



Development of a 3D-Printed Paper-Based Microfluidic Colorimetric Sensor for Nitrate Detection Using Silver Nanoparticles (AgNPs)

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ABSTRACT

Nitrate contamination in water is a serious environmental and public health issue worldwide. Conventional detection methods are accurate but expensive. This project develops a portable, low-cost 3D-printed paper-based microfluidic colorimetric sensor using silver nanoparticles (AgNPs) for nitrate detection. AgNPs were synthesized by chemical reduction and characterized using XRD, UV-Vis and FESEM. Two paper-based designs (dual-zone and Y-shaped) were fabricated on Whatman No.1 filter paper. A 3D-printed PLA holder supported the paper and guided capillary flow. Colour changes were measured using a blue LED and photodetector. Characterization confirmed crystalline, spherical AgNPs with an SPR peak at 400 nm. Both designs showed a linear response. The Y-shaped design gave a lower detection limit (66.54 ppm) than the dual-zone (92.20 ppm). The sensor successfully quantified nitrate and shows good potential as a low-cost platform.

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1. INTRODUCTION

Nitrate contamination from fertilizers, animal waste and industrial discharge has become a serious environmental issue [1]. High nitrate levels in water cause eutrophication and health problems such as methemoglobinemia [2]. Thus, monitoring nitrate is important for public safety. Conventional methods like ion chromatography and spectrophotometry are accurate but require expensive instruments and skilled operators [3]. These limitations make them unsuitable for on-site testing [4]. Paper-based microfluidic sensors offer a low-cost, pump-free alternative because they use capillary action [5]. Colorimetric detection allows visual observation [6]. Silver nanoparticles are widely used for colorimetric sensing due to their surface plasmon resonance (SPR) properties [7]. Nitrate ions cause AgNP aggregation, producing a visible colour change [8]. This project integrates 3D printing, paper microfluidics and AgNPs to create a portable nitrate sensor. A simple LED-photodetector setup quantifies colour intensity.

2. METHODOLOGY

2.1 Synthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) were synthesized via a chemical reduction method [9]. Sodium borohydride (NaBH₄) was employed as the reducing agent to convert silver ions (Ag⁺) into metallic silver (Ag⁰), while polyvinylpyrrolidone (PVP) was used as a stabilizing agent to prevent particle agglomeration. All solutions were prepared using deionized (DI) water to minimize interference from impurities.

The reagents were prepared as follows: NaBH₄ (0.1 g) was dissolved in 50 mL of DI water to form the reducing agent solution; AgNO₃ (0.16 g) was dissolved in 100 mL of DI water to serve as the silver ion source; and a 1% (w/v) PVP solution was prepared by dissolving 0.5 g of PVP in 50 mL of DI water.

For the synthesis, 20 mL of the AgNO₃ solution and 10 mL of the PVP solution were mixed, and DI water was added to bring the total volume to 100 mL. This mixture was placed on a magnetic stirrer and stirred continuously at room temperature. Subsequently, the NaBH₄ solution was added dropwise into the mixture at a rate of approximately one drop per second until a

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yellow coloration appeared, indicating the successful reduction of Ag^+ ions to AgNPs. The formation of AgNPs was preliminarily confirmed by observing the Tyndall effect using a laser beam, where visible light scattering along the beam path indicated the presence of dispersed nanoparticles in the colloidal solution. Table 1 summarizes the reagents used.

Table 1. Summary of reagents used in the synthesis of silver nanoparticles (AgNPs)

Reagent	Chemical Formula	Volume Used	Function of Synthesis
Sodium Borohydride	NaBH_4	Dropwise until colour change observe	Reducing agent to convert Ag^+ ions into metallic silver (Ag^0)
Silver Nitrate	AgNO_3	20 mL	Source of silver ions for nanoparticle formation
Polyvinylpyrrolidone	PVP	10 mL	Stabilizing agent to prevent nanoparticle aggregation and improve dispersion

2.2 Characterization of AgNPs

Three characterization techniques were employed. X-ray diffraction (XRD) was used to examine the crystalline structure. UV-Vis spectroscopy measured the surface plasmon resonance (SPR) peak. Field emission scanning electron microscopy (FESEM) revealed particle morphology.

