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Development of a Sensitive Spectrophotometric Method for Nickel Using Cinnamaldehyde - Thiosemicarbazone Ligand

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Abstract: ***The present study explores the synthesis, characterization, and spectrophotometric analysis of a Nickel (II) complex with cinnamaldehyde - thiosemicarbazone (Cin - TSC), a Schiff base ligand known for its chelating ability and potential bioactivity. Nickel, a transition metal of significant industrial and biological relevance, often requires selective and sensitive detection methods in various matrices. Traditional reagents for nickel estimation suffer from limitations due to interference by other metal ions. In contrast, thiosemicarbazones offer enhanced selectivity, stability, and analytical utility. In this study, the Ni (II) –Cin - TSC complex was prepared in ethanol under acidic conditions (pH 3), and its spectrophotometric properties were examined. The complex exhibited a yellow coloration with a maximum absorbance at 360 nm. The molar extinction coefficient at this wavelength was found to be 2.228×10^2 L mol^{-1} cm^{-1}. The stability of the complex was investigated, and it was observed to remain stable for up to 10–12 hours. Beer's law was obeyed in the concentration range of 8.48×10^{-5} M to 8.48×10^{-4} M of Ni (II). The Sandell's sensitivity was calculated to be 0.2648 µg cm^{-2}. The stoichiometry of the complex was determined by Job's method of continuous variation and the mole ratio method, both indicating a 1: 2 metal - to - ligand ratio. The degree of dissociation (α) was found to be 0.0364, and the dissociation constant (K) was calculated as 1.18×10^{-13}, indicating the high stability of the complex. The findings support the use of Cin - TSC as a reliable reagent for the spectrophotometric determination of nickel and provide insight into the structural and stability aspects of the complex. Applications: The Ni (II) –Cin - TSC complex was successfully applied for the quantitative determination of nickel content in commercially available Cadbury chocolate samples. Using the Beer's law calibration plot, the calculated values of nickel were found to be in close agreement with the experimentally observed values. This confirms the reliability and suitability of the Cin - TSC complex as an effective reagent for analytical applications, especially for nickel determination in food matrices.***

Keywords: Nickel (II), Thiosemicarbazone, Cinnamaldehyde, Spectrophotometry, Stability Constant, Schiff Base Complex, Dissociation Constant

1. Introduction

Nickel is a transition metal of great industrial and biological relevance, widely used in the manufacturing of stainless steel, electroplating, coinage, batteries, and catalysts [1]. Due to its broad utility and potential toxicity at elevated concentrations, the accurate determination and monitoring of nickel in various matrices is essential [2]. Among the various analytical methods available, spectrophotometric techniques are preferred for their simplicity, sensitivity, and cost - effectiveness [3].

Traditionally, reagents such as dimethylglyoxime (DMG), oximes, dithiozones, and azonaphthols have been employed for nickel determination. However, their selectivity and sensitivity often suffer from interferences by other metal ions [4]. To overcome these limitations, Schiff bases and thiosemicarbazones have emerged as promising ligands due to their strong metal - binding ability, tunable donor properties, and biological compatibility [5].

Thiosemicarbazones are versatile compounds known for their ability to form stable complexes with transition metals. Their applications span across analytical chemistry—where they are employed for metal ion detection—and pharmaceutical chemistry, owing to their antimicrobial, antiviral, and anticancer activities [6]. Their metal complexes often exhibit enhanced biological activity due to increased lipophilicity and cell membrane permeability [7].

In the present study, cinnamaldehyde thiosemicarbazone (Cin - TSC) is used as a novel reagent for the spectrophotometric determination of Ni (II). This ligand forms a yellow - colored complex with nickel in acidic media, making it suitable for analytical quantification [8]. The use of Cin - TSC not only improves sensitivity and selectivity but also contributes to the expanding library of biologically inspired ligands for metal detection [9].

