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Development of a Rapid Paper Test Kit Method Based on Silver Nanoparticles for Early Detection of Stroke Disease

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Abstract

Stroke is one of the leading causes of death worldwide, there's a need for rapid and affordable diagnostic tools. This study developed a paper test kit based on a colorimetric sensor utilizing silver nanoparticles (AgNp) synthesized via sodium borohydride ($NaBH_4$) reduction for cortisol detection as a biomarker related to stroke disease. The synthesized AgNp showed a distinct surface plasmon resonance peak at around 402 nm by UV-Vis spectrophotometry, confirming successful nanoparticle formation. Particle size analyzer (PSA) revealed uniform nanoscale distribution, with an average particle size of approximately 25 – 40 nm. The immobilization techniques of layer by layer (LBL) and immersion were compared for embedding AgNp onto the paper substrate. The LBL technique is more suitable for analytical precision and reproducibility, while the immersion technique is advantageous for rapid, large-area production of paper-based colorimetric sensors. The developed paper taper testing kit exhibited a gradual color change from yellow to brown with increasing cortisol concentration. The quantitative value as a linear correlation and sensitivity was 0.09984 and 0.069 μM/mL, respectively. The RGB value of the developed paper test kit is (210, 180, 140). The results highlight the potential of the AgNp-based paper sensor as rapid and portable analytical platform for cortisol detection.

Keywords: paper test kit, silver nanoparticles, cortisol, stroke, rapid method

INTRODUCTION

A stroke is a disease caused by the blockage or rupture of blood vessels that supply the brain, resulting in a deficiency of oxygen and nutrients to this organ (Donkor, 2018). This can lead to paralysis and even death in the affected individuals. So, stroke is a major global health concern and one of the leading causes of death worldwide. Globally, stroke is the second leading cause of death and a major contributor to disability. While the age-standardized mortality rate has been decreasing, the total number of stroke-related deaths has increased over the past three decades due to population growth and aging. In 2021, over 7 million people died from stroke worldwide (Feigin et al., 2025). In Indonesia, based on the 2023 Indonesian Health Survey Data, the prevalence of stroke in Indonesia reached 8.3 per 1,000 population, and Indonesia is ranked 7th in terms of stroke deaths in the world (Indonesian Ministry of Health, 2023). Also, stroke is a leading cause of death, with recent national estimates reporting approximately 300,000 annual deaths attributed to stroke (Zainuddin et al., 2025).

The burden of stroke is compounded by common risk factors such as hypertension, diabetes, and obesity, which are prominently prevalent in the Indonesian population and contribute to the high incidence and mortality rates. Early detection and rapid triage are critical because timely reperfusion and acute management dramatically reduce mortality and long-term disability, yet current diagnostic workflows rely heavily on neuroimaging and centralized laboratory assays that are often unavailable during the narrow therapeutic window in prehospital and low-resource settings (Feigin et al., 2025).

Various analytical methods have been developed for stroke detection and monitoring, including imaging-based, biochemical, and electrochemical approaches. Neuroimaging remains the gold standard for diagnostics, with computed tomography (CT) being widely used for the rapid identification of hemorrhagic stroke and magnetic resonance imaging (MRI) offering

superior sensitivity for ischemic stroke (Chaki & Woźniak, 2024). However, these imaging modalities are expensive, require expert personnel, and are often unavailable in rural or pre-hospital environments. In addition, magnetic resonance spectroscopy (MRS) and positron emission tomography (PET) have been explored for metabolic imaging of ischemic tissue, though their clinical use is limited by high cost and technical complexity (Malik et al., 2024).

In recent years, nanomaterial-based sensing platforms (like gold or silver nanoparticles) have emerged as promising alternatives for early stroke detection (Badi'ah, et al., 2023). These platforms demonstrate enhanced sensitivity, portability, and suitability for point-of-care testing (POCT) due to their optical, electrochemical, or plasmonic properties (Shajari, et al., 2023). Paper-based test kits integrated with nanoparticles offer promising alternatives by combining portability, affordability, and sensitivity.