2.3 Paper-Based Microfluidic Chip Fabrication

Two designs were cut from Whatman No.1 filter paper. The first was a dual-zone design consisting of one inlet (9 mm diameter) and one detection zone (9 mm diameter) connected by a straight channel (2 mm wide, 10 mm long). The second was a Y-shaped design with one inlet (10 mm diameter) connected to three detection zones (9 mm diameter each) by channels of 2 mm x 10 mm. Figure 1a shows the hand-drawn layout of the dual-zone chip, and Figure 1b shows the fabricated structure. Similarly, Figure 2a and Figure 2b illustrate the Y-shaped layout and the fabricated chip. Separate circular sensing pads (9 mm diameter) were cut and coated with 10 μL of AgNPs solution using a micropipette. Figure 3 illustrates the deposition process. The coated pads were dried at room temperature before being placed onto the detection zones.

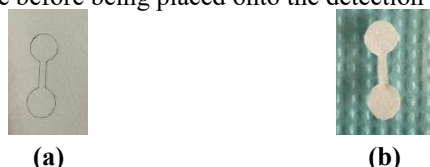


Fig. 1. Dual-zone paper-based microfluidic chip. (a) hand-drawn layout; (b) fabricated structure



Fig. 2. Y-shaped paper-based microfluidic chip. (a) hand-drawn layout; (b) fabricated structure



Fig. 3. Deposition of AgNPs solution onto circular pad

2.4 Flow Testing

Flow testing was performed using deionized water to evaluate the capillary flow behaviour of the paper-based microfluidic chips before nitrate detection. Deionized water was introduced at the inlet of each chip using a micropipette. The movement of the liquid along the channel was observed visually. No external pump was used. The test was repeated for both the dual-zone and Y-shaped designs to ensure that the paper channels allowed continuous liquid transport without leakage or blockage.

2.5 3D Printing of the Holder

A 3D-printed holder was designed using Shapr3D CAD software and fabricated by fused deposition modelling (FDM) with PLA filament [10]. The dual-zone holder has a rectangular base of 30 mm x 15 mm x 1 mm with a raised frame defining the inlet and detection zones. The Y-shaped holder has a square base of 45 mm x 45 mm x 1 mm with one inlet and three detection zones. Figure 4a and 4b shows the CAD model of the dual-zone holder and Y-shaped holder respectively. The actual printed casings are presented in Figure 5a (dual-zone) and 5b (Y-shaped). Figure 6a shows the assembled dual-zone device with the paper chip and AgNP-coated pad inside the holder, while Figure 6b shows the assembled Y-shaped device.

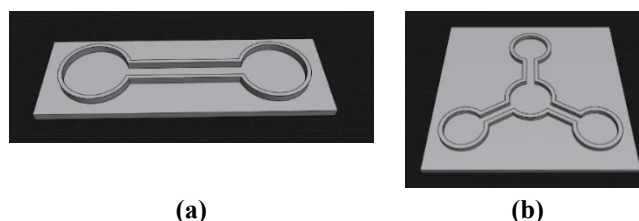


Fig. 4. CAD models of the 3D-printed holders. (a) Dual-zone; (b) Y-shaped



Fig. 5. Fabricated 3D-printed holders. (a) Dual-zone; (b) Y-shaped

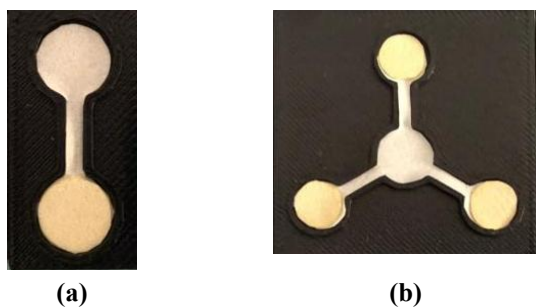


Fig. 6. Assembled paper-based microfluidic devices. (a) Dual-zone; (b) Y-shaped

2.6 Nitrate Detection and Optical Measurement

Nitrate solutions were prepared by serial dilution from a 1000 ppm stock solution. The stock solution was made by dissolving 0.274 g of silver nitrate (AgNO_3) in 100 mL of deionized water. From this stock, six different concentrations were prepared: 0, 100, 250, 500, 750 and 1000 ppm. The dilution was performed by mixing appropriate volumes of stock solution with deionized water to a final volume of 10 mL for each sample. Table 2 shows the preparation details. Figure 7 shows the six labelled samples (S1-S6) ready for testing.

