



An Isoniazid Based Schiff Base Sensor for Selective Detection of Pd²⁺ Ions

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Abstract

Here, we developed a novel isoniazid based fluorescent probe (**(E)-N'-(thiophen-2-ylmethylene)isonicotinohydrazide (TINH)**) through simple condensation reaction and employed for selective detection of Pd²⁺ ions with a low detection limit of 4.102×10^{-11} M. Among the many existing cations, **TINH** bound Pd²⁺ ions with an association affinity of 9.794×10^5 M⁻¹. Adding Pd²⁺ ions to ligand solution increased the absorption intensity in UV–Visible and quenched the emission intensity in fluorescence spectroscopic experiments. More importantly, this **TINH** complexed to Pd²⁺ ions in 1:1 stoichiometric ratio. To evaluate the stability of complexed system, pH experiments has been performed. The binding insights among the ligand and Pd²⁺ ions has been confirmed by IR spectroscopic and MASS spectrometric methods. Additionally, **TINH** also applied to real water samples for the identification and measurement of Pd²⁺ ions. Hence, this system could be highly applicable for detection of Pd²⁺ ions in environmental and industrial samples with in low detection range.

Keywords Palladium · UV–visible · Detection limit · Fluorescence · Binding insights · Job's plot

Introduction

The non-covalent interactions are the key concept for the host–guest chemistry [1]. In the recent years, researchers have mainly focused on chemosensing of such analytes

(cations, anions and neutral species) that may affect the biological, environmental and ecological systems [2–5]. The detection and quantification of such analytes using simple molecules have a great area of interest for researchers. However, the metal ions are crucial and overabundance or inadequacy of metal ions gives rise to serious threats to human beings [6]. Palladium (Pd) is a shiny, silvery white and rare metal in the Pt group which is highly resistant to corrosion so that it is used for making several items including jewelry, electronic items [7, 8]. Also used for dental fillings, alloys, catalytic converters for automobiles, energy producing fuel cells [9]. Pd acts as a catalyst and converts major environmental pollutants such as carbon monoxide and nitrogen oxides respectively into carbon dioxide and nitrogen gas [10]. It is also tremendously assisted to form difficult bonds in catalytic processes in coupling reactions like Suzuki Miyaura, Buchwald–Hartwig, Heck and Sonogashira reactions [11, 12]. Though even after utmost purification processes, Pd²⁺ ions often released as by-product along with desired product formation, these residual Pd²⁺ contents can cause serious threats to human life as it cause DNA degradation and adversely alters the cellular processes due to its coordination ability to sulphur containing proteins, vitamins [13, 14]. As per recommendations of WHO, the dietary intake limit of Pd is near about 15 µg per day and 5–10 ppm in drugs [15, 16]. That is why it is becoming necessary to

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develop highly selective and sensitive methods for precise detection and quantification of these hazardous Pd²⁺ ions from the ecological system.

The literature reports showed that traditional techniques such as AAS, AMS, ICP-AES, NAA, SPME-HPLC and HRF etc. has also been used for detection of Pd²⁺ ions [14, 17]. However, most of these techniques are time-consuming and require very costly, sophisticated instrumentation, handling problems, a large amount of samples is required. Hence, there is a great need to develop such chemosensors that can detect these ions selectively and sensitively, requires small amount of sample and less time consuming.

From literature, we come to know that most organic molecules used for Pd²⁺ selective binding have complex synthetic routes. In many published reports, one of the reactants is Rhodamine, has a large size with a complex structure. Also, we investigated that the researchers have used Isoniazid based chemosensors for detection of Zn²⁺, Hg²⁺, Al³⁺ and Cu²⁺ ions but not a single report for the selective detection of Pd²⁺ ions. Here, in this article we have developed a simple and cost effective Isoniazid based Schiff base chemosensor **(E)-N'-(thiophen-2-ylmethylene)isonicotinohydrazide (TINH)** having sulphur and nitrogen donor atoms for selective binding of Pd²⁺ ions. The synthesized molecule showed a low detection range with good association affinity for sensing of Pd²⁺ ions.

