Analytical model of graphene-based biosensors for bacteria detection

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In this research, a set of novel models based on field effect transistor (FET) structure using graphene have been proposed with the current–voltage (I–V) characteristics of graphene employed to model the sensing mechanism. It has been observed that the graphene device experiences a drastic increase in conductance when exposed to \textit{Escherichia coli} bacteria at 0–10\textsuperscript{4} cfu/mL concentrations. Hence, simplicity of the structure, fast response rate and high sensitivity of this nanoelectronic biosensor make it a more suitable device in screening and functional studies of antibacterial drugs and an ideal high-throughput platform that can detect any pathogenic bacteria. Accordingly, the proposed model exhibits a satisfactory agreement with the experimental data.

Keywords: graphene; \textit{E. coli} bacteria; biosensor; field effect transistor; I–V characteristics

1. Introduction

Current research in carbon-based materials has been conducted extensively to accommodate the advancing technology [1,2]. The discovery of a single atomic sheet of graphite layer, or graphene, by André Geim in 2004 has attracted the interest of researchers due to its superb electronic properties, which are technology-driven [3–6]. Single-layer graphene consists of sp\textsuperscript{2}-bonded carbon atoms that are arranged in the form of a hexagonal lattice (honeycomb lattice) in a two-dimensional (2D) structure comprising a thin layer of single carbon atoms. When compared with most conventional materials, graphene exhibits unique electronic properties [5,7–10]. In addition, graphene has a very high charge carrier mobility that could exceed 200,000 cm\textsuperscript{2}/Vs at room temperature. According to experimental transport measurement results, graphene can serve as an extremely attractive material for future use in nanoelectronic devices due to the extraordinary features of high electron mobility particularly in sensor applications [11–16].

The discovery of \textit{Escherichia coli} bacteria in human colon by Theodor Escherich (1885) was a significant breakthrough in public health sector; since then, the detection of the bacteria in different matrices and particularly in the environment has been a major issue [17–20].

Several biosensor structures have been reported for \textit{E. coli} sensing, such as optical biosensors, photodiode-based sensing [21] and integrated waveguide biosensors [22]. In addition, electro-chemical sensing techniques [23,24] or carbon nanotube biosensors based on field effect transistor (FET) structure have been described with very high bound of sensing [25–28]. Nonetheless, the greater parts of these experiments need the use of labels for detection and

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therefore, a clearer method is required. However, these biosensor fabrications are unmanageable or the limit of sensing is remarkably low. Thus, properties and definition of nanomaterials utilised as a part of nanobiotechnology for the \textit{E. coli} sensing is recorded in Table 1.

The introduction section explains a graphene-based nanoelectronic sensor and its extremely sensitive bacteria (\textit{E. coli}) detection (10 cfu/mL). In comparison with the earlier mentioned approaches, which are complex and time consuming, graphene-based nanoelectronics offers accurate and quick estimation.

\section{Experimental}

Chemical vapour deposition (CVD) method by using ethanol has been used to grow graphene film on copper foils. It was coated by a layer of small depth of polymethyl methacrylate (PMMA) dissolved in chlorobenzene. Then by using chemical etching method, graphene/PMMA was released from the copper foil. The drain and source (two electrodes) were so prepared as to pass through the graphene by using silver. Lastly, for electrode insulation, silicon rubber was used [20].

\textit{E. coli} (K12 ER2925) was bought from New England Biolab and it was cultured in LB (Luria Bertani) medium at 37°C. For $10^7$ cfu/mL density \textit{E. coli} production, culturing and colony counting methods were used. It was prepared for experimental work by diluting it in PBS solution (pH 7.2). The harvested \textit{E. coli} was stored at $-80^\circ$C.

A semiconductor device analyser was used for electrical measurement (Agilent, B1500A) and all measurements have been done under ambient conditions [20].

\section{Results and discussion}

The prototype of ion-sensitive field-effect transistor has indicated excellent performance for transistors, interconnects, electromechanical switches, infrared emitters and biosensors [29,30]. As shown in Figure 1, it appears similar to the electrolyte-gated FET. A graphene channel connects the source and drain electrodes [31,32]. When \textit{E. coli} bacteria come in contact with the surface or edge of graphene, the amount of carrier concentration changes due to the variability of the drain source current, which is a measurable parameter. The adsorbed molecules change the conductivity of the graphene from which the relation between conductivity and charge carrier density or carrier mobility can be derived and investigated [33–36].
An *E. coli* in contact with a film of antibody-functionalised graphene is illustrated in Figure 1a and 1b. This configuration focuses on checking the system response and finding the kinetics of bacteria binding. The sensor was kept in chambers with $10^4$ cfu/mL of *E. coli* [20,37]. As higher numbers of *E. coli* are caught by the graphene film antibodies, the conductance of the channel increases. Operating the graphene FET in the $p$-type region with zero gate voltage confirms that the graphene conductance increases as a result of higher levels of density caused by the high negativity of the walls of the bacteria.