The use of nanoparticles like AgNp exploits their unique optical and catalytic properties to provide a highly sensitive and specific colorimetric detection on a simple, cost-effective paper substrate (R, et al., 2020; Rusnaenah, et al., 2017a). This approach allows for point-of-care diagnostics in resource-limited settings. The novelty of this research lies in the integration of nanotechnology and paper-based platforms and the optimization of silver nanoparticle synthesis and functionalization for enhanced interaction with stroke biomarkers, which significantly improves detection limits and reduces response times compared to conventional assays. This innovative sensor holds significant potential for scalable, accessible stroke screening that can facilitate earlier clinical interventions and improved outcomes for the early detection of stroke biomarkers.

However, some studies have identified a new biomarker that may facilitate early stroke detection and does not require complex laboratory instruments like cortisol. Cortisol, one of the primary glucocorticoid hormones generated by the adrenal cortex, is commonly referred to as the stress hormone. Under normal conditions, cortisol levels are highest in the morning or 30 minutes after waking up and will gradually decrease towards noon and evening. The lowest cortisol levels occur at night before sleep, but the opposite can happen to people who work at night and sleep in the morning. Cortisol levels can also increase in response to physical stressors such as exercise, injury, and physiological stressors such as stress or fatigue (Knezevic, et al., 2023). High cortisol levels were found in stroke and hypertension patients compared to normal controls, wherein hypertension patients, serum cortisol levels reached 804±68 nmol/L and saliva samples reached 59.5±42 nmol/L (Dhull, et al., 2019)

In this work, we present the development of a simple analytical method that can later be used for monitoring stroke biomarkers. The novelty of our approach lies in several aspects, such as paper substrate integration and an AgNp-based colorimetric method. Together, this combination of paper-based platform, AgNp as colorimetric sensing, and cortisol biomarker constitutes a novel contribution to the field of stroke diagnostics. By demonstrating the feasibility of this approach, we aim to bridge the gap between advanced laboratory biosensing and truly deployable point-of-care tools for early stroke detection with a rapid and simple method. The following section describes the fabrication of the test kit, its analytical performance, and its potential role in the stroke care pathway.

METHODOLOGY

Instruments and Materials

The instrument used to test the wavelength and absorbance of AgNp were a UV-Visible Spectrophotometer (Shimadzu-1800). The size and distribution of AgNp produced were measured using a Particle Size Analyzer (PSA) (Malvern 1061025 from Germany).

Silver nitrate ($AgNO_3$) that used in this research from Sigma Aldrich with the CAS number 7761-88-8, sodium borohydride ($NaBH_4$) from Sigma Aldrich (CAS numbers 16940-66-2), and cortisol from Sigma Aldrich (CAS number 50-23-7) were the materials used in this study.

Procedure

Silver nanoparticle (AgNp) synthesis

$AgNO_3$ 1,0 mM was used to synthesize silver nanoparticles (AgNp) as the source of AgNp and 2,0 mM $NaBH_4$ as the reducing agent. $AgNO_3$ 1,0 mM solution was pipetted 10 mL at a time and added dropwise to 30 mL of a solution containing 2 mM $NaBH_4$. The mixing was done in an ice bath, and after that, the mixture was stirred for three minutes using a magnetic stirrer. Following that, the mixture is centrifuged for 15 minutes at 5000 rpm. The mixture was subsequently filtered through filter paper to yield a clear filtrate containing the synthesized silver nanoparticles (Badi'ah, 2021).

Silver nanoparticle characterization

A UV-Vis spectrophotometer set to 400 – 800 nm was used to characterize the maximum wavelength of silver nanoparticles. The PSA is another instrument to measure AgNp and estimate its distribution size.

Colorimetric cortisol sensor based on silver nanoparticles

Cortisol solution 2.0 mL was introduced into a test tube containing the prepared AgNp solution, and the pH was subsequently adjusted to 7 using HCl or NaOH. Then, observe the colour change that occurs.

Immobilization and application of silver nanoparticles as a paper test kit

Immobilization of silver nanoparticles as a paper test kit is performed by adsorption, which involves dropping 3 mL of silver nanoparticles onto Whatman 42 paper, which is then dried at room temperature for 24 hours. The immobilization method was carried out in two ways: the LBL or Layer by Layer technique, which was done by dropping the nanoparticles little by little, and the second technique, which was done by immersion. The sensor membrane is then attached to white PVC/PVDC material with 3M double tape. Thus, a paper test kit is produced and ready for use. Cortisol is dropped onto the silver nanoparticle-based test kit paper that has been produced. Then wait for a while until a colour change occurs.

