Graphene-based label-free electrochemical aptasensor for rapid and sensitive detection of foodborne pathogen

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Abstract Reduced graphene oxide (rGO) has emerged as a promising nanomaterial for reliable detection of pathogenic bacteria due to its exceptional properties such as ultrahigh electron transfer ability, large surface to volume ratio, biocompatibility, and its unique interactions with DNA bases of the aptamer. In this study, rGO-azophloxine (AP) nanocomposite aptasensor was developed for a sensitive, rapid, and robust detection of foodborne pathogens. Besides providing an excellent conductive and soluble rGO nanocomposite, the AP dye also acts as an electroactive indicator for redox reactions. The interaction of the label-free single-stranded deoxyribonucleic acid (ssDNA) aptamer with the test organism, Salmonella enterica serovar Typhimurium (S. Typhimurium), was monitored by differential pulse voltammetry analysis, and this aptasensor showed high sensitivity and selectivity for whole-cell bacteria detection. Under optimum conditions, this aptasensor exhibited a linear range of detection from $10^8$ to $10^1$ cfu mL$^{-1}$ with good linearity ($R^2 = 0.98$) and a detection limit of $10^1$ cfu mL$^{-1}$. Furthermore, the developed aptasensor was evaluated with non-Salmonella bacteria and artificially spiked chicken food sample with S. Typhimurium. The results demonstrated that the rGO-AP aptasensor possesses high potential to be adapted for the effective and rapid detection of a specific foodborne pathogen by an electrochemical approach.

Keywords Aptasensor · Azophloxine · Foodborne pathogen · Reduced graphene oxide · Salmonella enterica

Introduction

Nontyphoidal salmonellosis caused by Salmonella spp. is the most common foodborne disease globally and has a high impact on public health and socioeconomic status in both developed and developing countries [1, 2]. Approximately, 59.6 and 13.5% of Salmonellosis outbreaks associated with severe gastroenteritis were transmitted from food and human contact, respectively [3]. Salmonella Enteritidis is the predominant serovar that causes food poisoning worldwide especially in Asian countries (e.g., Japan, Korea, Singapore, and Thailand) [4–6]. In Malaysia, the major Salmonella serovars responsible for the foodborne outbreaks are S. Enteritidis and S. Typhimurium [7, 8] associated with poor hygiene in food handling and sanitation practices [9]. Thus, the development of fast, reliable, and sensitive detection techniques for the presence of foodborne pathogens is crucial to ensure a safe food supply and to reduce the outbreak of foodborne diseases [10].

The conventional culture methods require days (48 to 72 h) to confirm the presence of target pathogens due to the need for pre-enrichment of the food matrix and extensive culture plating procedures [11]. Therefore, molecular-based methods such as polymerase chain reaction (PCR) [12] and real-time PCR [13] were employed to complement the conventional time-consuming culture methods. PCR is an effective tool...
for detection of *Salmonella* by exponentially amplifying a DNA fragment or sequence of interest [12]. However, the PCR approach is a qualitative analysis leading to insufficient cell number quantitation and false-positive results due to non-specific amplification of DNA [14]. To rectify these issues, real-time PCR was applied to quantify DNA in real time, but it is laborious and requires costly reagents coupled with PCR-inhibitory effects in complex food matrices [10, 15].

By contrast, the intrinsic specificity and high affinity accompanied by high stability of RNA/DNA single-stranded aptamers rendered them particularly interesting candidates for novel detection probe in the diagnosis of pathogens using electronic biosensing platforms [16]. The interaction of the aptamer and its targets is based on their definite three-dimensional conformation [17, 18]; thus, aptamers have higher specificity enabling the discrimination of homologous targets with few DNA bases or amino acid mismatches [19, 20].

Recently, immense attention and effort have been channeled to the development of new biosensors for real-time detection of foodborne and waterborne pathogens. Electrochemical DNA sensors are miniaturized devices that allow rapid detection of foodborne pathogens with high affinity and specificity [21]. The most promising capability of these types of biosensors is the low detection limit in which it is capable to detect up to a single bacterial cell in 1–5 mL of the sample [22, 23].

