



Spectrophotometric and Fluorometric Studies of cobalt(II), zinc(II), nickel(II), iron(II) and copper(II) of Pyruvic acid 4-phenyl-3-thiosemicarbazone

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Abstract: The reagent pyruvic acid 4-phenyl-3-thiosemicarbazone (PAPTSC) was prepared by simple condensation reaction between pyruvic acid and 4-phenyl-3-thiosemicarbazide and characterized by Fourier transform-infra red (FT-IR), Ultraviolet-visible (UV-vis) spectroscopy, mass spectrometry and thermal analysis. The reactions of the reagent towards cobalt(II), zinc(II), nickel(II), iron(II) and copper(II) were examined spectrophotometrically and spectrofluorometrically. Experimental conditions were optimized in terms of reaction time, solution stability, pH and concentration of reagent per determination for maximum absorbance. Factors affecting fluorescence intensities were also optimized. Linear calibration range obeying Beer's law was within 5-25 µg/mL with molar absorptivity $0.119-0.171 \times 10^4 \text{ L.mol}^{-1}.\text{cm}^{-1}$ and fluorescence intensities of emission were within 666-724 nm with excitation within 332-361 nm respectively. The highly stable complexes once formed did not show any change in absorbance up to 24 hours. The method was used for the determination of copper from different copper wires and iron and zinc from pharmaceutical preparations.

Keywords: Metal complexes, Pyruvic acid 4-phenyl-3-thiosemicarbazone, Spectrofluorometry, Spectrophotometry.

1. INTRODUCTION

Thiosemicarbazones and phenylthiosemicarbazones are the compounds that have been studied for considerable time as complexing reagents and for their biological properties (Pelosi *et al.*, 2010). The biological studies are due to their antiviral, antibacterial, and antitumoral activities and the reports concerning these subjects keep on increasing (Pelosi *et al.*, 2010; Kulandaivelu *et al.*, 2011; Hall *et al.*, 2011; Sedra *et al.*, 2012). They form metal complexes by coordination with nitrogen and sulphur (Suvaranpu, *et al.*, 2012). The ligand containing sulphur as donor atom can form stable well characterized complexes with possibility of back coordination from metal ion under favorable conditions (Ali, *et al.*, 1974). The presence of nitrogen atom at suitable position along with sulphur tends to lower the solubility of the metal chelate by forming five membered chelate rings.

Thiosemicarbazones and phenylthiosemicarbazones containing sulphur and nitrogen as donor atoms have been extensively studied as complexing reagents (Greg *et al.*, 1988; Singh *et al.*, 1991). The reports concerning the metal complexation are mainly copper, nickel, manganese, cobalt, mercury, palladium or ruthenium (Kowol *et al.*, 2012; Akber *et al.*, 2012; Suni *et al.*, 2007; Xli *et al.*, 2010; Datta *et al.*, 2011; Malecki *et al.*, 2013). They are the group of compounds produced by condensing thiosemicarbazide with compounds containing carbon-oxygen double bond in

the presence of a few drops of glacial acetic acid. An overview of thiosemicarbazones has been reported by (Lobana *et al.*, 2009). Pyruvic acid is the simplest alpha keto acid and carboxylate ion of pyruvic acid is known as pyruvate and is involved in a number of metabolic pathways (Cody *et al.* 2000). Al-Karawi has reported the synthesis and characterization of sodium pyruvate thiosemicarbazone and the molecule coordinated divalent Fe, Co, Ni, or Cd with octahedral geometry (Al-Karawi *et al.*, 2012). (Affaan *et al.*, 2009) and (Wiecek *et al.*, 2008) reported biological studies, characterization and synthesis of organotin (IV) complexes of pyruvic acid thiosemicarbazone ligand. Copper (II) and Iron (III) metal chelates of pyruvic acid thiosemicarbazone are reported (Ferrari *et al.*, 1998; Diaz *et al.*, 1994; Timken *et al.* 1984). Chandra and Sharma reported Co(II) and Ni(II) complexes of phenyl pyruvic acid thiosemicarbazones (Chandra *et al.*, 1984). Thiosemicarbazones and their metal complexes are also reported to indicate fluorescence in visible region (Bharti *et al.*, 2016). The present work examines the reagent pyruvic acid 4-phenyl-3-thiosemicarbazone for spectrophotometric and spectrofluorometric studies of Zn(II), Cu(II), Fe(II), Co(II) and Ni(II) for the possible determination of the metal chelates.