Quantitative Analysis of Colorimetric Response

The quantitative evaluation of the paper-based sensor was conducted by measuring the change in colour intensity of the AgNp solution upon interaction with the cortisol. The test kit was prepared under identical conditions, and known concentrations of cortisol with 0.5 to 10 μM, were applied onto the paper substrate.

After incubation for 5 minutes, the resulting colour change was captured using a digital camera under controlled lighting conditions. The images were then analyzed using ImageJ software to extract the mean RGB intensity values from each concentration. The difference in red channel intensity (ΔR) was used as the analytical signal, as it exhibited the most significant change correlated with AgNp aggregation.

RESULTS AND DISCUSSION

Silver nanoparticle (AgNp) synthesis

The synthesis of silver nanoparticles (AgNp) was successfully achieved through a chemical reduction method using $AgNO_3$ as the precursor with $NaBH_4$ as the reducing agent. Upon the addition of $NaBH_4$, the reaction mixture immediately changed colour from colourless to pale yellow, indicating the initial formation of silver nuclei. The colour gradually deepened to a bright, clear yellow (Figure 1) within minutes, signifying the growth and stabilization of AgNp in the colloidal solution. This characteristic colour transition is a result of surface plasmon resonance (SPR) excitation, which arises from the collective oscillation of conduction electrons on the nanoparticle surface upon interaction with visible light (Abbas et al., 2024a; Alim-Al-Razy, et al., 2020a; Badi'ah, 2021; Sati, et al., 2025a). The reaction of AgNp formed is illustrated in Equation (1).

$$AgNO_3 + NaBH_4 \rightarrow Ag + 1/2B_2H_6 + NaNO_3 \quad (1)$$

This chemical reduction method is widely used because of its relatively simple and effective approach in producing AgNp.

Figure 1. Visual appearance of silver nanoparticles (AgNp) colloidal synthesized by the $NaBH_4$ reduction method.

The Characterization of Silver Nanoparticles

The synthesis of silver nanoparticles was characterized to confirm their optical properties, particle size distribution, and colloidal stability. UV-Visible spectrophotometry was first employed to monitor the formation and surface plasmon resonance (SPR) behaviour of the nanoparticles. The obtained absorption spectrum displayed a distinct and narrow SPR peak at 402 nm (Figure 2), which is a typical feature of spherical silver nanoparticles with diameters in the range of 10 – 30 nm (Ali et al., 2023; Mekuye, 2023). This absorption arises from the collective oscillation of conduction band electrons in response to incident light, a phenomenon strongly dependent on nanoparticle size, morphology, and dielectric environment (Pereira, et al., 2019).

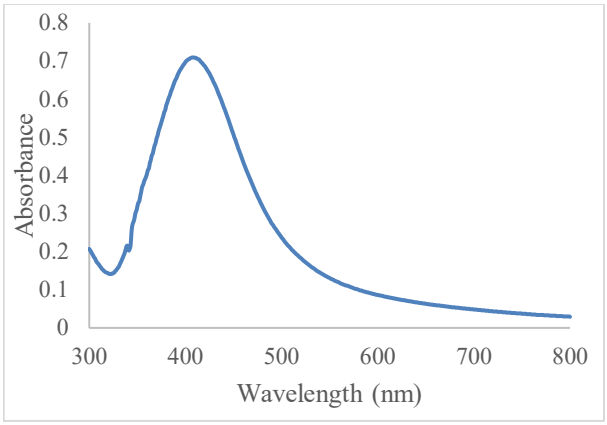


Figure 2. The UV-Vis spectra of silver nanoparticles

The absence of secondary peaks or broad shoulders beyond 500 nm indicated that the synthesized nanoparticles were well-dispersed with minimal aggregation. The sharpness and intensity of the absorption band confirmed that the reduction process using $NaBH_4$ effectively produced uniform nanoparticles with strong plasmonic properties. Similar spectral profiles have been reported for AgNp synthesized via rapid borohydride reduction, where the reaction kinetics favour the formation of small, monodisperse particles (Hastuti & Kim, 2024).

To further evaluate the distribution size of silver nanoparticles, a Particle Size Analyzer (PSA) was conducted using dynamic light scattering (DLS). The PSA results revealed an average particle size of approximately 25 – 40 nm (Figure 3), with a polydispersity index (PDI) of 0.28, indicating a narrow and uniform size distribution. The obtained PD value <0.3 suggests good mono dispersity and confirms that the synthesis conditions, particularly the rapid reduction by $NaBH_4$, were effective in minimizing particle coalescence during nucleation and growth (Danaei et al., 2018).

