

Comparative Study of Electrochemical Biosensors Based on Highly Efficient Mesoporous $\text{ZrO}_2\text{-Ag-G-SiO}_2$ and $\text{In}_2\text{O}_3\text{-G-SiO}_2$ for Rapid Recognition of *E. coli* O157:H7

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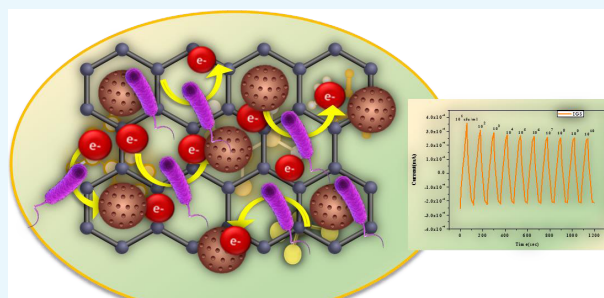


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ABSTRACT: Here, we reported an innovative and electrochemical biosensor for the rapid detection of *Escherichia coli* O157:H7. We fabricated the mesoporous $\text{ZrO}_2\text{-Ag-G-SiO}_2$ (ZAGS) and $\text{In}_2\text{O}_3\text{-G-SiO}_2$ (IGS) sensors, and cyclic voltammetry (CV) was employed to detect the bacteria. The development of these portable sensors addresses the challenges of conventional time-consuming and more expensive laboratory-based analyses. Hence, the biosensors were highly selective to detect *E. coli*. The sensor could recognize an individual *E. coli* cell in 1 μL of sample volume within 30 s. *E. coli* live cells tied down on sample nanoparticles worked toward the definite acquirement of *E. coli*. The high thickness of negative charge on the surface of *E. coli* cells effectively regulated the concentration of dominant part charge carriers in the mesoporous channel, allowing a continuous check of *E. coli* concentration in a known sample. The signal current decreased linearly, while the *E. coli* concentration increased from 1.0×10^1 to 1.0×10^{10} CFU/mL. ZAGS and IGS biosensors could detect *E. coli* in the range from 10^1 to 10^{10} CFU/mL. ZAGS and IGS biosensors in this investigation showed great specificity, reproducibility, stability, and selectivity and are expected to have a great impact on applications in the detection of foodborne pathogens.



E. Coli sensing

1. INTRODUCTION

Escherichia coli O157:H7 is the most widely recognized Shiga toxin-producing strain of *E. coli* and can cause disease with a minimum concentration of cells. Manifestations comprise severe stomach spasms, looseness of the bowels, nausea, or even lethal hemolytic uremic syndrome (HUS).^{1,2} Electrochemical biosensors, a branch that is used for rapid detection, good sensitivity, miniaturization potential, and mass production, have been demonstrated to be exceptionally encouraging in the biosensor field, with continuous progress in nanomaterials, for instance, nanoporous films,^{3,4} nanochains,⁵ nanotubes,^{6,7} microelectrodes,⁸ and screen-printed electrodes.^{9,10} Among the different nanomaterials considered,¹¹ graphene and its various forms, for example, graphene oxide (GO), reduced graphene oxide (RGO), and graphene quantum dot (GQD), have attracted attention for the progression of biosensors. Graphene is a single-atom-thick sheet of hexagonally organized, sp^2 -bonded carbon atoms occurring within a carbon material structure.¹² The two-dimensional (2D) properties of graphene with ultrahigh charge mobility give excellent electronic properties, having a large surface area (2630 m^2/g), which accommodates a high possibility of active sites for charge–biomolecular interactions, leading to sensing enhancement as well as supporting the desired functionalization to target biomolecules to improve selectivity.^{13–15} Meanwhile,

functionalized graphene is achieved by synthesis and preparation, for example, the carbon core structure can be oxidized forming GO, the reduced structure with vacancy defects is RGO, and structures a few nanometers in size with quantum phenomena are GQDs.^{16–18} ZrO_2 as a transducer is a promising material in the fabrication of a biosensor due to biocompatibility, phenomenal electrical and surface charge properties, and oxygen moieties.¹⁹ Silver nanoparticles (AgNPs) have low cost, nontoxicity, biocompatibility, and steady and high catalytic activity, helping us utilize it for biological sensing. In_2O_3 has been used for technological applications in optoelectronic devices and biosensors, owing to its high electric conductance, high transparency to visible light, and the strong interaction between certain biomolecules with In_2O_3 surfaces.²⁰ Mesoporous SiO_2 exhibits many advantageous characteristics, such as large surface and free volume, pore sizes that can be controlled, and tunable optical properties, and mesoporous SiO_2 -based biosensor directly

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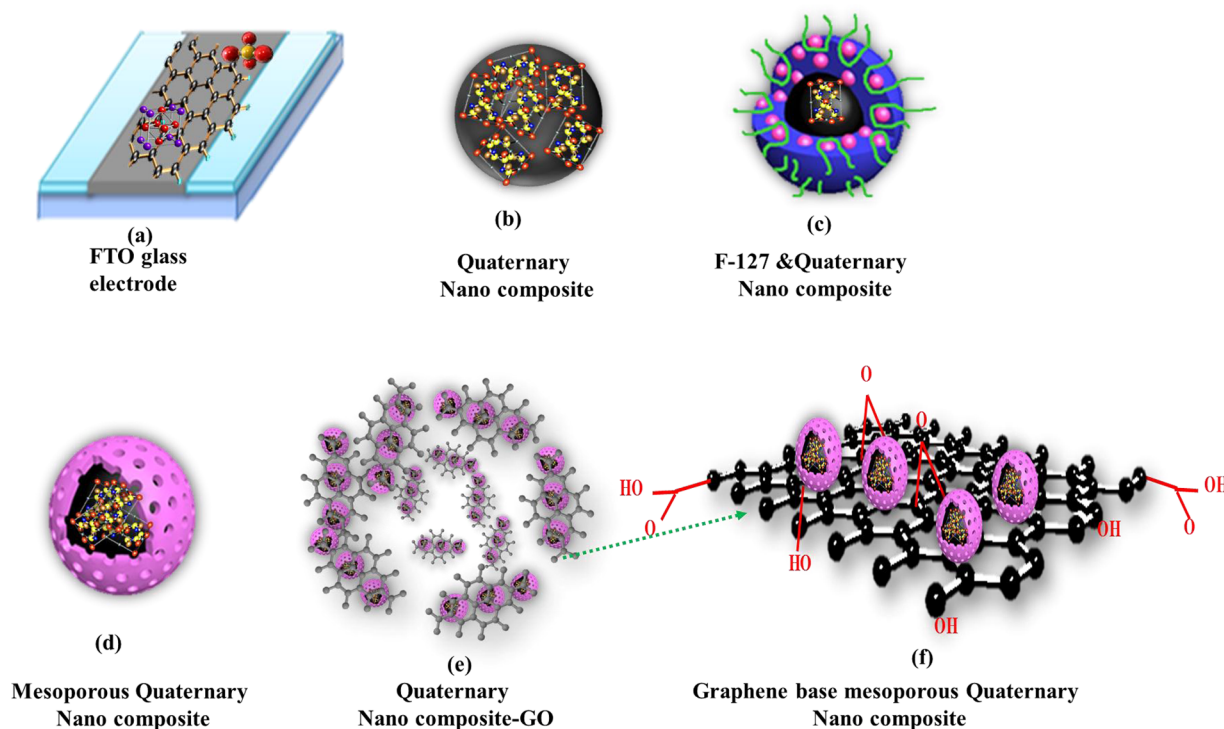
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Scheme 1. Synthesis Process of Graphene-Based Quaternary Mesoporous Nanocomposites



captures the target bacteria cells onto the porous surface. Therefore, monoclonal antibodies, which are specific to target bacteria, are immobilized onto the porous surface of a biosensor, which induces changes in the amplitude (intensity) of the sensor.^{21–27} The nanocomposites combining two or several different components are expected to further improve the deficient characteristics of each component, leading to a promising application in biosensing. In this comparative study, we fabricated two mesoporous nanocomposites, In_2O_3 -G-SiO₂ (IGS) (ternary) and ZrO_2 -Ag-G-SiO₂ (ZAGS) (quaternary), for detecting *E. coli*. We focus on the difference between the two mesoporous materials in ternary and quaternary type, which can be achieved through biosensing application individually. Considering that ZrO_2 -Ag-G-SiO₂ and In_2O_3 -G-SiO₂ have excellent physical properties, electrical properties, and also good electrochemical stability, they can make a steady sensing framework for the preparation of graphene-based quaternary nanocomposites. The objective of the present study was to set up a novel nonenzymatic sensor for the sensing of *E. coli*. Its sensitivity, linear range, detection limit, and stability were examined. The ZrO_2 -Ag-G-SiO₂ and In_2O_3 -G-SiO₂ nonenzyme sensors were very sensitive, entirely stable, and financially savvy. Also, samples have been effectively analyzed through developed sensors for *E. coli* sensing. This new concept has opened a new direction to construct rapid, delicate, and highly efficient electrochemical biosensors.

2. EXPERIMENTAL SECTION

2.1. Materials. Graphite powder flakes (99%), zirconium(IV) isopropoxide (70 wt % 1-propanol), and Pluronic F127 were procured from Sigma-Aldrich; ethylene glycol, AgNO_3 , HCl, phosphate buffer, NaOH, KOH, ethylene glycol, tetraethyl orthosilicate (TEOS), indium(III) chloride (InCl_3), urea, and sodium dodecylbenzene sulfonate (SDBS) were procured from Dae-Jung Chemical, Korea. Deionized

water (18.2 M Ω /cm) was utilized throughout the work. All other chemicals were of investigative grade and were used without further purification.

2.2. Synthesis Procedure. **2.2.1. Mesoporous SiO₂ Synthesis.** Initially, 1.0 g of triblock copolymer Pluronic F127 surfactant (Sigma-Aldrich) was added to a solution containing 15 mL of deionized water and 60 mL of 2 M HCl at 313 K with stirring until the copolymer was dissolved. At that point, 3.20 mL of tetraethyl orthosilicate (TEOS, Acros Organics) was added and stirred for 12 h at 313 K. The mixture was moved to a sealed container and heated to 373 K in an oven for 20 h. The resulting white precipitate was filtered, washed with water and ethanol, and dried at 338 K overnight. Finally, the copolymer was removed by calcination in air at 823 K for 3 h.

