Spectrophotometric Determination of Cobalt and Iron in Pharmaceutical Preparation Using 6-Methyl-2-Pyridinecarboxyaldehyde 4-Phenyl-3-Thiosemicarbazone as Chromogenic Reagent

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Summary: The reaction of new spectrophotometric reagent 6-methyl-2-pyridinecarboxaldehyde 4-phenyl-3-thiosemicarbazone (MPAPT) towards copper(II), nickel(II), cobalt(II), cobalt(III) cadmium(II), iron(II), bismuth(III), lead (II), mercury(II), palladium(II), platinum(IV) manganese (II), gold(III), silver(I) and zinc(II) have been studied spectrophotometrically. The colour reactions are rapid at optimized pH within 1-11 in methanol-water with molar absorptivity in the range of $0.36\text{-}4.4\times10^4$ $L.mole^{-1}$ cm^{-1}. The complexes are extractable in chloroform as metal chelates compounds. The cobalt(II) develops colour even at hydrochloric acid (0.8 M) in methanol-water and colour of iron(II) changes from yellow to green at pH 9, which is extractable in chloroform. Cobalt in large excess of iron could be determined in a mixture. The reagent has been used for the determination of cobalt and iron separately and in a mixture in pharmaceutical preparations with relative standard deviation (RSD) within 0.5-21%. The results have been compared using atomic absorption spectrometer.

Introduction

Thiosemicarbazone have received considerable attention in last a few decades, because of their potential use in pharmacology [1-3], as analytical reagent [4-10], as metal ion collectors [11] and as HPLC reagents for separation and determination of metal ions [12]. Thiosemicarbazones are known to be selective and sensitive reagents for cobalt, copper, nickel, palladium, iron and zinc. Their applications have been studied in samples such as cobalt in steel [13], zinc in potable water and insulin [14], copper in white metal, in blende and in waste water [15], cobalt and nickel in industrial catalysts [16] and nickel in river water.

The reagent picolinaldehyde thiosemicarbazone [17] and picolinaldehyde phenylthiosemicarbazone (PAPT) [18] have been reported as spectrophotometric reagents for metal ions. Incorporation of phenyl group in thiosemicarbazine has been reported [18] to have positive effect on the colour reactions towards metal ions. In the present work 6-methyl-2-pyridinecarboxaldehyde 4-phenyl-3-thiosemicarbazone (MPAPT) has been prepared to examine the effect of methyl group substitution adjacent to donor nitrogen atom on its colour reactions with metal ions and develop an analytical method for the determination of iron(II) and cobalt(II) in a mixture.

Results and Discussion

The reagent was easily prepared by simple condensation of 6-methyl-2-pyridinecarboxalde-hyde and 4-phenyl-3-thiosemicarbazide. The pro-duct was obtained in good yield (90% theoretical). The mass spectrum indicates a prominent mole-cular ion peak M^+ at m/z 269.8 (90%). The fragment peaks are observed at m/z 177.8 (20%) and 133.9(80%) due to

the loss of C_6H_5NH- and -C=5 followed by loss of N_2 to obtain base peak at m/z 106 corresponding to $[CH_3C_5H_3N\ C.H_2]^+$ fragment. The other main peaks are observed at m/z 93(80%) and 77(58) corresponding to $[CH_3.C_5H_4N]^+$ and phenyl groups.

The IR of the reagent indicates bands at 3320(m) and 3295(m) cm^{-1} due to NH stretching vibrations. The reagent indicates three bands at 1595(s), 1540(s), 1497(w) cm^{-1} due to C=N and C=C vibrations. A band is observed at 1385 (m) cm^{-1} due to -CH_3 group as could be expected from its structure (Fig. 1). The bands observed at 1250 and 1190 cm^{-1} have been assigned to C=S group [19].

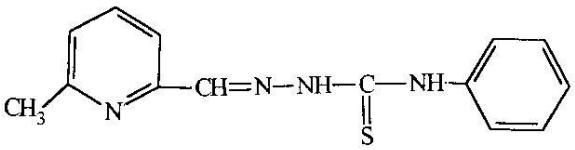

Fig. 1: Structural Diagram of Reagent.