With the rapid revolution in nanotechnology, inorganic nanomaterials (e.g., reduced graphene oxide (rGO), carbon nanotubes, silver, and gold) have been developed as sensing platforms in medical diagnostics and food forensic [24]. However, high production cost of gold and silver nanoparticles [25, 26] and nonbiocompatibility of carbon nanotubes [27] limit their usage in the construction of biosensor. Among these nanomaterials, rGO exhibits excellent physiochemical properties including high conductivity (~1000 S cm⁻¹) [28], large surface area (400–700 m² g⁻¹) [29], biocompatibility [30], and great mechanical strength (~130 GPa) [31]. Moreover, this 2D nanomaterial is composed of hexagonally arrayed sp²-bonded carbon atoms which facilitate its surface modifications through noncovalent interaction such as π-π stacking or hydrogen bonding between graphene and biomolecules for electroanalytical sensing applications [32]. The presence of versatile functional groups such as –OH, –COOH, and –CHO allows interaction with biomolecules to further improve the sensitivity of electrochemical biosensors [33, 34].

In a typical biosensing process, biological interactions are transduced as a mechanical, electrical, or optical signal. Specifically, electrochemical biosensors offer numerous advantages in medical and biotechnology fields by allowing direct transformation of a biological recognition to a readable electrical signal [35]. This electrochemical approach has a potential to be applied in biosensor for whole-cell bacteria detection because the outer membrane of bacteria itself is composed of conductive layers of macromolecules with electrochemical active groups that can react with the free ions in the electrolyte [36, 37]. Moreover, the development of label-free aptasensors provides a rapid, cost-effective, and direct detection of whole-cell bacteria in food matrices [10].

Up-to-date, electrochemical label-free aptasensors have been under extensive investigation. For instance, Hamidi-Asl et al. have successfully developed label-free gold-silver core shell NPs for the detection of *Escherichia coli* with a detection limit of approximately 90 cfu mL⁻¹ through differential pulse voltammetry (DPV) [38]. Sheikhzadeh et al. also reported a label-free electrochemical aptasensor (polypyrrole polymers conjugated aptamers) to detect *S. Typhimurium* through impedimetric measurements with a detection limit of 3 cfu mL⁻¹ [39]. Guo et al. [40] synthesized graphene-based label-free aptasensor functionalized with electroactive aromatic dyes that improve the solubility of graphene and also preserve its intrinsic properties (e.g., thermal, electrical, and mechanical properties).

In this study, we demonstrate for the first time the application of a new conductive layer consisting of rGO-azophloxine (AP) nanocomposite as the sensing platform for the detection of a foodborne pathogen, *S. Typhimurium*. The chemical doping of rGO with AP dye enriches its free charge-carrier density, thus potentially enhancing its solubility in hydrophilic medium and electrical conductivity. Furthermore, the excellent electroactive AP molecules eliminate the complex and expensive process of labeling and modification of aptamers. No report to the best of our knowledge is available for foodborne pathogen detection using electrochemical biosensor via label-free aptamers as linkers that assemble nanoparticles and specific to an outer membrane protein of a specific pathogen. This developed electrochemical aptasensor offers an excellent foodborne pathogen sensing system due to its unique advantages, such as simplicity, high sensitivity, good selectivity, and cost efficiency.

**Materials and methods**

**Apparatus**

The surface structure and morphology of rGO-AP sensing platform was characterized using a scanning electron microscope (SEM, FEI, Quanta FEG 650) at a working distance around 5 mm and a transmission electron microscope (TEM, Carl Zeiss, LEO LIBRA 120). The Fourier transform infrared spectroscopy (FTIR) was measured on Bruker IFS 66V/S, USA, and Raman spectra were studied using Renishaw inVia Raman microscope, UK. For electrochemical measurements, PGSTAT302N was used as electrochemical workstation (Metrohm AG, Switzerland) with a platinum wire and
silver/silver chloride (Ag/AgCl) as counter electrode and reference electrode, respectively. Glassy carbon electrodes (GCEs) were used as working electrodes.

**Materials and reagents**

Graphite powder, AP, ammonia solution (25 wt%), and hydrazine hydrate (80 wt%) were purchased from Sigma-Aldrich. Phosphate buffer solution (PBS) 10× (137 mM of sodium chloride, 2.7 mM of potassium chloride, and 10 mM of phosphate buffer, pH 7.4 at 25 °C) and Tris-hydrochloric acid buffer (20 mM L⁻¹ of Tris-HCl, 0.1 mol L⁻¹ of sodium chloride, and 5 mM L⁻¹ of magnesium chloride, pH 7.4 at 25 °C) were used as an electrolyte for the electrochemical detection of bacteria. DNA aptamer sequences (5′-TATGGCGGCGTCACCCGACGGGGACTTGACATTATGACAG-3′) were adapted from Joshi et al. [41]. DNA oligomers were dissolved in an appropriate amount of deionized distilled water to obtain the stock concentration of 100 μM. *S. Typhimurium, E. coli, Shigella dysenteriae, Vibrio cholerae,* and *Klebsiella pneumoniae* bacterial cultures used in this study were obtained from the culture collection of the Biomedical Science Laboratory, University of Malaya, Malaysia.

