Application of 2D nanomaterials in sensors for health and environmental monitoring

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A graphene quantum dots–glassy carbon electrode-based electrochemical sensor for monitoring malathion

Sanju Tanwar*1, Aditi Sharma2 and Dhirendra Mathur1

Abstract

Graphene quantum dots (GQDs) were made via a hydrothermal process with glucose as a precursor undergoing carbonization. Different spectroscopic techniques were used to analyze the optical characteristics of GQDs, including UV–visible, photoluminescence, FTIR, and Raman spectroscopy. Atomic force microscopy, transmission electron microscopy, and X-ray diffraction were used to characterize the morphological and structural properties of GQDs. An electrochemical sensor was developed by drop casting GQDs on a glassy carbon electrode (GCE). The sensor detects the organophosphate pesticide malathion in a selective and sensitive manner. Using cyclic voltammetry, the sensor’s oxidation–reduction behavior was investigated. Electrochemical impedance spectroscopy was conducted to study the electrochemical properties of the modified GQDs/GCE working electrode, which showed excellent charge transfer properties. We measured malathion in varying concentrations between 1 to 30 µM using differential pulse voltammetry, which resulted in a limit of detection of 0.62 nM. GQDs can thus be used to develop electrochemical sensors for the detection of pesticides in water.

Introduction

Global population growth makes food production more challenging, and pesticides are therefore used in agriculture in greater quantities than in the past to maintain and increase crop yields [1,2]. Pesticides containing organophosphates are widely used due to their availability as efficient, low-cost pesticides. It is important to recognize, however, that excessive pesticide use poses a negative impact on the environment and human health because of biomagnification and persistence [3]. One example of an organophosphate insecticide is malathion, which kills insects such as fleas and ants that attack plants. Malathion has been detected so far using chromatography [4,5], colorimetry [6], and mass spectrometry [7], although these methods are complicated and time-consuming and require expensive equipment with specialized expertise. It is therefore necessary to
develop a technology that can detect pesticides quickly, easily, and economically.

With electrochemical detection techniques, a wide range of pesticides can be detected by the fabrication of simple, cost-effective, rapid, and high-throughput portable devices [8]. The application of electrochemical methods in detection of pesticides has already been extensively studied [9-13]. Nanomaterials are ideal for electrochemical sensing because of their unique properties such as high chemical stability, thermal conductivity, electrical conductivity, and large surface-area to volume ratio to provide enhanced analyte interaction with the sensing surface [14]. Carbon-based nanomaterials and nanocomposites are being investigated for the electrochemical detection of a variety of pesticides, including organophosphates, organochlorines, and carbamates [15-17]. The use of graphene and its derivatives is widespread for electrochemical detection since 2D graphene sheets provide numerous electrochemical sites for the detection of target molecules, while electrons in the sp²-hybridized pₓ orbital have a faster electron transfer rate, which enhances response time and lowers the detection limit [18].

In an effort to combine the properties of carbon dots and graphene, graphene quantum dots (GQDs) with a size smaller than 100 nm and only a few layers of graphene (3 to 10 layers) have been developed as a new class of carbon nanomaterials [19]. Scientists have explored the possibilities of developing sensing devices based on graphene quantum dots in recent years [20-23]. In 2015, Dong et al. prepared an oxime-based sensor via attaching pralidoxime on a GQDs-modified GCE for detecting the organophosphorus pesticide fenthion [24]. In 2018, Sahub et al. worked on a biosensor platform consisting of graphene quantum dots functionalized with acetylcholinesterase and choline oxidase for the detection of the organophosphate pesticide dichlorvos [25]. In 2018, Qian Liu et al. developed a photo-electrochemical sensor with nitrogen-functionalized graphene quantum dots and 3D bismuth oxyiodine hybrid hollow microspheres for the detection of chlorpyrifos [26]. In 2020, Jiménez-López et al. worked on a fluorescent probe containing graphene quantum dots and silver nanoparticles for glyphosate detection [27]. In 2021, Xu Dan et al. developed a histidine-functionalized nickel/silver/graphene quantum dot/graphene hybrid for the colorimetric detection of malathion [28].

This paper describes the development of an electrochemical sensor based on a graphene quantum dot-modified glassy carbon electrode (GQDs/GCE) for the determination and quantification of the organophosphate pesticide malathion. Graphene quantum dots were synthesized hydrothermally using glucose as precursor. The glassy carbon electrode that served as working electrode in the electrochemical cell was modified with graphene quantum dots by drop casting. To evaluate the modified electrode’s oxidation/reduction behavior and charge transfer resistance, cyclic voltammetry and electrochemical impedance spectroscopy were performed. An investigation of the relationship between concentrations and peak currents was conducted using differential pulse voltammetry (DPV). In this study, the modified GQD electrodes were found to be effective sensing platforms for the electrochemical detection of organophosphate pesticides, particularly malathion.

Experimental Materials
Glucose (C₆H₁₂O₆), conc. sulfuric acid (98% H₂SO₄), potassium hexacyanoferrate(III) (C₄FeK₃N₆), and potassium chloride (KCl) were obtained from Fisher chemicals. Malathion (C₁₀H₁₉O₆PS₂) was obtained from Insecticides India Limited. Disodium phosphate (Na₂HPO₄·H₂O), monosodium phosphate (NaH₂PO₄), sodium hydroxide (NaOH), ethanol (C₂H₅OH), and isopropyl alcohol (C₃H₈O) were procured from Rankem chemicals. Nafion (C₉H₁₇O₇S) and activated charcoal were taken from Fisher Scientific. For all experimental work and the preparation of stock solutions, deionized (DI) water was used.

