

Development of an Electrochemical DNA Sensor: Investigation of DNA Adsorption on ITO Electrodes Modified with F-MWCNT/Polypyrrole/Gold Nanoparticles

Dhulfiqar S. Mutashar^{1,2*}, Wasan R. Saleh¹, Ilham A. Khalaf³

¹ Department of Physics, College of Science, University of Baghdad, Baghdad, Iraq

² Department of Applied Science, University of Technology, Baghdad, Iraq

³ Corporation of Research and Industrial Development, Baghdad, Iraq

ABSTRACT

Background: Electrochemical DNA sensors are emerging as sensitive and selective tools for biomedical diagnostics, enabling the detection of genetic mutations, infectious diseases, and cancer biomarkers. Surface modification of electrodes enhances DNA adsorption, improving sensor stability and performance. **Objective:** This study aimed to develop a high-performance electrochemical DNA sensor by modifying an indium tin oxide (ITO) electrode with fluorinated multi-walled carbon nanotubes (F-MWCNTs), polypyrrole (PPy), and gold nanoparticles (AuNPs), and to investigate its efficiency in DNA adsorption and detection. **Methods:** The ITO electrode was modified using a layer-by-layer electrochemical deposition method. The structural and morphological characteristics of the modified electrode were analyzed using scanning electron microscopy (SEM), Fourier-transform infrared spectroscopy (FTIR), and cyclic voltammetry (CV). DNA adsorption efficiency was assessed by differential pulse voltammetry (DPV) using methylene blue (MB) as an electrochemical indicator. The stability, sensitivity, and selectivity of the sensor for DNA detection were evaluated. **Results:** The F-MWCNT/PPy/AuNP-modified ITO electrode exhibited enhanced electrochemical conductivity, as confirmed by CV measurements. FTIR analysis confirmed successful surface functionalization, while SEM imaging showed a uniform and well-distributed nanocomposite layer. The sensor demonstrated high DNA adsorption capacity, with a significant increase in oxidation peak current upon DNA immobilization. The modified electrode exhibited excellent stability, sensitivity, and selectivity for DNA detection, with a low detection limit (LOD) and wide linear range. **Conclusion:** The F-MWCNT/PPy/AuNP-modified ITO electrode enhances DNA adsorption and provides a promising platform for electrochemical DNA sensing applications. This approach could be valuable for the early detection of genetic mutations and disease biomarkers in clinical diagnostics and biomedical research. Further studies are recommended to optimize sensor performance for real-world biological sample analysis.

Keywords: Immobilization, DNA Adsorption, Polypyrrole, Nanomaterials

INTRODUCTION

Electrochemical DNA sensors based on nanomaterials are a promising technology for detecting and analyzing DNA. These sensors offer several advantages, including rapid response, high sensitivity, low cost, and ease of use. Nanomaterials play an important function in enhancing the sensitivity of these sensors [1, 2]. Electrochemical DNA sensors based on multi-walled carbon nanotubes (MWCNTs) have been extensively studied in recent years. These sensors offer high sensitivity, specificity, and selectivity for the detection of DNA hybridization events. The unique electronic and structural properties of MWCNTs make them suitable for interfacing with biomolecules, such as DNA, resulting in the creation of high-performance DNA-sensing devices [3]. In addition, the functionalization of MWCNTs has further enhanced their performance in DNA sensing applications. For example, the incorporation of MWCNTs with other nanomaterials, such as graphene quantum dots (GQDs) and silver nanoparticles (AgNPs), has increased the electrocatalytic activity and detection limits for DNA sensors [4-6]. These developments in MWCNT-based DNA sensors have paved the road for their prospective application in a variety of disciplines, such as medical diagnostics, food analysis, and environmental monitoring [7]. DNA immobilization is a crucial step in various biological analysis techniques, including DNA-based biosensors [8]. There are several methods for immobilizing DNA, including physical absorption, Avidin/streptavidin-biotin interaction, and covalent chemical bonding immobilization [9]. The process of adsorption is considered the most straightforward technique employed

for immobilizing DNA probes onto the working electrode surface. This method does not necessitate the use of any form of chemical reagents or modification of DNA probes. The immobilization of DNA probes is achieved through the electrostatic adsorption between the negatively charged phosphate group of DNAs and the positively charged films-modified electrodes present on the working electrode surface. Previous studies have reported that the employment of chitosan and cationic polymeric films for DNA probe adsorption is purposed towards offering commendable biocompatibility and a high density of positively charged ions [10-12]. Various cationic polymeric films, including poly-L-lysine (PLL) [13], polypyrrole (PPy) [14], polyaniline [15], and polyethyleneimine [16], have been identified as potential DNA matrices for immobilization strategies by means of electrostatic adsorption. This study involves examining the adsorption of DNA on the modified electrode surface and its effect on the electrochemical properties of the electrode. The f-MWCNT/polypyrrole/gold nanoparticles composite is used to enhance the sensitivity and selectivity of the DNA electrochemical sensor.

