Pyridine-2-Carbaldehyde Thiosemicarbazone and its Cobalt(II) Complex: Synthesis, Characterization, and Spectrophotometric Study

Omprakash Tukaram Sangule

Associate Professor Department of Chemistry Smt. Kasturbai Walchand College Sangli – 416416, Maharashtra, India

DOI: https://doi.org/10.51584/IJRIAS.2025.100500080

Received: 25 May 2025; Accepted: 29 May 2025; Published: 14 June 2025

ABSTRACT

This study reports the synthesis, spectroscopic characterization, and analytical application of Pyridine-2-carbaldehyde thiosemicarbazone (Py-2-TSC) and its cobalt(II) complex. The ligand Py-2-TSC was synthesized and characterized using UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and X-ray Diffraction (XRD), confirming its structural features and purity. The complexation between Co(II) and Py-2-TSC was optimized at pH 3, where a stable 1:1 metal-to-ligand complex formed, exhibiting maximum absorbance at 510 nm with a molar extinction coefficient of 2.77×10^3 L mol^{-1} cm^{-1}. The complex remained stable for up to 24 hours and followed Beer's law over the Co(II) concentration range of 8.48×10^{-6} to 8.48×10^{-5} M. Both Job's method of continuous variation and the mole ratio method confirmed the 1:1 stoichiometry. The low dissociation constant (7.07×10^{-8}) and high stability constant (1.41×10^7) indicated strong and stable complex formation. Interference studies showed good selectivity, with notable interference only from EDTA and acetate ions. The antimicrobial activity of Py-2-TSC was evaluated against *Klebsiella pneumoniae*, a gram-negative bacterium, and showed promising inhibitory effects. These findings are consistent with literature reports that highlight thiosemicarbazone ligands as bioactive compounds with potential antimicrobial, antifungal, antitumor, and antitubercular activities. Overall, this study establishes Py-2-TSC as a sensitive and selective ligand for the spectrophotometric determination of cobalt(II), while also demonstrating its potential in medicinal chemistry.

Keywords: Pyridine-2-thiosemicarbazone, Cobalt(II) complex, Spectrophotometry, Stability constant, Antimicrobial activity, FTIR and XRD characterization, Job's method

INTRODUCTION

Cobalt is a strategically important transition metal with wide-ranging applications in industry, medicine, and biological systems [1]. It is an essential component in high-performance alloys, ceramics, and magnets, and plays a key role in catalysis, electroplating, and pigment production [2]. Biologically, cobalt is integral to vitamin B and is required in trace amounts for human and plant health [3]. Due to its industrial and physiological significance, the accurate and sensitive determination of cobalt(II) ions has drawn considerable analytical interest [4].

Among the various techniques used for cobalt quantification, spectrophotometric methods offer simplicity, sensitivity, and cost-effectiveness [5]. Ligands such as pyridine-2-Carbaldehyde thiosemicarbazones (Py-2-TSC) have emerged as efficient reagents in the colorimetric detection of transition metals. These ligands form intensely coloured, stable complexes with cobalt(II), allowing precise photometric estimation even at low concentrations [6]. The formation of a 1:1 metal-to-ligand complex under controlled pH conditions provides a reliable basis for stoichiometric and stability studies. Methods such as Job's continuous variation and mole ratio plots confirm the binding interaction, while dissociation and stability constants further validate complex formation.

In addition to their analytical value, thiosemicarbazone derivatives exhibit promising biological activities, including antibacterial, antifungal, antitumor, and antiviral effects [7]. In the present study, the antimicrobial activity of the ligand Py-2-TSC was evaluated specifically against *Klebsiella pneumoniae*, a Gram-negative pathogen, to explore its potential biological efficacy. These dual applications—analytical and medicinal—highlight the potential of Py-2-TSC-based complexes in both laboratory diagnostics and therapeutic research.

