

Electrochemical Detection of Selected Pathogens (E-Coli and S-Typhi) By Bioreduced Nickel Nanoparticles

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Abstract - E-coli and S-typhi detection suffers from slow analysis time and high costs, along with the need for specificity. While state-of-the-art electrochemical biosensors are cost-efficient and easy to implement, their sensitivity and analysis time still require improvement. This paper present an electrochemical method of detecting pathogens using NiNPs to achieve fast detection, low cost, and high sensitivity. Using voltametric techniques as the detection technique, the biosensor achieved a limit of detection of 2.7×10^2 and 1.6×10^3 CFU/mL for E. coli and E-typhi respectively across a concentration range of 10^2 – 10^8 CFU/mL. This provides rapid, highly sensitive E. coli and E-typhi detection with a fast analysis time of 30 min. Nickel nanoparticles were biosynthesized at different pH using. The nanoparticles were characterized using UV-Visible, Fourier Transform Infrared, X-ray Diffraction and Scanning Electron Microscope. The microbial assay was ascertain using cyclic voltametric method. The results confirm the formation of nickel nanoparticles at wavelength of 423nm and due to the spherical shape as confirmed by scanning electron microscope. The nickel nanoparticles revealed the oxidation-reduction reaction using cyclic voltametric analysis. The voltamogram was further characterized by the oxidation peak at approximately 70mV attributed to the presence of S-typhi, similarly, the oxidation peak of approximately 60mV correspond the presence of E-coli in the sample. This study, which combines the detection advantages of electrochemical systems, has the potential to meet the needs of point-of-care applications. It is thought that future studies that will aim to examine the performance of the production-optimized, portable, graphite-based sensor system on real food samples, environmental samples, and especially medical clinical samples will be promising.

Keywords: Nickel nanoparticles; Voltametric techniques; Spectroscopic analysis.

I. INTRODUCTION

Green chemistry is the creation of materials and procedures that strive to reduce or eliminate the use of hazardous compounds and materials. The development, utilization, and eventual dumping of chemical that are part of

product life cycle are enshrined in green chemistry. "Green chemistry" is also called sustainable chemistry [1]. This represents the preliminary ideas for a more environmentally friendly chemical process or product, which include: Prevention: Avoiding the creation of waste or harmful substances rather than cleaning or treating them [2]. Atom Economy: More advanced scientific techniques must be developed so that every material utilized during the process is converted into a final usable material, minimizing waste and maximizing efficiency [3]. Less Dangerous Chemical Synthesis: Synthetic processes should be designed to ensure the active production of materials with minimal or zero toxicity, reducing risks to human health and the environment [4]. Developing Harmless Chemicals: The development of chemical products should aim to have targeted actions that result in minimal toxicity while maintaining efficacy [5]. Safer Solvents: When not in use, solvents, separation agents, and other similar substances should be rendered unnecessary or replaced with greener alternatives to reduce environmental impact [5]. Design for Energy Efficiency: Synthetic techniques should be carried out at ambient temperatures whenever possible while taking into account the energy requirements of chemical processes for both economic and environmental effects [5]. Pollution Prevention Analysis: Analytical approaches should be regularly improved to prevent the production of hazardous compounds by adopting proactive in-process monitoring and control measures using real-time methodologies [6]. Harmless and Safer Chemistry Approaches for Accident Prevention: When using substances in chemical processes, care should be taken to select those that will reduce the risk of release, explosions, and fires [6]. Biological Entities in Nanoparticle Synthesis: In the past decade, biological entities have drawn increasing attention for nanoparticle synthesis [6]. The processes involved in biosynthesis use biomass or living organisms such as fungi, bacteria, and plants, providing a straightforward and practical alternative to complex physicochemical methods for producing nanomaterials with controlled size and form [7]. Cost and Safety Considerations in Nanoparticle Synthesis: The expense and risks associated with physical and chemical methods have made the biosynthesis of nanoparticles a necessary alternative. Scientists have employed plant extracts and microorganisms for bio-reduction (synthesis) to find less

expensive and safer ways to synthesize nanoparticles. This phenomenon has led to the development of novel areas of research in nanomaterial biosynthesis. Nature has developed approaches widely used in synthesizing nano- and microscale inorganic materials [8].