**Preparation of rGO-AP**

Graphene oxide (GO) was prepared from graphite powder using the modified Hummer’s method [24, 42]. The protocol for the rGO-AP synthesis was adopted from Guo et al. [43]. To synthesize rGO-AP, 2.5 mg of AP was dissolved in 10 mL of homogeneous stable GO dispersion (0.25 mg mL⁻¹) in water. This suspension was stirred for 30 min at room temperature. Then, 100 μL of ammonia solution was added to the mixture for pH adjustment. The reduction of GO was aided by the addition of 10 μL of hydrazine solution into the mixture. After vigorous shaking for several minutes, the mixture was refluxed for 4 h at 60 °C followed by filtration of the product with a nylon membrane (0.22 μm). The rGO-AP nanocomposite obtained by filtration can be readily dispersed in water by ultrasonication to obtain a series of concentrations ranging from 1 to 8 mg mL⁻¹.

**Preparation of the electrochemical aptasensor**

Firstly, the GCE was polished with 0.05 μm alumina powder and sonicated with acetone and deionized water, respectively. The electrodes were dried at room temperature. Then, 10 μL of rGO-AP solution was drop casted onto the surface of GCE and air dried to form rGO-AP/GCE. For immobilization, 5 μL of aptamer (ssDNA) with concentration ranging from 1 to 10 μmol L⁻¹ was dropped onto the rGO-AP/GCE platform and air dried for different durations (15 and 30 min and 1, 6, and 24 h) to obtain ssDNA/rGO-AP/GCE. This sensing platform was stored at 4 °C until further use.

**Electrochemical characterization of working electrodes and electrochemical detection of bacterial cells**

Cyclic voltammetry (CV) was used to investigate the conductivity of the sensing platform in 10× PBS at a scan rate of 100 mV s⁻¹. To prepare the biosensing platform, ssDNA/rGO-AP/GCE electrodes were incubated with serially diluted *S. Typhimurium* (STM) suspensions of different concentrations ranging from 10⁸ to 10¹ cfu mL⁻¹ for varying durations (1 to 20 min). The DPV study was conducted in Tris-HCl buffer solution to study the sensitivity and detection limit of the sensing platform (Fig. 1) with instrumental parameters, including pulse amplitude 0.025 V, pulse width 0.05 s, and pulse period 0.05 s. Lastly, for the selectivity test, different types of bacteria (10² cfu mL⁻¹) including *E. coli, Sh. dysenteriae, V. cholerae,* and *K. pneumoniae* were conducted by DPV studies. For the evaluation with food sample, raw chicken meat was artificially spiked with *S. Typhimurium* cultures (~10⁷–10⁸ cfu mL⁻¹) and then homogenized in buffer peptone water (BPW) (1:10, w/v) followed by incubation for 4 h at room temperature. Then, an aliquot of the homogenate was serially diluted in Tris-HCl buffer to obtain a bacterial suspension range of 10⁶–10¹ cfu mL⁻¹ and confirmed by viable plate count. The developed sensing bioelectrodes were incubated in the diluted bacterial suspension for 5 min followed by the electrochemical detection. Food homogenates from nonspiked chicken meat were used as negative controls. All the tests were repeated three times to ensure the reproducibility of the results.

The results obtained from the sensitivity test were validated by PCR using oligonucleotide primers targeting the outer

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Fig. 1 Schematic diagram of the three-electrode setup for electrochemical analysis
membrane protein C (ompC) of *Salmonella* as previously described by Alvarez et al. [44]. The PCR reagents (25 μL) consisted of 1× green GoTaq Flexi Buffer, 1.5 mmol L⁻¹ magnesium chloride (MgCl₂), 280 μmol L⁻¹ deoxynucleoside triphosphate (dNTP) mix, 0.4 μmol L⁻¹ of each primer pair, 1 U Taq DNA polymerase (Promega, Madison, USA), and approximately 100 ng of bacterial genomic DNA. The PCR assay was carried out in a thermal cycler using the following conditions: initial denaturation at 95 °C for 2 min; followed by 35 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min; with a final extension step of 72 °C for 5 min and then held at 4 °C.