Experimental Section

Materials and Instrumentation

The glassware used during experimental work was dried in an oven before being used. Highest purity analytical grade chemicals were used. Ordinary melting point apparatus was used for melting point measurements. For NMR spectra of chemosensor **TINH**, JEOL JNM ECZ400S (400 MHz) (Japan) was used by dissolving in DMSO-d₆ solvent. ABB MB3000 FTIR spectrophotometer (Japan) was used for collecting the IR spectral data. For Mass spectrum, Xevo spectrometer (Waters, USA) was used. The absorption spectra in the range 600 nm–200 nm were collected on LabIndia (3200) spectrophotometer. The fluorescent spectral data were recorded on Shimadzu-5301 pc spectrofluorophotometer in acetonitrile solvent. The pH data was recorded on a simple digital pH meter with the use of a diluted NaOH and HCl buffer solution system.

Analysis of Binding Ratio and Association Constant

Binding ratio of **TINH** and Pd²⁺ ion has been evaluated by using Job's plot and Benesi-Hildebrand plot methods. Association constant of interacting species was calculated

with the help of standard Benesi-Hildebrand plot and equation follows as $1/(A - A_0) = 1/(A_\infty - A_0) + 1/K (A_\infty - A_0) [C]$, where A₀ is the absorbance of ligand in the absence of linked metal ion, A is the absorbance calculated when ligand is attached to metal ion, A_∞ is the maximum absorbance in presence of metal ion and K is the association constant, [C] is the concentration of metal ion. The value of the association constant (K) has been calculated from the slope of the straight line of the plot of 1/(A-A₀) against 1/[C] [18].

Determination of LOD (Limit of Detection) and LOQ (Limit of Quantification)

LOD and LOQ of probe **TINH** has been determined by well known equations $3\sigma S^{-1}$ and $10\sigma S^{-1}$ respectively; where σ is the standard deviation value of blank measurement and S represents the slope of linear calibration plot [19].

Results and Discussion

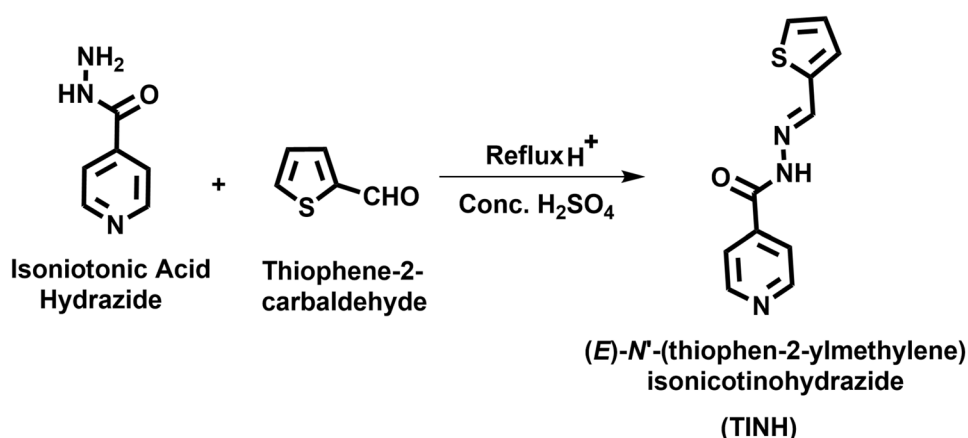
General Procedure for the Synthesis of Chemosensor (E)-N'-(thiophen-2-ylmethylene)isonicotinohydrazide (TINH)

The compound (E)-N'-(thiophen-2-ylmethylene)isonicotinohydrazide (**TINH**) has been synthesized by mixing the Isonicotinic acid hydrazide (0.3 g, 2.13 mmol) and Thiophene-2-carbaldehyde (0.24 g, 2.13 mmol) in ethanol and dichloromethane (DCM) solvent mixture in the presence of a catalytic amount of concentrated sulfuric acid under reflux conditions (Scheme 1) [20]. The reaction progress has been investigated using the TLC technique. After reaction completion, the solvent was evaporated from the reaction mixture and obtained a crude solid product. At last, this crude solid has been recrystallized in ethanol:acetonitrile (3:1, v/v) to obtained the final product as yellow colored solid powder.

