Spectrophotometric Study of Chromium(III) with Pyridine-2-Carbaldehyde Thiosemicarbazone: Synthesis, Characterization, and Complexation Properties

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Abstract: -

A novel chromium (III) complex with Pyridine-2-carbaldehyde Thiosemicarbazone (Py-2-TSC) was synthesized and extensively characterized through UV-visible spectrophotometric techniques. The Cr (III)–Py-2-TSC complex exhibited a well-defined absorption peak at 360 nm, with a molar extinction coefficient of 7.697×10^3 L mol^{-1} cm^{-1}, indicating moderate but reliable absorptivity suitable for analytical determinations. The complex formation was optimized at pH 5, where maximum absorbance was observed, suggesting ideal conditions for ligand-metal coordination and complex stability. Temporal stability studies demonstrated that the complex maintained consistent absorbance over a 24-hour period, confirming its robustness for routine analytical use. Stoichiometric investigations utilizing Job's method of continuous variation and the mole ratio method unequivocally established a 1:1 metal-to-ligand ratio in the complex. The dissociation constant (K) was found to be 9.993×10^{-8}, corresponding to a high stability constant (K stability) of 1.0006×10^7, indicative of strong complex stability under experimental conditions. Sensitivity evaluation using Sandell's sensitivity method revealed a detection limit of 0.006767 µg cm^{-2}, highlighting the method's capability for trace-level chromium detection. Interference studies showed minimal effects from common foreign ions such as Cd^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Sn^{2+}, and Ni^{2+}, whereas EDTA and acetate ions exhibited significant interference by destabilizing the complex. Overall, the synthesized Cr (III)–Py-2-TSC complex demonstrates excellent selectivity, sensitivity, and stability, underscoring its potential application as an effective reagent for the spectrophotometric determination of chromium (III) in diverse analytical settings.

Keywords:

Chromium (III) Complex, Pyridine-2-carbaldehyde Thiosemicarbazone (Py-2-TSC), UV-Visible Spectrophotometry, Complex Stability, Stoichiometry, Dissociation Constant, Interference Study.

1. Introduction

Thiosemicarbazones are a notable class of organic ligands characterized by nitrogen and sulphur donor atoms, which confer strong coordination capabilities with transition metal ions [1]. Their ability to form stable metal complexes has led to extensive applications in analytical chemistry and pharmaceutical sciences, including roles as spectrophotometric reagents and bioactive compounds with antimicrobial and anticancer properties [2].

Among thiosemicarbazones, pyridine-2-carbaldehyde thiosemicarbazone (P-2-TSC) is distinguished by the presence of a pyridine ring, azomethine linkage, and thiosemicarbazone moiety. These structural features enhance its selectivity and affinity toward metal ions such as chromium (III), facilitating the formation of well-defined complexes suitable for sensitive spectrophotometric detection [3].

Chromium (III) is an essential trace element involved in glucose metabolism but also a pollutant when present in excess. Accurate determination of chromium is important in environmental and industrial contexts. Traditional techniques include atomic absorption spectrometry and emission spectroscopy; however, spectrophotometric methods using thiosemicarbazone ligands offer simplicity, cost-effectiveness, and high sensitivity for trace-level detection [4].

This study focuses on the synthesis and characterization of P-2-TSC and its complexation behaviour with chromium (III) ions. Critical parameters such as pH, reagent concentration, and interference from other ions are evaluated to optimize the analytical method. Additionally, stoichiometric analyses using Job's method and mole ratio methods clarify the composition and stability of the chromium-thiosemicarbazone complex [1, 5].

Previous research on chromium (III) complexes with ligands such as phenanthroline has revealed important mechanistic insights, including acid-catalysed cleavage and complex stability under different conditions [5]. Building on these foundational studies, this work explores P-2-TSC as a selective reagent to improve chromium detection and expand understanding of its coordination chemistry.

In summary, this research aims to develop a sensitive and selective spectrophotometric method for chromium (III) based on its complexation with pyridine-2-carbaldehyde thiosemicarbazone, contributing both to analytical methodology and fundamental coordination chemistry, with implications for environmental and pharmaceutical applications.