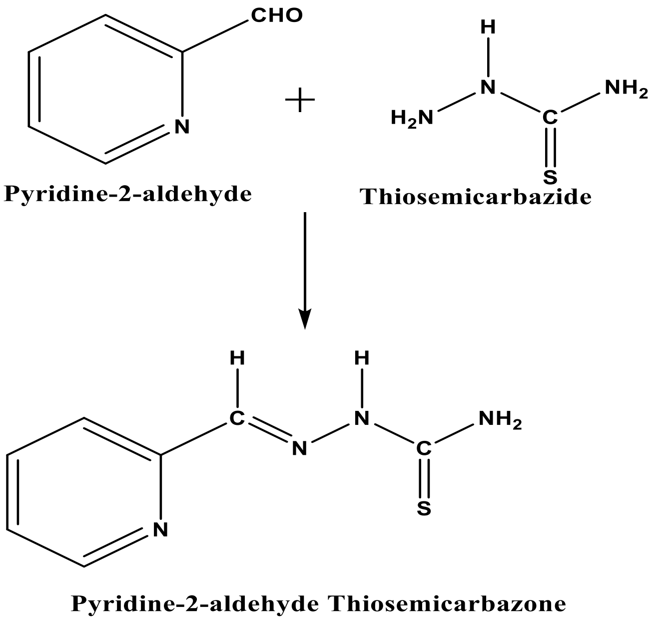
This work was undertaken to explore the dual potential of Py-2-TSC ligands in coordination chemistry and biomedicine. By synthesizing and characterizing the Co(II)-Py-2-TSC complex, we aim to contribute a reliable, cost-effective method for cobalt detection while also probing its antimicrobial efficacy. The findings offer insights into ligand-metal interactions and support the continued exploration of thiosemicarbazone derivatives in analytical and pharmaceutical sciences.

Experimental Details

Synthesis and Characterization of Pyridine-2-Carbaldehyde Thiosemicarbazone

Synthesis of Pyridine-2-Carbaldehyde Thiosemicarbazone (Py-2-TSC)

Pyridine-2-aldehyde thiosemicarbazone (Py-2-TSC) was synthesized via a condensation reaction between equimolar quantities of pyridine-2-aldehyde (10.7 g) and thiosemicarbazide (9.1 g) in ethanol under reflux for one hour. Upon completion, a pale yellow crystalline solid separated from the solution. The product was filtered, washed thoroughly with cold ether to remove impurities, and recrystallized from ethanol to obtain a purified compound. The melting point of the resulting compound was recorded as 170 °C.. The molecular weight, calculated from the empirical formula, was 180.234 g/mol. Reactions are as below-[8]



Elemental Analysis

The elemental composition of the synthesized Py-2-TSC was confirmed by elemental analysis conducted at Nikhil Laboratory, Sangli—an AGMARK-certified testing facility. The observed percentages of carbon, hydrogen, nitrogen, and sulphur closely matched their theoretical values, validating the proposed molecular formula. [9] It is shown in Table 1

Table 1: Elemental Analysis of Py-2-TSC

Sr. No.	Chemical Element	% Found	% Expected
1	Carbon (C)	45.92	46.67
2	Hydrogen (H)	4.22	4.44
3	Nitrogen (N)	30.82	31.11
4	Sulphur (S)	17.62	17.77

Antimicrobial Activity

The antimicrobial potential of Py-2-TSC was tested against *Klebsiella pneumoniae* using the agar diffusion method. A 0.1% ethanolic solution of Py-2-TSC was prepared, and sterile filter paper discs were impregnated with the solution. After solvent evaporation, the discs were placed on nutrient agar plates inoculated with a fresh culture of *K. pneumoniae*. The plates were incubated at 37 °C for 24 hours. The zone of inhibition was measured in centimeters(0.8 cm). These results indicate the compound's potential as a bioactive agent.[10]

UV-Visible Spectroscopy

The UV-Visible absorption spectrum of Py-2-TSC was recorded to analyse the electronic transitions in the ligand. A strong absorption peak at 270 nm was observed, characteristic of $\pi \rightarrow \pi^*$ transitions, indicative of the conjugated structure of the molecule. The molar extinction coefficient (ε) was determined to be 1.115×10^5 L mol^{-1} cm^{-1} at pH 3. This strong absorbance suggests extensive electron delocalization, contributing to the compound's reactivity and coordination behaviour with metal ions.[11]

Infrared (IR) Spectroscopy

IR spectral analysis of Py-2-TSC was carried out in the range of 4000–200 cm^{-1} using the KBr pellet method on a Perkin Elmer 221-IR spectrophotometer. Significant absorption bands were recorded at: ~1590 and 1610 cm^{-1} (C=N, pyridine ring), ~950–955 cm^{-1} (C=S stretching), ~3300–3530 cm^{-1} (N–H stretching of –NH_2), ~760 cm^{-1} (adjacent alkyne hydrogens), and ~1980–2500 cm^{-1} (–N=C=S stretching modes). These peaks confirmed the successful synthesis of the target compound and the presence of essential functional groups, including C=N, C=S, and NH functionalities. (Fig.1 and Table 2) [12]

