Bull. Chem. Soc. Ethiop. **2024**, 38(3), 591-603. ISSN 1011-3924
Printed in Ethiopia
DOI: https://dx.doi.org/10.4314/bcse.v38i3.4 Online ISSN 1726-801X

A SPECTROPHOTOMETRIC DETERMINATION AND THE QUANTUM CHEMICAL INVESTIGATION OF Pd(II)-3-HYDROXY-2-(4-METHOXYPHENYL)-4-OXO-4*H*-1-BENZOPYRAN COMPLEX

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(Received August 22, 2023; Revised January 29, 2024; Accepted January 30, 2024)

ABSTRACT. An analytical approach has been instigated for spectrophotometric inquisition of trace amounts of palladium in its bivalent oxidation state employing a novel 1-benzopyran derivative *viz.* A 1:2 [Pd(II):HMPB] light yellow complex is formed by a spontaneous interaction between Pd(II) and HMPB. The complex is quantitatively extracted from a weakly basic medium into dichloromethane absorbing prevalently and consistently at 405-425 nm. The method coheres to linearity up to 1.5 µg mL^{-1} of palladium(II). Analytical parameters such as the molar absorption coefficient (ε=3.011 × 10^{4} L mol^{-1} cm^{-1}), Sandell's sensitivity [0.0035 µg Pd(II) cm^{-2}], % RSD (0.62%) and the limit of detection [0.0147 µg Pd(II) mL^{-1}] indicate that the study satisfies all the criteria for good sensitivity, precision and accuracy. In addition, intervention with regard to numerous anions/complexing agents and cations of primary analytical importance has been performed to assess the method's flexibility and usefulness. The results show that the majority of them do not cause any interference during determination. To better understand chemistry of the prepared complex, spectroscopic quantum chemical studies including DFT and MEP mapping based on examination of the electronic characteristics of the complex in its most stable least energy conformation are used.

KEY WORDS: Palladium, 3-Hydroxy-2-(4-methoxyphenyl)-4-oxo-4*H*-1-benzopyran, Extractive spectrophotometric determination, DFT, MEP, Docking

INTRODUCTION

Palladium, one of the versatile members of platinum metals was discovered by William Hyde Wollaston in 1802. The metal derives its name from the word 'Pallas' meaning Greek goddess of wisdom. It is the least dense member of platinum group metals, existing as a white solid and exhibiting cubic crystal structure [1]. The study of palladium is one of the comprehensive and diverse areas of chemistry. This is attributed to the fact that the metal readily forms highly reactive adducts with many organic and inorganic molecules, thereby broadening the scope of application of the metal in extensive range of fields encompassing its utility in catalysis, industries and medicinal world [2, 3].

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Palladium catalysis has emerged as a vital technique for both standard and cutting-edge organic synthesis. The Heck reaction and associated chemistry have a distinctive place among the fundamental kinds of palladium-catalyzed conversions [4]. Owing to its ease of diffusion with H_2 gas palladium is used in catalytic converters of automobiles in industries. It also finds utility in making jewellery, medical and astronomical instruments. Besides this, the metal finds its importance as an alloying element wherein its alloys with metals like copper and silver are used for manufacturing electrical contacts. In the biological world as well, the metal extends its range of use as an antimicrobial and antineoplastic agent [5-9]. The overwhelming applications of the metal in various fields demand detailed study of the metal and its compounds. The trace measurement analysis involves the techniques *viz* gravimetric [10, 11], AAS [12, 13], X-ray fluorescence [14], NAA [15, 16], inductive coupled plasma-emission spectroscopy (ICP-ES) [17], inductive coupled plasma-mass spectrometry (ICP-MS) [18] and UV/VIS spectrophotometry [19-22]. However, due the limitation of these methods in terms of handling, maintenance and economy, their application for the purpose of analysis of the metal is less preferred. UV/Visible spectrophotometry, however, covers all the limitations of the other used techniques and has been effectively used for µg determination of the metal ions. In the current study, a simple and sensitive method, employing extraction technique has been worked out for spectrophotometric analysis of one of the transition metals, palladium(II), using a new reagent, 3-hydroxy-2-(4-methoxyphenyl)-4-oxo-4*H*-1-benzopyran (HMPB). The method is rapid, sensitive, selective, and gives highly reproducible results. Computational calculations additionally helped in confirming the geometrical, electrical and thermodynamic aspects of the studied molecules including HMPB and its complex with Pd(II) in an analogy with the previously reported study over transition metal complexes [23, 24]. Where, stability of the complex, intermolecular interactions and charge distribution inside the investigated molecules have been revealed by the FMO's values and MEP diagrams, the molecular docking studies assisted in analyzing the exact conformation of the molecule by finding the nature of interaction and binding process between ligands and active site proteins along with the location and proximity of the involved functional groups with highly supportive results, in accordance to the analytical findings. The method is considered quite advantageous from analytical point of view of the metal ion and can be directly used in presence of Cr(VI), Mo(VI), Fe(III), Sn(II) and other platinum metals which interferes seriously in other methods.

