ELECTROCHEMICAL SURFACE PLASMON RESONANCE DETECTION OF *ESCHERICHIA COLI* USING A GOLD NANOPARTICLE-MODIFIED ELECTRODE

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Public health depends on the quick and precise identification of Escherichia coli (E. coli), especially in areas like clinical diagnostics and food safety. In this work, a gold electrode modified with gold nanoparticles (AuNPs) produced by a green phytochemical process using broccoli (Brassica oleracea) leaf extract is used to build an electrochemical surface plasmon resonance (EC-SPR) detection platform. Direct application of the phytosynthesized AuNPs to commercial SPR disc electrodes improved their plasmonic and electrochemical characteristics. The developed EC-SPR platform offers several advantages over traditional methods, including rapid analysis, low sample volume, high sensitivity, and an environmentally friendly fabrication process.

Keywords: Electrochemical Surface Plasmon Resonance, Gold Nanoparticles, Green Synthesis, Escherichia coli Detection

1. Introduction

Rapid and accurate detection of *Escherichia coli* (*E. coli*) is a topic of interest in clinical diagnostics, environmental monitoring, and food safety. It has been shown that higher temperatures due to global warming have increased the multiplication of *E. coli* bacteria [1]. This could well stimulate the exploration of new detection methods. Though reliable, traditional detection methods, such as culture-based techniques and polymerase chain reaction (PCR) – often take quite a while to use, and many or require specialized equipment. Readjusted slightly in recent years, surface plasmon resonance (SPR) is an invaluable tool for real-time monitoring of interactions between biomolecules [2, 3].When electrochemical modulation is threaded together with it, electrochemically coupled SPR (EC-SPR) promises to deliver the greatest sensitivity and specificity because combining optical with electrochemical signals is just synergistically effective [4].

There are some benefits of using EC-SPR to detect *E. coli*: this combination can achieve greater sensitivity compared to traditional techniques.

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EC-SPR has an exceptionally brief response time, an essential attribute in preventing and controlling *E. coli* outbreaks. EC-SPR as a detection tool makes it possible to directly detect *E. coli*, simplifying analysis processes and minimizing potential errors. Sensors based on EC-SPR can be adapted to detect various strains of *E. coli* or even other pathogens. Because the EC-SPR technique is very sensitive, only very small sample volumes are required for detection. This makes it simple to collect samples of various kinds [5, 6].

Although the equipment is quite expensive, the investment will pay for itself over time through the ability to perform multiple tests quickly, without consumables that could greatly increase testing costs [7, 8]. Overall, the application of these combined EC-SPR techniques for *E. coli* detection can provide a fast, accurate and efficient solution, making them a useful tool in many fields such as food or microbiological diagnostics [9].

To aid in improving detection abilities of EC-SPR, the modification of gold electrode surface with nanomaterials was considered. Among them, gold nanoparticles (AuNPs) are popular due to their outstanding optical properties, biocompatibility and their high surface-to-volume ratio [10, 11]. Furthermore, AuNPs obtained by phytosynthesis with plant extracts is a substitute that avoids the use of toxic reducing agents. This aspect makes them more biocompatible and suitable for a wider range of applications. Broccoli (*Brassica oleracea var. italica*), rich in many phytochemicals such as flavonoids and glucosinolates, seemed an ideal choice for ecological synthesis of AuNPs [10, 11].

In this study, a gold disk electrode was subjected to electrochemical modification with phytosynthesized gold nanoparticles (AuNPs) obtained from broccoli leaves. The integration of these biologically derived nanostructures notably boosted the platform's electrochemical and plasmonic responses, enabling faster signal generation and achieving lower detection thresholds. The combination of sustainable nanotechnology with EC-SPR methods represents a promising route toward the innovation of modern diagnostic platforms.

2. Experimental

2.1 Materials and reagents

Gold nanoparticles (AuNPs) were obtained by green synthesis from dried broccoli leaves (*Brassica oleracea var. italica*). Chloroauric acid ($HAuCl_4$) (Merck KGaA, Germany) was used as the gold precursor. The synthesis method follows previous work reported by Fierăscu et al. or AuNPs and Ungureanu et al. for metallic nanoparticles obtained from cruciferous plants [12-14]. Luria Bertani (LB) (VWR Chemicals Avantor) broth was employed both as a bacterial growth medium and as a matrix for diluting AuNPs before electrode modification. The

Escherichia coli strain used for detection experiments was prepared and maintained as described by Ungureanu et all. [15].