**Results and discussion**

**The schematic procedure of aptasensor fabrication**

Figure 2 shows a facile approach for the synthesis of rGO-AP. Firstly, the GO with abundant oxygen groups can be easily functionalized with AP dye to form GO-AP composite through π-π interactions of the aromatic rings of the dye and GO [40, 45]. Moreover, there were electrostatic interactions (noncovalent charge transfer) between the cationic center of the dye and oxygen functionalities of GO [46]. The oxygen-rich groups of GO also permit the hydrogen bonding with the dye species [47]. The chemical structure of the AP dye is shown in Fig. 3. A single AP molecule possesses two hydrophilic sulfonate (−SO₃⁻) functional groups. Previous studies have shown that the dispersibility of GO in aqueous solutions increased when GO was functionalized with −SO₃⁻-containing compounds, e.g., sulfonic acid and 4-hydrazinobenzenesulfonic acid [48, 49]. Hence, the aromatic AP dye applied in this study could effectively exfoliate or solubilize GO and rGO nanosheets in water due to the presence of polar −SO₃⁻ functional group. With the aid of hydrazine, the GO-AP was successfully reduced to rGO nanosheets functionalized with negatively charged electroactive dye AP via π-π stacking interaction (rGO-AP). The electrostatic repulsion caused by the negative charge on the rGO-AP surface and the hydrophobic aromatic backbone of the AP dye stacked on rGO surface may overcome the aggregation of rGO in aqueous solutions [40, 50].

The water-soluble rGO-AP nanocomposite formed a stable thin film on GCE by drop casting and gave rise to a higher peak current. Then, DNA aptamer was immobilized on the rGO-AP/GCE through π-π interactions between nucleotide bases of DNA and rGO-AP surface [51] which is denoted as ssDNA/rGO-AP/GCE. When the ssDNA/rGO-AP/GCE was incubated with *S*. Typhimurium, the peak current decreased due to the steric hindrance created by bacterial cells. A considerable rise in peak current was observed with increasing concentration of the target bacteria. Therefore, the rGO-based label-free electrochemical aptasensor has been successfully developed for *Salmonella* detection as illustrated in Fig. 2.

![Fig. 2 Schematic diagram of the approach for fabrication of rGO-functionalized AP and electrochemical detection of bacteria](image)
Characterization of rGO-AP

Structural and morphological characterization

The structural and morphological analysis was performed using SEM and TEM respectively. As shown in the SEM image of Fig. 4A, a sheet-like graphene structure was formed and consistent with the TEM analysis.

Figure 4B depicts the TEM images of rGO-AP nanocomposite which reveals some multiple folds of rGO nanosheets with uniformly distributed spherical structure of the AP dye adsorbed on the rGO nanosheets. While scanning the whole Cu grid area during TEM analysis, the formed dyes were uniformly distributed on every rGO sheet. Moreover, the inset in Fig. 4B corresponded to the boxed area under higher magnification. The average thickness of the multilayer rGO-AP nanosheet examined using TEM was approximately 10 nm and this implied that the rGO-AP composite was produced in nanosize.

FTIR analysis

FTIR spectra were used to identify the functional groups present in the rGO-AP film. Figure 5A shows the FTIR spectra of AP, rGO-AP, rGO, and GO, respectively. Theoretically, GO has several essential acute peaks detected by FTIR patterns such as C=O stretching at 1731 cm\(^{-1}\), C–O–C stretching at 1224 cm\(^{-1}\), and C–O stretching at 1058 cm\(^{-1}\). These results confirmed that the oxygen molecules (O) occupied GO during the synthesis step. Furthermore, a broad and intense band of hydroxyl group (–OH) at 3250 cm\(^{-1}\) was observed from the FTIR pattern (Fig. 5A). Interestingly, a broadening of the transmittance value was observed indicating the increased diffusion rate of oxygen molecules into the graphite flakes to form a carbonyl group, a carboxylic group, and an epoxy group in the GO sample.