EXPERIMENTAL

Apparatus and reagents

The stock solution (1000 µg mL^{-1}) of Pd(II) was prepared by dissolving accurately measured quantity of palladium(II) chloride; $PdCl_2.2H_2O$ (CDH, AR), in minimum volume of concentrated HCl, raising the final volume up to 100 mL by using doubly distilled water. The hence prepared stock solution was standardized by dimethyl glyoxime method gravimetrically [25]. Proper dilution of the stock solution was done to prepare working solutions as per requirements. Ethyl alcohol was used as a solvent to dissolve and prepare 0.1% solution of 3-hydroxy-2-(4-methoxyphenyl)-4-oxo-4*H*-1-benzopyran (HMPB; m.p. 235 ºC). The reagent (Figure 1) was synthesized using a method similar to that of the AFO reaction [26, 27]. A fresh 1 M $NaHCO_3$ solution was used to set optimum basic conditions during the study. Highly pure DCM was used as an extractant. The solutions corresponding to various metal ions employed in interference investigation were prepared either in doubly-deionized water or dilute mineral acids in cases where hurdle in dissolution in former was observed.

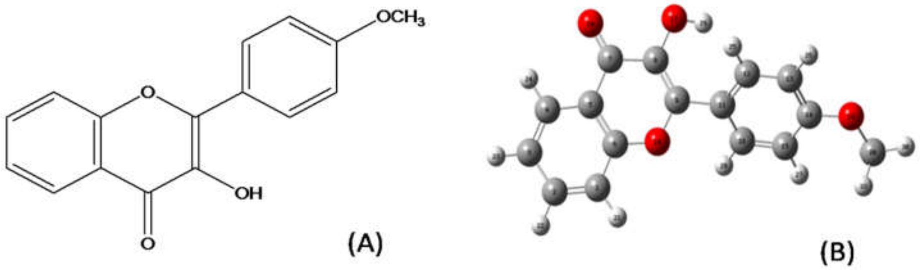

Figure 1. 2D [A] and optimized [B] structure of HMPB.

Analytical applicability of the proposed method has been tested by applying the strategy to numerous synthetic samples (some of them similar to the real samples of braggite, palladium alloy and white gold), the technical sample of palladium-charcoal catalyst and natural water samples (tap as well as well). The desired solutions were prepared by following the reported procedures [28].

The color intensity (absorbance) of the prepared extracts was recorded by employing EI-2375 UV/VIS- spectrophotometer using analogous quartz cells with 10 mm optical path distance.

Recommended procedure for extraction and determination of Pd(II)

Maintaining the ideal set of conditions corresponding to spectrophotometric analysis, to 1 mL of the working solution of Pd(II), 10 µg mL^{-1}, prepared from its stock solution by significant dilution with double deionized water and taken in a 125 mL separatory funnel, was added 1.2 mL of 0.1% HMPB in ethyl alcohol under 0.07 M basic conditions by adding 0.7 mL of 1 M $NaHCO_3$ solution. Aqueous phase volume was ultimately raised to 10 mL. The yellow colored complex thus formed was extracted into an equi-volume (10 mL) of dichloromethane by its constant shaking maintaining the time of equilibration as 30 s with occasional releasing of pressure and leading to the separation of two incompatible layers. The suspended water droplets present in the yellow colored organic extract were removed by using Whatman filter paper No. 41 pretreated with DCM. Finally, color intensity of the resulting yellow extract was evaluated at 415 nm in comparison to a reagent blank prepared under similar experimental conditions of procedure. The standard calibration curve between optical density and variable palladium concentrations was used to estimate the actual amount of divalent palladium.

