Progress in Chemical and Biochemical Research

Journal homepage: www.pcbiochemres.com

Original Research Article

Design and Fabrication of Modified DNA-Gp Nano-Biocomposite Electrode for Industrial Dye Measurement and Optical Confirmation

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ARTICLE INFO

Article history

Submitted: 2022-09-28
Revised: 2022-11-05
Accepted: 2022-12-15
Available online: 2022-12-23
Manuscript ID: PCBR-2210-1236
DOI: 10.22034/pcbr.2022.367576.1236

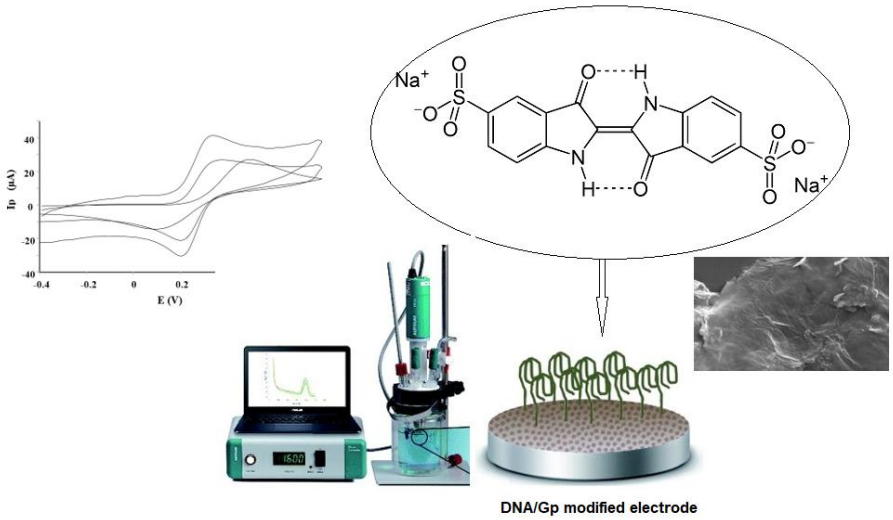
KEYWORDS

Biosensor
Electrochemistry
Dye measurement
Indigo carmine
Graphene
DNA

ABSTRACT

Indigo carmine dye is used in food and textile industries and has been proven to harm living organisms due to its binding to DNA in living organisms. In this research, a modified electrode by DNA/Gp nanocomposite on glassy carbon was designed and studied to measure this dye in actual samples. The results show the sensitivity of the modified electrode to this dye compared with the electrode without DNA/Gp nanocomposite. SEM, FT-IR, fluorescence, and electrochemical techniques were used to fabricate and examine nanocomposite and its reactions. The studies indicate that the electrochemical reaction is under adsorption control. The detection limit is 8.5×10^{-7} M, and the repeatability and stability of the electrode are at the optimal level.

GRAPHICAL ABSTRACT



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1- Introduction

Electrochemical biosensors have an extensive and exciting experience as analyte recognition and measurement devices. These devices have many advantages, including a low-cost and fast method, high sensitivity, miniaturization capability, and simple operation and recording of signals [1]. Several electrochemical assessments have been carried out to connect sensors and biosensors [2–4]. DNA as a biosensing element has received significant attention in recent years among the different biosensor types. DNA is a single- or double-stranded oligonucleotide with improved affinity to various targets, e. g. dyes [5]. Such as other sciences like pharmacy, advanced systems drug delivery, identification of new drugs, and assessment of biological activity have shown the promising results [6, 7].

Graphene is used in the construction and design of electrochemical sensors due to its high electrical conductivity and ability of electrocatalytic activity. Graphene has individual layers of carbon honeycomb network due to its excellent electrical and thermal conductivity properties. It has high density and mobility of charge carriers, optical conductivity, and mechanical properties. It has turned into unique material, so it is used in constructing and designing electrochemical sensors [8].

A bio-nano composite is obtained by combining DNA and graphene, which has both characteristics. Increasing the surface area and electrical conductivity of the used electrode increases the sensitivity and selectivity of the designed electrode [9].

Currently, environmental pollution is considered a global problem. Dyes are among the most dangerous groups of chemical compounds found in industrial wastewater, which are of considerable importance due to some reasons, such as reducing light penetration and causing disturbances in the photosynthesis process [10]. At the same time, they cause allergies, dermatitis, skin irritation, cancer, and genetic mutations in humans [11].

Colored waste produced by industries such as textile, paper, rubber, and plastic, if discharged into the environment before treatment, can cause many significant environmental problems. These dyes are usually chemically and photolytically stable. Likewise, due to having complex aromatic structures, they remain unchanged during the natural processes of biological decomposition and therefore cause turbidity and unpleasant odours [12].

In the past, indigo carmine was naturally obtained from plants, but today, its thousands of tons are produced and consumed artificially. This dye has very low toxicity through inhalation, but the average lethal dose of indigo is 9111 mg/kg for mammals [13–15]. Therefore, its measurement and removal from industrial and environmental effluents are crucial.

In addition to the electrochemical methods, dyes can be investigated using spectroscopic methods due to their very high quantum efficiency [16–20].

In this research, in addition to measuring indigo carmine dye by nano biosensor designed with DNA and graphene, the interaction between this dye and DNA was also studied by fluorescence spectroscopy.

2. Experimental

2.1. Methods and materials

Some of the materials and reagents used in this research include sodium nitrate, potassium permanganate, indigo carmine, sulfuric acid, hydrazine, hydrogen peroxide, hydrochloric acid, ethanol, iron III hexacyanide, potassium chloride, and sodium hydroxide purchased from Merck and they were used without purification. Buffer Tris hydrochloride, DNA, and graphite powder were obtained from Sigma and used the same way.

To examine the nanocomposite and its components, the FT-IR spectrum was taken. Scanning electron microscope (SEM) was used to understand the synthesis of graphene nanoparticles. Two-beam UV-Vis and fluorescence devices made by Perkin Elmer company were used to investigate the DNA solution and the interaction of dye and DNA.