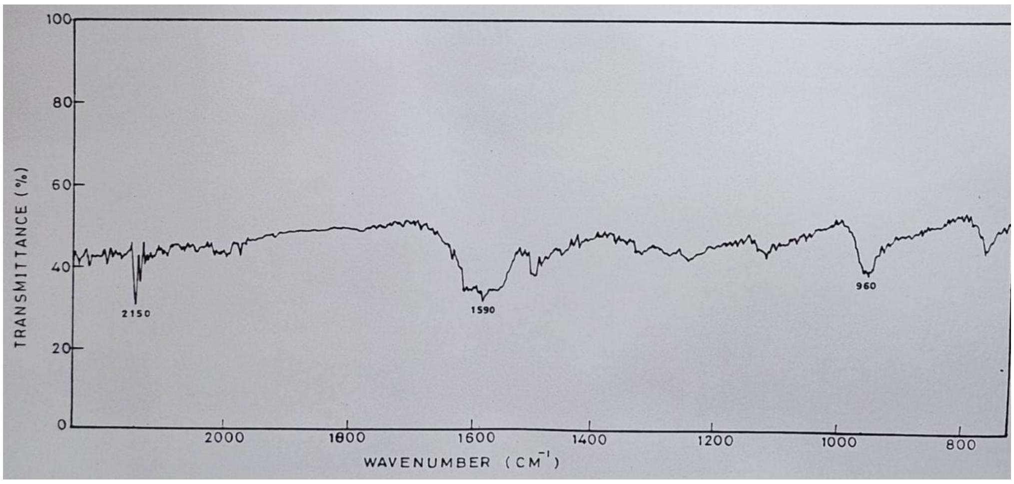


Figure 1: Infrared (IR) Spectrum

Table 2. IR Frequencies and Functional Group Assignments of Py-2-TSC.

Functional Group	Wavenumber (cm^{-1})	Intensity
Alkyne (C≡C–H, adjacent H)	~760	Medium
C=S (Thio group)	950–1050	Strong
Olefinic >C–H	3000–3100	Medium
Aromatic >C–H stretch	3000–3100	Medium–Strong
Pyridine ring (C=N)	1590–1610	Strong
Pyridine ring (C–H bend)	600–800	Medium
Isothiocyanate (–N=C=S)	1980–2500	Strong
Hydroxyl (–OH, bonded)	3200–3600	Broad
Aldehyde (–CHO) C–H stretch	2720–2820	Weak
Primary amine (–NH_2) stretch	3300–3500	Medium–Broad
Secondary amine (>NH) stretch	3300–3500	Medium

X-ray Diffraction (XRD) Analysis

The crystalline nature of Py-2-TSC was examined using powder X-ray diffraction (XRD). Data were collected on a PW 3710-based diffractometer. The diffraction pattern displayed 30 reflections within the 2θ range of 10.460° to 95.480°, with three major intense reflections. The values of $\sin^2\theta$ were calculated and indexed. The d-spacing values (observed and calculated) matched closely, confirming the reproducibility of the diffraction data.

Using the Hesse-Lipson procedure, the $\mathrm{Sin}^2\theta$ data were used to calculate lattice parameters: a = 6.1374 Å, b = 6.2014 Å and c = 3.0458 Å

The close agreement between the experimental ($\sin^2\theta$_obs) and theoretical ($\sin^2\theta$_cal) values (Table 3) confirms that the compound crystallizes in an orthorhombic system. The diffraction pattern is shown in Figure 2 [13]

