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Rapid, sensitive and specific electrochemical detection of *E. coli* using graphitized mesoporous carbon modified electrodes

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ABSTRACT

Escherichia coli (*E. coli*) is a highly pathogenic bacterium causing infections to the human body primarily from a variety of sources including food intake. As per the World Health Organization (WHO), the mortality and morbidity rate due to *E. coli* is significantly high, approximately 8%. Therefore, a real-time, rapid, user-friendly and interference-free *E. coli* detection system offers great clinical importance. In this work, a highly sensitive, specific, and simple method of electrochemical detection of *E. coli* has been demonstrated. The electrochemical system employs the conventional three-electrode configuration, wherein glassy carbon (GC), chemically modified with graphitized mesoporous carbon (GMC), has been employed as the working electrode. While using platinum and Ag/AgCl as counter and reference electrodes respectively, voltametric techniques were applied to obtain the interference mitigated response of *E. coli* detection. The GCE/GMC electrode showed excellent sensitivity and selective response towards *E. coli*in a linear detection range of 2.52×10^3 CFU/mL to 25.2×10^4 CFU/mL and 252CFU/mL to 2268CFU/mL respectively with a limit of detection (LOD) of 50.40CFU/mL. Further, the developed system was tested for interference with other bacteria and real samples, such as pond water, tap water, and deionized water. Appreciable recovery values and negligible interference were observed. The prepared electrode demonstrated promising results towards efficient, real-time, and rapid *E. coli* detection.

1. Introduction

Escherichia coli or *E. coli* bacterium is a rod-shaped bacteria class having a wide presence in the environment [1]. These bacteria are not only present in the environment but are also found i.e. contaminated food and untreated water, and even in the human intestine and animal gut. Although most of the *E. coli* bacteria are good bacteria and are considered to be symbiotic yet a few adversely affect human and animal health [2]. It can cause severe illnesses, such as urinary tract infections, respiratory illness and, bloodstream infections leading to complaints like stomach pain, cramps, fever, diarrhea, etc. [3]. Therefore, detection of the bacterial load is vital for human and animal health and surroundings like water-bodies, etc. [4]. The conventional method of viable cell counting takes more than 48 h for the detection of bacteria [5], whereby the probability of errors and inaccuracy is quite significant. Thus, a simple, rapid approach with high accuracy is required for qualitative and quantitative detection of *E. coli*.

The Electrochemical(EC) technique is the oldest and well-proven technique and offers a wide range of analytical possibilities. Because of quick response time, simple procedure, high sensitivity, and selectivity, EC-based sensing is widely employed for diversified applications. Different EC techniques used for the detection of bacteria include such as square wave anodic stripping voltammetry(SWASV), cyclic voltammetry(CV), and square wave voltammetry (SWV). Various advantages of the voltammetry technique include the possibility to identify the concentration of the *E. coli* without any separation or pre-treatment, high specificity and sensitivity, and amenability to miniaturization. The SWV technique [6] inculcates the features of other techniques and proves to be the most reliable and effective technique for electrochemical sensing of *E. coli* bacteria [7].

The available literature reports have demonstrated a three-electrode-based method for bacterial detection. Viswanathan et. al. reported an immunosensor based on nanocrystal bio conjugates and multi-walled carbon nanotube (MWCNT) over a screen-printed electrode for

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detection of food-borne pathogens such as *E. coli*, campylobacter, and salmonella [8]. Qi et al. suggested a signal amplification technique based on PDA (polydopamine)-mediated nanomaterial modification for the detection of Desulforibriocaledoiensis, and dopamine was enhanced by adding Fe3O4 @MnO2 nanoplates [9]. Li et al. described the synthesis of AUT (amine-terminated alkanethiol 11-amino-1-undecanethiol hydrochloride) as an immunosensor on a gold electrode surface and CHIT-MWNTs-SiO2 @THI was synthesized utilizing LBL (Layer by Layer) assembly for *E. coli*O157:H7, somatic (O), and flagellar (F) detection (H) [10], Zhao et al. developed an immunosensor to detect Vibrio parahaemolyticus foodborne pathogens using a screen-printed electrode covered with agarose/Nano-Au membrane and horseradish peroxidase (HRP) [11], Li et. al.developed detection system for *E. coli*O157:H7using Au-SiO_2 embedded on C_{60}/Fc/CHI–SH [12]. A summary of various reported works, electrochemical detection of *E. coli* using different electrodes, and voltammetry methods are presented in Table 1 for better illustration.

There are certain research gaps observed in the reported literature, such as smaller limit of detection (LOD), the additional need for immunosensor, longer assay time, complex surface modification, etc. Motivated with this, the present work demonstrates a conventional glassy carbon electrode (GCE) modified with graphitized mesoporous carbon (GMC) (*designated as GCE/GMC*), used for the detection of *E. coli* in pH 7 phosphate buffer solution (PBS) with a working volume of 5 mL. The GCE/GMC electrode is found to be an effective and reliable method in sensing *E. coli* bacteria. Various important parameters, such as repeatability, sensitivity, and stability, for the present system with *E-coli* bacteria, were perfectly achieved. The demonstrated sensor is easily scalable owing to the short preparation time, simplicity of the electrochemical method, and low preparation cost. Furthermore, interference from other bacteria was also examined such as Shewanella putrefaciens, Lactobacillus, Helicobacter pylori, and Bacillus anthracis [15] were considered. Real sample analysis with a tap, pond, and deionized water were also carried out. The platform exhibited excellent interference mitigated electro-catalytic oxidation of *E. coli* with good stability and reproducibility. Moreover, the LOD reported using this technique was observed to the lowest, amongst other techniques reported to date. Except for other features, and excellent linearity and sensitivity, to the best of the authors' knowledge, *E. coli* detection using such GCE/GMC electrode has not been reported yet.

Table 1
Comparison of various reported works showing electrochemical detection of *E. coli* using different electrodes and voltammetry method.