EXPERIMENTAL WORK

Materials

The used material in this work is, functionalized MWCNTs (f-MWCNTs) with 5.3-5.85% OH supplied by Nanostructured and

Amorphous Materials, Inc. (f-MWCNTs) (having an outer diameter of 8 nm, a length of 0.5-2 nm and a purity of >95.1 %). Gold nanoparticles (AuNPs) with purity of >99.97% APS: 28nm, bulk density $\sim 0.85 \text{ g/m}^3$, supplied by US Research Nanomaterials, Inc. Dimethyl sulfoxide (DMSO) solution of formula $(\text{CH}_3)_2\text{SO}$ with the molecular weight 78.14 (g/mol), purity 99.6% supplied by ALPHA CHEMIKA, India. While N, N Dimethylformamide (DMF) solution of

formula $\text{C}_3\text{H}_7\text{NO}$ with the molecular weight 73.08(g/mol), density 0.944 gm/ml (at 25 °C) used to prepare f-MWCNTs -PPy-AuNPs suspension, was supplied by Biosolve, USA Company. For electrolyte solution, Potassium ferrocyanide $(\text{Fe}(\text{CN})_6)^{-3/-4}$ of purity 99 % and Potassium chloride (KCl) of purity 99 % both from Sigma- Aldrich. 4-Aminothiophenol supplied by Biosynth. The chemicals were utilized as received, without further purification (Table 1).

Table 1: The subsequent DNA sequences implemented in the current study are as follows:

Probe DNA Targets (5'-3')	HS-GATACGAATGTCAGCTGACACG
Complementary DNA	HS-CTATGCTTACACGTCGACTGTGC
Non-complementary DNA	HS-CTTAGCAAACCTGGTGCACACACG

Preparation of (ITO/MWCNTs -PPy-AuNPs) modified electrode

For the preparation of the suspension of f-MWCNTs-AuNPs, a quantity of 2 mg of f-MWCNTs powder was combined with 2.0 mL of DMF and subjected to sonication for a duration of 15 minutes using a sonicated probe. Following this, 2 μg of AuNPs were introduced into the suspension and subjected to an additional 15 minutes of sonication to ensure a uniform distribution. The conductive polymer known as polypyrrole is produced through a simple and cost-effective in-situ chemical oxidative polymerization process, as outlined in references [17, 18].

A certain volume of the PPy solution is then extracted using a micro syringe and directly injected into the prepared suspension of (f-MWCNTs-AuNPs). This procedure serves to disperse f-MWCNTs aggregates and establish a small network. To achieve this, the mixture is sonicated for a duration of 20 minutes using an ultrasonic probe, as depicted in Figure 1. Subsequently, using a micropipette, 7 μL of the produced suspension was drop-cast onto the surface of the ITO. After drying at room temperature, the (ITO/MWCNTs-PPy-AuNPs) modified electrode was successfully built. Figure 2 shows the schematic diagram of the fabricated modified electrode

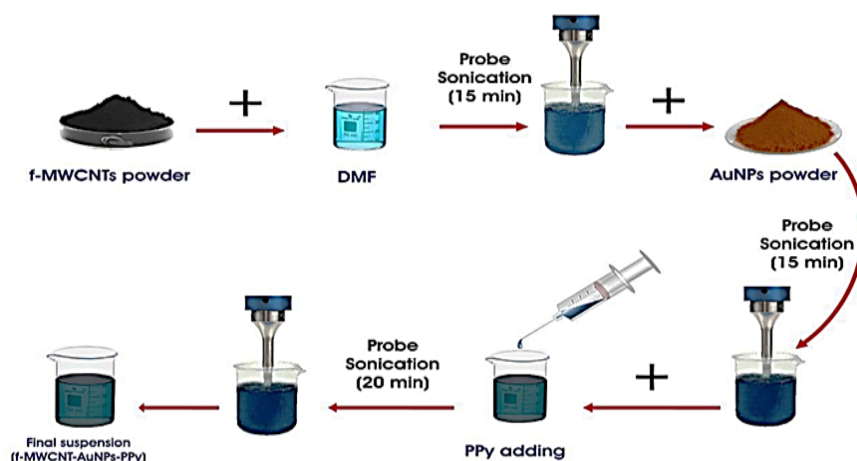


FIGURE 1: Schematic of the (f-MWCNTs-AuNPs-PPy) suspension preparation steps.