The reagent reacts with a number of metal ions to develop coloured solution in neutral to slightly acidic media immediately (Table-1). The complexes are easily extractable in chloroform. The colour of copper(II), nickel(II) cobalt(II), lead(II), cobalt(III), bismuth(III), iron(III) palladium(II), platinum(IV) and manganese(II) complexes in methanol-water are stable more than 24 hrs. Iron(II), mercury(II), gold(III) and zinc(II) when extracted in chloroform indicate solution stability > 6 hrs. The cadmium shows highest molar absorptivity of $4.4x10^4$ L $mole^{-1}$ cm^{-1} in methanol water with solution stability upto 2 hrs. Linear calibrations for copper(II), nickel(II), cobalt(II), cobalt(III), cadmium(II), manganese(II) and zinc(II) were observed at final concentration of 0.1-1.8 μg/ml; for iron(II), bismuth(III) mercury(II), lead(II), palladium(II), platinum(IV), silver(I) were obtained with 0.4-7.0 μg/ml but for gold(III) it was 5-50 μg/ml. Beer's law is obeyed by each of the coloured solution. The validity of calibration curves was tested by analyses of test solutions and relative % error was found with ± 0-1.5%.

The iron(III) develops yellow colour in aqueous-methanolic solution, but iron(II) in aqueous-methanolic media (pH 4-6) developed green colour, which changed to yellow and colour of the complex was not stable. The addition of ascorbic acid did not improve the solution stability. However after complexation, when the pH of the solution was raised to 9, it indicated green colour, which did not change to yellow, but some decrease in absorbance was noted. The colour of iron(II) complex when extracted in chloroform was observed highly stable and there was no sign in change in absorbance upto 12 hrs. It shows maximum absorbance at 654 nm with molar absorptivity of $7.26x10^3$ $L.mole^{-1}$ cm^{-1}. Beer's law is obeyed at a final concentration of 1-7 μg/ml in chloroform. The stoichiometry of the complex was investigated by variation of metal ligand ratio and it was observed to be 1:2 at pH 9.

Table-1: Quantitative spectrophotometeric studies of 6-methyl-2-pyridine-carboxaldehyde-4-phenyl-3-thiosemicarbazone

Metal ion	Solvent	pH of maximum absorbance	Colour	λ_{max} nm	$\varepsilon=10^3$ L $mole^{-1}$ cm^{-1}	Solution stability of complex
Ag(I)	methanol-water	6.0	yellow	377	9.71	> 4 hr
Au(III)	chloroform	4.0	yellow	388	3.54	> 24 hr
Mn(II)	chloroform	9.0	orange	410	41.31	> 24 hr
Cu(II)	methanol-water	6.75	Yellow	385	37.6	> 24 hr
Ni(II)	methanol-water	6.75	Yellow	383	35.5	> 24 hr
Fe(II)	Chloroform	9.00	Green	654	7.2	> 24 hr
Fe(III)	methanol-water	6.0	yellow	390	10.30	> 24 hr
Co(II)	methanol-water	6.00	Orange	392	39.3	> 24 hr
Co(III)	metanhol-water	6.00	Orange	392	39.4	> 24 hr
Zn(II)	Chloroform	8.00	Yellow	405	42.1	>24 hr
Hg(II)	Chloroform	6.50	Yellow	390	31.0	6 hr
Cd(II)	metahnol-water	6.75	Yellow	392	44.3	2 hr
Pb(II)	methanol-water	11.00	Yellow	388	31.0	> 24 hr
Pd(II)	methanol-water	7.50	Orange	382	22.4	> 24 hr
Pt(IV)	methanol-water	6.00	Yellow	390	20.1	> 24 hr
Bi(III)	methanol-water	6.00	Yellow	398	21.7	> 24 hr