All of the essential characteristic peaks of the rGO samples were weakened, and some hydroxyl functional groups were significantly dropped or disappeared compared with the GO sample. These phenomena clearly illustrated that oxygen functional groups such as C=O stretching, C–O–C stretching, C–O stretching, and hydroxyl groups were mostly removed and became rGO during the chemical reduction process using hydrazine. In addition, the C=C conjugation at 1568 cm\(^{-1}\) was detected on the rGO sample due to the sp\(^2\) hybridization. Meanwhile, a disappearance of the peak intensity of the O–H group in rGO sample at 3168 cm\(^{-1}\) was observed and approached zero when the AP was fully attached to the rGO. The main reason might be attributed to the implementation of rGO formation process for easy elimination of hydroxyl groups from the carbon basal plane and simple transformation to rGO monolayer during the attachment of AP stage.

The FTIR spectra of AP alone exhibited ring vibrations at 500–700 cm\(^{-1}\) attributed to CH bonds of aromatic rings. After
the chemical reduction to rGO and adsorption of AP dye on its surface, a significant change in FTIR spectra of the rGO-AP nanocomposite was observed as shown in Fig. 5A. For the rGO-AP spectra, it could be noticed that all of the ring vibrations of AP were visible at 502, 533, 600, and 734 cm\(^{-1}\) in agreement with a previous report \[43\]. Moreover, C–O/O–H stretching of AP was also found within the rGO-AP nanocomposite sample at 1025 and 1167 cm\(^{-1}\) followed by the bending vibration of C–H/O–H at 1429 cm\(^{-1}\). Interestingly, a significant decrease in the intensity of carboxyl group at 1739 cm\(^{-1}\) was observed which may be attributed to the electrostatic interaction between the nitrogen (cationic center) of the AP dye and COO\(^{-}\) of rGO \[46\]. This peak might have diminished due to the presence of delocalized electrons at the nitrogen center of the AP dye which affects the overall interactions and charge transfer with rGO \[52\]. Formation of a new peak was also observed in the spectra of the rGO-AP nanocomposite at 1571 cm\(^{-1}\) which may indicate the stretching vibration N=N (azo group) of the AP dye due to interaction with active functional groups \[53, 54\]. In addition, the presence of significant C=O stretching vibration (1683 cm\(^{-1}\)), C–H stretching vibration (2880–3000 cm\(^{-1}\)), and N–H stretching vibration (3700 cm\(^{-1}\)) further infers the attachment of AP dyes on rGO sheet.

**Raman spectroscopy analysis**

Disorder and defects in carbon-based materials were characterized using Raman spectroscopy. Figure 5B shows the Raman spectra for GO and rGO. The Raman spectra for GO and rGO manifest a successful reduction of GO to rGO relative to the changes in intensity of two important peaks, D and G. The Raman spectra for GO and rGO show two significant in-phase vibrations at 1349 cm\(^{-1}\) (D band) and 1593 cm\(^{-1}\) (G band) for rGO and at 1352 cm\(^{-1}\) (D band) and 1602 cm\(^{-1}\) (G band) for GO. The D band indicates the presence of disorder or structural defects in sp\(^2\)-hybridized carbon systems, whereas the G band obeys the first-order scattering of E\(_{2g}\) phonons due to the presence sp\(^2\)-hybridized carbon. The \(I_D/I_G\) ratio was used to measure structural disorder that occurs during the removal of oxygen groups attached to GO and size of sp\(^2\) ring clusters in a sp\(^3\)/sp\(^2\) hybrid network of carbon atoms.

As compared to GO, the chemically reduced rGO exhibits a stronger D band in the Raman spectra with higher \(I_D/I_G\) ratio due to the structural defects of graphene sheets. The defects were caused by distortion of bonds and increased formations of smaller size sp\(^2\) graphitic domains owing to the reduction process. However, a prominent G band proves that the crystallinity of the sample is preserved. After the surface functionalization of rGO with AP dye, the intensities and \(I_D/I_G\) ratio decreased to 0.61. The presence of D and G bands with lower intensities after functionalization indicates the recovery of conjugated graphitic domains of functionalized graphene surface with lesser defects and preserved the crystalline structure. Interestingly, the rGO-AP spectra not only exhibit G and D bands but also contain the bands of AP such as band N=N stretching vibrations (1406 cm\(^{-1}\)) of the azo compound, C–N symmetric stretch (1111 to 1208 cm\(^{-1}\)), very strong aromatic stretch.
quadrant stretch (1594 cm\(^{-1}\)), azo-benzene ring vibration (1489 cm\(^{-1}\)), phenyl-N vibration (1280 cm\(^{-1}\)), C–C stretching (1208 cm\(^{-1}\)), and a weak band of –SO\(_3^−\) groups (1172 cm\(^{-1}\)), indicating the successful attachment of AP with rGO [55, 56].