Literature reports support the relevance of thiosemicarbazones and Schiff base ligands in coordination chemistry. Halli et al. explored stability constants of nickel complexes with various Schiff base derivatives using potentiometric techniques [10]. Gurkhan studied the protonation constants and formation equilibria of substituted salicylidene derivatives with Ni (II) [11]. Nair and coworkers have extensively reported on mixed ligand complexes of nickel involving amino acids, pyridine - based acids, and sulfur - containing drugs, relevant to biological systems [12]. Schiff base metal complexes derived from thiophene, furfuraldehyde, and salicylaldehyde have been synthesized and evaluated for antimicrobial and structural properties by researchers including Mishra, Nag, and Satpatri [13].

Recent advances in ligand design emphasize bioinspired moieties like imidazole, benzimidazole, and thiosemicarbazones due to their structural resemblance to enzyme active sites and pharmacophores [14]. Choudhary and others demonstrated the preparation of Ni (II) complexes with substituted thiosemicarbazones [15], while Pleniceanu and

Mishra focused on their use in ion - selective electrodes and antimicrobial agents [16].

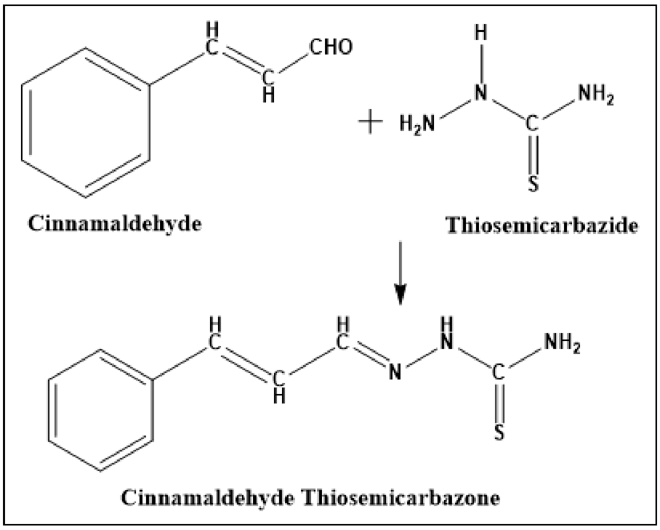
Given the pharmaceutical and analytical potential of thiosemicarbazone - based complexes, this study aims to synthesize and characterize the Ni (II) complex of cinnamaldehyde thiosemicarbazone and evaluate its spectrophotometric properties under optimized conditions. The stability, stoichiometry, and potential analytical applications of the complex are examined, contributing to the development of sensitive and selective methods for nickel detection. Moreover, the practical applicability of the Ni (II) –Cin - TSC complex was demonstrated through its use in the quantitative determination of nickel content in commercially available Cadbury chocolate samples. Utilizing the Beer’s law calibration plot, the experimentally determined nickel concentrations were found to be in close agreement with the calculated values. This consistency highlights the accuracy and reliability of the Cin - TSC complex, confirming its potential as an effective reagent for analytical applications, particularly for the selective and sensitive detection of nickel in complex food matrices.

2. Experimental

2.1 Synthesis and Characterization of Cinnamaldehyde Thiosemicarbazone

2.1.1 Synthesis of Cinnamaldehyde Thiosemicarbazone

Cinnamaldehyde thiosemicarbazone was prepared by refluxing equimolar quantities of cinnamaldehyde (C_6H_5–CH=CH–CHO, mol. wt.132.07) and thiosemicarbazide in a minimal amount of ethanol for one hour. [17]. The resulting yellow crystalline product was obtained with a melting point of 173°C. The product was recrystallized from ethanol. The molecular weight, determined from its molecular formula, is 205.284.



2.1.2 Elemental Analysis of Cinnamaldehyde - TSC

The elemental composition of Cinnamaldehyde - Thiosemicarbazone (Cinnamaldehyde - TSC) was determined and is presented in Table 1. The experimentally found values were in good agreement with the theoretically calculated values, confirming the proposed molecular formula and purity of the compound.

