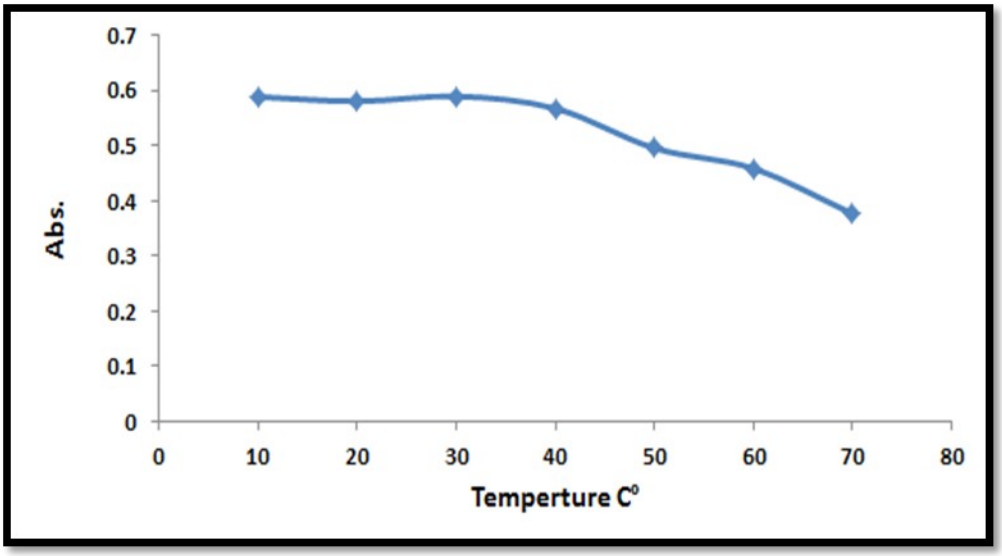


Fig .5:- Effect of temperatures on complex Co[APAN]$_2$

Calibration graph

The calibration equation for (0.1 – 2.5 µg ml^{-1}) Co (II) is :
$Y = 0.5637X + 0.0157$ ($R^2 = 0.9965$) .
Since the coloured complex is stable for 24 hrs , the method can be applied to large series of samples . The molar absorptivity and sandell' sensitivity are given in Table.1 .

Composition of the complex

The composition of complex was studied in the excess of reagent solution by the mole-ratio and Job s` methods Fig 6,7 . A break at a 1:2 (M:L) mole ratio suggested the formation of complex where M= Co(II) and L= APAN under the given condition.