All the voltametric tests were performed by μ-Autolab Type III (FRA2) Potentiostat/ galvanostat, a single-cell structure with three electrodes. The three-electrode system includes a glassy carbon electrode as a working electrode, a saturated calomel electrode (Ag/AgCl) as a reference electrode, and a platinum electrode as an auxiliary electrode; all three electrodes are made by Metrohm company.

2.2. Preparation of DNA solution

A thin strand of DNA fibre was separated, dissolved in a small glass container in double distilled water, and stored in a refrigerator at 4 °C for 24 hours to prepare the DNA solution. After that, the resulting solution was gently stirred for 20 minutes until all DNA was uniformLy dissolved in water. This solution was prepared once every 9 days. The absorption of DNA solution by UV-Vis device was read. The UV absorption ratio of DNA solution at 260/280 nm should be between 1.8 and 2, which indicates that the DNA is sufficiently free of protein. The concentration of the DNA stock solution used in this experiment was 30.5 μg/mL determined by the UV absorption spectroscopy at a molar absorption coefficient of 6600 $M.cm^{-2}$. All the solutions were prepared in Tris-HCl buffer with pH=7.4, and measurements were done at room temperature [21–23].

2.3. Synthesis of graphene oxide, graphene nanoparticles, and DNA-Gp nanocomposite

Graphite is oxidized in the presence of strong oxidants and will have oxygenated groups on both sides of its plates. Graphene oxide was synthesized by a modified method. In this method, a 1:5 ratio of sulfuric acid was added to phosphoric acid (40:360) to a mixture of 3 grams of graphite plates and 18 grams of potassium permanganate. The reaction mixture was stirred vigorously and heated for 12 hours at 50 °C until the oxidation reaction is complete. At the end of the reaction, the mixture was put in an ice bath and reduced its temperature to 0 °C . Then, 9 mL of H_2O_2 30% was slowly added until the colour of the resulting solution turns from dark brown to light brown. This solution is mixed with 200 mL of HCl 30% and 200 mL of water, and 211 mL of ethanol steeply and centrifuged it. After each washing cycle, the pH of the supernatant solution was measured until the pH of the solution approached neutral pH.

Graphene oxide is regenerated in the presence of a strong reductant, such as hydrazine, and turns into graphene. Graphene oxide lost its oxygen functional groups in the presence of hydrazine at 100 °C and was turned into oxygen-free graphene sheets. For graphene synthesis, 40 mL of 85% (W/W) hydrazine was added to 10 mL of graphene oxide; 2.5 mg/mL dissolved in water by a homogenizer, and heated the resulting mixture under reflux for 3 hours. Thereafter, the mixture was centrifuged 3 times with double-distilled water to wash and separate all the remaining hydrazine [1, 21].

For nanocomposite preparation, 100 mg of graphene prepared in the previous step was added to 40 mL of water. Graphene is a non-polar material that does not dissolve easily in water. Therefore, the obtained mixture was turned into a completely homogeneous mixture in the ultrasonic device, and then 10 mL of 2.5 mg/mL graphene homogenized with 20 mL of DNA solution was mixed with 6 mg/mL at 100 °C. The obtained mixture was stirred for half an hour. Next, the mixture was heated at the same temperature for 3 hours under reflux. Therefore, DNA/GP nanocomposite was created.

2.4. Preparation of modified electrode with DNA-Gp

Any contamination on the surface of the electrode increases the electron transfer resistance and slows down the reaction speed. Therefore, the surface is cleaned before using the electrode, which can be done physically (polishing) or chemically (for example, washing with solvent). The easiest way to activate the surface is to clean the electrode surface using shade materials such as alumina or diamond powder (with a grain size of 0.05-5 µm).

After cleaning the electrode surface and drying it completely, 5 µl of prepared DNA-Gp solution is placed on the electrode at room temperature, and then it is put in a suitable place for 24 hours to dry [9].

2.5. Interaction between indigo carmine dye and composite by electrochemical method

To identify and examine the surface of the modified electrode, the CV peak was taken from three unmodified Gp and DNA/Gp nanocomposite-modified electrodes in a 5 mM iron cyanide solution. Each modified electrode was made by dropping 5 µl of Gp or DNA/GO solution on the surface of the cleaned electrode and it was placed under the hood for 24 hours until it dries.

To examine the pH effect on the indigo carmine-nanocomposite interaction, first buffers with pH 3, 4, 6, 7, 8, and 9 were prepared. The CV peaks were taken in the absence and presence of indigo carmine. All buffer solutions have 0.1 M KCl and 10^{-3} M indigo carmine dye and are deoxygenized for 10 min by nitrogen gas.

To examine the dye concentration effect, 1-6 µM solutions by Tris-HCl buffer at pH=3 and 1 µM of KCl were prepared, and after 10 minutes of deoxygenation, the electrochemical peak was taken.

One of the most important parameters in electrochemical studies is the potential scanning rate. In the optimal conditions (2.7×10^{-6} M of indigo carmine, pH=3 of Tris-HCl buffer and 0.1 M KCl), at 0.01, 0.05, 0.1, 0.15, 0.2 and 1 V/s scan rate, CV peaks were taken.

For the stability study of the designed electrode, 25 mL of 10^{-6} M indigo carmine dye solution was poured into a flask (Tris-HCl buffer, KCl 1M). This solution was deoxygenated for 10 minutes. After that CV peak was taken, the electrode was placed in 1 M Tris HCl buffer for half an hour and washed with distilled water so that the composite was not removed from the electrode's surface. A CV peak was retaken in the previous solution, and this cycle was repeated several times.

Designed electrode reproducibility was studied; a solution with a 10^{-6} M concentration of the dye was poured into an electrochemical flask, and a CV peak by the modified electrode was taken. Then, the modified electrode was polished and modified again, and CV was taken from the solution and this was repeated for several times.

For the accuracy and precision checking of the designed electrode, a 1.5×10^{-6} M solution of indigo carmine dye at pH=3 and ionic strength of 1 M KCl was prepared, and the amount of dye was measured by DNA/Gp modified electrode.