Method	Electrode	Linear ranges (CFU/mL)	LODs (CFU/mL)	Ref.
SWASV	MWCNT–PAH/SPE	1×10^3 to 5×10^5	4×10^2	[8]
SWV	PDA/Fe_3O_4 @MnO_2–Fc-conA	1×10^3 to 1×10^8	–	[9]
CV	AUT/AuNP/CHIT/ MWNTs/SiO_2 @THI	4.12×10^2 to 4.12×10^5	2.50×10^2	[10]
CV	Agarose/AuNP	10^3 to 10^9	7.37×10^4	[11]
CV	CHI–SH/Fc/C_{60}/ Au–SiO_2/GOD/PtNCs	3.2×10^1 to 3.2×10^6	15	[12]
DPV	Cu-β-CD-GO/GCE	10–10^7	5	[13]
Amperometric	HRP-TMB/ H_2O_2/ AuNP	0.99×10^4 to 3.98×10^9	50	[14]
SWV	GCE/GMC	2.52×10^3 to 25.2×10^4	50.40	This work

SWASV: square wave anodic stripping voltammetry, MWCNT: multiwalled carbon nanotube, PAH: polyallylamine, SPE: screen printed electrode; SWV: square wave voltammetry, PDA: polydopamine, Fc: ferrocene, conA: concanavalin A, CV: cyclic voltammetry, AUT: amine-terminated alkanethiol 11-amino-1-undecanethiol hydrochloride, CHIT: chitosan, SiO2: silica, THI: thionine, AuNP: gold nanoparticles, CHI–SH: thiolated chitosan, C60: fullerene, Au–SiO2: Au nanoparticle coated SiO2 nanocomposites, GOD: glucose oxidase, PtNCs: platinum nano chains. DPV: Differential Pulse Voltammetry, Cu-β-CD-GO: Copper-β-cyclodextrin-graphene oxide, HRP: horseradish peroxides, TMB: 3,3′,5,5′- Tetramethyl benzidine, H_2O_2:Hydrogen peroxide, GCE: glassy carbon electrode, GMC: graphitized mesoporous carbon.

2. Experimental

2.1. Chemicals and reagents

The sodium phosphate dibasic dihydrate ($Na_2HPO_4.2\ H_2O$) and sodium phosphate monobasic anhydrous (NaH_2PO_4) were purchased from Avra chemicals. These two chemicals were used for making the PBS buffer of 0.1 M ionic strength which was used as a supporting electrolyte. GMC (50 nm and 99.95% purity) were procured from Sigma-Aldrich. Shewanella putrefaciens, Lactobacillus, Helicobacter pylori, and Bacillus anthracis were procured from the Biological Department of the BITS Pilani Hyderabad Campus and Deionized water was obtained from the Central Analytical Laboratory of our Institute.

2.2. Apparatus

A benchtop potentiostat (SP-300 from Biologic, France) was used for performing the CV and SWV experiments. A three electrodes system was used which comprises of disk-shaped glassy carbon electrode (3 mm diameter and 0.0707 cm^2 working surface area) as the working electrode. Platinum wire was used as the counter electrode and Ag/AgCl was used as the reference electrode. All of the experiments were carried out in a 5 mL cell [16].

2.3. Bacteria Preparation

*E. coli*strainDH5α was provided by the Department of Biological Sciences of our Institute. The cultured medium used was Lysogeny Broth (LB), which was prepared by taking 5 mL of LB media in 200 mL distilled water and autoclaved for 20 min at 15 psi at 121°C. The 0.5 gm yeast extract, 1 gm tryptone, and 1 gm NaCl were the constituents of the LB media. *E. Coli* was cultured by inoculating 200 μL of strain in 20 mL of LB media and incubated for 36 h with shaking at 180 rpm at 37 °C. The turbidity was checked and optical density (OD) measurements were carried out at a wavelength of 600 nm using the UV visible spectro photometers [17].

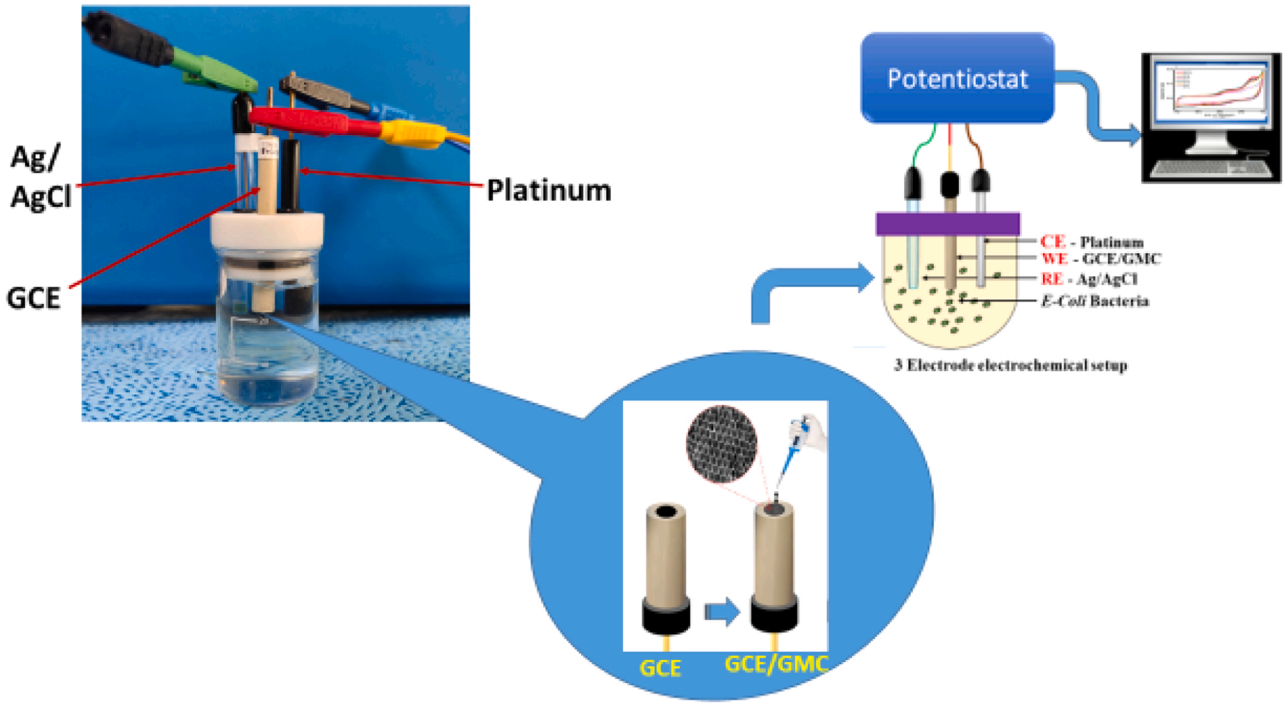
2.4. Electrode preparation

The GCE was mechanically and electrochemically pretreated and cleaned before modification. For the mechanical polishing, Al_2O_3 of 0.05 μm was used to polish the surface and then cleaned with distilled water. For electrochemical pretreatment, the polished GCE was kept in pH 7 phosphate buffer solution (PBS) and CV was run in a potential window − 1 to + 1.2 V to ensure no peak is obtained. 2 mg of GMC was dispersed in 500 μL of ethanol, sonicated for 15 min was used. Then,

5 μL of the dispersed solution was drop-casted onto the surface of GCE. Finally, the electrode was air-dried for 1 h. The electrochemical measurements were performed within the potential window of (−1 to 1) V and a scan rate of 50 mV/s using the modified electrode immersed in a neutral pH electrolyte of PBS, LB media, and *E. coli*. Scheme 1 illustrates the experimental procedure.