2.2. Apparatus

All experiments were carried out using an Autolab ESPRIT SPR (Metrohm AG) system. The electrochemical setup consisted of an SPR cuvette with a three-electrode configuration: a gold disk serving as the working electrode (d=3 mm), a silver/silver chloride (Ag/AgCl) reference electrode, and a platinum wire as the counter electrode. These SPR setup was connected to an Autolab PGSTAT 320N (Metrohm AG) potentiostat/galvanostat for electrochemical control.

Infrared (IR) spectroscopy measurements were performed using a Perkin Elmer Spectrum 100 FT-IR (Perkin Elmer, Waltham, MA, USA) equipped with an attenuated total reflectance (ATR) accessory. The spectra acquired was processed with the corresponding software, applying baseline correction and smoothing, with a resolution of 4 cm^{-1}.

2.3. Phytosynthesis of Gold Nanoparticles (AuNPs)

Gold nanoparticles were synthesized via a green chemistry route. This method leverages plant metabolites such as flavonoids, polyphenols, and thiol-containing compounds, which act as both reducing and capping agents, enabling the environmentally friendly production of metallic nanoparticles [16, 17]. Briefly, an aqueous extract was prepared from ground and dried broccoli leaves and used directly as both the reducing and stabilizing agent. This extract was mixed with a chloroauric acid solution at room temperature, initiating the reduction of Au^{3+} to metallic Au^{0} and resulting in a colloidal suspension of gold nanoparticles. The reaction mixture was incubated under ambient conditions until a visible colour change indicated nanoparticle formation.

2.4. Modification of SPR Electrodes

Prior to use, commercial SPR disc electrodes were rinsed thoroughly with LB medium. Subsequently, 10 µL of the synthesized AuNPs suspension (1 mM) was drop-cast onto the electrode surface. In some cases, dilutions of the nanoparticle solution in LB medium were used (ratios ranging from 1:4 to 1:6). The modified electrodes were used immediately after deposition, without drying or further treatment. After deposition, the SPR signal was monitored until it stabilized in order to proceed to the next stage.

3. Results and discussions

3.1. Electrochemical and SPR Evaluation of *E. coli* on gold electrode

The cyclic voltammetry graph recorded on unmodified gold electrode shows a notable increase in current starting around +0.15 V and becoming more

pronounced as the potential approaches +0.4 V, Fig. 1. This behaviour suggests an electrochemical interaction occurring in the system under electrode polarization. Given that the measurement was conducted in Luria Bertani medium in the presence of *E. coli*, the observed rise in current can be attributed to bacterial activity influenced by the applied potential.

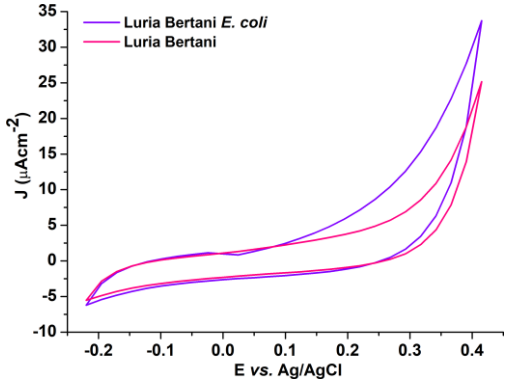

Fig. 1. Cyclic voltammetry of *E. coli* in Luria-Bertani medium on an unmodified Au electrode.

The voltammetric response reveals that a positive potential from +0.15 V up to +0.4 V represents a threshold potential at which electroactive behaviour becomes significant, possibly due to bacterial adherence, metabolic activity, or biofilm formation initiated or enhanced under these conditions. More specifically, the current increase may indicate that *E. coli* cells are responsive to this electrochemical stimulus, potentially through mechanisms involving membrane-associated redox processes or electron transfer pathways. The data support the hypothesis that *E. coli* can be attracted or activated at this potential, which may facilitate enhanced interaction with the electrode surface.

Moreover, previous studies have shown that bacterial membranes, including those of *E. coli*, carry a net negative charge under physiological conditions [18]. Therefore, applying a positive polarization (+0.4 V) enhances bacterial capture via electrostatic attraction, while negative polarization (-0.4 V) repels bacteria from the surface (Fig. 2. a)). During negative polarization, a clear decrease in SPR signal was observed, indicative of bacterial desorption (Fig. 2. b)). Conversely, the application of +0.4 V produced a significant increase in signal intensity, confirming the specific accumulation of *E. coli* at the electrode interface (Fig. 2. b)). This behaviour is consistent with previous findings that SPR intensity is modulated by the proximity and density of biological analytes at the gold surface [19].