2.2.2. Synthesis of In_2O_3 -G-SiO₂. The mesoporous In_2O_3 nanoparticles were dispersed in 7 mL of deionized water, followed by the addition of 0.07 wt % GO water suspension considering the proportion of coordinated In_2O_3 nanoparticles. GO was obtained from graphite using Hummer's method. The mixed solution was ultrasonicated for 20 min. This In_2O_3 -GO solution was then added dropwise to a 100 mL beaker containing silica powder at 10 and 20%. After stirring for 48 h, it was filtered, washed with 1 mL of methanol, and dried at 343 K. The furnace temperature was increased to 973 K at a speed of 10 °C/min and held at 973 K for 4 h. A light-yellow sample was obtained.

2.2.3. Synthesis of ZrO_2 -Ag-G-SiO₂. First, 6.5 g of Pluronic F127 was liquefied in 30.5 mL of ethanol, then 30.5 mL of the zirconium(IV) isopropoxide solution was added to 30.5 mL of ethanol and ethylene glycol with vigorous stirring. At this point, both solutions were mixed and stirred at 314 K for 1 h by adding 20.5 mL of H₂O dropwise to this mixture. Further, 3.5 g of AgNO_3 was liquefied in 10.5 mL of deionized water and then poured dropwise to the ZrO_2 solution under vigorous

stirring in the dark; the mixed solution was constantly stirred till the gel was formed. First, 0.333 g of graphene oxide (GO) was added into 250 mL of water and ultrasonicated for 35 min. Sonicated graphene oxide was transferred to the ZrO_2 -Ag solution and then stirred for 2 h at 374 K. The color of the precursor turned into a coffee color, showing the effective combination of G with Ag-combined ZrO_2 solution, forming a solution named E. The solution E was added dropwise to a beaker containing 0.3 g of SiO_2 powder and stirred at 374 K for 24 h and ultrasonicated for 1 h and 30 min. Then, the powder was filtered, washed with 1.5 mL of methanol, and dried at 338 K overnight. Next, it was calcined at 974 K at a speed of 283 K/min and held at 974 K for 5 h. Black color products were formed (Scheme 1).

2.3. Preparation of IGS and ZAGS Electrode. IGS and ZAGS thin films were developed using a traditional doctor-blade method.^{28–30} For the modified doctor-blade method, the synthesized ZrO_2 (ZS), ZrO_2 -Ag (ZA), ZrO_2 -Ag-G (ZAG), and ZrO_2 -Ag-G- SiO_2 (ZAGS) (Table S1) paste was prepared as follows: first, the synthesized material powder (1.1 g) was mixed with ethylcellulose and acetone (1.5 mL) in a mortar for 15 min. Fluorine-doped tin oxide (FTO) glass was shielded through the sample paste to make a thin film. Afterward, it was dried in the open air for 35 min. One drop of lubricating oil was put onto the film surface and stabilized at 374 K in the oven for 25 min to diminish cracks. A greasing oil/lubricating oil on the film will the enable sensor via prevention of contamination of surfaces at points where films may break.³¹

2.4. Characterization of the Materials. The phase structure and purity of the as-synthesized products were examined by X-ray diffraction (XRD; Rigaku, X-ray Diffractometer) with $\text{Cu K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$) at 40 kV, 30 mA over the 2θ range $20\text{--}70^\circ$. Morphologies were studied utilizing scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) analysis by utilizing SEM (JSM-76710F, JEOL, Tokyo, Japan), transmission electron microscopy (TEM) (JEM-4010, JEOL, Tokyo, Japan), and high-resolution TEM (HRTEM) (JSM-76710F, JEOL, Tokyo, Japan) operating at a 300 kV accelerating voltage. X-ray photoelectron spectroscopy (XPS), differential reflectance spectroscopy (DRS), and Raman spectroscopy (RAMAN) analyses were performed utilizing WI Tec. alpha300 series. Porous characterizations of ZAG and ZAGS structures were obtained with a full analysis of N_2 adsorption–desorption tests (BELSORP-max, BEL Japan Inc.). (PG201, Potentiostat, Galvanostat, VoltaLab, Radiometer, Denmark.)

2.5. Electrochemical Measurements. Cyclic voltammetry (CV) and measurements were performed under a three-electrode electrochemical setup to check the current and voltage profiles, where ZS, ZA, ZAG, and ZAGS were utilized as working electrodes, while platinum cathode and Ag/AgCl were used as the counter and reference electrodes, respectively. Electrochemical properties in commercial urine were examined at pH 7.0. As electrolytes, a phosphate buffer aqueous solution was used. The limit of detection (LOD) of the analyte was determined by the following equation^{32,33}

$$\text{LOD} = 3\text{SD}/N \quad (1)$$

where SD is the standard deviation of the analyte concentration determined from the current response of progressively including *E. coli* into the electrolyte and N is the slope of the calibration curve, which demonstrated the sensitivity of the electrode with a signal-to-noise ratio (SNR)

of 3. All analyses were completed by voltammetry (PG201, Potentiostat, Galvanostat, VoltaLab, Radiometer, Denmark).

3. RESULTS AND DISCUSSION

3.1. Characterization of the IGS and ZAGS Sample.

The average crystallite size of the as-provided sample was determined by the Scherrer equation demonstrated as follows

$$D = K \lambda / \beta \cos \theta \quad (2)$$

where D is the grain size, K is the Scherrer constant (generally 0.89), λ is the X-ray wavelength, β is the width of half of the diffraction peak, and θ is the Bragg diffraction angle. To determine the crystalline phase, structure, and chemical compositions of IGS and ZAGS structures, we utilized XRD analysis. Figure 1a shows the XRD patterns of the IGS sample.

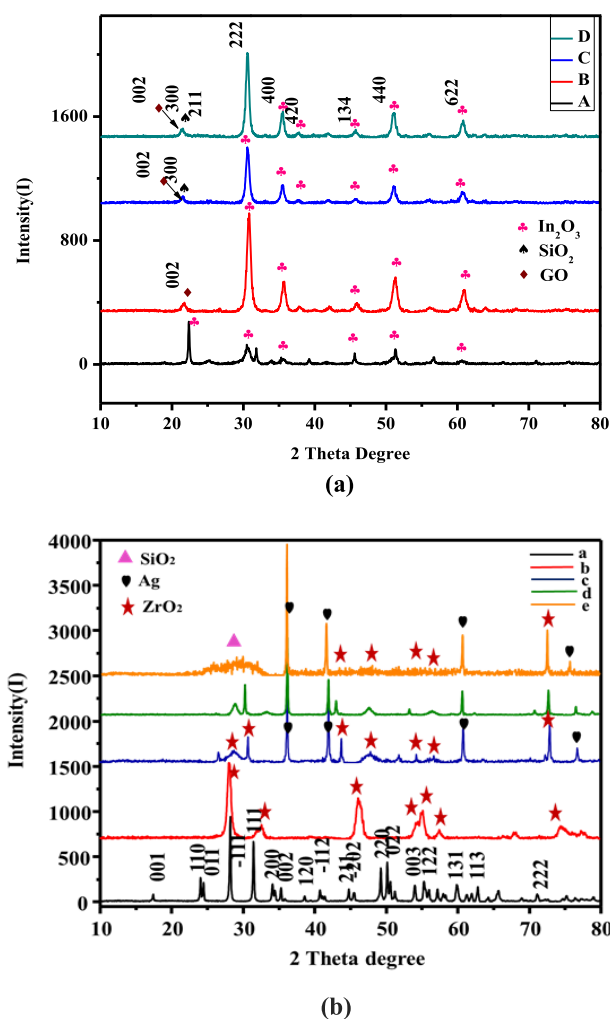


Figure 1. (a) XRD patterns of In_2O_3 (A), In_2O_3 -G (B), In_2O_3 -G- SiO_2 -10% (C), and In_2O_3 -G- SiO_2 -20% (D) and (b) commercial ZrO_2 (a), synthesized ZrO_2 (b), ZrO_2 -Ag (c), ZrO_2 -Ag-G (d), and ZrO_2 -Ag-G- SiO_2 (e).

It showed sharp peaks and no miscellaneous peaks. With the improvement of crystallites and the enhancement of crystallization, the peak intensities increased, which is designated to the great crystallinity of these samples.

Figure 1b shows the crystalline characteristic pattern of the ZAGS sample confirmed by the X-ray diffraction (XRD) method. As-synthesized ZrO_2 was shown at 24.8° with a lattice

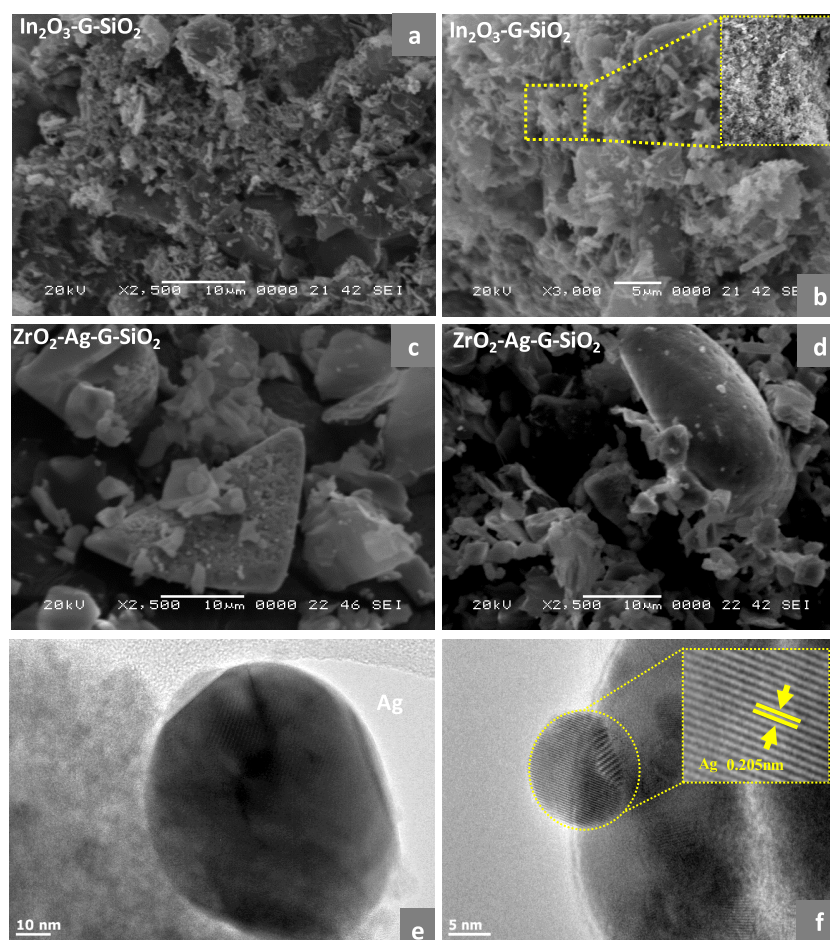


Figure 2. SEM images of In_2O_3 -G- SiO_2 (a, b) and ZrO_2 -Ag-G- SiO_2 (c, d); TEM and HRTEM image of Ag nanoparticle (e, f).