**Optimization of the sensing performance**

In order to obtain maximum sensing condition of the rGO-AP/GCE platform, four important parameters have been optimized in this study, namely the concentration of rGO-AP, concentration of aptamer, incubation time of aptamer, and bacterial cell immobilization. The effect of rGO-AP concentration loaded on GCE plays an important role to achieve a maximum amount of electrochemical signal as shown in Fig. 6A. The current density increased from 8 to 17 A m\(^{-2}\) with increasing concentration of rGO-AP and achieved maximum peak current at 3 mg mL\(^{-1}\). This could be due to the formation of a thicker film of rGO-AP nanocomposite (> 3 mg mL\(^{-1}\)) which decreased the electrical conductivity [43]. To get the optimum concentration of aptamer, the stock solution of the aptamer was diluted serially before the CV test. Maximum current density was obtained for the aptamer concentration of 5 \(\mu\)mol L\(^{-1}\) as shown in Fig. 6B. At higher concentrations of aptamer (> 5 \(\mu\)mol L\(^{-1}\)), the peak current dropped drastically which might due to the electrostatic repulsion created by negatively charged DNA molecules [57].

The hybridization of the aptamer with the rGO-AP/GCE sensing platform was studied as a function of incubation time. The electrochemical signal increased with incubation time and a complete hybridization of aptamer with the rGO sensing

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**Fig. 6 Effects of**

A rGO-AP concentration (mg mL\(^{-1}\)), B aptamer concentration (\(\mu\)mol L\(^{-1}\)), C aptamer incubation time (hour) and D bacterial incubation time (min) on the peak current measured with CV at a scan rate of 100 mV s\(^{-1}\).
platform was achieved in 1 h (Fig. 6C). Further increase in aptamer incubation time did not significantly affect the peak current. In this study, the incubation time with the bacterial suspension was also investigated. As shown in Fig. 6D, the electrochemical signal achieved a maximum current density after 5 min incubation time with bacteria cell suspension. Then, the peak current decreased dramatically which might be due to the saturation effect caused by bacterial cells blocking the current flow, leading to a decrease in electron transfer efficiency.

**Electrochemical analysis of working electrodes**

CV analysis was used to study the conductivity of rGO-AP/GCE under optimized conditions. Figure 7A shows the cyclic voltammograms of bare GCE, rGO/GCE, rGO-AP/GCE, and ssDNA/rGO-AP/GCE electrodes in 10× PBS (pH 7.0) at the scan rate of 100 mV s⁻¹. Redox peaks were absent in bare GCE and rGO/GCE, but a pair of redox peaks were observed for rGO-AP/GCE electrode which proved the redox reaction of AP dyes on the rGO surface. The AP dye has an innate electroactive property; thus, its attachment on the rGO surface increases the overall conductivity of the sensing platform. This AP dye also enhances a smoother flow of electrons on the rGO surface (transducer) to the electrolyte attributing an increase in peak current [43]. However, a subsequent increase in peak current was observed for ssDNA/rGO-AP/GCE electrode after aptamer immobilization due to the doping effect (direct charge transfer) between the adsorbed aptamer and rGO. Moreover, the high electron transferability nature of rGO [58] decreases the charge transfer resistance after DNA hybridization and increases the overall ionic conductance of the sensing platform [51, 59].

Figure 7B shows the DPV signals of bare GCE, rGO/GCE, rGO-AP/GCE, ssDNA/rGO-AP/GCE, and STM/ssDNA/rGO-AP/GCE electrodes in Tris-HCl buffer. The AP dye oxidized at −0.08 V indicated the interaction between ssDNA and bacterial cell. The oxidation peak was absent in bare GCE electrode, whereas upon the functionalization of the AP dye on the rGO surface (rGO-AP/GCE), a sharp increase in peak current was observed. The oxidation peak current at −0.08 V further increased with immobilization of aptamer on the sensing platform (ssDNA/rGO-AP/GCE) due to specific interaction of nucleobases of ssDNA with rGO-AP, increasing the electron flow across the electrolyte and contributing to higher peak current. However, binding of the target bacteria with respective aptamers caused a dramatic decline in peak current of the sensing platform STM/ssDNA/rGO-AP/GCE, indicating the hybridization event on rGO-AP/GCE sensing platform. The attachment of bacterial cells on the electrode surface will block the electron transfer process from the redox species (AP dye) resulting in the increase in resistance and subsequent decrease in current. The results obtained from DPV analysis were consistent with the CV studies.