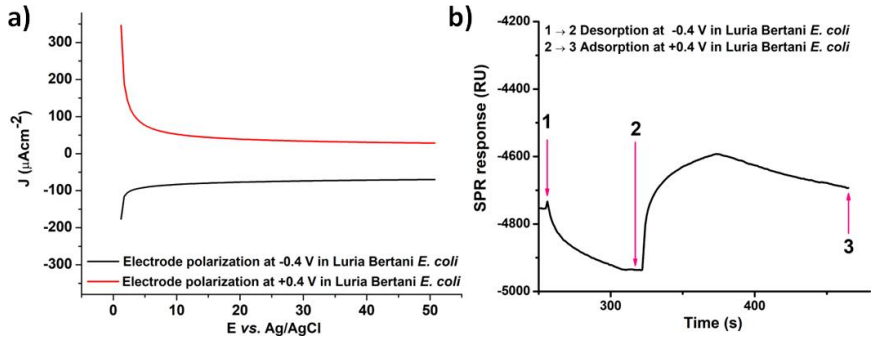

Fig. 2. EC-SPR coupling signals: a) Chronoamperometric curves corresponding to the desorption (-0.4 V) and adsorption (+0.4 V) of *E. coli* and b) SPR signal corresponding to the desorption (-0.4 V) and adsorption (+0.4 V) of *E. coli*.

3.2. Electrochemical and SPR Evaluation of *E. coli* on modified gold electrode with Phytosynthesized AuNPs

3.2.1 FT-IR Spectroscopic Characterization of Phytosynthesized AuNPs

Fourier-transform infrared (FT-IR) spectroscopy was used to recognize the functional groups present on the surface of gold nanoparticles (AuNPs) synthesized using broccoli (*Brassica oleracea*) leaf extract. The recorded FT-IR spectrum (Fig. 3.) displays several characteristic absorption bands, indicative of the phytochemicals involved in nanoparticle formation and stabilization.

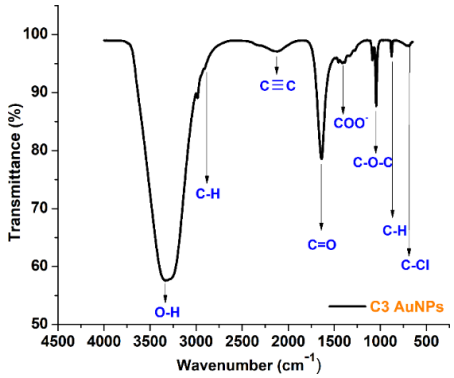

Fig. 3. FT-IR spectroscopy for the C3 AuNPs sample.

A broad band centred at **3285 cm⁻¹** is represented by O–H stretching vibrations, associated with hydroxyl groups from phenolic compounds and flavonoids. These groups act as reducing agents and contribute to nanoparticle stabilization via hydrogen bonding. The absorption at **2921 cm⁻¹** is assigned to aliphatic C–H stretching vibrations. This suggests the presence of organic moieties on the nanoparticle surface. A strong band at **1635 cm⁻¹** corresponds to

C=O stretching from carbonyl groups, likely originating from plant-derived proteins or polyphenolic acids. The band at **1384 cm^{-1}** is attributed to symmetric stretching of carboxylate (–COO^{-}) groups, confirming the involvement of organic acids as capping agents. Additionally, the signal at **1024 cm^{-1}** is assigned to C–O–C stretching vibrations, associated with glycosidic bonds or ester groups in plant polysaccharides.

A weak absorption in the **2550–2600 cm^{-1}** region may indicate the presence of thiol (–SH) groups (although this band is typically of low intensity and may overlap with broader O–H bands). Thiol groups are known for their strong affinity to gold surfaces, forming stable Au–S bonds. However, once the **–SH** group binds to the gold surface, the **S–H** stretching band often disappears from the FT-IR spectrum, due to the loss of the hydrogen atom and conversion into a covalent Au–S linkage.

