Supplementary

A Benzopyran-based Optical Sensor for Selective Trace Detection of Pd (II): Analytical and Computational Investigation

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Supplementary Information 1: Rationale behind the vacuum environment determination

Prior to the experimental analysis, the computational studies of the investigated complex were performed under vacuum conditions. The rationale behind the vacuum environment was to eliminate solvent–solute interactions and isolate the structural and electronic properties of the system under study. This standard model allows for direct comparison of fundamental parameters prior to introducing more complex solvent effect. In contrast, the experimental studies involving various solvents that influence its solubility, stability, and reactivity were performed. The conclusion of these studies was slight deviations between theoretical and experimental data due to the difference in their working environment. A clear baseline was observed on studying the vacuum-based approach that led to a trend which remained consistent in solvent studies.

Supplementary Information 2: Radical Scavenging activity of Pd (II)-HPC complex

DPPH radical scavenging assay interprets the compounds' antiradical properties. The complex's better efficacy was examined via analytical studies in order to measure its percentage RSA toward 2, 2-Diphenyl-1-picrylhydrazyl, DPPH [1-3].

The radical scavenging activity is mostly assessed by measuring the absorbance drop at 517 nm in organic solvents like methanol or ethanol. Prior to analysis, a stock solution of 10^{-3} M DPPH radicals in methanol were made specifically for the desired purpose. The absorbance value for the working solution of negative control (DPPH) was set at 1.00 ± 0.200 [4]. The stock solution of studied compounds was prepared by dissolving gallic acid,

HPC and its complex in methanol at a rate of 5 mg per 5 mL and covering the flask with aluminium foil to avoid its contact with light. In methanol, DPPH develops a vivid purple colour and results in a prominent absorption band at 517 nm. A change in colour from deep purple to yellow is adhered by DPPH on reacting with the compounds exhibiting antioxidant activity. From the stock solution of the positive control, ligand and its complex, several solutions of varying concentrations - 500, 250, 125, 62.5, and 31.25 µg mL^{-1} were prepared respectively for antioxidant potential studies. All the prepared solutions were incubated at 37 °C in the absence of light. Further each of the resultant solutions was compared to the prepared negative control using spectrophotometric optical density measurements at a wavelength of 517 nm.

Supplementary Information 3: Antimicrobial studies of Pd (II)-HPC complex

The bacterial and fungal strains were preserved on Nutrient Agar slants and after a 24 hours incubation period, the microorganisms were examined and subjected to the Agar well diffusion assay [5]. The antimicrobial activity is noted down in the form of their respective zone of inhibitions after specific incubation period.

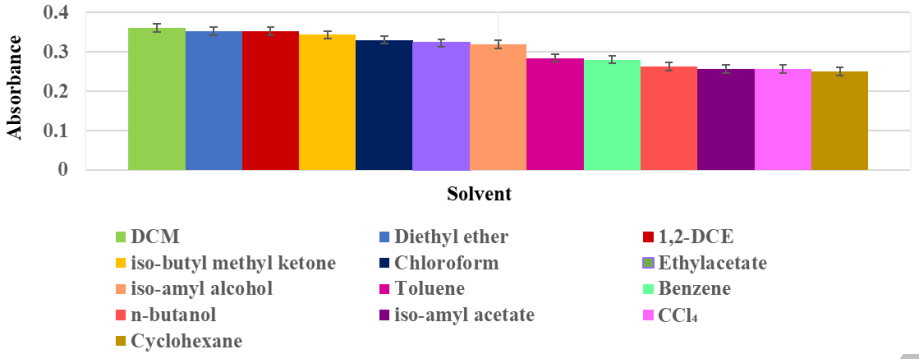
Agar Well Diffusion Assay

The agar well diffusion method was employed to identify the Pd (II)-HPC complex's antibacterial and antifungal properties [6,7].

