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

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

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ABSTRACT

3-Hydroxy-2-phenyl-4*H*-chromen-4-one (HPC), a chromogenic reagent acting as an optical sensor for the metal to be determined and having a sensitive impact on the spectrophotometric Pd (II) determination in the organic phase, has been studied in the present investigation. The ideal conditions for complexation were depicted by the various statistical evidences for instance standard deviation (SD = ± 0.00184), Sandell's sensitivity (S = 0.0055 μg cm^{-2}), detection limit (LOD = 0.1122 μg mL^{-1}) and regression coefficient (r = 0.9975). The attenuation coefficient of Pd (II)-HPC complex was 1.9159×10^4 L mol^{-1} cm^{-1} calculated at a wavelength range of 417-432 nm of the resulting stable binary yellow complex. Analytical findings support a square planar geometry of the investigated coordination complex. The theoretical studies for instance Density Functional Theory (DFT) have been conducted in order to enhance our comprehension about the complex's molecular geometry and its structural attributes. DFT, has a strong correlation with the analytical findings, proving that the studied complex behaves as a strong bioactive agent. The investigated complex was indeed subjected to antimicrobial and antioxidant studies, results of which reflected that the formed complex has a strong potential to act as a strong antimicrobial and a radical scavenging agent compared to ligand alone. Along with, the formed complex has been employed on commercial samples and has come out with remarkable sensitivity, selectivity, accuracy and precision, under set conditions of the procedure.

Keywords: Palladium, Chromen-4-one, Spectrophotometric determination, DFT and QTAIM studies, Antioxidant and Antimicrobial

INTRODUCTION

Over the last few years, benzopyran's coordination chemistry has garnered a lot of interest owing to its potential applicability. The study investigates sensitivity of these reagents as sensing materials for metal ions in an aqueous medium after photo excitation. The distinctive structures of benzopyran derivatives make them important in organic [1], inorganic [2-5], agricultural [6] and therapeutic [7] fields. One of the main dietary flavonoids [8] found in nature is 3-hydroxy-2-phenyl-4*H*-chromen-4-one (HPC). It has drawn the attention of many researchers because of its biological and pharmacological properties. It is frequently found in fruits and vegetables [9,10]. The mere existence of metal ions influences the biological activity of flavonoids [11,12]. The reagent, HPC has the potential to form complexes with different metal ions owing to the presence of two reactive sites- the hydroxy and oxo groups over the molecule [13,14]. Transition metals and their complexes are highly studied by inorganic chemists due to their multiplex electronic and stearic properties, which enable exchange of ligands with biomolecules.

The chemistry of transition elements, particularly platinum group metals (PGMs), is gaining interest due to their industrial applications and their significance in biological systems. These elements are used in solar energy conversion [15], phosphorescent dyes [16], luminescence-based sensors [17], photo catalysts [18], and electron transfer [19] triggers in biomolecules. Transition metals are a promising base for therapeutic studies. The complexes of transition metals can be controlled through ligand design and metal selection. Palladium, a rare platinum metal with atomic number 46, is a valuable resource in various industries. The study of materials with trace to high concentrations of palladium has led to improved detection and determination methods. In the recent decades, the utilization of molecular-based sensors has evolved as an appealing methodology, among numerous methods for trace determination of metal complexes. A wide range of probes based on transition metals, including Pt (II), Ru (II), Re (I), Ir (III), Cu (I), Au (I) and Os (II), have been produced in recent decades [20]. The significance of metal ion sensors in both the environment and physiology has garnered attention. These optical sensors help in detecting the metals out even in trace concentrations which then can be determined with the help of different instrumental techniques [21-23]. The development of analytical techniques, ideally straightforward, sensitive, and selective ones, is becoming more and more necessary in order to expand the range of applications for palladium and its compounds. Techniques like Neutron Activation Analysis (NAA) [24], Atomic Absorption Spectroscopy (AAS) [25], spectrofluorometric [26], voltammetry [27], flame emission spectrometry [28], sequential flow injection analysis [29], imprinted polymer-based method [30], Inductively Coupled

Plasma Optical Emission Spectroscopy (ICP-OES) [31], Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) [32], and individual catalytic method [33] have been employed. Expense of the instrumentation, technical expertise, and maintenance concerns are some of the obstacles that limit the broader use of these techniques. Many flexible UV/VIS [34] spectrophotometric techniques, however, have been employed to determine palladium at trace levels in its complexes in order to overcome the mentioned shortcomings. Although all of the Pd (II) detection techniques now in use yield plausible findings, their high infrastructure and time requirements make them impractical for analysing many samples. Conversely, selective optical sensors using UV/VIS spectrophotometry are highly beneficial for detection and determination of transition metals in a variety of samples, because they are quick, convenient, easy to use, affordable, and appropriate for monitoring.

The present approach signifies the role of HPC, a benzopyran derivative, acting as an optical sensor and helping further in trace Pd (II) determination as its coordination complex, using extractive organic phase UV/VIS-spectrophotometry and examining the antioxidant and antimicrobial efficacy of the thus developed optical sensor-based metal complex. The sensing mechanism of HPC in the presence of Pd (II) ions was validated by spectrophotometric measurements. DFT further provides insight into the electronic structural details and chemical reactivity of the Pd (II)-HPC complex, aligning with previous computational studies for similar metal complexes [35-37].

EXPERIMENTAL

Equipment: A UV/VIS spectrophotometer (EI-2375) equipped with identical 1 cm quartz cuvettes was used for all absorbance studies. All weight measurements were done using an exceptionally accurate electronic balance (KERRO Series P6B). To clean the calibrated glassware used in this study, it was left overnight soaked in diluted hydrochloric acid (HCl) followed by rinsing with double-distilled water. Vertical autoclave, burner and laminar air flow horizontal (4×2×2) and universal incubator were used for the antimicrobial studies.

Reagents and solutions: Deionized water and highly pure chemicals of Analytical grade were employed in the experiments. A 0.167g of precisely weighed palladium chloride, $PdCl_2$ (Sigma Aldrich; CAS : 7647-10-1) was dissolved in water with an addition of minimum amount of HCl and making up the volume with de-ionized water, in order to form a 100 mL stock solution of palladium with a strength of 1 mg Pd (II) mL^{-1}. A working solution with necessary concentrations, that is 100 μg mL^{-1} and 20 μg mL^{-1} were prepared by dilution of the metal's stock solution. Sodium bicarbonate as a reaction medium, while DCM (Sisco Research Laboratories Pvt. Ltd.; CAS:75-09-2) as an extractant were utilized in order to control the reaction conditions for complete complex formation. The HPC reagent, Figure 1, was purchased from Sigma Aldrich (CAS: 577-85-5), whose 0.2% (w/v) solution was prepared in acetone for complexation with the metal.