Altogether, the FT-IR data confirm that the phytosynthesized AuNPs are capped with plant-derived biomolecules containing hydroxyl, carboxyl, carbonyl, ether, and possibly thiol groups. These functionalities not only contribute to colloidal stability but also enhance the interaction of nanoparticles with the negatively charged lipopolysaccharides of *E. coli*, thus improving the sensitivity of the EC-SPR sensing system.

3.2.2. Gold electrode modification with Phytosynthesized AuNPs and EC-SPR signal

Gold electrode modification with Phytosynthesized AuNPs was performed according with 2.4. Electrochemical signal enhancement of modified gold electrode with phytosynthesized AuNPs is clearly observed in voltammograms from Fig. 4.

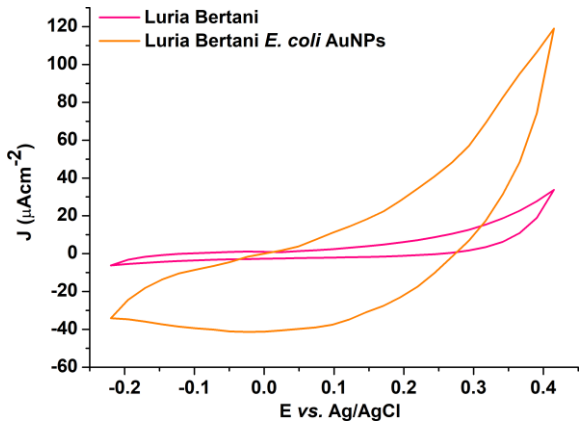

Fig. 4. Cyclic voltammetry of *E. coli* in Luria-Bertani medium on an AuNPs modified Au electrode.

Cyclic voltammetry of *E. coli* in Luria-Bertani medium revealed, at +0.4 V, a current density (J) of 117 μAcm^{-2} for AuNPs modified electrode (Fig. 4) comparing with 33 μAcm^{-2} for gold unmodified electrode (Fig. 1). In other words, the current density increased by approximately 3.55 times (254.55 %) due to the modification of the electrode with AuNPs. Thus, it can be concluded that the conductivity of the surface has increased. This aspect will further help in creating a highly efficient detection platform.

SEM imaging (Fig. 5. a)) revealed the formation of gold nanoparticle aggregates with sizes between 140-200 nm on the electrode surface.

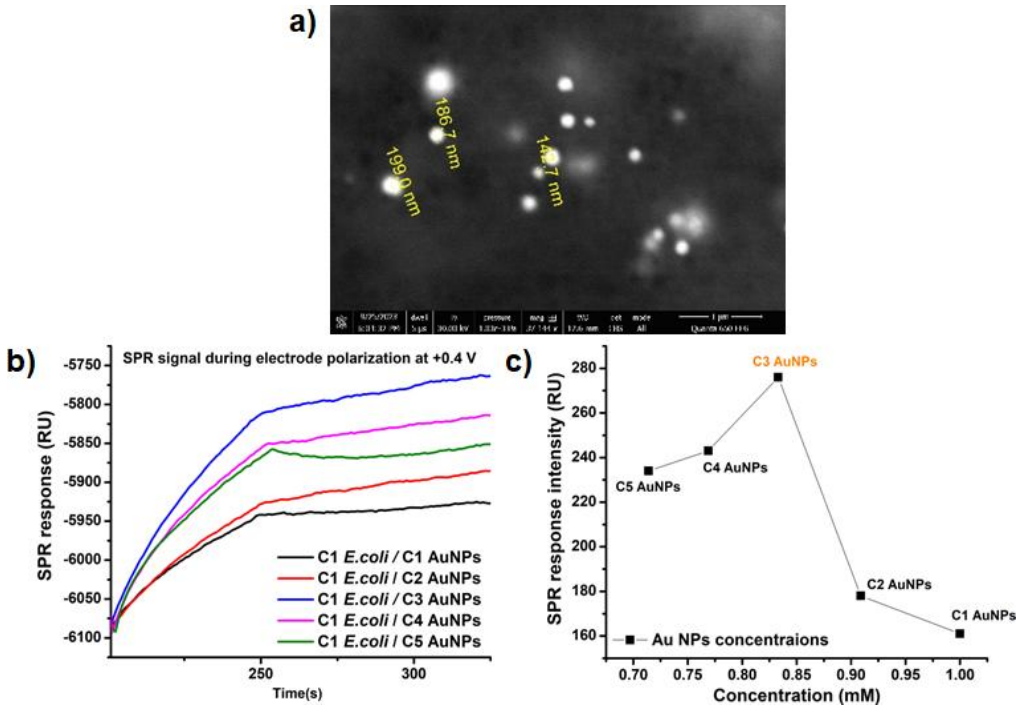

Fig. 5. a) SEM image of C3 AuNPs, b) SPR signal during chronoamperometry at +0.4 V and c) SPR signal intensity for each nanoparticle concentration.