2.5. Bacteria concentration

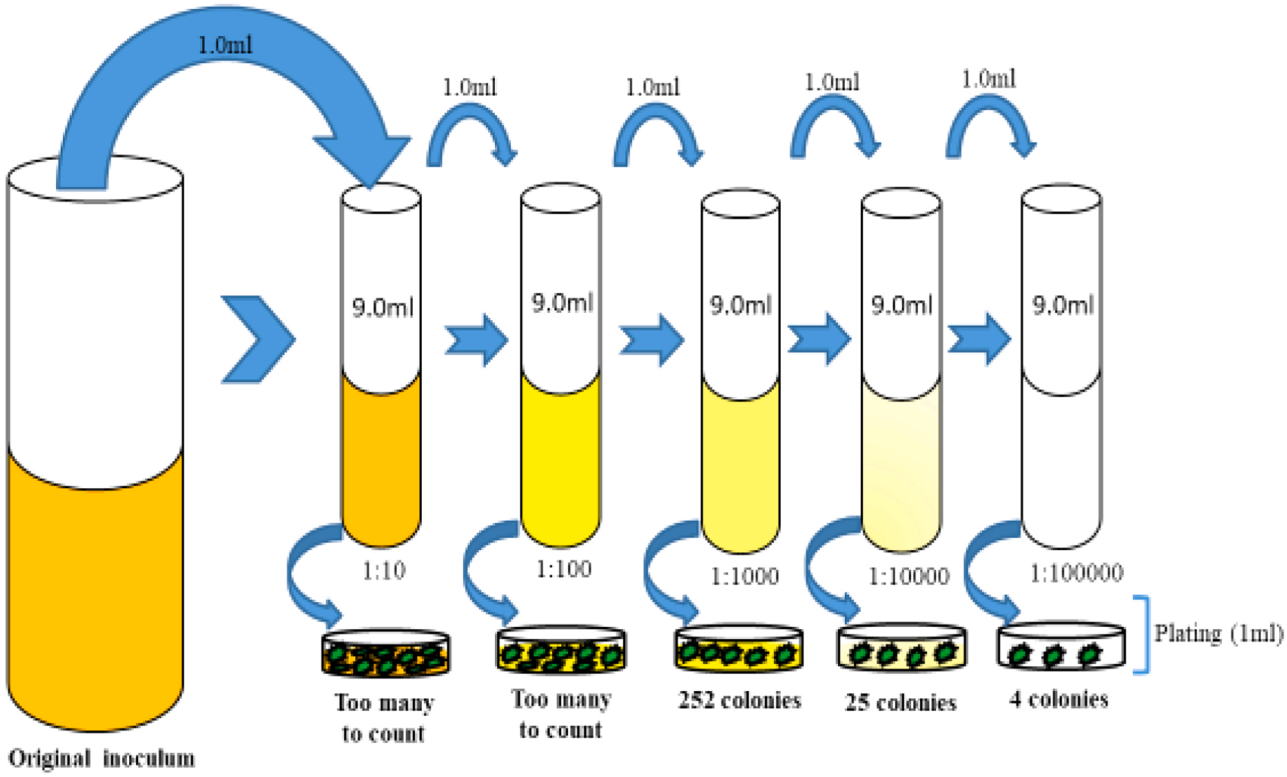
The colonies of *E. coli* were taken from the plate and inoculated into 1 mL of LB media centrifuge tubes. The tubes were placed in the incubator at 37 °C and were left overnight with shaking at 180 rpm. Then, 200 μL of *E. coli* was inoculated into the 20 mL of LB media centrifuge tubes. This culture was placed in a shaking incubator of 180 rpm at



Scheme 1. Real diagrammatic view and schematic representation of the electrode modification and electrochemical setup.

37 °C for 36 h. To count the cultured plate, the resulting cell suspension of *E. coli* bacteria has been used. The supernatant was discarded by centrifugation at 10000 rpm. Various *E. coli* bacteria concentrations (from 5 $\times 10^1$ CFU/mL to 2 $\times 10^2$CFU/mL) were made in the PBS buffer. Under the optimized conditions, the square wave voltammetry (SWV) technique was used for the determination of *E. coli* bacteria using the GCE/GMC electrode.

Generally, the unit used to denote *E. Coli* bacteria concentration is CFU/mL, (colony-forming unit/mL). CFU stands for colony-forming units, and it refers to the number of live bacteria found in a sample. A



Scheme 2. Schematic representation of the Serial dilution and the plating process.

viable bacterium means the bacteria that are growing or dividing in a sample. Due to higher bacteria concentration, first, the bacteria were diluted in the PBS to get the OD of 0.6. Then, the method known as Serial dilution or 10-fold dilution followed by the plating method was used to find the *E. coli* bacteria concentration. (Scheme 2).

Five centrifuge tubes were labeled with the dilution factor of 1:10, 1:100, 1:1000, 1:10000, 1:100000. The 1 mL pipette was used to introduce 9 mL of LB media into five labeled centrifuge tubes. Then inoculated 1 mL of cultured bacteria was placed into the first tube of LB media and the mixer was vortexed for 15 s. Again 1 mL of media from the first tube was transferred into the second tube and the mixer was vortexed for 15 s. The same process was repeated for the second, fourth, and fifth centrifuge tubes.

The plating method was carried out to count the number of colonies present in a sample. Five plates were prepared in a petri dish from nutrient agar media. 1 mL of liquid culture was taken from each diluted tube and spread into the labeled five plates of nutrient agar. All five plates were incubated overnight for 37°C. The colonies were counted from all these plates. The ideal range of colonies to be counted as (30−300) colonies in a sample. Less than 30 colonies resulted in the disguised result which was unable to justify and more than 300 colonies resulted in the merger of too many colonies that were difficult to count [18].