**Electrochemical detection of bacterial cells**

**Sensitivity test of ssDNA/rGO-AP/GCE electrode**

The changes of DPV signal of the working electrodes in the presence and absence of bacterial cells were investigated. Figure 8A shows the sensitivity test of ssDNA/rGO-AP/
GCE electrode at different concentrations of bacteria cell suspensions. The DPV signal was obtained using Tris-HCl buffer. As shown in Fig. 8A, a higher oxidation peak was obtained for the ssDNA/rGO-AP/GCE than rGO-AP/GCE indicating the hybridization event of the aptamer on the rGO surface. When the sensing platforms were incubated in Tris-HCl buffer solutions containing different concentrations of *S. Typhimurium*, the peak current decreased with decreasing concentration of bacterial cells. This may be due to the conformational change between the aptamer and bacterial cell causing a decrease in adsorption of the aptamer on the rGO surface, resulting in changes in the charge transfer [60, 61]. Moreover, the attachment of bacterial cell on the sensing electrode surface will block the electron transfer process from the redox molecule (AP) due to steric hindrance created by the bulky negatively charged bacterial cell [62]. The natural properties of the bacterial cell membrane which is its membrane capacitance (0.5–1.3 μF cm⁻²) and membrane resistance (10²–10⁸ Ω cm²) contribute to the steric hindrance and increase the electron transfer resistance [63, 64].

The peak current increased with the increasing complementary target concentration exhibiting a limit of detection up to 10⁴ cfu mL⁻¹. The logarithm relationship between the target bacterial concentration and oxidation peak current is plotted (Fig. 8B) and the linear relationship is described as follows:

\[ I = 0.10 \pm 0.01 \log c + 1.35 \pm 0.15 \]

with the correlation coefficient of \( R^2 = 0.98 \), where \( I \) is the current density (A m⁻²) and \( \log c \) is the logarithm of the target bacterial cell concentration. The increase in peak current was proportional to the increase in target cell concentration due to the conductivity of the bacterial cell membrane and cytoplasm. The conductivity of the bacterial cell membrane is approximately \( 10^{-7} \) S m⁻¹, whereas the conductivity of the interior of a cell can be as high as 1 S m⁻¹ due to the presence of charged molecules in the cytoplasm [65]. When the concentrations of bacterial cells increased, the number of cells packed densely on the electrode surface will also be increased. This will lead to the formation of a conductive channel by the negatively charged bacterial cell membranes and increases the current flow [66].

Overall, a comparison on the performances of the well-established DNA-based methods of detection and the aptasensor is summarized in Table 1. This comparison shows that the developed rGO-AP aptasensor exhibits high sensitivity with improved detection limit and allows rapid detection of *Salmonella*.

### Table 1  Comparison of the biosensor developed in this study with other detection methods

<table>
<thead>
<tr>
<th>Method used</th>
<th>Targeted bacteria</th>
<th>Limit of detection (cfu mL⁻¹)</th>
<th>Assay time (h)</th>
<th>Detectable range in food</th>
<th>Ref</th>
</tr>
</thead>
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<tr>
<td>Multiplex-PCR</td>
<td><em>Salmonella</em> spp.</td>
<td>( 10^3 )</td>
<td>3</td>
<td>( 10^5 ) cfu mL⁻¹</td>
<td>[67]</td>
</tr>
<tr>
<td>Quantitative PCR</td>
<td><em>Salmonella enterica</em></td>
<td>( 5 \times 10^2 )</td>
<td>3</td>
<td>( 7 \times 10^2 ) cfu mL⁻¹</td>
<td>[68]</td>
</tr>
<tr>
<td>LAMP</td>
<td><em>Salmonella</em> spp.</td>
<td>( 10^2 )</td>
<td>3</td>
<td>( 6.1 \times 10^2 ) cfu mL⁻¹</td>
<td>[69]</td>
</tr>
<tr>
<td>NASBA</td>
<td><em>S. Enteritidis</em></td>
<td>( 10^1 )</td>
<td>18</td>
<td>10⁴ cfu/25 g</td>
<td>[70]</td>
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<tr>
<td>Immunological assay</td>
<td><em>S. Typhimurium</em></td>
<td>( 10^1 )</td>
<td>2</td>
<td>( 10^1 ) cfu mL⁻¹</td>
<td>[71]</td>
</tr>
<tr>
<td>Potentiometric aptasensor</td>
<td><em>S. Typhimurium</em></td>
<td>( 10^1 )</td>
<td>10 min</td>
<td>( 10^1 ) cfu mL⁻¹</td>
<td>This work</td>
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**Fig. 8** A DPV of (a) ssDNA/rGO-AP/GCE, (b) \( 10^8 \) cfu mL⁻¹, (c) \( 10^6 \) cfu mL⁻¹, (d) \( 10^4 \) cfu mL⁻¹, (e) \( 10^2 \) cfu mL⁻¹, (f) \( 10^1 \) cfu mL⁻¹, and (g) rGO-AP/GCE in Tris-HCl buffer, pH 7.4. B Linear relationship between the current density and cell concentration (logarithm). C DPV of (a) *S. Typhimurium*, (b) *E. coli*, (c) *V. cholerae*, (d) *K. pneumoniae*, and (e) *Sh. dysenteriae* in Tris-HCl buffer, pH 7.4.
Selectivity test of ssDNA/rGO-AP/GCE electrode

