

Microwave and Conventional Synthesis of Palladium (II) Complex with, 2-(5-Bromo-2-oxoindolin-3-ylidene)hydrazine-1-carbothioamide, Characterization and Study of Microbiological Activity

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Abstract

In the present study, both Schiff base ligand [2-(5-Bromo -2-Oxoindolin-3-ylidene)-1-Hydrazine Carbothioamide], [HBITSC] and its complex with Palladium (II) have been synthesized by conventional and microwave assisted reactions. As compared to conventional method, better yield of the desired product is obtained by microwave assisted reactions. The solid metal complex formed is brown in colour and non - hygroscopic in nature. The authenticity of this ligand and its complex has been established on the basis of elemental analysis, molar conductance, magnetic susceptibility, electronic spectra, IR spectroscopy.

These studies showed that this Schiff base ligand exhibits bidentate nature and coordinate to the metal atom through azomethine nitrogen and thioketo sulphur. On the basis of spectral analysis studies, geometry of complex was proposed to be square planar. Non - electrolytic nature of complex is revealed by the molar conductivity data. On the basis of above studies, ratio of metal to ligand proposed to be 1:2 i.e. two ligands were suggested to be coordinated to palladium atom. The ligand and metal complex have been screened for their microbiological activity.

Keywords: Microwave synthesis, Square planar geometry, Schiff base HBITSC, Microbiological activity.

Introduction

Thiosemicarbazones have aroused considerable interest in the field of chemistry and biology due to their antibacterial, antifungal, antimalarial, antineoplastic and antiviral activities.¹⁻⁵ Microwave-assisted synthesis is a branch of green chemistry. Microwave irradiated reactions under solvent free or less solvent conditions are attractive offering reduced pollution, low cost and offer high yields together with simplicity in processing and handling.⁶⁻⁸

In the present study, we have successfully explored the possibility of a green route and synthesized Schiff base ligand derived from 5-Bromoisatin and thiosemicarbazide, [2-(5-Bromo -2-Oxoindolin-3-ylidene)-1-Hydrazine

Carbothioamide] [HBITSC] and its complex with Pd(II). Both ligand and metal complex have been synthesized by both conventional and microwave methods for its comparison.

The complex is coloured and stable in air at room temperature. The structure of the ligand and complex was characterized by electronic spectra, elemental analysis, molar conductance, NMR and IR spectroscopy which helps to determine the geometry and mode of bonding. Ligand and its metal complex were also screened for the microbiological activities.

Material and Methods

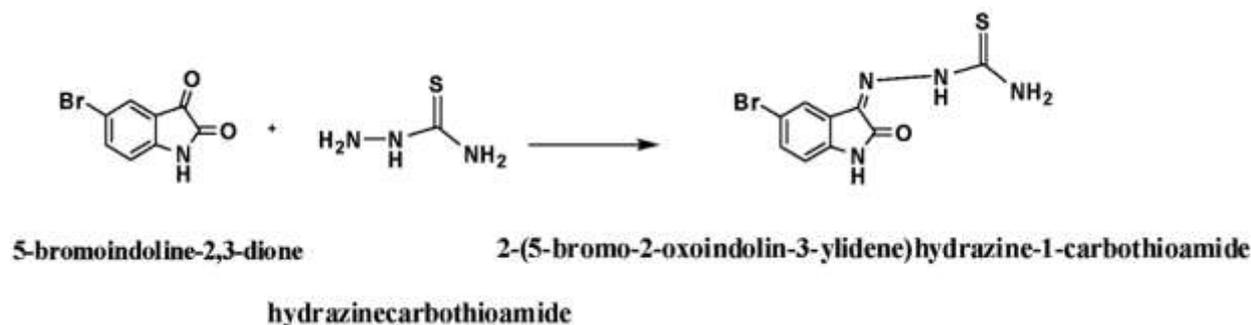
All solvents and chemicals used were of AR grade. The percentage compositions of (C, H, N, O) elements of the compounds were determined by using an element analyzer C, H, N, O model Flash EA 1112 series. For the confirmation of presence of various functional groups present in ligand and complex, Infrared spectra were recorded using SHIMADZU IR. By using ELICO SL -159 UV -Visible Spectrophotometer, the electronic spectrum of complex was recorded which was followed by melting point determination of ligand.