The formula used was,

Number of bacteria (CFU)/mL = (number of colonies on plate × reciprocal of the dilution of the sample) / volume of the culture plate

CFU/mL (experiment) = (252 × 1000) / 1 =252000 CFU/mL= $2{\cdot}52\times10^5$CFU/mL

2.6. Scanning electron microscopy (SEM) sample preparation

Firstly, 70 mg Glassy Carbon powder was taken on the petri dish and 30 μL of baby oil was poured into it. Stirring was done to make a thick paste out of it. A small amount of paste was taken and put into the Glass substrate. To dry the paste, the glass substrate was put into the oven at 100 Celsius for around 2 h. After then, 10 μL of GMC solution was drop cast on the Glass substrate having Glassy carbon paste. Finally, it was left for the air to dry for 1 h. Thus, the sample was ready to carry out the SEM study.

3. Results and discussion

3.1. Electrocatalytic activity of the E. Coli

To understand the electrochemical oxidation of *E-Coli*, CV and SWV was observed in PBS and *E. coli* solution, whereby a distinct oxidation peak was observed using the CV and SWV techniques for *E. coli* bacteria. Using the CV technique, an anodic peak at $E^0 = 0.749$ V was observed. Similarly, using the SWV technique an oxidation peak at $E^0 = 0.742$Vvs Ag/AgCl was observed. Fig. 1(a) illustrates the CV response of GCE/GMC in various concentrations of *E. coli*in 0.1 M PBS buffer at a scan rate of 50 mV/s. The electrochemical characterization of LB media and *E. coli*

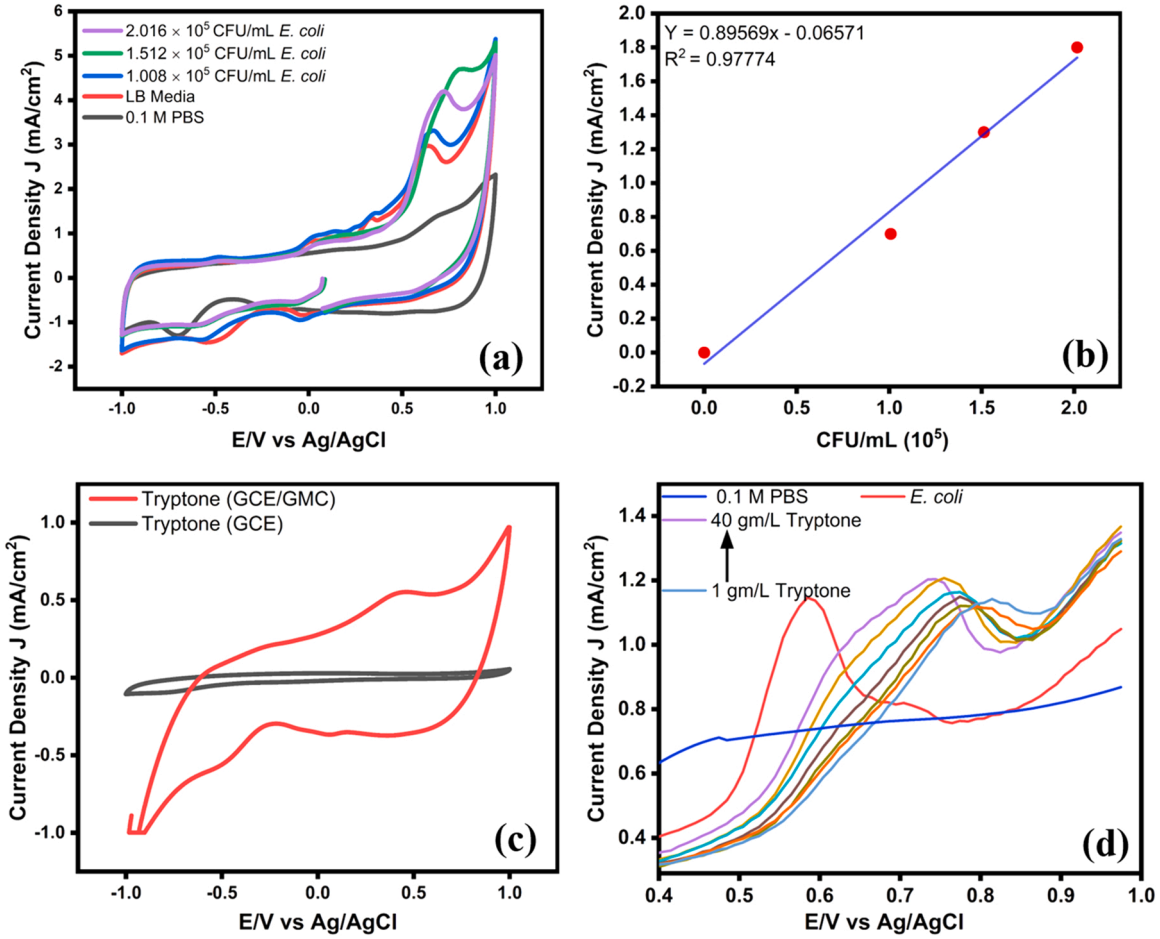


Fig. 1. (a). CV Response of GCE/GMC in pH 7 PBS, LB and various concentrations of *E. coli*50 mV/s vs. Ag/AgCl. (b) Corresponding linear plot of peak current density vs *E. coli* concentration from 1.008 × 10^5 CFU/mL to 2.016 × 10^5 CFU/mL by subtracting the background current density. (c) CV Response of GCE/GMC of Tryptone 50 mV/s vs. Ag/AgCl. (d)SWV Response of varying Tryptone concentration from 1 gm/L to 40 gm/L with a constant concentration of the *E. coli*.

was performed and no significant peak was observed for GCE/GMC electrode in PBS. However, GCE/GMC in a mixture of PBS and LB media gave a feeble peak. Upon addition of bacterial culture of various concentrations, a significant increase in the oxidation peak current density was observed authenticating that the peak current density was directly proportional to the bacterial concentration.

The electrochemical sensing [19] of *E. coli* gets enhanced when the GCE was modified with GMC resulting in a larger peak current density, better peak shape, and lower potential because of the good electron transfers ability. While during reverse scanning, a negligible amount of peak was observed, the electron transfers between the electrode surface and the probe molecule were hampered by the poorly conductive electrode. The continuous scanning was carried out on increasing the *E. coli* concentration to study the mechanism involved in the electrochemical sensing of bacteria. On increasing the *E. coli* concentration, the peak current density gets increased and moved to a higher potential. Fig. 1(b) is the corresponding linear fit graph manifesting that the increase in anodic peak current density is directly proportional to the increase in concentration.

The LB media has a composition of tryptone, yeast extract, and NaCl which is possibly contributing to the peak obtained. The addition of bacteria in the solution is enhancing the peak due to an increase in the electron transfer mechanism by the influence of bacteria. The increase in current density value is due to the conductivity of bacterial cell membranes and cytoplasm [20]. For a better understanding, a control experiment with tryptone alone is performed with GCE/GMC and a feeble peak was observed. However, this peak slightly shifts plausibly due to yeast extract and NaCl.