2.6. Fluorescence method investigations

To determine λ_{ex}, a three-dimensional spectrum of indigo carmine dye was taken. Therefore, 10 µl of indigo carmine dye (10^{-6} M) was dissolved in 5 mL of Tris-HCl buffer (0.1 M), and then a 3D spectrum was taken from it, which was obtained from λ_{em}=450-750 nm.

The optimal time interaction between the indigo carmine dye and the DNA/Gp nanocomposite was obtained. 3 mg of the nanocomposite was poured into 5 mL of 0.1 M Tris-HCl buffer. It was sonicated until dissolved the nanocomposite. After that, 25 µl of 10^{-3} M indigo carmine dye was added to the composite solution. At appropriate time intervals, the supernatant from the solution was taken by centrifugation, and the fluorescence spectrum was recorded and remixed.

To study the pH effect by optical method, 2.5 mL of Tris-HCl buffer with pH equal to 3, 5, 7.4, 9,

and 10 were added in five reaction vessels, and 1 mg of DNA/Gp nanocomposite was dissolved in them along with 15 µl of 10^{-3} M indigo carmine dye solution. After 24 hours, fluorescence was taken from them.

To examine the effect of ionic strength on the indigo carmine dye interaction, solutions with a concentration of 5.2×10^{-6} M of dye were prepared. KCl adjusted the ionic strength to 0.05, 0.07, 0.1, 0.5, and 1 M, and the amount of dye interaction was measured by fluorescence.

3. Results and Discussion

3.1. Spectral investigations

In the infrared spectrometry method, bond vibration is investigated, caused by the change of bond length and bond angle in molecules to identify organic compounds and functional groups. The molecules' constant dipole moment's vibration creates an electric field that varies with time and causes its reciprocal change with radiation. Therefore, it is necessary that its dipole moment changes during the vibration, so most substances except non-polar substances such as Cl_2 have absorption in this spectrum range.

Graphene oxide (GO) was prepared by a modified method from graphite oxide. Although it is impossible to determine the exact structure of GO, it is a structure of a continuous network of graphene interrupted by epoxy, alcohol, ketone, and carbonyl groups on both sides of the plane and its edges. Sulfuric acid is used to oxidize graphite plates, and potassium permanganate oxidizes the remaining unoxidized graphite plates. Using the FT-IR spectrum, the functional groups have been identified, which include: O-H stretching vibration of 3432 cm^{-1}, CH_2 stretching vibration of 2923 cm^{-1}, C=O stretching vibration of 1714 cm^{-1}, C=C stretching vibration of unoxidized carbon-carbon bond the residue of graphite oxide with sp^2 hybrid is 1629 cm^{-1}, the C=O stretching vibration of the carboxyl group is 1429 cm^{-1}, the C-O stretching vibration of the hydroxyl group is 1026 cm^{-1}, and the C-O-C stretching vibration of epoxy is 873 cm^{-1}. Figure (1A) [9] depicts the FT-IR spectrum of graphene oxide.

The functional groups of graphene oxide are regenerated by reaction with hydrazine. OH, groups are reduced, and then the carboxylic acid groups replace the OH group, and the rings of the epoxy groups are opened. Therefore, the intensity of epoxy and hydroxy groups decreases. Using the FT-IR spectrum, functional groups have been identified, which include O-H stretching vibration 3567 cm^{-1}, CH_2 stretching vibration 2925 cm^{-1}, C=O stretching vibration 1737 cm^{-1}, C=C stretching vibration 1616 cm^{-1}, carboxyl C-O stretching vibration 1502 cm^{-1}, C-O stretching vibration of the hydroxyl group is 856 cm^{-1}, and C-O-C stretching vibration of epoxy is 580 cm^{-1}. The FT-IR spectrum of pure graphene is displayed in Figure (1B) [24].

Nucleic acids, like carbohydrates and proteins, are polymers. The monomer units of nucleic acids are called nucleotides. Each nucleotide comprises three parts; 1- a 5-carbon ribose sugar or deoxyribose RNA-DNA, 2- one to three phosphate groups, and 3- a nitrogenous organic base purine or pyrimidine.

The FT-IR spectrum has identified functional groups. N-H stretching vibration at 3590 cm^{-1}, O-H stretching vibration related to the bond between guanine bases with cytosine or adenine with thymine at 3342 cm^{-1}, C=C stretching vibration related to 5-carbon sugar 1638 cm^{-1}, FT-IR spectrum of DNA is demonstrated in Figure (1D) [24]. The composite containing pure graphene and DNA was identified as containing all the peaks related to pure graphene and DNA seen; only their intensities changed, indicating that the composite was well-synthesized. The FT-IR spectrum of the DNA/Gp composite is given in Figure (1C).

The most crucial factor in the study of nanomaterials is to study these materials' shape, dimensions, and morphology. Three different

techniques are used in the imaging of nanomaterials. The optical methods are rarely used in nanomaterials because of the low separation power of these methods due to the enormous wavelength of light photons.

The SEM image in Figure 2 shows that the graphene sheets are well-formed. Individual graphene sheets on the nanometre scale are visible in the image, indicating their successful synthesis.