The combination of UV-Vis and PSA analyses provides complementary evidence for the success of nanoparticle synthesis. The strong and sharp plasmonic peak around 400 nm aligns with the measured particle size from PSA, both supporting the formation of small, stable, and uniformly distributed AgNp. These characteristics are crucial for ensuring reproducibility for optical responses in subsequent colorimetric sensing applications, as particle aggregation or broad size variation could lead to inconsistent spectral behaviour and reduced analytical sensitivity (Martínez, et al., 2024)

In summary, the UV-Vis and PSA characterization results confirm that the $NaBH_4$-based reduction method efficiently produces silver nanoparticles with desirable optical and physical properties suitable for use in colorimetric biosensing platforms. The well-defined SPR band and narrow particle size distribution highlight the reproducibility and stability of the synthesis process, forming a reliable foundation for subsequent sensor fabrication and performance evaluation.

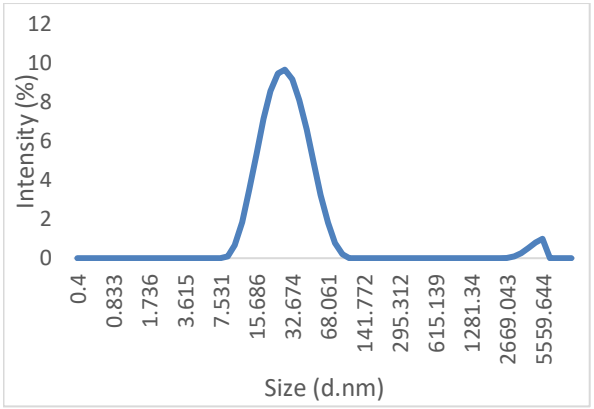


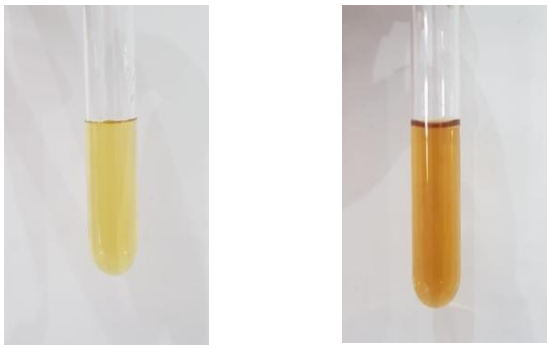
Figure 3. Size distribution of silver nanoparticles

Colorimetric cortisol sensor based on silver nanoparticles

The development of a colorimetric sensor utilizing silver nanoparticles demonstrated a rapid and sensitive response toward cortisol detection. Upon the addition of cortisol to the AgNp colloidal solution, a visible colour transition from yellow to brownish was observed (Figure 4), indicating aggregation of silver nanoparticles. This colour change is attributed to the interaction between cortisol molecules and the surface of AgNp, leading to alterations in the localized surface plasmon resonance (LSPR) behaviour (Badi'ah et al., 2023; Badi'ah, et al., 2023). The phenomenon was further confirmed through UV-Vis spectrophotometric analysis, which showed a significant decrease in the characteristic AgNp absorbance peak at approximately 400 nm, accompanied by a slight redshift to around 500 nm at higher cortisol concentration.

The cortisol molecules interact with the nanoparticle surface via hydroxyl and carbonyl functional groups, which can form hydrogen bonds or weak coordination with the silver surface, causing partial aggregation of nanoparticles (Badi'ah et al., 2023; Shama et al., 2023). These interactions disrupt the surface charge balance and reduce the electrostatic repulsion between particles, resulting in visible colour changes that can be exploited for quantitative analysis. Such LSPR-based aggregation mechanisms have been widely reported for other biomolecules, confirming the

potential of AgNp as a sensitive optical transducer in biosensing applications (Heredia-Cancino, et al., 2021; Ozaki, et al., 2021).



A B

Figure 4. The visual color of (A) Silver nanoparticles colloidal and (B) silver nanoparticles after reaction with cortisol.