plane of (110). All diffraction peaks were well-indexed (JCPDS 37-1484). After being modified with Ag and SiO_2 , the ZrO_2 -Ag-G- SiO_2 spectrum demonstrated all diffraction peaks along the extra peaks, corresponding to (JCPDS 65-2871) and (JCPDS 39-1425), respectively, approving the pure crystalline nature of the material.

The morphology of IGS and ZAGS was considered by scanning electron microscopy (SEM). Figure 2a,b shows SEM images of IGS. The results showed that a heterogeneous structure could be formed by controlling the particle size and morphology. Figure 2a demonstrates pure In_2O_3 and G comprised of expansive sporadic spheres. After introducing SiO_2 , large amounts of nanocrystals were present on In_2O_3 surfaces. Figure 2b shows that the particle size was increased slightly, and the total of nanoparticles became sporadic. Figure 2c,d confirmed that ZAGS was combined. These figures revealed that nano-flake-like structures of ZAGS were uniformly distributed. Such flake-like nanostructured geometry prompted a rough surface of the electrode, which enhances the electrode performance on account of its high surface area, better surface-to-volume ratio, and disclosure of more electrocatalytically active sites on ZAGS. Figure 2e shows the TEM image of Ag nanoparticle and shows continuous single directional lattice fringes. HRTEM image is shown in Figure 2f. The marked d -spacing of 0.205 nm corresponds to the interplanar distance of $\text{Ag}\{200\}$ lattice planes. The HRTEM results further confirmed that the Ag nanoparticles adopt a single-crystal structure. Ag nanoparticles show equal distribu-

tion in TEM micrographs. They can increase the surface area of the sensor and are highly conductive, facilitating the charge transfer process. After the addition of AgNPs on the electrode surface with a large surface area, the sensor was used to detect *E. coli* with high sensitivity, where *E. coli* is immobilized on the biosensor surface through its mesoporous structure.

Figure 3a shows that the Raman spectra of IGS and ZAGS structures are polarized parallel (E) and perpendicular ($E_\perp$) to their growth direction. The crossed polarization configuration demonstrates the cooperation among In_2O_3 , ZrO_2 , and G. This was affirmed by the Raman spectral analysis. For IGS, two distinct peaks were situated at 1450 and 1550 cm^{-1} . These intensity peaks could be recognized at unadulterated In_2O_3 vibrational modes. As expected, G showed two prominent G and D bands generally attributed to the E_{2g} phonon of sp^2 bonds of carbon atoms and a breathing method of κ -point phonons of A_{1g} symmetry, respectively.³⁴ Lorentzian line shape analysis of these polarization-dependent relative intensities in In_2O_3 spectra showed that the Raman determination rules remained unaltered. The validity of these determination guidelines for this situation is related to the nanowire's great crystalline quality. For single crystals, phonons are expected to experience a long-range order, causing phonons to conserve the translational symmetry. As shown in Figure 3b, as-synthesized G indicated two peaks at 1331 and 1573 cm^{-1} corresponding to the D band and the C–C bond stretching frequency (G band), respectively. Generally, the intensity proportion of the D and G bands (I_D/I_G) is utilized to assess

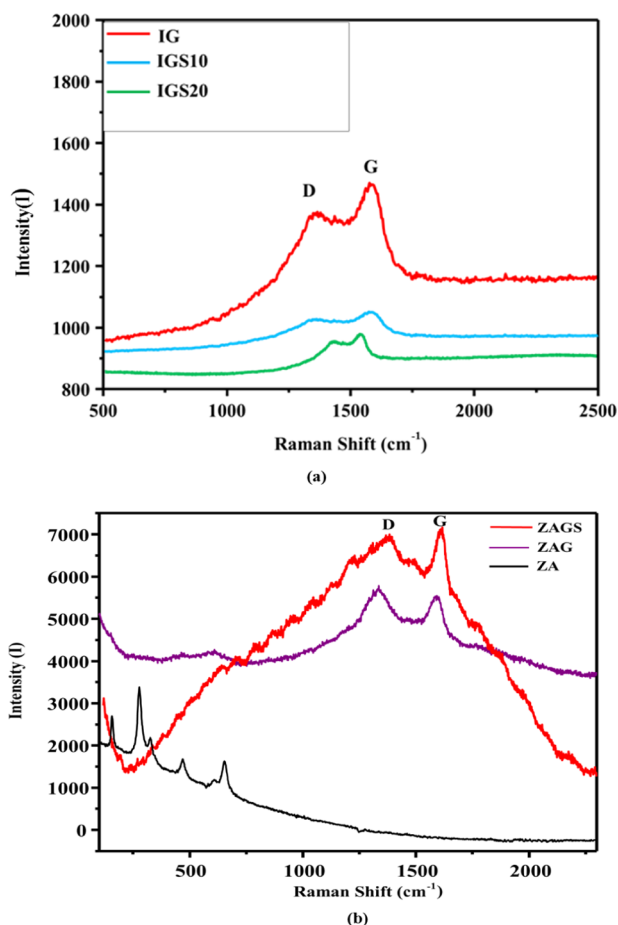


Figure 3. (a) Raman spectra of In_2O_3 -G (IG), In_2O_3 -G- SiO_2 10% (IGS10), In_2O_3 -G- SiO_2 20% (IGS20) and (b) ZrO_2 -Ag (ZA), ZrO_2 -Ag-G (ZAG), and ZrO_2 -Ag-G- SiO_2 (ZAGS).

the degree of disorder and the typical size of sp^2 areas. In this study, the value of I_D/I_G was calculated to be 0.94.

Figure 4 shows the resultant absorbance of UV-DRS. DRS data recorded for IGS and ZAGS structures were changed over to absorption spectra by the Kubelka–Munk (K–M) theory. Their respective band gaps (E_g) were evaluated using the following equation³⁵

$$(\alpha h\nu)^{1/2} = A(h\nu - E_g) \quad (3)$$

where α is the molar absorption coefficient determined as $\alpha = (1 - R)^2/2R$, $h\nu$ is the incident light frequency, A is the proportionality constant, and E_g is the band gap of the material. Table 1 illustrates the data from $(\alpha h\nu)^{1/2}$ as a function of photon energy. Figure 4a demonstrates that band gap in IGS nanocomposite was in the range of 1.25 eV, which was smaller than that of In_2O_3 , IG, or IGS (i.e., 3.0, 2.10, and 2.0 eV, respectively). Figure 4b revealed that band gaps decreased from 4.79 eV for ZC to 3.25 eV for the ZS sample. On further investigation, the band gap of Ag-modified ZA decreased to 3.11 eV. After progressively combining with graphene, the band gap of the ZAG changed to 2.61 eV. Surprisingly, the band gaps remarkably change to 2.00 eV in the ZAGS after combining through mesoporous SiO_2 . Valence band (VB) and conduction band (CB) potentials of all of the samples were calculated based on the following equations³⁶

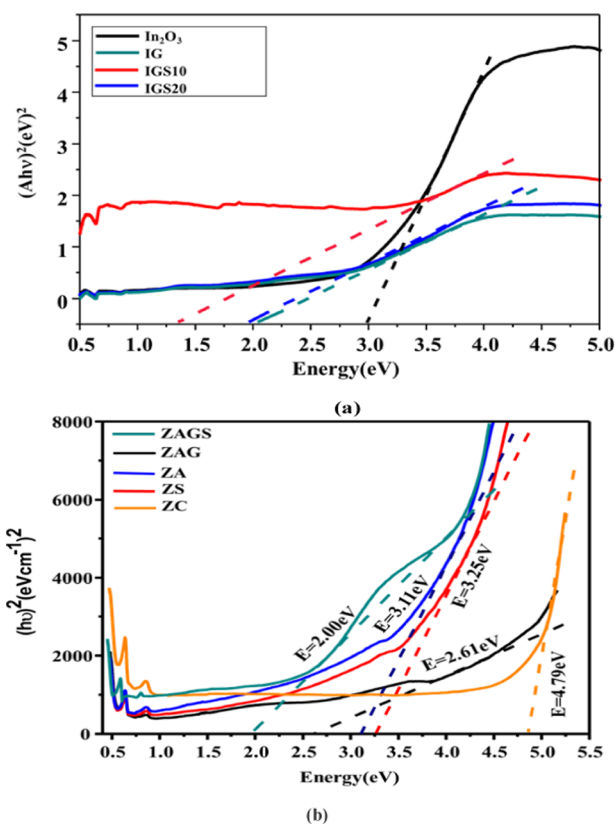


Figure 4. (a) DRS data of In_2O_3 , In_2O_3 -G (IG), In_2O_3 -G- SiO_2 10% (IGS10), and In_2O_3 -G- SiO_2 20% (IGS20) and (b) commercial ZrO_2 (ZC), synthesized ZrO_2 (ZS), ZrO_2 -Ag (ZA), ZrO_2 -Ag-G (ZAG), and ZrO_2 -Ag-G- SiO_2 (ZAGS).