Characterization Data

FTIR data: (Fig. S1) (cm⁻¹) 3210 (-NH), 1658 (>C=O), 1574, 1279, 847, 748; **¹H NMR data:** (Figs. S2 & S3) (400 MHz, DMSO-d₆) δ 12.04 (s, 1H, -NH), 8.80–8.79 (m, 2H, Ar-H), 8.69 (s, 1H, -CH), 7.83–7.81 (m, 2H, Ar-H), 7.73–7.71 (d, 1H, J = 5.2 Hz, Ar-H), 7.54–7.52 (m, 1H, Ar-H), 7.18–7.16 (dd, 1H, J = 5.2 Hz, J = 3.6 Hz, Ar-H); **¹³C NMR data:** (Fig. S4) (101 MHz) δ 161.4, 150.3, 144.0, 140.4, 138.7, 131.5, 129.4, 127.9, 121.4; **ESI-MS data:** (Fig. S5) m/z, observed 232.0546 [M + 1]⁺.

Scheme 1 Synthesis of (E)-N-(thiophen-2-ylmethylene)isonicotinohydrazide (TINH)



Metal Binding Experiments; Absorption and Emission Spectral Studies

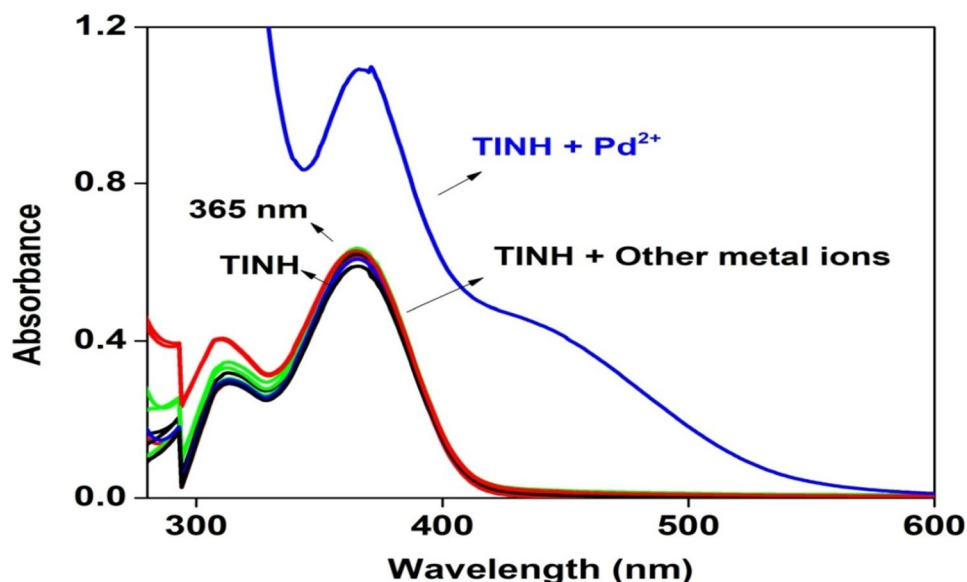
Absorption Spectral Study

UV–Visible spectral technique has been employed to determine the metal selective characteristics of chemosensor **TINH**. For this, the absorption spectra of ligand **TINH** and ligand in presence of different cations have been recorded in acetonitrile:H₂O (1:1 v/v) solvent media. The spectral investigations showed that the 20 μM solution of ligand exhibited an absorption maxima wavelength at 365 nm. This was attributed to $n\text{-}\pi^*$ electronic transitions in the ligand molecule (Fig. 1). In order to check the metal selective behavior of **TINH** towards different metal ions, the UV–Visible spectra of

TINH with acetate and nitrate salts of metal ions (20 μM) including Pb^{2+} , La^{3+} , Y^{3+} , Fe^{2+} , Fe^{3+} , Al^{3+} , Pr^{3+} , Pd^{2+} , Th^{4+} , Ca^{2+} , Li^+ , Cr^{3+} , Hg^{2+} , Dy^{3+} , Mn^{2+} , K^+ , Cd^{2+} , Zn^{2+} , UO_2^{2+} and Mo^{2+} ions have been recorded. The outcomes of experiment revealed that ligand demonstrate a remarkable response only towards Pd^{2+} ion with a red shift from 365 to 372 nm (Fig. 1). However, the presence of other test ions in solution with ligand **TINH** didn't exhibit any colorimetric or spectral changes.

In addition, the interference of other metal ion to complexed species has been conducted with help of UV–Visible experiments. For this, 5-fold of different cations were added to the solution of the **TINH-Pd**²⁺ system and spectra were recorded and analyzed (Fig. 2). The recorded spectral data showed that none of the added metal ions interfered with the binding between the **TINH-Pd**²⁺ system.