Melting points were determined in an open capillary tube. To measure the molar conductivity, methanol was used as solvent and measurement was made on ELICO SL - 303 model.

Synthesis of 2-(5-Bromo -2-oxoindolin-3-ylidene)-1-hydrazine carbothioamide [HBITSC] by conventional method:

Ethanol solution of 5-Bromoisatin and thiosemicarbazide in equimolar amount was refluxed for 4-5 hours to prepare Schiff base ligand HBITSC after optimizing reaction conditions and reaction time.

The progress of reaction was monitored on thin layer chromatography (TLC) using petroleum ether: ethyl acetate (7:3 ml) eluent. The reaction mixture was then cooled and a sharp yellow crystalline product obtained was collected by filtration (70%, yield) and purified by recrystallization using aqueous ethanol (procedure recommended by Vogel.⁹ The pure product was characterized by elemental and spectral analysis.



Microwave assisted synthesis of 2-(5-Bromo -2-oxoindolin-3-ylidene)-1-hydrazine carbothioamide [HBITSC]: 5-Bromoisatin and thiosemicarbazide in equimolar amount was grinded thoroughly in mortar and pestle. The reaction mixture was transferred in small Borosil beaker. Then 3 mL of dry ethanol was added to it as a solvent. Reaction mixture was irradiated with microwave (180 W) for 3min. (six pulses each of 30 sec.) The progress of the reaction was checked by means of TLC.

After completion of the reaction, the reaction mixture was poured into ice cold water. The solid crude product was precipitated out of the reaction mixture immediately which was filtered and dried properly. Then it was recrystallized from hot ethanol to get pure product. The pure product was characterized by elemental and spectral analysis. Yield was 85%.

Preparation of complex with Schiff base ligand (HBITSC)- Conventional method: Aqueous solution of palladium chloride was mixed with ligand (HBITSC) solution prepared in DMF in molar ratio of 1:2. to prepare palladium (II) complex. The optimum pH for complex formation is 3.0, therefore during reaction, pH was maintained at 3.0 using sodium acetate buffer. The reaction mixture was refluxed on boiling water bath for 2-3hrs. By means of TLC, the progress of the reaction was continuously checked. After completion of the reaction, reaction mixture was cooled. The solid crude product was precipitated. It was filtered, washed with ether and dried in vacuum desiccator over anhydrous CaCl_2 (yield 70%).

Preparation of complex with Schiff base ligand (HBITSC)- Microwave method: Mixture of aqueous solution of palladium chloride and ligand (HBITSC) solution prepared in dimethyl formamide solvent in molar ratio of 1:2 is taken in small Borosil beaker to prepare palladium (II) complex. pH was maintained at 3.0 using sodium acetate buffer at pH=3. Reaction mixture was irradiated with microwave (180 W) for 6 min (6 pulses each of 1min.)

The progress of the reaction was monitored by TLC. After completion of the reaction, reaction mixture was cooled. The solid crude product was filtered, washed with ether and dried in vacuum desiccator over anhydrous CaCl_2 (yield 80 %).

The reactions were completed in a short time (6 min) with better yields as obtained in the conventional procedure.

Biological Studies: Schiff base and its Pd (II) complex were screened *in vitro* for antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*. The antibacterial activity was evaluated using agar plate method. Sterile Mueller Hinton agar plates were used for bacterial test and Sabourauds agar plates for testing fungus activity. Agar plate's surface was inoculated by spreading a standardized inoculum (24 hrs old culture, optical density 0.1) of the test microorganism over entire surface.