Table 1. Texture Properties of the Obtained IG, IGS10, IGS20, ZC, ZS, ZAG, and ZAGS

samples	BET surface area (m^2/g)	total pore volume (cm^3/g)	average pore diameter (nm)	band gap energy (eV)
IG	29.688	0.1039	13.995	2.10
IGS20	253.79	0.281	4.4284	1.25
ZC				4.79
ZS				3.25
ZA				3.11
ZAG	8.6593	0.012273	5.6691	2.61
ZAGS	9.1703	0.020549	8.9632	2.00

$$E_{\text{CB}} = X - E_e - \frac{1}{2}E_g \quad (4)$$

$$E_{\text{VB}} = E_{\text{CB}} + E_g \quad (5)$$

Here, E_{VB} and E_{CB} are valence and conduction band edge potentials, respectively. X is the electronegativity of the semiconductor, E_e is the energy of free electrons on the hydrogen scale, and E_g is the band gap energy of the semiconductor.

Figure 5 demonstrates N_2 adsorption–desorption isotherms of mesoporous IGS and ZAGS. The perceived Brunauer–Emmett–Teller (BET) surface area was $253.79 \text{ m}^2/\text{g}$ for IGS mesoporous materials. The absolute pore volume of IGS was $0.281 \text{ cm}^3/\text{g}$ and the average pore diameter was 4.4284 nm . It demonstrated that the surface area parameters of the IGS sample were most elevated among these samples (Table 2).

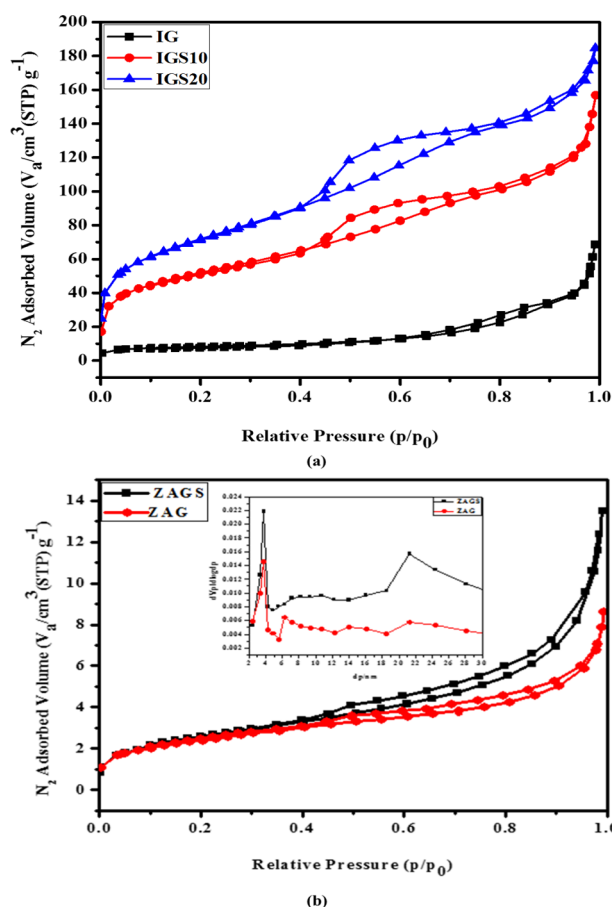


Figure 5. (a) Nitrogen adsorption–desorption isotherms of In_2O_3 -G (IG), In_2O_3 -G-SiO₂ 10% (IGS10), and In_2O_3 -G-SiO₂ 20% (IGS20) and (b) ZrO_2 -Ag-G (ZAG) and ZrO_2 -Ag-G-SiO₂ (ZAGS).

Figure 5b shows that the ZAGS samples exhibit typical type IV isotherms, demonstrating that the materials had mesopores. Isotherms of samples exhibit a steep H2 type hysteresis loop at a relative pressure (P/P_0) in the range between 0.6 and 0.9. The hysteresis loops gradually shift to a higher relative pressure (P/P_0) from ZAG to ZAGS, suggesting that these mesopores increased including mesoporous SiO₂. A mesopore diameter as large as 5.67 nm could be observed for ZAG. When combining through mesoporous SiO₂, it continues to increase to 8.96 nm in ZAGS and the BET surface area also increased from 8.66 to 9.17 m²/g (Table 2).

Figure 6 shows the X-ray photoelectron spectroscopic (XPS) investigation results, which further affirm the above theory. It is revealed that In, O, C, and Si components exist in all samples. The XPS survey spectrum of IGS (Figure 6a) showed peaks

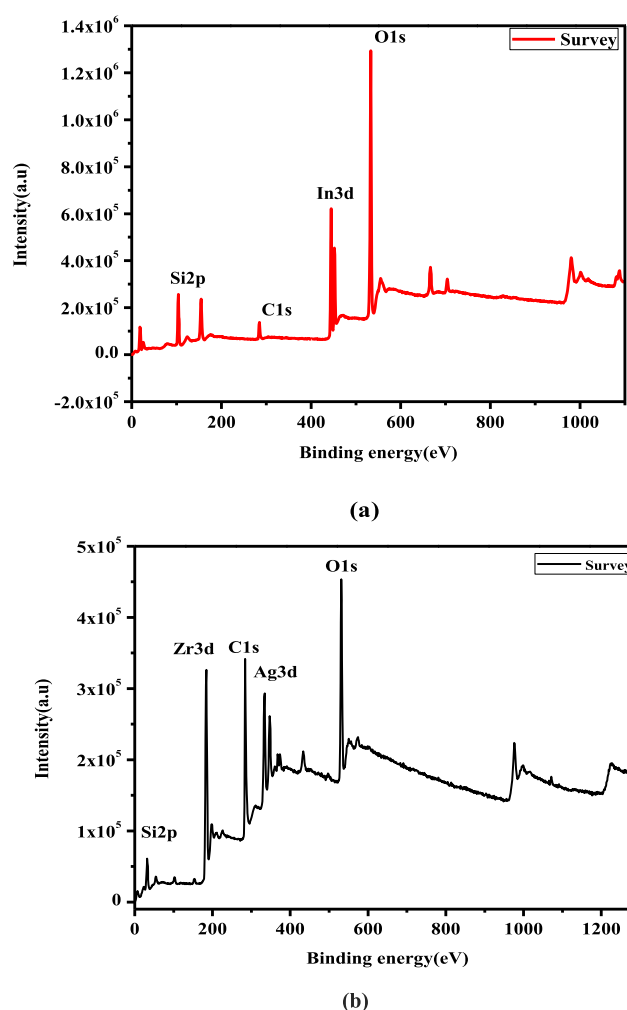


Figure 6. (a) XPS spectra of In_2O_3 -G-SiO₂ 20% (IGS) and (b) ZrO_2 -Ag-G-SiO₂ (ZAGS).

corresponding to In, O, C, and Si atoms. Mass concentrations of these four atoms (In, O, C, and Si) were 277.6342, 15.9994, 12.0107, and 28.0855, respectively. These results suggested that In^{3+} ions bonded with unsaturated oxygen and Cl^- ions. Thus, the $\text{O}-\text{In}-\text{Cl}_x$ ($x = 1$ or 2) structure was formed on the surface of the In_2O_3 -SiO₂ sample. The XPS spectrum for In 3d is shown in Figure 8e. Spin–orbital splits for In 3d_{5/2} and In 3d_{3/2} XPS peaks also had characteristic double peaks centered at binding energies of 444.1 and 451.7 eV, respectively. These were relegated to In^{3+} ions.⁴³ Figure 6b shows that the complete spectrum of ZrO_2 -Ag-G-SiO₂ shows the presence of Si, Zr, C, Ag, and O atoms attributed to effective modification. The corresponding high-resolution spectra with respect to C 1s

Table 2. Performance Comparison between Biosensors for the Detection of *E. coli* O157:H7

developed sensor	particles	targeted bacteria	LOD (CFU/mL)	ref
colorimetric sensor	dopamine Fe ₃ O ₄ NPs	<i>E. coli</i> XL 1	10 ⁴	37
electrochemical immunosensor	GNPs chitosan	<i>Salmonella</i> and <i>E. coli</i>	10 ⁵	38
electrochemical impedance	GNPs antibody	<i>E. coli</i>	10 ³	39
mass spectroscopy	magnetic Fe ₃ O ₄	Gram-positive/negative bacteria	10 ⁴	40
electrochemical immunofunctionalized	magnetic nanoparticle	<i>E. coli</i> O157:H7	10 ³	41
electrochemical biosensor	1-Cyst-Fe ₃ O ₄ NPs	<i>E. coli</i>	10 ⁵	42
mesoporous electrochemical biosensor	ZrO ₂ -Ag-G-SiO ₂ and In ₂ O ₃ -G-SiO ₂	<i>E. coli</i> O157:H7	10 ¹⁰	this work

signal 284.5 eV as a reference binding energy in Figure S1a recognized to C–C, bonds of graphene. As shown in Figure S1b, Si 2p peaks were found at 103.2 eV. These peaks located at 184.3 eV were harmonized to Zr 3d in Figure S1c. The total results of the XPS study confirmed that all surface chemical compositions of ZAGS were found in the as-prepared nanocomposite.