Fig. 1 Absorption spectral results of **TINH** (20 μM) in the presence of Pb^{2+} , La^{3+} , Y^{3+} , Fe^{2+} , Fe^{3+} , Al^{3+} , Pr^{3+} , Pd^{2+} , Th^{4+} , Ca^{2+} , Li^+ , Cr^{3+} , Hg^{2+} , Dy^{3+} , Mn^{2+} , K^+ , Cd^{2+} , Zn^{2+} , UO_2^{2+} and Mo^{2+} ions in acetonitrile:H₂O (1:1, v/v) solvent media



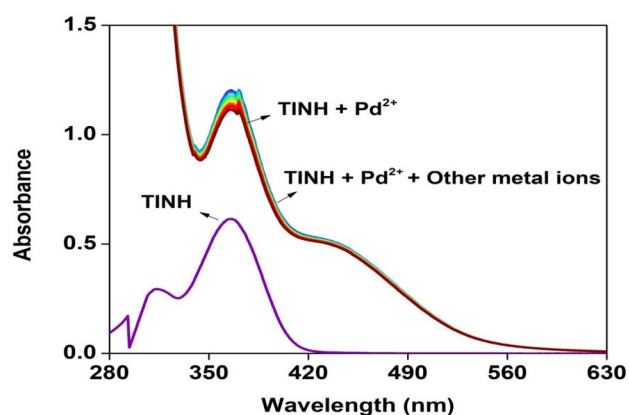
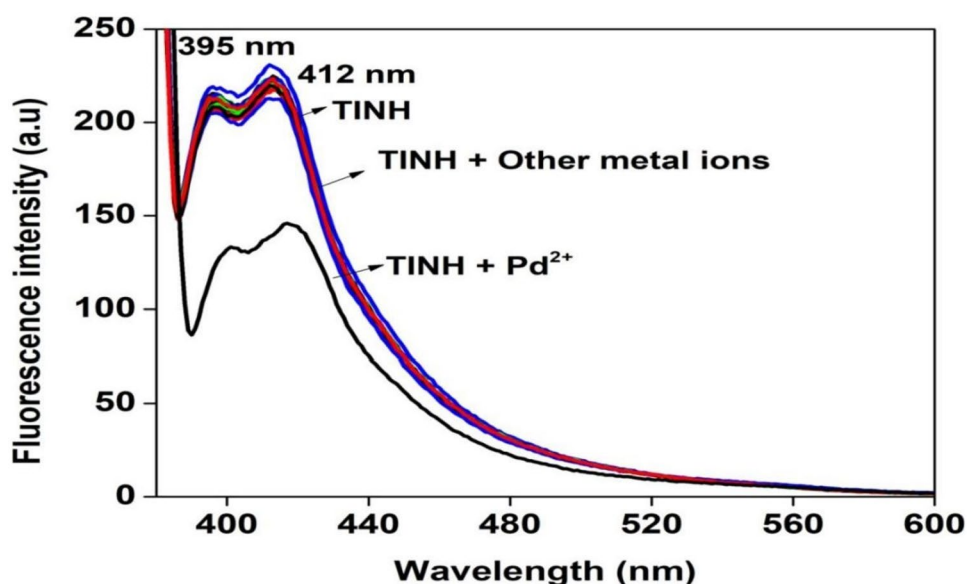


Fig. 2 Metal interference study showing the effect of other metal ions (Pb^{2+} , La^{3+} , Y^{3+} , Fe^{2+} , Fe^{3+} , Al^{3+} , Pr^{3+} , Th^{4+} , Ca^{2+} , Li^{+} , Cr^{3+} , Hg^{2+} , Dy^{3+} , Mn^{2+} , K^{+} , Cd^{2+} , Zn^{2+} , UO_2^{2+} and Mo^{2+} ions) on the binding of **TINH-Pd²⁺**

Emission Spectral Study

The emission spectra of **TINH** and **TINH** in presence of different cations were recorded in acetonitrile:H₂O (1:1 v/v) solution. The 20 μM solution of the ligand **TINH** exhibited two emission maxima at 395 nm and 412 nm due to the photo induced electron transfer process (PET) in the ligand molecule [21]. The experimental outcomes confirms that the addition of various acetate and nitrate salts of metal ions (20 μM) including Pb^{2+} , La^{3+} , Y^{3+} , Fe^{2+} , Fe^{3+} , Al^{3+} , Pr^{3+} , Th^{4+} , Ca^{2+} , Li^{+} , Cr^{3+} , Hg^{2+} , Dy^{3+} ,