Then, in aseptic condition a hole with a diameter of 6 mm was punched with a sterile cork borer and a volume (20 μL) of the test compound solution (with different concentrations) was introduced into the well. The Petri dishes were incubated at 37°C / at R.T. for 48 hrs. The test compounds diffuse into the agar. Generally, if test sample has antimicrobial or antifungal activity, then it inhibits germination and growth of the test microorganism. The diameters of inhibition growth zones were measured as a zone of inhibition in mm.

Results and Discussion

The Pd(II) HBITSC complex is brown colored solid and stable at room temperature. Molar ratio of Pd (II) HBITSC complex is 1:2. The analytical and physical data of ligand and Pd (II) HBITSC complex are given in table 1.

Infrared spectral analysis: SHIMADZU IR Affinity 1S. Spectrometer ($4000\text{-}400\text{cm}^{-1}$) was used to record Infrared spectra of ligand and Pd (II) HBITSC complex. Fig. 1 shows an IR spectrum of ligand and fig. 2 shows an IR spectrum of Pd (II) HBITSC complex. Infrared spectra help in identification of important functional groups. Comparison of the spectral data of both is summarized in table 2.

IR spectrum of the ligand shows broad band for (C=N) stretching of azomethine group¹⁰ at 1603.89cm^{-1} . The same band was shifted at 1608.64cm^{-1} i.e. at higher frequency in Pd(II) complex. This indicates that azomethine nitrogen has donated electron density to metal and is coordinated to metal atom on complexation.^{11,12} By taking into account structure of ligand and metal complex, change in position of the characteristic peaks such as C=N, C=S etc and appearance of new peaks help us in conformation of structure.

Table 1
The Analytical and physical data of ligand and its Pd(II) HBITSC complex

Compound (Colour)	Mol. Wt	M.P. °C	% Elemental Analysis Found (Calculated)								λ_m Ohm ⁻¹ cm ² mol ⁻¹
			C	H	N	Br	O	S	Cl	M	
HBITSC (Yellow)	299.64	278	36.42 (36.10)	2.46 (2.67)	18.5 (18.72)	26.71 (26.7)	4.99 (5.2)	10.92 (10.6)	-	-	-
[Pd(HBITSC) ₂]Cl ₂ (Dark Brown)	776.8	289	27.13 (27.845)	1.998 (2.06)	13.81 (14.44)	21.2 (20.6)	4.32 (4.13)	8.49 (8.25)	9.23 (9.15)	13.67 (13.72)	31.42