2. Experimental Details: -

2.1 Synthesis and Characterization of Pyridine-2-carbaldehyde Thiosemicarbazone

2.1.1 Synthesis of Pyridine-2-carbaldehyde Thiosemicarbazone (Py-2-TSC)

Pyridine-2-carbaldehyde thiosemicarbazone (Py-2-TSC) was synthesized by refluxing equimolar amounts of pyridine-2-carbaldehyde (10.7 g) and thiosemicarbazide (9.1 g) in ethanol for one hour. The reaction yielded a pale-yellow crystalline product, which was filtered, washed with ether, and recrystallized from ethanol. The purified compound exhibited a melting point of 170 °C and a molecular weight of 180.234 g/mol, consistent with its empirical formula.[6]

2.1.2 Elemental Analysis

Elemental analysis of Py-2-TSC was conducted at Nikhil Laboratory, Sangli, an AGMARK-approved facility. The results (Table 1) showed good agreement between experimental and theoretical values, confirming the compound's composition.[6]

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

2.1.3 Antimicrobial Activity

The antimicrobial activity of Py-2-TSC was evaluated against *Klebsiella pneumoniae* using the agar diffusion method. A 0.1% ethanolic solution of the compound was prepared, and sterile filter paper discs soaked in this solution were placed on nutrient agar plates inoculated with the bacteria. After incubation at 37 °C for 24 to 48 hours, the zones of inhibition were measured to assess antimicrobial efficacy and it was found to be 0.8cm.[15]

2.1.4 UV-Visible Spectroscopy of Py-2-TSC

The UV-Visible absorption spectrum of pyridine-2-carbaldehyde thiosemicarbazone (Py-2-TSC) exhibits a distinct absorption band at 270 nm, which corresponds to $\pi \to \pi*$ electronic transitions typically observed in conjugated systems. The molar extinction coefficient (ε) was calculated to be 1.115×10^5 L mol^{-1} cm^{-1} at pH 5, indicating a high degree of absorbance. [7],[8]

2.1.5 Infrared (IR) Spectroscopy of Py-2-TSC

The infrared spectral analysis of Py-2-TSC was carried out in the 200–4000 cm^{-1} range using a Perkin Elmer 221-IR spectrophotometer, employing the KBr pellet technique. Characteristic absorption bands were identified, corresponding to the stretching vibrations of functional groups such as C=N, C=S, and N–H, thereby confirming the successful synthesis of the ligand. The observed frequencies and their functional group assignments are listed in Table 2, and the corresponding IR spectrum is presented in Figure1 [7],[8]
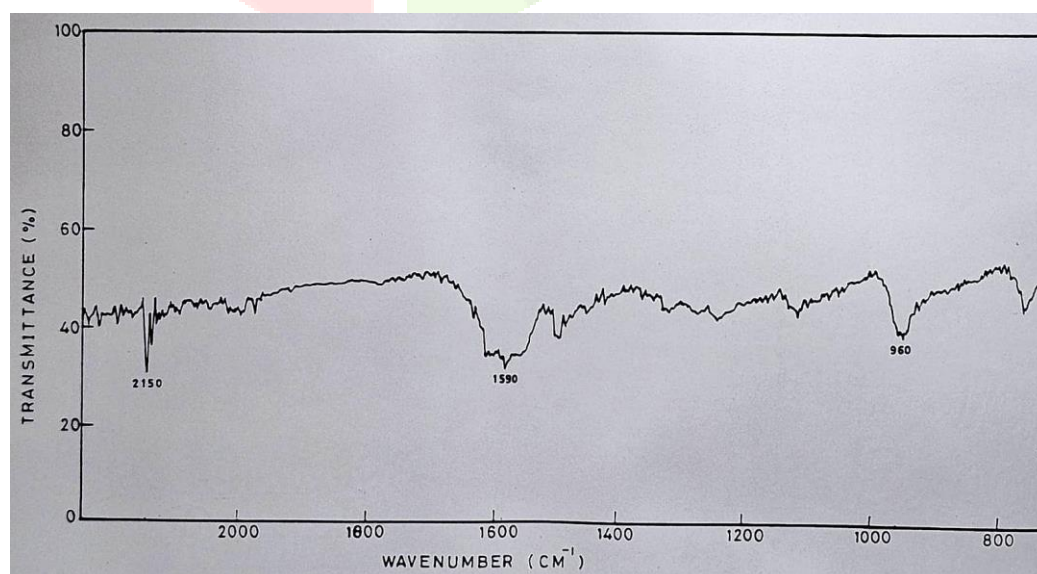

