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Original scientific paper

ZnO@Fe₃O₄ nanoparticles decorated porous reduced graphene oxide-based electrochemical sensor for the detection of palbociclib anticancer drug in human plasma

Saba Kermanpour¹ , Milad Arab-Nozari² , Fereshteh Chekin^{3,4} and Pardis Zaboli¹

¹Department of Pharmacy, Am. C., Islamic Azad University, Amol, Iran

²Department of Toxicology and Pharmacology, Am. C., Islamic Azad University, Amol, Iran

³Department of Chemistry, Am. C., Islamic Azad University, Amol, Iran

⁴Innovation and Management Research Center, AA. C., Islamic Azad University, Amol, Iran

Corresponding Author: ✉ fereshtehchekin@iaau.ac.ir; Tel/Fax: +98-121-2517087

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Abstract

In this work, a porous reduced graphene oxide (prGO) material was synthesized via an environmentally benign green synthesis strategy using *Thymus kotschyanus* plant extract as a reducing agent. A magnetic hybrid platform based on Fe₃O₄ and ZnO nanoparticles-prGO (ZnO@Fe₃O₄-prGO) was prepared and its physicochemical and morphological properties were systematically characterized using UV-Vis spectroscopy, Fourier transform infrared spectroscopy, Brunauer-Emmett-Teller surface area analysis, X-ray diffraction, field emission scanning electron microscopy, and voltammetry analyses. The ZnO@Fe₃O₄-prGO was modified on a carbon paste electrode (ZnO@Fe₃O₄-prGO/CPE) for the electroanalytical determination of palbociclib. Square wave voltammetry revealed a linear dynamic range between 0.05 and 4 μM and a detection limit of 37 nM. The synergistic incorporation of ZnO and Fe₃O₄ nanoparticles onto prGO sheets enhanced π-electron delocalization, increased electroactive site density, and improved electron transfer kinetics. The ZnO@Fe₃O₄-prGO/CPE sensor showed excellent selectivity and reproducibility, with recoveries of 92.5 to 105.0 % (RSD 1.9 to 4.2 %) in spiked human plasmas. These findings demonstrate that the eco-friendly ZnO@Fe₃O₄-prGO nanohybrid is a sustainable, robust, and highly sensitive platform for pharmaceutical detection and on-site electrochemical actuation applications.

Keywords

Drug detection; voltammetry; modified electrode; hybrid oxides; nanoparticles; plasma samples

Introduction

Palbociclib (Pal), a potent and highly selective CDK 4/6 inhibitor, has shown promising growth inhibitory activity against various types of tumours such as hepatocellular, renal cell, and non-small cell lung carcinoma, neuroblastoma, multiple myeloma, oesophageal adenocarcinoma and melanoma. Pal has been most extensively investigated in the field of breast cancer [1]. Pal, marketed as Ibrance, is an anti-cancer medication designed to combat HR-positive and HER2-negative breast cancer [2]. Pal is a piperazine pyridopyrimidine that is [6-Acetyl-8-cyclopentyl-5-methyl-2-[[5-[(piperazin-1-yl)pyrindin-2-yl]amino]pyrido [2,3-d]-pyrimidin-7(8H)-one] and acts in the cell cycle machinery. It is a second-generation cyclin-dependent kinase inhibitor selected from a group of pyridopyrimidine compounds due to its favourable physical and pharmaceutical properties [3]. At the same time, we should also pay more attention to the adverse effects caused by Pal, an anticancer drug. The adverse effects of Pal vary across trials and can be divided into treatment-emergent adverse events and treatment-related adverse events. The effects of Pal are not limited to tumour cells. It produces similar effects in normal cells [4]. Hence, in cancer therapy, dose regulation of this drug is crucial.

The various analytical methods based on high-performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC-MS/MS), ultra-performance liquid chromatography (UPLC), solid-phase extraction coupled to liquid chromatography-mass spectrometry (SPE-LC-MS), UV-Vis spectroscopy, electrophoresis, spectrofluorimetry and electrochemistry were reported for the quantification of Pal drug in biological fluids with other drugs [5-7]. Electrochemical techniques can be applied straightforwardly to solve many pharmaceutical problems with high selectivity, sensitivity, accuracy, and precision, often in remarkably reproducible ways [8,9]. In electrochemical sensors, high overpotential and low redox current limit drug determination. Therefore, sensor modification is considered the most promising approach for drug monitoring and is commonly used to overcome these limitations [10]. Modifiers such as molecularly imprinted polymers (MIPs) [11] and boron-doped diamond electrodes (BDDEs) [12] have been suggested for electrochemical sensors for Pal detection.

ZnO is a low-cost, safe, non-toxic semiconductor material with good mechanical strength and high thermal stability, and it could provide large surface area and superior electron mobility, making it attractive for electrochemical sensor applications [13]. However, its electron transport capacity is limited. To overcome this, ZnO can be combined with other metal oxides such as iron oxide (Fe₃O₄) to improve the electrocatalytic performance of the sensor. Magnetic nanoparticles, particularly Fe₃O₄ nanoparticles, are promising materials for sensor applications due to their inherent biocompatibility, magnetic properties and large surface area [14]. They are also easily affected by external fields, providing a high signal-to-noise ratio in biological samples and enabling easy extraction; more importantly, they have excellent electronic and magnetic properties due to fast electron-hopping transfer between Fe³⁺ and Fe²⁺ [15,16]. However, they also have shortcomings, including poor stability and variability due to high reactivity, a tendency to agglomerate due to nanoscale effects and magnetic attraction, and poor electrical conductivity, which limit their use in electrochemical sensors. Also, the stability of ZnO in phosphate buffer solution and alkaline solution is significantly insufficient, which may interfere with sensor stability and limit sensor applications in neutral and alkaline conditions. Additionally, metal oxide nanomaterials aggregate on the electrode surface, reducing effective surface area and sensor performance [17]. Large-surface-area metal oxide nanoparticles doped into carbon nanomaterials are considered a promising solution to overcome these limitations.

Porous carbon materials have been shown to be excellent electrode materials for developing sensitive bioelectrochemical sensors [18,19]. They offer unique advantages for electrochemical sensors,

as their porosity increases the electrode's specific surface area, allowing immobilization of a high number of ligands required to enhance sensor sensitivity. In addition, they promote analyte diffusion through interconnected pores, enabling faster sensing. Recent studies demonstrated that porous reduced graphene oxide (prGO) has gained remarkable attention due to its large surface area, outstanding electrochemical capability and distinctive hydrophilic behaviour. In addition, because of its unique physical and chemical properties, prGO can decrease the aggregation of electro-active materials by anchoring metal oxide nanoparticles [9,20]. As a result, an accurate electrochemical sensor is required to analyse Pal in biological samples using voltammetry on a ZnO@Fe₃O₄-prGO-modified carbon paste electrode.