RESULTS AND DISCUSSION

Spectrophotometric studies

Absorption spectrum

A yellow coloured 1:2 (M:L) complex extractable into dichloromethane (DCM) is formed by the interaction of HMPB with divalent palladium. The formed complex exhibits absorption maximum in the range of wavelength 405-425 nm whereas the reagent blank manifested negligible optical density in the same wavelength region (Figure 2). λ_{max} of 415 nm was selected for further measurements of optical density in consideration with the congruous (stability > 24 h) and maximum absorbance attained by the complex.

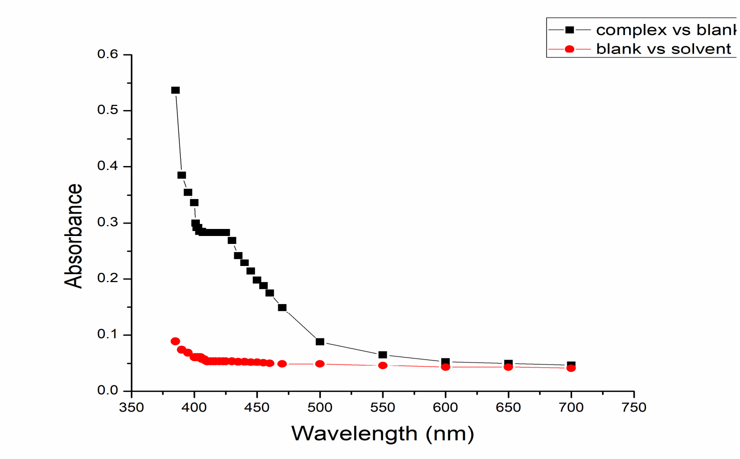

Figure 2. Dependence of color intensity of Pd(II)-HMPB complex on wavelength (1 μg Pd(II) mL^{-1}, other conditions same as are mentioned in the procedure).

Choice of extraction solvent

The extraction behaviour of Pd(II)-HMPB complex was studied into a number of organic solvents. Extraction of the complex decreases in the order: DCM > isobutyl methyl ketone > ethyl acetate > 1,2-dichloroethane > isoamyl acetate > chloroform > carbon tetrachloride > benzene > toluene > cyclohexane > amyl alcohol. While using cyclohexane and amyl alcohol as the extraction solvent, no phase separation was observed. Benzene and toluene extracted the complex to a very small extent. Phase separation was clear in DCM and isobutyl methyl ketone but complex was not stable in isobutyl methyl ketone as absorbance falls continuously in it. A single extraction with equal volume (10 mL) of DCM under optimized conditions is sufficient for quantitative recovery of Pd(II)-HMPB complex. The optical density remained unchanged for more than 24 h in DCM.

Choice of the medium and its concentration

The impact of different reaction media including ammonia, $NaHCO_3$ Na_2CO_3, HCl, H_2SO_4, $HClO_4$, H_3PO_4 and CH_3COOH (0.07 M concentration in each case) on complexation was investigated in terms of complex's absorbance in DCM and studied against reagent blank in respective case. The absorbance of the complex was found to possess relatively lower values under acidic conditions and was observed to decrease in the order: $NaHCO_3 > Na_2CO_3 > NH_3 > CH_3COOH > H_3PO_4 > HCl > H_2SO_4 > HClO_4$. Therefore, sodium bicarbonate was found out to be efficacious with maximal optical density (0.283) as evidenced in this medium at a wavelength of 415 nm. Additionally, the greatest colour intensity and hence complete complexation was observed when extractions were conducted using 0.04-0.10 M $NaHCO_3$. An appreciable descending optical density was observed on further addition of $NaHCO_3$. Hence, an amount of 0.07 M of $NaHCO_3$ was selected as an ideal concentration for complexation.

Effect of [HMPB]

While studying the effect of [HMPB] on complexation, a gradual rise in optical density was noted up to the addition of 0.9 mL 0.1% (w/v) solution of it in ethyl alcohol. A maximal and most congruous color intensity was observed between 0.9-1.5 mL of HMPB added, followed by a decline thereafter. Hence, further research was carried out utilizing 1.2 mL of 0.1% (w/v) solution of ligand in ethyl alcohol.

Table 1. Consequences of various parameters on Pd(II)-HMPB complex.