The effect of Tryptone concentration has been studied with constant *E. coli* concentration as shown in Fig. 1(d). Fig. 1(d) depicts the effect of varying the Tryptone concentration with a constant concentration of the *E. coli.* The concentration of the Tryptone was varied from 1 gm/L to 40 gm/L and Square Wave Response was recorded. As the concentration of tryptone is varied, the current is linearly increased with constant E. Coli. A shift in peak is observed. However, when E Coli concentration is increased, the peak current increases linearly at the potential slightly different from tryptone alone.

3.2. Effect of scan rate

The electron transfers process of the working electrode GCE modified with GMC was observed by carrying out the relationship between the peak current density and scan rate in the range of (10−150) mV/s. The reduction peak observed around − 0.5 ± 2 V is due to GMC and dissolved oxygen in PBS. The same peak is observed in the plain PBS as well. By using the following equation,

$i_{pa} = 2.99 \times 10^5\, n[(1-\alpha)n_a]^{1/2} ACD^{1/2} v^{1/2}$, [21] and it is found that the diffusion-controlled reaction takes place with the diffusion coefficient is calculated as $12.6164 \times 10^{-28} cm^2 sec^{-1}$.

wherein n = total no of electrons, A=electrode surface area (0.07065 cm^2), C = concentration of *E.coli*(2.016×10^5 CFU/mL), υ = scan rate (150 mV/s), n_a = no of electrons involved in rate-determining step, α = transfer coefficient(0.5), I_P = peak current (0.25) mA.

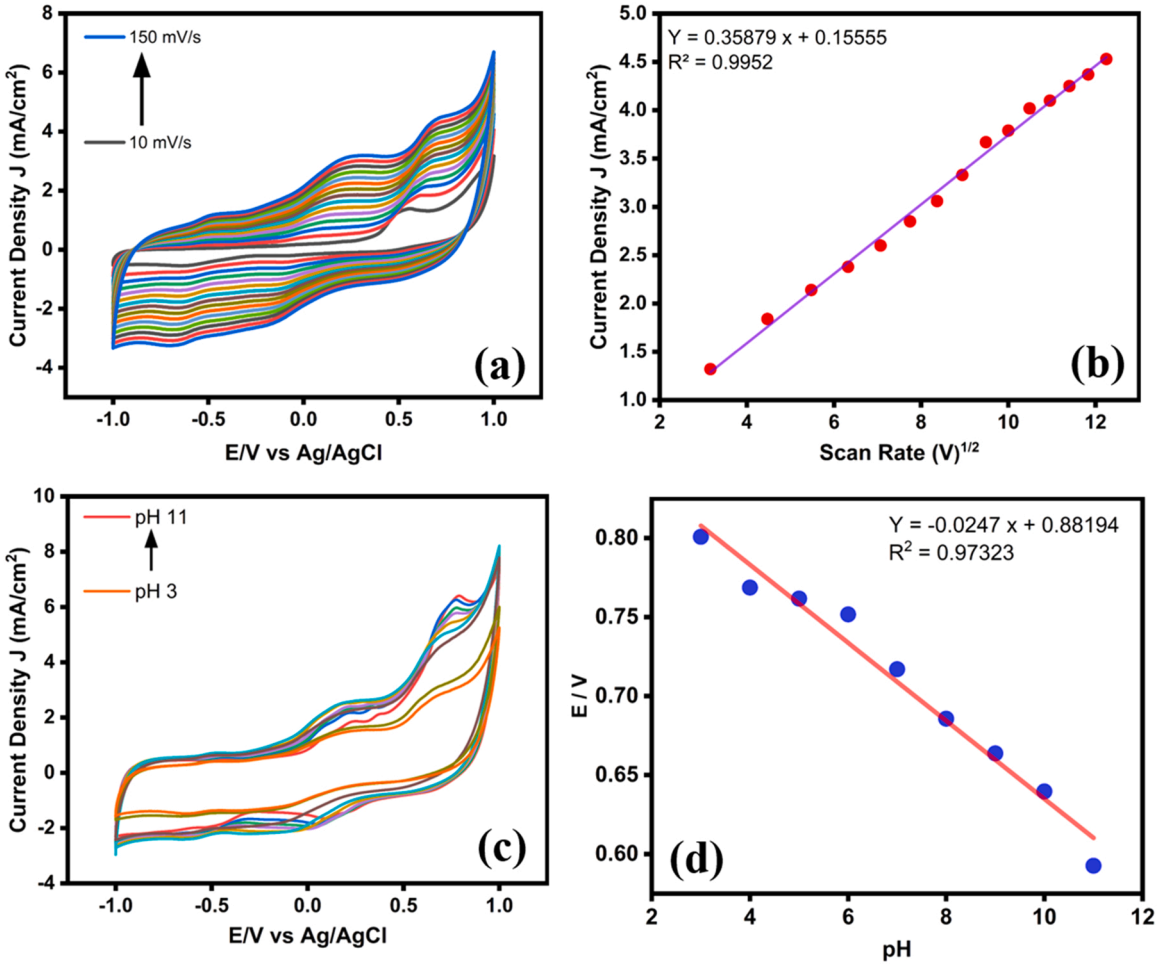


Fig. 2. (a) Effect of varying scan rate in*E. coli*PBS solution (10−150) mV/s; (b) Corresponding plot of I vs $\upsilon^{1/2}$; (c) Comparative CV response of *E. Coli in* various pH (3−11); (d) Corresponding plot of E^0 vs pH.

3.3. Effect of pH

The effect of solution pH was analyzed on the CV response of various pH (3−11) PBS buffer was examined. Fig. 2(c) illustrates the CV response of GCE/GMC in pH (3−11) bacterial solution. The response depicts that the increase in oxidation peak current density on increasing the pH from 3 to 11. The plot of E^0 vs. pH was linearly observed with a negative slope of 0.0247 mV pH^{-1}. Here, the participation of electrons