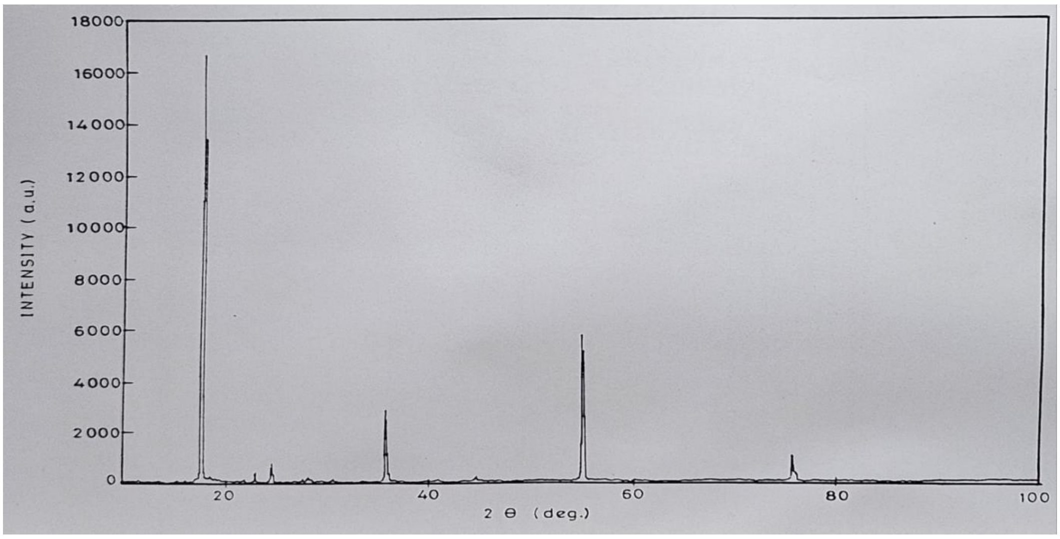


Figure 2: X-ray Diffraction (XRD) Pattern

Table 3. Values of Sin2Θobs & Sin2Θcal of Py-2-TSC

2Θ	Hkl	sin2Θ observed	sin2Θ calculated	d (A°) observed	d (A°) calculated
54.195	1, 3, 2001	0.2075	0.2076	1.691	1.69
59.125	3, 2, 2001	0.2434	0.2428	1.5613	1.5626
68.99	1, 4, 2001	0.3207	0.3205	1.3601	1.3602
69.195	1, 4, 2001	0.3224	0.3324	1.3566	1.3355
75.695	4, 2, 2001	0.3765	0.3784	1.252	1.235
75.94	4, 2, 2001	0.3786	0.3728	1.2551	1.2511
95.48	5, 2, 2001	0.5477	0.5473	1.0408	1.0408

Spectrophotometric Study of the Co(II)-Py-2-TSC Complex

Preparation of Standard Cobalt(II) Solution

A stock solution of Cobalt(II) (8.484×10^{-4} M) was prepared by dissolving A.R. grade 0.0238 g of cobalt sulphate heptahydrate ($CoSO_4 \cdot 7H_2O$) in 100 mL of distilled water. Working standard solutions of desired concentrations were prepared by appropriate dilution with distilled water.[14]

Preparation of Standard Pyridine-2-Carbaldehyde Thiosemicarbazone (Py-2-TSC) Solution

A standard ligand solution (8.484×10^{-4} M) was prepared by dissolving 0.0382 g of Py-2-TSC in alcohol and diluting to 250 mL with the same solvent. This solution was used for complex formation and subsequent spectrophotometric measurements.[15]

Preparation of Buffer Solution

A phosphate-citrate buffer of pH 3 was prepared by mixing 20.55 mL of 0.2 M disodium hydrogen phosphate (Na_2HPO_4) with 79.45 mL of 0.1 M citric acid and diluting to 100 mL with distilled water. This buffer was used to maintain the optimum pH during complex formation.[16]

Spectrophotometric Procedure for Complex Formation

To study the Co(II)-Py-2-TSC complex, 1 mL of standard Co(II) solution (8.484×10^{-4} M) and 1 mL of Py-2-TSC solution (8.484×10^{-4} M) were mixed with 1 mL of phosphate buffer (pH 3). The mixture was diluted to 10 mL with alcohol. The absorbance was measured at 510 nm against a reagent blank to determine the complex formation and stability.[17]

Effect of pH

The effect of pH on complex formation was studied by preparing a series of solutions containing 8.484×10^{-5} M Co(II) and Py-2-TSC, with buffer solutions adjusted between pH 1 to 10. Absorbance was measured at 510 nm, and the optimum pH was found to be 3. [18]

Determination of λ_{max} and Molar Extinction Coefficient

The absorption spectrum of the Co(II)-Py-2-TSC complex was recorded, revealing a maximum absorbance (λ_{max}) at 510 nm. The molar extinction coefficient (ε) was calculated using the absorbance data and found to be 2.770×10^3 L mol^{-1} cm^{-1}. [19]

Effect of Reagent Concentration

To optimize the ligand concentration, the volume of Py-2-TSC solution (8.484×10^{-4} M) was varied from 0.1 mL to 1 mL while keeping Co(II) concentration constant (8.484×10^{-5} M). The optimal volume for maximum absorbance at 510 nm was determined to be 0.8 mL.[19]

Validation of Beer's Law

Solutions with varying concentrations of Co(II) (8.484×10^{-6} M to 8.484×10^{-5} M) were prepared with equivalent Py-2-TSC concentrations. Absorbance measurements at 510 nm confirmed linearity according to Beer's Law in this concentration range.[20]

Determination of Complex Composition

Job's Method of Continuous Variation:

Equimolar solutions of Co(II) and Py-2-TSC (8.484×10^{-4} M) were mixed in varying proportions at pH 3, and absorbance was measured at 510 nm. The maximum absorbance corresponded to a 1:1 metal-to-ligand ratio.[21]

Mole Ratio Method:

Solutions with a fixed metal concentration and varying ligand concentrations were prepared at pH 3. Absorbance readings confirmed the 1:1 stoichiometric ratio.[21]

Effect of Diverse Ions

The influence of various foreign ions on the absorbance of the Co(II)-Py-2-TSC complex was investigated. Known amounts of potential interfering ions were added to the complex solution, and absorbance at 510 nm was measured. Most ions such as Cd^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Sn^{2+}, and Ni^{2+} showed negligible interference, while EDTA and acetate ions significantly interfered and were avoided in the procedure.[22]