Basicity w.r.t. aqueous phase (M)^a	0.005	0.01	0.03	0.04-0.10	0.11	0.12	0.14	0.15	0.20
Absorbance	0.207	0.227	0.235	0.283	0.245	0.227	0.212	0.205	0.193
HMPB (mL)^b	0.1	0.3	0.5	0.7	0.8	0.9-1.5	1.7	1.9	
Absorbance	0.150	0.200	0.240	0.243	0.254	0.283	0.277	0.256	
Contact time (s)^c	5	10	15-120						
Absorbance	0.236	0.243	0.283						

Conditions: ^{a}Pd(II) = 10 µg; HMPB [0.1% in ethyl alcohol] = 1.0 mL; aqueous volume = organic volume = 10 mL; extraction solvent = DCM; time of contact = 30 s; λ_{max} = 415 nm. ^{b}$NaHCO_3$ = 0.04-0.10 M; remaining conditions the same as in (a) excluding a strength variation in HMPB; HMPB = 3-Hydroxy-2-(4-methoxyphenyl)-4-oxo-4*H*-1-benzopyran. ^{c}HMPB [0.1% (w/v) in ethyl alcohol] = 1.2 mL; remaining conditions the same as in (b) excluding variation in the contact time.

Effect of contact time

Under the ambient set of conditions, the optical density of Pd(II)-HMPB complex formed in water phase after equilibration with an equi-volume DCM enhanced initially and then obtained a steady and maximum for 15-120 s of shaking. Hence, 30 s was identified as the optimal contact time to achieve equilibrium for each extraction performed during further analysis.

From the above analysis, the ambient conditions rendering maximal, reproducible and persistent values of the optical density were decided and incorporated in the proposed methodology (Table 1).

Effect of diverse ions

For the sake of assessing the selectivity and subsequently commercial viability of the newly structured Pd(II)-HMPB complex, the impact of numerous important and analytically significant complex forming reagents (anions) and cations was examined. Prior to adding reagents to maintain the standard conditions for complex formation, the foreign ions were added to Pd(II) solution to monitor degree of interference by their presence (Figures 3A and 3B). Among anions, added in mg/10 mL amounts (except H_2O_2 added in mL), only oxalate was found to interfere. From the studied cations, the interference shown by vanadium(V) and iron(III) was nullified by incorporating suitable complexing or masking agents. Up to 0.5 mg per 10 mL of Fe(III) had been masked by adding 50 mg of EDTA ‘disodium salt as the masking agent, sodium phosphate, 20 mg was used to remove the interfering effect of up to 0.1 mg per 10 mL of V(V) when added prior to addition of other reagents.

Optical and analytical parameters

The adherence to Beer’s law of Pd(II)-HMPB system was analyzed by noting optical density of the divalent metal ion of variable concentrations. The obtained results fitted well into the equation of straight line wherefrom the standard determination range of Pd(II) was found to be 0.0-1.5 µg mL^{-1}. However, the ideal concentration range of determination as inferred from Ringbom’s plot [29] was found to be 0.8-1.3 µg Pd(II) mL^{-1}. The linear regression equation being Y = 0.283 X + 0.001 [Y = absorbance, X = µg Pd(II) mL^{-1}] with the regression coefficient of 0.9886 and detection limit of the procedure as 0.0147 µg Pd(II) mL^{-1}. Molar absorption coefficient and Sandell’s sensitivity of the complex are calculated to be 3.011×10^4 L mol^{-1} cm^{-1} and 0.0035 µg Pd(II) cm^{-2}, respectively at 415 nm. The reproducibility of the method had been tested by performing ten sets of experiments under standard conditions of the procedure; the % relative standard deviation found, respectively was 0.62%.

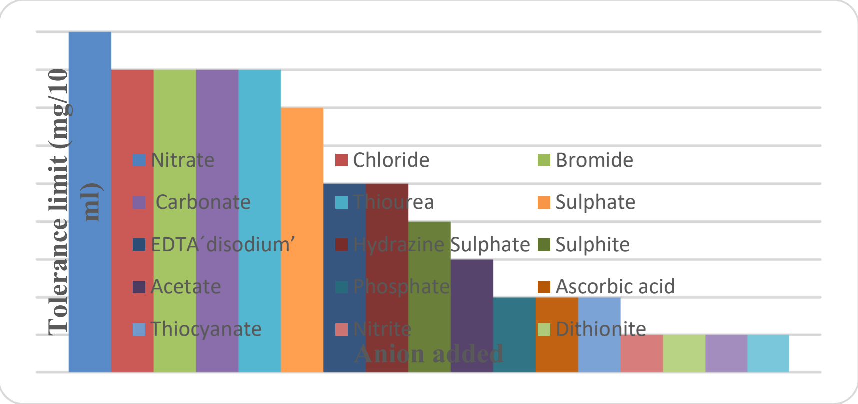

Figure 3A. Effect of complexing agents on Pd (II)-HMPB complex.