2. MATERIAL AND METHODS

2.1. Chemicals.

4-phenyl-3-thiosemicarbazide (Fluka, Switzerland), Pyruvic acid (Sigma Aldrich, Switzerland), methanol

(99%) (E-Merck, Germany), hydrochloric acid (37%) (Fluka, Switzerland) were used. Reagent grade salts of copper(II)chloride, ferrous ammonium sulfate, cobalt nitrate and nickel chloride (E-Merck, Germany) were used. Buffer solutions were prepared from the chemicals, acetic acid, potassium chloride, ammonium acetate, sodium acetate, sodium tetraborate, boric acid, sodium carbonate, sodium bicarbonate, ammonia solution and ammonium chloride (E-Merck, Germany).

The solutions of buffer at unit interval were made from the following, ammonium chloride (1M) ammonia solution (pH10), sodium bicarbonate (1M) sodium carbonate saturated (pH9), boric acid-sodium tetraborate (1M) (pH 8-9), ammonium acetate (1M), (pH 7), acetic acid (1M) - sodium acetate (1M), (pH 3-6) and potassium chloride (1M) adjusted with hydrochloric acid (0.1M) (pH 1-2). The stock solutions of metal ions (Cu, Fe, Ni, Co, Zn) containing 1mg/mL were prepared from appropriate reagent grade salts. A few drops of acid were added before make up the volume to the mark.

2.2. Equipments.

Orion 5 Star pH meter (Orion Private Ltd, Boston MA USA), with glass electrode and internal reference electrode was used to measure pH of solutions. Fourier transform-infra red (FT-IR) spectrum was recorded on an Avatar 330 FT-IR ThermoNicolet, (Thermo Electron corporation USA) with attenuated total reflectance (ATR) accessory (smart partner) within the range of 4000-600 cm^{-1} . The mass spectrum of the reagent was recorded at HEJ Research Institute of Chemistry, University of Karachi.

Differential thermal analysis (DTA) and Thermogravimetric analysis (TGA) of the reagent was recorded with Pyris Diamond Thermogravimetric/Differential Thermal Analyzer (TG/DTA) (Elmer Perkin Technology, Japan), between room temperature to 600 $^{\circ}\text{C}$ with a heating rate 10 $^{\circ}\text{C}/\text{min}$ with a nitrogen flow rate of 50 mL/min. TGA was recorded against alumina as reference.

Spectrophotometric studies were carried out on double beam (Elmer Perkin35 spectrophotometer, Singapore) with dual 1cm quartz cuvettes. Ultraviolet win lab computer software was used to control spectrophotometer. Steady-state fluorescence excitation and emission spectra were measured using Spectrofluorometer (Shimadzu, Kyoto, Japan), Model RT-5301 PC. Atomic absorption studies were carried out at Elmer PerkinAA-800 (Elmer Perkin, Singapore) atomic absorption spectrometer coupled with air-acetylene flame standard burner head and was operated

at the conditions recommended by the manufacturer. The analysis was carried out at least in triplicate ($n=3$) with integration time 3S and delay time 3S.

2.3. Synthesis of reagent pyruvic acid 4-phenyl-3-thiosemicarbazone (PAPTSC)

4-phenyl-3-thiosemicarbazide (0.01 mole) dissolved in 50mL methanol was added to pyruvic acid (0.01 mole) dissolved in 15mL methanol. A few drops of hydrochloric acid (1M) were added to the solution and contents were refluxed on water bath for two hrs. The solution was cooled at 5 $^{\circ}\text{C}$ overnight and the precipitates formed were filtered and recrystallized from methanol. Melting point was observed at 171 $^{\circ}\text{C}$.

2.4. Spectrophotometric procedure

The solution (0.5-2.5mL) containing Ni(II), Zn(II), Fe(II), Co(II) and Cu(II) (5-25 μg each) separately was transferred to 10mL volumetric flask and was added 1.5mL of reagent pyruvic acid 4-phenyl-3-thiosemicarbazone solution (0.1% in methanol). 1 mL of appropriate buffer solution for each metal complex was then added and contents were mixed well. The volume of the solution was made up to the mark (10mL) with water or methanol. The spectrophotometric studies in terms of absorption spectra of the solutions were recorded within 500-240 nm against reagent blank.