The results demonstrate that the AgNp synthesis by $NaBH_4$ is highly suitable for cortisol sensing due to their small particle size and stable colloidal nature, which enhances surface reactivity and optical sensitivity. The visible and measurable spectral changes upon cortisol interaction confirm the feasibility of the AgNp-based colorimetric sensor as a simple, rapid, and effective diagnostic platform for clinical and field applications. These findings align with prior studies using noble metal nanoparticles for hormone and biomarker detection, validating the effectiveness of plasmonic nanomaterials in point-of-care diagnostics.

Immobilization and application of silver nanoparticles as a paper test kit

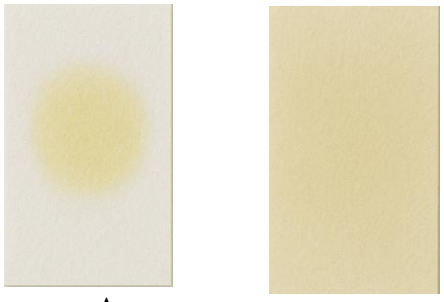
In this study, the immobilization of silver nanoparticles onto cellulose-based paper substrates was carried out using two distinct approaches, like layer by layer (LBL) deposition method and the immersion technique. Both methods aimed to optimize nanoparticle attachment, uniformity, and stability on the paper matrix to develop a reliable colorimetric paper test kit for cortisol detection.

In the LBL technique, small aliquots of the synthesized AgNp colloid were dropped sequentially onto the filter paper surface and allowed to dry between each application layer. This process enhanced nanoparticle density and ensured better surface coverage, resulting in a homogeneous yellow colour characteristic of AgNp. The repeated deposition cycles promoted stronger physical adsorption and possible hydrogen bonding between AgNp and the hydroxyl groups of cellulose fibers, thereby improving immobilization stability (Nawae, et al., 2018)

In contrast, the immersion method involved dipping the paper substrate directly into the AgNp colloidal suspension for a defined period, followed by air drying. During immersion, nanoparticles were physically adsorbed onto and within the porous cellulose structure through capillary-driven diffusion. This technique resulted in deeper penetration in nanoparticles into the paper matrix, producing a slightly darker hue than the LBL method. However, in some cases, the immersion method led to minor aggregation due to prolonged exposure and local nanoparticle accumulation (Ahmed, et al., 2016)

A comparative analysis between the two methods (Figure 5) revealed that the LBL technique provides better surface uniformity and colour consistency, while the immersion method offered higher nanoparticle loading capacity. The LBL approach minimized aggregation and yielded more reproducible optical responses, making it suitable for quantitative applications such as cortisol detection, where consistent colorimetric readouts are critical. Meanwhile, the immersion approach may be advantageous for large-scale fabrication due to its simplicity and faster processing.

Overall, these results indicate that both the LBL and immersion techniques are viable for immobilizing AgNp onto paper, but each offers distinct advantages depending on the intended application. The LBL technique is more suitable for analytical precision and reproducibility, while the immersion technique is advantageous for rapid, large-area production of paper-based colorimetric sensors. The successful immobilization of AgNp onto paper substrates establishes a solid foundation for developing low-cost, portable, and easy to use point of care diagnostic kits for cortisol and other clinically relevant biomarkers.



A B

Figure 5. Immobilization of silver nanoparticles paper test kit using the layer-by-layer (LBL) technique (A) and the immersion technique (B)

The working principle of silver nanoparticles as a test kit paper for cortisol detection is that the paper test kit, which has been immobilized with silver nanoparticles exposure a colour change when interacting with cortisol. The colour change that occurs on the test paper kit is from yellow to brown. The application of AgNp as a paper test kit for cortisol detection has been successfully carried out, as shown in Figure 6 using the LBL or layer-by-layer technique and Figure 7 using the immersion technique. The colour change on the test kit paper occurs in about 5 minutes.