Solutions for analytical studies

Diverse ions: To investigate impacts induced by the majority of the analytically significant cations and anions/complexing agents, the necessary solutions were made from their available Na/K salts by dissolving them either in de-ionized water or mineral acids.

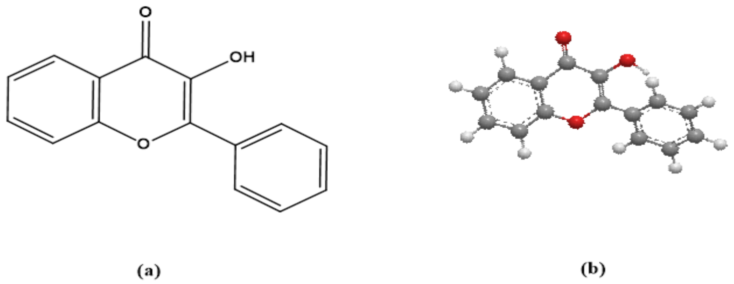
Samples

Synthetic mixtures: The synthetic samples were prepared by mixing microgram amounts of palladium (II) with milligram amounts of other metal ions in suitable proportions. The International Critical Tables of Numerical Data, Physics, Chemistry and Technology [38] provided the canonical data that was used to prepare synthetic mixtures and reference alloys (Table 5) to remove any uncertainty in alloy composition and to bring our study into compliance with internationally recognized standards. The alloys produced were not only chemically accurate but also equivalent to those utilized in advanced spectroscopic investigations.

Pd charcoal catalyst: In order to prepare Pd/C catalyst solution for analytical applications, accurately weighed catalyst sample was dissolved in aqua regia and heated. After filtering out charcoal, excess of nitric acid was removed by heating with concentrated HCl to dryness. The residue obtained was then treated with double-ionized water and dilute HCl. It is then exposed to palladium (II) determination in accordance to the proposed strategy.

Solutions for biological studies: 1mM stock solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich;CAS:1898-66-4) having strength of 1000μg mL^{-1} and Gallic acid (CDH Fine Chemical Ltd.; CAS: 5995-86-8) of varying concentration were procured and used to check antioxidant efficacy of the studied compounds. Methanol was used as a solvent for the studies.

For antimicrobial studies, Nutrient broth (HIMEDIA), Agar (HIMEDIA; CAS: 9002-18-0), DMSO (Sisco Research Laboratories Pvt. Ltd.; CAS: 67-68-5), Bacterial and fungal strains (IMTECH, Chandigarh, India).



(a) (b)

Fig. 1. 3-Hydroxy-2-phenyl-4*H*-chromen-4-one (a) 2D (b) 3D view

Recommended protocol for determination of Pd (II)-HPC complex

Spectrophotometric analysis: To attain precise and impeccable outcomes, the spectrophotometric analysis was executed in the most efficient way possible. A working solution of 20 $\mu g\ mL^{-1}$ of the metal under investigation was prepared for analysis, by diluting its stock solution with the proper volumes of double-deionized water. The complexation process enmeshed pipetting out 1ml of the working solution in a 125mL of separatory funnel with successive addition of 1 mL of 1M sodium bicarbonate ($NaHCO_3$)followed by 1 mL of 0.2% 3-hydroxy-2-phenyl-4*H*-chromen-4-one (HPC) reagent in acetone. The final aqueous volume was made to 10 mL with the double-deionized water. A yellow-coloured complex was promptly formed on addition of acetonic solution of the prepared reagent, which was extracted with an equi-volume of dichloromethane (DCM) with a constant shaking for a period of 45 seconds resulting in formation of two +separate layers. The lower organic layer was filtered using Whatman paper 41 and collected in order to record its optical density at 425 nm. The precise amount of divalent palladium (II) was calculated using a calibration curve between optical density and different palladium concentrations.

Theoretical investigations: The computational investigation assists in identifying the compound's related Frontier molecular orbitals, FMOs (HOMO and LUMO), minimal energy molecular organizations, and other structural characteristics [39]. By using the Gaussian 16 package [40], a comprehension of the quantum chemical description and the associated computations of reagent and its corresponding Pd (II) complex was gained. Furthermore, the examined complex was subjected to both the NBO [41, 42] and QTAIM [43] analyses using PBE/def2-TZVP level of theory. The rationale for using a vacuum environment in contrast to the experimental conditions, which involved numerous solvents is discussed in Supplementary Information 1.

DPPH radical scavenging assay is a widely used methodology for assessing the scavenging activities under *in vitro* conditions was used to study HPC and its complex, in view of the antiradical characteristics possessed by metal complexes based on chromone-4-ones

(Supplementary Information 2).With the goal to assess the antimicrobial abilities of Pd (II)-HPC complex, four bacterial and one fungal strains were opted based on their clinical relevance. The strains that have been selected entails, Gram-positive bacteria [*Staphylococcus aureus* (MTCC-96) and *Bacillus subtilis* (MTCC-121)]; Gram-negative bacteria [*Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 74)]; Fungal strain [*Candida albicans* (CTCC 10231)] (Supplementary Information 3).

RESULTS AND DISCUSSION

Spectrophotometric analysis

When HPC was reacted with palladium in divalent state, a yellow-coloured sparingly soluble complex resulted in aqueous phase that was quantitatively extracted into DCM, absorbing visible radiations with maximum optical density in the range of 417-432 nm (Figure 2; Curve A) In the same range, there was quite low absorbance shown by the reagent (Figure 2; Curve B). In order to pursue further, a wavelength of 425nm was selected due to the consistent absorbance and absorption maximum of the complex at it.

The varying experimental conditions being maintained, while studying various parameters one after the other, taking 20 µg Pd per 10mL aqueous phase in each case, stoichiometry of the complex, optical and statistical characteristics are mentioned below.