Thus it may be suggested that the ligands acts as tridentate complexing reagents. The presence of copper(II), nickel(II), lead(II), zinc(II), silver(I), gold(III), palladium(II), platinum(IV) iron(III), mercury(II), oxovanadium(IV), manganese(II), cobalt(II), cobalt (III), magnesium(II), calcium(II), beryllium (II), barium(II), citrate, tertrate and phosphate on the extraction of iron(II) was investigated. It was observed that their concentration (5 μg/ml) similar to that of iron(II), indicated relative % error within 2.0%. Pharmaceutical preparations Fefol Capsules and TriHemic 600 tablet were analysed (Table-2). The preparations were also analysed using flame atomic absorption spectrometry and close correlations were observed with relative deviation (R.D) within 0.88 to 3.1%.

In order to examine the effect of methyl group substituted in MPAPT on the iron(II) complex, the absorption spectra of iron(II) complexes of PAPT and MPAPT were recorded (Fig. 2), some enhancement in molar absorptivity with bathochromatic shift of 11 nm is observed due to methyl substitution in MPAPT.

Gomez Ariza *et al* [18] have used PAPT for the determination of cobalt in acidic solution, where large excess of iron did not affect the determination of cobalt. Thus the determination of cobalt using MPAPT from acidic solution was considered. It was observed that cobalt(II) complex of MPAPT indicates maximum sensitivity at pH 6 (Fig. 3) with molar absorptivity of 3.93×10^4 L.mole^{-1} cm^{-1} at 392 nm, but at pH 1.a decrease in molar absorptivity to 1.95×10^4 L.mole^{-1} cm^{-1} was observed with bathochromatic shift in λ_{max} to 428 nm. Further increase upto 0.8M hydrochloric acid did not affect the absorbance of cobalt(II). The colour of cobalt (II) complex at pH 6 was completely extractable in chloroform, but at pH 1 it was only partially extractable. Nickel(II), lead(II), cadmium(II), mercury(II), zinc(II) and bismuth(III) at concentration (5 μg/ml) with cobalt(II) (1 μg/ml) in aqueous methanolic solution at pH 1, did not interfere. Copper(II) and iron(II) only enhanced the absorption, but increasing the final concentration of hydrochloric to 0.8 M, eliminated the interfering effect of copper and iron(II) upto five times the concentration of cobalt(II) and relative % error was found within 1.5%. The reagent MPAPT was used for the determination of cobalt in Cytamen and Bevidox injections and cobalt and iron contents in Incremin syrup. Cobalt was analysed as cobalt(II) in aqueous methanol at a final concentration of hydrochloric acid (0.8M), but the cobalt contents were low thus it was spiked with 4 μg of cobalt. Iron(II) was determined by solvent extraction in chloroform. The metal contents were also determined by atomic absorption spectrometry and

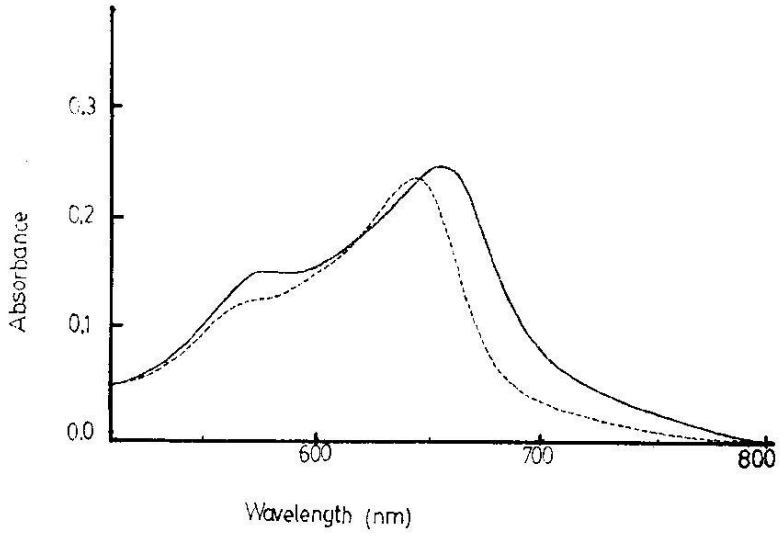

Fig. 2: Absorption spectra of iron(II) complexes in chloroform at a final 2 μg/ml ----PAPT and .....MPAT.