For the dual-zone paper-based microfluidic device, 30 μL of each nitrate solution was introduced dropwise into the inlet zone using a micropipette. For the Y-shaped device, 90 μL was used to ensure that all three channels were filled and the liquid reached each detection zone uniformly. After adding the sample, the paper chip was left at room temperature for 24 hours to allow complete colour development at the AgNP-coated detection zones. This waiting period ensured that the colour change reaction reached a stable endpoint before measurement.

Table 2. Preparation of nitrate solutions by serial dilution

Sample	Stock solution (mL)	DI water (mL)	Total volume (mL)	Concentration (ppm)
1	0	10	10	0
2	1	9	10	100
3	2.5	7.5	10	250
4	5	5	10	500
5	7.5	2.5	10	750
6	10	0	10	1000

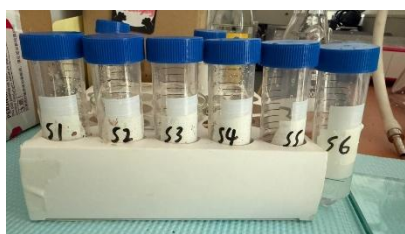


Fig. 7. Prepared nitrate standard solutions (S1-S6) with different concentrations

For optical measurement, a blue LED (powered by a 3.7 V battery with a 220 Ω resistor) illuminated the detection zone. A photodiode with a 10 k Ω resistor detected the reflected light intensity and converted it into a voltage output (in mV) measured with a digital multimeter. Figure 8 shows the LED

illumination setup, Figure 9 shows the photodiode circuit and Figure 10 shows the complete measurement system.

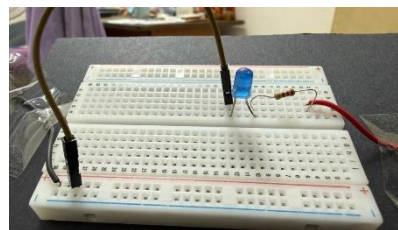


Fig. 8. Blue LED illumination setup

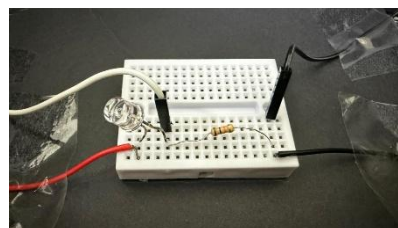


Fig. 9. Photodiode circuit setup

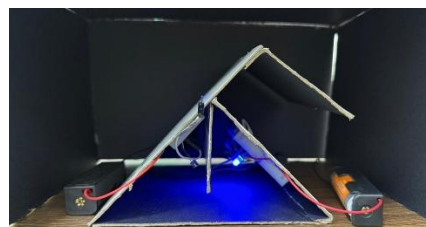


Fig. 10. Complete optical measurement setup

For each nitrate concentration, three voltage readings were taken from the detection zone (s). In the Y-shaped device, readings from all three detection zones were recorded and averaged. The average voltage for each concentration was then used to construct the calibration curve.

3. RESULTS AND DISCUSSION

3.1 Characterization of AgNPs

The successful formation of AgNPs was further confirmed by the Tyndall effect: when a laser beam was passed through the solution, visible light scattering was observed, as shown in Figure 11a (the synthesized AgNPs solution) and Figure 11b (the Tyndall effect).

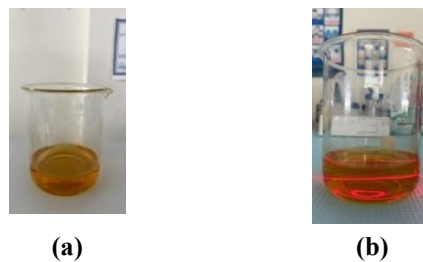


Fig. 11. Characterization of synthesized AgNPs. (a) Synthesized AgNPs solution; (b) Tyndall effect

The XRD pattern in Figure 12 shows distinct peaks at 2θ values of 38.13°, 44.33° and 64.41°, corresponding to the (111),

(200) and (220) crystal planes of face-centred cubic (FCC) silver [11]. This confirms the crystalline nature of the synthesized AgNPs. The UV-Vis spectrum in Figure 13 exhibits a strong absorption peak at approximately 400 nm, which is the characteristic surface plasmon resonance (SPR) of silver nanoparticles [12]. The presence of a single, well-defined peak indicates good dispersion and stability. The FESEM image in Figure 14 reveals that the AgNPs are spherical and nanoscale in size [12].