Experimental

Chemicals and apparatus

Palbociclib (C₂₄H₂₉N₇O₂), zinc acetate dihydrate ((CH₃CO₂)₂Zn·2H₂O), iron(II) sulphate hepta hydrate (FeSO₄·7H₂O) and potassium hexacyanoferrate(II) ([K₄Fe(CN)₆]), potassium permanganate (KMnO₄), hydrogen peroxide (H₂O₂), sulfuric acid (H₂SO₄), potassium hydroxide (KOH), phosphoric acid (H₃PO₄), sodium dihydrogen phosphate (NaH₂PO₄), disodium hydrogen phosphate (Na₂HPO₄) and sodium phosphate (Na₃PO₄) were purchased from Sigma-Aldrich. Graphene oxide (GO) powder was obtained from Iranian Nanomaterials Pioneers. The *Thymus kotschyanus* plant, which grows in Azerbaijan, was used in this work. Distilled water was used for sample preparation.

Electrochemical assays were performed using a Sama 500-c potentiostat/galvanostat (Iran). A cell consisting of a working electrode (carbon paste electrode (CPE, with geometrical area 0.28 cm²) and modified CPE, a reference electrode (Ag|AgCl|3M KCl), and an auxiliary electrode (platinum wire) was used for electrochemical measurements. A 0.1 M phosphate-buffered solution (PBS) with different pH values containing 0.1 M KCl was used as the electrolyte. X-ray diffraction (XRD) measurements were carried out using a Bruker D8-Advance X-ray diffractometer (Germany). UV-Vis assays were carried out using a UV-Vis spectrophotometer (UV-1900, Shimadzu Co., Japan). The morphology and topography were analysed by MIRATESCAN-XMU field emission-scanning electron microscope (FE-SEM, Czech Republic) combined with an energy-dispersive X-ray spectroscopy (EDS) machine. All release measurements were carried out at room temperature. FT-IR spectra of samples were recorded by IR Tracer-100, Shimadzu Co. (Japan) in the range of 4000 to 400 cm⁻¹. Nitrogen adsorption-desorption analysis disclosed the porosity characteristics of the ZnO@Fe₃O₄-prGO hybrid on a BET BELSORP Mini II equipment (Japan).

Synthesis of ZnO@Fe₃O₄-porous reduced graphene oxide nanohybrid

The reduced graphene oxide (rGO) and porous reduced graphene oxide (prGO) were prepared according to previous work [21]. 2 g of ground *Thymus kotschyanus* plant in 50 mL of distilled water was sonicated in an ultrasound bath (60 kHz frequency) at 40 °C for 30 min. The mixture was filtered to separate the extract using Whatman filter paper and stored at 4 °C. 30 mg of GO nanostructure was sonicated in 30 mL of distilled water for 30 min at room temperature and 20 mL of *Thymus kotschyanus* extract was added to the GO solution. The mixture was refluxed for 5 h at 60 °C. The solid product (rGO) was filtrated, washed with water, and dried in the oven at 80 °C for 6 h. 15 mg of rGO nanostructure was sonicated in 20 mL of 30 % H₂O₂ for 30 min and refluxed for 8 h at 50 °C. The product (prGO) was filtered, washed with water and dried in the oven at 80 °C for 8 h. 10 mg of prGO was sonicated in 20 mL of water for 1 h and 15 mL of FeSO₄·7H₂O solution (0.1 M) was added to the prGO suspension under stirring. The pH of the solution was adjusted to 10 by 1 M NaOH solution, transferred to a Teflon-lined stainless steel

autoclave, and heated at 200 °C for 6 h. The product (Fe₃O₄-prGO) was washed by water and then dried at 60 °C overnight. For preparation of ZnO@Fe₃O₄-prGO nanohybrid, 10 mg of Fe₃O₄-prGO nanocomposite was sonicated in 50 mL of distilled water for 30 min and added slowly to 20 mL of 0.1 M zinc acetate dihydrate solution with stirring for 30 min at room temperature. The mixture was added dropwise to 0.2 M KOH. The final mixture was stirred for 2 h at 80 °C. Then, the ZnO@Fe₃O₄-prGO product was centrifuged, washed with water, and dried in an oven at 100 °C for 6 h.

Preparation of modified sensor

The CPE was prepared as described in our previous reports [22,23]. The graphite powder plus paraffin was hand-mixed until a uniformly wetted paste was obtained. The carbon paste was then packed into a glass tube (internal radius of 3 mm). Electrical contact was made with a copper wire. The electrode surface was renewed by polishing it on weighing paper. 1 mg of ZnO@Fe₃O₄-prGO was added to 1 mL of ethanol and sonicated for 10 min. Then, 3 µL of ZnO@Fe₃O₄-prGO solution was drop-casted onto CPE and dried at room temperature. The fabricated sensor was depicted as ZnO@Fe₃O₄-prGO/CPE. For comparative purposes, the prGO/CPE and Fe₃O₄-prGO/CPE sensors were prepared using the same method.

Analysis of serum plasma samples

To prepare the plasma samples, 1 mL of plasma was diluted with 9 mL of 0.1 M PBS (pH 7.0) and mixed until homogeneous. Known concentrations of Pal drug were spiked into the plasma mixture. Finally, all prepared plasma samples were analysed by the voltammetry method. The plasma samples were also determined by UV-Vis spectroscopy at 361 nm by diluting 500 µL of plasma in 4 mL of 0.1 M PBS (pH 7.0).

Results and discussion

Characterization of ZnO@Fe₃O₄- porous reduced graphene oxide nanohybrid

Initially, UV-Vis spectroscopy was used to verify the formation of ZnO@Fe₃O₄-prGO nanohybrid in the wavelength region 300–500 nm. Figure 1A shows UV-Vis spectra of prGO and ZnO@Fe₃O₄-prGO nanostructures.