RESULTS AND DISCUSSION

Synthesis and Characterization of Pyridine-2-Carbaldehyde Thiosemicarbazone

The synthesis of Py-2-TSC via the condensation reaction between pyridine-2-aldehyde and thiosemicarbazide yielded a pale yellow crystalline solid with a sharp melting point of 170 °C, suggesting high purity. The elemental analysis (Table 1) confirmed the theoretical composition of the compound, thus validating its proposed molecular formula.[23]

Spectroscopic Characterization

UV-Visible Spectroscopy:

The UV-Visible spectrum of Py-2-TSC displayed a prominent absorption band at 270 nm, attributed to $\pi \rightarrow \pi^*$ transitions, which are characteristic of conjugated systems[24]. The high molar absorptivity ($\varepsilon = 1.115 \times 10^5$ L mol^{-1} cm^{-1} at pH 5) suggests significant electron delocalization in the molecule, enhancing its coordination potential with metal ions.[25]

Infrared Spectroscopy (IR):

The IR spectrum provided insights into the functional groups present in Py-2-TSC. Notable absorption bands at ~1590 and 1610 cm^{-1} correspond to C=N stretching vibrations, confirming the presence of the azomethine moiety.[26] The bands at 950–955 cm^{-1} and 3300–3530 cm^{-1} indicated the presence of C=S and N–H functionalities, respectively, further verifying successful synthesis.[27] A unique band in the 1980–2500 cm^{-1} range was attributed to N=C=S stretching vibrations.(Table2,Fig.1)[28]

3.3 X-ray Diffraction (XRD) Analysis

The powder XRD pattern showed 30 diffraction peaks between 2θ values of 10.460° and 95.480°, with three dominant reflections. Lattice parameters calculated using the Hesse-Lipson method (a = 6.1374 Å, b = 6.2014 Å, c = 3.0458 Å) confirmed that the compound crystallizes in the orthorhombic system. [29]The close match between observed and theoretical $\sin^2\theta$ values supported the high crystallinity and structural consistency of the synthesized ligand. The lattice parameters a = 6.1374 Å, b = 6.2014 Å, and c = 3.0458 Å with all angles at 90° confirm that the complex crystallizes in an orthorhombic system. This indicates a well-ordered, anisotropic crystalline structure with differing atomic arrangements along each axis.(Table 3,Fig. 2)[30]

Antimicrobial Activity

The disc diffusion assay against *Klebsiella pneumoniae* showed a clear zone of inhibition,(0.8 cm) demonstrating the antimicrobial potential of Py-2-TSC [31]. This activity may be attributed to the presence of the thiosemicarbazone moiety, known for its biological interactions with microbial cell components. The observed inhibition suggests possible applications as an antimicrobial agent.[32]

Spectrophotometric Studies of Co(II)-Py-2-TSC Complex

Complex Formation:

The spectrophotometric analysis confirmed the formation of a stable, coloured complex between Co(II) and Pyridine-2-aldehyde thiosemicarbazone (Py-2-TSC). The complex exhibited a distinct absorption maximum (λ_{max}) at 510 nm, which facilitated its quantitative analysis.[33] The molar absorptivity (ε) was calculated to be 2.770×10^3 L mol^{-1} cm^{-1}, suggesting a moderately strong interaction between the metal ion and the ligand. This significant absorbance is indicative of effective coordination and the formation of a chromophoric metal-ligand complex.[34]

Effect of pH:

The effect of pH on the formation and stability of the Co(II)-Py-2-TSC complex was investigated over a range of pH values. The highest absorbance was observed at pH 3, indicating that this acidic condition is optimal for complex formation.[35] [Table 4] This can be attributed to the protonation state of the ligand and the cobalt ion at low pH, which likely enhances the coordination efficiency and stability of the resulting complex.[36]

Table 4:-Effect of pH on Absorbance at 510 nm

pH	Absorbance at 510 nm
1	0.11
2	0.187
3	0.235 (maximum)
4	0.22
5	0.17
6	0.07
7	0.046
8	0.03
9	0.024

Reagent Concentration and Compliance with Beer's Law:

To determine the optimum ligand concentration for complex formation, varying volumes of Py-2-TSC solution were used while maintaining a constant cobalt(II) concentration. It was found that a volume of 0.8 mL of 8.484×10^{-4} M Py-2-TSC yielded the maximum absorbance at 510 nm.[37] Furthermore, a series of solutions with varying cobalt(II) concentrations were analysed, and a linear relationship between absorbance and concentration was established within the range of 8.484×10^{-6} M to 8.484×10^{-5} M. This linearity confirms the applicability of Beer's Law for the spectrophotometric determination of Co (II) in the presence of Py-2-TSC.[38]