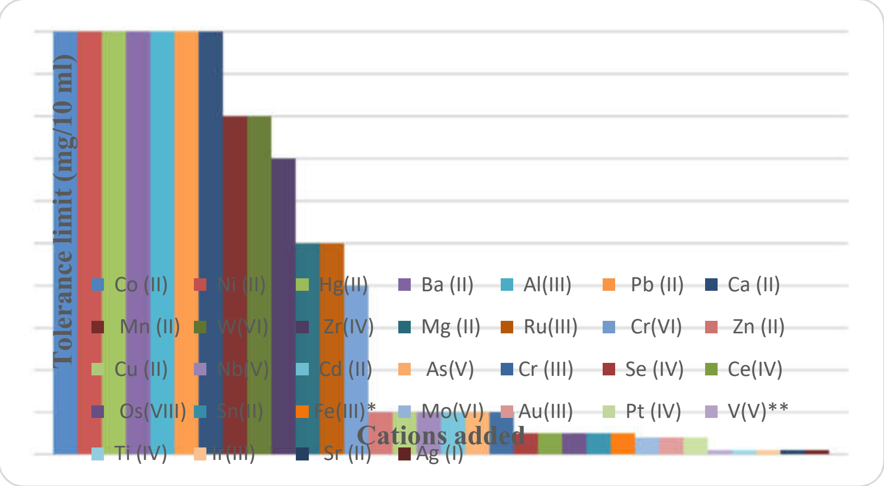

Figure 3B. Effect of cations on Pd (II)-HMPB complex.

Stoichiometry of the complex

Job’s method of continuous variations [30] as modified for a two phase system by Vosburgh and Cooper [31] and the mole ratio approach [32] were used to estimate out 1:2 [Pd(II):HMPB] stoichiometry of the studied complex. Figure 4 depicts the outcomes of Job’s method [A] and hence proposed optimized geometry [B] of the studied complex.

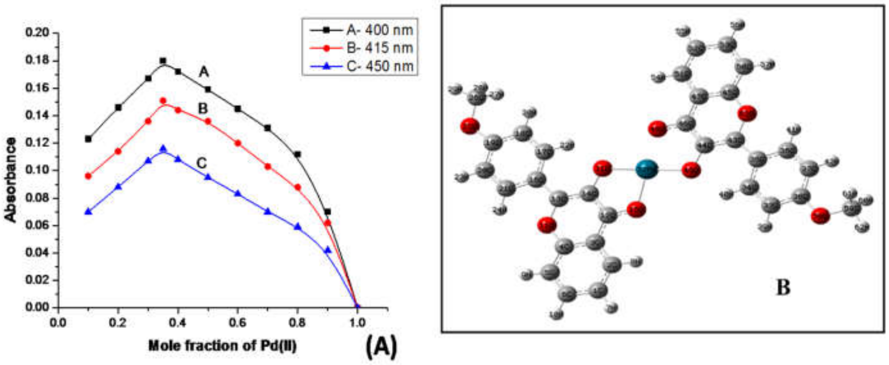

Figure 4. Job's method of continuous variations **[A]** and optimized proposed structure **[B]** of Pd(II)-HMPB complex.

Quantum chemical parameters

Molecular geometry analysis

Geometry of the ligand and complex molecules and the numbering of the atoms were obtained using the Gaussian 09 and GAUSSVIEW [33] softwares, as indicated, respectively in Figure 1B and 4B. The most optimal structural parameters (bond length and bond angle) were evaluated using DFT (B3LYP) using the 6-311G (d,p) (for ligand) and LANL2DZ (for complex) basis sets [34, 35] (SI 1). The longest bond length among the ligand was between C13 and C16, measuring 1.4578 Å. The O31-H32 distance in the ligand has the shortest bond length at 0.9861 Å, while the longest Pd32-O48 and shortest bond length C34-H40 among the complex were 2.0565 Å and 1.0836 Å, respectively. Similar to this, the bond angle range of complex was (O48-Pd32-O49) 81.8466° - (C35-C43-C44) 127.7594°, whereas bond angle value of ligand fall in between (O25-C26-H29) 105.0302° - (C14-C13-C16) 129.4699°.

