

Antibacterial Activity and DNA Interaction of the Pd(bpy)(ONO₂)₂ Coordination Complex

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Abstract

Antibacterial activity of Pd(bpy)(ONO₂)₂ complex (bpy = bipyridine) was studied by disc diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. The compound was found to be active against the bacteria. DNA binding constant of that complex was studied with extracted DNA from lemon by UV-Vis spectroscopy. The calculated binding strength was found to be 2.09×10^6 which is significantly higher than that of the [Pd(bpy)(OH₂)₂]²⁺ complex with CT-DNA.

Keywords: Palladium complex, DNA binding, absorption titration, antibacterial activity, disk diffusion method

1. Introduction

The chemistry of transition metal complexes has received much attention in recent years due to their rational design and synthesis in coordination chemistry, also because of their potential applications as functional materials, enzymatic reaction mechanism and in bioinorganic chemistry. The transition metal complexes are applied in various activities such as anticancer, antibiotic, antimicrobial and antifungal agents [1-4]. The interaction with DNA and antibacterial activity of palladium (II) and platinum (II) complexes having chelating ligands such as N–N-diamines, O–S-donor, N–S-amino-thioether, diaminoacids, dicarboxylic acids and dithiocarbamates have been extensively studied in the past few years [1, 5-10].

There are several methods available by which one can study the binding of DNA with metal complexes such as spectroscopic studies (absorption spectroscopy, emission spectroscopy, ¹H NMR spectroscopy, resonance Raman spectroscopy, linear dichroism spectra, circular dichroism, fluorescence spectroscopy) [2, 5], voltametric studies (cyclic voltammetry, differential pulse voltammetry) [1], viscometric studies [2, 4], thermal denaturation studies [1], electrophoresis measurements [1]. Absorption titration can monitor the interaction of a metal complex and DNA by UV-Vis spectrophotometry. In general, hypochromism and redshift are associated with the intercalative binding of the complex to the helix, due to strong stacking interactions between the aromatic chromophore of the complex and the base pairs of DNA [11]. DNA binding study of some palladium complexes by UV-Vis spectrophotometry has been reported [1, 2].

Disk diffusion, broth dilution and agar dilution methods are widely used as antibacterial susceptibility testing methods. Disk diffusion refers to the diffusion of an antimicrobial agent of a specified concentration from disks, tablets or strips, into the solid culture medium that has been seeded with the selected inoculum isolated in a pure culture. Disk diffusion is based on the determination of an inhibition zone proportional to the bacterial susceptibility to the antimicrobial present in the disk. Annular radius (the shortest distance from the edge of the disc to the edge of confluent growth) indicates the antibacterial properties of antibacterial agents. Antibacterial having annular diameter ≥ 12 mm shows susceptible properties and annular diameter with < 12 mm shows resistant properties [12]. Antibacterial activity of some ionic palladium complexes has been reported by disk diffusion method [1].

In this work, we have synthesized Pd (II) complex Pd(bpy)(ONO₂)₂ by adapting the literature method [5]. The UV-Vis absorption properties of this bipyridyl metal complex have been utilized to monitor the interaction processes with DNA. Antibacterial activity of the complex has been studied against two Gram positive bacteria

(*Staphylococcus aureus*, and *Enterococcus faecalis*) and three Gram negative bacteria (*Escherichia coli*, *Klebsiella oxytoca* and *Pseudomonas aeruginosa*) by disk diffusion method.

2. Materials and Methods

2.1. Materials and Reagents

Palladium (II) chloride was obtained from Johnson Matthey, England; 2, 2' - bipyridine and silver nitrate were purchased from Merck, Germany. The starting complex $[\text{Pd}(\text{bpy})\text{Cl}_2]$ was prepared by literature methods [13]. All solvents used were of the analar grade. DNA was extracted by salt extraction method from lemon [14]. DNA-binding experiments were carried out in tris-HCl buffer solution (50 mM NaCl, 5 mM Tris-HCl, pH 7.1). Tris-HCl buffer was prepared using deionized double distilled water. Solutions of extracted DNA in buffer gave a ratio of UV-Vis absorbance of 1.5:1 at 260 and 280 nm, which indicated that DNA was mixed with some amount of protein. The concentration of DNA was determined spectrophotometrically ($\epsilon_{260} = 6600 \text{ M}^{-1} \text{ cm}^{-1}$) [1].