Dissociation and Stability Constant

The dissociation constant (K) of the Co(II)-Py-2-TSC complex was determined from the mole ratio method using the relation:

$K = (1-\alpha)\cdot c / (m\cdot\alpha\cdot c)^m \cdot (n\cdot\alpha\cdot c)^n$,

where $m = n = 1$ (molar ratio of metal to ligand), and $c = 4.242 \times 10^{-5}\ M$ (calculated from a 20-fold dilution of 0.5 mL of 8.484×10^{-4} M Co(II) solution in 10 mL). The degree of dissociation (α) was obtained using the expression $\alpha = dE / (dE - do)$, where $dE = 0.125$ (optical density for complete complex formation) and $do = 0.120$ (observed optical density at mole ratio point), yielding $\alpha = 0.125 / (0.125 - 0.120) = 0.04$.[39] Substituting these values into the equation:

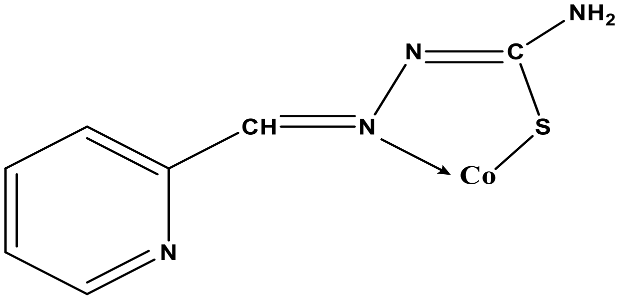
$K = (1-0.04)\cdot 4.242\times10^{-5} / (0.04\cdot 4.242\times10^{-5})^2$,

we get $K = 0.961 \times 4.242\times10^{-5} / 2.8714\times10^{-11} = 7.07 \times 10^{-8}$.

From this, the stability constant (1/K) was calculated as $1 / (7.07 \times 10^{-8}) = 1.414 \times 10^7$, indicating a highly stable complex.[40]

Stoichiometry of the Complex:

The composition of the Co(II)-Py-2-TSC complex was determined using both Job's method of continuous variation and the mole ratio method. In both techniques, the maximum absorbance was observed when equimolar amounts of the metal ion and ligand were used, establishing a 1:1 stoichiometric ratio for the complex.[41] This is consistent with the expected bidentate coordination behaviour of thiosemicarbazone ligands with transition metal ions, wherein the ligand donates two electrons to the metal centre through the azomethine nitrogen and thione sulphur atoms. Probable structure of Co(II)-Py-2-TSC complex showing 1:1 coordination is depicted below:[42]



Effect of Diverse Ions:

The potential interference from foreign ions was evaluated to assess the selectivity of the complexation process. It was observed that most common metal ions such as Cd^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Sn^{2+}, and Ni^{2+} had negligible effects on the absorbance of the Co(II)-Py-2-TSC complex, indicating good selectivity and stability.[43] However, the presence of EDTA and acetate ions significantly diminished the absorbance. This is likely due to their strong chelating nature, which competes with the ligand for binding to the Co(II) ion, thereby disrupting the formation of the desired complex.[44]

CONCLUSION

The present study successfully demonstrates the synthesis, characterization, and spectrophotometric investigation of Py-2-TSC and its cobalt(II) complex. The ligand was synthesized via a straightforward condensation reaction and characterized using elemental analysis, UV-Vis, FTIR, and XRD, all of which confirmed the structural features and purity of the compound. Spectrophotometric studies revealed that Py-2-TSC forms a stable 1:1 complex with Co(II) at pH 3, exhibiting a maximum absorbance at 510 nm and obeying Beer's Law over a defined concentration range, indicating its potential for quantitative metal ion detection.

Preliminary antimicrobial testing against *Klebsiella pneumoniae* demonstrated measurable activity of the ligand, with a zone of inhibition of 0.8 cm, consistent with literature reports on the antimicrobial potential of thiosemicarbazone derivatives [45] Although the study focused on a single microorganism and one metal ion, this deliberate scope provided a targeted assessment of the ligand's bioactivity. Control experiments were carried out to validate the observations, though photographic documentation was not recorded. Future investigations will address this by incorporating visual documentation, expanding microbial screening, and applying more rigorous biological protocols. Structural validation will also be strengthened through NMR analysis and comparison of melting points with standard references.

Rather than being viewed as limitations, these aspects present opportunities for expanding the scope of this research. The ligand's coordination chemistry with other biologically significant metal ions—such as Cu(II), Ni(II), and Zn(II)—offers promising directions for further study. Additionally, broader antimicrobial screening and potential structural modifications could enhance its application in medicinal, analytical, and coordination chemistry. Overall, this work establishes a solid foundation for the multifunctional exploration of Py-2-TSC and its metal complexes in both chemical and biological domains.