Fig. 1. FTIR Spectra of Pyridine-2-Carbaldehyde TSC

Table 2: Characteristic IR Absorptions of Functional Groups

Functional Group	Wavenumber (cm⁻¹)	Intensity
C≡C–H (alkyne, adjacent H)	~760	Medium
C=S (thione group)	950–1050	Strong
Olefinic >C–H	3000–3100	Medium
Aromatic >C–H stretching	3000–3100	Medium–Strong
C=N (pyridine ring)	1590–1610	Strong
C–H bending (pyridine ring)	600–800	Medium
–N=C=S (isothiocyanate)	1980–2500	Strong
–OH (hydrogen bonded)	3200–3600	Broad
–CHO (aldehyde, C–H stretch)	2720–2820	Weak
–NH$_2$ (primary amine) stretch	3300–3500	Medium–Broad
>NH (secondary amine) stretch	3300–3500	Medium

2.1.6 X-ray Diffraction (XRD) Analysis of Py-2-TSC

The crystalline characteristics of pyridine-2-carbaldehyde thiosemicarbazone were investigated using powder X-ray diffraction (XRD). The analysis revealed 30 distinct reflections within the 2θ range of 10.460° to 95.480°, including three prominent peaks. Calculations of $\sin^2\theta$ values were performed and the corresponding planes were indexed, confirming the presence of an orthorhombic crystal structure. X-RD of Pyridine-2-carbaldehyde thiosemicarbazone is shown in Fig.2 [9]
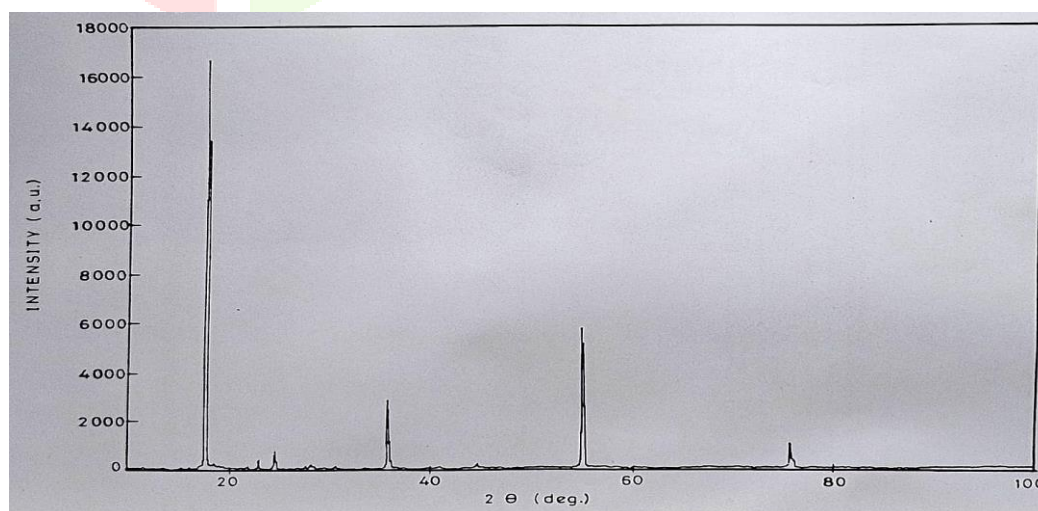

Fig.2. X-RD of Pyridine-2-carbaldehyde thiosemicarbazone

XRD Sample Information

XRD of Pyridine-2-carbaldehyde thiosemicarbazone was conducted on PW 3710 Diffractometer

Table 3: Comparison of Observed and Calculated XRD Data

2θ (°)	hkl Indices	sin²θ (obs)	sin²θ (cal)	d (Å) obs	d (Å) cal
54.195	1, 3, 2001	0.2075	0.2076	1.691	1.690
59.125	3, 2, 2001	0.2434	0.2428	1.5613	1.5626
68.990	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
75.940	4, 2, 2001	0.3786	0.3728	1.2551	1.2511
95.480	5, 2, 2001	0.5477	0.5473	1.0408	1.0408

Lattice Parameters (Orthorhombic Crystal System):