2.2. Apparatus and Measuring Techniques

Elemental analyses were performed in elemental Analyzer System Vario EL III Element Analyzer, at the Analytical Service Cell, BCSIR Laboratories, Dhaka. The Infrared spectrum of complex was recorded on KBr pellets with a SHIMADZU IR spectrometer (Model No-Prestige 21). UV-Visible spectra of the complex and absorption titration experiments of DNA and complex were studied in a SHIMADZU UV-Visible spectrophotometer (Model No: UV-1800 PC), from 200 nm to 800 nm ranges. All apparatus were used in antibacterial test after sterilizing. Melting points of the complex was obtained with an electro thermal melting point apparatus GALLENKAMP made in England. Bacteria were cultured and antibacterial test were performed in incubator and laminar air flow.

2.3. Synthesis of $[\text{Pd}(\text{bpy})(\text{ONO}_2)_2]$ Complex

The complex $[\text{Pd}(\text{bpy})(\text{ONO}_2)_2]$ was prepared by adapting the synthetic route reported in the literature [5]. $[\text{Pd}(\text{bpy})\text{Cl}_2]$ (50mg, 0.15mmol) was suspended in doubly distilled water, and equivalent amount of AgNO_3 was added to it with constant stirring. The reaction mixture was heated with stirring under dark for 6h at 60°C and for 16 h at room temperature. After the completion of the reaction the AgCl precipitate was separated from the solution by a centrifuge machine. The filtrate was evaporated at $40\text{--}50^\circ\text{C}$ to complete dryness, and a yellowish green $[\text{Pd}(\text{bpy})(\text{ONO}_2)_2]$ complex was obtained. The complex was washed with acetone and dried under vacuum. Yield 80.5 mg (93%). Anal. Found: C, 32.18; H, 1.548; N, 14.05% Calc. for $\text{C}_{10}\text{H}_8\text{N}_4\text{O}_6\text{Pd}$: C, 31.07; H, 2.09; N, 14.49%; IR (cm^{-1}): ν (aromatic C-H), 3082; ν (C=N) and (aromatic C=C), 1606, 1566 and 1423; ν (coordinated nitrates), 1490, 1269 and 984; ν (combination bands for nitrates), 1753 and 1736; UV-Vis: $\lambda_{\text{max}} = 206 \text{ nm}$ and 304 nm

2.4. DNA Binding Studies by Absorption Titration

Proteins are separated from DNA by extraction with chloroform. Absorption titration experiments were carried out by varying the DNA concentration at 3.2, 6.4, 16 and $19.2 \mu\text{M}$ and maintaining the complex concentration constant at $5.6 \mu\text{M}$. The reference solution was the tris-HCl buffer solution. The sample solution was scanned in the range of 200–800 nm. The absorption data were analyzed for an evaluation of the intrinsic binding constant, K_b of the complex with DNA.

2.5. Antibacterial Studies

2.5.1. Preparation of the Culture Media (bacteria solution)

Bacterium was grown in nutrient broth which was prepared by mixing 0.15g beef extract, 0.25 g peptone and 0.25g NaCl in 50 mL of distilled water. The broth was heated for 15 minute for complete dissolution, and was autoclaved for 15 minute. Then single colony of *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* bacteria was added in 20 mL broth separately and incubated at 37°C for 24 hours.

2.5.2. Preparation of Sample Disk

A stock solution of 20 mg mL^{-1} was made by dissolving compound in distilled water. Paper discs of Whatman filter paper (0.45 micro phore) of uniform diameter (5 mm) and thickness (1mm) were sterilized. 10 micro liters of stock solution ($200 \mu\text{g}$ sample) were soaked in each disk.

2.5.3. Preparation of Agar Plates

The media was made up by dissolving bacteriological nutrient agar (3.2 g) in 100 mL distilled water. The mixture was autoclaved for 15 min at 120°C and then dispensed onto sterilized Petri dishes, allowed to solidify and then used for inoculation.

2.5.4. Procedure of Inoculation

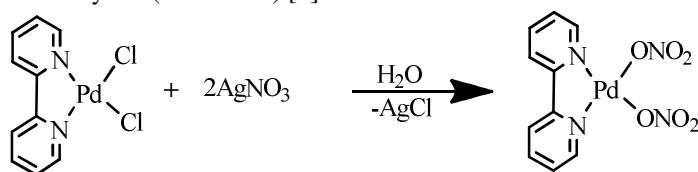
Inoculation was done with the help of micropipette with sterilized tips; 25 µl of activated strain was placed onto the surface of an agar plate, and spread evenly over the surface by means of a sterilized bent glass rod [12].