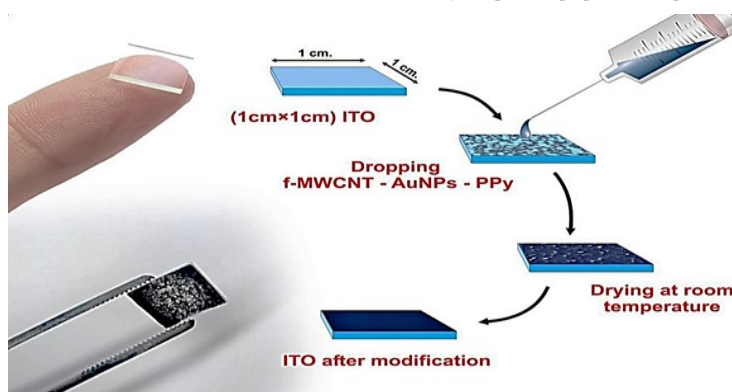


FIGURE 2: Schematic of the proposed ITO electrode modification steps.

Results and Discussion

Physical studies

The XRD pattern of the generated f-MWCNT-G-AuNPs-PPy composite is shown in Figure 3. The XRD pattern displays a wide peak

at $2\theta = 23.5^\circ$, indicating that PPy is amorphous [19]. Carbon has a prominent peak in the spectra of f-MWCNTs at $2\theta = 26.6^\circ$ Miller indices (002) (JCPDS file 96-1061). Diffraction peaks for AuNPs were detected at $2\theta = 37^\circ$, $2\theta = 46.42^\circ$, $2\theta = 63.42^\circ$, and $2\theta = 78.48^\circ$ corresponding to (111), (200), (220), and (311) respectively (JCPDS

file 89-3697). As a consequence, it was hypothesized that - stacking between the PPy chains and graphene planes occurs [20].

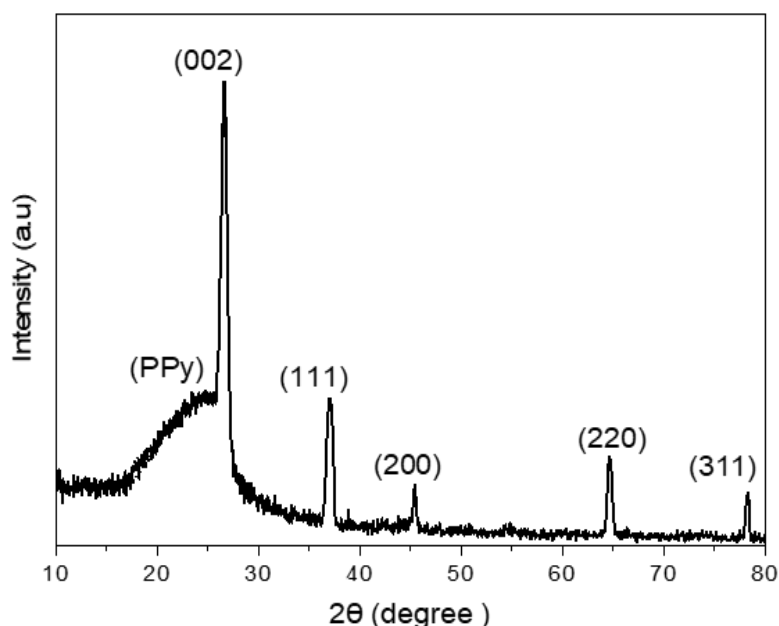


FIGURE 3: XRD pattern of f-MWCNTs-AuNPs-PPy.

The proportionality of capacitance of the ITO electrode material to its surface area is a well-established fact. Upon conducting a comparison between f-MWCNTs-AuNPs and f-MWCNTs-PPy-AuNPs, it has come to light that the nanocomposites of f-MWCNTs-AuNPs demonstrate a synergistic impact as a result of their elevated conductivity and surface area. The mesoporous structure of PPy further adds to this effect and results in an enhanced capacitance of PPy-coated f-MWCNTs-AuNPs nanocomposites. Figure 4 depicts the field emission scanning electron microscopy (FE-SEM) images that

have been collected at a greater magnification to thoroughly analyze the coating state of the f-MWCNTs-AuNPs by PPy. In Figure 4a, a homogeneous f-MWCNT layer is observed with well-dispersed AuNPs on the modified ITO electrode's surface. Figure 4b, on the other hand, displays a thick covering of PPy on the f-MWCNTs-AuNPs nanocomposite. This phenomenon is caused by the presence of more pyrrole throughout the in place polymerization process. This occurrence promotes the formation of PPy on the surface of the f-MWCNTs-AuNPs, resulting in the formation of a thick coating.