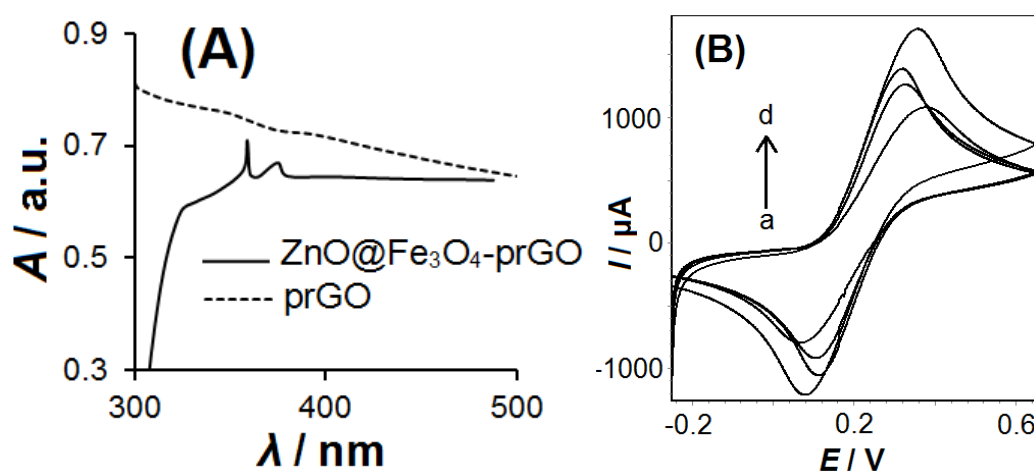


Figure 1. (A) UV-Vis spectra of prGO and ZnO@Fe₃O₄-prGO aqueous solutions (1 mg mL⁻¹), (B) cyclic voltammograms of CPE (a), prGO/CPE (b), Fe₃O₄-prGO/CPE (c) and ZnO@Fe₃O₄-prGO/CPE (d) in 0.1 M PBS (pH 7.0) at 100 mV s⁻¹

As shown, no peak was observed for the prGO nanosheets, while the ZnO@Fe₃O₄-prGO nanohybrid exhibited peaks at 359 nm and 375 nm in good agreement with ZnO and Fe₃O₄ nanoparticles,

respectively. The electrochemical characterization of ZnO@Fe₃O₄-prGO was assessed by cyclic voltammetry (CV), which enables identification of the potential values of the redox moieties and provides information about charge-transfer efficiency. Figure 1B shows the cyclic voltammograms of CPE, prGO/CPE, Fe₃O₄-prGO/CPE and ZnO@Fe₃O₄-prGO/CPE in 0.1 M PBS (pH 7.0) containing 10 mM [Fe(CN)₆]^{3-/4-}. Peak-to-peak separations (ΔE) were 300, 190, 191 and 245 mV for CPE, prGO/CPE, Fe₃O₄-prGO/CPE and ZnO@Fe₃O₄-prGO/CPE, respectively, while corresponding anodic peak currents were 1250.2, 1288.5, 1422.6 and 1729.2 μ A. The highest current for ZnO@Fe₃O₄-prGO/CPE indicates enhanced electrocatalytic activity, attributed to a larger active surface area and improved conductivity, which together promote faster electron transfer.

To rationalize this enhanced response and available electroactive sites, the electroactive surface area (ECSA) was determined from CVs at various scan rates using the Randles-Ševčík Equation (1):

$$I = 2.69 \times 10^5 n^{3/2} D^{1/2} A C \nu^{1/2} \quad (1)$$

where I , n , D , A , C and ν represent the peak current, number of electrons transferred, diffusion coefficient (7.6×10^{-6} cm² s⁻¹), active electrode surface area, analyte concentration and scan rate, respectively. ECSAs were calculated as 0.08, 0.11, 0.13, and 0.17 cm² for CPE, prGO@CPE, Fe₃O₄-prGO/CPE and ZnO@Fe₃O₄-prGO/CPE, respectively. The largest ECSA of ZnO@Fe₃O₄-prGO/CPE supports higher charge transfer and more efficient electrochemical reactions.

The morphological and compositional features of the synthesized materials were jointly assessed using FE-SEM and EDS. In the FE-SEM image, the surface of prGO (Figure 2a) appears crumpled and shows sheet-like structures. Similar features have been observed for the synthesis of prGO in the literature [21,24]. In contrast, the image of Fe₃O₄-prGO (Figure 2b) shows that prGO sheets are decorated by sphere-shaped Fe₃O₄ nanoparticles with a diameter in the range 43 to 59 nm. The ZnO@Fe₃O₄-prGO hybrid shows sphere- and pyramid-like shape nanoparticles on prGO sheets in good agreement with Fe₃O₄ and ZnO nanoparticles, respectively. Also, the morphological observation of the ZnO@Fe₃O₄-prGO hybrid indicates an almost homogeneous structure and particles with less aggregation. Complementary EDS analysis revealed the successful incorporation of Fe (30.91 %) and Zn (4.64 %) atoms into the carbon (40.29 %) and oxygen (24.11 %) framework of prGO (Figure 2d), highlighting the efficient formation of ZnO and Fe₃O₄ nanoparticles on prGO sheets.

The crystalline architecture of the ZnO@Fe₃O₄-prGO hybrid was systematically characterized by XRD analysis (Figure 3A). The XRD diffractogram revealed distinct reflections at 2θ of 22.1 and 43.3°, corresponding to the (002) and (100) planes of prGO sheets, respectively [23]. Also, the reflections at 2θ of 30.3, 30.8, 32.4, 35.5, 35.8, 50.5, 53.7, 57.1, 62.6, 62.8 and 74.3° were observed in the spectrum. The reflections of 30.3, 35.5, 53.7, 57.1 and 62.8° could be indexed to the (220), (311), (422), (511) and (440) planes of Fe₃O₄ nanoparticles [25], respectively, while the diffraction peaks at 30.8, 32.4, 35.8, 50.5, 62.6 and 74.3° belong to the (100), (002), (101), (110), (112) and (202) planes of the wurtzite hexagonal zinc oxide (JCPDS36-1451) [26].

The FT-IR pattern of ZnO@Fe₃O₄-prGO hybrid was presented in the range 400 to 4000 cm⁻¹ (Figure 3B). The peaks of 469 and 589 cm⁻¹ are related to the Fe-O band [27], while the peak of 910 cm⁻¹ is related to the Zn-O band [28]. The peaks at 1083, 1398, 1577, 2351, and 3361 cm⁻¹ may correspond to C-C, C-O, C=O, C-H, and O-H groups, respectively [29]. The observed peaks confirm conjugation of Fe₃O₄ and ZnO nanoparticles with prGO sheets and the formation of a ZnO@Fe₃O₄-prGO hybrid.