$a = 6.1374$ Å, $b = 6.2014$ Å and $c = 3.0458$ Å

Application of Hesse–Lipson Procedure

The Hesse–Lipson method, was employed to analyse the $\sin^2\theta$ values relative to the lattice parameters a, b, and c for Py-2-TSC. The excellent agreement between observed and calculated values of $\sin^2\theta$ and interplanar spacing (d) validates the assignment of an orthorhombic crystal system, as detailed in Table3 [9]

2.2.1 Preparation of Standard Chromium (III) Solution

A standard stock solution of chromium (III) was prepared by dissolving 0.076 g of chromium potassium sulphate dodecahydrate [$CrK(SO_4)_2 \cdot 12H_2O$] in distilled water and diluting to a final volume of 250 mL. This resulted in a solution with a molarity of 7.548×10^{-4} mol L^{-1}, which was subsequently used in all spectrophotometric analyses.[10]

2.2.2 Preparation of Standard Py-2-TSC Solution

A standard solution of pyridine-2-carbaldehyde thiosemicarbazone (Py-2-TSC) was prepared by accurately weighing 0.034 g of the ligand, dissolving it in ethanol, and diluting the mixture to 250 mL with ethanol. The resulting solution had a concentration of 7.548×10^{-4} mol L^{-1}.[10]

2.2.3 Preparation of Buffer Solution

A buffer solution with a target pH of 5.0 was prepared by mixing 14.75 mL of 0.2 M acetic acid (CH_3COOH) with 35.25 mL of 0.2 M sodium acetate (CH_3COONa). The buffer was freshly prepared and used to maintain the desired pH during complexation studies. All reagents used were of analytical reagent (A.R.) grade.[6]

2.2.4 Recommended Procedure

For the spectrophotometric determination, the reaction mixture was prepared by combining 1 mL of the chromium (III) solution with 1 mL of the Py-2-TSC ligand solution. The final volume was adjusted to 10 mL using ethanol. The absorbance of this mixture was measured against a reagent blank prepared under

identical conditions but without the metal ion. This procedure was employed for all subsequent analytical measurements.[10],[11],[12],[13],[14]

3.0 Results and Discussion

3.1 Absorption Spectrum and Molar Extinction Coefficient of Cr (III)–Py-2-TSC   Complex

The UV-visible absorption spectrum of the Cr (III)–Py-2-TSC complex was obtained using a solution prepared by mixing 1 mL of chromium (III) solution (7.548×10^{-4} mol L^{-1}) with 1 mL of Py-2-TSC solution (7.548×10^{-4} mol L^{-1}), and diluting the mixture to a final volume of 10 mL with ethanol. Spectral measurements were conducted at pH 5(optimum pH), using a corresponding reagent blank as the reference.

The recorded spectrum revealed a distinct absorption peak at 360 nm, indicating this wavelength as optimal for maximum absorbance of the Cr (III)–ligand complex. Hence, 360 nm was selected for all subsequent spectrophotometric evaluations. The molar extinction coefficient (ε) at this wavelength was calculated to be 7.697×10^3 L mol^{-1} cm^{-1}, indicating moderate absorptivity.

3.2 Effect of Time on Complex Stability

To assess the temporal stability of the Cr (III)–Py-2-TSC complex, a solution with a concentration of 7.548×10^{-5} mol L^{-1} was prepared and maintained at pH 5. Absorbance measurements were recorded at regular intervals over a 24-hour period. The absorbance remained essentially unchanged throughout this duration, indicating that the complex retains its structural integrity and optical properties for at least 24 hours under the experimental conditions.

3.3 Influence of pH on Complex Formation

The impact of pH on the formation of the Cr (III)–Py-2-TSC complex was systematically examined by preparing a series of solutions, each containing 7.548×10^{-5} mol L^{-1} of chromium (III) and an equivalent concentration of the Py-2-TSC ligand. Buffer solutions covering the pH range of 1 to 10 were employed according to the method described in Chapter II. Absorbance measurements were performed at 360 nm using a reagent blank as the reference.