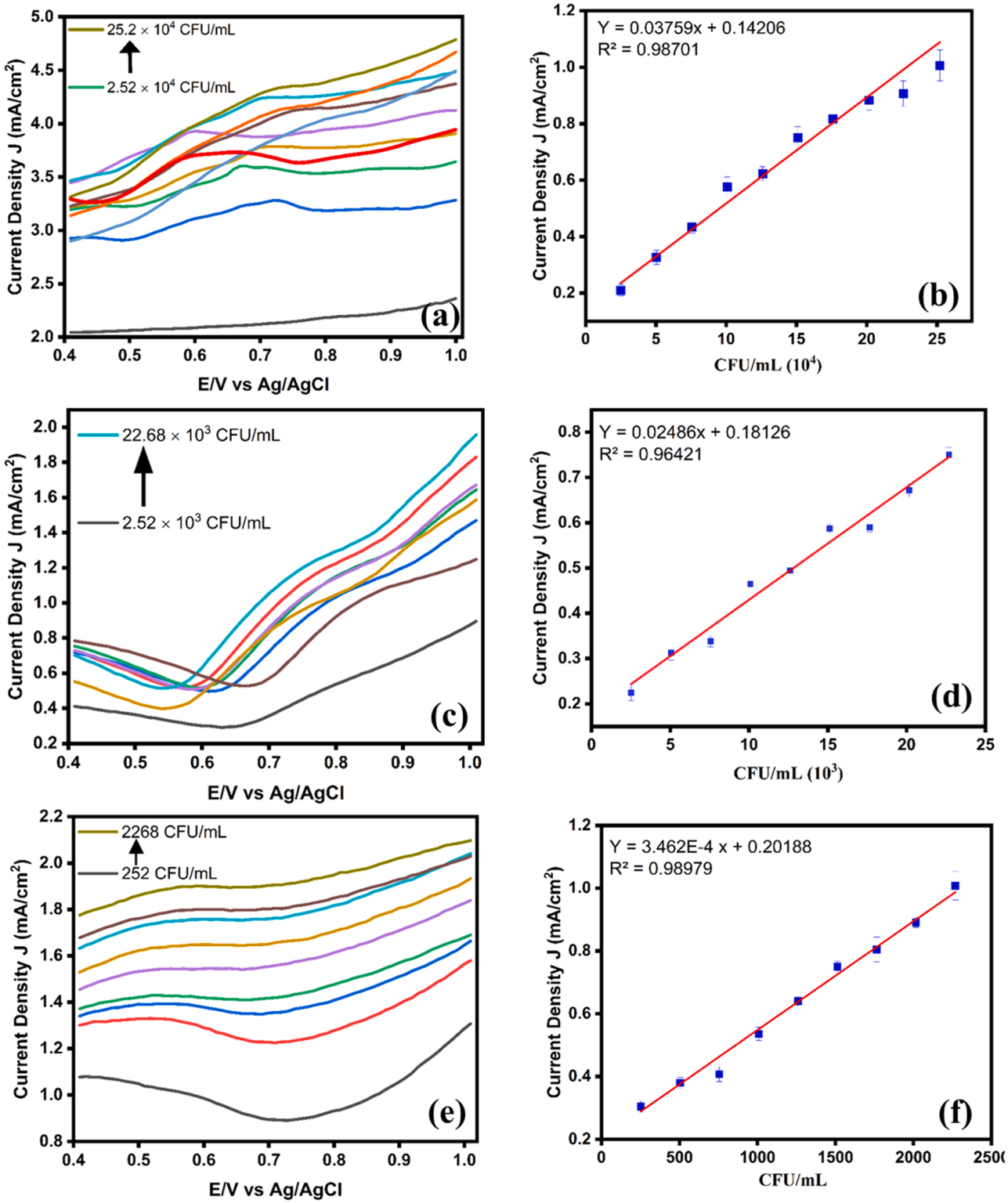


Fig. 3. (a) SWV response of the *E. coli various* concentrations (2.52–25.2) × 10^4 CFU/mL; (b) Corresponding linear graph for *E. coli* concentration (2.52–25.2) × 10^4 CFU/mLby subtracting the background current density.;(c) SWV response of the *E. Coli* corresponding to lower concentrations (2.52–22.68) × 10^3 CFU/mL;(d) Corresponding calibration graph for *E. coli* concentration (2.52–22.68) × 10^3 CFU/mLby subtracting the background current density. (e) Square wave voltammetry response of the *E. coli* concentration (252–2268) CFU/mL. (f) Corresponding calibration graph for *E. coli* concentration (252–2268) CFU/mLby subtracting the background current density.

and protons was approximately equal which characterizes the Nernstian reaction [22].

3.4. Effect of E. coli concentration

The concentrations of *E. coli* were varied from 2.52×10^4 CFU/mL to 25.2×10^4 CFU/mL and the SWV technique was used to characterize the peak current density within the optimized parameter ranges. These parameters were defined as, Initial potential (E_i) = 0.4 V, Final potential (E_o) = 1 V, Pulse height 25.0 mV, Pulses width= 50.0 ms, and Step height = 10.0 mV. Fig. 3(a) illustrates the SWV responses. Here, the peak current density gets increase because of an increase in the *E. coli* bacteria concentration. In Fig. 3(a), the lowest read line in red is GCE/GMC which is in only phosphate buffer solution, hence, a flat curve is obtained. Whereas, the next line, green, is GCE/GMC in LB media alone without bacteria, wherein, the slight bump is visible. Once the bacteria culture was added, the peak grew gradually upon increasing the bacterial concentration. Therefore, this signifies that the peak observed was due to the presence of bacteria. The lowest current density obtained from the green line, (blank) was subtracted from all the consecutive current densities of the concentration, and a baseline-corrected peak value was plotted for the linear graph.

Fig. 3(b) is the corresponding calibration plot. The given plot was made by subtracting the peak current density of LB media to each *E. coli* concentration. Fig. 3(b) showed good linearity of 96%. This signified the number of active sites present on the electrode [23].

E. coli concentration was further varied from 2.52×10^3 CFU/mL to 22.68×10^3 CFU/mL. The peak current density was calculated again using the SWV technique. As illustrated in Fig. 3(c), the current density gets increases, as the *E. coli* bacteria concentration increases. The actual current density of *E. coli* was drawn by subtracting the peak current density of the LB media. The corresponding base line corrected plot between the concentration of bacteria and peak current density is shown in Fig. 3(d) where linearity was observed with a slope of 0.02486 and an accuracy of 96.42%.

For the calculation of the LOD, *E. coli* was further diluted and concentration was lowered from the (252–2268) CFU/mL. This can be illustrated in Fig. 3(e), where the current density has been increased on increasing the *E. coli* concentration. The corresponding calibration graph has been plotted by subtracting the background current density as shown in Fig. 3(f). The slope of 0.0003462 and accuracy of 98.97% were obtained.

The LOD was calculated by taking the peak average current density from the triplicated experiments. The slope was estimated by plotting the graph between the peak average current density and *E. coli* bacteria concentration. Standard deviation was calculated from the current density values derived from the triplicated experiments. Thus, theoretical LOD was obtained as50.40 CFU/mL. Further, the presence of *E. coli* was detected for better selectivity and further used for real-time application.