2.5.5. Application of Disks

Sample disks and antibiotic disks were applied in each earlier inoculated agar plates and incubated at 37°C for 24 h. The zone of inhibition (diameter) was then measured (in mm) around the sample and standard antibiotic disk. Antibiotic imipenem (IPM) was used against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella oxytoca*, and *Enterococcus faecalis* bacteria and ciprofloxacin (CIP) was used against *Pseudomonas aeruginosa* bacteria as standard antibiotic disk. The antibacterial results of the compound were compared with the standard antibiotic disc.

3. Results and Discussion

Reaction between $[\text{Pd}(\text{bpy})\text{Cl}_2]$ and AgNO_3 in water resulted the formation of yellowish green $[\text{Pd}(\text{bpy})(\text{ONO}_2)_2]$ complex in 93% yield (**scheme-1**) [5].



Scheme 1 Synthesis of $[\text{Pd}(\text{bpy})(\text{ONO}_2)_2]$ complex

Physical properties and elemental analysis data of the prepared compound are given in **Table 1**.

Table 1: Physical Properties and Elemental Analysis Data of $[\text{Pd}(\text{bpy})(\text{ONO}_2)_2]$ Complex

Color	Soluble in	Insoluble in	Elemental Analysis	
			Obtained value (%)	Calculated value (%)
Yellowish green	H ₂ O, DMF, DMSO, MeOH	n-Hexane, CH ₂ Cl ₂ , CHCl ₃ , acetone, EtOH	C: 32.18; H: 1.548; N,,: 14.05	C: 31.07; H: 2.09; N: 14.49

3.1. IR Spectra

The IR spectrum of the complex shows stretching bands at 3082 cm⁻¹ for aromatic C-H, 1606, 1566 and 1423 cm⁻¹ for C=N and C=C stretches for bipyridine ligand, 1490, 1269 and 984 cm⁻¹ for coordinated nitrates groups. Two combination bands at 1753 and 1736 cm⁻¹ are also observed for the coordinated nitrate groups (**Figure 1**). J. A. Walmsley *et al.* reported the similar IR spectrum of this complex [15].