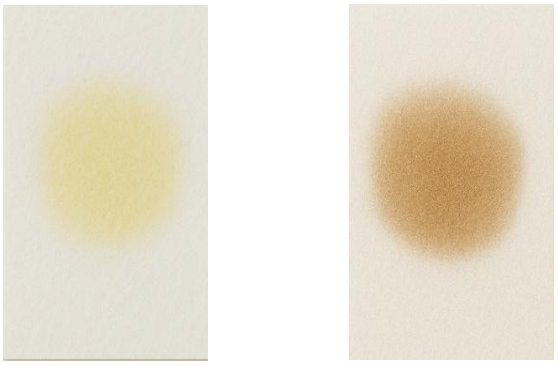


Figure 6. Paper test kit with layer-by-layer technique before adding cortisol (A) and after adding cortisol sample (B)

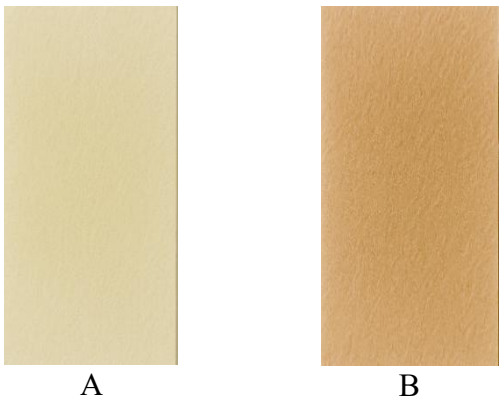


Figure 7. Paper test kit with immersion technique before adding cortisol (A) and after adding cortisol sample (B)

Quantitative Analysis of Colorimetric Response

The quantitative evaluation of the colorimetric response was conducted to assess the sensitivity and reliability of the AgNp-based paper test kit for cortisol detection. Upon exposure to cortisol, a distinct colour transition was observed from light brown to dark brown, correlated to AgNp aggregation induced by the analyte nanoparticle interaction. The colour intensity was quantitatively analyzed using UV-Vis spectrophotometry. As cortisol concentration increased, a gradual red-shift and broadening of the SPR peak were recorded, indicating nanoparticle aggregation and changes in interparticle distance (Kumar, et al., 2025)

The linearity of AgNPs for cortisol detection was assessed by observing their optical responses through a variation of cortisol from 0.5 – 10 µM, showing a strong linear correlation (R^2 = 0.9984) as shown in Figure 8. This result demonstrates that the colorimetric signal can be used for the precise quantification of analyte concentration within the physiological range of cortisol levels. The assay's sensitivity was indicated by the calibration curve's slope, demonstrating that the colorimetric response escalated proportionally with cortisol concentration as a result of increasing interparticle crosslinking among the AgNp. The curve calibration (Figure 8) indicated that the absorbance ratio varied dramatically with increasing cortisol concentration, with a sensitivity value of 0.069 µM/mL, confirming the probe's strong responsiveness to changes in cortisol concentration. Additionally, digital image processing using RGB analysis was performed on the paper test kit to support quantitative evaluation. The RGB value of this paper test kit is (210, 180, 140).

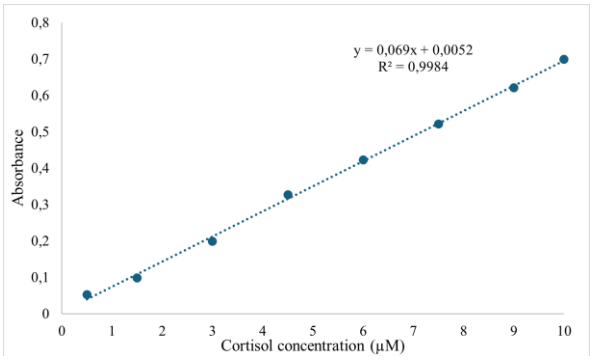


Figure 8. The linear calibration curves the relationship between absorbance and cortisol variation concentration

Overall, the quantitative analysis confirmed that the developed AgNp paper sensor provides a rapid, sensitive, and visually interpretable platform for cortisol detection. The results validate the strong plasmonic response of silver nanoparticles as a reliable

transduction mechanism for colorimetric biosensing and have potential for early screening of stroke biomarkers.

CONCLUSION

In this research, have done successfully developed of silver nanoparticles (AgNp) for the development of a paper test kit aimed at cortisol detection for early stroke assessment. The immobilization technique of silver nanoparticles onto a paper test kit was carried out using two distinct approaches, like like layer by layer (LBL) deposition method and the immersion technique. The LBL technique is more suitable for analytical precision and reproducibility, while the immersion technique is advantageous for rapid, large-area production of paper-based colorimetric sensors. The quantitative value as a linear correlation and sensitivity was 0.09984 and 0.069 µM/mL, respectively, and the RGB values are 210, 180, and 140.

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