Optimum Conditions for Pd (II)-HPC complex

An array of non-aqueous solvents was studied among which were DCM, diethyl ether, 1, 2-dichloroethane, iso-butyl methyl ketone, chloroform, ethyl acetate, iso-amyl alcohol, toluene, benzene, n-butanol, iso-amyl acetate, carbon tetrachloride and cyclohexane in an attempt to extract the Pd (II)-HPC complex. Extraction of the complex was observed to decrease in the same order (Supplementary Figure 1) as mentioned, while using variable solvents. It was noticed that a single extraction with equal volume (10 mL) of DCM was sufficient to give quantitative extraction. The optical density remained unchanged for more than 2 days in DCM.

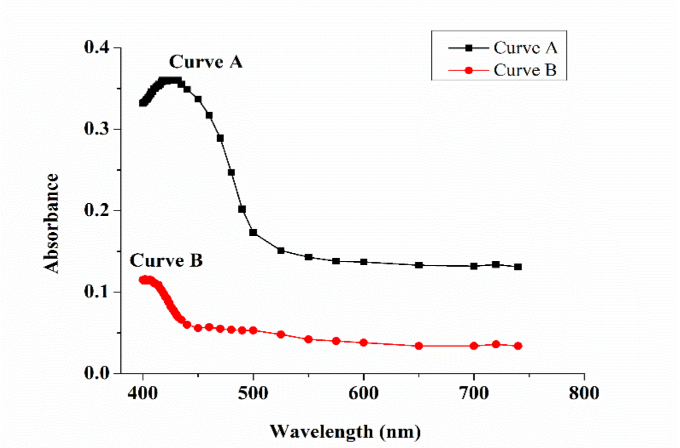


Fig. 2. Absorption spectrum under optimal conditions

The impact of various reaction media on complexation between Pd (II) and HPC in the DCM was investigated. The best medium for assessing complexation was a weakly basic, $NaHCO_3$, which displayed the maximum optical density at 425 nm as is indicated in Supplementary Figure 2. Maximum colour intensity was seen when extractions were carried out using 0.05-0.20 M $NaHCO_3$ (Figure 3a), signifying that full complexation had taken place. However, as more $NaHCO_3$ was added, a noticeable drop in optical density was noted.

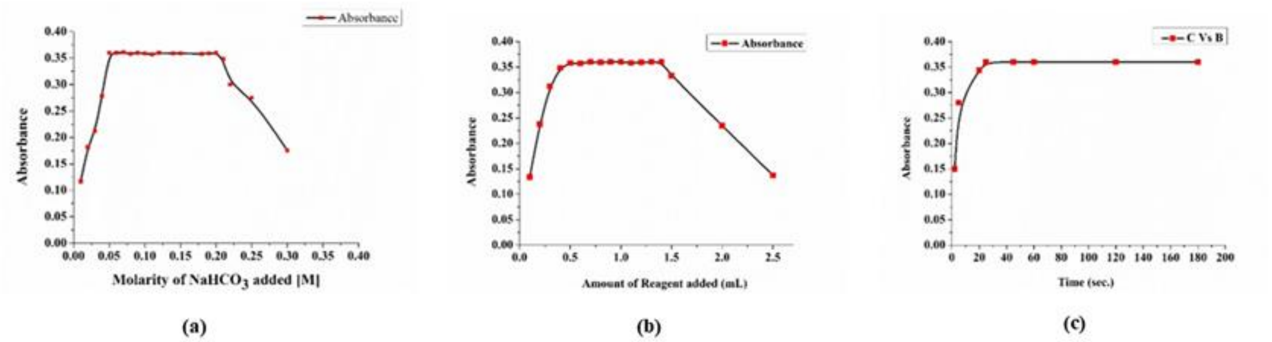


Fig.3. Optimization of reaction parameters

(a) Pd (II) = 20 µg; HPC [0.2% solution in acetone] = 1 mL; Equal volume of aqueous phase and organic phase = 10 mL; Equilibration time = 45 sec.; $\lambda_{max.}$ *= 425 nm*

(b) 0.1 M sodium bicarbonate; other conditions are the same as in (a) excepting variation in HPC concentration

(c) HPC [0.2% solution in acetone] = 1mL; other conditions are the same as in (b) excepting variation in equilibration time

While examining the effect of ligand (HPC) concentration on the complexation process, a steady increase in absorbance became apparent upon increasing additions of an acetonic solution of HPC at a concentration 0.2% (w/v). The most intense and consistent absorbance value was found in the range 0.5-1.4 mL of the reagent (Figure 3b). Higher concentration, however, decreases the absorbance as a slight turbidity is developed in the organic phase of reagent blank. Hence, 1.0 mL of 0.2% in acetone reagent is selected for further studies. The reagent solution prepared in ethanol gives lower value of the absorbance. Shaking the sample solution containing the complex at optimum conditions, once for 2 sec. with an equal volume (10 mL) of solvent (DCM) gives an absorbance value of 0.150, which increased to a maximum (0.360) for 25-180 sec. contact time (Figure 3c). This shows that 45 sec. contact time is sufficient in this determination procedure.

Investigations on interference

In order to assess the commercial adaptability of the studied Pd (II)-HPC complex and its selectivity, a total of 21 anions/complexing agents and 34 cations were examined for their involvement using the recommended approach. By taking 20 µg Pd (II) per 10 mL aqueous phase under proposed conditions, the impact of various ions was investigated. The variety of anions/complexing agents (Supplementary Table 1) and cations (Supplementary Table 2) when applied prior to the reagent's addition caused <1% error. Al, Co, Ti, Bi interfered seriously causing >1% error. However, encroachment due to Co (II), Al (III), Ti (IV) and Bi (III) was eliminated in presence of respective masking agents as mentioned in Supplementary Table 2. However surprisingly, Ni (II) interferes seriously with the determination.