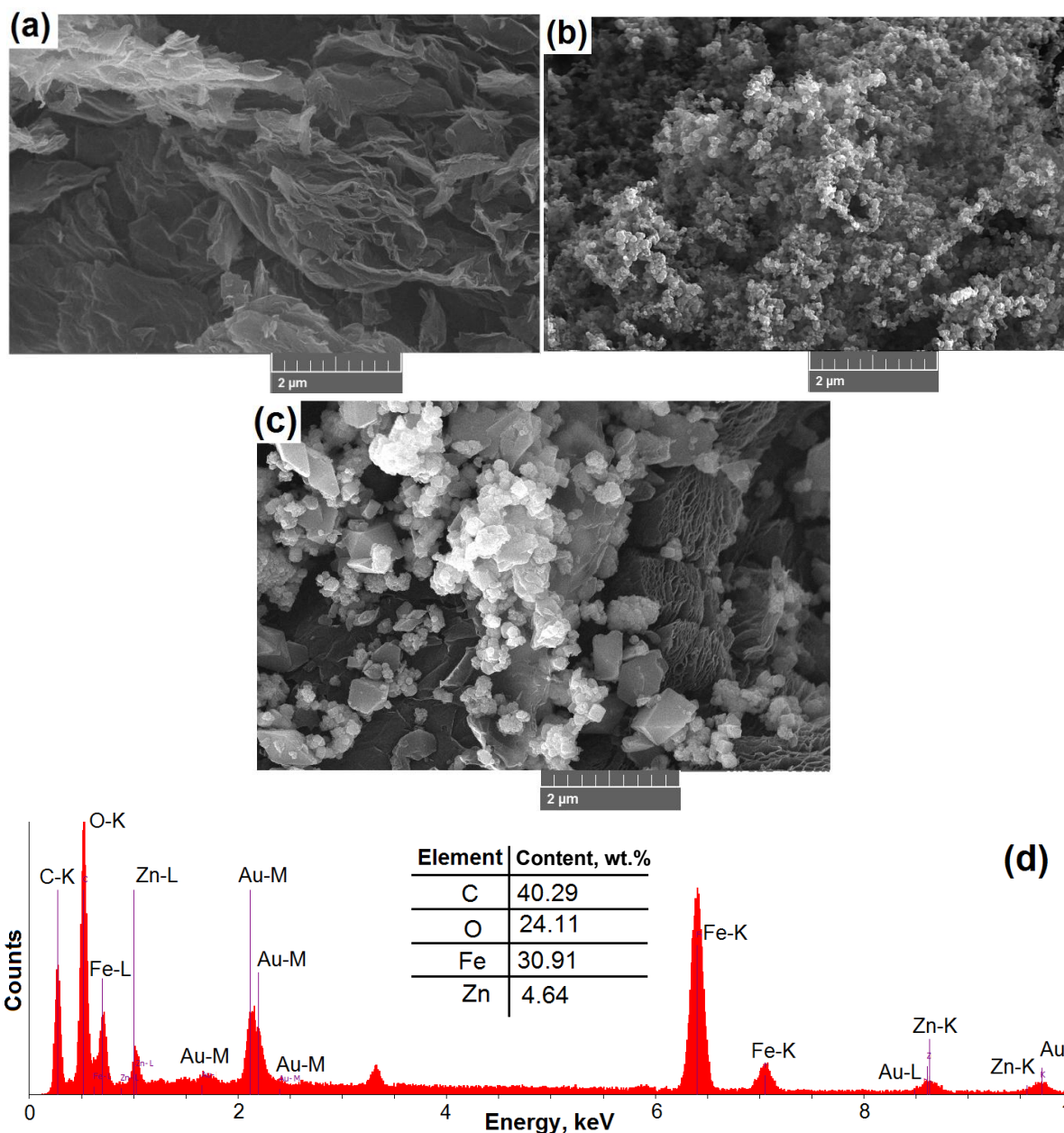


Figure 2. FE-SEM images of (a) prGO, (b) Fe₃O₄-prGO and (c) ZnO@Fe₃O₄-prGO nanohybrid; (d) EDS pattern of ZnO@Fe₃O₄-prGO

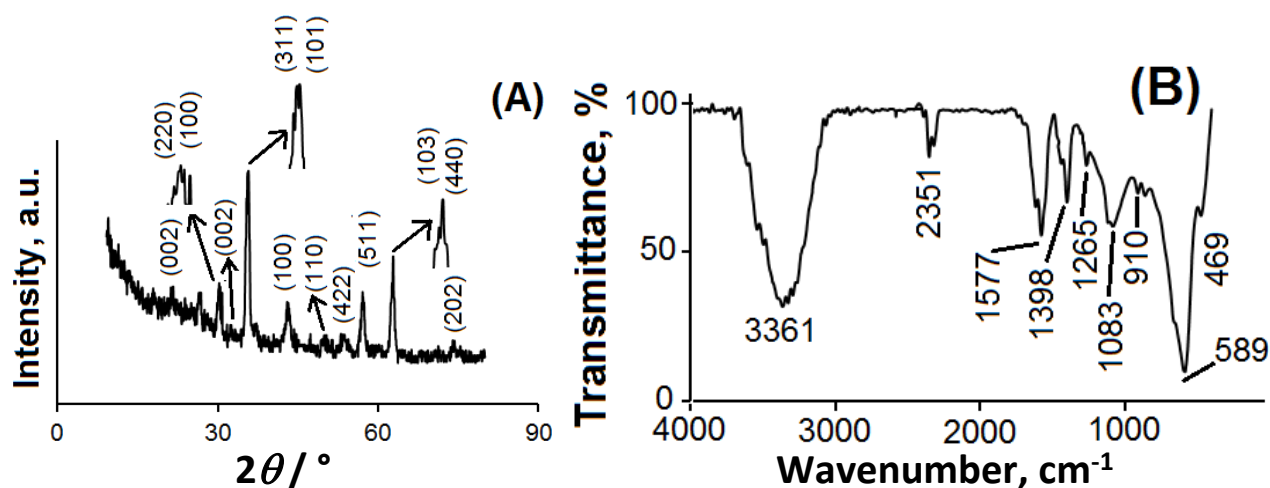


Figure 3. (A) XRD spectrum and (B) FT-IR pattern of ZnO@Fe₃O₄-prGO

N_2 sorption isotherm of the $ZnO@Fe_3O_4$ -prGO hybrid is represented in Figure 4A. According to IUPAC classification, this curve indicates a porous structure and a high specific surface area for the $ZnO@Fe_3O_4$ -prGO hybrid. BET specific surface area, volume of all pores and average pore size calculated from the Barrett-Joyner-Halenda (BJH) pore size distribution curve were defined as $408.74 \text{ m}^2 \text{ g}^{-1}$, $0.211 \text{ m}^3 \text{ g}^{-1}$ and 2.06 nm , respectively.