Table-2: Analysis of Pharmaceutical Products

S.No.	Product	Metal ion	Amount Found by Spectrometric metal (C.V.%)	Amount Found by F.A.A. Method (C.V.%)	% Relative Devation
1.	Fefol Capsul	Fe	55.0 mg/ 0.415 g (1.2)	56.7 mg/0.415 g (2.2)	3.09
2.	TriHemic 600 Tablet	Fe	114 mg/ 1.2064 g (2.6)	113 mg/1.2064 g (1.1)	0.88
3.	Cytamen injection	Co	43.25 ug/ 1.598 g (2.0)	42.5 ug/ 1.598 g (0.8)	1.76
4.	Bevidex Injection	Co	43.12/ 3.265 g (2.1)	43.0 µg/ 3.265 g(0.7)	0.28
5.	Incremin Syrup	Co	0.88 ug/5 ml (1.5)	0.81 ug/5 ml (1.1)	8.6
		Fe	27.38 mg/5 ml (1.5)	27.31 mg/5 ml (1.3)	0.26

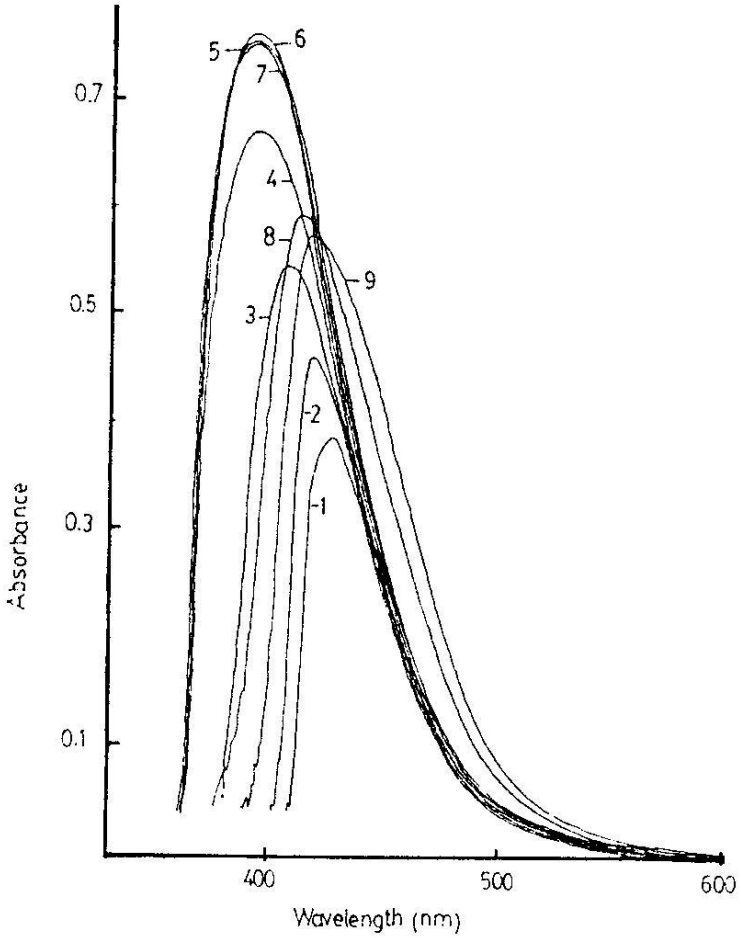

Fig. 3: Effect of pH 1,2,3,4,5,6,7,8,9 on the absorption spectrum of cobalt(II) complex concentration 1 µg/ml in methanol water.

the results in the Table-2 indicates relative deviation (R.D) within 0.26-1.76% except for cobalt contents in incremin syrup where R.D was observed 8.6%.