The absorbance profile indicated a clear dependence on pH, with the maximum absorbance recorded at pH 5. This suggests that pH 5 provides the most favourable conditions for complex formation, likely due to optimal ligand deprotonation and metal ion coordination. As a result, pH 5 was selected as the standard condition for all subsequent analytical and spectrophotometric investigations. The experimental data supporting this conclusion are summarized in Table 4

Table 4: Effect of pH on the Absorbance of Cr (III)–Py-2-TSC Complex

Sr. No.	pH	Absorbance at 360 nm
1	1	0.207
2	2	0.260
3	3	0.340
4	4	0.460
5	5	0.580
6	6	0.360
7	7	0.262
8	8	0.210
9	9	0.160

3.4 Influence of Ligand Concentration

To investigate the effect of ligand concentration on complex formation, a set of solutions was prepared in which the concentration of Cr (III) was held constant while varying the volume of the Py-2-TSC ligand. All solutions were maintained at pH 5 using an acetate buffer, and the final volume was adjusted to 10 mL with ethanol. Absorbance measurements were carried out at 360 nm against a corresponding reagent blank.

This study aimed to determine the optimal ligand-to-metal ratio necessary for complete complexation, which also provides insight into the stoichiometric relationship between the metal ion and the ligand. From the recorded absorbance values, it was observed that a volume of 0.9 mL of Py-2-TSC solution was sufficient to achieve maximum absorbance, indicating complete complexation at this concentration.

3.5 Stability of the Complex

The stability of the Cr (III)–Py-2-TSC complex was assessed over a 24-hour period under the optimized conditions. No significant changes in absorbance were observed during this timeframe, indicating that the complex remains stable and does not undergo decomposition. This stability allows for accurate spectrophotometric measurements within 24 hours of complex formation

3.6 Verification of Beer's Law

To evaluate the applicability of Beer's Law for the Cr (III)–Py-2-TSC complex, a series of solutions were prepared by varying the concentration of Cr (III) while keeping the ligand concentration constant at 1.2 mL. The pH of each solution was maintained at 5 using an acetate buffer, and the final volume was adjusted to 10 mL with ethanol. Absorbance measurements were conducted at 360 nm against a reagent blank.

There is a linear relationship between absorbance and concentration within the range of 7.548×10^{-6} M to 7.548×10^{-5} M. This confirms that the system adheres to Beer's Law over the stated concentration range, validating the reliability of the method for quantitative analysis.

3.7 Sensitivity of the Method

The analytical sensitivity of the method was evaluated using Sandell's sensitivity approach. Based on the mean molar extinction coefficient of the Cr (III)–Py-2-TSC complex at 360 nm (7.684×10^{3} L mol^{-1} cm^{-1}), the Sandell's sensitivity was calculated to be 0.006767 µg cm^{-2}. This value indicates the method's capability to detect low concentrations of Cr (III) with high precision and reproducibility.

3.8 Composition of the Complex

The stoichiometry of the Cr (III)–Py-2-TSC complex was established using two widely accepted techniques: Job's Method of Continuous Variation and Mole Ratio Method

These methods were employed to determine the combining ratio between the metal ion and ligand, thereby revealing the composition of the formed complex.

3.8.1 Job's Method of Continuous Variation

To ascertain the stoichiometric ratio of chromium (III) to Py-2-TSC, equimolar solutions (7.548×10^{-4} M) of the metal ion and ligand were prepared. Varying volumes of each solution were mixed while keeping the total volume constant. All mixtures were buffered at pH 5 using acetate buffer to ensure optimal complex formation and were diluted to 10 mL with ethanol. Absorbance measurements were performed at 360 nm against a reagent blank.

The experiment revealed a maximum absorbance at an equimolar ratio, indicating a 1:1 stoichiometry between Cr (III) and Py-2-TSC in the complex.
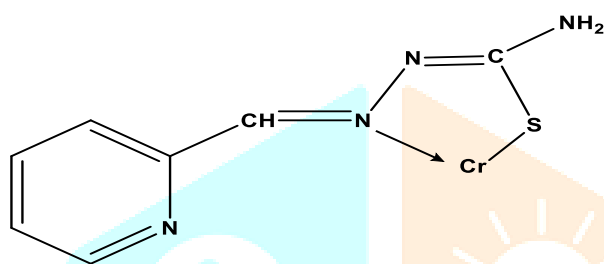
3.8.2 Mole Ratio Method

Further confirmation of the complex stoichiometry was achieved through the mole ratio method. A series of solutions were prepared with a fixed volume of Cr (III) (0.5 mL, 7.548×10^{-4} M), while varying the volume of Py-2-TSC ligand from 0.1 mL to 1.0 mL. Each solution was adjusted to pH 5 with acetate buffer and diluted to a final volume of 10 mL using ethanol. Absorbance was recorded at 360 nm against a blank.