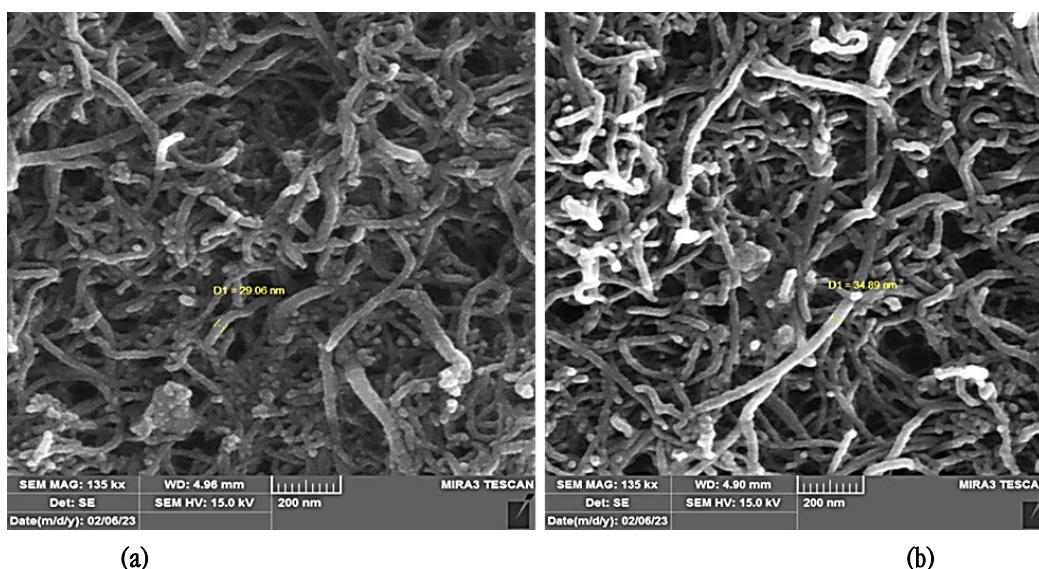


FIGURE 4: SEM images of (a): f-MWCNTs-AuNPs and (b) f-MWCNTs-AuNPs-PPy films deposited on ITO conductive glass.

The produced suspension's FTIR spectra were measured in the 500-4000 cm^{-1} range using a Thermo Nicolet Avatar 360 FT-IR Spectrometer. The FTIR spectra of the modified electrode are presented in Figure 3. Peaks of the spectrum located at 1093 cm^{-1} , 1376 cm^{-1} , and 1674 cm^{-1} might be ascribed to C-H (alkene bending), $\text{C}\equiv\text{C}$ and C-N stretching, and antisymmetric and symmetric ring-stretching modes,

respectively.

The wide peak at 3500 cm^{-1} in the spectral region belongs to the stretching vibration of the O-H carbonyl and hydroxyl functional groups. Furthermore, the weak peaks around 2935 cm^{-1} , 1256 cm^{-1} , and 659 cm^{-1} belong to C-N vibration, C-C stretching, and C=H bending, respectively. This is consistent with the findings of [21].

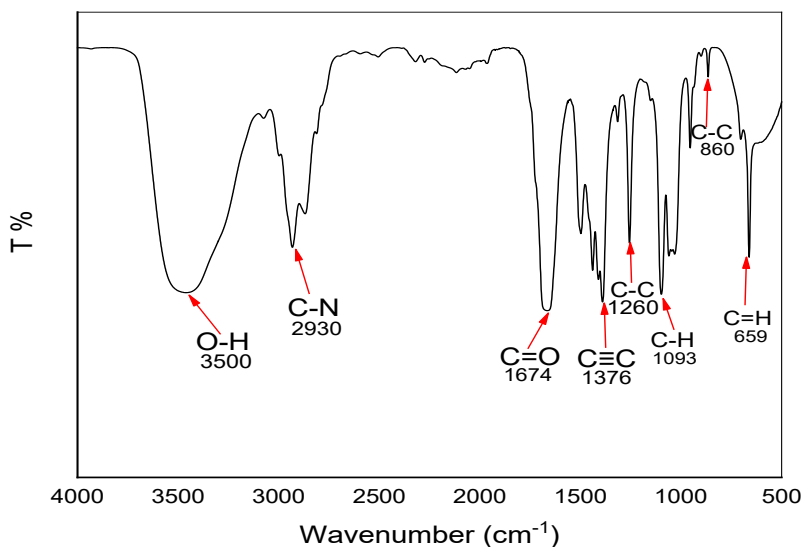
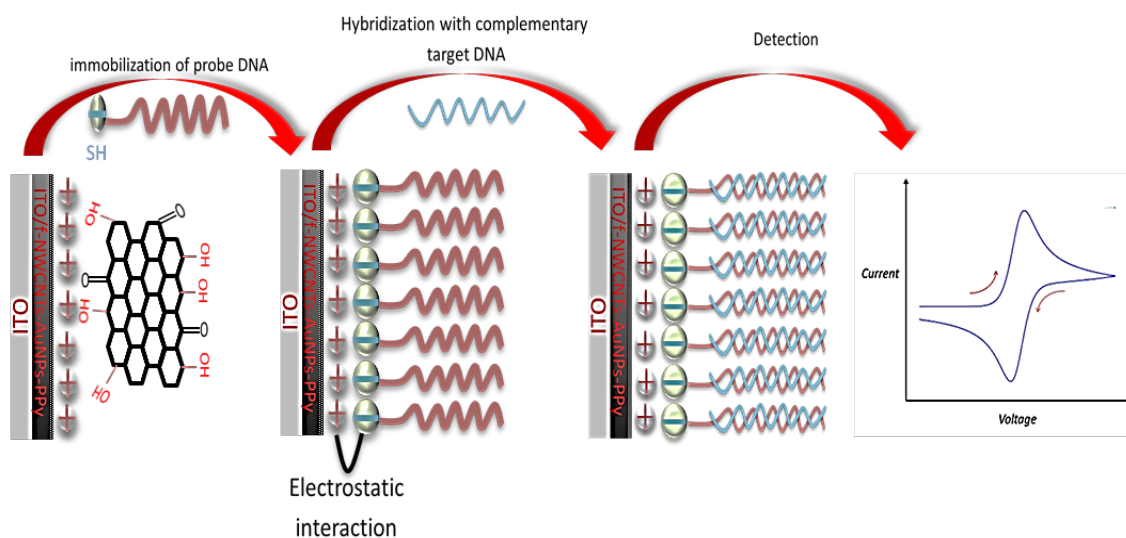