Electrochemical oxidation of palbociclib

The electrochemical oxidation of Pal drug was comprehensively examined on different electrode platforms using CV in $0.1 \text{ M PBS (pH 7.0)}$ containing $3 \mu\text{M}$ Pal at a scan rate of 100 mV s^{-1} (Figure 4B). The CV profiles consistently displayed well-defined anodic peaks without corresponding cathodic signals, confirming the irreversible nature of the oxidation process. The peak currents obtained for CPE, prGO/CPE, Fe_3O_4 -prGO/CPE and $ZnO@Fe_3O_4$ -prGO/CPE were 3.9 , 8.8 , 15.5 and $21.1 \mu\text{A}$, respectively. The markedly enhanced response of $ZnO@Fe_3O_4$ -prGO/CPE underscores its superior electrocatalytic activity, which can be ascribed to accelerated electron-transfer kinetics and the higher density of electroactive sites imparted by dual Fe_3O_4 and ZnO nanoparticle conjugation. These findings clearly demonstrate the pivotal role of surface functionalization in modulating electrode performance, where $ZnO@Fe_3O_4$ -prGO/CPE provides both efficient charge transport pathways and a greater abundance of catalytically active centres, thereby significantly improving sensitivity toward Pal detection.

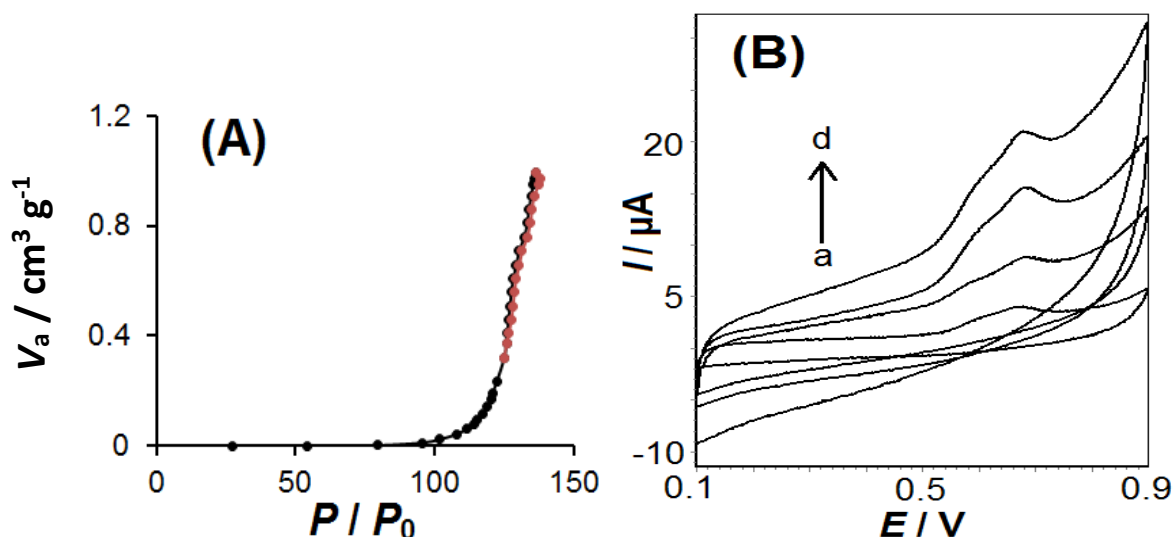


Figure 4. (A) Nitrogen adsorption-desorption isotherm (V_a -monolayer adsorbed gas quantity) of $ZnO@Fe_3O_4$ -prGO hybrid, (B) cyclic voltammograms of $3 \mu\text{M}$ Pal in $0.1 \text{ M PBS (pH 7.0)}$ and 0.1 M KCl as supporting electrolyte at the surface of (a) CPE, (b) prGO/CPE, (c) Fe_3O_4 -prGO/CPE and (d) $ZnO@Fe_3O_4$ -prGO/CPE at a scan rate of 100 mV s^{-1}

Effect of scan rate

Building on these findings, it was essential to further probe the kinetic aspects of the electrooxidation process, as scan rate is a critical parameter that directly governs both electron transfer and mass transport phenomena. Variations in scan rate not only influence the diffusion of analyte molecules toward the electrode surface but also modulate adsorption/desorption dynamics, thereby providing key mechanistic insights. To this end, CV tests were performed for $ZnO@Fe_3O_4$ -prGO/CPE in $0.1 \text{ M PBS (pH 7.0)}$ containing $1 \mu\text{M}$ Pal at scan rates ranging from 25 to 200 mV s^{-1} (Figure 5A). As illustrated in Figure 5B, the anodic peak current increased proportionally with scan rate, accompanied by a positive shift in peak potential, indicating an adsorption-controlled

reaction. Further, the adsorption behaviour of Pal on ZnO@Fe₃O₄-prGO/CPE makes the adsorption time (t) a crucial parameter for detection. The oxidation current also improved with increasing time up to 60 s (Figure 5C). So, the electrode surface is saturated at 60 s.