Table 1: Elemental Analysis of Cinnamaldehyde - TSC

Sr. No.	Chemical Element	Percentage Found (%)	Expected (%)
1	Carbon	58.22	58.53
2	Hydrogen	5.1	5.36
3	Sulphur	15.3	15.6
4	Nitrogen	20.22	20.48

2.1.3 Antimicrobial Activity of Cinnamaldehyde - TSC

The samples were prepared at a concentration of 100 mg/mL in alcohol. The molten nutrient medium was poured into Petri plates and allowed to solidify. A 24 - hour - old culture of *Klebsiella pneumoniae* was spread over the solidified medium using a nichrome wire loop. Sterile filter paper discs were immersed into the test solution using sterile forceps. After allowing the solvent to evaporate, the discs were placed on the inoculated plates. [18]

The plates were incubated at 37°C for 24 and 48 hours, and the zone of inhibition was measured. The antimicrobial activity is recorded and measured in term of zone of inhibition and it was found to be 0.60 cm.

2.1.4 Absorption Spectra of Cinnamaldehyde - TSC in the UV - Visible Region

The absorption spectra of Cinnamaldehyde - Thiosemicarbazone (2.439×10^{-5} M) were recorded in ethanol at pH 4, using an acetate buffer. A strong absorption band was observed at 330 nm, with a molar extinction coefficient (ε) of 1.250×10^5 L·mol^{-1}·cm^{-1}. [19]

Since the visible range starts from approximately 330 nm, the observed absorption confirms that Cinnamaldehyde - TSC absorbs in the UV region.

2.1.5 Infrared Spectra of Cinnamaldehyde - TSC

The IR spectrum was recorded in the range of 4000–200 cm^{-1} using a Perkin Elmer 221 IR spectrophotometer with the KBr pellet technique [20]. The characteristic absorption bands observed are presented in Table 2 (Fig.1).

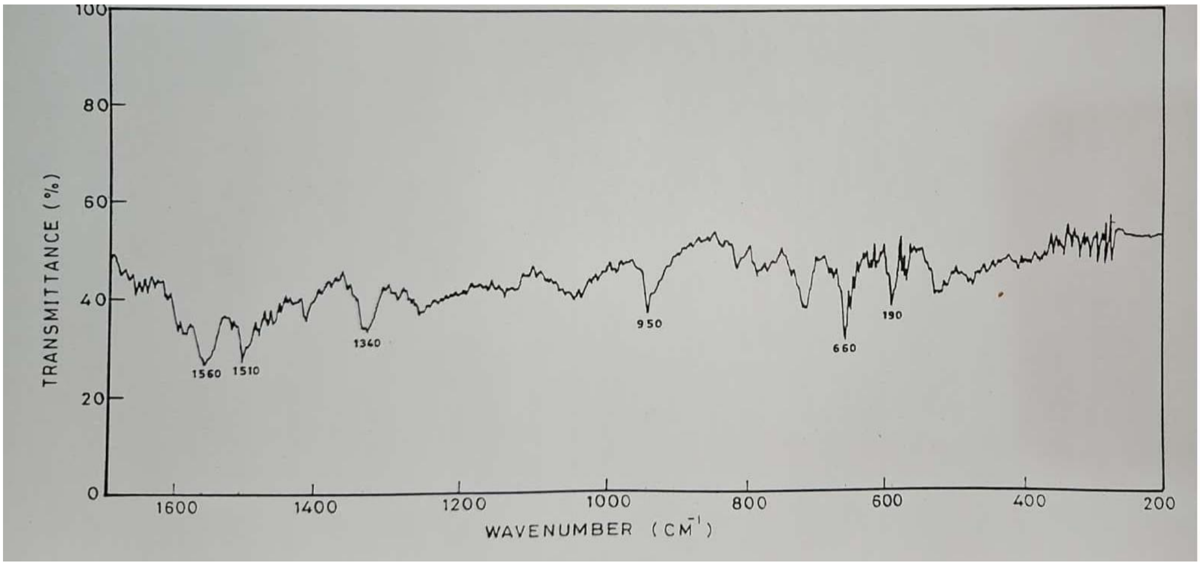


Figure 1: IR Spectra of Cinnamaldehyde - Thiosemicarbazone.