Synthesis of graphene quantum dots
Graphene quantum dots (GQDs) were synthesized using glucose as a precursor material via a hydrothermal route [29] with some modifications. Glucose (2 g) was dissolved in 20 mL DI water and filtered for the removal of undissolved particles through Whatman filter paper. In the above solution, 20 mL of conc. H₂SO₄ was added dropwise until it turned brownish under constant stirring. The hydrothermal treatment was conducted by heating the 40 mL suspension at 200 °C for 5 h in a 50 mL poly(tetrafluoroethylene)-lined autoclave. Washing with DI water was carried out in order to remove the acid from the resulting black suspension once it had been cooled to room temperature. NaOH solution was subsequently used to neutralize the solid collected after centrifugation. To obtain the GQDs, the final black suspension was filtered through a 0.22 µm syringe filter.

Fabrication of the electrochemical sensor
A mirror-like surface was first achieved on the bare GCE by polishing it with 0.3 and 0.05 µm alumina powder. In the next step the GCE was sonicated in ethanol and rinsed with DI water to remove surface impurities. The GQDs-based ink was prepared in a glass vial with four components, that is 15 mg activated charcoal as a conductivity enhancer, 15 mg GQDs as modifying agent, 25 µL Nafion as binder, and 1 mL isopropyl alcohol as solvent. All components were sonicated for 30 min to create a homogeneous mixture that could be utilized for the
modification of the bare GCE [29]. The final step was to drop cast 5 μL of the GQDs dispersion on the GCE surface and allow it to dry at room temperature. A GQDs-modified working electrode (GQDs/GCE) was obtained, which will be used as an electrochemical nanosensor in further studies for malathion detection (Figure 1).

Characterization

FTIR, Raman, UV–vis, and fluorescence spectroscopy measurements were carried out to determine the optical properties of GQDs. A Perkin Elmer LAMBDA 750 spectrophotometer was used to record the UV–visible absorbance spectrum. The luminescence characteristics of the GQDs were investigated using a Perkin Elmer LS 55 fluorescence spectrometer. On a Perkin Elmer FT-IR Spectrum 2, FTIR spectra were measured in the range of 500–4000 cm⁻¹ by making KBr pellets of the sample. At room temperature, an AIRIX STR 500 laser Raman spectrometer was used with Ar laser excitation at 532 nm. A Panalytical X-Pert Pro diffractometer with Cu Kα radiation (λ = 1.5418 Å) was used for investigating the structural properties of GQDs. Morphology and size of GQDs were confirmed with data obtained from a Bruker AFM analyzer atomic force microscope and a FEI Tecnai G2 20 S-TWIN transmission electron microscope.

Electrochemical measurements

GQDs/GCE, Ag/AgCl, and a platinum wire were used as working, reference, and counter electrode, respectively, in all electrochemical experiments, conducted on a SP Biologic 150 electrochemical workstation at room temperature. A solution of 0.1 M KCl containing 0.05 M K₃[Fe(CN)₆] was used as an electrolyte to analyze the oxidation–reduction behavior of the working electrode through cyclic voltammetry. For detection and quantification of pesticides, differential pulse voltammetry experiments were conducted with 0.1 M phosphate-buffered saline at pH 7.

Results and Discussion

Characterization of graphene quantum dots

The UV–vis absorption spectrum of the GQDs in distilled water is depicted in Figure 2a, which shows two prominent absorption peaks around 270 and 320 nm, in agreement with the data previously reported [30,31]. The shoulder at 270 nm is probably caused by π–π* transition of the C=C bonds, and the absorption hump at 320 nm is likely caused by n–π* transitions of the C=O bonds. As shown in Figure 2b, when the graphene quantum dot suspension was excited at 320 nm, the photoluminescence (PL) spectrum of GQDs showed a strong peak around 425 nm, similar to those reported for GQDs [32]. When excited
at wavelengths between 320 and 420 nm, the PL peak shifts from 420 nm (violet) to 520 nm (green), and the PL intensity also decreases significantly. Therefore, it can be inferred that not only quantum size effects, but also defects on the surface, contribute to the PL in GQDs.

Size and morphology of GQDs were characterized using TEM and AFM. The TEM micrographs shown in Figure 3a confirm the formation of evenly dispersed GQDs with almost spherical shape. Figure 3b shows the size distribution and the log-normal fit, from which a mean of 12.75 nm and a full width at half maximum (FWHM) of 15.41 nm were obtained. The GQDs vary in size from 5 to 40 nm, with the highest number of dots having a size in the 10–20 nm range. The HRTEM image of the GQDs in Figure 3c shows their crystalline structure. The lattice spacing obtained is 0.34 nm, which can be related to the (002) crystal planes of GQDs. Figure 3d shows an AFM image of the synthesized GQDs. The x axis and the y axis in the inset of the AFM image show the horizontal distance and vertical distance, respectively, covered by the GQDs. The variation in size of the GQDs can be determined from the x axis, while from the y axis, the thickness of the GQDs can be obtained. The average thickness of the GQDs is about 2.8 nm, which indicates the presence of 8–9 graphene layers, assuming an interlayer distance of 0.33 nm [33].