Fig. 3 Emission spectral results of **TINH** (20 μM) in the presence of Pb^{2+} , La^{3+} , Y^{3+} , Fe^{2+} , Fe^{3+} , Al^{3+} , Pr^{3+} , Pd^{2+} , Th^{4+} , Ca^{2+} , Li^{+} , Cr^{3+} , Hg^{2+} , Dy^{3+} , Mn^{2+} , K^{+} , Cd^{2+} , Zn^{2+} , UO_2^{2+} and Mo^{2+} ions in acetonitrile:H₂O (1:1, v/v) solvent media



Mn^{2+} , K^{+} , Cd^{2+} , Zn^{2+} , UO_2^{2+} and Mo^{2+} ions to ligand **TINH** didn't affect the emission intensity of ligand band except for Pd^{2+} ions. However, the addition of Pd^{2+} ions to **TINH**, caused a quenching in emission intensity of the band for ligand molecule (Fig. 3).

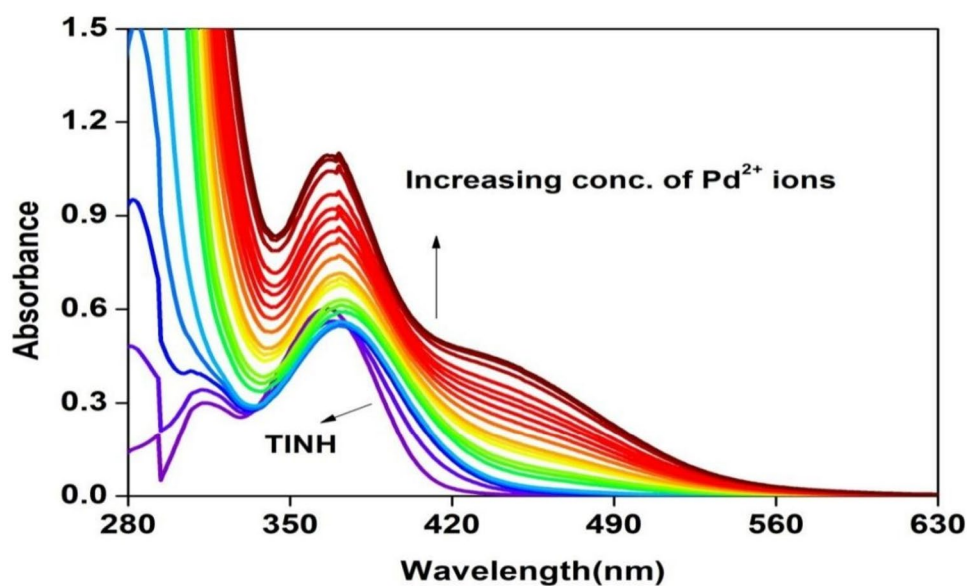
Effect of pH

The effect of pH changes on the binding of **TINH** and Pd^{2+} ions were analyzed by recording the UV-Visible and Fluorescence data of complexed system at wavelength 365 nm and 412 nm respectively. For this, different solutions of **TINH-Pd²⁺** system have been prepared with varying pH range from 2–12, using a pH meter and HCl (1 M) and NaOH (1 M) solutions. The spectral data revealed that the **TINH-Pd²⁺** binding showed a decrease (in UV-Visible spectra) in spectral intensity at a pH range from 2 to 5. This instability in binding, may be due to the less stability of imine bond in pH range (less than 6). Also, a decrease in fluorescence intensity has been observed for complexation from a pH of 2 to 7. The intensity of complexation increases at higher pH may be due to formation of metal ion hydroxides. The experiment demonstrated that the ligand significantly bound to Pd^{2+} ions at physiological and higher pH range (Figs. S6 & S7) [22].

UV-Visible and Fluorescence Titrations

UV-Visible titrations have been performed to investigate the binding insights among sensor **TINH** with Pd^{2+} ions

Fig. 4 UV–Visible titration spectral results of **TINH** with increasing conc. of Pd^{2+} ions



[23]. The gradual increase in the concentration of Pd^{2+} ions ($0\ \mu\text{M}$ to $100\ \mu\text{M}$) to the ligand **TINH** solution caused the enhancement in the absorption intensity at $395\ \text{nm}$ (Fig. 4).