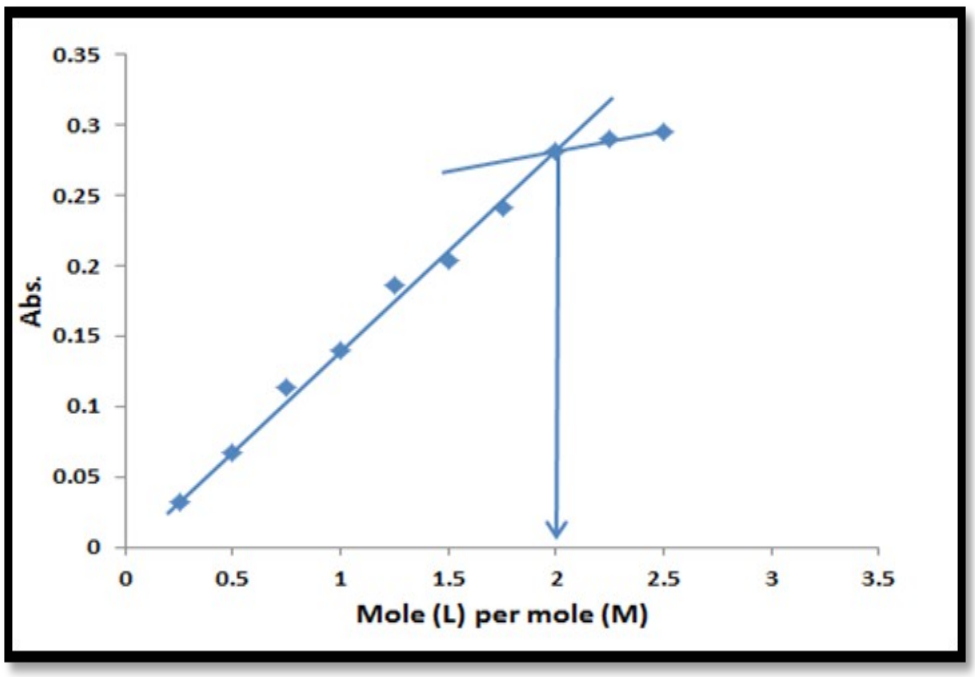


Fig 6:- mole-ratio method for $Co[APAN]_2$ complex

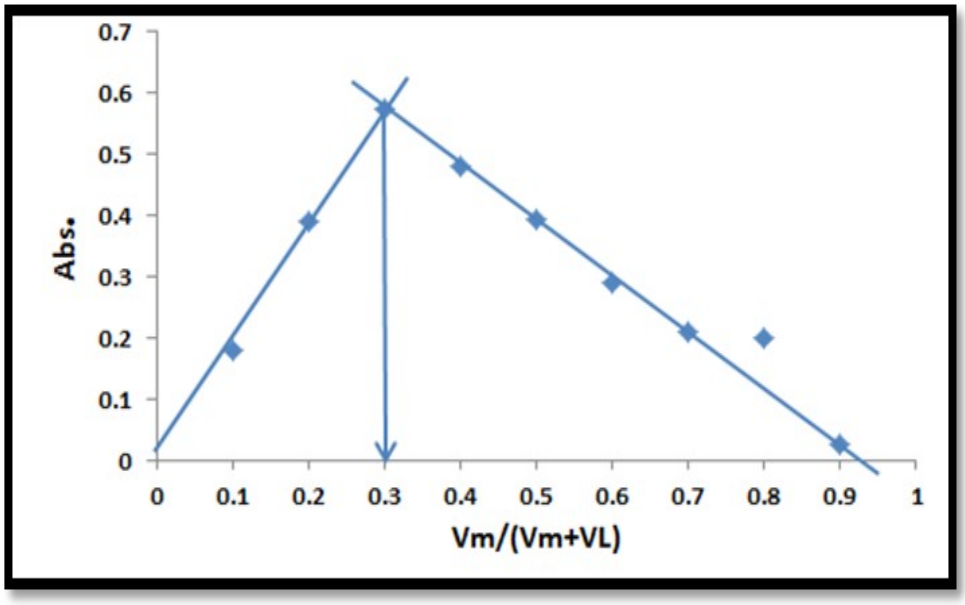


Fig 7:- Job s` method for Co[APAN]$_2$ complex

Conductivity measurements

The solubility of the complexes in dimethy sulfoxide and ethanol permitted of the molar conductivity of 10^{-3} M solution at 25 °C and by comparison, the electrolytic nature for complexe. The low values of the molar conductance data listed in Table 2 indicate that the complexes are non electrolytes.

Table2. Conductivity values of complex

Complex	Molar conductivity, S. mole^{-1} .m^2 DMSO	Molar conductivity, S mole^{-1}. m^2 Ethanol
Co (APAN)	17.1	11.7

Interferences

The effect of diverse ions in the determination metal ion was studied . To test of diverse ions were determined by the general procedure , in the presence of their respective foreign ions . The metal ion can be determined in the presence of a 8 or more fold excess of cation and anion Table.3. In the experiment , a certain amount of standard Co (II) solution , coexisting ion solution and masking agent (or absence of masking agent) were added . It is found that all the studied ions

interfere seriously . However , their interferences are masked efficiently by addition 1.0 ml of 0.1 M of (Na_2HPO_4 , $NaNO_2$).

Table.3 :- Effect of foreign ions

Seq	foreign ions	Conc.µg/ml	E%
1-	Hg^{2+}	10	13
2-	Mn^{2+}	10	12
3-	Cd^{2+}	10	15
4-	Fe^{3+}	10	13
5-	Zn^{2+}	10	21
6-	Pd^{2+}	10	11
7-	Cu^{2+}	10	27
8-	Ni^{2+}	10	25
9-	So_4^{2-}	10	0.1
10-	CO_3^{2-}	10	0.9
11-	Br^{1-}	10	0.7