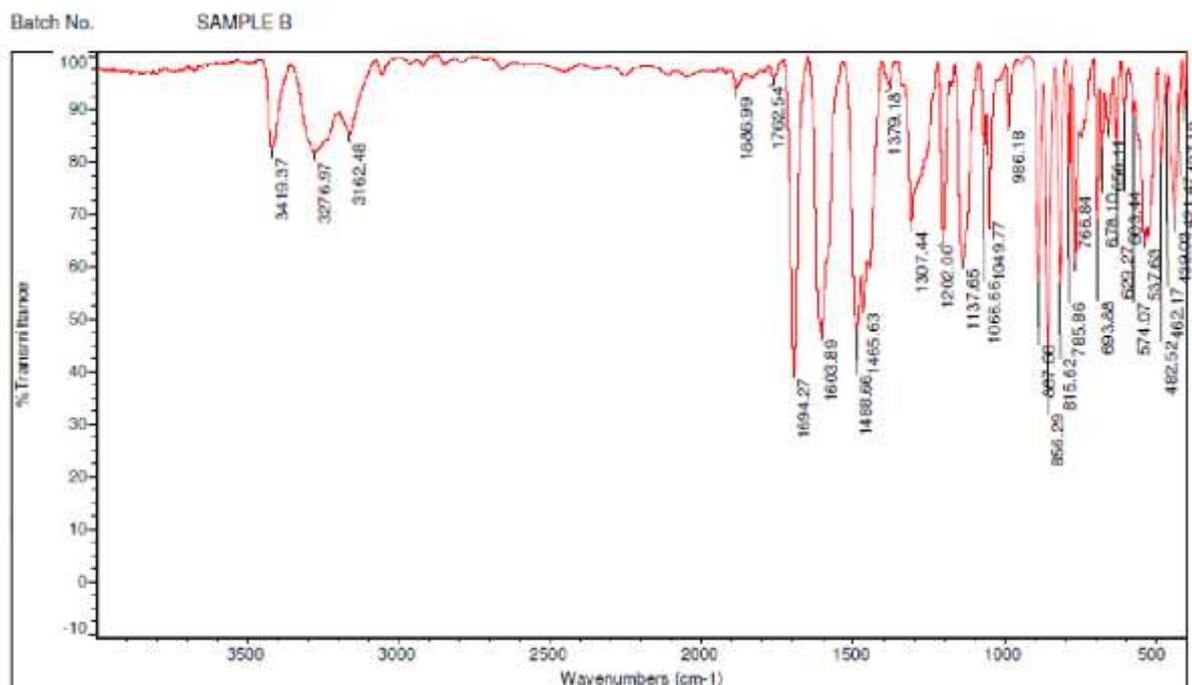


Figure 1: IR spectrum of ligand [HBITSC]