3.2. Electrochemical Property Measurements of Electrodes. The electrochemical analysis for two types of working electrodes (IGS and ZAGS electrodes) performed under a three-electrode cell system with Pt wire as the counter electrode and Ag/AgCl as a reference electrode within the potential range of -0.3 to $+0.2$ V is shown in Figure 7. The

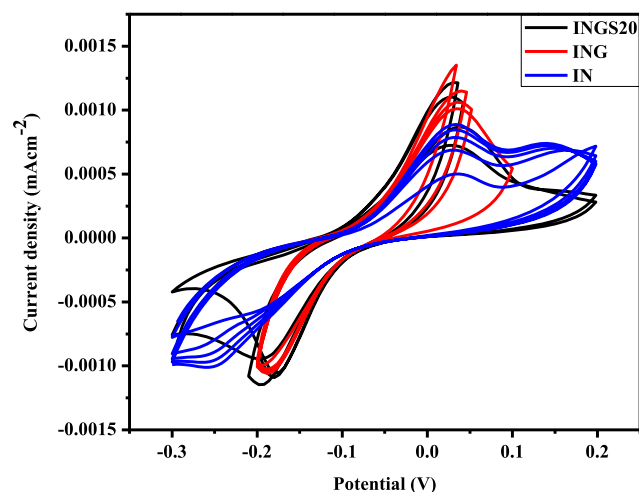


Figure 7. Cyclic voltammogram measurement with In_2O_3 (IN), In_2O_3 -G (ING), and In_2O_3 -G- SiO_2 20% (ING20) samples without *E. coli*.

anodic part of the voltammogram was characterized by the occurrence of the anodic peaks corresponding to the electro-oxidation of the phosphate-buffered saline (PBS), while the cathodic part of the cyclic voltammogram was characterized by the occurrence of cathodic peaks corresponding to the electroreduction of the electrolyte. The IGS electrode is measured in the electrolyte and an oxidation peak was observed, which confirms that these electrodes have specific catalytic properties. IGS electrodes showed a well-defined oxidation peak at the potential of $+0.1$ V and presented a substantial rise in anodic current density of 0.0015 mA/cm^2 . This indicates that the redox peaks in the curve can be attributed to the direct electron transfer behaviors of IGS and ZAGS nanocomposite films.

3.3. Electrochemical Measurements on Immobilized Bacteria. Figure 8 shows that the current of ZAGS was lower than that of IGS because of the unique structure of the quaternary nanocomposite. Here, *E. coli* was immobilized on the electrodes' surface. Figure 8a,b demonstrates a progression of CV estimations completed in IGS and ZAGS electrodes with *E. coli* immobilized from their solutions in lysogeny broth (LB) (stock solution). The two diagrams show IGS and ZAGS electrochemical responses related to characteristic oxidation and reduction peaks. The current was about 0.0002 mA/cm^2 for IGS and 0.0003 mA/cm^2 for ZAGS, which diminishes with the introduction of *E. coli* due to the bacteria-adsorbed surface acting as an insulator to decline the current. The current was lowest when there were *E. coli*. The reason was that the *E. coli*

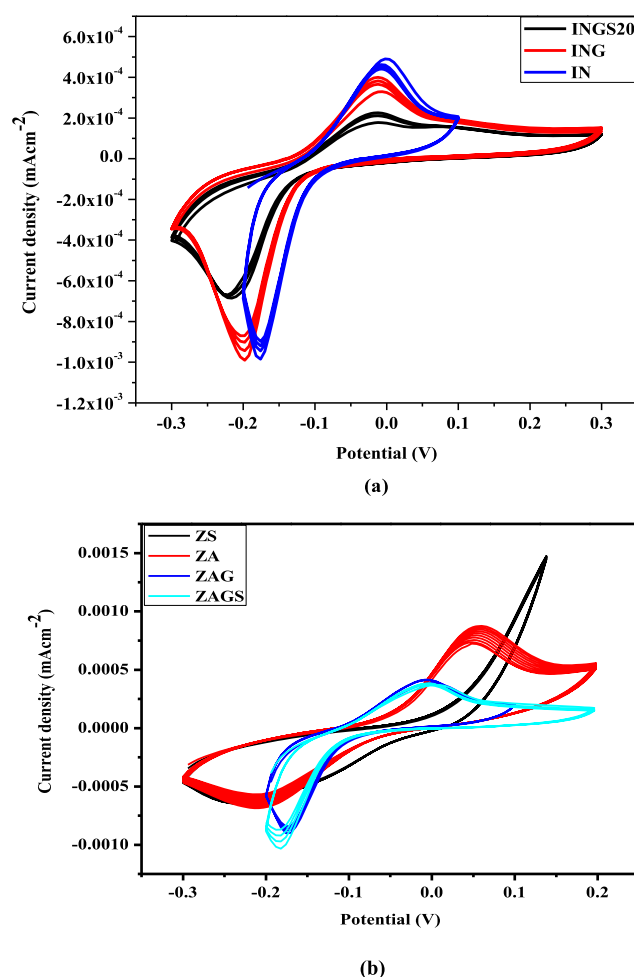


Figure 8. (a) Cyclic voltammogram measurement in the presence of *E. coli* for In_2O_3 (IN), In_2O_3 -G (ING), and In_2O_3 -G- SiO_2 20% (ING20) and (b) synthesized ZrO_2 (ZS), ZrO_2 -Ag (ZA), ZrO_2 -Ag-G (ZAG), and ZrO_2 -Ag-G- SiO_2 (ZAGS).

entrapped the surface of the electrode and blocked the permeating channel. As *E. coli* was not electroactive, it could hinder the electron transfer rate, resulting in bigger electron transfer resistance. Therefore, ZAGS had high sensitivity.

The previous investigations are based on the improvement of microbe-based inhibition sensors, which is far from actual sensor improvement. Real sensor advancement utilizes bacteria immobilized on the electrode surface. In this work, *E. coli* was immobilized on the surface of IGS and ZAGS electrodes. Figure 9 shows the current variation with different concentrations of *E. coli* by IGS and ZAGS electrodes with *E. coli* immobilized from their solutions of different concentrations in LB broth (stock solution concentration of 10^1 – 10^{10} CFU/mL). The two diagrams show the electrochemical responses in IGS and ZAGS. As shown in Figure 9a,b, the estimated current was gradually decreased on increasing the concentration of *E. coli*. Negative currents usually correspond to reduction peaks for cathodic reactions (negative currents). An anodic current is a type of partial current that refers to the electrons entering an electrode in an electrode reaction. To understand the full current of an electrode reaction, it is necessary to consider the cathodic current, which is the flow of electrons leaving an electrode. Here, i - t curve showed the baseline current that means the cathodic current

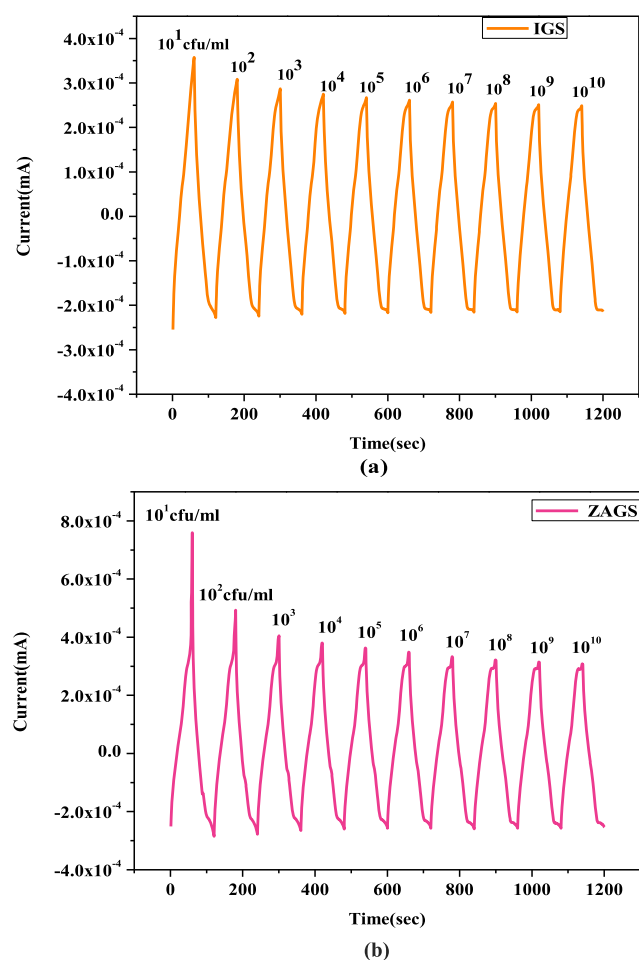


Figure 9. (a) Concentration dependency (i - t curve) of *E. coli* by In_2O_3 -G-SiO₂ (IGS) and (b) ZrO_2 -Ag-G-SiO₂ (ZAGS) sensor.

(negative current) is constantly linear, which corresponds that the electrode does not tend to lose an electron. The spikes of current are in the positive range, which means that electrons enter the electrode. On the other hand, in each cycle of cyclic voltammogram, with increasing *E. coli* concentration the peak current started falling because *E. coli* hindered the electron transfer to the electrode. From the spike of current in Figure S2, the calibration plot linearity range of the biosensor is demonstrated. *E. coli* blocks the electron transfer of the electrode through the immobilization of the electrode, thus increasing the resistivity of the sensor with increasing concentration of *E. coli* rather than conductivity. The cathode current I_c at -0.3 V increases with a low concentration of *E. coli*. Estimations of $\Delta I_c/I_c$ for *E. coli* reduce progressively with the increase of concentration, while *E. coli* are unaffected by the electrode in a wide concentration.

3.4. Specificity of the Sensor. Here, the selectivity of IGS and ZAGS sensor for *E. coli*, compared to other bacteria strains, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella enterica*, (each at 10^9 CFU/mL), was employed, and the PBS sample was set as the reagent blank. However, there was no significant variation in the current response between the bacterial samples and the reagent control except *E. coli*, which is shown in Figure 10. For *E. coli*, a significant change in the current response was observed. Therefore, we can conclude that the sensors have good specificity. Figure S3 demonstrates the SEM and TEM images of ZAGS and IGS

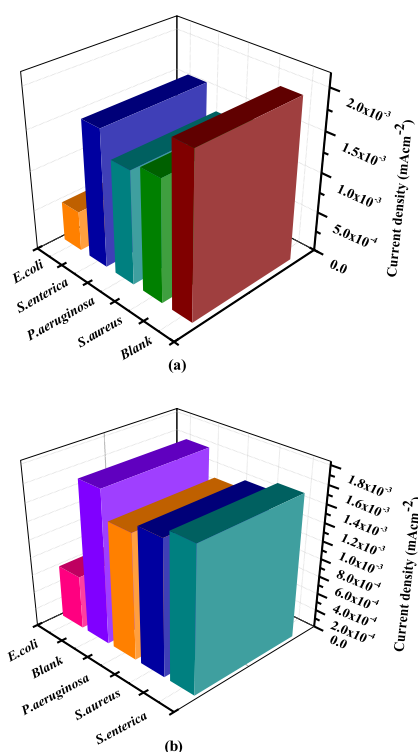


Figure 10. Selectivity test of *E. coli* with ZrO_2 -Ag-G-SiO₂ (ZAGS) sensor (a) and In_2O_3 -G-SiO₂ (IGS) sensor (b).

sensors exposed after the electrochemical test. Compared to Figure 2, it was found that no observable structural changes occurred in the sensor surface of the composites.