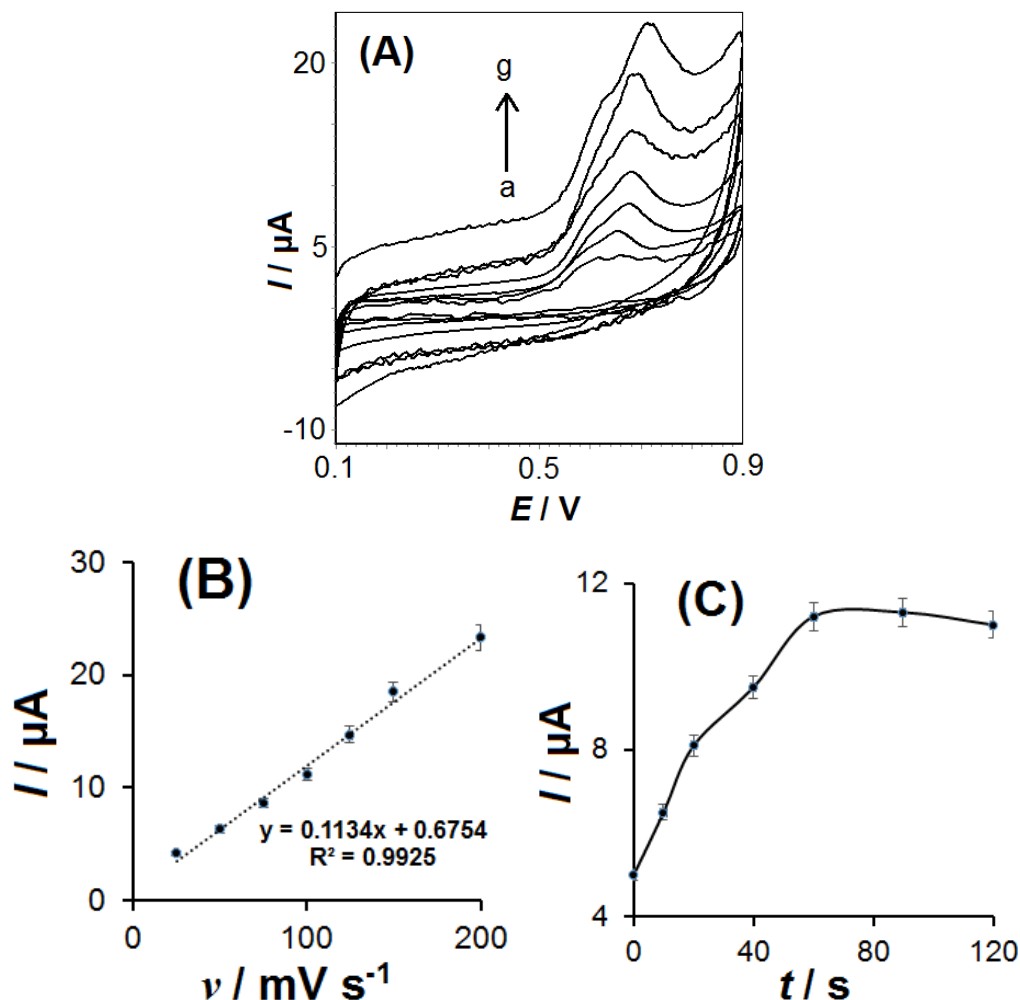


Figure 5. (A) Cyclic voltammograms of 1 μM Pal in 0.1 M PBS (pH 7.0) and 0.1 M KCl as supporting electrolyte at the surface of ZnO@Fe₃O₄-prGO/CPE at various scan rates: (a) 25, (b) 50, (c) 75, (d) 100, (e) 125, (f) 150 and (g) 200 mV s⁻¹; (B) plot of peak currents vs. scan rate, (C) dependence of adsorption time on oxidation peak current of 0.1 μM Pal

Effect of pH

The pH of the solution is a decisive parameter in electrochemical systems, as it governs proton–electron coupling, electrode surface interactions, and analyte behaviour. Establishing the optimum pH is therefore essential for maximizing sensor performance in terms of sensitivity and selectivity. To assess this effect, cyclic voltammograms of ZnO@Fe₃O₄-prGO/CPE were recorded in 0.1 M PBS (pH 4.0–8.0) containing 5 μM Pal at a scan rate of 100 mV s⁻¹ (Figure 6A). The anodic current increased from pH 4.0 to 7.0, reaching a maximum at pH 7.0, and decreased at higher values (Figure 6B). Thus, pH 7.0 was identified as the optimum medium for Pal oxidation. With increasing pH, the peak potential shifted in the negative direction alongside changes in current, reflecting the involvement of protons in the oxidation process. A linear correlation between the anodic peak potential (E_p) and pH was established by the equation $E_{pa} = 0.052\text{pH} + 1.04$ ($R^2 = 0.996$), indicating an equal number of protons and electrons according to Scheme 1.

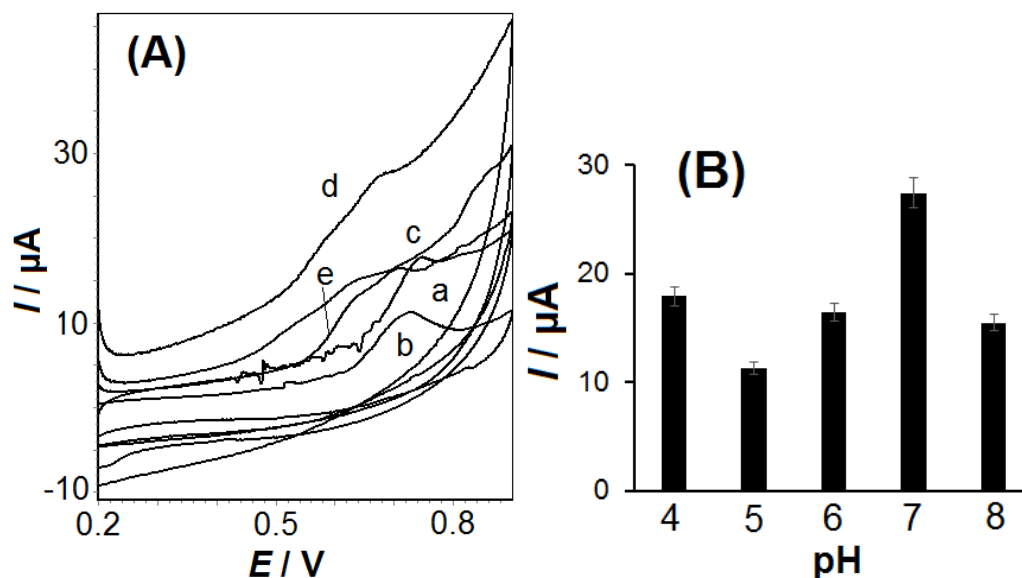
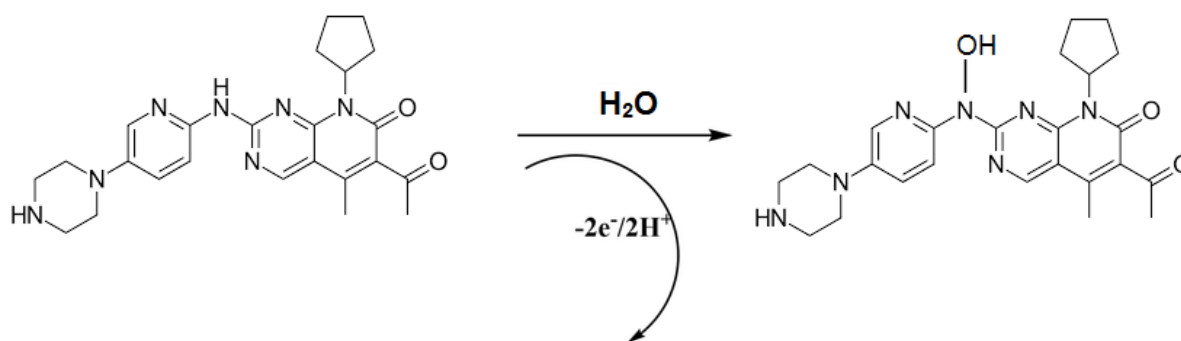