FIGURE 5: FTIR spectra of (f-MWCNTs-PPy-AuNPs).

Electrochemical studies

The modified electrode, composed of f-MWCNTs-AuNPs-PPy/ITO, underwent an initial washing process using deionized water. This was done prior to the immobilization of the probe double-stranded (ds-DNA). Subsequently, the electrode was dried in ambient air conditions at room temperature. Afterward, the immobilization process of 10 μ l of a solution containing a dsDNA probe (ds-DNA, 5 ng/10 μ l) was conducted on the electrode that was prepared using f-MWCNTs-AuNPs-PPy/ITO. Later, the electrode was left undisturbed in a humid chamber at a temperature of 25 $^{\circ}$ C for a duration of 3 hours. The immobilization of probe dsDNA onto f-MWCNTs-AuNPs-PPy/ITO

modified electrode occurs an electrostatic interaction between positively charged f-MWCNTs-AuNPs-PPy/ITO and negatively charged dsDNA. The thiol molecule has a larger negative charge density than the dsDNA's negatively charged phosphate backbone. Therefore, under pH conditions of approximately 7.0, the negatively charged thiol group displays a greater affinity towards the positively charged f-MWCNTs-AuNPs-PPy/ITO thin film, as opposed to the phosphate group of dsDNA [22]. The complete operational process for immobilizing (dsDNA) is illustrated in figure 5. To eliminate the unattached probe from the bioelectrode, a sequence of rinses using TE buffer were executed, subsequently leading to desiccation in the surrounding atmosphere.



Scheme 1: A systematic portrayal of the immobilizing mechanism employed for the SH probe dsDNA on the f-MWCNTs-AuNPs-PPy/ITO modified electrode.

In this study, voltammetric measurements were done with an IRASOI PGS-10 potentiostat/galvanostat operated by LMS-27 software. In electrochemical testing, a 200 mL electrochemical cell with a modified ITO WE, an Ag/AgCl reference electrode as a working electrode (RE), and a platinum wire as counter electrode (CE) were used. Electrochemical Impedance Spectroscopy (EIS) investigations were carried out with a PGS-18 potentiostat. Figure 4 shows the Cyclic Voltammetry (CV) of the bare ITO and modified electrodes in 10 mM

$[\text{Fe}(\text{CN})_6]^{-3/-4}$ in 0.1 M KCl solution, at (-0.5 – 1.2) V potential range with a scan rate of 100 mV/s. The bare ITO electrode exhibited very low capacitive current and no Faradic current in the applied potential range.

The CV curve for the f-MWCNTs-AuNPs-PPy/ITO modified electrode shows that the oxidation peak current has a magnitude of approximately 0.0028 mA, while the redox peak separation voltage has

a wide range of about 0.43 V. However, upon immobilizing the dsDNA probe on the modified electrode surface of f-MWCNTs-AuNPs-PPy/ITO, a reduction in peak oxidation current of approximately 0.0024 mA was observed. This reduction can be attributed to the effective binding of the dsDNA probe to the electrode surface, which hinders charge transportation by blocking the conducting sites of the f-MWCNTs-AuNPs-PPy/ITO modified electrode with insulating probe DNA molecules. Furthermore, subsequent to the process of hybridization between the dsDNA and its corresponding complementary target, there

is an additional reduction observed in the redox peak currents, reaching a magnitude of 0.0020 mA. The determination of hybridization efficiency is accomplished by calculating the ratio of the coverage of the hybridized target to the coverage of immobilized probes. Therefore, cyclic voltammetry is utilized to estimate the surface coverages of f-MWCNTs-AuNPs-PPy/ITO and f-MWCNTs-AuNPs-PPy/ITO dsDNA bioelectrodes. This technique provides insight into the overall coverage of the species that is absorbed on the bioelectrode's surface, which is then carried out using the Brown-Anson model [22].