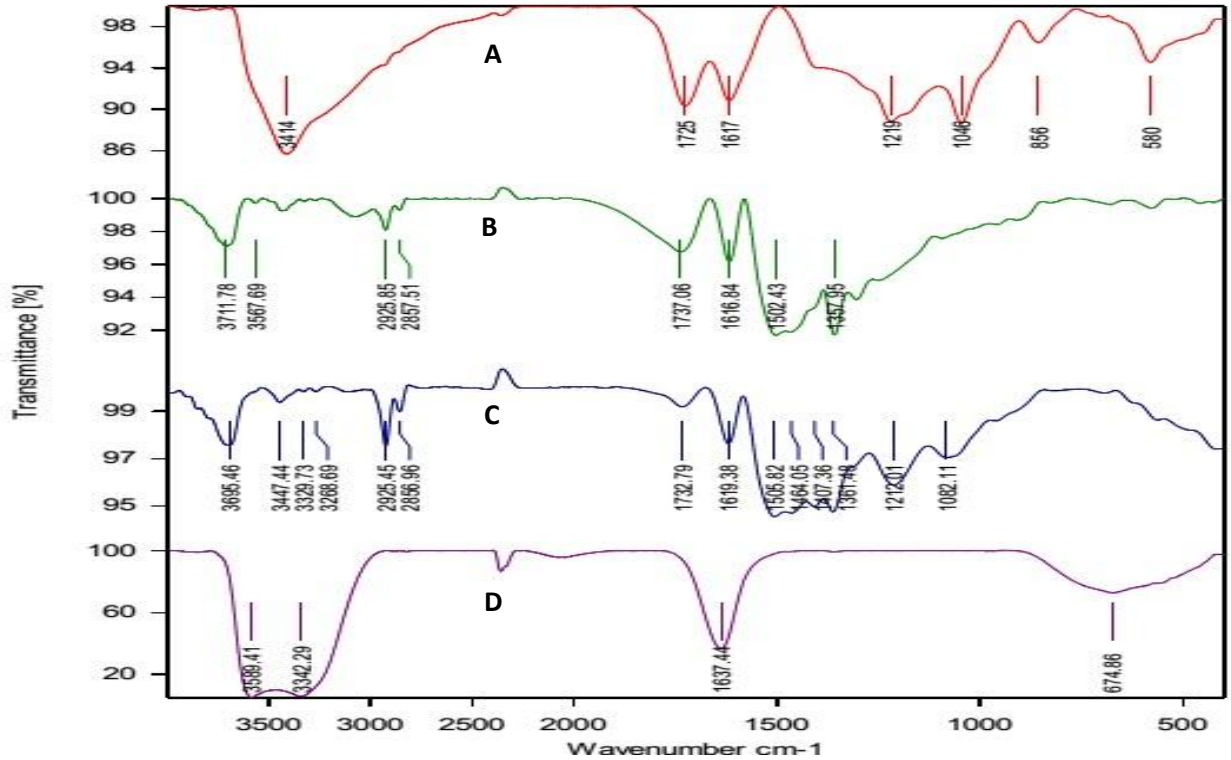


Fig. 1. FT-IR spectrum of DNA/Gp composite, A) graphene oxide, B) pure graphene, C) composite, and D) DNA.

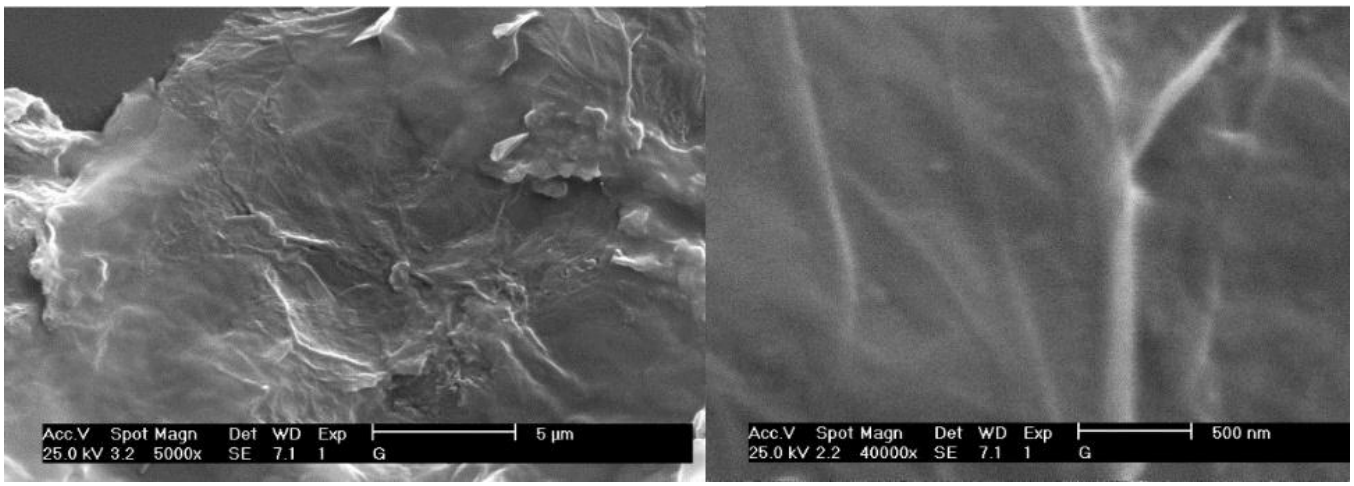


Fig. 2. SEM electron microscope image of graphene.

The DNA strands in the graphene sheets were shown of the composite on the surface of the modified electrode, and an SEM photo was taken, which can be seen in Figure 3. Evidently, the DNA strands are well placed on the graphene sheets on the surface of the modified electrode.

As can be seen in Figure 4, DNA has an absorbance with a maximum of 261 nm. Graphene has also the maximum absorption at the wavelength of 271 nm. When these two are mixed, and the DNA/Gp nanocomposite is obtained, an absorption peak with the maximum absorption at 272 nm wavelength is obtained due to the electron transfer and covalent transfer between graphene and DNA, which causes an average absorption at this wavelength.