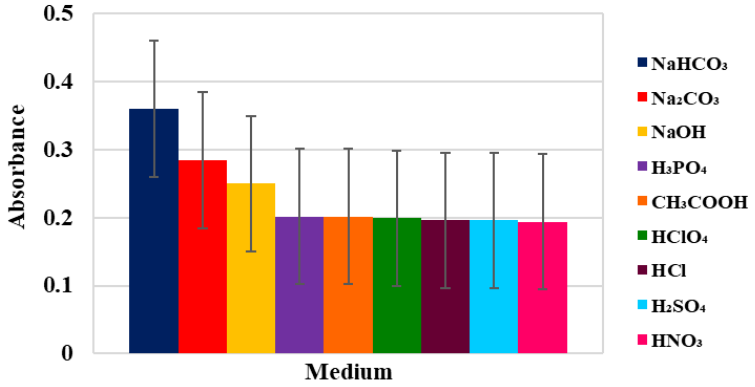
In accordance with the 0.5 McFarland standards, the suspensions of chosen bacteria and fungus were combined in sterile saline (0.9% NaCl) using 16-hour-preserved cultures and adjusted to 1.5×10^{8} cfu mL^{-1}. A 15–20 mL of Nutrient Agar medium was poured into the Petri plates, in succession to swabbing the plates with 100 µL of the bacterial and fungal cultures, thereafter left for 15 minutes to allow for adsorption over the media. A sterile cork-borer was used to create 8 mm-diameter agar wells into the seeded agar plates, which were then filled with 100 µL of HPC and Pd (II)-HPC complex in accordance with their respective molecular weights.

To encourage microbial growth, each plate was incubated for 24 hours at 37°C. The zone of growth inhibition, that incorporates well width, against the test organisms was measured with the help of a zone reader, commonly known as Hi Antibiotic Zone Scale, to assess the complex's antimicrobial activity [8].

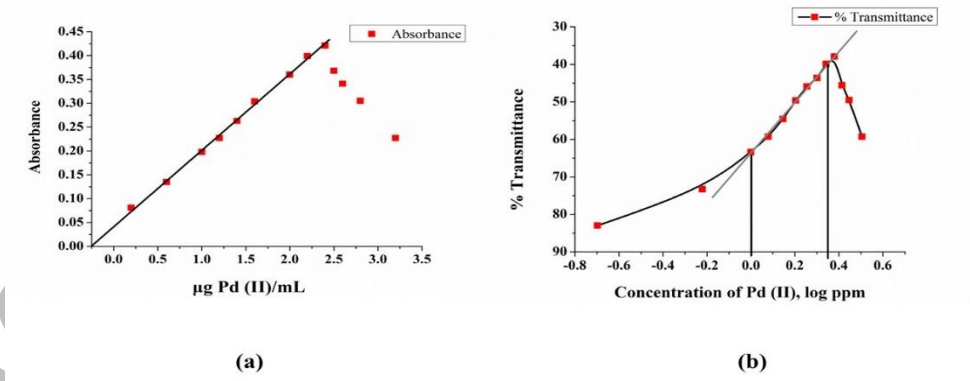
I. Supplementary Figures



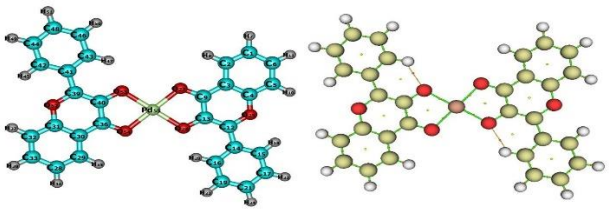
Supplementary Fig. 1. Effect of solvents



Supplementary Fig. 2. Effect of reaction medium



Supplementary Fig. 3. Optical parameters (a) Beer's Law (b) Ringbom's plot at 425 nm



Supplementary Fig. 4. Molecular graph of Pd (II)-HPC complex showing Pd-O G_{BCP}/V_{BCP} values between 0.5 and 0 indicating its covalent character

II. Supplementary Tables

Supplementary Table 1. Effect of anions/ complexing agents on Pd (II)-HPC complex