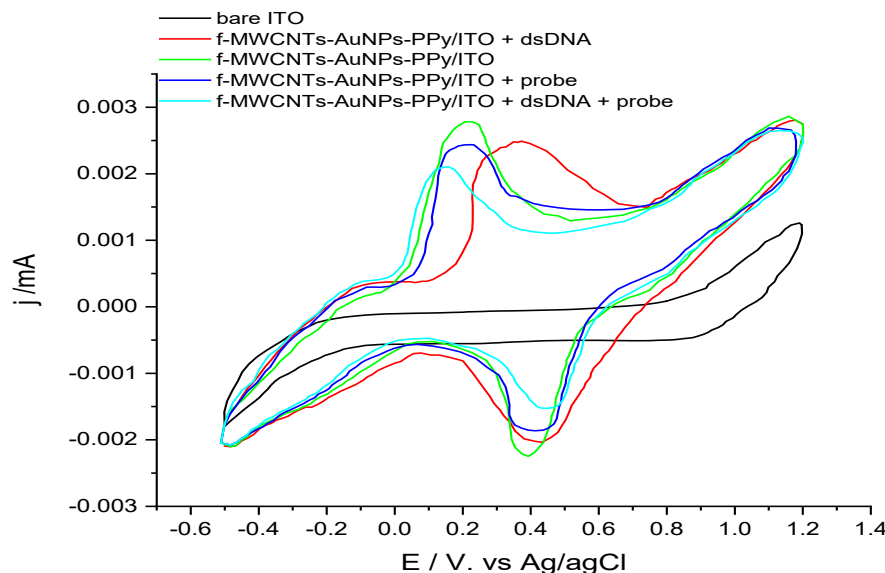


Figure 6: Cyclic voltammograms for bare ITO, f-MWCNTs-AuNPs-PPy/ITO, f-MWCNTs-AuNPs-PPy/ITO + probe, f-MWCNTs-AuNPs-PPy/ITO + dsDNA, f-MWCNTs-AuNPs-PPy/ITO + probe + dsDNA at scan rate 100 mV/s.

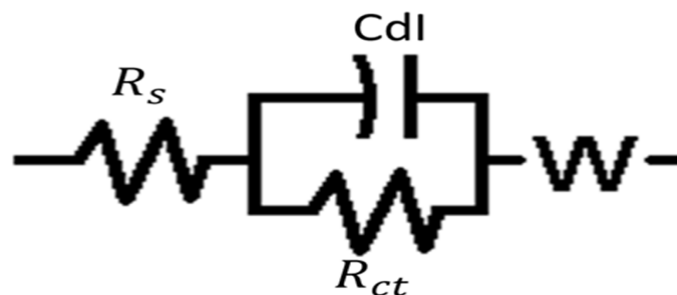


Figure 7: Equivalent electrical circuit used in EIS fitting data for electrodes.

In Electrochemical Impedance Spectroscopy (EIS), data collected from measurements are fitted to an equivalent electrical circuit model specified circuit to identify its characteristics. An equivalent electrical circuit was built employing ZSimpWin software using the impedance spectra. Randle's circuit represents the electrical circuit that serves as a model for the impedance spectra obtained through experimentation (Figure. 7) [23]. The interface between the electrode and the solution is composed of a parallel combination of resistance to charge transfer (R_{ct}) and a capacitor with a double layer (Cdl), along with the presence of resistance in the solution (R_s). When the frequency is low, impedance referred to as the Warburg impedance (Z_w) emerges due to the movement of the redox couple to and from the electrode. Nyquist plots, shown in Figure 8, were obtained at 0.2 V at a frequency range (0.1 - 100) KHz. In all of the prepared electrodes' electrochemical impedance spectroscopy (EIS) spectra, a distinct semicircle is readily observable. The magnitude of this semicircle is indicative of the

electron-transfer resistance (R_{ct}), thereby granting valuable understanding into the electron-transfer kinetics of the redox probe at the interface connecting the electrode and the electrolyte. The resistance associated with the transfer of charge (R_{ct}) was determined to be 79 Ω for the bare ITO electrode. In contrast, the R_{ct} for the f-MWCNTs-AuNPs-PPy/ITO modified electrode was approximately 29 Ω , which signifies the superior electron transfer capabilities of the f-MWCNTs-AuNPs-PPy/ITO based matrix in comparison to the ITO matrix. Upon the immobilization of dsDNA on the modified electrode consisting of f-MWCNTs-AuNPs-PPy/ITO, a notable increase in the R_{ct} value of up to 53 Ω is observed. The rise in the aforementioned value can be ascribed to the hindrance of electron movement, which is a direct result of the existence of DNA backbones that are negatively charged due to phosphatic composition. The $[\text{Fe}(\text{CN})_6]^{-3/-4}$ ions play a significant role in hindering the transfer of electrons, thereby confirming the successful immobilization of DNA.