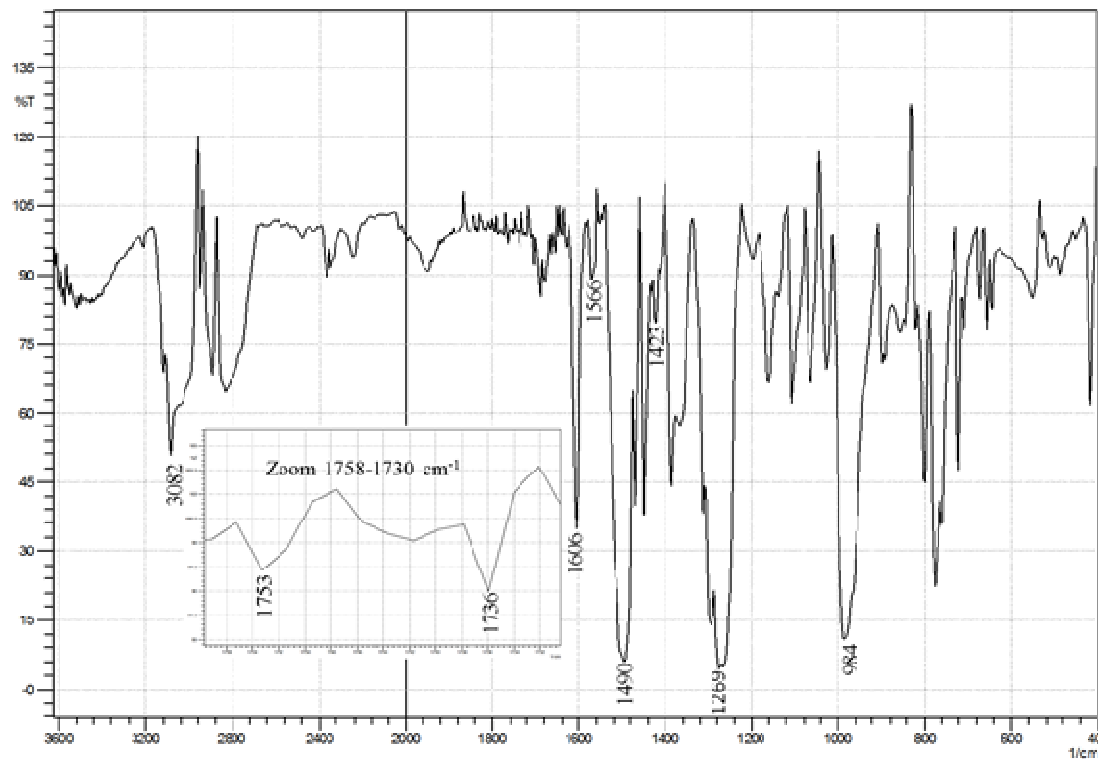


Figure 1: IR Spectrum of $[\text{Pd}(\text{bpy})(\text{ONO}_2)_2]$ complex

3.2. UV-Vis Spectra

The UV-Visible spectrum of the complex in water showed absorption bands at 206 nm and 304 nm due to $\pi - \pi^*$ and metal to ligand charge transfer, respectively (**Figure 2**). S. Wimmer *et al.* reported the UV-Visible spectrum of similar compound $[\{\text{Pd}(\text{bpy})(\mu\text{-OH})\}_2][\text{NO}_3]_2$ which shows absorption bands at 244 nm and 307 nm [16].

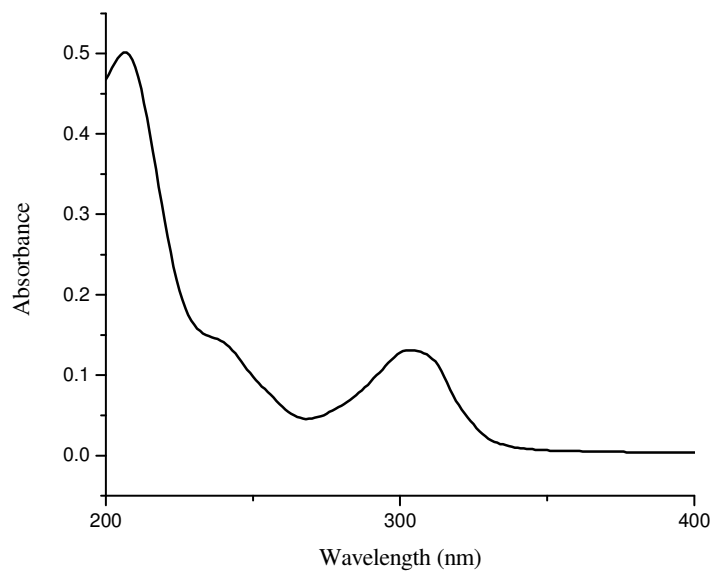


Figure 2: UV-Vis Spectrum of aqueous solution of $[\text{Pd}(\text{bpy})(\text{ONO}_2)_2]$ complex

3.3. DNA Binding Studies by Absorption Titration Experiments

One of the most common techniques in DNA-binding studies of metal complexes is electronic absorption spectroscopy. The magnitude of spectral perturbation is an evidence for DNA-binding. The absorption titrations were carried out by using a fixed amount of each metal complex ($5.6\mu\text{M}$) with increasing concentrations of DNA in the range from $3.2\text{--}19.2\mu\text{M}$. The reference solution was the buffer solution used in the study. The absorption spectra of the complex with increasing concentrations of DNA are given in **Figure 3**. On increasing the concentration of DNA, the absorption bands of the complexes at 304 nm was affected, resulting in hyperchromic shift. The absorption intensity is increased due to the fact that the purine and pyrimidine DNA-bases are exposed because of binding of the complexes to DNA [1].