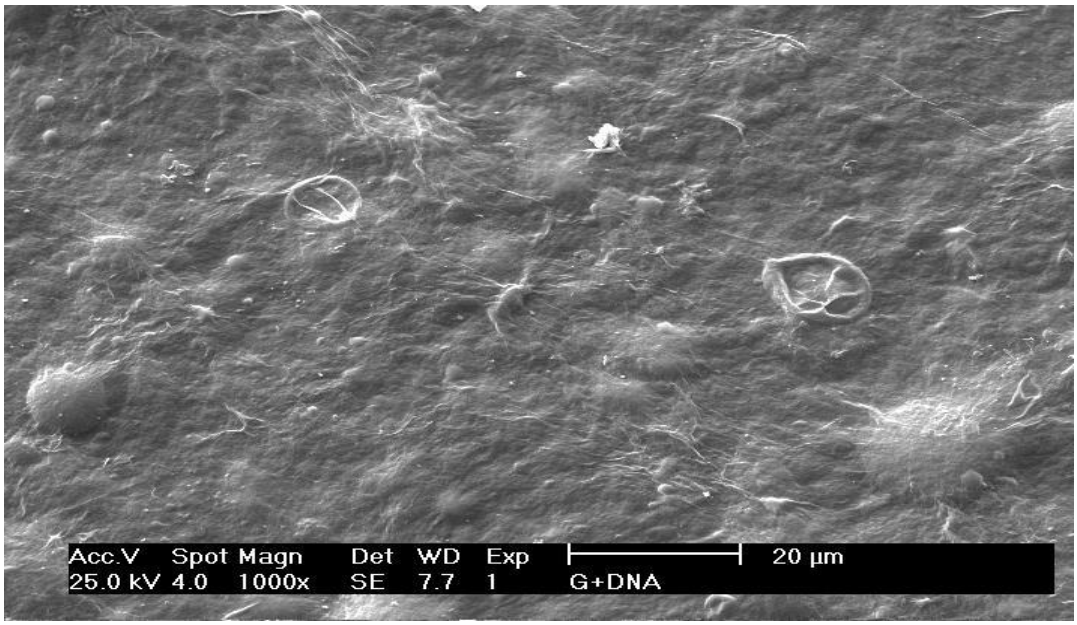


Fig. 3. SEM electron microscope image of DNA/Gp composite.

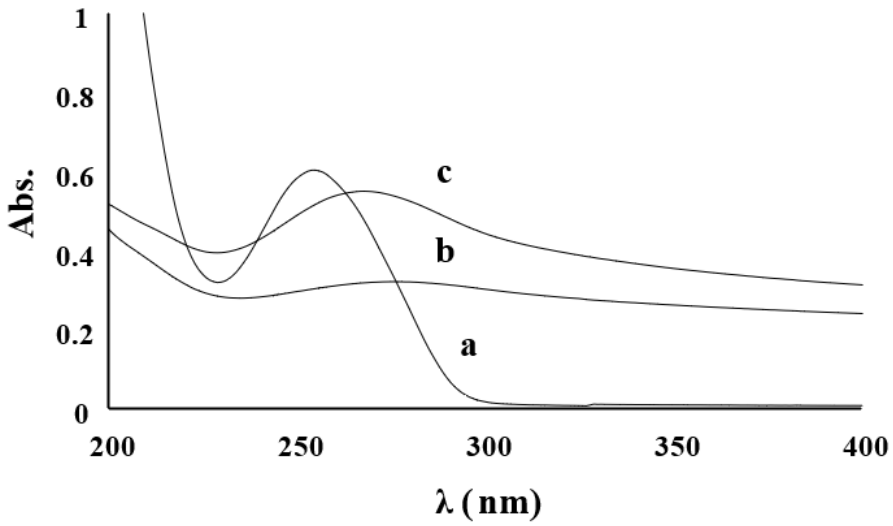


Fig. 4. UV-Vis spectrum of a) DNA, b) Gp, and c) DNA/Gp composite.

3.2. Investigating electrochemical studies

Cyclic voltammetry is a powerful technique in electrochemical studies that provides much information about the potential and electrochemical processes taking place on the surface [25, 26].

Cyclic voltammetry method in $Fe(CN)_6^{3-/4-}$ system is an excellent method to investigate the characteristics of the modified electrode surface.

As can be seen in Figure 5, the unmodified electrode has an oxidation-reduction peak of $Fe(CN)_6^{3-/4}$. After modifying the Gp's glassy carbon electrode, Gp is a conductor of electricity. As a result, it dramatically accelerates the transfer of electrons and causes an increase in the intensity of the current. Another point is that the oxidation-reduction peak of the Gp electrode moves towards negative values, which indicates the ease of oxidation. This observation shows that Gp increased the current and made the oxidation-reduction reaction easier. When the DNA/Gp is on the surface of the glassy carbon electrode, the current is reduced due to the repulsion of the negative charge of DNA and the negative charge of $Fe(CN)_6^{3-/4-}$. In addition, the oxidation peak has shifted towards positive potentials, which indicates that the reaction is complicated due to the electrostatic repulsion.

The modified electrode with DNA/Gp composite is first placed in Tris-HCl buffer, its CV peak is recorded, and then its CV peak is recorded in the buffer solution and the presence of indigo carmine. According to Figure 6, the modified electrode has a peak in the Tris-HCl buffer. The groups that cause the modified electrode to peak include the carboxylic groups on the surface of the graphene sheets and DNA. When indigo carmine is added to the modified electrode, the redox peak decreases due to the binding of indigo carmine to the electrode surface due to the sulfur groups present in its structure and the inhibition of electrochemical reactions on the electrode surface. Thus, there is a decrease of the current in the oxidation and a reduced half-reaction. In addition, the peak of the electrode shifts towards the positive potentials.