Table 2: IR Spectra and Expected Functional Group

Sr. No.	Frequency (cm^{-1})	Expected Functional Group
1	655	C–S stretching or deformation
2	715	Out - of - plane =C–H bending (aromatic ring)
3	950	C=S stretching (thiocarbonyl group)
4	1335	C–N stretching or CH_2 bending
5	1410	C–N stretching or CH_2 bending
6	1500	C=S coupled with N–H bending
7	1555	C=N stretching (azomethine group)
8	2580	N–C=S stretching
9	3000	C–H stretching of mono - substituted aromatic ring
10	3200	N–H stretching (overlapped with hydrogen bonding)
11	3330	Aromatic C–H stretching; intermolecular hydrogen bonding (possibly O–H/N–H)
12	3520	N–H symmetric and asymmetric stretching of $–NH_2$ (primary amine group)

The IR spectral analysis of the compound reveals several characteristic absorptions that confirm the presence of key functional groups typically associated with thiosemicarbazone derivatives. A low - frequency band at 655 cm^{-1} is attributed to C–S stretching or deformation, while the absorption at 715 cm^{-1} likely arises from out - of - plane =C–H bending vibrations, characteristic of aromatic systems. A strong peak observed at 950 cm^{-1} corresponds to C=S stretching, providing evidence for the thiocarbonyl group. The medium - intensity bands at 1335 cm^{-1} and 1410 cm^{-1}, though unassigned directly in the table, may originate from C–N stretching or CH_2 bending modes. The absorption at 1500 cm^{-1} is associated with C=S vibrations coupled with N–H bending, further supporting the presence of a thiosemicarbazone moiety. A distinct band at 1555 cm^{-1} is consistent with the C=N stretching vibration of the azomethine group formed by condensation of thiosemicarbazide with cinnamaldehyde. Additionally, the band at 2580 cm^{-1} is indicative of N–C=S stretching, a hallmark of thiourea and thiosemicarbazone derivatives.

In the higher frequency region, the absorption around 3000 cm^{-1} suggests the presence of a mono - substituted aromatic ring, while a broader band near 3200 cm^{-1} may result from N–H stretching overlapped with hydrogen bonding interactions. A sharper peak at 3330 cm^{-1} is attributed to aromatic C–H stretching, with possible contributions from intermolecular hydrogen bonding involving N–H or O–H groups. The band at 3520 cm^{-1} corresponds to symmetric and asymmetric N–H stretching vibrations of the primary amine ($–NH_2$) group. Together, these features confirm the presence of azomethine (C=N), thiocarbonyl (C=S), amine ($–NH_2$), and aromatic functionalities. The observed absorptions align well with the expected structure of a cinnamaldehyde - derived thiosemicarbazone ligand, supporting successful Schiff base formation and retention of thiosemicarbazide functionality.

2.1.6 X - Rd Of Cinnamaldehyde

X - ray Diffraction Analysis (Based on Hesse - Lipson Procedure):

The X - ray diffraction data for the synthesized cinnamaldehyde thiosemicarbazone ligand were analyzed using the Hesse - Lipson procedure. The calculated values of $\sin^2\theta$ for the diffraction peaks labeled A, B, and C were found to closely match the experimentally observed $\sin^2\theta$ values. Furthermore, the interplanar spacings (d - values), determined using Bragg's Law, showed excellent agreement with the observed values. This consistency in $\sin^2\theta$ and d - spacing data strongly supports that the compound crystallizes in the orthorhombic crystal system, characterized by mutually perpendicular axes of unequal lengths ($a \neq b \neq c$; $\alpha = \beta = \gamma = 90°$).