Figure 6. (A) Cyclic voltammograms of 5 μM Pal in 0.1 M PBS and 0.1 M KCl as supporting electrolyte at the surface of ZnO@Fe₃O₄-prGO/CPE at various pH: (a) 4.0, (b) 5.0, (c) 6.0, (d) 7.0 and (e) 8.0 at scan rate of 100 mV s⁻¹, (B) plot of peak current vs. pH



Scheme 1. Electrochemical oxidation mechanism of Pal

Electrochemical determination of palbociclib

Under the optimized experimental conditions, Pal was quantitatively determined using square wave voltammetry (SWV). SWV responses were recorded in 0.1 M PBS (pH 7.0) containing different concentrations of Pal (Figure 7A).

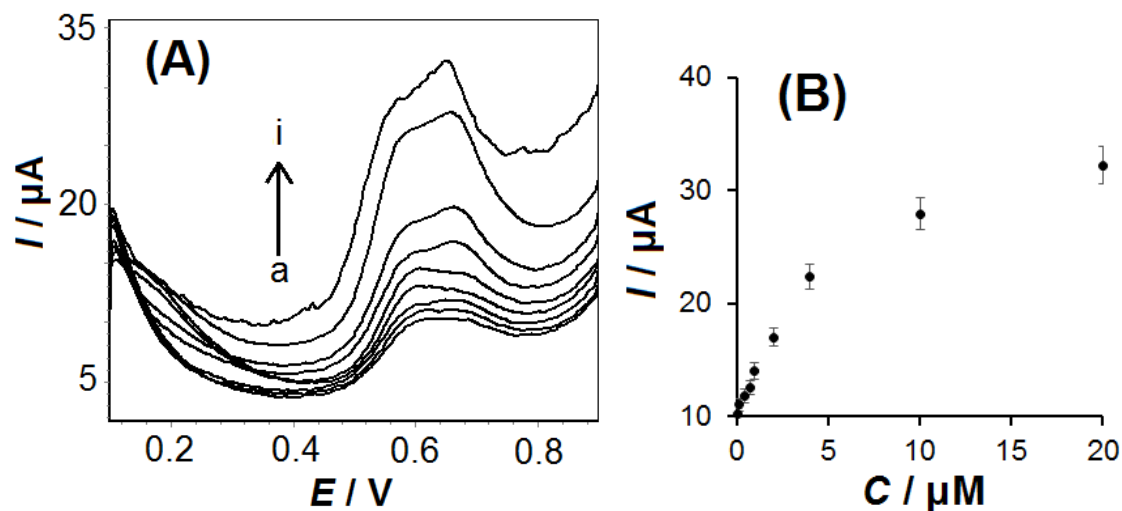


Figure 7. (A) Square wave voltammograms of ZnO@Fe₃O₄-prGO/CPE in the presence of (a) 0.05, (b) 0.1, (c) 0.4, (d) 0.7, (e) 1, (f) 2, (g) 4, (h) 10 and (i) 20 μM Pal, (B) calibration curve of peak currents vs. C_{Pal}

As the concentration of Pal increased, the oxidation peak current exhibited a proportional enhancement, indicating a well-defined electrochemical response of the sensor toward the analyte. The calibration plot constructed from the oxidation peak current versus Pal concentration displayed excellent linearity in the range of 0.05 to 4.00 μM , described by the regression equation $I = 10.47 + 3.04C_{\text{Pal}}$ (Figure 7B). The limit of detection (LOD) was 37 nM, with a sensitivity of 3.04 $\mu\text{A } \mu\text{M}^{-1}$. These results clearly demonstrate the superior analytical performance of the proposed electrochemical sensor. As summarized in Table 1, the developed platform provides a lower or comparable LOD and higher sensitivity than those reported for previously published Pal determinations, highlighting its potential for sensitive and reliable detection in real-sample applications.

Table 1. Analytical parameters for determination of Pal drug using different methods

Method	Linear range	LOD	Sample	Ref.
UV-Vis	1-20 $\mu\text{g mL}^{-1}$	0.02 $\mu\text{g mL}^{-1}$	Tablet	[30]
UV-Vis	2-10 mg mL^{-1}	0.0782 mg mL^{-1}	Drug	[31]
LC-MS/MS ^a	72.8-185.5 ng mL^{-1}	120 ng mL^{-1}	Drug	[32]
Electrophoresis	10.0-100.0 $\mu\text{g mL}^{-1}$	0.11 $\mu\text{g mL}^{-1}$	Tablet	[33]
MIP-Voltammetry	0.025-0.25 nM	0.003 nM	Serum	[11]
DPV ^b	0.01-1 μM	2.28 nM	Serum	[12]
SW-ADSV ^c	0.1-1.0 μM	0.09 nM	Drug	[34]
SWV	0.05-4.0 μM	37 nM	Drug	This work

^aliquid chromatography-tandem mass spectrometry, ^bdifferential pulse voltammetry, ^csquare wave-adsorptive differential stripping voltammetry

Evaluation of interference, repeatability, reproducibility and stability

Selectivity is a key parameter affecting the performance of electrochemical sensors, especially in complex matrices. The interference tests were carried out by adding some common interfering species such as cysteine, glutathione, lysine, glucose, Na⁺, K⁺, Cl⁻, ascorbic acid and sucrose as common interferences in biological samples. These interferences and Pal were successively added to 0.1 M PBS solution (pH 7.0), and current responses were recorded at 0.69 V (Figure 8A). Negligible responses were observed for interferences even at tenfold higher concentrations. These results demonstrate the high selectivity of the developed ZnO@Fe₃O₄-prGO/CPE sensor for Pal detection, highlighting its potential for practical applications.