Then, to augment the sensitivity of the detection system, electrochemical control was introduced into the SPR platform, leading to the development of an EC-SPR setup. Electrochemical surface plasmon resonance (EC-SPR) measurements were carried out to detect *E. coli*. The detection sequence involved:

1. **Baseline recording**: SPR signal was measured after rinsing the unmodified electrode with LB medium.

2. **Electrode functionalization**: 10 µL of AuNPs solution with different concentrations (*Table 1*) was applied; the SPR signal was recorded again.

Table 1

The labeling of each AuNPs concentration.

Sample	AuNPs Concentration (mM)
C1 AuNPs	1.000
C2 AuNPs	0.909
C3 AuNPs	0.833
C4 AuNPs	0.769
C5 AuNPs	0.714

3. **Sample addition**: 10 µL of *E. coli* suspension was dropped onto the electrode.
4. **Electrode polarization at -0.4 V**: This step aimed to repel loosely bound or non-specifically adsorbed bacterial cells; the corresponding EC-SPR signals were recorded (Fig. 1. a)).
5. **Electrode polarization at +0.4 V**: The electrode was positively polarized to promote electrostatic attraction of negatively charged bacterial membranes, and the EC-SPR signals were again recorded (Fig. 1. a)).
6. **Calibration curve**: This will be achieved through polarizing the electrode at +0.4 V, and the signal intensity difference will be measured between the beginning (point 2, Fig. 2. b)) and the end of the polarization (point 3, Fig. 2. b)).

Different concentrations of AuNPs were tested to determine the optimal modification level for the electrode (*Table 1*). As illustrated in Fig. 5. c), the SPR signal enhancement after +0.4 V polarization increased with nanoparticle concentration, reaching a peak at 0.833 mM. At higher concentrations, a reduction in signal was noted, likely due to nanoparticle agglomeration, which reduces the available surface area and impairs efficient bacterial interaction - an effect previously documented in nanoparticle-based biosensors [20]. The optimal concentration (0.833 mM) thus reflects a balance between high surface functionality and colloidal stability.

3.3. EC-SPR response sensitivity and linearity in *E. coli* detection on modified gold electrode

The analytical performance of the EC-SPR system was evaluated using *E. coli* suspensions at concentrations ranging from 0.454×10^5 to 0.830×10^5 CFU/mL (*Table 2*).

The change in SPR signal intensity recorded as the difference between signal values before and after positive polarization demonstrated a direct correlation with bacterial concentration.

Table 2

The labeling of each *E. coli* bacteria concentration.

Sample	*E. coli* Bacteria Concentration (CFU/mL)
C1 *E.coli*	0.830×10^5
C2 *E.coli*	0.625×10^5
C3 *E.coli*	0.555×10^5
C4 *E.coli*	0.500×10^5
C5 *E.coli*	0.454×10^5

Fig. 5. a) shows the SPR signal intensity as a function of *E. coli* concentration, on unmodified electrode polarized at +0.4 V. It can be observed that the response is completely nonlinear, suggesting that *E. coli* cannot attach to unmodified Au electrode.

In contrast with the previous unmodified electrode, for AuNPs modified electrode, the SPR signal intensity as a function of *E. coli* concentration shows a linear response, with a coefficient of determination (R^2) of 0.996, confirming a high quantitative capability, Fig. 5. c). The calibration curve for the detection of *E. coli* using AuNPs modified electrode polarized at +0.4 V exhibits a strong linear relationship between the SPR response intensity (RU) and the bacterial

$$x = 0.0043x - 178.99 \tag{1}$$

where y represents the SPR response intensity and x the *E. coli* concentration.

The sensitivity of the surface was calculated from the slope of the calibration curve. The value obtained is 0.0043 RU/(CFU/mL). The very high correlation coefficient (R^2 = 0.996) shows a good linearity within this concentration range. To evaluate the analytical performance of the EC-SPR detection platform, the limit of detection (LoD) and the limit of quantification (LoQ) were calculated based on the calibration data and the formulas presented in a study were used [21]. A linear regression model was performed for the SPR response as a function of *E. coli* concentration, resulting in a slope of 0.0043 RU/(CFU/mL) and a residual standard deviation (σ) of 3.70 RU.