3.5. Sensing Performance. IGS and ZAGS sensors are made on FTO electrodes with a mesoporous material as the conducting channel. In IGS and ZAGS, the mesoporous material links the source and the channel electrodes. Under ordinary conditions, GO is electrically insulating because of the existence of immersed sp^3 bonds, the high density of electronegative oxygen atoms attached to carbon, and different deformities that increase the energy gap in the electron density of states. The conductivity of GO depends on its chemical and atomic structure because of the degree of structural disorder arising due to substantial sp^3 carbon fraction. GO-based films are found to be of an insulating nature with a sheet resistance of around $10^4 \Omega/\text{sq}$. The insulating nature of GO can be linked to the concentration of sp^3 C-O bonds, resulting in a decrease of electron transfer and leading to the disruption of the percolating pathway of the sp^2 carbon cluster.⁴⁴ After thermal reduction, because of bigger sp^2 domains, the mesoporous material and graphene turn out to be electrically conducting and the IGS and ZAGS sensors demonstrate resistance between 50 and 150 k Ω . The sensing of *E. coli* is subjected to a balance of electrical conductivity of the mesoporous material and IGS and ZAGS sensors. It does not react to the increase of the analyte. We additionally considered the impact of the distinctive thickness of the FTO glass layer (1, 2, 3, and 4 nm) on the appearance of the IGS and ZAGS sensors. The dimension of the coating was 5 mm and the thickness of the coating was about 0.1, 0.3, and 0.5 mm and had uniform thickness and dimensions. The performance was quite different between the film thicknesses of 0.1 and 0.5 mm. IGS and ZAGS sensors with a film thickness of 0.3 mm demonstrated the biggest response.⁴⁵ For sensing tests, the sensor was

exposed to different concentrations of *E. coli* in DI water (10^1 – 10^{10} CFU/mL). The test sample volume of 1 μ L was utilized for experiments. The real number of *E. coli* identified on the sensor relies upon both the concentration of *E. coli* and the sample volume utilized for the experiment. Even though the lowest concentration of the *E. coli* sample utilized for detection was 10^1 CFU/mL, considering calculations, the sensor could identify a *E. coli* cell. The sensing of *E. coli* cells by IGS and ZAGS sensors from the sample is reflected by the alteration in the electrical conductivity of the mesoporous channel. There was a linear reduction in current after the expansion of *E. coli* aliquot from 10^1 to 10^{10} CFU/mL. The IGS and ZAGS sensors had a quick response time for *E. coli* identification, which is one of the significant benefits of sensors. This was recognized as the fast diffusion of ions from the liquid drop on the surface of the device to the contact region.⁴⁶ Although *E. coli* is bigger than ions, the low volume of the sample reduces the diffusion length and allows rapid investigation of samples. The described techniques for *E. coli* recognition generally include at least 30 min of incubation time after various sensor design steps.⁴⁷ The FTO glass/mesoporous IGS and ZAGS was used as a control analysis. The increase of *E. coli* did not produce any signal, demonstrating that the *E. coli* IGS and ZAGS sensors respond simply after binding with *E. coli*. The conductivity of sensing mechanism between the FTO glass layer and the mesoporous IGS and ZAGS channel is regulated after interacting with negatively charged *E. coli* cells. The mesoporous layer is the potential electron reservoir for free electrons since oxygen vacancies in FTO glass fail to locate the corresponding empty O_2 p conduction band states. The 1–2 μ m measured *E. coli*, which is rich in negative charge on its surface, is in the region close to the surface of the electron-rich FTO glass layer. This puff of negative charge further decreases the electron-donating capacity of FTO glass. To re-establish its insulating property, the oxygen-deficient domain boundary of FTO glass carries on like a local electron donor and mesoporous IGS and ZAGS as recipients.⁴⁷ The majority carriers (i.e., holes) in the mesoporous IGS and ZAGS merge with these electrons. This decline in the concentration of majority charge carriers diminishes the current flow between sources and channels. Moreover, the sensing mechanism can be associated with the gating impact of *E. coli* cells present on the sensor surface. The cell loses positive charge, which is instigated by adsorption of negatively charged *E. coli* cells. The dimensions of the positive charge on cells diminish with the increasing concentration of *E. coli*. This diminished positive charge of cells shows that ZAGS and IGS are less negatively charged since the counteracting agent itself can function as a dielectric medium. Subsequently, the concentration of holes in the mesoporous IGS and ZAGS from the gate-insulator/semiconductor interface, i.e., the current flow between the source and channel, declines because of the adsorption of *E. coli*. Over an edge concentration of 10^{10} CFU/mL, there are abundant *E. coli* cells in the sample solution. As opposed to coming in direct contact with the FTO glass layer, *E. coli* cells can be stacked over one another. The viewed outcome is because of the increasing *E. coli* cells in the sample solution, which decreases the conductivity of the sensor. This decrease in conductivity can be ascribed to the surface layer of *E. coli* cells. This created more holes in the mesoporous IGS and ZAGS channels along these lines, increasing the active site of IGS and ZAGS sensors. In this way, there are contending impacts wherein it prompts a decline in the current (10^1 – 10^{10} CFU/mL). By following the graph for

real-time detection of *E. coli*, the impact of solution resistivity on IGS and ZAGS sensor is due to the increasing concentration of *E. coli* that progressively declines after the increase of 10^{10} CFU/mL of cells. This implies that *E. coli* is adsorbed on the surface of electrodes and acts as an insulating layer, diminishing the current. The current response of the manufactured electrochemical sensor to various concentrations of *E. coli* and controlled phosphate buffer solution was analyzed. The targeted *E. coli* was recognized by estimating the electrochemical signal of the IGS and ZAGS samples. It has been described that the electrochemical recognition technique proposes rapid signal transduction requiring a low cost. The catalytic current diminished linearly with the increasing concentration of *E. coli* from the scope of $10^1 \times 10^{10}$ with a coefficient ($r^2 = 0.879$). The absolute time needed from the manufacture of the biosensor to the detection of *E. coli* was 75 min. In this way, this technique sensed *E. coli* in a lower concentration of 10^1 CFU/mL and with the maximum range of 10^{10} CFU/mL. At the point when *E. coli* was added, it was observed that the state of the CV curve changed significantly. The *E. coli* were cultured for 16 h at 37 °C and then transferred with PBS buffer for sensing. Despite this, the *E. coli* were distinguished by the developed sensor directly after the culture was grown. The leaving time was 2 s, and scanning time for two portions was 10 s. It was affirmed that the electrochemical signal was diminished by adding various concentrations of *E. coli*. These results demonstrate that the IGS and ZAGS samples are very delicate and sensitive for *E. coli* detection. The linear correlation between distinctive bacterial concentration and signal-to-noise ratio is $r^2 = 0.879$. The SNR was determined with the peak current of IGS and ZAGS sample to each concentration of *E. coli*. Then, it was isolated by the current 0.1 V of the blank, which was utilized as the signal of the electrochemical sensor. The signal-to-noise proportions of 10^1 , 10^2 , 10^3 , 10^4 , and 10^5 CFU/mL were 0.64, 1.27, 1.40, 1.50, and 1.68 μ A, respectively. The manufacture and experimental time of the targeted *E. coli* is 75 min. Thus, a sustainable method takes less time in contrast to the recently announced report (Table 2).^{37–42} Subsequently, the most minimal concentration was 10^1 and the highest concentration was 10^{10} CFU/mL. This study demonstrates that the IGS and ZAGS sample is an efficient method to deal with very sensitive recognition materials for selected bacteria. The 10 scans keep running on the blank electrode without *E. coli* concentration, at which point the standard deviation of $\sigma = 0.0087$ of 10 scan runs of blank electrode response was determined, and then, there was an equation of limit of detection (LOD) with three times of the standard deviation equation ($3 \times \sigma$)/ m , where m , the decided slope (0.879) of the determined outcomes, was a limited deviation. We also investigated the stability of the ZAGS and IGS sensor under ambient conditions, as shown in Figure 11. Furthermore, the ZAGS and IGS electrode permitted exact control of the consistency of the film, which thus added to the stability of the sensor. When conserved at a storage temperature of 4 °C, IGS and ZAGS sensors did not demonstrate any major fading in the experiment following 7, 14, 21, and 28 days of fabrication. PBS can help the total recovery of the sensor surface without distressing IGS and ZAGS activity. The stability of such *E. coli* IGS and ZAGS sensors as far as reusability goes needs further analysis. The sensing tests were done at 25 °C. Increase in temperature will particularly influence the conductivity of mesoporous nanocomposites. Since our sensor was prepared for room-

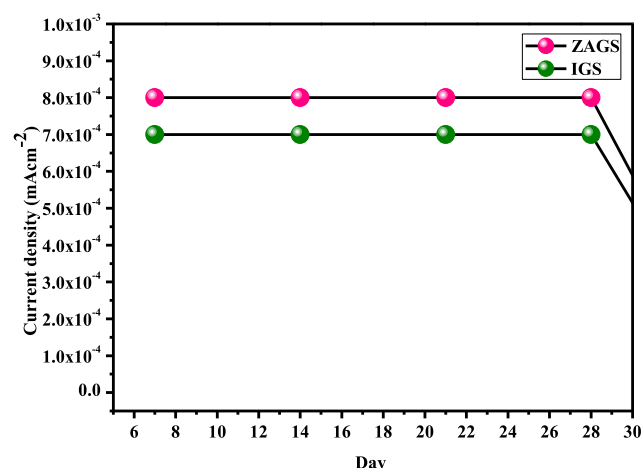


Figure 11. Stability test of $\text{ZrO}_2\text{-Ag-G-SiO}_2$ (ZAGS) and $\text{In}_2\text{O}_3\text{-G-SiO}_2$ (IGS) sensor.

temperature investigation, we did not expand our analysis for a range of temperatures.