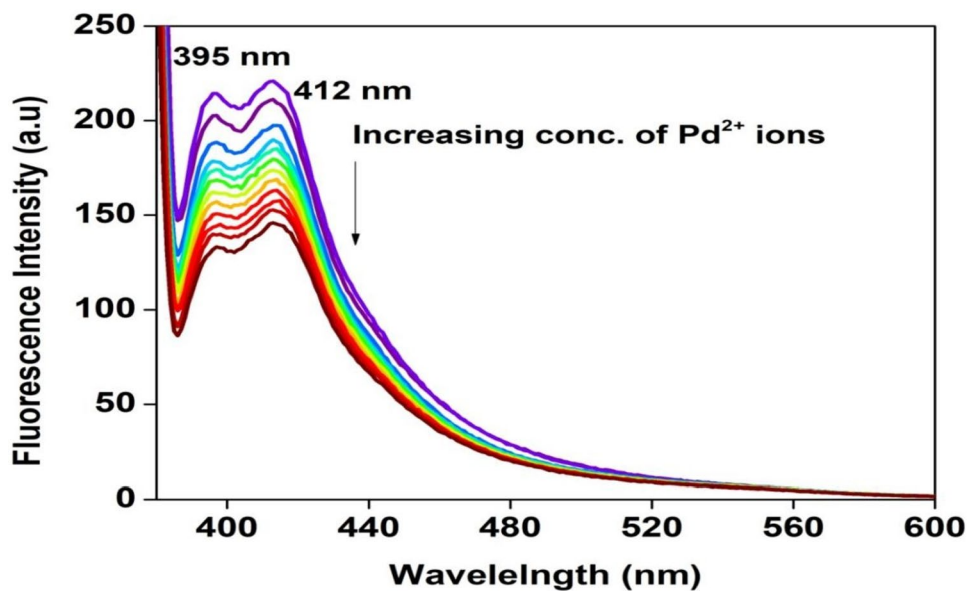
Furthermore, the effect on fluorescence intensity of **TINH** with the increasing concentration of Pd^{2+} ions has been investigated through fluorescence titration method. Emission spectral results showed that sequential increase in concentration of Pd^{2+} ions ($0\ \mu\text{M}$ to $100\ \mu\text{M}$) to **TINH** solution quenched the emission intensity of **TINH** at $412\ \text{nm}$ and $395\ \text{nm}$ (Fig. 5).

Binding Analytical Parameters

Job's Plot and Benesi-Hildebrand Studies

To check the binding ratio among the complexing system, Job's plot method has been employed [24]. For this, ten solutions of varying mole fractions have been prepared and UV–Visible spectra at a fixed wavelength have been recorded. A graph of absorbance v/s mole fraction has been plotted by using the absorption data at constant

Fig. 5 Fluorescence titrations spectral results of **TINH** with increasing amount of Pd^{2+} ions



y	-1.021 X + 1.600
Pearson's r	-0.9993
Adj. R-Square	0.9986
Association constant	$9.794 \times 10^5 \text{ M}^{-1}$

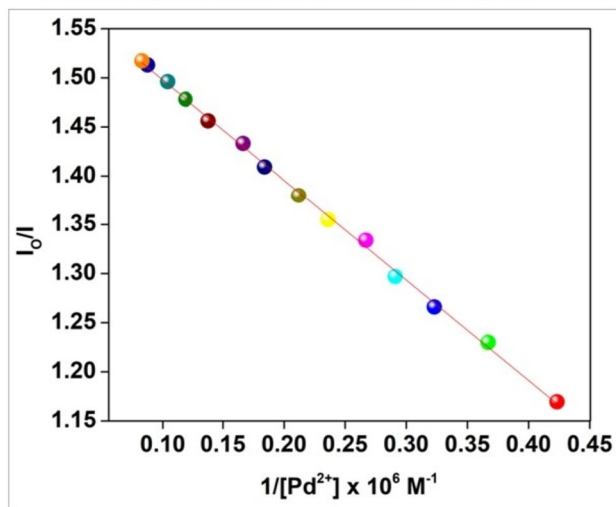


Fig. 6 Benesi–Hildebrand plot of **TINH-Pd²⁺** complex

wavelength (Fig. S8). The deviation in absorbance maximum in graph at 0.5 identify a 1:1 binding stoichiometry for the binding of **TINH-Pd²⁺** system.