Experimental

Preparation of 6-methyl-2-pyridinecarboxaldehyde 4-phenyl-3-thiosemicarbazsone (MPAPT)

4-Phenyl-3-thiosemicarbazide (1.38 g) dissolved in 4.0 ml ethanol water (1:1) was added to 6-methyl-2-pyridinecarboxaldehyde (1 g) which was dissolved in 20 ml ethanol. The mixture was added 0.3 ml glacial acetic acid and was refluxed for 45 min. The mixture subsequently cooled at 5°C over night. The yellow crystalline material was recrystallized from ethanol m.p. 170°C. Calculated for $C_{14}H_{14}N_4S$, requires % C=62.22, H=5.18, N=20.74; found % C=62.30, H=5.16, N=20.86. Mass spectrum (rel intensity %) indicates M^+ at m/z 269.8(90) and fragment peaks at m/z 177.8(20), 133.7(80), 106(100). IR in KBr in cm^{-1} (rel.intensity) indicates bands at 3320(s), 3290(m) for NH, 1600(s), 1590(vs), 1540(vs), 1500(m), C=C and C=N, 1250(s), 1190(s) - C=S, 1380(s), ($-CH_3$).

Reagents and Solutions

Solutions containing (1 mg/ml) of metal ion were prepared from $FeSO_4.(NH_4)_2SO_4.6H_2O$, $(CH_3COO)_2Co.4H_2O$, $NiSO_4.6H_2O$, $CuSO_4.5H_2O$, $(CH_3COO)_2$ $Pb.3H_2O$, $CdCl_2.H_2O$, $HgSO_4$, $Zn(CH_3COO)_2.2H_2O$, Bi_2O_3, $PdCl_2$, K_2PtCl_4, $MnSO_4.H_2O$, $AgNO_3$ (E. Merck) and $AuCl_3$. Cobalt(II) solution (10 ml) containing 1 μg/ml was added nitric acid (65%) (2 ml) and was heated to near dryness and residues was dissolved in water (100 ml) to obtain cobalt(III) (100 μg/ml). Freshly prepared cobalt(III) solution was used for spectrophotometric studies. Hydrochloric acid (37%) (Fluka) and nitric acid (65%) (Fluka) were used. 2-pyridinecarboxaldehyde-(Aldrich), 6-methyl-2-pyridinecarboxaldehyde (Aldrich), 4-phenyl-3-thiosemicarbazide (Fluka) were used. The reagent picolinaldehyde-4-phenyl-3-thiosemicarbazide (PAPT) was prepared as reported [18].

Buffer solution at unit interval within pH range 1-11 were prepared from the following. Hydrochloric acid (0.1 M) sodium chloride (1M), acetic acid (1M), sodium acetate (1M), sodium bicarbonate (1M), sodium carbonate (saturated solution), ammonium acetate (1M), ammonium chloride (1M) and ammonia (37%). All the reagents used were GR grade from E. Merck or Puriss from Fluka. The reagents were used without further purification. $AuCl_3$ solution was prepared by dissolving appropriate amount of pure gold in hydrochloric acid and nitric acid (3:1 v/v).

A. Analytical procedure

Metal ion solution (1-2 ml) containing (0-70 μg), reagent solution (1 ml. 0.2% m/v in methanol) and appropriate buffer solution (2 ml) were transferred into 10 ml volumetric flask. The volume was adjusted to mark with methanol. The absorption spectrum was recorded in visible region within 700-350 nm against reagent blank in same solvent system.

B. Solvent extraction procedure

Solution (1-2 ml) containing metal ion (0-70 μg) was transferred to separating funnel and reagents were added as A. The solvent extraction was carried out with 5 ml chloroform and organic layer was collected in 10 ml volumetric flask. The extraction was repeated with chloroform (3 ml). The final volume was adjusted with chloroform.

Variation in absorbance with pH was investigated by adding 2 ml of different buffer solution in pH range 1-11 following the procedure A or B. The absorption spectrum of the solution was recorded periodically to evaluate solution stability of the complex.