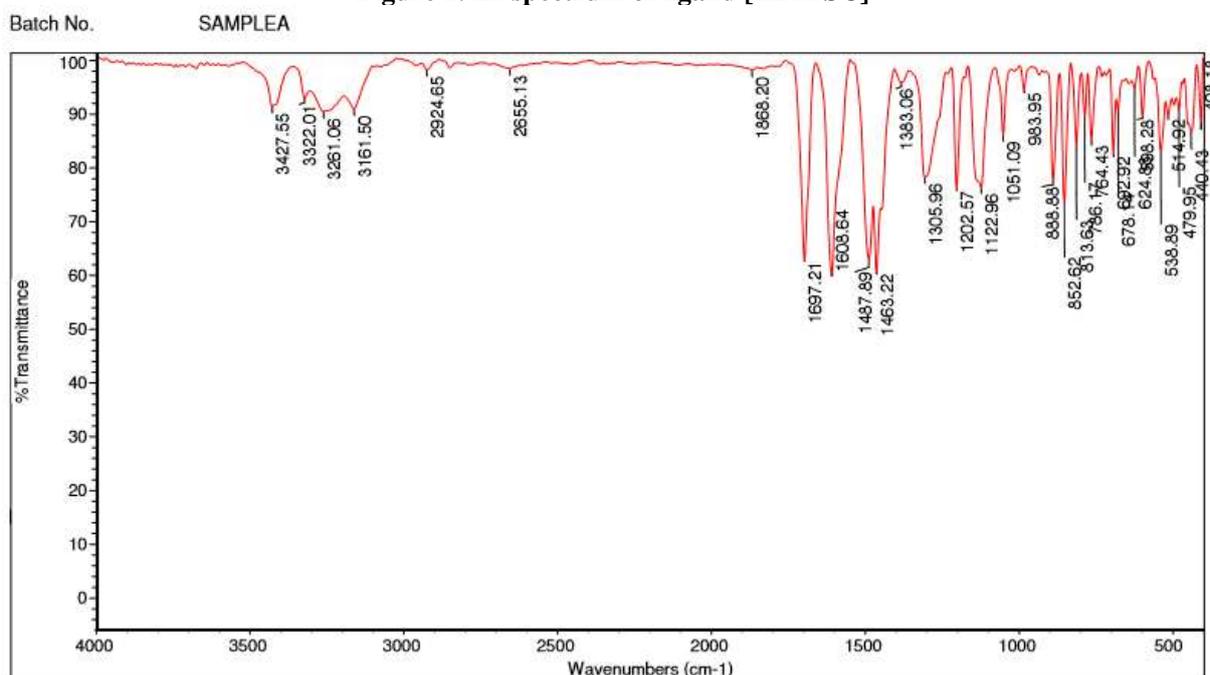


Figure 2: Infrared spectrum of Pd (II)-{HBITSC} complex

The second important band observed in the IR spectrum of ligand is at 856.20cm^{-1} (strong band). This is due to the (C=S) stretching vibration. This band is also found to be shifted but at lower frequency 852.62cm^{-1} in spectra of complex. The shifting in position of band from 856.20cm^{-1} to 852.62cm^{-1} confirms the coordination of thioketo sulphur to metal atom.¹¹⁻¹³

After studying both spectrums in detail, it was observed that two new bands have appeared in the region of $440\text{-}600\text{cm}^{-1}$ in the complex and were absent in the spectrum of ligand. One band corresponding to $\nu(\text{M-S})$ stretching frequencies 440.43cm^{-1} region and the other band between 514.00cm^{-1} are because of stretching frequencies $\nu(\text{M-N})$ respectively.

After studying above spectral details, we suggest that ligand behaves as bidentate ligand and gets coordinated to metal ion through azomethine nitrogen and thio keto Sulphur (Table 2, figure 1 and 2)

Molar conductance: To measure molar conductance of Pd (II) complex, ELICO Conductivity meter (cell constant 1.0cm^{-1}) is used. Measurement was carried out at room temperature and $1 \times 10^{-3}\text{M}$ solution of Pd (II) complex in methanol was used (Table 1). The non - electrolytic nature of the complex was revealed by comparing this value with known molar conductivities.¹⁴

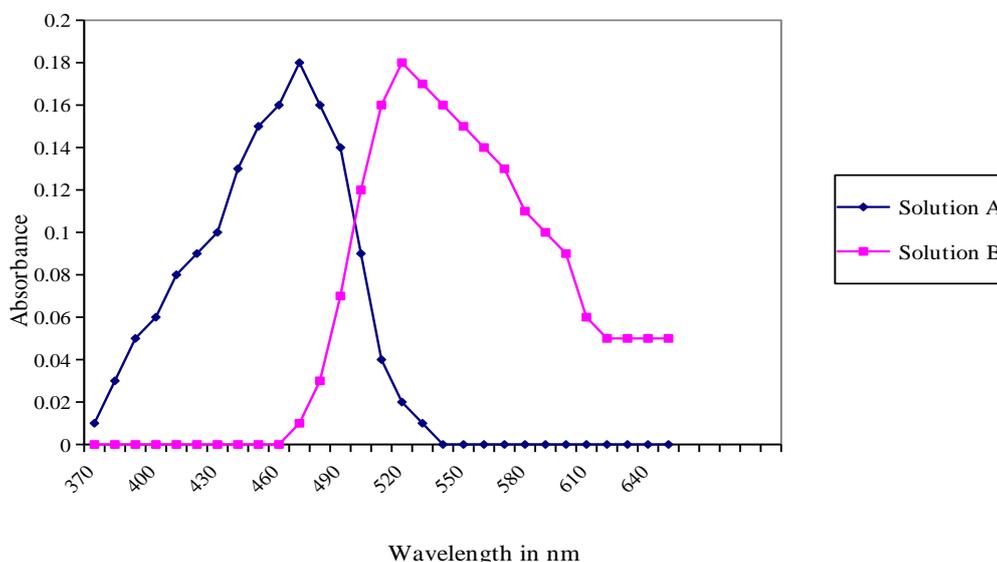
Electronic Spectra of Complex: To decide the geometry of the metal complex, electronic spectra play very important role. They also give idea about the nature of ligand field around metal atom. The electronic structures of transition metal ions are extremely varied since they occur in variable structural environments which can be identified with UV-Visible spectroscopy. The electronic spectra were recorded on ELICO SL - 159 UV-Visible Spectrophotometer. Square planner geometry is confirmed for the Pd (II) complex^{14,15} as it displays band at 19230.00cm^{-1} (520nm). This band may be assigned to $^1\text{A}_{1g} \rightarrow ^1\text{B}_{1g}(\nu_2)$ transitions (Figure 3).