The XRD pattern of the synthesized GQDs is shown in Figure 4a. A broad diffraction peak at 24.08° is obtained, which corresponds to the (002) crystal planes of the GQDs with a d spacing of 0.369 nm [34]. It can be inferred from the higher d spacing value of GQDs that oxygen containing functional groups are still present in GQDs even after hydrothermal treatment. Due to the nanoscale size of GQDs and a small number of graphene layers, the diffraction peak appears broad [35]. Using

Figure 3: (a) TEM image, (b) size distribution along with log-normal fit, (c) HRTEM image, and (d) AFM image of GQDs.
the FWHM of the diffraction peak, an average crystallite size of 2.69 nm was calculated for the synthesized GQDs using the Debye–Scherrer formula, \( D = \frac{0.9\lambda}{\beta \cdot \cos \theta} \), where \( D \) is the average crystallite size of the synthesized GQDs, \( \lambda \) is the X-ray wavelength, \( \theta \) is the Bragg diffraction angle, and \( \beta \) is the FWHM. The elemental analysis of GQDs from EDX measurements is shown in Figure 4b. The EDX spectrum shows the presence of only carbon and oxygen in the GQDs with 82.35 atom % carbon and 17.65 atom % oxygen. Figure 5a shows the functional groups present on the surface of the GQDs measured using FTIR infrared spectroscopy. The broad absorption band at 3430 cm\(^{-1}\) corresponds to stretching vibrations of O–H bonds [36], which impart hydrophilicity to GQDs to form a dispersion in water. Similarly, the peaks at 2923 and 2850 cm\(^{-1}\) may be assigned to C–H stretching vibrations, the peaks at 2358, 1040, and 1158 cm\(^{-1}\) to C–O stretching vibrations, the peaks at 1625 cm\(^{-1}\) to C=C vibrations, and the peaks at 1380 cm\(^{-1}\) to C–H vibrations of alkyl groups [37]. It can be inferred that the surface of GQDs is passivated by surface groups that occur during the carbonization of glucose. The Raman spectrum of the GQDs in the spectral range of 1000–2000 cm\(^{-1}\) without any baseline correction displays typical D (ca. 1385 cm\(^{-1}\)) and G bands (ca. 1585 cm\(^{-1}\)) with an excitation wavelength of 532 nm as shown in Figure 5b, resembling those of a standard graphitic structure [38]. As a result of defects in the sp\(^2\)-hybridized GQDs structure, the D band occurs due to transverse optical (TO) phonons about the \( k \) point of the Brillouin zone, while the G band arises from vibrations in rings of sp\(^2\)-hybridized atoms inside the GQDs.

**Electrochemical studies**

**Electrochemical impedance spectroscopy**

In order to investigate the charge transfer on the electrode surfaces, electrochemical impedance spectroscopy (EIS) was used with the redox probe ferrocyanide. In EIS spectra, the semicircle component represents the charge transfer resistance (\( R_{ct} \)) at the surface of the electrode. The Nyquist plots of the GQDs/
GCE and the bare GCE are shown in Figure 6 in 0.1 M KCl solution containing 0.05 M [Fe(CN)₆]³⁻/⁴⁻. The bare GCE electrode exhibits a semicircle with a resistance of about 12.71 kΩ. After modification with GQDs, the Rₑ value decreases to about 9.98 kΩ. It can be inferred that, as a result of an increase in conductivity, K₃Fe(CN)₆ can reach the electrode surface more easily.

Cyclic voltammetry
The redox electrochemical behavior of the bare GCE and GQDs/GCE electrodes was first evaluated using cyclic voltammetry in 0.1 M KCl containing 0.05 M [Fe(CN)₆]³⁻/⁴⁻ at a scan rate of 100 mV·s⁻¹, as shown in Figure 7a. The bare GCE exhibits well-defined anodic and cathodic redox peaks at 1.45 and 0.16 V, respectively. In the cathodic direction, GQDs/GCE exhibits a peak at ~0.76 V, which can be assigned to the reduction of [Fe³⁺(CN)₆]³⁻ to [Fe²⁺(CN)₆]⁴⁻. In the anodic direction, GQDs/GCE exhibits two peaks, one at 1.14 V due to the oxidation of [Fe²⁺(CN)₆]⁴⁻ to [Fe³⁺(CN)₆]³⁻ and a second one at 2.27 V due to an oxidation of Fe³⁺(CN)₆³⁻ to [Fe⁴⁺(CN)₆]²⁻ as
reported in [39]. As a result of the modification with GQDs, electron transfer was improved, resulting in a higher peak current and an electron-conducting channel on the modified electrode, showing an increase in peak current from 0.037 to 0.39 mA.

**Effect of scan rate**

Figure 7b shows cyclic voltammetry results of the GQDs/GCE electrode to study the interfacial kinetics from 20 mV·s⁻¹ scan rate to 400 mV·s⁻¹ scan rate. The increase in the square root of scan rates led to a linear increase in peak current for anodic and cathodic reactions, as shown in Figure 7c. For scan rates of 20 to 400 mV·s⁻¹, an incremental scan rate results in a more positive anodic peak and a more negative cathodic peak, suggesting that the redox reaction is a reversible process. Moreover, Figure 7d shows that peak shift and scan rate have a linear relationship, indicating that electrochemical reactions at the electrode are diffusion-controlled, and the linear relationship \( R² = 0.9674 \) between peak height and scan rate suggests an enhanced electrochemical activity.

**Electrochemical detection of malathion**

Using the modified working GQDs/GCE electrode as electrochemical sensor, a differential pulse voltammetry (DPV) analysis was conducted with various concentrations of malathion in 0.1 M PBS (pH 7) at a scan rate of 50 mV·s⁻¹. Different concentrations of malathion were detected. The oxidative desulfurization of malathion into malaoxon (Figure 8) results in a current peak (centered at +1.9 V) at the GQDs/GCE electrode.