3.5. Interference effect

Interference effect [24,25] from various bacteria, such as Shewanella putrefaciens, Lactobacillus, Helicobacter pylori, and Bacillus anthracis of 1.008×10^5 CFU/mL concentration was performed to check the selectivity of the device in the presence of 1.008×10^5CFU/mL *E. coli*. A negligible amount of interference was observed from various other bacteria. As can be seen in Fig. 4(a) all the other bacteria gave responses at a slightly different potential than that of *E. coli* and *E.coli* gave predominant peak current value. Furthermore, as can be seen in Fig. 4(b). the bar graph depicts that simultaneous addition of other bacteria gave less reduction in peak current density < 5%.

3.6. Real sample analysis

The standard addition approach [26] was used to test the various real samples like tap water, pond water, and deionized water. Here, GCE/GMC was subjected to various spiked concentrations of *E. coli* and the approximate concentration of *E. coli* was calculated based on the average current density values obtained by triplicated experiments. Fig. 5a–c are the SWV responses of the standard addition method. The obtained results are illustrated in Table 2. The recovery values signify that the *E. coli* was selectively detected in the real sample of tap water, pond water, and deionized water [27].

Following that, real samples of water were taken in the 10 mL of the filtrate. This solution was analyzed with the developed method, and SWV was performed. The standard addition approach was used with 1.008×10^5 CFU/mL, 1.512×10^5 CFU/mL, and 2.016×10^5 CFU/mL. *E. coli* and SWV were conducted once again. This procedure was performed three times in the linear region to notice an increase in peak current density values as *E. coli* concentration increased. At

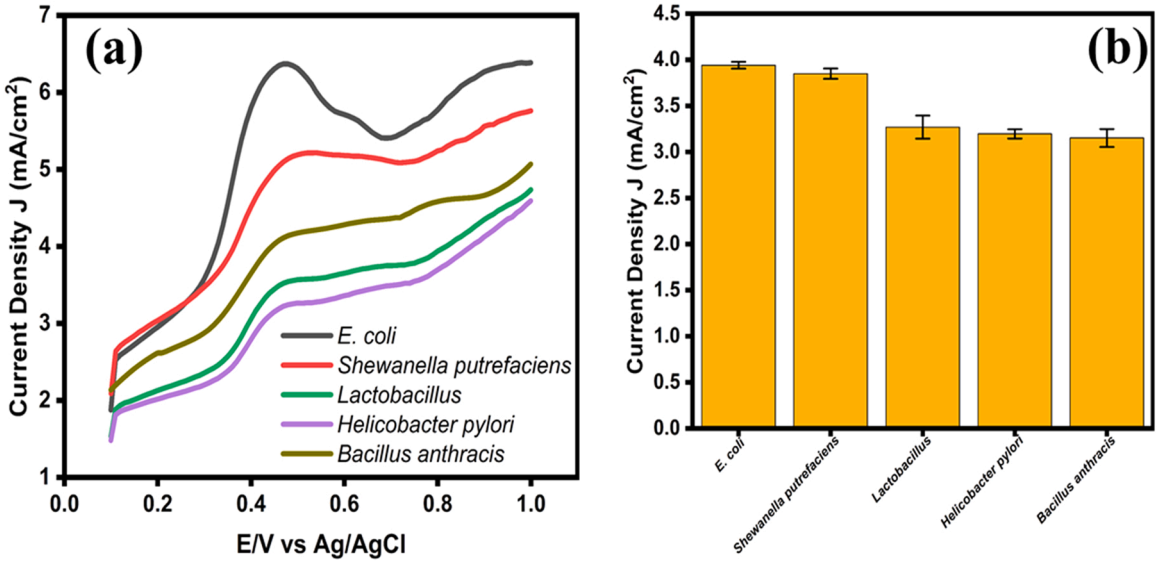


Fig. 4. (a) SWV response of various bacteria of 1.008×10^5 CFU/mL concentration; (b) Bar graph representation for various bacteria of 1.008×10^5 CFU/mL concentration.