^1H NMR: During complex formation, many times deprotonation occurs which can be confirmed by NMR spectra. ^1H NMR spectra further confirms coordination of thiosemicarbazones in the Pd (II) complex (Table 3). The proton peaks of N-H group at δ 11.32 ppm and 12.25 ppm in complex remain same as in the ligand, it suggested that deprotonation does not occur and it has also shown keto form of the ligands.^{14,16} The multiplet as strong band in the region 6.87-7.82 ppm of aromatic ring protons, also shifted downfield in the complex^{14,16} (Table 3).

On the basis of experimentally obtained data, the probable structure of the complex can be shown as in fig. 3. The present study clearly indicates that the Pd(II), forms stable coordination compound with ligand with the ratio 1:2 as in figure 4.

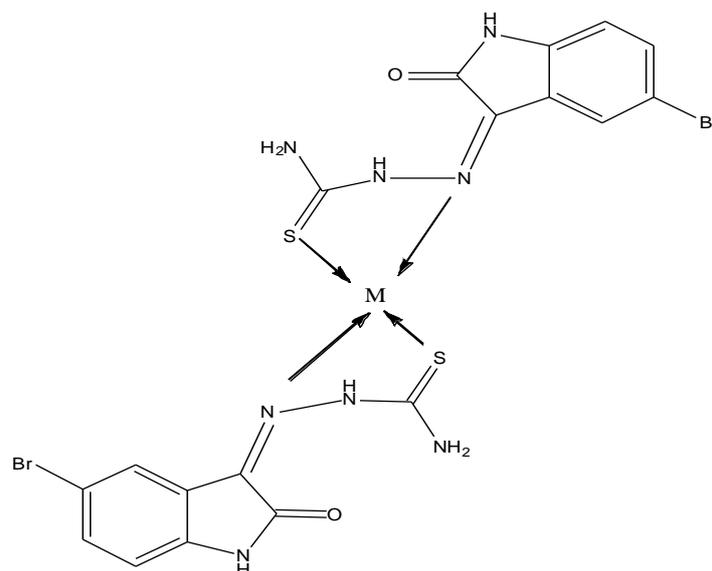
Table 2
The Important IR bands of Ligand and Pd Metal Complexes

Compound	$\nu(\text{C=N})$ cm^{-1}	$\nu(\text{C-S})$ stretching cm^{-1}	$\nu(\text{M-N})$ cm^{-1}	$\nu(\text{M-S})$ cm^{-1}
HBITSC	1603.89	856.2	-	-
$[\text{Pd}(\text{HBITSC})_2]\text{Cl}_2$	1608.64	852.62	514.00	440.43



Sample A= Reagent, Sample B= Pd (II) complex

Figure 3: Absorbance spectra of HBITSC and Pd-HBITSC complex

STRUCTURE OF ML_2

M = Pd(II)