Salts used	Anion/Complexing agent	Tolerance Limit (mg/10mL)
Sodium bicarbonate	HCO_3^-	100
Ascorbic acid	$C_6H_8O_6$	100
Sodium chloride	Cl^-	90
Sodium sulphite	SO_3^{2-}	80
Sodium nitrate	NO_3^-	80
Potassium nitrite	NO_2^-	50
Sodium fluoride	F^-	50
Thiourea	$SC(NH_2)_2$	50
Sodium sulphate	SO_4^{2-}	10
Potassium bromide	Br^-	10
Sodium dithionite	$S_2O_4^{2-}$	08
Sodium acetate	$C_2H_3O_2^-$	05
Hydrazine sulphate	$N_2H_6SO_4$	01
Sodium carbonate	CO_3^{2-}	01
Potassium iodide	I^-	01
Potassium oxalate*	$(C_2O_4)^{2-}$	01
Hydrogen Peroxide (30%)**	H_2O_2	01
Glycerol**	$C_3H_8O_3$	01
Potassium thiocyanate	$[SCN]^-$	0.5
EDTA "disodium salt"	$C_{10}H_{14}N_2Na_2O_8$	0.5
Sodium potassium tartrate	$C_4H_4O_6^{2-}$	0.1

*Interferes seriously even in trace concentration
**Added in mL

Supplementary Table 2. Effect of cations on Pd (II)-HPC complex

Salt used	Cation studied	Tolerance limit, mg/10mL	Salt used	Cation studied	Tolerance limit, mg/10mL
$CoCl_2.6H_2O$	Co (II)[a]	10	$CrCl_3$	Cr (III)	01
$NiSO_4.6H_2O$	Ni (II)	10	Na_2SeO_4	Se (IV)	0.5
$BaCl_2.H_2O$	Ba (II)	10	$RuCl_3$	Ru (III)	0.1
$PbNO_3$	Pb (II)	10	Nb_2O_5	Nb (V)	0.1
$AlCl_3$	Al (III)[b]	10	OsO_4	Os (VIII)	0.1
$HgCl_2$	Hg (II)	10	$SnCl_2$	Sn (II)	0.1
$ZnCl_2$	Zn (II)	10	$Na_2WO_4.2H_2O$	W (VI)	0.1
$CdCl_2$	Cd (II)	10	H_2PtCl_2	Pt (IV)	0.1
$CuSO_4.5H_2O$	Cu (II)	10	$NaVO_3$	V (V)	0.1
$CaCl_2$	Ca (II)	08	$(NH_4)_2MoO_4$	Mo (VI)	0.1
$MgCl_2$	Mg (II)	08	$AuCl_3$	Au (III)	0.1
$AgNO_3$	Ag (I)	08	$IrCl_3$	Ir (III)	0.1
$MnCl_2.4H_2O$	Mn (II)	05	$(NH_4)_4[Ce(SO_4)_4].2H_2O$	Ce (IV)	0.1
Na_2HAsO_4	As (V)	05	TiO_2	Ti (IV)[d]	0.1
$SrSO_4$	Sr (II)	05	$FeCl_3$	Fe (III)	0.05
$Bi_2(SO_4)_3$	Bi (III)[c]	02	$ZrOCl_2.8H_2O$	Zr (IV)	0.05
$K_2Cr_2O_7$	Cr (VI)	02	$FeSO_4.7H_2O$	Fe (II)	0.05

[a]Co (II) and [d]Ti (IV) masked with 1 mL hydrogen peroxide
[b]Al (III) masked with 50 mg fluoride
[c]Bi (III) masked with 0.1 mg sodium potassium tartrate

Supplementary Table 3. Radical scavenging analysis of HPC and its Pd(II) complex

Concentration (μg mL^{-1})	% RSA		
	Gallic acid	Pd (II)-HPC	HPC
500	80	74.92	66.66
250	74.3	59.75	52.27
125	65.7	56.43	43.64
62.5	52.2	49.66	36.43
31.25	47.6	32.73	28.75

IC_{50}: Gallic acid (46.8 μg mL^{-1}); Pd (II)-HPC (62.9 μg mL^{-1}); HPC (240 μg mL^{-1})

Supplementary Table 4. Antimicrobial activity of HPC and its complex showing their zone of inhibition

Microbial strain	Zone of Inhibition (mm)[a]	
	HPC	Pd (II)-HPC complex
Bacillus subtilis	22±0.28	24±0.11
Staphylococcus aureus	21±0.57	22±0.26
Escherichia coli	18±0.48	26±0.12
Pseudomonas aeruginosa	17±0.11	22±0.15
Candida albicans	17±0.20	21±0.66

[a]Values, mean of triplicates (± SD)

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