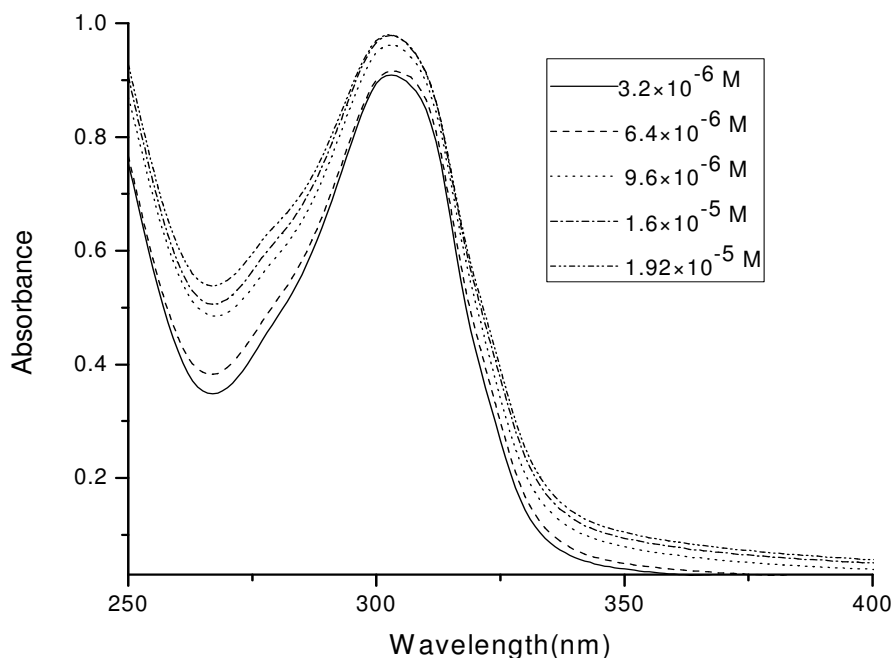


Figure 3: UV-Vis spectra of $[\text{Pd}(\text{bpy})(\text{ONO}_2)_2]$ complex after addition of DNA in different molar concentration.

In order to illustrate the binding strength of the palladium (II) complex with DNA, the intrinsic binding constant K_b was determined from the spectral titration data. It can be calculated by monitoring the changes in absorbance at the corresponding λ_{max} with increasing concentrations of DNA, using the following Eq.:

$$[\text{DNA}]/(\epsilon_a - \epsilon_f) = [\text{DNA}]/(\epsilon_b - \epsilon_f) + 1/K_b(\epsilon_b - \epsilon_f)$$

Where $[\text{DNA}]$ is the concentration of DNA in base pairs, ϵ_f , ϵ_a , and ϵ_b correspond to the extinction coefficients, respectively, for the free complex, for each addition of DNA to the complex and for the palladium (II) complex in fully bound form [1, 2].

A plot of $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ versus $[\text{DNA}]$ gives K_b , in **Figure 4** as the ratio of the slope to the intercept. From the $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ versus $[\text{DNA}]$ plots, the intrinsic binding constant K_b for complex was 2.09×10^6 , reveal a strong binding to lemon DNA. M. S. Mohamed *et al.* reported that binding constant K_b for similar complex $[\text{Pd}(\text{bpy})(\text{OH}_2)_2]^{2+}$ was 3.78×10^3 with protein free CT-DNA [1]. Intrinsic binding constant of the $[\text{Pd}(\text{bpy})(\text{ONO}_2)_2]$ complex is higher than that of the $[\text{Pd}(\text{bpy})(\text{OH}_2)_2]^{2+}$.

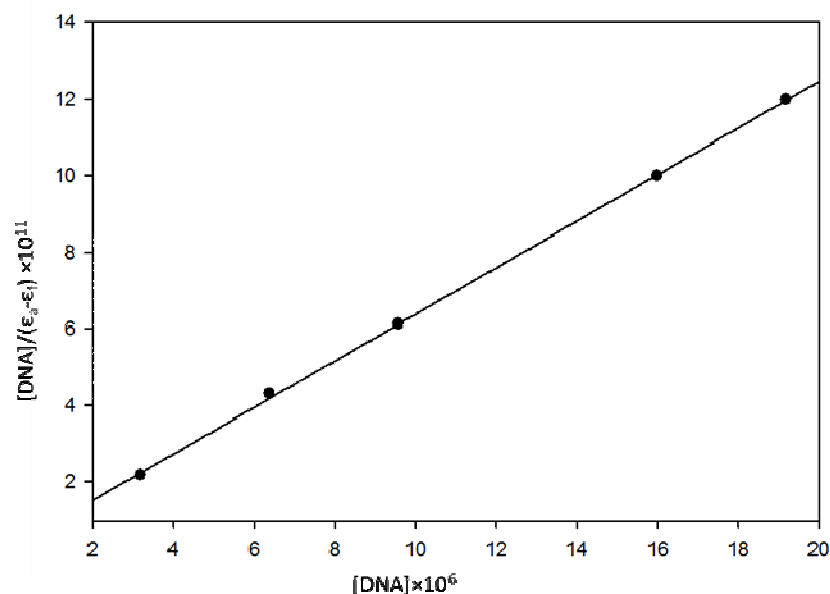


Figure 4: Plot of $[DNA]/(\epsilon_a - \epsilon_f)$ Vs $[DNA]$ to determine the intrinsic binding constant K_b

3.4. Antibacterial Activity

The testing of antibacterial activity of this compound was studied against five bacteria. We tested against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*, and compared them with the standard antibiotics (corresponding bacteria) (**Table 2**).