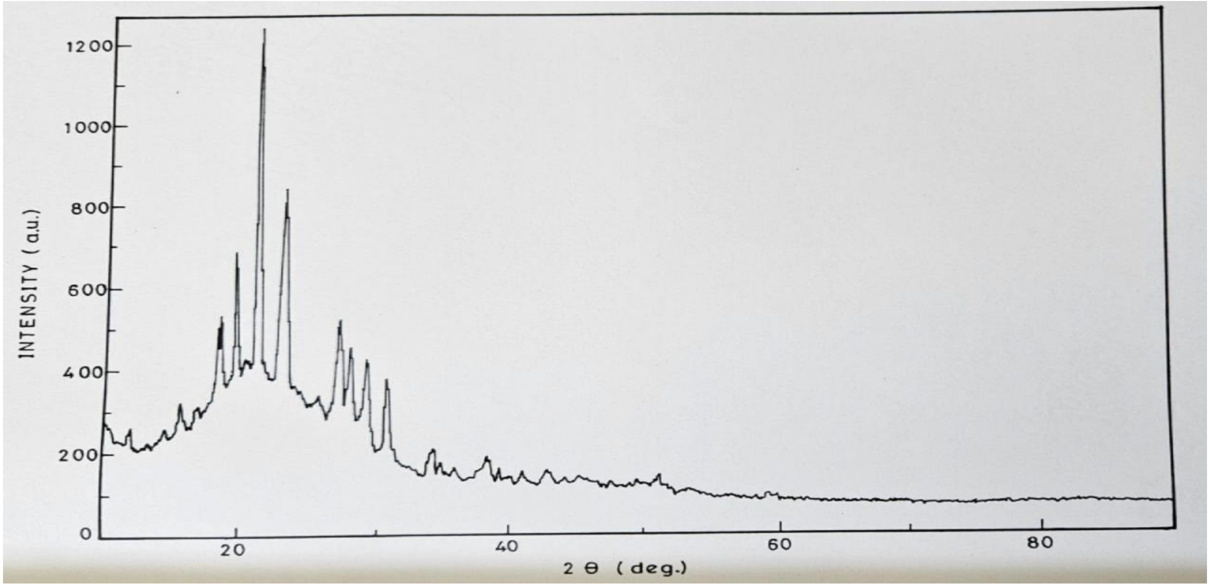


Figure 2: X - RD of Cinnamaldehyde - Thiosemicarbazone.

The X - ray diffraction (XRD) pattern of cinnamaldehyde thiosemicarbazone is shown in Fig.2, which confirms the crystalline nature of the compound and supports its orthorhombic crystal system based on the observed diffraction peaks.

2.2 Study of Nickel - Cinnamaldehyde Thiosemicarbazone Complex

2.2.1 Preparation of Standard Nickel Solution

Stock solution of Nickel (II) was prepared by dissolving 0.595 g of $NiSO_4$ in distilled water and then diluting to 250 mL with distilled water. The molarity of the stock solution was 8.48×10^{-3} M.

2.2.2 Preparation of Cinnamaldehyde - Thiosemicarbazone (Cin - TSC) Solution

A standard solution of cinnamaldehyde - thiosemicarbazone (Cin - TSC) was prepared by dissolving 0.435 g of the ligand in 250 mL of absolute ethanol. The molarity of the resulting solution was calculated to be 8.48×10^{-3} M. [21]

2.2.3 Preparation of Buffer Solution

A buffer solution of pH 3.0 was prepared by mixing 20.55 mL of 0.2 N disodium hydrogen phosphate (Na_2HPO_4) with 79.45 mL of 0.1 N citric acid solution. [22] All reagents and chemicals employed in this study were of Analytical Reagent (AR) grade.