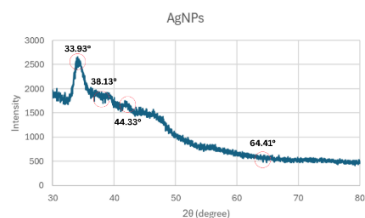


Fig. 12. XRD patterns of AgNPs

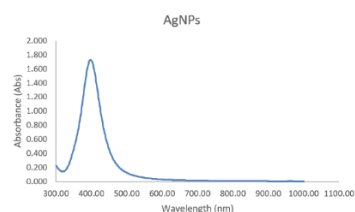


Fig. 13. UV-Vis absorption spectrum of AgNPs

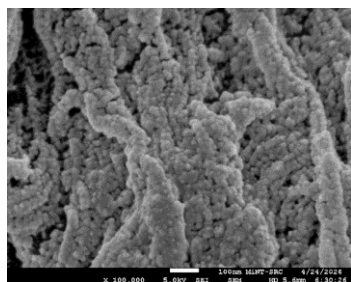


Fig. 14. FESEM image of AgNPs

3.2 Paper-Based Microfluidic Chip Performance

The capillary flow behaviour of the fabricated paper chips was evaluated using deionized water. Figure 15a and 15b shows the flow test results for the dual-zone and Y-shaped device respectively. The liquid was successfully transported from the inlet zone to the detection zone through capillary action, indicating proper channel function. The liquid distributed uniformly into all three detection zones in the Y-shaped design, confirming that the Y-shaped design enabled stable and uniform capillary-driven flow across multiple channels. No leakage and overflow was observed in both designs. These results demonstrate that both paper-based microfluidic chips were suitable for delivering sample liquid to the AgNP-coated detection zones without external pumping.



Fig. 15. Flow behaviour of paper-based microfluidic devices using deionized water. (a) Dual-zone; (b) Y-shaped

3.3 Performance of the 3D-Printed Holder

The 3D-printed PLA holder was used to support the paper chip during experiments. Figure 16a (assembled dual-zone device) and Figure 16b (assembled Y-shaped device) show the integration of the paper chip and AgNP-coated pads within the holder. During testing, the holder kept the chip in a stable, flat position and prevented bending or movement when liquid was introduced. The alignment of the inlet and detection zones was maintained consistently. The rigid structure helped maintain consistent liquid flow through the microfluidic channels. Thus, the 3D-printed holder effectively improved mechanical stability and ease of handling compared to using bare paper chips.

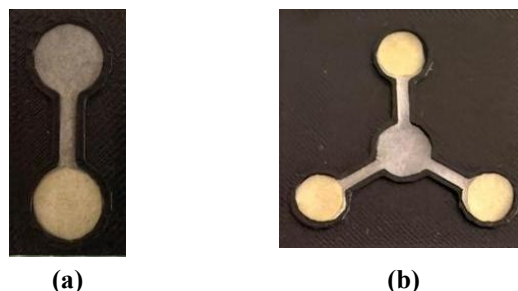


Fig. 16. Assembled paper-based microfluidic devices integrated with the 3D-printed holder. (a) Dual-zone; (b) Y-shaped

3.4 Colorimetric Response to Nitrate

When nitrate solutions were introduced, a visible colour change from yellow to dark brown was observed at the AgNP-coated detection zones. Figure 17 shows the colour response of the dual-zone device at increasing nitrate concentrations, while Figure 18 presents the response of the Y-shaped device at corresponding nitrate concentrations. In both cases, colour intensity increased with nitrate concentration. This change is attributed to nitrate-induced aggregation of AgNPs, which alters the SPR properties.