2.5. Spectrofluorometric procedure

To an aliquot of working standard solutions of Ni(II), Zn(II), Fe(II), Co(II) and Cu(II) containing (5-25 μg each) separately was added 1.5mL reagent pyruvic acid 4-phenyl-3-thiosemicarbazone solution (0.1% in methanol), 1 mL of suitable buffer was then added to each metal chelate. The contents were mixed well and methanol was used to adjust volume of the solution to 10mL mark. The fluorescence intensities of the solutions were measured on spectrofluorometer after adjusting the excitation wave length within 800-220 nm. The blank determination without addition of analyte was also prepared simultaneously and was used to adjust background fluorescence intensity.

2.6. Analysis of real samples

Determination of Copper

Different copper wires (Pakistan Cable, Supreme Delux, Fiji Star) were purchased from local market of Hyderabad, Sindh, Pakistan. 0.5 gram was dissolved in aqua regia (1 mL HNO_3 and 3 mL HCL) by heating slowly to near dryness. The residue was added 1 mL hydrochloric acid (37%) and again heated gently to near dryness. The residue was dissolved in distilled water, filtered and volume was adjusted to 100mL. The solution 1mL was further diluted to 10mL and solution 1 mL was transferred to 10mL volumetric flask and spectrophotometric analytical procedure was followed.

2.7. Analysis of pharmaceutical preparations

Determination of Zinc and Iron

Three tablets of each Polybion forte (Merck Sereno, Quetta, Pakistan), Surbex-Z (Abbott Laboratories, Karachi, Pakistan) and Fefol and Fefolvit (Glaxo Smith Kline, Karachi, Pakistan) containing 22.5mg per tablet zinc or 47mg per tablet iron were crushed separately to a fine powder. The powder corresponding to one tablet was weighed and digested in aqua regia hydrochloric acid and nitric acid (3:1 v/v). The contents were heated gently to near dryness and was added 1mL hydrochloric acid (37%). The contents were again heated to near dryness. The residue was dissolved in water, filtered and volume was made to 100mL with water and spectrophotometric analytical procedure was followed. The quantitation was made from the external calibration curve using linear regression equation based on $y = mx + c$.

3. RESULTS AND DISCUSSION

The reagent pyruvic acid 4-phenyl-3-thiosemicarbazone (PAPTSC) was easily prepared in good yield by simple condensation reaction between pyruvic acid and 4-phenyl-3-thiosemicarbazide in slightly acidic medium.

The mass spectrum indicated molecular ion peak (M^+) at m/z 237.1 and at m/z (219.1) and 192.1 due to loss of H_2O and $-COOH$ groups respectively. The base peak was obtained at m/z 135.1 corresponding to the fraction $C_6H_5NHC=S$. (Fig. 1).

The FT-IR spectrum also supported the structure with peak at cm^{-1} at 3358 for OH/NH , at $1744\ cm^{-1}$ for $C=O$, $1635\ cm^{-1}$ for $C=N$ and 1447 for phenyl group (Fig. 2).

TGA of the reagent indicated weight loss from $171\ ^\circ C$ with loss of 90% up to $300^\circ C$. The weight loss was observed in two stages with initial weight loss of 45% up to $210^\circ C$ and secondary loss of 45% up to $300^\circ C$ (Fig. 3).

The reagent pyruvic acid 4-phenyl-3-thiosemicarbazone (PAPTSC) reacts with Zinc(II), Nickel(II), Cobalt(II), Iron (II), and Copper (II) to develop yellow colors immediately in slightly acidic to slightly basic medium. The reactions were examined spectrophotometrically and the experimental conditions

were optimized in terms of pH and reagent concentration per determination. The effect of buffers in terms of pH was checked within 1-10 at unit interval and 0.5-2.5mL of (0.1% reagent solution in methanol) at an interval of 0.5mL. The maximum absorbance of metal complexes was considered as optimum condition. Zinc(II) and Iron(II) indicated maximum absorbance at pH6, Copper(II) at pH8 and Cobalt(II) and Nickel(II) at pH9 with maximum absorbance within 332-361nm.

The complexes indicated high sensitivity with molar absorptivity of $0.119-0.171 \times 10^4\ L.mol^{-1}.cm^{-1}$. The absorbance was recorded against concentration for each element with coefficient of determination (R^2) for cobalt 0.996, copper 0.997, nickel 0.998, zinc 0.998 and iron 0.999 (Table-1) obeying Beer's law within 5-25 $\mu g/mL$. The color reactions were repeatable in terms of the wave length of maximum absorbance and absorbance of the solutions with relative standard deviation within 2.5-3.7% ($n=4$).