2.2.4 Recommended Procedure

To a 1 mL aliquot of nickel (II) solution, a drop of concentrated sulfuric acid (H_2SO_4) was added to acidify the medium. Subsequently, 1 mL of the buffer solution (pH 3.0) was introduced, followed by the addition of 1 mL of the prepared Cin - TSC solution. The final volume was made up to 10 mL using absolute ethanol. The absorbance of the resulting solution was recorded at various wavelengths against a reagent blank under identical conditions. [23]

2.2.5 Analytical Study Overview:

The analytical investigation of the Nickel–Cinnamaldehyde Thiosemicarbazone complex includes the evaluation of various physicochemical parameters. The study encompassed:

- Effect of pH on the stability and formation of the complex, [24]
- Effect of reagent concentrations to determine optimal complexation conditions,
- Verification of Lambert - Beer's Law to confirm linear absorbance - concentration relationship,
- Job's Method of Continuous Variations and Mole Ratio Method to ascertain the stoichiometry of the complex [25],
- Study of Diverse Ion Effects to evaluate selectivity and interference tolerance of the method.

From the experimental data, the formation constant (stability constant), dissociation constant, and Sandell's sensitivity of the complex were also calculated.

3. Result and discussion

3.1 Absorption Spectra and Molar Extinction Coefficient of Ni (II) –Cinnamaldehyde - TSC Complex

The absorption spectrum of the Ni (II) –Cinnamaldehyde - TSC complex was recorded using a solution containing equimolar concentrations (8.48×10^{-3} M) of Ni (II) and cinnamaldehyde - thiosemicarbazone. The solution was prepared by acidifying 1 mL of Ni (II) solution with a drop of concentrated H_2SO_4, followed by the addition of 1 mL buffer (pH 3) and 1 mL of the ligand solution. Absorbance was measured against a reagent blank.

It was observed that the complex exhibited a maximum absorbance (λ_{max}) at 360 nm. The molar extinction coefficient (ε) of the Ni (II) –Cin - TSC complex at this wavelength was found to be 2.228×10^2 L mol^{-1} cm^{-1}.

3.2 Effect of Time on Absorbance

To investigate the stability of the Ni (II) –Cinnamaldehyde - TSC complex over time, the complex was prepared as described above at pH 3, and the absorbance was monitored at regular intervals. It was noted that the intensity of the yellow coloration gradually diminished, and a significant decrease in absorbance was observed after 10–12 hours, indicating instability of the complex over prolonged periods.

Based on these findings, it was concluded that freshly prepared solutions must be used for reliable spectrophotometric measurements.

3.3 Effect of pH on Complex Formation

The effect of pH on the formation and absorbance of the Ni (II) –Cinnamaldehyde - TSC complex was studied by preparing a series of solutions containing Ni (II) and the ligand (both at 8.48×10^{-3} M) across a pH range of 1 to 10. Buffer solutions for the required pH values were prepared according to the standard procedure. [21]

Absorbance measurements were recorded at 360 nm against corresponding reagent blanks. The results indicated that the maximum absorbance occurred at pH 3, suggesting that this is the optimum pH for complex formation.

3.4 Effect of Ligand Concentration

To evaluate the effect of ligand concentration on complex formation, a series of solutions were prepared with a constant concentration of Ni (II) and varying volumes of cinnamaldehyde - thiosemicarbazone ligand, all at pH 3, and the final volume was adjusted to 10 mL with ethanol.

The absorbance was measured at 360 nm against a reagent blank. From the results, it was found that the optimum volume of ligand required for complete complexation with Ni (II) was 2.4 mL. Beyond this volume, no significant increase in absorbance was observed, indicating saturation.

3.5 Stability of the Complex

The stability of the Ni (II) –Cinnamaldehyde - Thiosemicarbazone complex was evaluated by monitoring the absorbance of the freshly prepared solution over time. It was observed that the complex remains stable for up to 10–12 hours, beyond which noticeable fading of color and decrease in absorbance occur. Therefore, all spectrophotometric measurements were conducted using freshly prepared solutions to ensure accuracy and reproducibility.

3.6 Validity of Beer's Law

To assess the applicability of Beer's Law, a series of solutions were prepared containing varying concentrations of Ni (II), while maintaining a constant volume (2.5 mL) of the ligand solution at pH 3. Each solution was diluted to 10 mL with absolute ethanol, and the absorbance was recorded at 360 nm against a reagent blank.

The results, confirm that Beer's Law is obeyed over the concentration range of 8.48×10^{-5} M to 8.48×10^{-4} M for Ni (II).