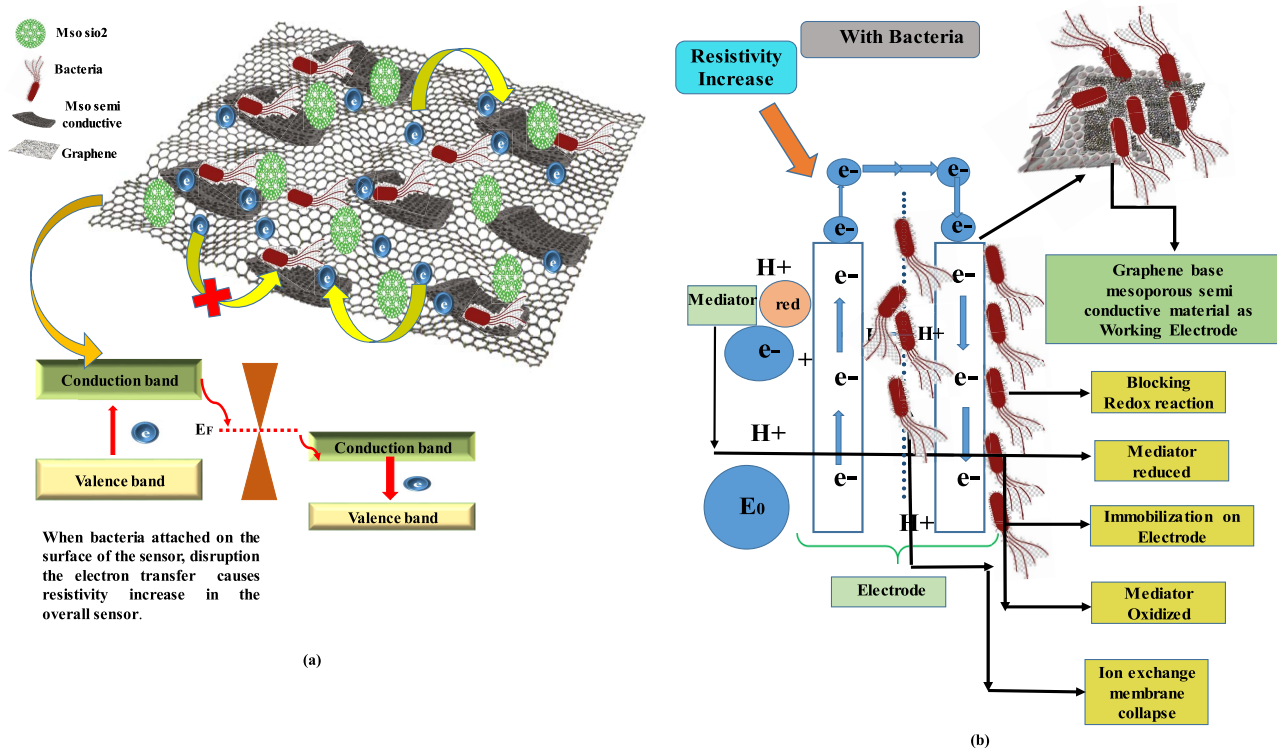
3.6. Mechanism of *E. coli* Sensing. The electrocatalytic effect of electrodes on the electrochemical decline was examined. IGS and ZAGS sensors in 0.1 M phosphate buffer support at pH 7.4 at an applied potential of -0.2 V were studied. An alternate effect was seen when the IGS and ZAGS electrodes were stored overnight in 0.1 M phosphate buffer. This effect is supposed to be the alteration in the hydration of the thin film. To demonstrate that was not just a collection impact because of the existence of the thin film, a comparable investigation was made with a cellulose layer to immobilize the

thin film at the surface of the electrode. At the point when the biosensor was left overnight in a buffer solution with *E. coli* (the response yet going), the difference between the first and the second determination was observed. Various concentrations of *E. coli* were immobilized at the surface. Increasing the concentration of *E. coli* at the surface of the electrode (10^1 – 10^{10} CFU/mL) improved the recognition of the location of 1.17×10^{-7} M and the linear domain somewhere in the range of 1.96×10^{-5} and 2.55×10^{-4} M of the improved electrode. The loss of the biosensor's conductivity in time could be due to the inactivation or to the loss of the redox reaction from the biosensor's surface. Figure 7 shows the regular i – t curves of IGS and ZAGS to increase the resistivity. Several *E. coli* were captured at the surface of the electrode. *E. coli* blocks the ion change membrane through the immobilization of the electrode. Thus, electron transfer is disrupted and the resistivity of the sensor rather than conductivity is increased. Better residuals with the increase of time could be clarified by film hydration. Better repeatability with time was also observed (Scheme 2).

4. CONCLUSIONS

In this study, we have demonstrated a comparative electrochemical-based label-free, specific, sensitive detection of the waterborne pathogen *E. coli*. The electrochemical method for the ZAGS and IGS sensors is more sensitive, faster, and simpler than the conventional methods used to detect living bacteria in solution. The ZAGS and IGS biosensors detect *E. coli* in a lower concentration of 10^1 CFU/mL and with the maximum range of 10^{10} CFU/mL and it outputs accurate quantitative results. Future developments of this electrode

Scheme 2. (a) Bacteria-Sensing Mechanism^a



^aElectron transfer disruption and increase of resistivity of the sensor due to *E. coli* captured at the surface of the electrode. (b) *E. coli* blocks the ion change membrane through the immobilization of the electrode.

must include reducing detection limits, increasing the selectivity of resistant bacteria, integrating this analytical method into paper-based devices, and detecting drug resistance in real and complex samples to identify different bacteria. By observing the cells adhered to the ZAGS and IGS sensors, this method can provide a platform to study the properties of a single cell after separation and/or attachment onto the sensor surface. This approach can also be applied to study other biomolecules. We believe that this method provides a robust potential for simple and specific detection of *E. coli* and antibiotic-resistant *E. coli*.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsomega.0c00895>.

XPS spectra of C 1s, O 1s, Si 2p, In 3d, Zr 3d, and Ag 3d (Figure S1); calibration curve of In₂O₃-G-SiO₂ (IGS) sensor and ZrO₂-Ag-G-SiO₂ (ZAGS) sensor (Figure S2); SEM and TEM images of In₂O₃-G-SiO₂ and ZrO₂-Ag-G-SiO₂ (Figure S3); nomenclature samples (Table S1) (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) Xu, M.; Wang, R.; Li, Y. An electrochemical biosensor for rapid detection of *E. coli* O157: H7 with highly efficient bi-functional glucose oxidase-polydopamine nanocomposites and Prussian blue modified screen-printed interdigitated electrodes. *Analyst* **2016**, *141*, 5441–5449.
- (2) Griffin, P. M.; Tauxe, R. V. The epidemiology of infections caused by *Escherichia coli* O157: H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* **1991**, *13*, 60–98.
- (3) Joung, C.-K.; Kim, H.-N.; Lim, M.-C.; Jeon, T.-J.; Kim, H.-Y.; Kim, Y.-R. A nanoporous membrane-based impedimetric immunosensor for label-free detection of pathogenic bacteria in whole milk. *Biosens. Bioelectron.* **2013**, *44*, 210–215.
- (4) Tan, F.; Leung, P. H. M.; Liu, Z.-b.; Zhang, Y.; Xiao, L.; Ye, W.; Zhang, X.; Yi, L.; Yang, M. A PDMS microfluidic impedance immunosensor for *E. coli* O157: H7 and *Staphylococcus aureus* detection via antibody-immobilized nanoporous membrane. *Sens. Actuators, B* **2011**, *159*, 328–335.
- (5) Li, Y.; Fang, L.; Cheng, P.; Deng, J.; Jiang, L.; Huang, H.; Zheng, J. An electrochemical immunosensor for sensitive detection of *Escherichia coli* O157: H7 using C60 based biocompatible platform and enzyme functionalized Pt nanochains tracing tag. *Biosens. Bioelectron.* **2013**, *49*, 485–491.
- (6) Maurer, E. I.; Comfort, K. K.; Hussain, S. M.; Schlager, J. J.; Mukhopadhyay, S. M. Novel platform development using an assembly of carbon nanotube, nanogold and immobilized RNA capture element towards rapid, selective sensing of bacteria. *Sensors* **2012**, *12*, 8135–8144.
- (7) Varshney, M.; Li, Y.; Srinivasan, B.; Tung, S. A label-free, microfluidics and interdigitated array microelectrode-based impedance biosensor in combination with nanoparticles immunoseparation for detection of *Escherichia coli* O157: H7 in food samples. *Sens. Actuators, B* **2007**, *128*, 99–107.
- (8) Li, Z.; Fu, Y.; Fang, W.; Li, Y. Electrochemical impedance immunosensor based on self-assembled monolayers for rapid detection of *Escherichia coli* O157: H7 with signal amplification using lectin. *Sensors* **2015**, *15*, 19212–19224.
- (9) Xu, M.; Wang, R.; Li, Y. Rapid detection of *Escherichia coli* O157: H7 and *Salmonella Typhimurium* in foods using an electrochemical immunosensor based on screen-printed interdigitated microelectrode and immunomagnetic separation. *Talanta* **2016**, *148*, 200–208.
- (10) Setterington, E. B.; Alocilja, E. C. Electrochemical biosensor for rapid and sensitive detection of magnetically extracted bacterial pathogens. *Biosensors* **2012**, *2*, 15–31.
- (11) Holzinger, M.; Le Goff, A.; Cosnier, S. Nanomaterials for biosensing applications: a review. *Front. Chem.* **2014**, *2*, No. 63.
- (12) Bianco, A.; Cheng, H.-M.; Enoki, T.; Gogotsi, Y.; Hurt, R. H.; Koratkar, N.; Kyotani, T.; Monthieux, M.; Park, C. R.; Tascon, J. M. D.; Zhang, J. All in the graphene family—A recommended nomenclature for two-dimensional carbon materials. *Carbon* **2013**, *65*, 1–6.
- (13) Stoller, M. D.; Park, S.; Zhu, Y.; An, J.; Ruoff, R. S. Graphene-based ultracapacitors. *Nano Lett.* **2008**, *8*, 3498–3502.
- (14) Kim, J.; Ishihara, M.; Koga, Y.; Tsugawa, K.; Hasegawa, M.; Iijima, S. Low-temperature synthesis of large-area graphene-based transparent conductive films using surface wave plasma chemical vapor deposition. *Appl. Phys. Lett.* **2011**, *98*, No. 091502.
- (15) Hummers, W. S., Jr.; Offeman, R. E. Preparation of graphitic oxide. *J. Am. Chem. Soc.* **1958**, *80*, 1339.
- (16) Chua, C. K.; Pumera, M. Chemical reduction of graphene oxide: a synthetic chemistry viewpoint. *Chem. Soc. Rev.* **2014**, *43*, 291–312.
- (17) Suvarnaphaet, P.; Pechprasarn, S. Graphene-based materials for biosensors: a review. *Sensors* **2017**, *17*, No. 2161.
- (18) Liu, R.; Wang, Y.; Li, B.; Liu, B.; Ma, H.; Li, D.; Dong, L.; Li, F.; Chen, X.; Yin, X. VXC-72R/ZrO₂/GCE-Based Electrochemical Sensor for the High-Sensitivity Detection of Methyl Parathion. *Materials* **2019**, *12*, No. 3637.
- (19) Kumar, S.; Sharma, J. G.; Maji, S.; Malhotra, B. D. Nanostructured zirconia decorated reduced graphene oxide based efficient biosensing platform for non-invasive oral cancer detection. *Biosens. Bioelectron.* **2016**, *78*, 497–504.
- (20) Qurashi, A.; Irfan, M. F.; Alam, M. W. In₂O₃ nanostructures and their chemical and biosensor applications. *Arabian J. Sci. Eng.* **2010**, *35*, 126–145.
- (21) Massad-Ivanir, N.; Shtenberg, G.; Segal, E.; et al. Optical detection of *E. coli* bacteria by mesoporous silicon biosensors. *J. Visualized Exp.* **2013**, *81*, No. 50805.