Optical features and analytical figures of excellence

In order to evaluate the coherence to Beer's law, optical density values of the complex for a series of metal ion solutions with different concentrations were examined to have a linear relation between the two, confirming that Pd (II) can be studied within the 0.0-2.4 µg

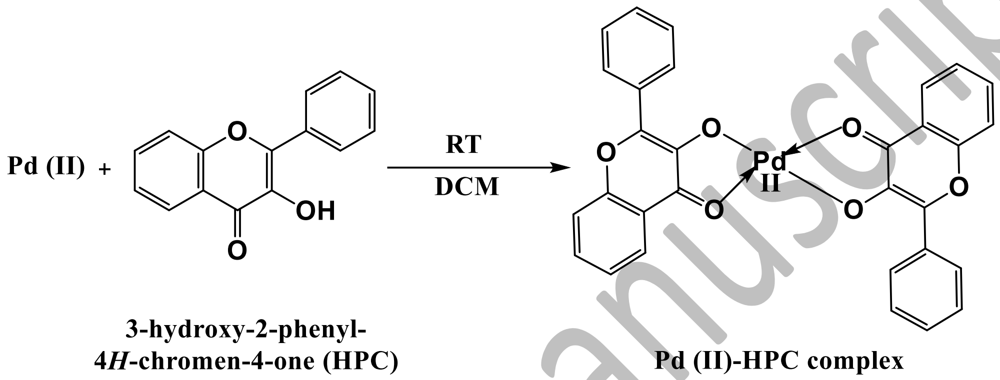
Table 1. Optical and analytical figures of excellence

Variable	Calculated Values
Maximum absorption wavelength (nm)	417-432
Molar attenuation coefficient (L $mol^{-1}cm^{-1}$)	1.9159×10^4
Sandell’s sensitivity (µg cm^{-2})	0.0055
Beer’s Law (Linearity range) (µg mL^{-1})	0.0-2.4
Ringbom’s optimum range (µg mL^{-1})	1.0-2.2
Linear regression equation	$y = 0.1598x + 0.0425$
Regression coefficient	0.9975
Detection Limit (LOD) (µg mL^{-1})	0.1122
Standard deviation	0.00184
RSD (%)	0.51
Stoichiometry of the complex [M:L]	1:2
Stability of the complex	>2 days

mL^{-1}concentration range (Supplementary Figure 3a). Using Ringbom's plot, the ideal concentration range for determination was assessed to be 1.0-2.2 μg Pd (II) mL^{-1} (Supplementary Figure 3b).The optical features and analytical parameters of excellence associated with the investigated compound are as mentioned in Table 1.

Stoichiometry of the complex under investigation

A 1:2 [M: L] stoichiometry was established between Pd (II) and HPC using the conventional Job's continuous variations [44,45] and mole ratio [46] methods to propose structure of the studied complex as shown in scheme 1.



Scheme 1. Synthesis of Pd (II)-HPC complex

Computational Chemistry

The optimized structure of the obtained complex is given in Figure 4. The structure belongs to the C_{2h} symmetry point group and has a planar conformation. The Pd-O bond length is 2.01 Å and O-Pd-O bond angle is 82°. The MO analysis results show that the HOMO is mainly on the Pd and O atoms and the C=C bond. While the LUMO is distributed over the entire ligand.

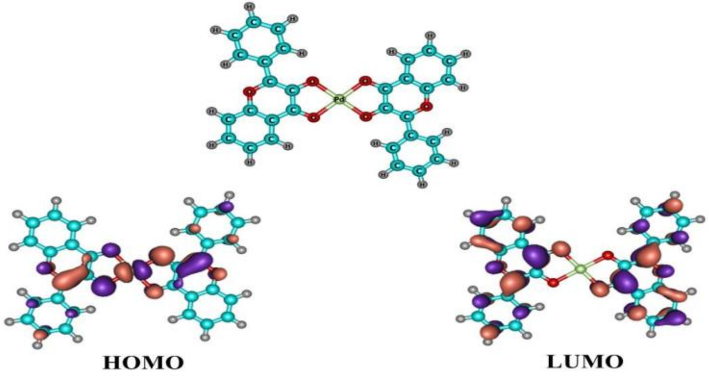


Fig. 4. Optimized structure and frontier MOs of the Pd (II)-HPC complex at the PBE/def2-TZVP level of theory

The absorption spectrum of the resulting complex has an absorption maximum at 414 nm, with an oscillator strength f = 0.3799. This shift is related to the charge transfer from MO131 to MO134, seen in Figure 5.

Electronic characteristics

Frontier molecular orbitals play a crucial role in determining the electronic properties of systems namely, the HOMO-LUMO energy gap (E_{gap}), electron affinity (A), ionization potential (I), chemical potential (μ), chemical hardness (η), electronegativity (χ), chemical softness (S) and electrophilicity index (ω) [47,48]. The energy of HOMO-LUMO is a reactivity marker, indicating a molecule's willingness to give and receive electrons under favourable conditions. Negative values (E_{HOMO} and E_{LUMO}) indicate stability, while positive values of E_{gap} indicate good stability and charge transfer. The ionization potential and energy gap values indicate the compound's capacity to donate electrons, leading to stabilization of free radicals.

Both the negative μ and the positive χ indicated that the compounds might take in electrons from the surroundings, which would lower their energy. Using chemical hardness and electronegativity, the global electrophilicity index (ω) was computed to measure the energy stabilization in the molecule [49]. The various quantum chemical parameters of the studied complex are as shown in Table 2.

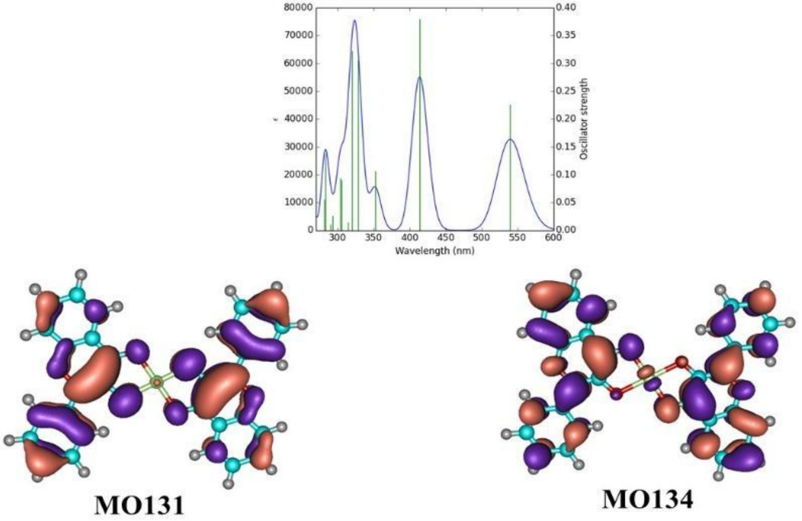


Fig. 5. Absorption spectrum of the Pd (II)-HPC complex at $\lambda_{max.}$ 414 nm, with an oscillator strength *f* = 0.3799

More stable complexes with higher perturbation energy values signify greater charge transfer from ligand to metal.