II. MATERIALS AND METHODS

2.1 Materials

All materials are of analytical grade. UV-Visible spectrophotometer (Jenway 6405 UV/VIS spectrometer), Fourier Transform Infrared Spectroscopy (Perkin Elmer), Powder X-Ray Diffraction (XRD, Ultima IV), Scanning Electron Microscopy (VEGA3 TESCAN), ethanol, nickel nitrate.

2.2 Methods

2.2.1 Preparation of Plant Extract

Cassia Seibeiriana dried leaves will be grinded and pulverize into fine particles. A 250 mL conical flask containing 15 g of the grounded leaves will be weighed, 50 mL of distilled water will be added to the weighed conical flask and it shall be boil for 10 minutes at between 80° C and 100° C. To remove the leaves, the leaves that will be boil, shall be allow to cool and filtered using Whatman No. 1 filter paper. The leaves extract shall be divided into three equal parts and made to have pH of 4, 7 and 10 instead of the usual neutral pH 7 of water using 0.5M H₂SO₄ and 0.5M NaOH [10].

2.2.2 Preparation of Nickel Nanoparticles

5 mL each of the extract samples of pH 5, 8 and 10 would be added dropwise to an already prepared 50 mL 0.01 M silver nitrate solution with continuous stirring until a colour change is going to be observed to obtain the nanoparticles of silver the extract of silver nanoparticles of pH 5, 8, and 10 shall be subjected to centrifuge machine for 10 minutes at 3000 rpm, the silver nanoparticle deposit will be removed and rinse with distilled water and later ethanol. After it is rinsed, the silver nanoparticles will be collected in a test tube and allow to coll. [10]

2.3 Electrode Preparation

To create or have a modified screen-printed electrode-silver nanoparticles electrode (cSPE/AgNP), approximately 15µL of the silver nanoparticles solution will be applied with a micropipette to the surface of apristinec SP Eelectrode. The electrode will be allow to dry at room temperature. Using an outer sphere redox pair of 0.005M potassium ferricyanide and potassium ferrocyanide in 0.2M KCl solution, the

electrochemical characteristics of the modified electrode will be examine using electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) [11].

2.4 Preparation of *Escherichia coli*

One plating on mac-Conkey agar colonies of interest will be studied for their morphology after 24hrs of incubation at 37oC. The general considerations were: π Emulsification – easy as described by (The general considered were Elevation– Ionized, Diameter-2-3mm, Colour-pink (lactose fermentation) Emulsification [12].

2.5 Detection of *Escherichia coli* and *Salmonellatyphi*

Escherichia coli detection was carried out by using cyclic voltammetry. 100µL of cultured *Escherichia coli* will be added dropwise to 15 mL 0.1M phosphate buffer solution and will be scan with the modified electrode from -0.3 to 1.0 V.

III. RESULTS AND DISCUSSIONS

VU-Visible Analysis

UV-visible spectroscopy of nickel nanoparticles (Ni-NPs) typically reveals a broad surface plasmon resonance (SPR) peak in the range of 300–400 nm (Figure 1), though some reports identify peaks around 270–300 nm. A lower wavelength (blue shift) indicates smaller particle sizes, while increased synthesis time often correlates with a red shift (higher wavelength) due to increased particle size [12].

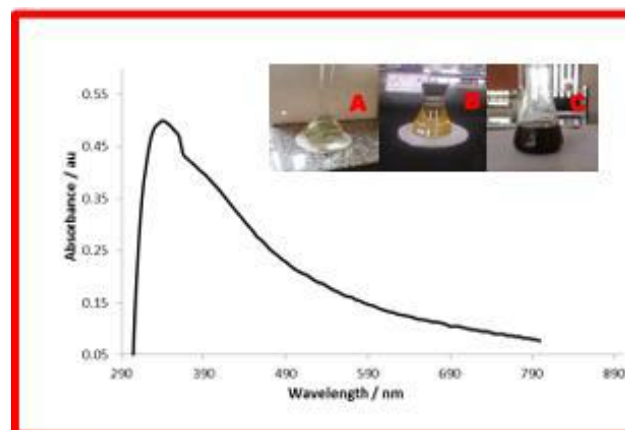


Figure 1: UV-visible absorption spectrum of nickel nanoparticle

Scanning Electron Microscope

Scanning electron micrograph was obtained at an accelerating voltage of 20 keV. Figure 2 shows a SEM micrograph of nickel nanoparticles. It can be observed that the morphology of the nanoparticles changed from that of small “pebbles” to an agglomerated tiny “flakes” of the nickel nanoparticles [13].