The photoluminescence properties of metal chelates were studied at room temperature in methanol or water. All the complexes in the solution phase were excited in the range of wavelength 400-300nm. The fluorescence intensities of different concentrations (5-25 $\mu g/mL$) of metal complexes were measured. The emissions were observed as a broad and intense band within 800-600nm, attributed to the spin allowed lig and centered $\pi \rightarrow \pi^*$ bands in ultraviolet region of the excitation spectra. The intense emission maxima of complexes were observed at 666, 672 684, 686, 724nm for Ni, Co, Cu, Fe and Zn respectively. The fluorescence data are given in the (Table 2).

The method was applied to real samples for the determination of copper from copper wires with % recovery 95-97 with relative standard deviation within 2.5-3.7%. Zinc and iron contents containing labeled amount 22.5 mg and 47mg per tablet were analyzed from pharmaceutical preparations. The amount found for zinc was within 20.5-21.2 with RSD within 2.02-2.61% and 45.6-46.5 with relative standard deviation 2.32-3.34 respectively (Table 3).

The samples were also analyzed using flame atomic absorption spectrophotometer and good agreement was observed with spectrophotometric procedure.

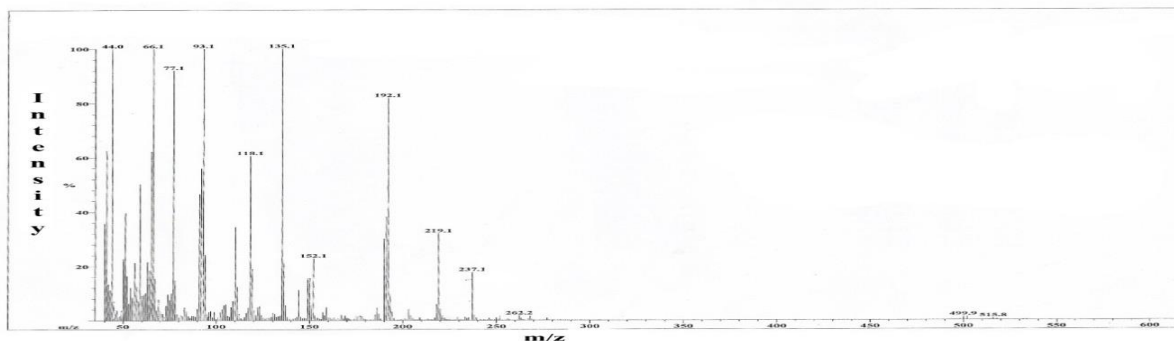


Fig. 1. Mass spectrogram of reagent pyruvic acid 4-phenyl-3-thiosemicarbazone.

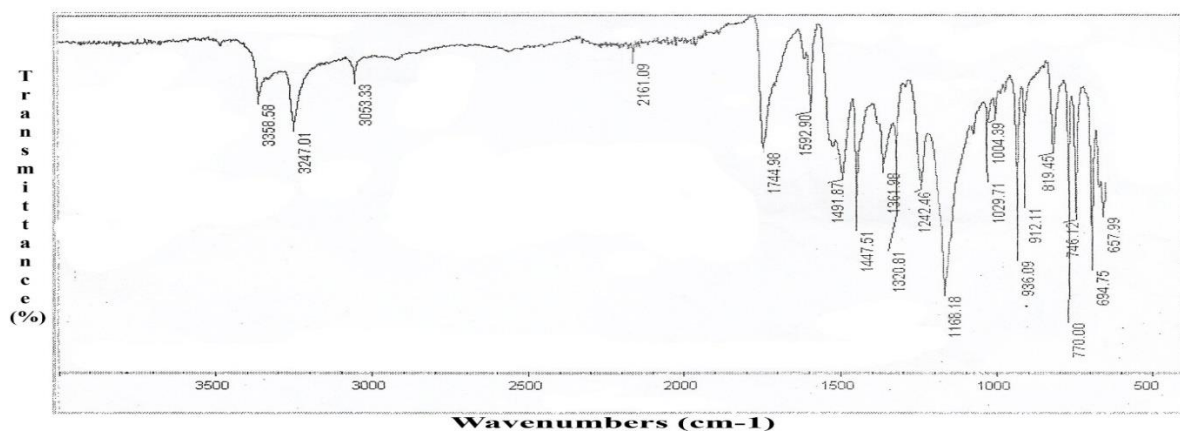


Fig. 2. FT-IR spectra of reagent pyruvic acid 4-phenyl-3-thiosemicarbazone.