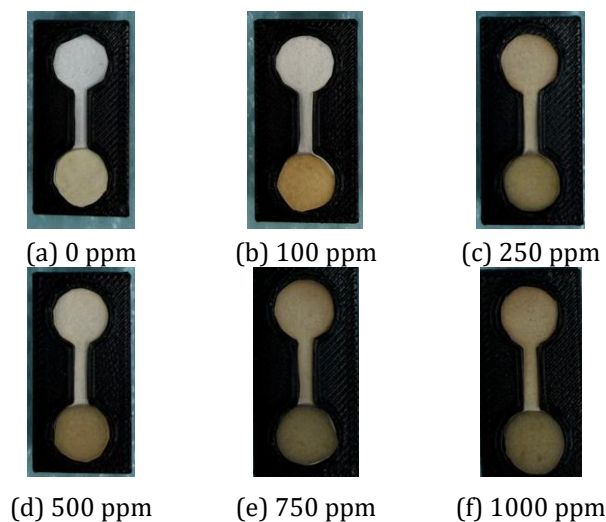


Fig. 17. Colour response of the dual-zone device at corresponding nitrate concentrations

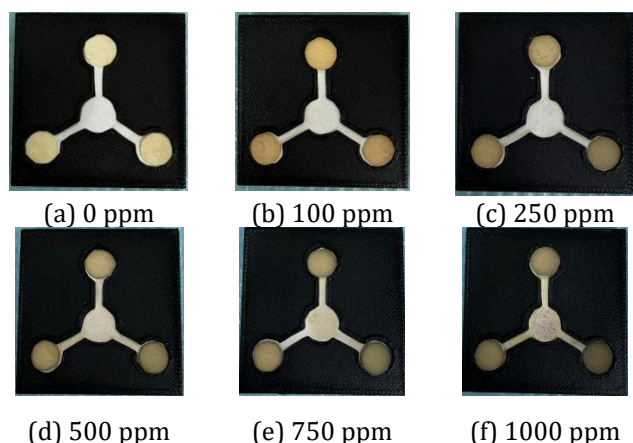


Fig. 18. Colour response of the Y-shaped device at corresponding nitrate concentrations

3.5 LED-Photodetector Measurements and Calibration Curves

The LED-photodetector system quantified the colour intensity as output voltage. As the detection zone became darker with higher nitrate concentration, less light was reflected to the photodiode, resulting in a lower voltage.

3.5.1 Dual-Zone Device

For the dual-zone paper-based microfluidic device, voltage measurements were repeated three times for each nitrate concentration. Table 3 lists the recorded voltage values, and the corresponding curve is shown in Figure 19.

Table 3. Voltage readings of the dual-zone paper-based microfluidic device at different nitrate concentrations

Concentration (ppm)	Trial 1 (mV)	Trial 2 (mV)	Trial 3 (mV)	Average Voltage (mV)
0	28.10	27.90	28.40	28.13
100	26.50	26.20	26.40	26.37
250	26.30	26.00	26.60	26.30
500	21.60	21.40	21.90	21.63
750	20.90	20.60	21.10	20.87
1000	20.30	20.10	20.50	20.30

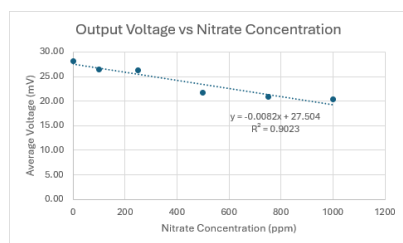


Fig. 19. Calibration curve of the dual-zone paper-based microfluidic device

As presented in Table 3, the average voltage decreased from 28.13 mV at 0 ppm (blank) to 20.30 mV at 1000 ppm. The calibration curve (Figure 19) follows a linear relationship with R² value of 0.9023. The limit of detection (LOD) was calculated using Equation (1), where σ is the standard deviation of the blank (0 ppm) measurements and m is the slope of the

calibration curve. For the dual-zone device, $\sigma = 0.252$ mV and $m = 0.0082$ mV/ppm, giving an LOD of 92.20 ppm.

$$LOD = \frac{3\sigma}{m} \quad (1)$$

3.5.2 Y-Shaped Device

For the Y-shaped device, voltage readings were recorded from three detection zones for each nitrate concentration, and the average value was calculated. Table 4 summarizes the voltage readings, and Figure 20 yields an R² value of 0.8456. Using Equation (1) with $\sigma = 0.346$ mV and $m = 0.0156$ mV/ppm, the LOD is calculated as 66.54 ppm.

Compared to the dual-zone design, the Y-shaped device exhibits higher sensitivity (steeper slope) and a lower detection limit. This improvement is likely due to more uniform liquid distribution, averaging of three readings to reduce random error and the slightly larger inlet that facilitates smoother capillary flow.