The ability of the aptasensor in distinguishing between S. Typhimurium and non-S. Salmonella bacterial cell was also studied as shown in Fig. 8C. The DPV data showed that the aptamer was only specific for S. Typhimurium as compared to other foodborne bacteria, namely E. coli, Sh. dysenteriae, V. cholerae, and K. pneumoniae. The redox peaks were visible in all bacterial cultures due to the presence of electroactive dye AP and conductivity of bacterial cell membranes. However, all the current peaks except for S. Typhimurium were found below the detection limit of the biosensor.

Evaluation of the of ssDNA/rGO-AP/GCE electrode in food sample

This aptasensor system was tested with artificially spiked chicken meat with three different concentrations (10^1, 10^2, and 10^4 cfu mL^{-1}) of S. Typhimurium. The DPV signals were recorded in Tris-HCl buffer. The measured DPV signal exhibited increased peak currents corresponding to the increase in target cells as shown in Fig. 9. This aptasensor can detect bacterial concentration ranging from 10^1 to 10^4 cfu mL^{-1} in the artificially spiked chicken sample. As shown in Table 2, the reading exhibited a similar response with the linear relationship between the current density and cell concentration of ssDNA/rGO-AP/GCE sensing platform tested in pure bacterial cultures. There was no significant difference (P > 0.05, t test analysis) between the current density obtained from different concentrations of bacteria from the cell culture suspension and from the food homogenates of the spiked sample.

Reproducibility and stability of the aptasensor

The reproducibility of the aptasensor was investigated by parallel measurements of the conductivity, sensitivity, and selectivity in Tris-HCl buffer. The relative standard deviation (RSD) was 1.28% (n = 3), revealing acceptable reproducibility for the detection of bacteria. This aptasensor also showed a good stability up to 20 days with increase in current response by 5.1% when stored in ultrapure water at 4 °C.

Validation of experimental results with the PCR method

The results obtained by the aptasensor for the sensitivity test were validated with PCR assay as shown in Fig. 10. PCR amplification of the ompC of S. Typhimurium gave a DNA fragment of 204 bp. DNA extracted from the different concentrations of bacterial cell density (10^8 – 10^1 cfu mL^{-1}) was tested with PCR. The results obtained showed that the detection limit of PCR was only up to 10^2 cfu mL^{-1} as compared to the detection limit of the aptasensor which was 10^1 cfu mL^{-1}. This shows that aptasensing is more sensitive than PCR.
Conclusion

In summary, a facile electrochemical aptasensor has been successfully developed for the first time by incorporating rGO-AP nanocomposite coupled with label-free DNA aptamer for the electrochemical detection of S. Typhimurium. This rGO-AP nanocomposite is biocompatible with aptamer and exhibits excellent electrochemical activity due to the enhanced electron transfer of AP. This biosensor showed high sensitivity for detecting S. Typhimurium with a detection limit of 10^1 cfu mL^-1. Thus, this new aptasensor with the proposed electrochemical assay shows a promising and cost-effective approach for application in food safety control.

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Compliance with ethical standards

The authors declare that they have no conflict of interest.

References


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