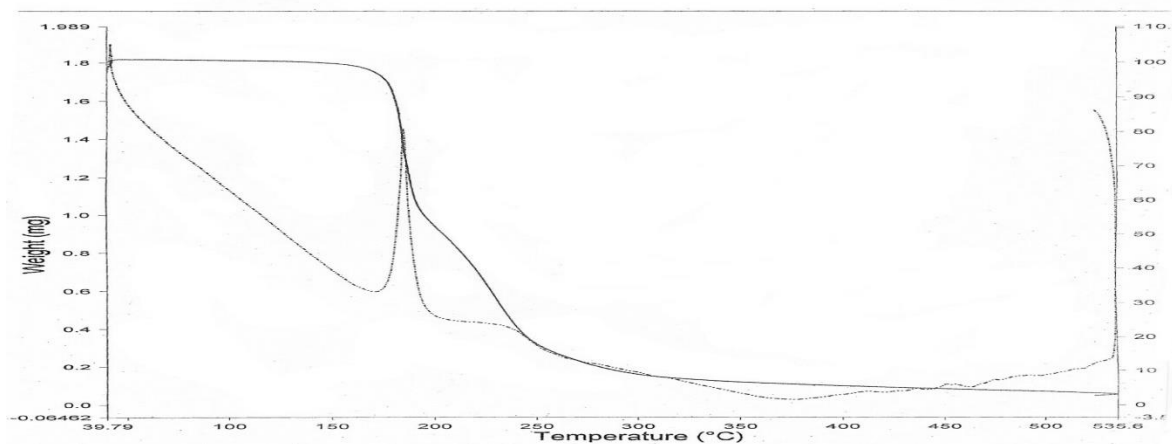


Fig. 3. Thermo gravimetric spectra of pyruvic acid 4-phenyl-3-thiosemicarbazone

Table 1. Quantitative determination of Pyruvic acid Phenylthiosemicarbazone with metals by UV-Visible spectrophotometry.

Metal	λ_{max} (nm)	Linear calibration $\mu\text{g/ml}$	pH	Molar absorptivity (ϵ) L/cm/mol	Regression equation ($Y=mx+c$)	R^2
Fe	342	5-25	6.0	17161	$Y=0.302x+0.003$	0.999
Cu	341	5-25	8.0	11919	$Y=0.181x+0.002$	0.997
Co	336	5-25	9.0	16252	$Y=0.278x-0.005$	0.996
Ni	332	5-25	9.0	15235	$Y=0.250x+0.007$	0.998
Zn	361	5-25	6.0	13315	$Y=0.199x+0.002$	0.998

Table 2. Quantitative determination of Pyruvic acid Phenylthiosemicarbazone with metals by spectrofluorometry.

Metal	Excitation $\lambda_{\text{max}}(\text{nm})$	Emission $\lambda_{\text{max}}(\text{nm})$	Linear calibration $\mu\text{g/ml}$
Fe	342	686	5-25
Cu	341	684	5-25
Co	336	672	5-25
Ni	332	666	5-25
Zn	361	724	5-25

Table3.Determination of iron and zinc from pharmaceutical preparation by UV-Visible spectrophotometry.

Name of product	Metal	Labeled amount (mg/Tab)	Found amount (mg/Tab) n=4 (RSD)	% RSD
Fefol	Iron	47	45.6 (2.32)	2.17
Fefolvit	Iron	47	46.5 (3.34)	1.73
Polybion Forte	Zinc	22.5	21.1 (2.61)	3.5
Surbex-Z	Zinc	22.5	20.5 (2.02)	3.75

4.**CONCLUSION**

The reagent pyruvic acid 4-phenyl-3-thiosemicarbazone(PAPTSC) has been synthesized from pyruvic acid and 4-phenyl-3-thiosemicarbazide by condensation reaction. It was characterized by FT-IR and mass spectrometry and examined as spectrophotometric and spectrofluorometric reagent for metal ions. Various experimental conditions and factors affecting fluorescence intensities were also optimized. All metal chelates were excited in the range of maximum wavelength 400-300 nm and emissions as broad and intense band within 800-600 nm. The developed procedure was successfully applied for the analysis of zinc and iron from pharmaceutical preparations and copper from different copper wires.

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