Figure 4: Structure of complex

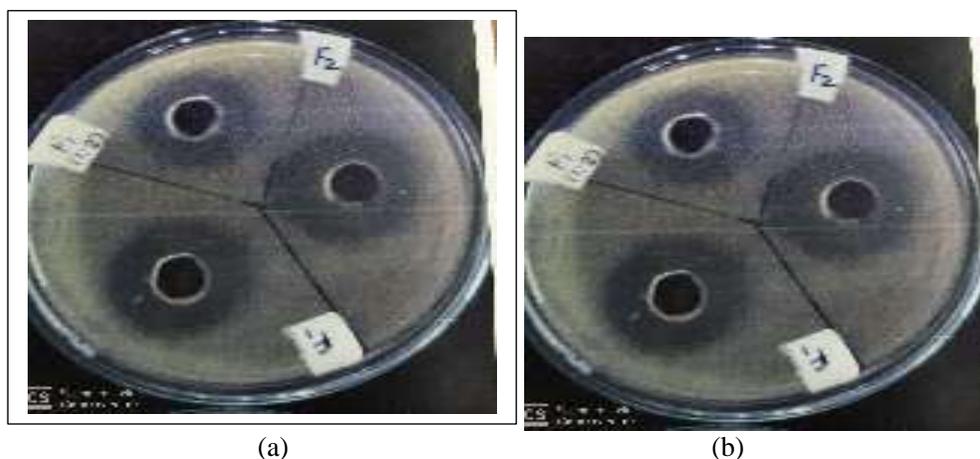
Figure 5: Inhabitation zone plates (a) *Pseudomonas aeruginosa* (b) *Staphylococcus aureus*

Table 3
NMR spectral data (δ , ppm) of the thiosemicarbazones and the Pd complex

Compound	$\delta(\text{N-H})$	$\delta(\text{N-H})$	$\delta(\text{Ar-H})$
HBITS	12.21	11.31	6.77-7.68
$[\text{Pd}(\text{HBITS})_2]\text{Cl}_2$	12.21	11.31	6.87-7.82

Biological activity: Antibacterial activity of Schiff base ligand and its Pd (II) -HBITS complex was studied. Study was conducted for antibacterial activity against gram positive bacteria (*staphylococcus aureus*), gram negative bacteria (*P.aeruginosa*). Even antifungal activity against (*Candida albicans*) was studied. A measurement of zone of inhibition in mm helps in comparative study of the microbiological activity of ligand and the Pd (II) -HBITS complexes. This data from table 4 indicates that Pd (II) complex shows higher antibacterial activity than that of the free ligand. The increase in the biological activity of Pd (II)

complex was explained on the basis of overtones concept and chelation theory.

On chelation as there is overlap of the ligand orbital with metal orbitals and partial sharing of positive charges of Pd (II) with donor groups, the polarity of the Pd (II) reduced to a greater extent. Because of delocalization of electrons over the whole chelate ring increases, lipophilicity¹⁷ of the complex increases which results into the penetration of the complex into lipid membrane. This leads to blocking of the metal sites on enzymes of microorganism.

Table 4
Zone of inhibition of growth in millimeters after 48 hours of incubation

Compound	Concentration	Name of micro organism	
		P.a (Gm-ve)	S.a (Gm+ve)
HBITSC	A	08 mm	12mm
[Pd (HBITSC) ₂ Cl ₂]	A	10 mm	14 mm
[Pd (HBITSC) ₂ Cl ₂]	B	12 mm	15 mm
[Pd (HBITSC) ₂ Cl ₂]	C	14 mm	16 mm
SOLVENT		-	-

A=10.0mg/1.0ml

B=20.0mg/1.0ml

C= 30.0mg/1.0ml

Conclusion

It can be concluded from the study that Schiff base ligand 2-(5-Bromo -2-Oxoindolin-3-ylidene)-1-Hydrazine Carbothioamide, [HBITSC] acts as a very good bidentate complexing agent towards Pd (II) during complexation. Analytical data of complex is in good agreement with its molecular formula. By comparing electronic, IR and NMR spectral data of the ligand with that of its Pd (II) - HBITSC complex, the involvement of azomethine (N), thioketo (S) of the bidentate Schiff base to the Pd (II) was confirmed.

The appearance of new bands due to $\nu(M-N)$ and $\nu(M-S)$ in the Pd (II) - HBITSC complex further confirms the coordination of this Schiff-base with Pd (II). Complex of Pd (II) with HBITSC shows diamagnetic behaviour^{14,15} and its geometry was proposed to be square planer in nature on the basis of the electronic spectra^{14,15}. On the basis of these findings, the structures have been proposed for the complex which are in good agreement with theoretical consideration.

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