Table 4. Voltage readings of the Y-shaped paper-based microfluidic device at different nitrate concentrations

Concentration (ppm)	Detection Zone 1 (mV)	Detection Zone 2 (mV)	Detection Zone 3 (mV)	Average Voltage (mV)
0	38.70	38.10	38.70	38.50
100	30.80	30.50	31.90	31.07
250	28.20	28.00	28.90	28.37
500	26.40	26.80	26.50	26.57
750	19.70	21.30	21.60	20.87
1000	21.30	21.40	21.00	21.23

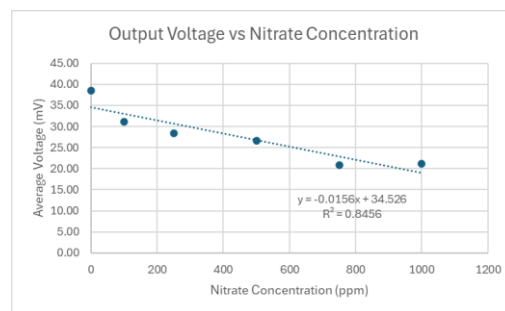


Fig. 20. Calibration curve of the Y-shaped paper-based microfluidic device

3.6 Discussion

Both the dual-zone and Y-shaped paper-based microfluidic designs exhibited a linear relationship between nitrate concentration and output voltage, confirming that the LED-photodetector system can quantitatively detect nitrate via colour intensity changes. As the nitrate concentration increased, the AgNP-coated detection zone became darker, reducing the reflected light reaching the photodiode and thereby producing a lower voltage output. This behaviour is consistent with the principle that interaction between nitrate ions and AgNPs alters the surface plasmon resonance of the nanoparticles, leading to a visible colour change.

The Y-shaped design outperformed the dual-zone design, achieving a lower limit of detection (LOD) of 66.54 ppm

compared to 92.20 ppm for the dual-zone device. The higher sensitivity of the Y-shaped device indicated by its steeper calibration slope (0.0156 mV/ppm vs. 0.0082 mV/ppm) can be attributed to several factors. First, the Y-shaped configuration distributes the sample from a single inlet to three detection zones, allowing three voltage readings to be averaged for every test which reduced random error. Second, its slightly larger inlet (10 mm diameter) facilitates more uniform liquid distribution into the three channels. Third, the multi-zone design promotes more consistent capillary flow.

At very high nitrate concentrations (750-1000 ppm), the change in output voltage became smaller, suggesting a saturation effect. This is likely due to limited active sites on the AgNP-coated paper. The sensor has a finite working range which should be considered for future applications.

Manual cutting of the paper chips introduced minor dimensional variability, which could affect flow consistency. However, the Y-shaped device's multiple detection zones helped compensate for this by averaging readings. Although the reaction required one day for complete colour development, limit the real-time use, the system still successfully demonstrated a low-cost, portable approach for nitrate quantification.

4. CONCLUSION

This project successfully developed a 3D-printed paper-based microfluidic colorimetric sensor for nitrate detection using silver nanoparticles. The synthesis of AgNPs via chemical reduction was confirmed by visual colour change and the Tyndall effect. Characterization using XRD, UV-Vis spectroscopy and FESEM verified that the synthesized AgNPs are crystalline, exhibited a near-spherical morphology and showed a strong SPR absorption peak at 400 nm. Both the dual-zone and Y-shaped paper microfluidic designs were fabricated from Whatman No.1 filter paper and successfully transported nitrate solutions via capillary action without external pumps. The 3D-printed PLA holder provided mechanical stability and proper alignment of the paper chips. The LED-photodetector system effectively quantified nitrate concentration by converting colour intensity changes into voltage outputs. The Y-shaped design showed better sensitivity and a lower detection limit (66.54 ppm) compared to the dual-zone design (92.20 ppm). All three project objectives were achieved. The developed sensor demonstrates good potential as a portable, low-cost platform for environmental nitrate monitoring.

5. FUTURE WORK

Several improvements can be made in future studies. First, the synthesis parameters of AgNPs (reagent concentration, temperature) can be optimized to improve sensitivity. Second, smartphone-based image analysis could be integrated for easier colour quantification. Third, the sensor should be tested with real water samples (river water, agricultural wastewater) to evaluate performance under field conditions. Fourth, using potassium nitrate (KNO₃) or sodium nitrate (NaNO₃) instead of silver nitrate would provide better standardization. Finally, a signal amplifier could be added to increase the voltage output range.

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