The most important donor-acceptor interactions are displayed in Table 3 along with the associated perturbation energy values. The interaction between the ligand's lone pair of electrons on its oxygen atoms as donor and the Pd (II) metal's antibonding orbitals as acceptor is responsible for the metal-ligand complex's remarkable donor-acceptor interactions. Strong interactions were examined by Pd (II)-HPC complex from LP O26 to LP* Pd55 and LP O53 to LP* Pd55 with perturbation energy values of 73.25 kcal mol^{-1}.

Table 2. Quantum chemical studies

Sr. No.	**Electronic properties (eV)**	**Pd (II)-HPC Complex**
1	E_{HOMO}	-4.46
2	E_{LUMO}	-3.10
3	Energy gap (E_{gap})	1.36
4	Electron affinity (A)	1.70
5	Ionization potential (I)	6.11
6	Chemical potential (μ)	-3.91
7	Chemical hardness (η)	2.20
8	Electronegativity (χ)	3.91
9	Chemical softness (S)	0.45
10	Electrophilicity index (ω)	-1.77

Table 3. The key donor-acceptor interactions for the Pd (II)-HPC complex, along with the associated perturbation energy values

Donor to acceptor transition		**Perturbation energy values (kcal mol^{-1})**
LP O26	**LP* Pd55**	73.25
LP O27	**LP* Pd55**	57.77
LP O53	**LP* Pd55**	73.25
LP O54	**LP* Pd55**	57.77

Quantum Theory of Atoms in Molecules (QTAIM) study

Bader's topological QTAIM studies [50] can be used to examine the nature and strength of metal-ligand interactions. Supplementary Figure 4 shows the Pd (II)-HPC molecular graph, showing the locations of bond critical points (BCPs) and associated bond paths between the ligand's oxygen atoms (O26, O27, O53 and O54) and the Pd (II) metal. The topological parameters studies at BCPs involved are reported in Table 4. By examining the sign of Laplacian values, one can ascertain the intensity of intermolecular interactions [50-53].

Table 4. Topological characteristics at the BCPs of Pd (II)-HPC complex

Bond critical points (BCP)	**Electron density,** ($\rho(r))\rho(r)$)	**Laplacian,** $\nabla^2\rho(r)$ $\nabla^2\rho(r)$	**Lagrangian energy density,** $G(r)G(r)$	**Potential energy density,** $V(r)$ $V(r)$	G_{BCP}/V_{BCP}
Pd55−O53	0.104	0.465	0.137	-0.158	0.867
Pd55−O54	0.104	0.486	0.142	-0.162	0.876
Pd55−O27	0.104	0.486	0.142	-0.162	0.876
Pd55−O26	0.104	0.465	0.137	-0.158	0.867

One useful criterion for identifying the type of interaction is the ratio of G_{BCP}/V_{BCP}. The varying ratios demonstrate different type on interactions such as covalent ($0.5 < G_{BCP}/V_{BCP} > 0$), electrostatic with partially covalent ($1 < G_{BCP}/V_{BCP} < 0.5$) and non-covalent ($G_{BCP}/V_{BCP} > 1$) interactions [54]. The calculated G_{BCP}/V_{BCP} values are represented in Table 4, showing Pd-O values ranging between 0.5 and 0, that means the resulting interactions are covalent in character.

Applications of Pd (II)-HPC complex

Analytical, antioxidant, and antimicrobial applications

The amount of palladium was ascertained using a few synthetic mixtures and analytically important palladium alloys such as palladium charcoal catalyst, palau I, white gold, Cooper's alloy, palladium alloy and palladium gold. Commercial adaptability of the method was deduced by analysing different samples of varying concentrations resulting in reproducibility and satisfactory results as indicated in Table 5. The method is simple and takes only 2-3 min for a single determination.

Table 5. Analysis of alloys and synthetic mixtures

Palladium Alloys		
Matrix Compositions	Pd (II) studied (μg10 mL^{-1})	Pd (II) found (μg10 mL^{-1})^{b}
White gold: Pd (0.75), Au (0.2)	20	20.05 ± 0.05
Palladium alloy: Pd (0.75), Ag (0.010)	20	19.94 ± 0.08
Palau I: Pd (0.01), Au (0.04)	10	10.05 ± 0.03
Cooper's alloy: Pd (7.5), Ag (0.021), Co^{c} (0.0015)	10	10.10 ± 0.06
Palladium gold: Au (0.042), Co^{c} (0.003)	10	9.94 ± 0.08
Palladium charcoal catalyst	5*	5.17 ± 0.07
Synthetic Mixtures		
Pt (0.1), Sr (0.5)	20	19.89 ± 0.06
Cu (0.10), Zn (0.05), Al^{d} (0.8)	16	15.90 ±0.05
Ce (0.5), Zn (0.2), Pb (3)	10	9.89 ± 0.09
Ru (0.1), Au (0.01), Ir (0.01)	10	10.05 ± 0.06
Fe (0.05), Cr (0.003), V (0.001)	6	6.08 ± 0.06

**Certified value*

[a] *Figures in parenthesis indicate the presence of metal ion in mg 10 mL^{-1}*

[b] *Average of triplicate analysis (± SD)*

[c] *Co (II) masked with 0.1mL H_2O_2*

[d] *Al (III) masked using Na-F*

Radical scavenging analysis

The decolorization of DPPH was used to investigate the antioxidant activity of the ligand and its complex at different concentrations. With an increase in the concentration, an increase in the percentage of RSA was observed. At 500 μg mL^{-1} concentration, the maximum scavenging activity of ligand and the Pd (II)-HPC complex was noticed with a % RSA of 66.66% and 74.92%, respectively. The order for % RSA is gallic acid, Pd (II)-HPC followed by that of HPC (Supplementary Table 3 and Figure 6). The Pd (II)-HPC complex can be better utilized as an antioxidant since it has greater scavenging action than the free ligand.

Antimicrobial Studies

In accordance with the opted approach - Gram-positive, Gram-negative bacteria and Fungus are selected to assess the antimicrobial potential of the newly synthesized Pd (II)-HPC complex by determining their respective zones of inhibition.

Supplementary Table 4 and Figure 7 illustrate that the analytically synthesized compound exhibits a highly significant zone of growth inhibition against each of the microbial strains. It inhibits the bacterial cultures within the zone of inhibition, which is 21-26 mm.