The DPV study in Figure 9a shows an increase in the oxidation peak current as the concentration of malathion increases from 1 to 30 µM, suggesting that the GQDs/GCE electrode is sensitive towards malathion. As shown in Figure 9b, the linear regression equation of peak current and concentration for malathion detection is:

\[
I_p (\mu A) = 1.5989c_{mal} (\mu M) + 0.4503 \quad (R^2 = 0.9944)
\]

Using the equation \( kS_b/m \) [35], where \( S_b \) represents standard deviation of the peak current of the blank, \( m \) represents the slope of the calibration plot, \( k \) has a value of 3, the limit of detection (LOD) was calculated to be 0.62 nM. In a similar manner, the limit of quantitation (LOQ) of 2.06 nM was calculated using a \( k \) value of 10. A comparison of the GQDs/GCE electrode with other existing electrodes for malathion detection is presented in Table 1. It indicates that the proposed GQDs-based electrode has a lower detection limit than other electrodes. The modified GQDs/GCE working electrode has an increased surface area to volume ratio due to the small size of GQDs. In addition, the dif-
Table 1: Comparison of sensing parameters for the electrochemical detection of malathion using different electrode materials.a

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Technique</th>
<th>Limit of detection</th>
<th>Linear range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AChE-ZrO$_2$/CHIT composite film/GCE</td>
<td>CV</td>
<td>$1.3 \times 10^{-6}$ M</td>
<td>$1.0 \times 10^{-8}$ to $5.9 \times 10^{-7}$ M</td>
<td>[40]</td>
</tr>
<tr>
<td>AChE/PAn-PPy-MWCNTs/GCE</td>
<td>CV</td>
<td>1.0 ng·mL$^{-1}$</td>
<td>0.01 to 0.5 µg·mL$^{-1}$ and 1 to 25 µg·mL$^{-1}$</td>
<td>[41]</td>
</tr>
<tr>
<td>Tyr/nano-Pt/graphene/GCE</td>
<td>chronoamperometry</td>
<td>3 ppb</td>
<td>5 to 30 ppb</td>
<td>[42]</td>
</tr>
<tr>
<td>PANI-ES/SWCNTs/graphite</td>
<td>DPV</td>
<td>$2.0 \times 10^{-7}$ M</td>
<td>(2.0 to 14) $\times 10^{-7}$ M</td>
<td>[43]</td>
</tr>
<tr>
<td>PLaE-CS/AuNPs-GNs/GCE</td>
<td>DPV</td>
<td>1.51 nM</td>
<td>1.5 to 1513.5 nM</td>
<td>[44]</td>
</tr>
<tr>
<td>poly(TTP)/AChE/GCE</td>
<td>CV</td>
<td>4.08 nM</td>
<td>9.99 to 99.01 nM</td>
<td>[45]</td>
</tr>
<tr>
<td>mitochondria-modified paper-based electrodes</td>
<td>CV</td>
<td>20 nM</td>
<td>20 to 60 nM</td>
<td>[46]</td>
</tr>
<tr>
<td>AuNP-CS-IL/PGE</td>
<td>SWV</td>
<td>0.68 nM</td>
<td>0.89 to 5.94 nM and 5.94 to 44.6 nM</td>
<td>[47]</td>
</tr>
<tr>
<td>CHIT-g-PANI</td>
<td>potentiometry</td>
<td>3.8 µM</td>
<td>2.0 to 62.5 µM</td>
<td>[48]</td>
</tr>
<tr>
<td>CuFe$_2$O$_4$-rGO/GCE</td>
<td>SWV</td>
<td>0.992 ± 0.007 ppm</td>
<td>0.5 to 8 ppm</td>
<td>[49]</td>
</tr>
<tr>
<td>FTO/PAn/Ppy/CRGO</td>
<td>DPV</td>
<td>0.8 ng·mL$^{-1}$</td>
<td>500 to 2 $\times 10^4$ ng·mL$^{-1}$</td>
<td>[50]</td>
</tr>
<tr>
<td>GQDs/GCE</td>
<td>DPV</td>
<td>0.62 nM</td>
<td>1–30 µM</td>
<td>present work</td>
</tr>
</tbody>
</table>


Different functional groups present on the GQDs surfaces provide additional active sites, which increase sensitivity and lower the limit of detection.

Interference study
The selectivity of the GQDs/GCE electrode towards malathion was examined by studying interfering effects in the presence of the organophosphate pesticide glyphosate. The DPV measurements showed that adding 0.1 µM glyphosate in the electrolytic solution containing 1 µM malathion shows no alteration in the peak potential for malathion detection (Figure 10).

Conclusion
Graphene quantum dots (size range 5 to 40 nm) were chemically synthesized by using glucose as a precursor in a hydrothermal method. This paper describes the fabrication of an electrochemical nanosensor by modifying a bare glassy carbon electrode with GQDs. The oxidation–reduction behavior of the GQDs/GCE electrode was studied using cyclic voltammetry. Electrochemical impedance spectroscopy showed an increased charge transfer of the modified electrode. Differential pulse voltammetry analysis was performed to detect the organophosphate pesticide malathion. From 1 to 30 µM, a linear relationship was observed between the peak current and the malathion concentration. A detection limit of 0.62 nM was determined, and an interference study showed that the developed electrochemical sensor is selective for malathion. The sensor’s selectivity for malathion can be attributed to its surface composition of different functional groups providing specific sites for pesticide molecules. These results demonstrate that the GQDs/GCE electrochemical sensor is capable of detecting malathion over a wide linear range with low detection limits and high selectivity. The graphene-based nanosensor described here could be used in future to develop portable monitoring systems for water contamination.
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Author Contributions
Sanju Tanwar: conceptualization, research methodology, experimental design, electrochemical investigation, original draft writing and proofreading; Aditi Sharma: characterization and investigation; Dhirendra Mathur: review and supervision.

Conflicts of Interest
There are no conflicts to declare.