Molecular electrostatic potential (MEP)

The MEP is a beneficial identifier to explain the electrophilic attack, hydrogen bonding interactions, and nucleophilic reactive sites. The 3D visualization of MEP can also provide insights into molecular interactions and distribution of charge inside the molecule. Gauss View 5.0 software was used to perform MEP at B3LYP/6-311++G(d,p) and LANL2DZ to determine locations of electrophiles and nucleophiles' reactivity (Figure 5). Different colours were used to denote the different electrostatic potentials at the surfaces, with red, blue and green denoting the maximum negative, maximum positive and close to zero regions of electrostatic potential, respectively. Red < orange < yellow < green < blue are the colours in ascending order of electrical potential. The MEP trace clearly showed the areas where electrophilic and nucleophilic assaults may potentially occur. The ligand's electrostatic potential ranges from -5.593 to +5.593 a.u., however, that of complex is between -6.371 a.u. and +6371 a u. One can infer the hydrophilic and hydrophobic nature, the ability to produce chemical complexes and the intermolecular interaction by analyzing the MEP surface, all of which are highly useful details for medicinal applications. As a result, MEP surface analysis makes it simple to qualitatively get reactive regions of ligand and the complex under study.

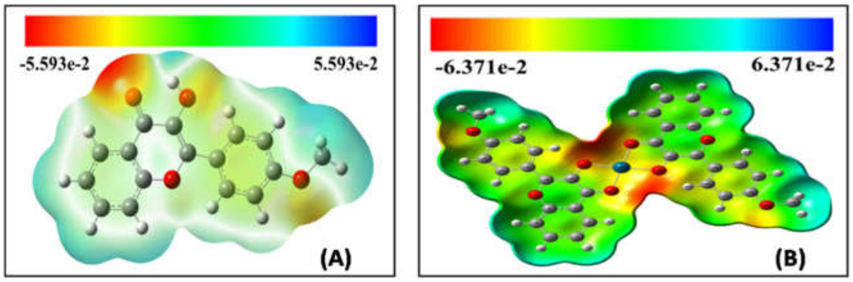

Figure 5. MEP diagram of Ligand (A) and complex (B).

Frontier molecular orbital (FMO) analysis

The HOMO, LUMO and frontier orbital gaps, the important components of quantum chemistry, serve as examples of the chemical reactivity and kinetic stability of compounds. The HOMOs are primarily electron donors, whereas LUMOs are primarily electron acceptors. These orbitals are crucial in determining the electrical properties of the molecule and largely contribute to its chemical stability. Chemical reactions are allowed if the FMOs are set up so that the same signed lobes overlap. The energy differential between HOMO and the LUMO orbitals is known as the gap energy ΔE. This one measures a molecule's chemical activity and polarizability. It promotes charge transfer within the molecule. The chemical reactivity and kinetic stability of a molecule can be estimated using this quantity of energy. When the gap is large, it is difficult for electrons to go to the higher energy state, making the molecule harder and less reactive. On the other hand, when the flow is simple, a soft, receptive molecule is produced. The complex and ligand formed utilizing TD-B3LYP/6-311++G(d,p) and LANL2DZ level of theory are depicted in 3D plots in Figures 6A and 6B. The color red designates the positive phase, whereas the color green designates the negative phase. The energy difference between HOMO and the LUMO is 2.5913 eV in the complex and 3.6109 eV in the ligand, as illustrated in figure 6. Chemical stability is influenced by chemical hardness as well as the band gap. The chemical hardness values of the ligand and complex are 1.8054 and 1.2956, respectively, as shown in Table 2 indicating that the compound is chemically stable. Similar to this, it was determined that the complex's electronegativity was 3.7946 and that the ligand was 4.0673. Electronegativity indicates how attracted an atom is to electrons in a covalent bond. Using the B3LYP technique, the ligand and complex electrophilicity indices were 4.5815 and 5.5568, respectively. The significant energy transformation between HOMO and the LUMO is indicated by the moderate values.