12-	Cl^{1-}	10	2
13-	F^{1-}	10	0.2
14-	$S_2O_8^{2-}$	10	0.4

FT.IR of reagent and it's complex

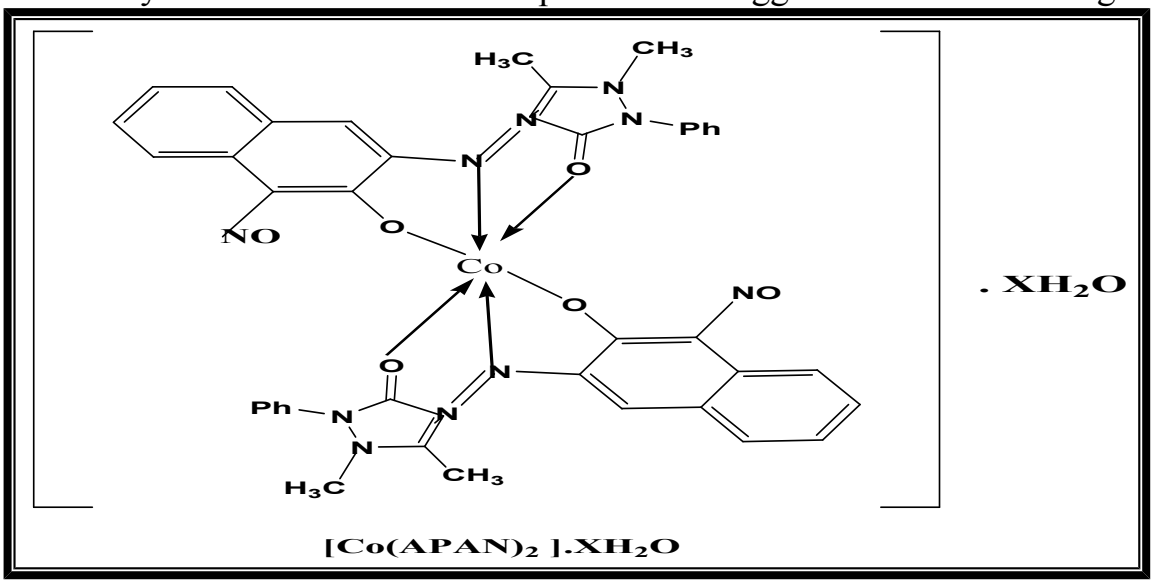
The FT.IR of the free ligand and it's metal chelate were carried out in the (400-4000) cm^{-1}Range . The IR bands of the (APAN) and its Co (II) complex with their probable assignment are given in Table.4 . The IR spectrum of ligand shows a broad band at 3425 cm^{-1}, which can be attributed to the naphtholic OH group . However, the υ(N=N) stretching band in the free ligand is observed at 1535 cm^{-1}. This band is shifted to lower with low intensity at 1519 cm^{-1} frequency value upon complexation suggesting chelation via the (M-N) [22] . The IR spectrum of the ligand revealed a sharp band at 1650 cm^{-1} due to υ(C=O) of pyrazole azo . This band is shifted to higher with low intensity at 1681 cm^{-1} frequency value upon complexation [23]. The bonding of oxygen to the metal ion is provided by the occurrence of band at 447 cm^{-1} as the result of υ(M-O) [24,25].

Table. 4:Selected FT.IR data of (APAP) and it's complex with Cu (II)

Compound	**υ (OH)**	**υ (C=N)**	**υ (C-H) arom.**	**υ (N=N)**	**υ (C=C)**	**υ (C=O)**	**υ (M-O)**	**υ (M-N)**
HL	3425 m	1697 s	3062 m	1535 m	1627 s	1650 s	_	_
[Co (L)]	3409 m	-	3062 m	1519 m	1666 s	1681 s	447 w	408 w

S: sharp ; m: medium ; w: weak

On the basis of the FT.IR, stoichiometric, and elemental analysis molar conductivity data the structure of complex can be suggested as the following :-.



structure of Complex

Applications

1-Determination of Cobalt (II) in practical samples .

To determine the accuracy and precision of the method , Cobalt (II) was determined at two different concentrations with different interferences ions and masked these ions by using masking reagent . The results are shown in Table.5 , indicate that satisfactory precision and accuracy could be attained with proposed method .

Table.5:- Determination of Co (II) in synthetic samples

Amount taken of Co (II) p.p.m	Recovery%	RSD%
1	99.8	0.66
2	99.85	0.48

2-Determination of Cobalt (II) in raw milk samples .

10 ml of raw milk was added drop wise to a heated crucible to evaporate it without frothing then heated strongly to 450-500° C for one hr after the moisture has been removed . We took utmost care avoid loss by sputtering . The dark ash was dissolved in the minimum of strong nitric acid and evaporated .Then dissolved in the minimum of dilute nitric acid once again . After filtering the ash and making up the filtrate volume to 10 ml Sample was left for 60 min and then ready for UV-Visible spectrophotometric analysis and atomic absorption [26] Table 6.

Table 6. Co(II) levels (µg.g-1) in milk raw sample

Ion in milk raw	Amount found by our Spectrophotometric method, $\mu g.g^{-1}$	Amount found by atomic absorption method, $\mu g.g^{-1}$
Co (II)	**0.211**	**0.192**

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المستخلص

تم تطوير طريقة طيفية جديدة و سريعة و حساسة في تقدير الكميات الضئيلة للكوبلت الثنائي ، إعتمدت الطريقة على أساس تكوين معقد 2:1 (فلز – كاشف) مع الكاشف 3-(4′-انتي بايرايل آزو) -1-نايتروزو-2-نفثول (APAN) ، يمتلك المعقد أعلى قمة إمتصاص عند الطول الموجي (432) نانوميتر و معامل إمتصاص مولاري 3.3 10^4 X لتر. مول$^{-1}$. سم$^{-1}$. كانت العلاقة الخطية بين الإمتصاص عند الطول الموجي الأعظم و التراكيز تتراوح بين (0.1 – 2.5) جزء في المليون أما الدقة و الضبط للطريقة فقد أعطت نتائج تراوحت ما بين 0.60 % بالنسبة إلى 0.2 RSD % بالنسبة إلى الخطأ النسبي . و لبيان إنتقائية الطريقة تم دراسة تأثير عدد من الأيونات السالبة و الموجبة على إمتصاصية المعقد المتكون . كان ثابت الإستقرار للمعقد تحت الظروف الفضلى و درجة حرارة الغرفة 10^8 1 X لتر. مول$^{-1}$ ، طبقت الطريقة المقترحة و بنجاح على نماذج لمزيج محضر مختبريا وكذلك نماذج الحليب الخام.