ORCID® IDs
Sanju Tanwar - https://orcid.org/0000-0002-9005-2941

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New application of bimetallic Ag/Pt nanoplates in a colorimetric biosensor for specific detection of *E*. *coli* in water

Azam Bagheri Pebdeni¹, Mohammad N. AL-Baiati² and Morteza Hosseini*¹,³

Full Research Paper

Address:
¹Nanobiosensors lab, Department of Life Science Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran, Iran, ²Department of Chemistry, College of Education for Pur Science, University of Kerbala, Karabal, Iraq and ³Department of Pharmaceutical Biomaterials and Medicinal Biomaterials Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

Email:
Morteza Hosseini* - hosseini_m@ut.ac.ir

* Corresponding author

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Abstract

A fast and sensitive aptasensor was developed using nanoplates with peroxidase activity as a novel approach. *E*. *coli* detection is described using a silver/platinum nanoplate (Ag/Pt NPL) that interacts with an oligonucleotide aptamer as a bioreceptor. The size of the Ag/Pt NPLs was about 42 nm according to the FE-SEM images. The EDS result indicates that a thin layer of Pt ions was coated on the surface of the Ag NPLs. This nanobiosensor has the ability to specifically bind to *E*. *coli*, increasing the peroxidase activity of the apt-Ag/Pt NPL. Finally, the blue color of the solution in the contaminated water samples was increased in the presence of 3,3′,5,5′-tetramethylbenzidine (TMB) as a substrate and H₂O₂. The assay can be completed in 30 min and the presence of *E*. *coli* levels can be distinguished with the naked eye. The absorbance at 652 nm is proportional to pathogen concentration from 10 to 10⁸ CFU·mL⁻¹, with a detection limit of 10 CFU·mL⁻¹. The percent recovery for the water samples spiked with *E*. *coli* is 95%. The developed assay should serve as a general platform for detecting other pathogenic bacteria which affect water and food quality. The proposed *E*. *coli* detection strategy has appealing characteristics such as high sensitivity, simple operation, short testing time, and low cost.

Introduction

One of the most dangerous foodborne infections is caused by *E*. *coli*. It can induce hemorrhagic colitis, hemolytic uremic syndrome, and thrombocytopenic purpura. *E*. *coli* is responsible for more than 2 million acute illness cases worldwide each year [1,2]. Water quality testing is based on the presence of *E*. *coli*, which is still regarded as the best indication of fecal contamination [3,4]. There are several methods for detecting bacteria (e.g., ELISA and PCR) with colony counting serving as
the gold standard. However, these procedures need a significant amount of time, skilled operators, and costly equipment. As a result, for effective medical treatment with minimal detection time, a selective and sensitive detection strategy is required [5,6]. New nanoparticles for fast bacterial detection can be fabricated.

Colorimetric biosensors have become popular in analytical applications due to their high sensitivity, convenience, and ease of signal readout [7,8]. These biosensors have been extensively utilized in pathogen identification, primarily because of their ability to rapidly display results in visible color [9,10]. Nanomaterials have improved the ability to detect pathogens in water and food by enhancing signals and sensitivity. These materials, which encompass nanoparticles, nanorods, nanowires, and nanoclusters, can be combined with aptamers, antibodies, enzymes, and other ligands to display unique physical, chemical, and optical traits. The colorimetric method typically depends on the enzymatic properties, aggregation, and dispersion of these nanomaterials, which can be influenced by the presence of aptamers, antibodies, and other agents [4]. Among the various colorimetric test techniques, the enzymatic conversion of a chromogenic substrate, such as 3,3′,5,5′-tetramethylbenzidine (TMB), is widely employed. This conversion process can generate vibrant products when H2O2 is present [11,12]. Colorimetric biosensors often use chromogenic substrates such as TMB, ABTS, and o-phenylenediamine (OPD) to produce a visual readout signal through peroxidase mimetic activity. The TMB is preferred due to its reliance on a single organic substrate, eliminating the need for a helper molecule. Studies have shown TMB to be non-mutagenic, but it may still be carcinogenic. However, the low solubility of TMB in water requires modifications to increase its solubility [7]. Due to its low cost, simplicity, fast response, and the lack of expensive equipment required, the peroxidase-like activity has garnered significant attention in the detection of harmful microorganisms. The naked eye can easily observe the blue hue resulting from the catalytic oxidation of TMB by H2O2 on a paper-based device. As a result, it is highly suitable for the generation of novel and portable biosensors [13]. The color shift produced by an enzyme-catalyzed substrate reaction is an appealing option for developing colorimetric-based biosensors to detect targets such as pathogens [14]. Functional nanomaterials with catalytic activity similar to enzymes (nanozymes) reveal substantial benefits over natural enzymes, such as ultrahigh environmental stability, appropriate catalytic activity, and ease of prototyping [15,16]. We created plate-like silver nanoparticles (i.e., silver nanoplates, Ag NPLs) covered with a layer of Pt atoms to improve the peroxide activity of NPLs, and use them as colorimetric biosensor materials. Metallic NPLs were employed in a variety of applications, including antibacterial activity [17-19], hazardous dye removal [20], photocatalytic degradation and bactericidal action [21], sensors and biosensors [22-25], and as electrocatalysts [26]. Aptamers are single-stranded DNA or RNA oligonucleotides that attach to their targets with great affinity and specificity. Aptamers have high stability in a variety of environments and during long-term storage and can be manufactured using normal oligonucleotide chemical synthesis processes, with minor chemical changes if necessary. Aptamers are thus regarded as a good baroreceptor in a variety of biological applications [27,28]. Colorimetric paper-based biosensors are the most popular and appealing since the presence of a specific pathogen can be easily observed by a simple change in color, which can be distinguished easily with the naked eye eliminating any use of expensive and complex types of equipment [29-31]. In this research, we provide a novel and highly sensitive nanobiosensor as well as a paper-based analytical equipment for detecting E. coli. The E. coli could be trapped by the aptamer-NPL to create bacteria–aptamers–Ag/Pt NPL complexes in which the aptamers effectively change on the surface of Ag/Pt NPL. Surprisingly, in the conventional TMB and H2O2 color reaction, the bacteria–aptamers–Ag/Pt NPL complex displayed a substantially greater color yield (Scheme 1). The aptamer-NPL complex was able to distinguish E. coli from other foodborne pathogens, as evidenced by a significantly different signal. This detection principle was applied to contaminated water, where E. coli was successfully detected using the developed colorimetric sensor. The sensor offers a straightforward, sensitive, and dependable method for detecting pathogens and ensuring water safety.