- (22) Ambrosi, A.; Chua, C. K.; Bonanni, A.; Pumera, M. Electrochemistry of graphene and related materials. *Chem. Rev.* **2014**, *114*, 7150–7188.
- (23) Viswanathan, S.; Narayanan, T. N.; Aran, K.; Fink, K. D.; Paredes, J.; Ajayan, P. M.; Filipek, S.; Misztal, P.; Tekin, H. C.; Inci, F.; Demirci, U.; et al. Graphene–protein field effect biosensors: glucose sensing. *Mater. Today* **2015**, *18*, 513–522.
- (24) Shang, N. G.; Papakonstantinou, P.; McMullan, M.; Chu, M.; Stamboulis, A.; Potenza, A.; Dhesi, S. S.; Marchetto, H. Catalyst-free efficient growth, orientation and biosensing properties of multilayer graphene nanoflake films with sharp edge planes. *Adv. Funct. Mater.* **2008**, *18*, 3506–3514.
- (25) Bonanni, A.; Chua, C. K.; Zhao, G.; Sofer, Z.; Pumera, M. Inherently electroactive graphene oxide nanoplatelets as labels for single nucleotide polymorphism detection. *ACS Nano* **2012**, *6*, 8546–8551.
- (26) Liu, B.; Sun, Z.; Zhang, X.; Liu, J. Mechanisms of DNA sensing on graphene oxide. *Anal. Chem.* **2013**, *85*, 7987–7993.
- (27) Dontschuk, N.; Stacey, A.; Tadich, A.; Rietwyk, K. J.; Schenk, A.; Edmonds, M. T.; Shimoni, O.; Pakes, C. I.; Prawer, S.; Cervenka, J. A graphene field-effect transistor as a molecule-specific probe of DNA nucleobases. *Nat. Commun.* **2015**, *6*, No. 6563.
- (28) Bollella, P.; Fusco, G.; Tortolini, C.; Sanzò, G.; Favero, G.; Gorton, L.; Antiochia, R. Beyond graphene: electrochemical sensors and biosensors for biomarkers detection. *Biosens. Bioelectron.* **2017**, *89*, 152–166.
- (29) Singh, M.; Holzinger, M.; Tabrizian, M.; Winters, S.; Berner, N. C.; Cosnier, S.; Duesberg, G. S. Noncovalently functionalized monolayer graphene for sensitivity enhancement of surface plasmon resonance immunosensors. *J. Am. Chem. Soc.* **2015**, *137*, 2800–2803.
- (30) Nguyen, T.-V.; Lee, H.-C.; Yang, O.-B. The effect of pre-thermal treatment of TiO₂ nanoparticles on the performances of dye-sensitized solar cells. *Sol. Energy Mater. Sol. Cells* **2006**, *90*, 967–981.
- (31) Kleinert, J.; Srinivasan, V.; Rival, A.; Delattre, C.; Velez, O. D.; Pamula, V. K. The dynamics and stability of lubricating oil films during droplet transport by electrowetting in microfluidic devices. *Biomicrofluidics* **2015**, *9*, No. 3034104.
- (32) Zhou, Y.; Zheng, H.; Chen, X.; Zhang, L.; Wang, K.; Guo, J.; Huang, Z.; Zhang, B.; Huang, W.; Jin, K.; Tonghai, D. The *Schistosoma japonicum* genome reveals features of host-parasite interplay. *Nature* **2009**, *460*, 345.
- (33) Zhan, B.; Liu, C.; Chen, H.; Shi, H.; Wang, L.; Chen, P.; Huang, W.; Dong, X. Free-standing electrochemical electrode based on Ni(OH)₂/D graphene foam for nonenzymatic glucose detection. *Nanoscale* **2014**, *6*, 7424–7429.
- (34) Kudin, K. N.; Ozbas, B.; Schniepp, H. C.; Prud'Homme, R. K.; Aksay, I. A.; Car, R. Raman spectra of graphite oxide and functionalized graphene sheets. *Nano Lett.* **2008**, *8*, 36–41.
- (35) Yang, Z.; Hu, K.; Meng, X.; Tao, Q.; Dong, J.; Liu, B.; Lu, Q.; Zhang, H.; Sundqvist, B.; Zhu, P.; Yao, M.; Liu, B. b. Tuning the band gap and the nitrogen content in carbon nitride materials by high temperature treatment at high pressure. *Carbon* **2018**, *130*, 170–177.
- (36) Tauc, J.; Grigorovici, R.; Vancu, A. Optical properties and electronic structure of amorphous germanium. *Phys. Status Solidi B* **1966**, *15*, 627–637.
- (37) Mumtaz, S.; Wang, L.-S.; Hussain, S. Z.; Abdullah, M.; Huma, Z.; Iqbal, Z.; Creran, B.; Rotello, V. M.; Hussain, I. Dopamine coated Fe₃O₄ nanoparticles as enzyme mimics for the sensitive detection of bacteria. *Chem. Comm.* **2017**, *53*, 12306–12308.
- (38) Xiang, C.; Li, R.; Adhikari, B.; She, Z.; Li, Y.; Kraatz, H. B. Sensitive electrochemical detection of *Salmonella* with chitosan–gold nanoparticles composite film. *Talanta* **2015**, *140*, 122–127.
- (39) Maalouf, R.; Fournier-Wirth, C.; Coste, J.; Chebib, H.; Saikali, Y.; Vittori, O.; Errachid, A.; Cloarec, J.-P.; Martelet, C.; Jaffrezic-Renault, N. Label-free detection of bacteria by electrochemical impedance spectroscopy: comparison to surface plasmon resonance. *Anal. Chem.* **2007**, *79*, 4879–4886.
- (40) Reddy, P. M.; Chang, K.-C.; Liu, Z.-J.; Chen, C.-T.; Ho, Y.-P. Functionalized magnetic iron oxide (Fe₃O₄) nanoparticles for capturing gram-positive and gram-negative bacteria. *J. Biomed. Nanotechnol.* **2014**, *10*, 1429–1439.
- (41) Settingington, E. B.; Alocilja, E. C. Electrochemical biosensor for rapid and sensitive detection of magnetically extracted bacterial pathogens. *Biosensors* **2012**, *2*, 15–31.
- (42) Panhwar, S.; Hassan, S. S.; Mahar, R. B.; Carlson, K.; Rajput, M. u. H.; Talpur, M. Y. Highly Sensitive and Selective Electrochemical Sensor for Detection of *Escherichia coli* by Using L-Cysteine Functionalized Iron Nanoparticles. *J. Electrochem. Soc.* **2019**, *166*, 227.
- (43) Poznyak, S. K.; Golubev, A. N.; Kulak, A. I. Correlation between surface properties and photocatalytic and photoelectrochemical activity of In₂O₃ nanocrystalline films and powders. *Surf. Sci.* **2000**, *454–456*, 396–401.
- (44) Biswas, M. R. U. D.; Oh, W.-C. Comparative study on gas sensing by a Schottky diode electrode prepared with graphene–semiconductor–polymer nanocomposites. *RSC Adv.* **2019**, *9*, 11484–11492.
- (45) Mitra, A.; Barick, B.; Mohapatra, J.; Sharma, H.; Meena, S. S.; Aslam, M. M. Large tunneling magnetoresistance in octahedral Fe₃O₄ nanoparticles. *AIP Adv.* **2016**, *6*, No. 055007.
- (46) Zhao, X.; Hilliard, L. R.; Mechery, S. J.; Wang, Y.; Bagwe, R. P.; Jin, S.; Tan, W. A rapid bioassay for single bacterial cell quantitation using bioconjugated nanoparticles. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15027–15032.
- (47) Zhu, G.; Lin, Z. H.; Jing, Q.; Bai, P.; Pan, C.; Yang, Y.; Zhou, Y.; Wang, Z. L. Toward large-scale energy harvesting by a nanoparticle-enhanced triboelectric nanogenerator. *Nano Lett.* **2013**, *13*, 847–853.