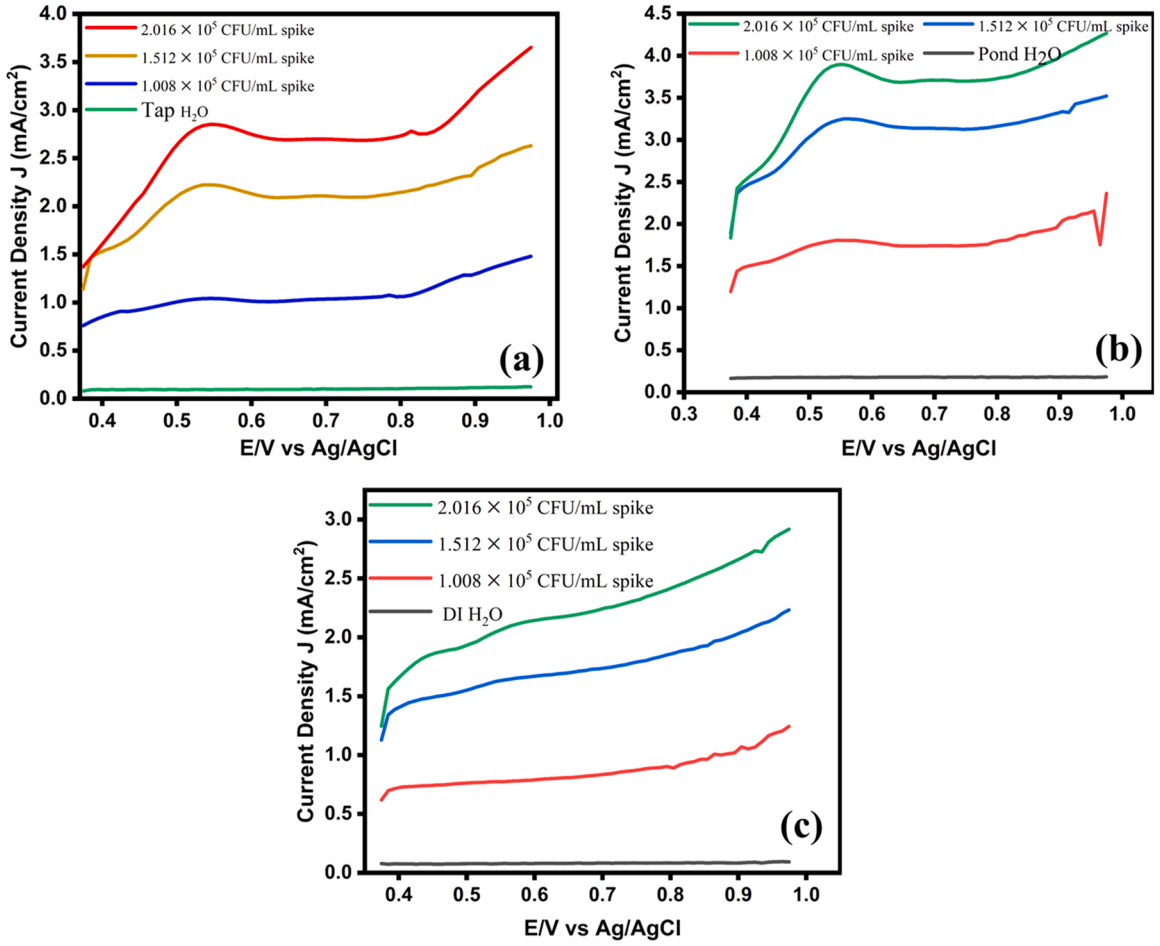


Fig. 5. (a) Square wave voltammetry response of *E. coli* in Tap water. (b) Square wave voltammetry response of *E. coli* in Pond water. (c) Square wave voltammetry response of *E. coli* in DI water.

Table 2
Real Sample Analysis of *E. coli* with Tap, Pond, and DI Water.

Sample	J1 (mA/cm^2)	J2 (mA/cm^2)	J3 (mA/cm^2)	Average (mA/cm^2)	SD	Added (A) ($\times 10^5$) CFU/mL	Found (F) ($\times 10^5$) CFU/mL	Recovery (A/F *100) %
Tap H_2O	1.39	1.19	1.18	1.25	0.01	1.008	0.94	93.2
	1.85	1.47	1.33	1.55	0.022	1.512	1.54	101
	0.97	0.95	0.73	0.88	0.011	2.016	1.93	95.7
Pond H_2O	1.44	1.46	1.43	1.44	0.001	1.008	1.03	102
	2.01	2.24	2.10	2.12	0.009	1.512	1.48	98
	2.36	3.02	2.77	2.72	0.027	2.016	1.99	99
DI H_2O	0.73	0.94	0.98	0.88	0.011	1.008	0.99	98
	1.12	1.07	1.29	1.16	0.009	1.512	1.54	101
	1.52	1.51	1.59	1.54	0.004	2.016	2.036	100

1.008×10^5CFU/mL, 1.512×10^5CFU/mL, and 2.016×10^5CFU/mL *E. Coli* concentrations, significant recovery values were found for different kinds of water samples.

3.7. Microscopic characterization

SEM study was carried out to study the shape and size of various nanomaterials and also to calculate the value of components quantitatively [28]. Fig. 6(a)–(d) shows the particle shape and size for the Graphitized Mesoporous Carbon. The abundance amount of carbon can be seen on the glassy carbon electrode substrate. To carry out the SEM images,

4. Conclusion

The present work reports an *E. Coli* GCE/GMC modified electrode for *E.coli* detection. All the experiments were performed with a standard three-electrode system. It was observed that the electrochemical signals of *E. coli* bacteria increased with an increase in their concentration. A distinct oxidation peak found at 0.749 V, 0.742 V (E^0 V v/s Ag/AgCl) using CV and SWV techniques respectively. A further effect of scan rate, pH, and concentration was also analyzed. The limit of detection was estimated as50.4 CFU/mL using the SWV technique. An interference study was carried out to understand the interaction of *E. coli* with various bio-chemicals like Xanthine, Hypoxanthine, Uric acid, Dopamine, and Ascorbic acid. Finally, real sample analysis was carried out in tap, pond, and deionized water showing good reliability and recovery.

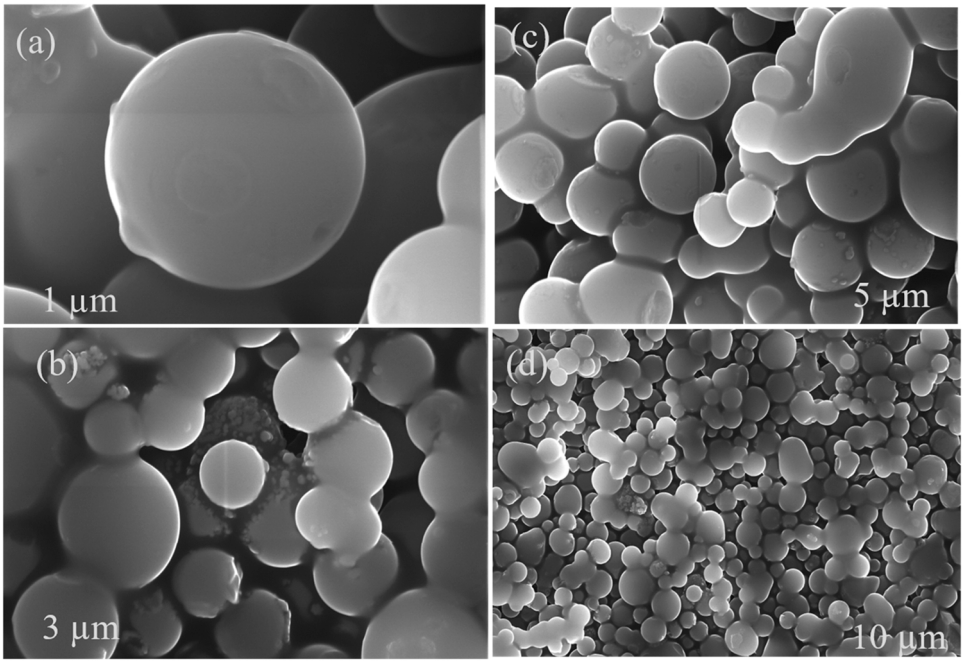


Fig. 6. SEM images of the GMC with different magnifications on the Glassy Carbon powder.

Because of the quick preparation time, simple modification process, and low preparation cost, the proposed sensor is easily scalable. Future research in this direction can pave way for the development of nanostructured electrochemical sensors for diagnostics and real-time monitoring of *E. coli* growth in diverse fields.

CRediT authorship contribution statement

Manish Rishi: Conceptualization, Data curation, Formal analysis, Writing – original draft. **Khairunnisa Amreen:** Data curation, Formal analysis, Writing – review & editing. **Jaligam Murali Mohan:** Data curation, Writing – review & editing. **Arshad Javed:** Project administration, Resources, Writing – review & editing. **Satish Kumar Dubey:** Project administration, Resources, Writing – review & editing. **Sanket Goel:** Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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