Results and Discussion

Characterization of Ag/Pt NPL and apt-NPL

Field-emission scanning electron microscopy (FE-SEM) was used to investigate the form and shapes of NPLs. The size of the NPLs is approx. 42 nm. Figure 1a shows a combination of truncated triangular and circular plates of Ag/Pt NPLs. The NPLs were evenly distributed and shaped in the form of discs or triangles. Energy-dispersive X-ray spectroscopy (EDS) was used to examine Ag and Pt on the synthesized NPLs (Figure 1b). The percentage of Pt is smaller than that of Ag, indicating that a thin layer of Pt ions has been coated on the surface of Ag NPLs. In the aqueous solution, the Ag/Pt NPLs appear bluish purple. To predict the secondary structure of the oligonucleotides the UNAfold program was employed, as illustrated in Supporting Information File 1, Figure S1.

Peroxidase-like activity of Ag/Pt NPLs

Supporting Information File 1, Figure S2a illustrated a comparison of the inherent peroxidase activity of Ag/Pt NPLs. The intrinsic peroxidase activity of Ag/Pt NPLs is substantially greater than those of silver or platinum alone. When compared
Scheme 1: Schematic representation of the detection of *E. coli* by Ag/Pt NPL as a peroxidase nanozyme in solution and paper based microfluidic device.

Figure 1: a) The SEM image AND b) EDS analysis of Ag/Pt NPL.

to Ag NPLs or Pt NPLs alone, the bimetallic NPLs demonstrated higher peroxidase-like activity. We synthesized Ag NPLs and Pt NPLs under the same conditions as bimetallic NPLs. After adding TMB and H$_2$O$_2$, we compared the peroxidase activity of the three NPLs at 652 nm. The catalytic activity of Ag NPLs, Pt NPLs, and Ag/Pt NPLs demonstrate the superiority of bimetallic NPLs over the others in terms of peroxidase-like catalytic activity. These nanostructures were successfully used to make a colorimetric aptasensor for *E. coli* sensing. The substrate used to examine the peroxidase-like activity of the Ag/Pt NPLs was TMB since it may be oxidized by H$_2$O$_2$ to create a blue-colored product during the peroxidase-like catalysis. The Ag/Pt NPLs were synthesized by reducing Ag and Pt using ascorbic acid as the reducing agent in the synthesis of NPs. The chromogenic TMB was oxidized in the presence of Ag/Pt NPLs, resulting in a noticeable blue hue. As shown in Supporting Information File 1, Figure S2a, the absorbance intensity at 652 nm, which is the characteristic peak of oxidized TMB, considerably rose after the addition of Ag/Pt NPLs. The best concentration of TMB and H$_2$O$_2$ that resulted in the maximum peroxide activity of NPLs throughout the optimal period of 4 min was chosen (Supporting Information File 1, Figure S2b). Different concentrations of TMB were used as the substrate when the H$_2$O$_2$ concentration was adjusted to 1 M. As indicated in Supporting Information File 1, Figure S3a, 10 mM of TMB has the maximum peroxide activity of NPL throughout 4 min. The varied concentrations of H$_2$O$_2$ (0.5–3 mM) were examined after the concentration of TMB was set at 10 mM.
peroxidase activity of NPL is maximum at 1 M $\text{H}_2\text{O}_2$ (Supporting Information File 1, Figure S3b). The stability of the NPLs in different buffers was tested and the results showed suitable peroxidase activity of this nanozyme in various solutions (Supporting Information File 1, Figure S3c). The shelf-life of the aptasensor is at least six months; storage at 4 °C is necessary. The peroxidase activity of the Ag/Pt NPLs enhanced following the addition of the aptamer. Using DNA to functionalize NPLs can increase not only target recognition but also enzyme activity, which is a useful technique for developing biosensors for target detection. Furthermore, the electrostatic and intermolecular interactions of aptamer–TMB increased the substrate affinity of NPLs. As a result, the catalytic efficacy of Ag/Pt NPL was improved, resulting in a deeper blue signal (Figure 2a, Figure 2b) [32,33]. The zeta potentials of Ag/Pt NPLs, aptamer-NPLs, aptamer-NPLs coupled with $E. \text{coli}$, and $E. \text{coli}$ alone were measured (Figure 2c). The surface charges of all NPLs were negative due to dispersion agents such as sodium citrate and ascorbic acid, according to measurements of their zeta potentials. The NPLs had a negative charge of $-4.4$ mV before introducing the aptamers, which changed to $-6$ mV. A reduction in the zeta potential of the aptamer-Ag/Pt NPLs revealed that the aptamer was effectively changed on the surface of NPLs. In comparison to $E. \text{coli}$ and aptamer-NPLs, the negative charge of aptamer-NPL- $E. \text{coli}$ reduced to $-10$ mV after incubation time, indicating that the particular aptasensor was gathered around the whole cell of $E. \text{coli}$, implying that aptamer-NPLs might successfully capture the target bacterium.
Principles of *E. coli* detection

After adding TMB and H$_2$O$_2$, a particular quantity of *E. coli* was introduced to the produced aptamer-Ag/Pt NPLs, and the changes in peroxidase-like activity were measured. According to Figure 3a and Figure 3b, the peroxidase-like activity of NPLs was enhanced after the addition of a particular target to the aptamer-NPL solution. At the same time, the control was light blue. This technique indicated that the aptamer, as a specific bioreceptor immobilized on the NPLs, specifically attached to *E. coli*. The standard curve was generated in concentrations ranging from 10 to 10$^8$ CFU·mL$^{-1}$ using the linear regression equation $y = 0.0638x + 0.3508$ ($R^2 = 0.9678$). The sensitivity of the NPLs was studied, and its detection limit (LOD) was calculated to be 10 CFU·mL$^{-1}$ ($S/N$) after a 30 min detection time with the naked eye, indicating that the concentration of *E. coli* in the sample was growing (Figure 3c).