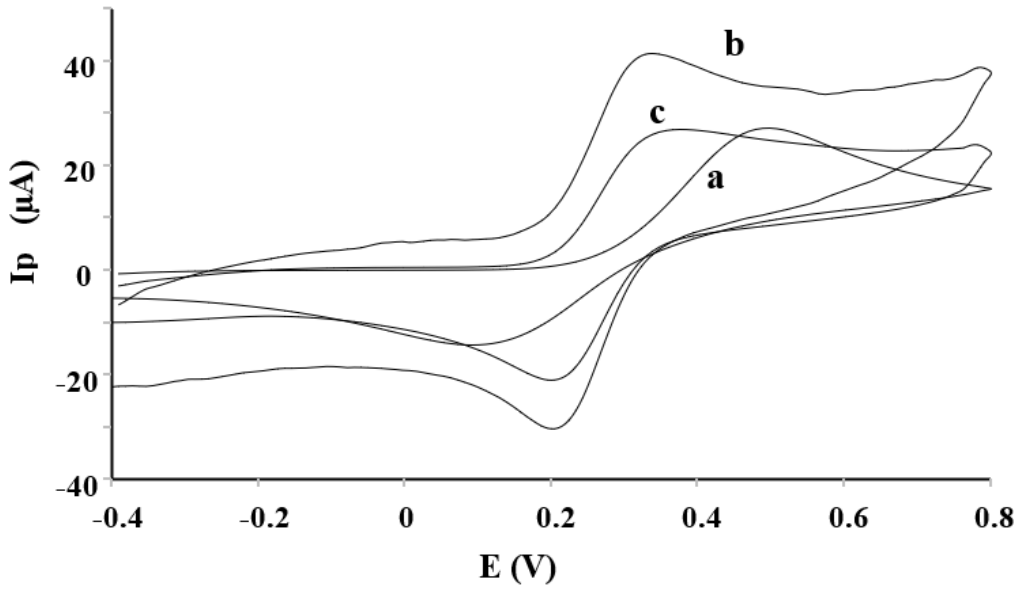


Fig. 5. Cyclic voltammetry of: a) unmodified electrode, b) modified glassy carbon electrode by Gp, and c) modified electrode by DNA/Gp. KCl 0.1 M, 5 mM $Fe(CN)_6^{3-/4-}$.

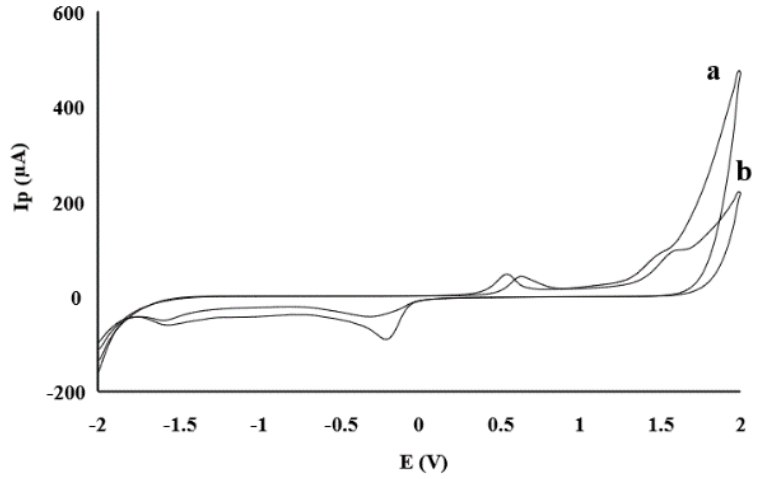


Fig. 6. Cyclic voltammetry of modified electrode with DNA/Gp composite in Tris-HCl in (a) the presence and (b) absence of indigo carmine.

When pH of the solution changes, according to the structure (Figure 7), indigo carmine is converted into an anion in alkaline conditions by losing the protons attached to the nitrogen group. DNA has a negative charge in alkaline conditions because DNA bases have a negative charge by losing hydrogens attached to amino groups. For the sake of the negative charge of indigo carmine dye and DNA, electrostatic repulsion between the dye and DNA occurs. Thus, peak intensity will be changed very small in alkaline conditions.

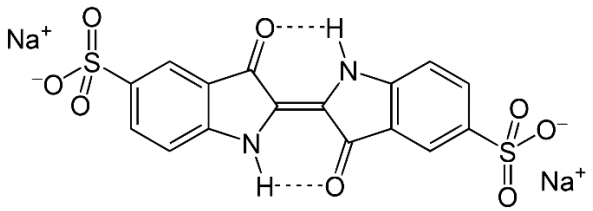


Fig. 7. Indigo carmine structure.

However, due to the protonation of DNA and indigo carmine dye in acidic environments, the negative charge is lost, and the repulsion is reduced. At the most extreme pH, with DNA becoming positive, there is much less electrostatic repulsion between neutral indigo carmine dye and positive DNA, and the chance of hydrogen interaction increases. Therefore, in acidic environments compared to alkaline environments, the interaction between dye and DNA is more significant, and the decrease in current intensity will be greater. Therefore, pH = 3 was chosen as the optimal pH (Figure 8).

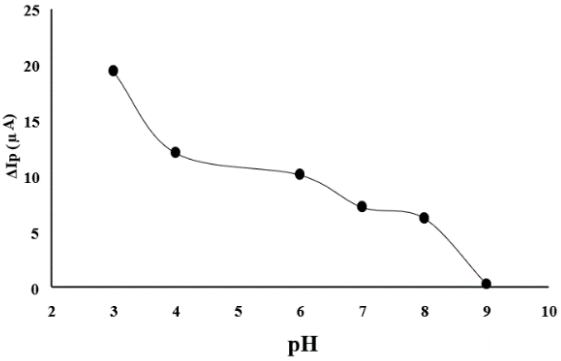
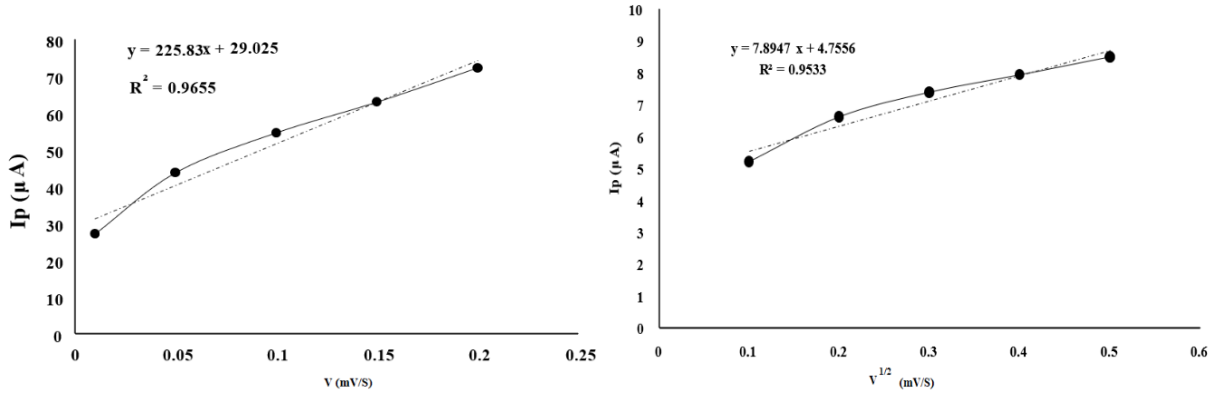


Fig. 8. Peak current diagram of the modified electrode in the presence of indigo carmine at different pH.