The data obtained from the fluorescence titrations have been used to draw a plot called Benesi-Hildebrand plot [18]. The Benesi-Hildebrand equation has been applied to derive the association affinity among the ligand and analyte. The linearity in the graph showed 1:1 binding for the complexing system (Fig. 6). However, the association affinity for **TINH-Pd²⁺** complex comes out to be $9.794 \times 10^5 \text{ M}^{-1}$ (Fig. 6).

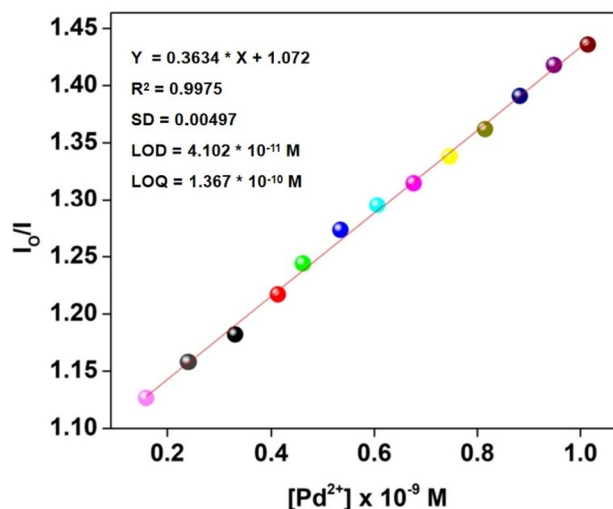


Fig. 7 LOD and LOQ calibration curve and values for **TINH-Pd²⁺** complex

Determination of LOD (Limit of Detection) and LOQ (Limit of Quantification)

Linear correlation curve against emission intensity I_0/I vs increasing concentration of Pd^{2+} ions was plotted using fluorescence titration data of **TINH** with Pd^{2+} ions (Fig. 7) [19]. From the curve, LOD and LOQ values were determined using the equations $3\sigma/S$ and $10\sigma/S$ respectively. The LOD value was found to be $4.102 \times 10^{-11} \text{ M}$ and LOQ was $1.367 \times 10^{-10} \text{ M}$. Further, these values were found relevant and acceptable in comparison with the literature (Table 1).

Table 1 A table of comparison for quantitative data of various Pd^{2+} chemosensors

Sr No.	Method	Sensor	LOD	LOQ	Ref.
1	Fluorescence	Coumarinyl-rhodamine based	$18.8 \times 10^{-9} \text{ M}$	—	[25]
2	Hybrid colorimetric fluorescent	Rhodamine bonded azophenol based	$450 \times 10^{-9} \text{ M}$	—	[26]
3	Fluorescence	Rhodamine based allyl-ether	$50 \times 10^{-9} \text{ M}$	—	[27]
4	Fluorescence	Rhodamine-ethynyl-benzaldehyde	$191 \times 10^{-9} \text{ M}$	—	[28]
5	Fluorescence	Rhodamine appended oxime	$15 \times 10^{-9} \text{ M}$	—	[29]
6	Fluorescence	Rhodamine-benzimidazole	$21 \times 10^{-9} \text{ M}$	—	[30]
7	Fluorescence	Rhodamine based	$190 \times 10^{-9} \text{ M}$	—	[31]
8	Fluorescence	Rhodamine hydrazide based	$1.70 \times 10^{-6} \text{ M}$	—	[32]
9	Fluorescence	Tetraphenylimidazole	$9.2 \times 10^{-7} \text{ M}$	—	[33]
10	Colorimetric and Fluorescence	2-Hydroxy-naphthaldehyde	$9.8 \times 10^{-7} \text{ M}$	$3.2 \times 10^{-6} \text{ M}$	[14]
11	Fluorescence	Quinoline and thiophene	$6.4 \times 10^{-7} \text{ M}$	—	[34]
12	Colorimetric	Salicylaldehyde based	$0.076 \times 10^{-6} \text{ M}$	—	[35]
13	Colorimetric	Rhodamine based	$4.2 \times 10^{-9} \text{ M}$	—	[36]
14	Fluorescence	Isoniazid based	$4.102 \times 10^{-11} \text{ M}$	$1.367 \times 10^{-10} \text{ M}$	This work