C. Determination of Cobalt in Cytamen and Bevidox injection

Cytamen injection (1.0 ml, 1.598 g) (Glaxo Laboratories (Pak) Karachi) or Bevidox injection (3 ml, 3.2650 g) (Abbott Lab. (Pak) Karachi) was transferred to crucible and was added nitric acid (65%) (5 ml) and hydrochloric acid (37%) (10 ml). The mixture was heated gently to dryness. The residue was heated strongly on flame for 2 hrs. The dry ash was dissolved in water and volume was adjusted to 25 ml. Solution (2 ml) was transferred to volumetric flask (10 ml) and was added ascorbic acid (1 ml. 1% m/v in water), hydrochloric acid (1 M) (2 ml) and reagent solution (1 ml, 0.2% m/v in methanol). The volume was adjusted to 10 ml with

methanol. Amount of cobalt was evaluated from calibration curve prepared from cobalt(II).

D. Determination of Iron in Fefol Capsule and TriHemic 600 tablet

Fefol capsule (0.415 g) (SK&F (Pak) Karachi) and TriHemic 600 tablet (1.2641 g) (Lederle Lab. (Pak) Karachi) was transferred to a beaker and was added hydrochloric acid (37%) (10 ml) and nitric acid (65%) (5 ml). The contents were heated gently to near dryness and more hydrochloric acid (5 ml) was added and contents were again heated to near dryness. The residue was dissolved in water and volume of Fefol capsule was adjusted to 100 ml and solution (2 ml) was further diluted to 50 ml. In case of TriHemic tablet volume was adjusted to 250 ml and solution (2.5 ml) was further diluted to 50 ml. Solution (1 ml) was transferred to separating funnel and was added freshly prepared ascorbic acid (1 ml, 1% m/v in water), reagent solution (1 ml, 0.2% m/v in methanol) sodium carbonate-bicarbonate buffer pH 9 (2 ml) and extraction procedure was followed as B.

E. Determination of Cobalt and Iron in a Syrup

Well mixed incremin syrup (20 ml) (Lederle Laboratories Division, Cynamaid (Pak) Ltd), was added $KHSO_4$ (2 gm) and was gently heated on hot plate until effervescences were evolved. The dark residue was strongly heated on flame till white product was obtained. The residue was dissolved in water and volume was adjusted to 25 ml.

Two solutions (4 ml) each was concentrated to 0.5 ml and a solution was spiked with 4 μg of cobalt(II) and was added ascorbic acid (1 ml, 1% m/v in water), reagent solution (1 ml, 0.2% m/v in methanol) and hydrochloric acid (2 ml, 4M). The volume was adjusted to 10 ml with methanol. The absorbance was recorded at 428 nm against reagent blank within 15 min. The amount of cobalt was evaluated from the calibration curve and standard addition technique. For iron determination solution (1 ml) was diluted to 100 ml and 1 ml was transferred to separating funnel and was added freshly prepared ascorbic acid (1 ml, 1% m/v in water) and procedure was followed as B.

Elemental microanalysis and mass spectrum of the reagent were recorded at HEJ Research Institute of Chemistry, University of Karachi.

Spectrophotometric studies were carried out on Hitachi 220 spectrophotometer. IR in KBr was recorded on Perkin Elmer 1430 infrared spectrophotometer. Varian Spectr AA-20 atomic absorption spectrometer with standard burner head for air-acetylene flame was used. Cobalt and iron were determined at 240.7 nm and 248.3 nm at the conditions recommended by the manufacturer. The analyses were carried out in triplicate with integration time 3 sec.

Conclusion

New spectrophotometric reagent MPAPT has been reported for the selective determination of iron(II) after extraction of iron(II) complex in chloroform from alkaline solution pH 9, and cobalt(II) in acidic aqueous methanolic solution. A large excess of iron did not affect the determination of cobalt. The methods have been used for determination of cobalt and iron in a mixture in pharmaceutical preparation and good correlation have been observed with that of atomic absorption spectrometry.

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