To detect *E. coli* bacteria, the 1-pyrenebutanoic acid succinimidyl ester is used as a linker. The succinimidyl ester group at the end covalently reacts with the amino group on the antibody and pyrene group at another end binds to the graphene surface through strong $\pi-\pi$ interaction. It has been confirmed by experimental results that conductance of graphene is a function of carrier density and mobility. In other words, changes in electron density and/or charge carriers by absorption of *E. coli* linker molecules or ions in graphene change the conductance [38].
The modelling of biosensor based on graphene is described in the supplementary material (S1). Based on that, the rate of changes in conductivity depending on the *E. coli* concentration or the performance of biosensor was assessed.

To further examine their functionality, the graphene devices were kept in closed chambers with various *E. coli* concentrations to allow the bacteria to grow and multiply. The graphene devices were then washed with a PBS solution of pH 7.2. This is to enable the production of the intended final *E. coli* concentration \([20]\). Next, they were electrically tested by measuring the I–V characteristics with zero voltage between the solution and the gate. The corresponding I–V curves of a single graphene device before and after being exposed to *E. coli* bacteria at concentrations between 0 and \(10^4\) cfu/mL are illustrated in Figure 2(a)–2(f).

![Figure 2](image1.png)

**Figure 2.** The current–voltage characteristics for different *E. coli* concentrations.
The current–voltage characteristics of the proposed model for biosensor based on graphene in comparison with results from experimental data are illustrated in Figure 2 (a)–2(f). It can be observed from the results that the charge transfer between E. coli (0–10,000 cfu/mL) and graphene causes the current to increase. An acceptable agreement between extracted data and the suggested model are clearly illustrated by the figures. In the proposed model, different E. coli concentrations are shown in terms of the control parameter ($\psi$) as presented in Table 2.

Figure 3 shows the evaluation of the rate increment in conductance of graphene affected by several bacteria concentrations.

As can be seen, the concentration of E. coli as low as 10 cfu/mL could be erroneously detected. This method is several steps lower than the earlier stated approaches used by SWCNT-network FETs [39], polymerase series response [40] and external plasmon intensification [41]. Particularly, 10 cfu/mL created 3.25 ± 0.43% increment in graphene-based sensor conductance ($n = 6$ devices), which relates to the existing increment of ~1.17 µA at $V_{ds} = 0.2$ V (notably greater than the existing noise of (0.02 µA)).

In Table 3, the validation data for the analytical model are presented. MSE signifies mean square error, $R^2$ is $R$-squared, $Q^2$ is cross-validated, RSS is residual sum of squares,
TSS is total sum of squares, SSE is the sum of squared residuals and PRESS is predictive error sum of squares.

Data validation is the process of determining the degree to which a simulation model and its associated data are an accurate representation of the real world from the perspective of the intended uses of the model. As shown in Table 3, the validation data for analytical model is calculated for different E. coli concentrations (0–10,000 cfu/mL).

4. Conclusion
Three different models were developed by this research using artificial neural network, support vector regression and analytical method to characterise the current–voltage relation of a graphene-based sensor for E. coli bacteria. The graphene component shows a measureable sensitivity when in contact with E. coli as its conductance changes. Hence, this behaviour is proposed to be used in the detection of this type of bacteria. Also, a bacteria concentration control parameter (ψ) is introduced in the derivation of the analytical model and is calculated iteratively. The created sensor is label-free, rapid, extremely sensitive and selective biosensor for detecting bacteria E. coli with a very low sensing limit of 10 cfu/mL. The proposed model would enhance a realistic understanding of the biosensor performance under exposure to E. coli, thereby minimising the need for empirical experiments.

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Supplemental data
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Table 3. Validation data for analytical model at different concentration of E. coli.

| E. coli concentration | 0     | 10    | 100   | 1000  | 10,000 |
|-----------------------|-------|-------|-------|-------|--------|
| MSE                   | 0.0128| 0.00009| 0.0008| 0.0003| 0.0072 |
| $R^2$                 | 0.9411| 0.9986| 0.9915| 0.9966| 0.9613 |
| $Q^2$                 | 0.7951| 0.9979| 0.9811| 0.9910| 0.8334 |
| RSS                   | 0.0913| 0.0016| 0.0108| 0.0039| 0.0429 |
| TSS                   | 1.5512| 1.1619| 1.2765| 1.1479| 1.1079 |
| SSE                   | 0.2681| 0.0021| 0.0182| 0.0081| 0.1443 |
| PRESS                 | 0.3178| 0.0024| 0.0241| 0.0103| 0.1846 |
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