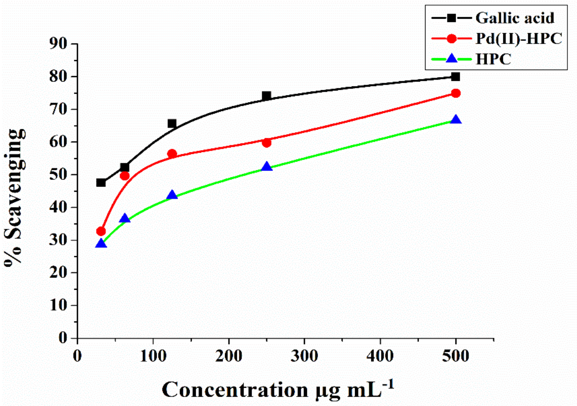


Fig. 6. % Radical Scavenging Activity of (a) Gallic acid in black (b) Pd (II)-HPC in red (c) HPC in green

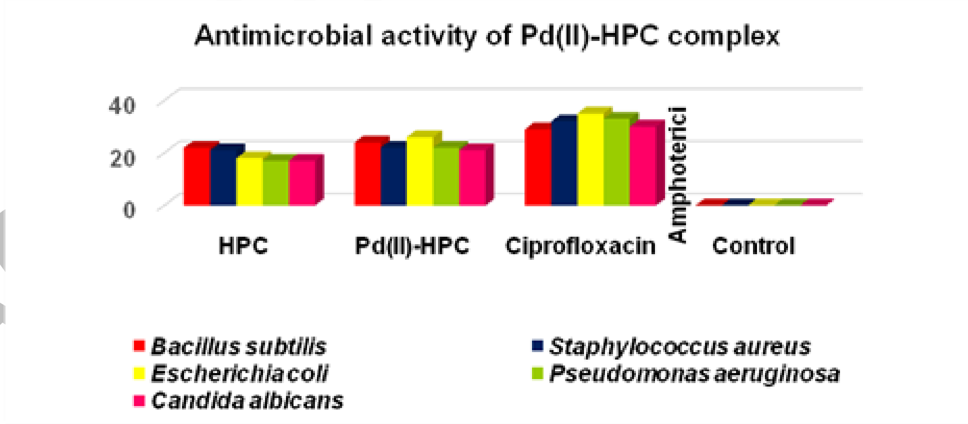


Fig. 7. *In-vitro* antimicrobial activity with DMSO acting as negative control and Ciprofloxacin (Bacterial) and Amphotericin (Fungal) as positive controls

The zone of inhibition for the reagent, HPC is found to be less in comparison to the studied Pd (II)-HPC complex measuring in the range of 17-22 mm. It is, therefore, deduced that the Pd (II)-HPC complex shows a remarkable antimicrobial activity.

CONCLUSION

In the present study, a chromogenic ligand, HPC was detected as an optical sensor for the micro level spectrophotometric analysis of Pd (II) in sodium bicarbonate mediated organic phase and was found to form a robust yellow coloured complex at a wavelength of 425 nm. The study's precision and reproducibility were evaluated by the RSD (0.51%) and correlation coefficient values (0.9975). In compliance with Job's approach and mole ratio method, the complex's stoichiometric ratio was deduced to be 1:2 [Pd (II): HPC], revealing a square planar geometry. The optimized geometry, quantum chemical behaviour and metal-ligand interactions of the investigated Pd (II)-HPC complex had been studied by employing DFT. Apart from being a sensitive spectrophotometric method, it is noteworthy to mention that the present study successfully elucidates the therapeutic potential of Pd (II)-HPC complex. As wholesome, this research involving theoretical and therapeutic studies indeed will act as a futuristic pathway for the synthesis of novel metallodrugs via incorporating the therapeutically active chromogenic agents to the divalent palladium metal.

AUTHOR CONTRIBUTIONS

TA: Investigation, analysis, writing, editing, NA: Review & editing, Supervision, resources. KD: Data curation. RK and NTS: Computational studies, writing – review & editing.

DECLARATION OF COMPETING INTEREST

The authors assert that they have no known financial or personal relationships that could have potentially influenced the work presented in this paper.

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REFERENCES

1. Mostafa A.M., Hanafy A.I., El-Rahman N. M. A., El-Sayed M.M. (2012). New palladium (II) complexes bearing benzopyran derivative ligands: Synthesis and characterization. *Egypt. J. Chem.*, **55** (1), 57-72. https://doi.org/10.21608/ejchem.2012.1131
2. Mohmad M., Agnihotri N., Kumar V., Sharma U., Kumar R. *et al*. (2023) A novel analytical, bioanalytical and theoretical approach to the platinum (II) – 3-hydroxy-2-(4-methoxyphenyl)-4*H*-chromen-4-one complex. *Results Chem.*, **5**, 100767. https://doi.org/10.1016/j.rechem.2023.100767
3. Kaur N., Agnihotri N., Agnihotri R. (2019). 3-Hydroxy-2-[2'-(5'-methylthienyl)]-4-oxo-4H -1-benzopyran for the spectrophotometric determination of tungsten (VI) and palladium (II). *Vietnam J. Chem.,***57**(6), 686–695. https://doi.org/10.1002/vjch.201900069
4. Dhonchak C., Agnihotri N., Azam M., Javed S., Muthu S., *et al.* (2023) Theoretical investigations into the spectrophotometrically analyzed niobium (V)-6-chloro-3-hydroxy-7-methyl-2-(2'-thienyl)-4*H*-chromen-4-one complex. *Pol. J. Chem. Technol.*, **25**(3), 63-70. https://doi.org/10.2478/pjct-2023-0026
5. Mohmad M., Agnihotri N., Kumar V., Azam M., Wabaidur S.M., *et al.* (2022) Radical scavenging capacity, antibacterial activity, and quantum chemical aspects of the spectrophotometrically investigated iridium (III) complex with benzopyran derivative. *Front. pharmacol.*,**13**, 945323. https://doi.org/10.3389/fphar.2022.945323
6. Liu X., Zhang Y., Zou Y., Yan C., Chen J. (2024). Recent advances and outlook of benzopyran derivatives in the discovery of agricultural chemicals. *J. Agric. Food Chem.*,**72**(22), 12300-12318. https://doi.org/10.1021/acs.jafc.3c09244
7. Tiwari A.K., Singh M.V. (2023). Insights into the origin and therapeutic implications of benzopyran and their derivatives. *Chemistry Select*, **8**(20), e202300220. https://doi.org/10.1002/slct.202300220
8. Terahara N. (2015). Flavonoids in Foods: A Review, *Nat. Prod. Commun.,* **10**(3), 1934578X1501000. http://dx.doi.org/10.1177/1934578X150100033
9. Panche A.N., Diwan A. D., Chandra S. R. (2016). Flavonoids: an overview, *J. Nutr. Sci.,* **5**, e47. https://doi.org/10.1017/jns.2016.41