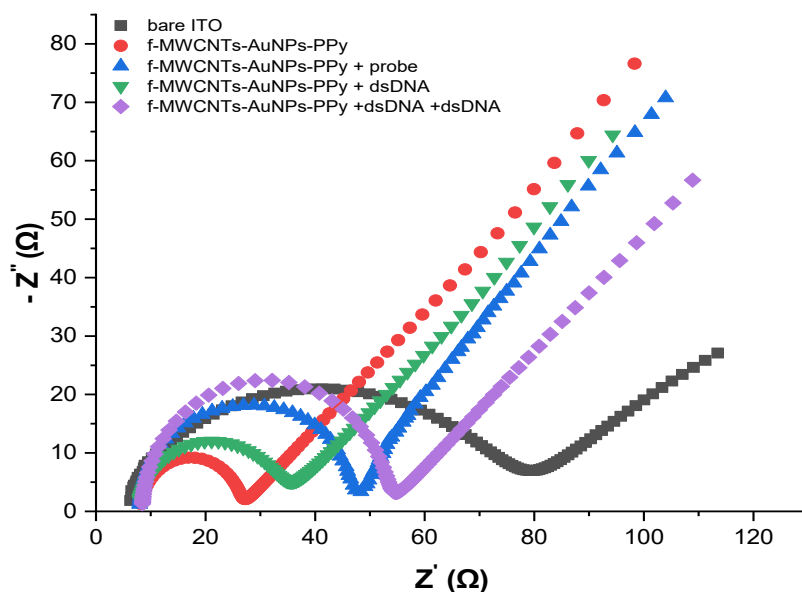


Figure 8: Nyquist diagrams of the bare ITO, f-MWCNTs-AuNPs-PPy/ITO, f-MWCNTs-AuNPs-PPy/ITO + probe, f-MWCNTs-AuNPs-PPy/ITO + dsDNA, f-MWCNTs-AuNPs-PPy/ITO + probe + dsDNA in (10 mM) $[\text{Fe}(\text{CN})_6]^{-3/-4}$ in (0.1 M) KCl.

Conclusions

This study delves into the factors underlying the dynamic correlation between R_{ct} and of f-MWCNTs-AuNPs-PPy, and concludes that the dominant forces for the presence or absence of dsDNA on the modified electrode surface are electrostatic interaction between dsDNA and PPy, as well as attractive motion. In other words, manipulating the proportion of PPy in the composites allows for control over the tendency of dsDNA movement, which is advantageous for the development of innovative biosensors. Furthermore, it can be inferred

that the increase in charge transfer resistance stems from the dsDNA remaining on the surface with a higher concentration of PPy, while the decrease in response can be attributed to the dsDNA detaching from the sensor.