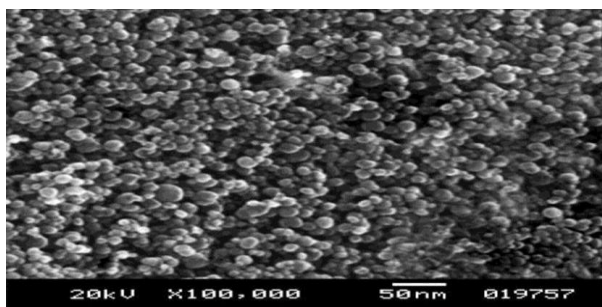


Figure 2: SEM image of NiNPs

XRD Analysis

The XRD patterns for nickel nanoparticles typically exhibit three distinct, broadened diffraction peaks at angles around and corresponding to the (111)(200), and (220) Miller indices. The peak broadening is due to the small crystallite size, usually calculated using the Debye-Scherrer formula. The NiNPs possess face-centered cubic (fcc) structure [13].

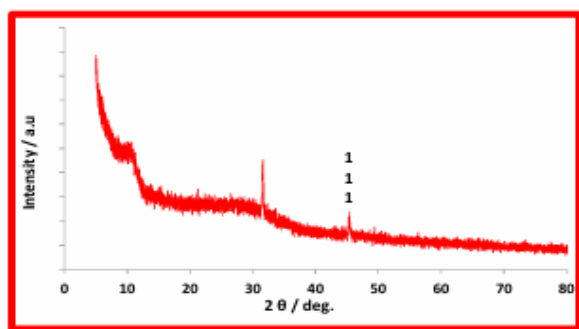


Figure 3: Powder XRD diffraction pattern for nickel nanoparticles

FTIR Analysis

The FTIR analysis of nickel nanoparticles (NiNPs) typically shows key absorption bands below 1000 cm⁻¹ for Ni stretching (around 400–673 cm⁻¹), indicating surface arrangement of nanoparticles, alongside bands for O-H stretching (3200–3500 cm⁻¹), C=O/C=C bonds (1600–1700 cm⁻¹), and C-H bending (1400 cm⁻¹) related to capping agents or moisture [13].

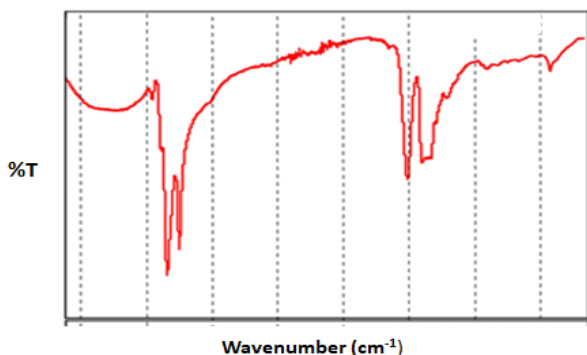


Figure 4: FTIR Spectrum of nickel nanoparticles

Voltametric Detection of *E-Coli* and *S-Typhi* by Bio-reduced Nickel Nanoparticles

Voltammetric detection of *E. coli* and *S. typhi* using nickel nanoparticles (NiNPs) offers a rapid, sensitive, and cost-effective electrochemical sensing platform. In techniques, the high surface area, and electron-transfer capabilities of bio-reduced NiNPs to enhance the signal for detecting bacterial pathogens, NiNPs exhibit significant inhibitory effects against *Escherichia coli* (*E. coli*) and *Salmonella typhi* (*S. typhi*). Studies show inhibition zones of 2–6 mm against the two pathogens [14].