3.7 Sandell's Sensitivity

The Sandell's sensitivity of the Ni (II) –Cinnamaldehyde - TSC complex was calculated in accordance with the procedure outlined by Sandell. Based on the average molar extinction coefficient ($\varepsilon = 2.216 \times 10^2$ L mol^{-1} cm^{-1}) at 360 nm, the Sandell's sensitivity was determined to be 0.2648 µg cm^{-2}. This value indicates the minimum amount of Ni (II) that can be detected per unit area using the proposed method.

3.8 Stoichiometry of the Metal–Ligand Complex

3.8.1 Job's Method of Continuous Variation

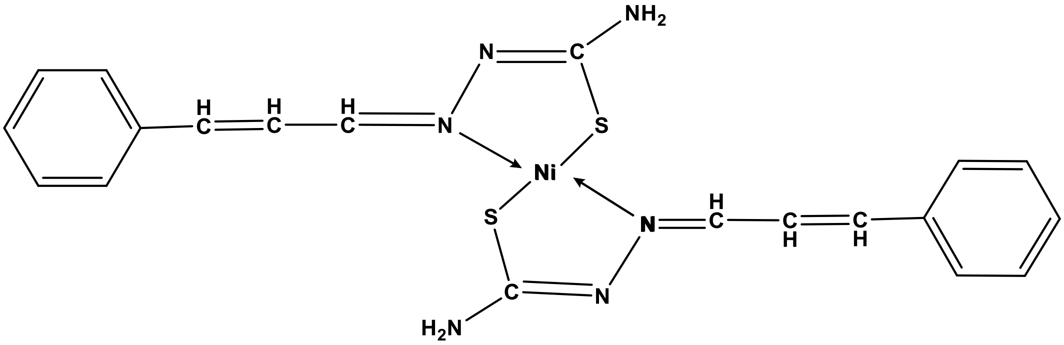
To study Job's method of continuous variation, various solutions were prepared containing different amounts of metal and reagent (in equimolar proportions) at the optimum pH 3. All solutions were diluted to 10 mL with alcohol, and absorbance was measured at 360 nm against a reagent blank. The metal - to - reagent ratio is **1: 2**.

3.8.2 Mole Ratio Method

For the mole ratio method, different solutions containing the same amount of metal but varying amounts of ligand were prepared at an optimum pH (pH 3). All solutions were diluted to 10 mL with absolute alcohol, and absorbance was measured at 360 nm against a reagent blank. The metal - to - reagent break at **1: 2** confirms the result obtained from Job's method.

3.9 Proposed Structure

Hence, the probable structure for the Ni (II) – Cinnamaldehyde - TSC complex is shown below:



Structure shows a square planar coordination complex where Ni (II) is chelated by two thiosemicarbazone ligands via azomethine nitrogen and thione sulfur atoms, forming a stable 1: 2 complex.

3.10 Degree of Dissociation (α)

The degree of dissociation (α) of the Ni (II) –Cinnamaldehyde – Thiosemicarbazone complex was calculated using the following relation:

$$\alpha = (dE - do) / dE$$

Where:

- dE = Extrapolated optical density corresponding to complete complex formation = 0.192
- do = Observed optical density at the stoichiometric molar ratio = 0.185

Substituting the values:

$\alpha = (0.192 - 0.185) / 0.192$
$\alpha = 0.007 / 0.192$
$\alpha = 0.0364$

Thus, the degree of dissociation of the complex is **0.0364**.

3.11 Dissociation Constant (K) or Instability Constant

The dissociation constant **K** of the complex, also referred to as the instability constant, was calculated using data from the mole ratio method and applying the following general equation:

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

Where:

- m = Number of moles of metal ion (Ni^{2+}) = 1
- n = Number of moles of ligand (Cin - TSC) = 2
- α = Degree of dissociation = 0.0364
- c = Total concentration of the complex = 8.48×10^{-4} M

Substituting the values:

$K = [(1 \times 0.0364 \times 8.48\times10^{-4})^{1} \times (2 \times 0.0364 \times 8.48\times10^{-4})^{2}] / [(1 - 0.0364) x [8.48\times10^{-4}]$
$K=1.443\times10^{-10}$

The low stability constant ($K = 1.443 \times 10^{-10}$) suggests weak but specific interaction in the moderately stable Ni (II) – Cinnamaldehyde - TSC complex.