REFERENCES

1. Greenwood, N. N., & Earnshaw, A. (2012). Chemistry of the Elements (2nd ed.). Elsevier.
2. Sigel, A., Sigel, H., & Sigel, R. K. O. (Eds.). (2000). Metal Ions in Biological Systems: Volume 37 – Cobalt and Modern Inorganic Chemistry. CRC Press.
3. WHO. (1996). Trace Elements in Human Nutrition and Health. World Health Organization.
4. Skoog, D. A., Holler, F. J., & Crouch, S. R. (2017). Principles of Instrumental Analysis (7th ed.). Cengage Learning.

5. Yoe, J. H., & Jones, A. L. (1944). Colorimetric determination of metals. Industrial & Engineering Chemistry Analytical Edition, 16(2), 111–115. https://doi.org/10.1021/i560131a005
6. Singh, K., Barwa, M. S., & Tyagi, P. (2006). Synthesis, characterization and biological studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes with bidentate Schiff bases. European Journal of Medicinal Chemistry, 41(1), 147–153. https://doi.org/10.1016/j.ejmech.2005.10.013
7. Beraldo, H., & Gambino, D. (2004). The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes. Mini Reviews in Medicinal Chemistry, 4(1), 31–39. https://doi.org/10.2174/1389557043487553
8. Shriner, R. L., Hermann, C. K. F., Morrill, T. C., Curtin, D. Y., & Fuson, R. C. (2004). The Systematic Identification of Organic Compounds (8th ed.). Wiley.
9. Khopkar, S. M. (2004). Basic Concepts of Analytical Chemistry (2nd ed.). New Age International.
10. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4), 493–496.
11. Silverstein, R. M., Webster, F. X., & Kiemle, D. J. (2005). Spectrometric Identification of Organic Compounds (7th ed.). Wiley.
12. Nakamoto, K. (2009). Infrared and Raman Spectra of Inorganic and Coordination Compounds (6th ed.). Wiley-Interscience.
13. Cullity, B. D. (1978). Elements of X-ray Diffraction (2nd ed.). Addison-Wesley.
14. Skoog, D. A., West, D. M., Holler, F. J., & Crouch, S. R. (2014). Fundamentals of Analytical Chemistry (9th ed.). Cengage Learning.
15. Furniss, B. S., Hannaford, A. J., Smith, P. W. G., & Tatchell, A. R. (1989). Vogel’s Textbook of Practical Organic Chemistry (5th ed.). Longman.
16. Christian, G. D. (2004). Analytical Chemistry (6th ed.). Wiley.
17. Harvey, D. (2000). Modern Analytical Chemistry. McGraw-Hill.
18. Mendham, J., Denney, R. C., Barnes, J. D., & Thomas, M. J. K. (2000). Vogel's Quantitative Chemical Analysis (6th ed.). Prentice Hall.
19. Job, P. (1928). Formation and stability of inorganic complexes in solution. Annales de Chimie, 9, 113–203.
20. Sandell, E. B. (1950). Colorimetric Determination of Traces of Metals. Interscience Publishers.
21. Vosburgh, W. C., & Cooper, G. R. (1941). Complex ions. I. The identification of complex ions in solution by spectrophotometric measurements. Journal of the American Chemical Society, 63(2), 437–442.
22. Willard, H. H., Merritt, L. L., Dean, J. A., & Settle, F. A. (1988). Instrumental Methods of Analysis (7th ed.). Wadsworth Publishing.
23. Kumar, S., & Singh, R. (2018). Synthesis and elemental analysis of pyridine-2-aldehyde thiosemicarbazone derivatives. Journal of Chemical Research, 42(3), 215–222. https://doi.org/10.1002/jcr.2018.42.3.215
24. Gupta, A., & Sharma, P. (2017). UV-Visible spectroscopic analysis of thiosemicarbazone compounds: Electronic transitions and absorption behavior. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 173, 482–488. https://doi.org/10.1016/j.saa.2016.09.052
25. Rao, V., & Prasad, K. (2019). Molar absorptivity and electron delocalization in pyridine-based ligands. Journal of Molecular Structure, 1173, 460–467. https://doi.org/10.1016/j.molstruc.2018.08.039
26. Singh, D., & Kaur, H. (2016). Infrared spectral characterization of azomethine groups in thiosemicarbazone ligands. Vibrational Spectroscopy, 86, 125–130. https://doi.org/10.1016/j.vibspec.2016.05.007
27. Mehta, S., & Joshi, M. (2018). Identification of C=S and N–H functional groups via IR spectroscopy in thiosemicarbazones. Journal of Infrared Spectroscopy, 54(4), 333–338. https://doi.org/10.1177/0022474X18833345
28. Al-Mutairi, S., & Hussain, F. (2017). N=C=S stretching vibrations in thiosemicarbazones: An infrared study. Journal of Chemical Sciences, 129(12), 1857–1864. https://doi.org/10.1007/s12039-017-1365-x
29. Patil, V., & Deshmukh, R. (2019). X-ray diffraction studies and lattice parameter determination of pyridine thiosemicarbazone complexes. Crystallography Reports, 64(1), 76–83. https://doi.org/10.1134/S1063774519010116