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Reference

- [1] F. M. Ahmed and S. M. Hassan, "Optical and AC Electrical Properties for polypyrrole and polypyrrole/Graphene (ppy/gn) Nanocomposites," *Iraqi Journal of physics*, vol. 19, no. 51, pp. 72-78, 2021.
- [2] M. Alagappan, S. Immanuel, R. Sivasubramanian, and A. Kandaswamy, "Development of cholesterol biosensor using Au nanoparticles decorated f-MWCNT covered with polypyrrole network," *Arabian Journal of Chemistry*, vol. 13, no. 1, pp. 2001-2010, 2020.
- [3] A. A. Ansari, R. Singh, G. Sumana, and B. Malhotra, "Sol-gel derived nano-structured zinc oxide film for sexually transmitted disease sensor," *Analyst*, vol. 134, no. 5, pp. 997-1002, 2009.
- [4] A. P. Brown and F. C. Anson, "Cyclic and differential pulse voltammetric behavior of reactants confined to the electrode surface," *Analytical Chemistry*, vol. 49, no. 11, pp. 1589-1595, 1977.
- [5] E. E. Bruzaca *et al.*, "Electrochemical sensor based on multi-walled carbon nanotubes for imidacloprid determination," *Analytical Methods*, vol. 13, no. 18, pp. 2124-2136, 2021.
- [6] S. Cajigas, D. Alzate, and J. Orozco, "Gold nanoparticle/DNA-based nanobioconjugate for electrochemical detection of Zika virus," *Microchimica Acta*, vol. 187, pp. 1-10, 2020.
- [7] R. Elshafey, G. F. Abo-Sobehy, and A.-E. Radi, "Graphene oxide/graphene quantum dots: A platform for probing ds-DNA-dimethoate interaction and dimethoate sensing," *Journal of Electroanalytical Chemistry*, vol. 899, p. 115678, 2021.
- [8] D. Feng *et al.*, "Electrochemical DNA sensor for sensitive BRCA1 detection based on DNA tetrahedral-structured probe and poly-adenine mediated gold nanoparticles," *Biosensors*, vol. 10, no. 7, p. 78, 2020.
- [9] H. R. A. Hasanjani and K. Zarei, "DNA/Au-Pt bimetallic nanoparticles/graphene oxide-chitosan composites modified pencil graphite electrode used as an electrochemical biosensor for sub-picomolar detection of anti-HIV drug zidovudine," *Microchemical Journal*, vol. 164, p. 106005, 2021.
- [10] M. Jawad, S. Majid, E. Al-Ajaj, and M. Suhail, "Preparation and Characterization of Poly (1-vinylpyrrolidone-co-vinyl acetate)/Poly (methyl methacrylate) Polymer Electrolyte based on TPAI and KI," *Adv. Phys. Theor. Appl.*, vol. 29, pp. 14-22, 2014.
- [11] H. Jin *et al.*, "A label-free impedance-based electrochemical sensor based on self-assembled dendritic DNA nanostructures for Pb²⁺ detection," *Bioelectrochemistry*, vol. 149, p. 108312, 2023.
- [12] T. Kurinamaru, N. Kojima, and R. Kurita, "Immobilization of DNA on Biosensing Devices with Nitrogen Mustard-Modified Linkers," *Current Protocols in Nucleic Acid Chemistry*, vol. 77, no. 1, p. e85, 2019.
- [13] Y. Liu *et al.*, "In situ detection of melatonin and pyridoxine in plants using a CuO-poly (L-lysine)/graphene-based electrochemical sensor," *ACS sustainable chemistry & engineering*, vol. 7, no. 24, pp. 19537-19545, 2019.
- [14] C. Madhusudhan, K. Mahendra, B. Madhukar, T. Somesh, and M. Faisal, "Multifunctional polypyrrole/multi-walled carbon nanotube composite material: dielectric, humidity sensing and broadband EMI shielding properties," *Polymer Science, Series B*, vol. 63, no. 3, pp. 280-290, 2021.
- [15] L. Pilan and M. Raicopol, "Electrochemical DNA biosensors based on carbon nanomaterials," in *Carbon Related Materials: Commemoration for Nobel Laureate*

Professor Suzuki Special Symposium at IUMRS-ICAM2017, 2021: Springer, pp. 209-247.

- [16] A. Srinivasan, P. Ranjani, and N. Rajendran, "Electrochemical polymerization of pyrrole over AZ31 Mg alloy for biomedical applications," *Electrochimica Acta*, vol. 88, pp. 310-321, 2013.
- [17] A. Y. Taradh and W. R. Saleh, "Fabrication and Characterization of Functionalized Multi-Wall Carbon Nanotubes Flexible Network Modified by a Layer of Polypyrrole Conductive Polymer and Metallic Nanoparticles," *Nano Hybrids and Composites*, vol. 36, pp. 21-33, 2022.
- [18] M. Tommalieh, "Electrical conductivity characterization of chitosan/poly (vinyl alcohol) doped by bismuth oxide nanoparticles," *Composites Communications*, vol. 25, p. 100692, 2021.
- [19] L. T. Tran, H. V. Tran, H. T. M. Dang, A. Van Nguyen, T. H. Tran, and C. D. Huynh, "Electrosynthesis of electrochemically reduced graphene oxide/polyaniline nanowire/silver nanoflower nanocomposite for development of a highly sensitive electrochemical DNA sensor," *RSC advances*, vol. 11, no. 32, pp. 19470-19481, 2021.
- [20] J. Wang, H. Zhou, J. Liu, J. He, J. Liu, and W. Yang, "Electrochemical detection of DNA by formation of efficient electron transfer pathways through adsorbing gold nanoparticles to DNA modified electrodes," *Microchemical Journal*, vol. 169, p. 106581, 2021.
- [21] G. Wei *et al.*, "Immobilization-Free Electrochemical DNA Sensor based on signal cascade amplification strategy," *Biotechnology and Applied Biochemistry*, vol. 69, no. 3, pp. 1036-1046, 2022.
- [22] Q. Wu, Y. Zhang, Q. Yang, N. Yuan, and W. Zhang, "Review of electrochemical DNA biosensors for detecting food borne pathogens," *Sensors*, vol. 19, no. 22, p. 4916, 2019.
- [23] H. Zhao, Q. Liu, J. Wang, A. Huang, B. Qiu, and Z. Lin, "Electrochemical aptamer sensor based on metal ion-labeled polyethyleneimine gold nanoparticles for simultaneous detection of multiple disease markers," *Electrochimica Acta*, vol. 399, p. 139423, 2021.



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