3.12 Diverse Ion Effect

The effect of diverse ions was investigated by mixing Nickel (II) (8.48×10^{-3} M) with cinnamaldehyde thiosemicarbazone (Cin - TSC) (8.48×10^{-3} M) at pH 3. To assess the selectivity of the method, various foreign metal ions were introduced into the reaction mixture and their potential interference was evaluated.

Interference by foreign ions is considered tolerable when it causes less than 2% deviation in absorbance values.

The following metal ions exhibited negligible interference, indicating the high selectivity of the Ni (II) –Cin - TSC system: Cd^{2+}, Fe^{3+}, Co^{2+}, Sn^{2+}, Hg^{2+}, and Mg^{2+}. In contrast, EDTA (ethylenediaminetetraacetic acid) and acetate ions significantly interfered with complex formation and must be strictly excluded from the system for accurate analysis.

4. Conclusion

The present investigation demonstrates that cinnamaldehyde - thiosemicarbazone (Cin - TSC), a Schiff base ligand with recognized pharmaceutical and analytical relevance, serves as a highly effective chromogenic reagent for the spectrophotometric determination of Nickel (II) ions. The Ni (II) –Cin - TSC complex forms readily in an alcoholic medium at pH 3, yielding a distinct yellow - colored complex with a maximum absorbance at 360 nm. Stoichiometric studies using Job's method and the mole ratio method revealed a 1: 2 metal - to - ligand ratio, indicating coordination through the azomethine nitrogen and thione sulfur donor atoms.

The method follows Beer's law in the concentration range of 8.48×10^{-5} M to 8.48×10^{-4} M, with a molar extinction coefficient (ε) of 2.228×10^{2} L mol^{-1} cm^{-1} and a Sandell's sensitivity of 0.2648 µg cm^{-2}, suggesting moderate sensitivity suitable for micro - analytical purposes. The low dissociation constant ($K = 1.18 \times 10^{-13}$) and the degree of dissociation ($\alpha = 0.0364$) further confirm the high stability of the complex, supporting its use in reliable and reproducible nickel estimation.

Moreover, the practical applicability of the Ni (II) –Cin - TSC complex was successfully demonstrated by determining nickel content in commercially available Cadbury chocolate samples. The experimentally obtained values closely matched those calculated using the Beer's law calibration plot, confirming the accuracy, selectivity, and utility of the method in real sample analysis. This validates the complex's potential for routine use in analytical laboratories, especially where detection of nickel in complex food matrices is required.

5. Limitations

1) While the method exhibits selectivity toward Ni (II), potential interference from other transition metal ions in multi - element matrices remains a concern, necessitating further masking studies.
2) The complex exhibits limited temporal stability (10–12 hours), requiring timely measurement, which may hinder automation or high - throughput processing.

6. Future Scope

Future work may focus on:

- Extending the method to the analysis of Ni (II) in environmental, biological, and industrial samples containing multiple metal ions.
- Immobilizing the Cin - TSC reagent on solid supports or incorporating it into optical or electrochemical sensor platforms for on - site nickel detection.
- Derivatizing Cin - TSC to enhance its sensitivity, selectivity, and linear dynamic range for broader analytical applications.

7. Significance of the Study

This study significantly contributes to the field of analytical coordination chemistry by introducing a simple, cost - effective, and moderately sensitive spectrophotometric method for nickel determination. Its successful application in food sample analysis and its potential adaptability for field and industrial use support the development of green, reagent - based alternatives to traditional instrumental methods. The method holds promise for use in resource - limited educational and analytical laboratories, bridging the gap between reagent synthesis and real - world analytical performance.

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