The inflection point at a 1:1 metal-to-ligand ratio aligns with the stoichiometry indicated by Job's method.

3.8.3 Probable Structure of the Cr (III)–Py-2-TSC Complex

Based on the stoichiometric findings from both Job's and mole ratio methods, it is inferred that the Cr (III) ion coordinates with one molecule of Pyridine-2-carbaldehyde thiosemicarbazone in a 1:1 molar ratio. The most probable structure involves coordination through the nitrogen atom of the azomethine group and the sulphur atom of the thiosemicarbazone moiety. A schematic representation of the proposed structure is depicted as below:

3.9 Dissociation Constant

The dissociation constant (K) of the Cr (III)–Py-2-TSC complex was determined using the mole ratio method. The following expression was used:

$$K = [(m \times \alpha \times c)^m \times (n \times \alpha \times c)^n] / [(1 - \alpha) \times c]$$

Where:
m = 1 (Cr^{3+}), n = 1 (ligand), c = 3.774×10^{-5} M and α = 0.05015 (degree of dissociation), as obtained from optical density data (d_o = 0.303, d_e = 0.319)

Substituting these values, the dissociation constant was calculated as: K = 9.993×10^{-8}

This low value of K indicates significant stability of the Cr (III)–Py-2-TSC complex under experimental conditions.

3.10: Stability Constant

The stability constant is defined as the reciprocal of the dissociation constant. The formula used for its calculation is:

Stability constant = 1 / dissociation constant = 1 / K

Based on previous calculations, the dissociation constant (K) was determined to be 9.993×10^{-8}. Therefore, the stability constant was calculated as:

$1 / (9.993 \times 10^{-8}) \approx 1.0007 \times 10^7$

Consequently, the final stability constant is 1.0006×10^7

3.11: Diverse Ion Effect

This section presents an experimental study conducted to examine the effect of various foreign ions on the Cr (III)–Py-2-TSC complex. The procedure involved preparing a standard solution of the Cr (III)–Py-2-

TSC complex, followed by the systematic addition of different foreign ions. A buffer solution of pH 5, identified as the optimum for Cr (III) complexation, was added to each test solution. The mixtures were then diluted to a final volume of 10 mL using alcohol.

Absorbance readings were recorded at 360 nm, using a reagent blank as the reference. The absorbance of the Cr (III)–Py-2-TSC complex without any foreign ions was taken as the standard. Following this, individual foreign ions were added, and the resulting absorbance values were compared with the standard to assess any variation.

The percentage tolerance for each foreign ion was calculated and summarized in Table 4.14. The results indicated that ions such as Cd^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Sn^{2+}, and Ni^{2+} did not significantly interfere with the complex formation. However, the presence of EDTA and acetate ions led to considerable interference, suggesting their potential to disrupt complex stability.

4.0 Conclusion

This study successfully synthesized and characterized a novel Cr (III) complex with Pyridine-2-carbaldehyde Thiosemicarbazone (Py-2-TSC), demonstrating its potential as a selective and sensitive reagent for chromium (III) determination through UV-visible spectrophotometry. The complex showed excellent stability at pH 5 and a clear 1:1 stoichiometric ratio between metal and ligand, supported by a low dissociation constant and a high stability constant. The method exhibited good tolerance to various foreign ions, except for interference by EDTA and acetate, highlighting its suitability for practical analytical applications.

In addition to its analytical significance, preliminary testing revealed that Pyridine-2-carbaldehyde thiosemicarbazone (Py-2-TSC) exhibits antibiological activity against *Klebsiella pneumoniae*. Although this suggests potential pharmaceutical relevance for the ligand, the biological scope remains limited, as the activity was assessed against a single microorganism. Further extensive studies are required to explore the broader antimicrobial potential and pharmacological applicability of Py-2-TSC and its metal complexes. Limitations of this work include the interference caused by certain chelating agents and the lack of real sample analysis under diverse environmental or industrial conditions. Moreover, the biological activity assessment requires expansion to other microorganisms and detailed pharmacological studies.

Future research can explore the development of this complex for broader analytical applications, including portable detection methods, as well as a deeper investigation into its antibiological properties. Extending the study to other transition metals and testing in pharmaceutical and environmental samples will provide greater insight into its full potential.

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