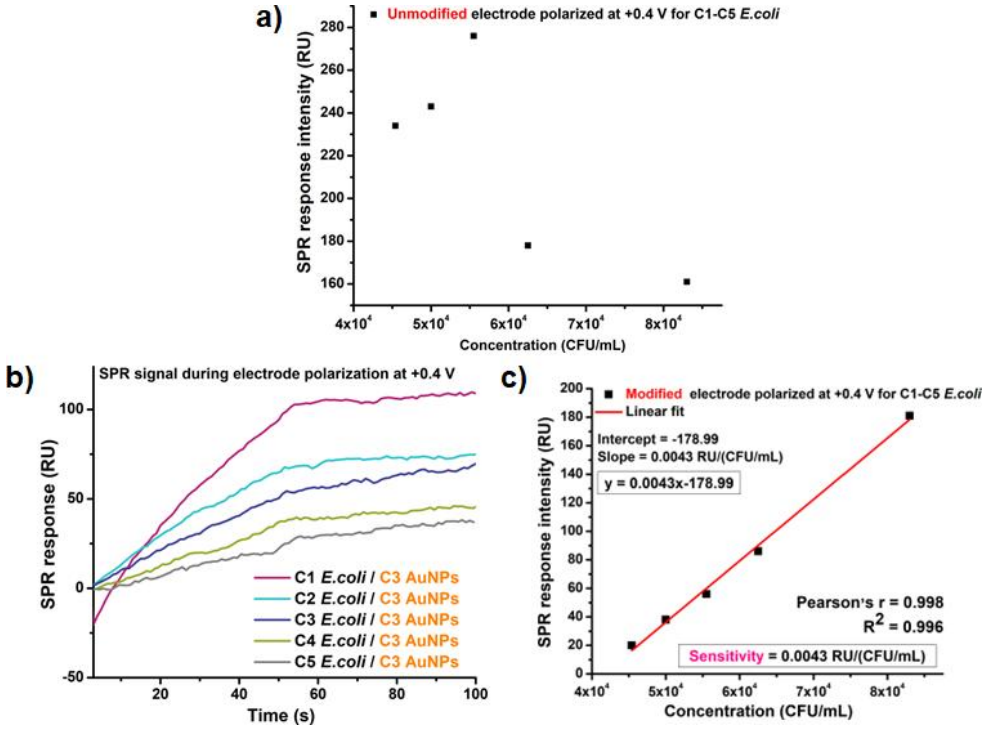

Fig. 5. a) SPR response intensity for each bacterial concentration without nanoparticles, b) SPR signal for each bacterial concentration in the presence of the C3 AuNPs sample, c) Calibration curve for the SPR signal from b).

Based on these calculations, a limit of detection (LoD) of 0.454 × 10^5 CFU/mL and a limit of quantification (LoQ) of 0.830 × 10^5 CFU/mL were obtained. These values are clear evidence for the suitability of the system for detecting low concentrations and volumes of *E. coli* in aqueous samples. Such a linear response is essential for real-world applications, where accurate quantification of bacterial contamination is very important. The sensitivity and correlation obtained are comparable or superior to the values reported in other EC-SPR detection systems for *E. coli* detection [10,11]. It can be noted that these performances were achieved without the use of biological recognition elements, such as antibodies or aptamers. This achievement simplifies the testing and improves lifetime and robustness of the detection platform obtained.

4. Conclusions

This study presents an EC-SPR detection platform enhanced by modifying a gold disc SPR electrode with gold nanoparticles (AuNPs) synthesized via a green approach using *Brassica oleracea* (broccoli) leaf extract. FT-IR analysis revealed that these biosynthesized AuNPs were capped with biomolecules from

the extract, including functional groups facilitating strong interactions with the negatively charged *E. coli* membrane and contributing to improved detection sensitivity.

The integration of electrochemical control into the SPR platform provided additional functionality. For +0.4 V polarized AuNPs modified electrode, calibration experiments revealed a robust linear relationship between SPR signal and *E. coli* concentration, with a good LoD and LoQ confirming the system suitability for detecting low bacterial loads.

With further testing in real-world matrices, this label-free detection platform holds strong potential for deployment in food safety monitoring, environmental testing, and rapid diagnostic applications.

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