If the sample color is too subtle, we can enter the amount of gray intensity of the sample in the calibration curve and calculate the concentration of the target in the tested sample. The color of the sample must always be checked against the control. The aptamer-Ag/Pt NPL solution can greatly increase the intensity of the distinctive peak of the catalytic system. The bacteria were introduced to a solution containing TMB and H$_2$O$_2$ to validate the phenomenon, and no alterations in color were detected, as shown in Figure 2 (light blue color curve). These findings showed that *E. coli* lacked peroxidase activity. However, the introduction of bacteria in the catalytic aptasensor can enhance the creation of the blue product. The resultant agent is generated by the specific binding of the aptamer to the surface of the bacteria and the accumulation of numerous biosensors around the target leads to an increase in the intensity of the blue color. As a result, we attempted to explain the phenomenon and used it to develop a biosensor for detecting pathogens. To qualify the concentration of bacteria, the blue product in the solution was employed as a signal reporter. This study also demonstrated that enriching bacteria with aptamer-NPL can increase peroxidase-like activity. Table 1 compares the proposed colorimetric aptasensor to different previously published *E. coli* detection methods. The findings were compared to those of previous specified situations, proving that this technique gives an increased dynamic range and appropriate LOD. This biosensor offers several advantages such as quick reaction, high sensitivity, ease of use, low cost, and the capacity to do analysis for an extended period of time. Also, this aptasensor can function well on a paper-based platform and, as the cost of the paper is low, the production of this biosensor compared to many similar biosensors is lower.
Evaluation of selectivity

Table 1: Comparison of the analytical performances between the method presented in this paper and the reported methods for the detection of *E. coli*.

<table>
<thead>
<tr>
<th>Method</th>
<th>Nanostructure</th>
<th>Bioreceptor</th>
<th>Analytical range (CFU·mL⁻¹)</th>
<th>LOD (CFU·mL⁻¹)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERS-LFA strips</td>
<td>core–shell of Au–Ag Yb, Tb, Fe-doped NaYF4 NPs</td>
<td>antibody label-free</td>
<td>6.94 × 10⁸–10⁹</td>
<td>6.94 × 10¹</td>
<td>[34]</td>
</tr>
<tr>
<td>fluorescent</td>
<td></td>
<td></td>
<td>100–10⁹</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>colorimetric</td>
<td>papain-AuNCs</td>
<td>aptamer</td>
<td>10²–10⁶</td>
<td>5.6 × 10²</td>
<td>[36]</td>
</tr>
<tr>
<td>immunochromatographic assay</td>
<td>Au/Pt NPs</td>
<td>mAb</td>
<td>1.93 × 10⁴–1.93 × 10⁸</td>
<td>1.83 × 10⁴</td>
<td>[37]</td>
</tr>
<tr>
<td>colorimetric</td>
<td>magnetic probe</td>
<td>T7 bacteriophage</td>
<td>–</td>
<td>1 × 10⁴</td>
<td>[38]</td>
</tr>
<tr>
<td>fluorescent</td>
<td>GO–Fe₃O₄</td>
<td>aptamer</td>
<td>10³–10⁷</td>
<td>467</td>
<td>[39]</td>
</tr>
<tr>
<td>colorimetric paper-based</td>
<td>Ag/Pt NPL</td>
<td>aptamer</td>
<td>10–10⁸</td>
<td>10</td>
<td>this work</td>
</tr>
<tr>
<td>microfluidic device</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Tap water and lake water were chosen as candidates to evaluate the potential of this colorimetric biosensor in real sample analysis. The lake water sample was collected from Bandar Anzali wetlands (Gilan, Iran). The *E. coli* presence was examined by spiking known amounts of the bacteria in water samples. *E. coli* thresholds in drinking water is well below 10⁴ CFU·mL⁻¹, which is the limit of the colorimetric test [36]. Thus, our biosensor is very sensitive for detection of *E. coli* in drinking water and for analyzing the food safety. The percent recovery of the target bacteria was calculated as the ratio of the concentration of the spiked target bacteria in the real samples to the bacteria concentration in the method (measured concentration). The relative standard deviation (RSD) was obtained by multiplying the standard deviation by 100 and dividing the result by the average. As shown in Supporting Information File 1, Table S1, the recovery percentage ranged from 94 to 103 percent, emphasizing the effectiveness and accuracy of the biosensor in detecting *E. coli* in drinking water. A paper platform was optimized to execute the aptamer-based assay with the goal of producing a fast and portable biosensor. The paper-based microfluidic system can result in an increased sensitivity due to the shorter amount of sample used without any need for dilution, separation, or pre-treatment through various stages.

Paper-based colorimetric detection of *E. coli* in water as a real sample

The suggested biosensor paper-based model was meant to monitor the change in blue color with different concentrations of *E. coli*. The vivid blue hue showed an increase in bacteria concentrations after the addition of TMB and H₂O₂ to the paper-chip containing the aptasensor and *E. coli*. Due to the increase in aptamer–bacteria interaction based on paper-chip pores, this paper-based biosensor was not time-dependent and showed a fast color-change response in the absence or presence of specific target (Figure 4). The intensity of the blue color, calculated with the ImageJ software, shows a desirable linear correlation with the logarithm of *E. coli* concentration, as shown in Figure 4. In the equations, "y" was the intensity of the response value and "x" is the log concentration of *E. coli*. The color was blue, and the *E. coli* concentration was high. The outcomes confirmed this. Each paper-based sensor is usable once, but the preparation of the paper-based sensor is not complex and shows suitable reproducibility. This paper-based method can accurately distinguish between different *E. coli* concentrations, making it a quick and convenient method for testing.
of 10 CFU·mL$^{-1}$, this system offers a rapid, sensitive, and portable biosensor for preventing *E. coli* contamination and resolving public health concerns in the future.