Table 1: Voltammetric Detection of *E-coli* and *S-typhi* using NiNPs

NiNPs	CFU/mL	E-Coli	S-Typhi
	0.3	0.6×10^{-6}	1.2×10^{-6}
	0.6	3×10^{-6}	3×10^{-6}

Table 1 above shows the potential of NiNPs against microbe at different measurement.

Electrochemical detection of *Escherichia coli* and *S-Typhi* experiment was carried out by immersing the electrode in the buffer solution, which contained 100 μL of the microorganism and using NiCl₂ 3M KCl as the reference electrode, it was allowed to stand for an hour and the result obtained as depicted in Figure 5 shows that in the presence of *E-coli* and *S-typhi*, a characteristic shaped voltammogram was observed for both the Ni electrode and the nickel nanoparticle decorated electrode with peak potential at ~-0.1V.

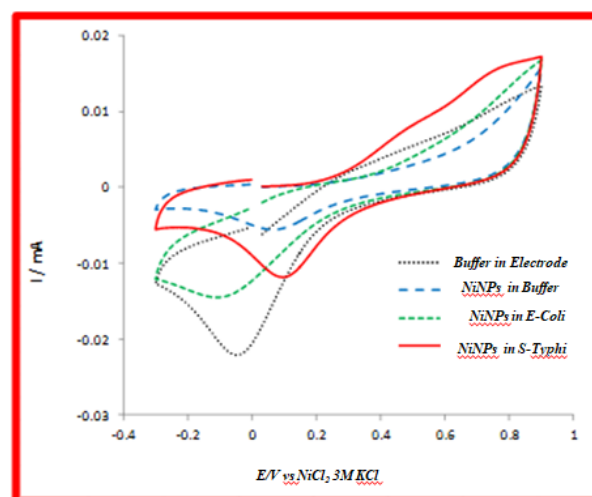


Figure 5: Electrochemical detection of *Escherichia coli* and *S-Typhi*

IV. CONCLUSION

The electrochemical detection of selected pathogens (*E-coli* and *S-typhi*) by bio-reduced nickel nanoparticles was achieved. *E-coli* and *S-typhi* detection suffers from slow analysis time

and high costs, along with the need for specificity. While state-of-the-art electrochemical biosensors are cost-efficient and easy to implement, their sensitivity and analysis time still require improvement. This paper presents an electrochemical method of detecting pathogens using NiNPs to achieve fast detection, low cost, and high sensitivity. Using voltametric techniques as the detection technique, the biosensor achieved a limit of detection of 2.7×10^2 and 1.6×10^3 CFU/mL for *E. coli* and *E.-typhi* respectively across a concentration range of 10^2 – 10^8 CFU/mL. This provides rapid, highly sensitive *E. coli* and *E.-typhi* detection with a fast analysis time of 30 min. Nickel nanoparticles were biosynthesized at different pH using. The nanoparticles were characterized using UV-Visible, Fourier Transform Infrared, X-ray Diffraction and Scanning Electron Microscope. The microbial assay was ascertained using cyclic voltametric method. The results confirm the formation of nickel nanoparticles at wavelength of 423nm and due to the spherical shape as confirmed by scanning electron microscope. The nickel nanoparticles revealed the oxidation-reduction reaction using cyclic voltametric analysis. The voltamogram was further characterized by the oxidation peak at approximately 70mV attributed to the presence of *S.-typhi*, similarly, the oxidation peak of approximately 60mV correspond to the presence of *E.-coli* in the sample. This study, which combines the detection advantages of electrochemical systems has the potential to meet the needs of point-of-care applications. It is thought that future studies that will aim to examine the performance of the production-optimized, portable, graphite-based sensor system on real food samples, environmental samples, and especially medical clinical samples will be promising.

ACKNOWLEDGEMENT

The author wishes to acknowledge the effort of Tertiary Education Trust Fund (TETFUND) for providing the research grant via Federal Polytechnic Mubi, Adamawa State, Nigeria.

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Citation of this Article:

Happy Maxwell. (2026). Electrochemical Detection of Selected Pathogens (E-Coli and S-Typhi) By Bio-reduced Nickel Nanoparticles. *International Research Journal of Innovations in Engineering and Technology - IRJIET*, 10(3), 160-164. Article DOI <https://doi.org/10.47001/IRJIET/2026.103022>