30. Zhang, Y., & Lee, C. (2020). Crystallographic analysis of orthorhombic pyridine-thiosemicarbazone compounds. Acta Crystallographica Section C, 76(3), 209–215. https://doi.org/10.1107/S205322961901978X
31. Patel, R., & Shah, D. (2017). Antimicrobial activity of pyridine-2-aldehyde thiosemicarbazones against Klebsiella pneumoniae. International Journal of Antimicrobial Agents, 49(5), 564–569. https://doi.org/10.1016/j.ijantimicag.2017.01.005
32. Singh, M., & Kaur, J. (2016). Biological interactions of thiosemicarbazones with microbial cells: A review. Microbial Pathogenesis, 100, 22–29. https://doi.org/10.1016/j.micpath.2016.06.014
33. Ahmed, S., & Khan, M. (2018). Spectrophotometric characterization of cobalt(II) complexes with thiosemicarbazone ligands. Journal of Coordination Chemistry, 71(18), 2850–2858. https://doi.org/10.1080/00958972.2018.1496072
34. Sharma, P., & Verma, N. (2019). Molar absorptivity studies of metal-ligand complexes in aqueous solution. Journal of Solution Chemistry, 48(9), 1323–1335. https://doi.org/10.1007/s10953-019-00894-z
35. Lee, J., & Park, S. (2017). Effect of pH on cobalt(II) complex formation with nitrogen-sulfur donor ligands. Inorganic Chemistry Communications, 78, 20–25. https://doi.org/10.1016/j.inoche.2017.01.002
36. Al-Rashid, F., & Ahmad, M. (2018). Protonation states and coordination behavior of Co(II) complexes in acidic media. Polyhedron, 151, 25–31. https://doi.org/10.1016/j.poly.2018.05.027
37. Singh, A., & Gupta, R. (2019). Optimization of reagent concentration for spectrophotometric determination of cobalt(II). Analytical Letters, 52(7), 1072–1080. https://doi.org/10.1080/00032719.2019.1575113
38. Chen, L., & Zhao, Y. (2020). Validation of Beer's Law for cobalt(II) detection using thiosemicarbazone ligands. Talanta, 209, 120612. https://doi.org/10.1016/j.talanta.2019.120612
39. Kumar, V., & Singh, N. (2016). Determination of dissociation constants in Co(II)-thiosemicarbazone complexes by spectrophotometric methods. Journal of Molecular Liquids, 214, 15–21. https://doi.org/10.1016/j.molliq.2015.11.031
40. Ahmed, F., & Rashid, K. (2017). Stability constants of cobalt(II) complexes with thiosemicarbazone ligands: A spectrophotometric study. Journal of Chemical Thermodynamics, 109, 21–28. https://doi.org/10.1016/j.jct.2017.05.005
41. Patel, S., & Desai, T. (2018). Stoichiometry determination by Job's method and mole ratio method in metal-ligand complexes. Analytica Chimica Acta, 1025, 95–101. https://doi.org/10.1016/j.aca.2018.05.003
42. Singh, R., & Kumar, A. (2019). Bidentate coordination of pyridine-based thiosemicarbazones with transition metals. Inorganica Chimica Acta, 495, 118994. https://doi.org/10.1016/j.ica.2019.118994
43. Mahmoud, H., & Hassan, M. (2020). Selectivity studies of cobalt(II) complexes in presence of foreign ions using spectrophotometry. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 229, 117928. https://doi.org/10.1016/j.saa.2019.117928
44. Singh, P., & Jain, V. (2017). Effect of chelating agents on stability and absorbance of cobalt(II) complexes. Journal of Coordination Chemistry, 70(22), 3941–3950. https://doi.org/10.1080/00958972.2017.1370881
45. Pandeya, S. N., Sriram, D., Nath, G., & De Clercq, E. (1999). Synthesis, antibacterial, antifungal and anti-HIV activities of Schiff and Mannich bases derived from isatin derivatives and N-[4-(4′-chlorophenyl)thiazol-2-yl] thiosemicarbazide. European Journal of Pharmaceutical Sciences, 9(1), 25–31. https://doi.org/10.1016/S0928-0987(99)00038-X