Table 2: Antibacterial Activity Data for the [Pd(bpy)(ONO₂)₂] Complex

Bacteria	Gram reaction	Inhibition zone diameter (mm)	
		Complex	Antibiotic
<i>Escherichia coli</i>	G ⁻	14	30
<i>Enterococcus faecalis</i>	G ⁺	12	26
<i>Klebsiella oxytoca</i>	G ⁻	14	32
<i>Staphylococcus aureus</i>	G ⁺	20	36

It was observed that the complex have inhibitor reactivity against all tested bacteria and the complex shows highest antibacterial activity against Gram positive *Staphylococcus aureus* bacteria (**Figure 5**). Antibacterial activity of complex [Pd(bpy)(OH₂)₂]²⁺ shows similar tend as the complex studied here [1].

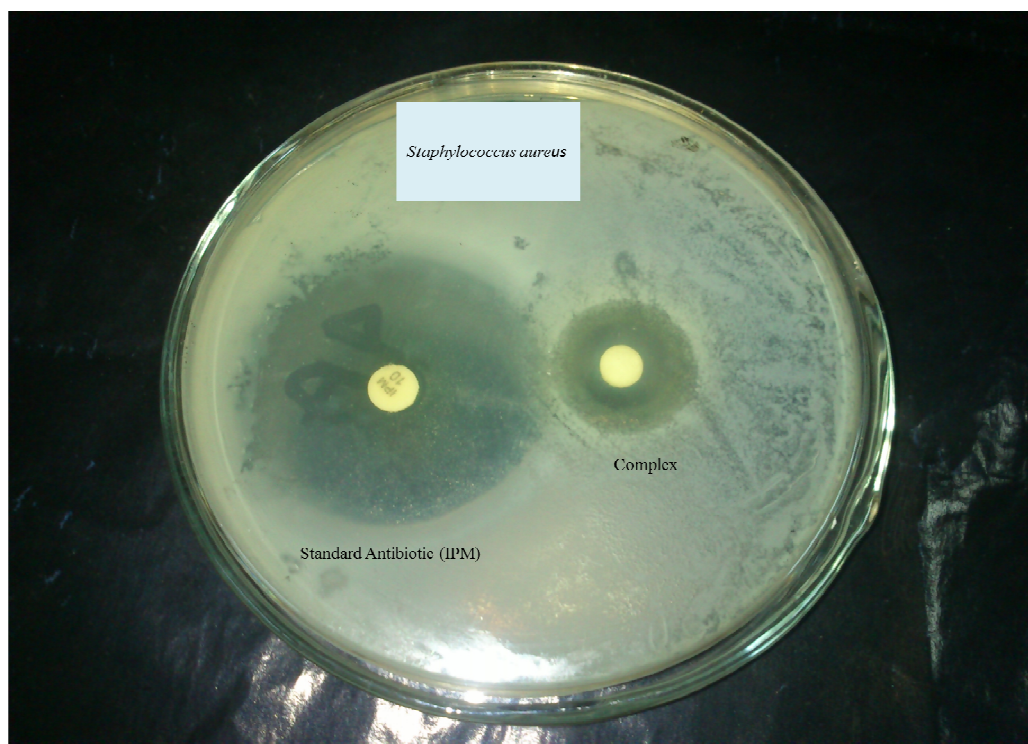


Figure 5 Antibacterial activity of $[\text{Pd}(\text{bpy})(\text{ONO}_2)_2]$ complex and standard antibiotic (IPM) against *Staphylococcus aureus* in the form of inhibition zone

4. Conclusion

The present study describes the DNA interaction and antibacterial activity of $\text{Pd}(\text{bpy})(\text{ONO}_2)_2$ complex. The intrinsic binding strength K_b of this metal complex with DNA was determined by UV-Vis spectrophotometry and antibacterial activity of this metal complex studied by disk diffusion method. The complex exhibits strong binding ability to DNA and good antibacterial activity.

5. Acknowledgements

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6. References

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