### Experimental Materials

Silver nitrate (AgNO$_3$), potassium tetrachloroplatinate(II), ascorbic acid, TMB, H$_2$O$_2$ (for determining peroxidase-like activity), and sodium hydroxide were purchased from Sigma. Tris-EDTA and HCl (for TE buffer production) were purchased from Merck. The aptamer for identifying *E. coli* (5’-CCC CCC CCC CCG GAC GCT TAT GCC TTG CCA TCT ACA GAG CAG GTG TGA CGG-3’) was acquired from Pishgam (Tehran, Iran). Lyophilized bacterial strains and bacterial culture medium were supplied from Baharafshan Institute of Research and Development (Tehran, Iran). All other substances were of analytical reagent grade and were used as supplied, with no further purification. The treatment was carried out with sterile deionized water.

### Apparatus

Perkin-Elmer lambda 25 UV–vis spectrometer was employed for UV–vis absorption measurement in the range of 200–800 nm. The morphology and shape of NPLs were studied using a field-emission scanning electron microscope (Supra 400VP, Zeiss, Oberkochen, Germany). Dynamic light scattering experiments were carried out at room temperature using a Zetasizer Nano-ZS90 Malvern. The elementary compositions of the samples were determined using EDS on a Tescan energy-dispersive spectrometer. The ImageJ software was used to de-
termine the gray intensity of the blue color of the paper-based microfluidic device.

Synthesis of Ag/Pt NPLs
A modified chemical reduction method was used to create Ag NPLs [26,38]. The aqueous solution synthesis of Ag-Pt NPL is described below. To begin, 50 L of 0.05 M AgNO₃ aqueous solution was mixed with 10 mL of 2.5 × 10⁻⁴ M sodium citrate aqueous solution. After that, 25 µL of 0.1 M ascorbic acid solution was gradually added to a stirred mixture of sodium citrate and AgNO₃. The purple Ag seed solution was then obtained. In addition, 10 mL of 0.05 M AgNO₃ aqueous solution was mixed with 20 mL of 0.1 M hexadecyltrimethylammonium bromide (CTAB) aqueous solution. Slowly, 10 mL of 0.1 M ascorbic acid and 0.408 mL of the prepared Ag seed solution were dropped into the CTAB aqueous solution. The Ag nanotemplates were circular. After adding 0.8 mL of 2 M NaOH aqueous solution, the circular Ag nanotemplates were prepared. To lessen the interaction of free CTAB with the synthesis of the circular Ag-Pt NPLs, 200 mL of the synthesized Ag circular NPLs was precipitated by centrifugation at 4000 rpm and dispersed in DI water (3 mL). To complement the Ag NPLs with Pt, at a fixed controlled temperature (60 °C), 8.3 µL of K₂PtCl₄ solution was added to 3 mL of stirred Ag NPL solution. The circular Ag-Pt NPLs were obtained after 70 min.

Bacterial culture
In a similar manner to what has been show in reference [5], Gram-positive and negative strains of pathogenic bacteria such as, S. aureus (ATCC 29213), E. coli (ATCC 35218), Pseudomonas aeruginosa (ATCC 10145), Salmonella typhimurium (ATCC 14028), and Bacillus subtilis (ATCC 168) were cultured in sterile Luria-Bertani broth (LB broth) and incubated at 37 °C overnight. After centrifugation, the bacteria were transferred to nutrient agar plates and cultured for 24 h at 37 °C. The absorbance of several bacterial colonies of diverse strains was measured using UV–vis spectroscopy at a wavelength of 600 nm (OD600). The gold standard method of plate counting was used to estimate the number of bacterial cells.

Detection of E. coli for bacterial assays
Following the optimization of the experimental settings, various concentrations of E. coli (10⁻¹⁰–10⁹ CFU·mL⁻¹) were combined with the aptamer-Ag/Pt NPL solution and gently shaken at room temperature. The peroxidase activity of NPLs in the presence of TMB and H₂O₂ was evaluated using a UV–vis spectrophotometer after the incubation period was shortened.

Analysis in real samples
Tap water and lake water were used as real samples to evaluate the analytical efficacy of the aptasensor. To do this, different quantities of E. coli were spiked in real samples which were previously sterilized. Finally, the spiked samples were mixed with the aptasensor and incubated for 30 min as the optimum incubation time. The peroxidase-like activity of the aptasensor was ultimately demonstrated in the presence of TMB and H₂O₂.

Paper-based device
Whatman filter paper No. 1 is considered as an appropriate paper for paper-based analysis. A PVC sheet was affixed to the back of each paper to prevent solution penetration through the bottom of the sheets (similarly to reference [12]). To conduct the analysis, 3 µL of aptamer-NPL was dropped on the paper in the first stage, and after drying, this procedure was repeated two more times to get a total amount of 9 µL of aptamer-NPL on each deposition zone. Following that, one drop of the E. coli-contaminated sample was put onto a paper chip. The final stage in the paper evaluation was to add 6 µL of H₂O₂ and 6 µL of TMB to paper chips. The ImageJ software was utilized to evaluate the intensity of the resulting blue color.

Supporting Information
Supporting Information contains the simulated structures of the aptamers and additional experimental information.

Supporting Information File 1
Additional figures and tables.
[https://www.beilstein-journals.org/bjnano/content/supplementary/2190-4286-15-9-S1.pdf]

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ORCID® iDs
Mohammad N. Al-Baiati - https://orcid.org/0000-0001-6947-2629
Morteza Hosseini - https://orcid.org/0000-0002-1492-7443

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