Table 2. Local descriptors of ligand (HMPB) and complex [Pd(II)-HMPB].

Parameter	Energy values	
	(HMPB)	[Pd(II)-HMPB]
E_{Homo} (eV)	-5.8732	-5.0903
E_{Lumo} (eV)	-2.2623	-2.4990
Ionization potential	5.8732	5.0903
Electron affinity	2.2623	2.4990
Energy gap (eV)	3.6109	2.5913
Electronegativity	4.0673	3.7946
Chemical potential	-4.0673	-3.7946
Chemical hardness	1.8054	1.2956
Chemical softness	0.5538	0.7718
Electrophilicity index	4.5815	5.5568

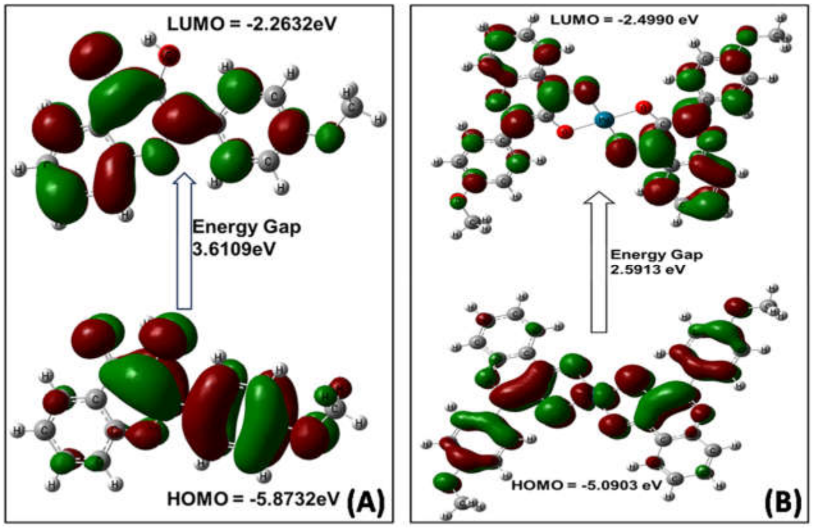

Figure 6. (A) HOMO-LUMO composition of the FMOs of ligand. (B) HOMO-LUMO composition of the FMOs of the complex.

Molecular docking

Docking studies use the precise geometry of the enzyme site structure to analyze the molecule in an exact conformation pose in the active site. In addition to predicting the location and proximity of the functional groups involved, it also predicts the type of the interaction and binding process between ligands and active site proteins. In order to select an optimal ligand for additional research, the interaction patterns of ligands and known medications binding to the target protein are compared. The Swiss ADME-Target prediction, an online tool (http://www.swisstargetprediction.ch/) was utilized to dock the study's ligand (HMPB) with an appropriate protein and downloading the protein data in PDB format from RSC PDB (https://www.rcsb.org/structure/). 5OMY [36] was the suitable protein downloaded from RSC PDB for studied ligand. Figure 7A and 7B show 3D interaction of ligand with their suitable protein. Chimera 1.14 software [37] was used for minimizing the ligand energy. MD simulation was carried out by AutoDock vina [38] with a ligand having minimum energy. AutoDock is a semi-flexible docking software consisting of two parts one is AutoGrid and the other is AutoDock. AutoGrid is used to calculate lattice energy level, on the other hand, AutoDock is defined as a search tool to determine optimal conformation and score. AutoDock Tools is a graphical user interface program that was used to formulate, run and analyze the MD simulation. Remove all the heteroatoms from the complex protein and make it ligand-free. Prepare the protein for docking by adding atomic charges, solvation parameters and polar hydrogens. Assigned gasteiger charges and merged non polar hydrogens. MD simulation was found to work on active binding sites of structures. The evaluated binding energy was -8.6 kcal mol^{-1} and inhibition constant 0.4924. The observed H-bond length was found to be 2.629 Å. The bioactive nature of the molecule was confirmed based on binding energies. BIOVIA discovery studio tool software was used for plotting 2D-interactions of ligand with predicted protein, which were shown in Figure 7A and 7B. Low binding energies evaluation reveals that the used ligands are biologically active.