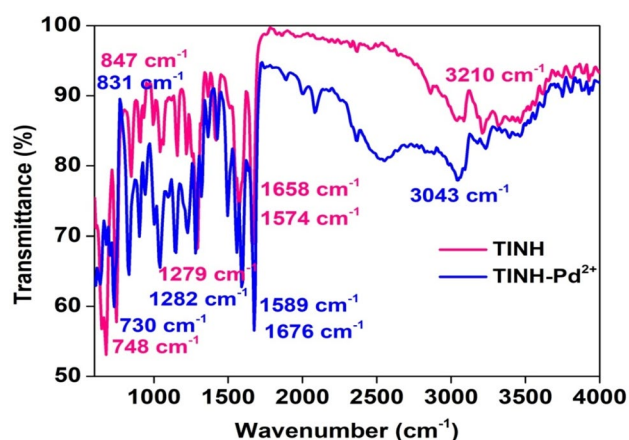


Fig. 8 Comparison IR study among the **TINH** and **TINH-Pd²⁺** complex

IR and Mass Spectral Study of Complex

To further check the binding insights of ligand, the IR spectra of ligand **TINH** and **TINH-Pd²⁺** complex have been recorded in solid-state and compared. The shifting in IR bands (in cm^{-1}) of **TINH** from 3210, 1658, 1574, 1279, 847, 748 to 3043, 1676, 1589, 1282, 831 and 730 were observed (Fig. 8). The shifting in the bands may be due to interactions among the lone pairs of nitrogen atom of imine and sulphur atom of thiophene to Pd^{2+} ions [37]. Also, the Fig. S5 showed the presence of an intense molecular ion peak at m/z 232.0546 corresponding to $[\text{TINH} + 1]^+$ in the mass spectrum of ligand confirmed the formation of a predictable outcome. However, the Fig. S9 demonstrated that the formation of **TINH-Pd²⁺** species displayed a molecular ion peak at m/z 338.1665 corresponding to $[\text{TINH} + \text{Pd}^{2+} + 1]^+$, which supported the expected 1:1 stoichiometric ratio of ligand **TINH** and Pd^{2+} ions.

Applicability of Chemosensor **TINH** to Real Water Samples

Fluorescence techniques have been employed for investigating the practical utility of chemosensor **TINH** through real sample analysis, which was accomplished with ground and tap water samples. For this, different water samples with known concentration of Pd^{2+} ions have been analyzed with **TINH** (Table S1). From the results, it has been observed that the chemosensor **TINH** could be applicable for quantitative analysis of Pd^{2+} ions in real water samples [38].

Conclusion

In conclusion, the presented work explored the metal sensing properties of a simple isoniazid based Schiff base sensor (**E**)-**N'**-(thiophen-2-ylmethylene)

isonicotinohydrazide (TINH). The synthesized molecule selectively bound the Pd^{2+} ions upto a detection limit of 4.102×10^{-11} M and with a LOQ of 1.367×10^{-10} M. The binding modes 1:1 complexation stoichiometry has been confirmed by Job's plot method. The stability and sensitivity of the **TINH** and **TINH-Pd²⁺** system has been investigated using pH tests. Moreover, binding analytical parameters (LOD, LOQ) were compared with literature and found acceptable. **TINH** selectively identify the Pd^{2+} metal ion using the standard addition method has been validated in real water sample, highlighting its potential field advantage. Thus, the sensor **TINH** could be applied as a potential tool for detecting Pd^{2+} ions in bio-medical and environmental applications.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10895-023-03491-x>.

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Author Contributions Jasbir Singh: Conceptualization, Investigation, Experimental, Resources, Formal analysis; Shubham Saini: Experimental, Formal analysis & Editing; Ravish K. Chauhan: Resources, Formal analysis; Writing- Original draft preparation; Pallavi Bhardwaj: Conceptualization, Investigation, Resources, Formal analysis; Ashwani Kumar: Writing- Original draft preparation, Formal analysis; Virender: Experimental, Writing- Original draft preparation, Formal analysis.

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Availability of Data and Materials Data and materials are available on demand.

Declarations

Ethical Approval No such data applies to human and/ or animal studies. This work is a novel and has not been published in any journal or thesis work.

Competing Interests The authors declare no competing interests.

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