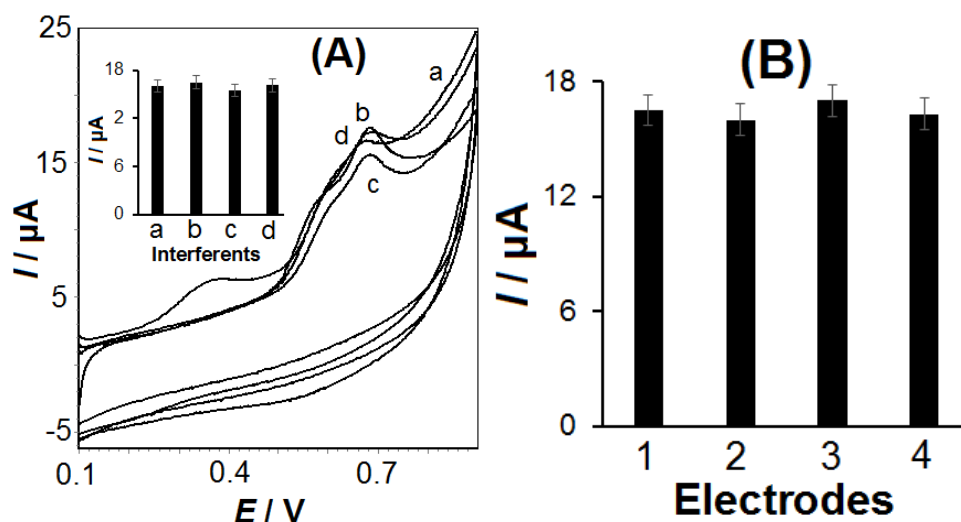


Figure 8. (A) CVs of ZnO@Fe₃O₄-prGO/CPE electrodes in 0.1 M PBS (pH 7.0) upon addition of (a) 4 μM Pal, (b) 4 μM Pal + 10 μM ascorbic acid, (c) 4 μM Pal + 10 μM glucose and (d) 4 μM Pal + 10 μM Na⁺. Inset: plot of peak current vs. interferences, (B) plot of peak current for 4 different ZnO@Fe₃O₄-prGO/CPE electrodes fabricated using the same procedure

The reproducibility of the ZnO@Fe₃O₄-prGO/CPE electrode was evaluated by employing four independently prepared electrodes under identical experimental conditions (Figure 8B). The results showed a relative standard deviation (RSD, %) of 2.29 % for the anodic peak current responses, demonstrating excellent reproducibility of the electrode fabrication procedure. The repeatability and intermediate precision were investigated by measuring five successive tests for 4 μ M Pal. The RSD values were estimated to be 3.4 and 5.2% for one day (intra-assay) and seven days (inter-assay), respectively, indicating excellent repeatability.

The long-term stability of the proposed sensor was systematically evaluated over a 20-day period. The results demonstrated that the electrode retained more than 82 % of its initial response, highlighting its remarkable stability and confirming its suitability for reliable electrochemical sensing applications.

Assay of plasma samples

To evaluate the applicability and validity of the proposed sensor, recovery experiments were monitored with Pal analysis in known amounts of human plasma. The mean recoveries and RSD values of Pal were found to be 92.5 to 105.0 % and 1.9 to 4.2 %, respectively (Table 2). Moreover, Pal analysis in human plasma was performed by UV-Vis spectroscopy (Figures 9A and 9B). The data reported in Table 2 showed that the electrochemical results don't significantly differ from UV-Vis spectroscopy. This highlights the strong potential of the ZnO@Fe₃O₄-prGO/CPE sensor for pharmaceutical monitoring in clinical diagnostics and other biological and environmental matrices.

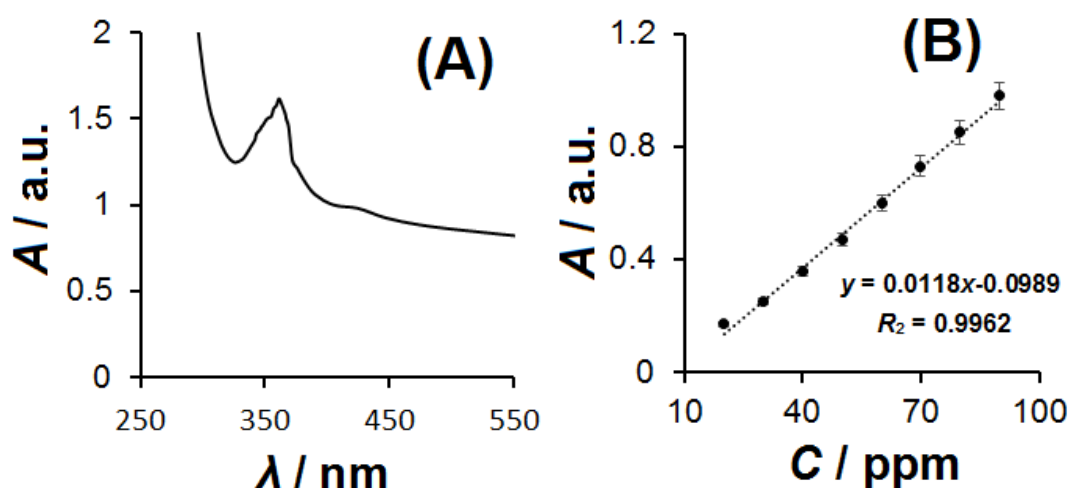


Figure 9. (A) UV-Vis spectrum of 2 mg mL⁻¹ Pal, (B) the calibration curve of Pal absorbance to its concentration

Table 2. Determination of Pal in plasma samples at surface of ZnO@Fe₃O₄-prGO/CPE in 0.1 M PBS (pH 7.0)

Sample	Content, μ M		Mean recovery, % (n=3)	
	Added	Found	Proposed method	UV method
Plasma 1	2.0	1.9	95.0 $\pm$ 3.7	97.8 $\pm$ 3.3
Plasma 1	4.0	4.1	102.5 $\pm$ 1.9	95.4 $\pm$ 2.6
Plasma 2	2.0	2.1	105.0 $\pm$ 4.2	93.9 $\pm$ 1.8
Plasma 2	4.0	3.7	92.5 $\pm$ 2.4	101.1 $\pm$ 4.5

Conclusion

The ZnO@Fe₃O₄-prGO nanohybrid was synthesized *via an* environmentally friendly process using *Thymus kotschymanus* plant extract as a reducing and stabilizing agent and applied to modify carbon paste electrodes for Pal detection. The ZnO@Fe₃O₄-prGO/CPE sensor exhibited a broad linear range (0.05 to 4.00 μ M), high sensitivity (3.04 μ A μ M⁻¹), a low LOD (37 nM), high reproducibility and repeatability, and long-term stability. Validation with human plasma confirmed excellent recoveries

(92.5 to 105.0 %) and RSD values below 5 %, demonstrating its clinical relevance. Although tested only in plasma, the selectivity of the ZnO@Fe₃O₄-prGO/CPE platform highlights its potential for more applications in pharmaceutical and environmental monitoring.

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