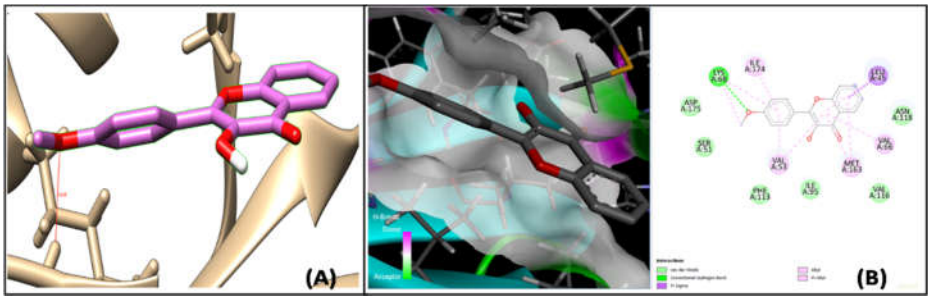

Figure 7. 3D (A) and 2D (B) docking image of ligand docked into the binding site of 5OMY.

Analytical utility

The analytical applicability of the method is validated by its usefulness in precise determination of palladium in numerous synthetic mixtures. The application of the technique is further widened by its effective application for palladium determination in several real samples of palladium including some of its important alloys, the technical sample of palladium-charcoal catalyst and water samples (Table 3). The proposed method for microgram level determination of the metal is highly cost effective, simple to handle, rapid, accurate, precise, sensitive, selective having wider tolerance limits of diverse ions thereby increasing its scope of application and compares adequately with earlier reported methods of palladium determination [19].

Table 3. Analysis of miscellaneous samples by the studied methodology.

S.No.	Composition of matrix[a]	Pd(II) added (µg/10 mL)	Pd(II) found (µg/10 mL)[b]
1.	Co (5), Zn (0.5), Sn (0.1)	6	6.02
2.	Pt (0.2), Ir (0.1), Os (0.4)	7	7.06
3.	Ni (5), Zn (0.8), Sn (0.2)	5	5.05
4.	Fe^{III} (0.2)*, Cr^{VI} (3), Al (5)	10	10.13
5.	W(8), Ru (4), Os (0.1)	12	12.16
6.	Ir (0.1), Ag(0.1), Pt(0.4)	9	9.06
7.	V (0.1)**, Ti(0.1), Ir (0.1)	14	13.83
8.	Pt(0.045), Ni(0.01)[c]	11	11.11
9.	Ag(0.006)[c]	15	14.91
10.	Au(0.16)[d]	10	9.97
11.	Palladium charcoal catalyst	5***	4.96
12.	Water sample: Tap well	10 10	10.16 9.91

[a]mg/10 mL quantity of metal ion in parentheses. [b]Mean of three replicate analyses. [c]Composition analogous to braggite, palladium alloy and white gold respectively, *In presence of 50 mg EDTA,**In presence of 20 mg sodium phosphate, ***Certified value.

CONCLUSION

A sodium bicarbonate mediated spectrophotometric analysis for the trace level determination of palladium(II) following its complexation with 3-hydroxy-2-(4-methoxyphenyl)-4-oxo-4*H*-1-benzopyran (HMPB) resulting in the production of a light yellow colored complex, has been fortuitously carried out. The composition of Pd(II)-HMPB complex has been validated as 1:2 [Pd(II):HMPB] by employing Job's continuous variations and mole-ratio methods. The coherence to linearity is shown up to 1.5 µg Pd(II) mL^{-1} at 415 nm. The selectivity, sensitivity, precision, accuracy and cost-effectiveness have been amply depicted and emphasized by the respective values of the various optical and statistical parameters studied during course of conduction of the present investigation including its intervention studies, application to synthetic and natural mixtures and technical sample of palladium charcoal catalyst, molar absorption coefficient and Sandell's sensitivity. The optimized geometry of the proposed structure and structural and electronic details have been examined by an insight into DFT and MEP studies employed. In nutshell, the present study is highly a novel addition of the analytical applications and computational studies to the ongoing spectrophotometric investigations. For detecting trace amounts of palladium in the divalent state, the suggested method outperforms the previously documented spectrophotometric method being more effective and reliable [19-22].

ACKNOWLEDGMENTS

The authors acknowledge the financial support through the Researchers Supporting Project number (RSP2024R147), King Saud University, Riyadh, Saudi Arabia. Authors are thankful to the authorities, MMDU, Mullana for lab facilities.

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