The modified DNA/Gp electrode in Tris-HCl buffer solution and indigo carmine dye has an oxidation-reduction peak with increasing intensity of scan rate. In addition to increase the intensity of the current, it also moves towards higher potentials (Figure 9).



A B

Fig. 9. Changes in the intensity of the oxidation peak current to a) scanning rate and b) the square of the scanning rate of the DNA/Gp modified electrode potential in the presence of indigo carmine dye.

The electrode current relative to the scanning rate was used to study the electrode surface absorption mechanism. The comparison of Figures 9A and 9B concluded that according to the higher R^2, the current is under the absorption control. The stability of the designed electrode has been investigated to prove the number of times that can be used to measure indigo carmine. The modified electrode with DNA/Gp is placed in a solution containing Tris-HCl buffer and indigo carmine dye solution, and a peak is taken from it. To clean the analyte on the electrode surface, place it in a buffer solution for 30 minutes and wash it with distilled water so that the composite is not removed from the electrode surface. However, the indigo carmine on the surface of the electrode is separated. Then a DPV peak is retaken inside the previous solution. This action is repeated five times. After examining the data, the standard deviation value for 5 data equals 5.94. For the first three data, the standard deviation is equal to 3.8. Given that 3.8 is much less than 5%, 5.94 is greater than 5%. Therefore, the repeatability of this electrode is three times. So, this electrode can be used three times without error (Figure 10).

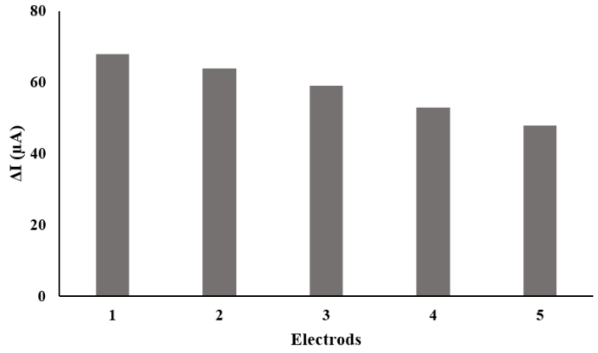


Fig. 10. Stability study graph according to the DNA/Gp modified electrode number in 0.1 M Tris-HCl buffer.

Reproducibility has been investigated to prove that the electrodes of the same barouche are made equally.

This study investigated reproducibility by making the modified DNA/Gp electrode four times and measuring equal amounts of indigo carmine dye in Tris-HCl buffer. After getting the first desired peak, the electrode was utterly polished, and the electrode was modified again for measurement.

After examining the data, using equation 1, the value of the standard deviation of the reproducibility of the electrode manufacturing was obtained (SD = 1.4198).

$$s = \sqrt{\frac{\sum(X_i - \ddot{x})^2}{n-1}} \quad (1)$$

As can be seen, the standard deviation calculated after making the electrode four times is less than 1.9. Therefore, making the electrode with the same method has been repeatable and successful.

To draw the calibration curve related to indigo carmine dye measurement, different concentrations of indigo carmine were added to the electrochemical cell containing Tris-HCl buffer. A peak was taken from the solutions using the modified DNA/Gp electrode. The electrode has a negative signal, meaning the current decreases as the analyte concentration increases. As shown in Figure 11, the electrode's response was studied in the concentration interval between 1 to 6 µM; the electrode becomes insensitive to indigo carmine from the 5.5 µM onwards. The cause of this phenomenon is the saturation of the surface concerning indigo carmine and the lack of response to higher concentrations of indigo carmine. As a result, the electrode can be used in concentrations lower than 5.5 µM of indigo carmine.

The statistical methods for determining and comparing detection limits are also important. The most commonly accepted qualitative definition for *detection limit* is the minimum concentration or weight of a species that can be detected with a certain degree of accuracy. To calculate the detection limit empirically, we use the following equation:

$$DL = \frac{3S_b}{m} \quad (2)$$

The Sb value is obtained with the help of the difference between the actual concentration values and the calculated concentrations with the help of the line equation.

$$S_b = \sqrt{\frac{\sum(c - c')}{n-1}} \quad (3)$$

This study's detection limit of indigo carmine dye concentration is 8.5×10^{-7} M [25,27].

To measure indigo carmine in an actual sample by the standard addition method, 25 µl of indigo carmine dye with a concentration of 10^{-3} M was added to 10 mL of water selected as an accurate sample.

After taking the CV peak from the resulting solutions and drawing the curve, the difference between the electrode current in the buffer and the solution containing the analyte concerning the added indigo carmine concentration, the required curve was obtained. By putting a zero value instead of y in the equation of the resulting line, the concentration of indigo carmine in the accurate sample was equal to 1.98×10^{-6} M.

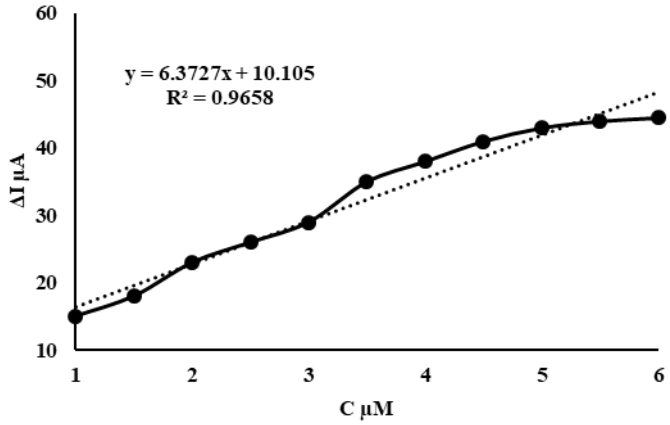


Fig. 11. Calibration curve of modified DNA/Gp electrode in different concentrations of indigo carmine dye.