10. Harborne J. B. (1986). Plant flavonoids in biology and medicine. Biochemical, pharmacological, and structure-activity relationships. Proceedings of a symposium. Buffalo, New York, July 22-26, 1985. Prog Clin Biol Res. 1986; 213:1-592. PMID: 3714734.
11. Herić A., Dibranin N., Martić L., Hodžić E., Zahirović A., *et al.* (2024) Ruthenium-based complexes as anti-tumor agents. *J. Health Sci.*,**14** (2),70-83. http://dx.doi.org/10.17532/jhsci.2024.2693
12. Zahirović A., Fočak M., Fetahović S., Tüzün B., Višnjevac A., *et al.* (2024) Hydrazone-flavonol based oxidovanadium (V) complexes: Synthesis, characterization and antihyperglycemic activity of chloro derivative *in vivo*. *J. Inorg. Biochem.*, **258**, 112637. https://doi.org/10.1016/j.jinorgbio.2024.112637
13. Lapouge C., Dangleterre L., Cornard J.-P. (2006). Spectroscopic and theoretical studies of the Zn (II) chelation with hydroxyflavones.*J. Phys. Chem. A.*,**110** (45), 12494–12500. https://doi.org/10.1021/jp064362q
14. Radu C., Olteanu A.A., Aramă C.C., Uivaroși V. (2024). Hydroxyflavone complexes with biogenic and abiogenic metals. FARMACIA, **72** (4), 751-764. https://doi.org/10.31925/farmacia.2024.4.3
15. Pawlus K., Jarosz T. (2022). Transition metal coordination compounds as novel materials for dye sensitized solar cells. *Appl. Sci.*,**12** (7), 3442. https://doi.org/10.3390/app12073442
16. Chou P.-T., Chi Y. (2007). Phosphorescent dyes for organic light-emitting diodes. *Eur. J. Chem.*,**13**(2), 380-395. https://doi.org/10.1002/chem.200601272
17. Sathish V., Ramdass A., Velayudham M., Lu K.-L., Thanasekaran P., *et al.* (2017) Development of luminescent sensors based on transition metal complexes for the detection of nitroexplosives. *Dalton Trans.*, **46** (48), 16738-16769. https://doi.org/10.1039/C7DT02790G
18. Cieśla P., Kocot P., Mytych P., Stasicka Z. (2004). Homogeneous photocatalysis by transition metal complexes in the environment. *J. Mol. Catal. A Chem.*, **224** (1-2), 17-33. https://doi.org/10.1016/j.molcata.2004.08.043
19. Ferraro V.,Adam C.R., Vranic A., Bräse S. (2023). Recent advances of transition metal complexes for photopolymerization and 3D printing under visible light. *Adv. Funct. Mater.*, **34** (20), 2302157. https://doi.org/10.1002/adfm.202302157

20. Alfonso M., Garrido E., Sancenón F., Máñez R.M. (2020). Metal complexes as sensors. Reference module in chemistry. *Mol. Sci. Chem. Eng.* 139, 26, 8808–8811. https://doi.org/10.1016/b978-0-12-409547-2.14910-8
21. Chakraborty S., Ravindran V., Nidheesh P. V., Rayalu S. (2020). Optical sensing of copper and its removal by different environmental technologies. *Mol. Sci. Chem. Eng.*, **5**(34), 10432–10474. https://doi.org/10.1002/slct.202002113
22. Žurga N., Moragues T., DeMello A.J. (2023). A re-evaluation of the role of coumarin C460 as a fluorescent Pd (II) sensor. *J Photochem Photobiol B*, **247**,112783. https://doi.org/10.1016/j.jphotobiol.2023.112783
23. Khan D., Shaily. (2023). Coumarin-based fluorescent sensors. *Appl. Organomet. Chem.*, **37**(7), e7138. https://doi.org/10.1002/aoc.7138
24. Jaine J.E., Mucalo M.R. (2018). Rapid determination of rhodium, palladium, and platinum in supported metal catalysts using multivariate analysis of laser induced breakdown spectroscopy data. *Spectrochim. Acta, B At. spectrosc.,***145**, 58-63. https://doi.org/10.1016/j.sab.2018.04.009
25. Tokalıoğlu Ş., Oymak T., Kartal Ş. (2004). Determination of palladium in various samples by atomic absorption spectrometry after preconcentration with dimethylglyoxime on silica gel. *Anal. Chim. Acta,***511**(2), 255–260. https://doi.org/10.1016/j.aca.2004.02.015
26. Petruci J. F. da S., Cardoso A. A. (2013). A new palladium chelate compound for determination of sulfide. *Microchem. J.,* **106**, 368–372. https://doi.org/10.1016/j.microc.2012.09.009
27. Van der Horst C., Silwana B., Iwuoha E., Somerset V. (2012). Stripping voltametric determination of palladium, platinum and rhodium in freshwater and sediment samples from South African water resources. *J Environ Sci Health A,* **47** (13), 2084–2093. https://doi.org/10.1080/10934529.2012.695986
28. Campbell M.H. (1968). Rapid determination of rhodium and palladium using liquid-liquid extraction with tricaprylmonomethylam-monium chloride and flame photometry. *Anal. Chem.*, **40** (1), 6-9. https://doi.org/10.1021/ac60257a047
29. Oguma K. (2002). Flow-injection simultaneous determination of iron (III) and copper (II) and of iron (III) and palladium (II) based on photochemical reactions of thiocyanato-complexes. *Talanta*, **58** (6), 1077–1087.