3.3. Fluorescence studies

To confirm the results obtained by the electrochemical method and further study, the interaction of the indigo carmine dye with DNA by fluorescence spectroscopy method was examined as one of the most sensitive and selective methods [9]. In this method, the interaction time between the indigo carmine dye and the DNA/Gp composite was taken to section 2, the interaction at different times in the fluorescence spectrum. As can be seen in Figure 12, the indigo carmine fluorescence intensity decreases due to the absorption by the DNA/Gp nanocomposite, and the maximum decrease occurs after 24 hours. After this time, no decrease in fluorescence intensity is observed. 24 hours was chosen as the optimal time for the subsequent experiments.

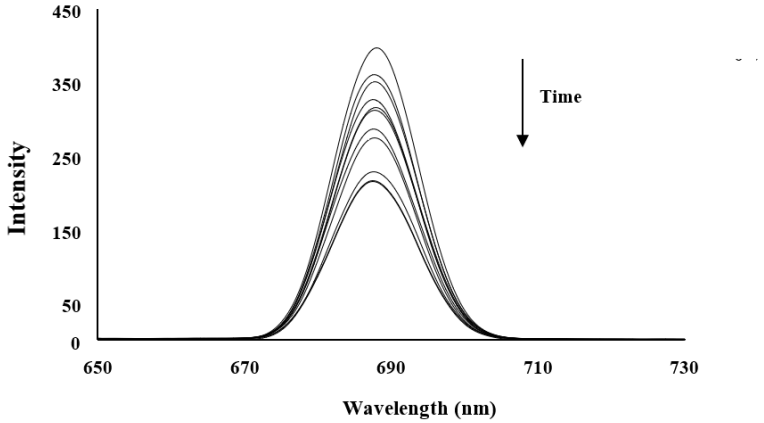


Fig. 12. Fluorescence of DNA/Gp nanocomposite solution and 0.1 M Tris_HCl buffer, 1 µM indigo carmine dye in 1 to 29 hours.

To obtain the best pH for the interaction between the indigo carmine dye and DNA/Gp composite, different solutions with pH between 3 and 10 were prepared from indigo carmine dye and composite. After 24 hours, the decrease in fluorescence absorption was measured. According to Figure 13, it is concluded that the most significant difference is observed at pH=3. Therefore, pH = 3 is the optimum pH in the interaction of indigo carmine dye and nanocomposite.

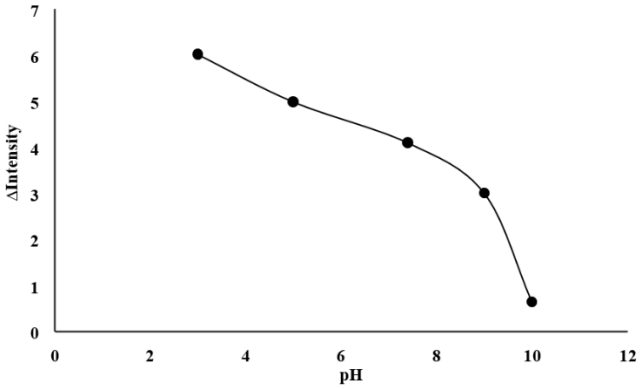


Fig. 13. Fluorescence intensity difference graph according to pH.

Due to the indigo carmine and DNA's structure, there is the most negligible repulsion and the highest chance of interaction between the dye and the composite in an acidic environment. Therefore, the most significant decrease in fluorescence intensity is observed at pH=3 [28].

The ionic strength effect on indigo carmine dye removal was investigated. Solutions with a concentration of 5.2×10^{-6} M of the dye were prepared. KCl adjusted the ionic strength in the ionic strength of 0.05, 0.07, 0.1, 0.5, and 1 M regulation. The dye removal rate in the presence of nanocomposite by fluorescence method was studied. According to Figure 14, as the ionic strength increases, the intensity of interaction between indigo carmine dye and DNA/Gp composite decreases. As a result, we have the highest interaction at the lowest ionic strength. With the increase of ionic strength due to the charge of dye, nanocomposite, and electrostatic repulsion, the intensity of fluorescence increases, so the interaction decreases [27].

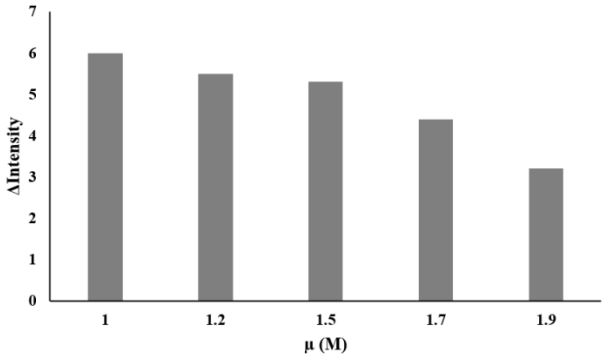


Fig. 14. Diagram of fluorescence intensity difference according to the change of ionic strength of the solution.

4- Conclusion

This research concluded that these nanomaterials were synthesized correctly, based on the investigations carried out by measuring various parameters, including FT-IR spectra and SEM photographs of graphite, graphite oxide, and graphene. The modified electrode was designed by DNA-GO nanocomposite to measure indigo carmine dye and investigate their interaction. The study of the pH effect found that in an acidic environment, with an increase in the H^+ concentration, the current due to oxidation-reduction decreases. In the study of the potential scan rate, it was found that the electrochemical

reaction is under absorption control, and the repeatability and stability of the electrode are optimal. In the investigation of the linear range, it was found that the detection limit of indigo carmine is 8.5×10^{-7} M. In the investigation of the measurement of the accurate sample by the standard increase method, the concentration of indigo carmine in the accurate sample was 1.98×10^{-6} M. The obtained results were also confirmed by the optical spectroscopy.

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HOW TO CITE THIS ARTICLE

Hossein Peyman*. Design and Fabrication of Modified DNA-Gp Nano-Biocomposite Electrode for Industrial Dye Measurement and Optical Confirmation. Prog. Chem. Biochem. Res, 5(4) (2022) 391-405.

DOI: 10.22034/pcbr.2022.367576.1236
URL: http://www.pcbiochemres.com/article_163512.html