https://doi.org/10.1016/s0039-9140(02)00427-7

30. Kalal H.S., Taghiof M., Hoveidi H., Pakizvand N., Vahidi H., *et al.* (2013) The Pre-concentration and determination of iridium and palladium in environmental water by imprinted polymer-based method. *Int. J. Environ. Sci. Technol.,* **10**, 1091–1102. https://doi.org/10.1007/s13762-013-0308-y
31. Gao R., Zhang N. (2015) ICP-OES Determination of palladium in palladium jewellery alloys using yttrium internal standard. *At. Spectrosc.,* **36** (5), 216-220. http://doi.org/10.46770.AS.2015.05.005
32. Hann S., Rudolph E., Köllensperger G., Reiter C. (n.d.). (2006). Analysis of palladium by high resolution ICP-MS. *Palladium Emissions in the Environment*, 73–82. https://doi.org/10.1007/3-540-29220-9_5
33. Jabbari A., Barzegar M., Mohammadi M. (2005). Catalytic kinetic spectrophotometric determination of palladium (II) and its application to the determination of traces of propylthiouracil. *Indian J. Chem.,* **44A** (6), 1215-1218.
34. Goswami A.K. (2023). Spectrophotometric determination of palladium & platinum: Methods & reagents (1st ed.). CRC Press. https://doi.org/10.1201/9781003276418
35. Muhammed R.A., Abdullah B. H., Rahman H. S. (2024). Synthesis, cytotoxic, antibacterial, antioxidant activities, DFT, and docking of novel complexes of palladium (II) containing a thiourea derivative and diphosphines. *J. Mol. Struct.*, **1295**,136519. https://doi.org/10.1016/j.molstruc.2023.136519
36. Arora T., Garima Km., Kumar V., Javed S., Azam M., *et al.* (2024) Spectrophotometric, quantum chemical and molecular docking investigations of 4*H*-1-benzopyran-derived Pd (II) complexes. *Bull. Chem. Soc. Ethiop.*, **38** (5),1311-1327. https://dx.doi.org/10.4314/bcse.v38i5.10
37. Agnihotri N., Mohini, Al-Resayes S. I., Javed S., Azam M., *et al.* (2024) A spectrophotometric determination and the quantum chemical investigation of Pd (II)-3-hydroxy-2-(4-methoxyphenyl)-4-oxo-4H-1-benzopyran complex. *Bull. Chem. Soc. Ethiop.*, **38** (3),591-603. https://doi.org/10.4314/bcse.v38i3.4
38. International Critical Tables of Numerical Data, Physics, Chemistry and Technology, National Research Council: Washington, D.C., 1926–1933; Vols. I–VII.
39. Bougossa S., Mhadhbi N., Ahmed A. B., Hamdi M., Elghniji K., *et al.* (2024) Design of a new palladium (II) halide complex as a bio-active material: synthesis, physico-chemical studies, DFT-computations and evaluation of anti-inflammatory, antioxidant and anti-

gastric damage activities. *RSC Adv.*, **14** (25), 17413-17433. https://doi.org/10.1039/D4RA02984D

40. Frisch M.J., Truck G.W., Schlegel H.B., Scuseria G. E., Robb M. A., *et al.* (2004) Gaussian 03(Revision B.04), Gaussian, Inc., Wallingford CT.
41. Shahangi F., Chermahini A.N., Farrokhpour H., Teimouri A. (2015). Selective complexation of alkaline earth metal ions with nanotubular cyclopeptides: DFT theoretical study. *RSC Advances*, **5**, 2305-2317. https://doi.org/10.1039/C4RA08302D
42. Armakovic S., Armakovic J.S., Setrajcic J.P., Holodko V. (2014). Aromaticity, response and nonlinear optical properties of sumanene modified with boron and nitrogen atoms. *J. Mol. Model.,* **20**, 2538-2551. https://doi.org/10.1007/s00894-014-2538-4
43. Henkelman G., Arnaldsson A., Jónsson -H. (2006). A fast and robust algorithm for Bader decomposition of charge density. *Comput. Mater. Sci.*, **36**, 354-360. https://doi.org/10.1016/j.commaatsci.2005.04.010
44. Job P. (1928). Formation and stability of inorganic complexes in solution. *Ann. Chim.*, **9**, 113-203.
45. Vosburgh W.C., Cooper G.R. (1941). Complex Ions I. The identification of complex ions in solution by spectrophotometric measurements. *J. Am. Chem. Soc.,***63** (2), 437-442. https://doi.org/10.1021/ja01847a025
46. Yoe J.H., Jones A.L. (1944). Colorimetric determination of iron with disodium-1,2-dihydroxybenzene-3,5- disulfonate. *Indian Chem. Eng.*, **16** (2), 111-115. https://doi.org/10.1021/i560126a015
47. Allah M.A.A.H., Balakit A.A., Salman H.I., Abdulridha A.A., Sert Y. (2022). New heterocyclic compound as carbon steel corrosion inhibitor in 1 M H_2SO_4, high efficiency at low concentration. *Mat. Sci. Eng.*, 2034588. https://doi.org/10.1080/01694243.2022.2034588
48. Abdulridha A.A., Allah M.A.A.H., Makki S.Q., Sert Y., Salman H.E., *et al.* (2020) Corrosion inhibition of carbon steel in 1 M H_2SO_4 using new azo schiff compound: Electrochemical, gravimetric, adsorption, surface and DFT studies. *J. Mol. Liq.*, **315**,113690. https://doi.org/10.1016/j.molliq.2020.113690
49. Parr R.G., Szentpály L.V., Liu S. (1999). Electrophilicity index. *J. Am. Chem. Soc.*, 121 (9) 1922-1924. https://doi.org/10.1021/ja983494x

50. Henkelman G., Arnaldsson A., Jónsson -H. (2006). A fast and robust algorithm for Bader decomposition of charge density. *Comput. Mater. Sci.*, **36**, 354-360. https://doi.org/10.1016/j.commaatsci.2005.04.010
51. Soliman S.M., Barakat A. (2016). Decomposition of intermolecular interactions in the crystal structure of some diacetyl platinum (II) complexes: Combined Hirshfeld, AIM, and NBO analyses. *Molecules*, **21**, 1669. https://doi.org/10.3390/molecules21121669
52. Ganji M.D., Larijani H.T., hods R.A., M. Mehdizadeh. (2018). First principles and molecular dynamics simulation studies of functionalisation of Au_{32} golden fullerene with amino acids. *Sci Rep*, **8**, 11400. https://doi.org/10.1038/s41598-018-29887-5
53. Shahnazari G.H., Ganji M.D. (2021). Understanding structural and molecular properties of complexes of nucleobases and Au_{13} golden nanocluster by DFT calculations and DFT-MD simulation. *Sci Rep*, **11**, 435. https://doi.org/10.1038/s41598-020-80161-z
54. Niknam P., Jamehbozorgi S., Rezvani M., Izadkhah V. (2022). Understanding delivery and adsorption of Flutamide drug with ZnONS based on: Dispersion-corrected DFT calculations and MD simulations. *Phys. E: Low-Dimens. Syst. Nanostr*, **135**, 114937. https://doi.org/10.1016/j.physe.2021.114937