# Paper Analytical Devices Immobilized Organic Nanocrystal Combined Silver Nanoparticles for Simple and Rapid Heavy Metal Ion Detection

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**Abstract.** Important parameters in assessing the quality of clean water include the concentration of dissolved heavy metals, such as lead (Pb), which can negatively affect both aquatic organisms and humans when present above the threshold level. Conventional methods such as AAS, ICP-MS, and ICP-OES are widely used for Pb detection in water but require complex sample preparation and lengthy analysis times. Paper-based Analytical Devices (PADs) are increasingly employed in combination with nanoparticles (NPs) to enhance detection sensitivity. In this study, silver nanoparticles (AgNPs) were synthesized using red dragon fruit peel extract as the reducing and capping agent, while dithizone nanoparticles (DTZ-NPs) were prepared via the reprecipitation method and subsequently immobilized onto PADs. The synthesized NPs were characterized using PSA, revealing average sizes of 289.2 nm for AgNPs and 371 nm for DTZ-NPs. Optimization of the PADs was carried out by varying the nanoparticle ratio and the volume of nanoparticles immobilized, with the optimum condition indicated by the greatest change in color intensity (ΔE). The best performance was obtained at an AgNPs:DTZ-NPs ratio of 1:2 with a nanoparticle volume of 3 mL immobilized on the PADs. Under these conditions, the fabricated PADs successfully responded to Pb²⁺ ions at ppm levels.

# INTRODUCTION

Lead (Pb2+) is one of the most hazardous heavy metals, capable of accumulating in the environment and within living organisms [1]. Due to its extensive use, Pb2+ contamination has been detected in soil, water, plants, and food. Anthropogenic activities such as fossil fuel combustion and mining further contribute to the release of Pb2+-containing waste into the environment. According to the World Health Organization (WHO), exposure to Pb2+ can cause severe health effects, including anemia, cardiovascular and renal disorders, cognitive impairment, seizures, coma, and even death. Therefore, regular monitoring of Pb2+ levels is of critical importance.

Several methods are available for detecting Pb2+ levels, including Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma Mass Spectrometry (ICP-MS), X-ray fluorescence, anodic stripping voltammetry, and spectrophotometry [2]. However, these techniques often have drawbacks, such as high instrumentation costs, laborious sample preparation, the need for trained personnel, and lengthy analysis times [3]. To address these limitations, simple, rapid, and cost-effective methods that enable in situ analysis without compromising detection accuracy are being developed, among which Paper-based Analytical Devices (PADs) represent a promising approach.

Paper-based Analytical Devices (PADs) are a paper-based detection technology capable of manipulating small liquid volumes (10-6 to 10-9 L) [1]. Since their introduction by the Whitesides Group in 2007, research on the development and application of PADs has expanded significantly, driven by their low cost, simplicity, ease of fabrication, and rapid analysis capability. PADs are commonly employed in colorimetric assays, where detection is based on visible color changes. However, their main limitations lie in sensitivity and selectivity. To overcome these challenges, PADs can be integrated with nanoparticles [4].

Nanoparticles are particles with sizes ranging from 1 to 1000 nm, which confer a very large surface area [5]. This property increases the probability of interactions between nanoparticles and analytes, thereby enhancing detection performance. In addition, the optical properties of nanoparticles—which can be tuned by controlling their size, shape, dielectric constant, and inter-particle distance—make them widely applicable in heavy metal analysis, even at highly dilute concentrations. Among metal nanoparticles, gold (AuNPs) and silver nanoparticles (AgNPs) are most extensively reported as sensors, with AgNPs receiving greater attention than AuNPs due to their lower cost [6]. Moreover, silver ions in AgNPs can form relatively strong covalent bonds with other substances and provide a high surface area, further supporting their sensing performance [7].

AgNPs can be synthesized through various methods; however, the chemicals typically employed in these processes are often toxic and produce environmentally hazardous byproducts. Green synthesis offers a sustainable alternative by utilizing microorganisms or plant extracts that act both as reducing and capping agents. Several studies have demonstrated the green synthesis of AgNPs from natural sources, including dragon fruit peel extract, which contains phenolic compounds capable of reducing Ag(I) to Ag(0) [8]. To further improve the selectivity of PADs for Pb2+ detection, AgNPs can be combined with dithizone (1,5-diphenylthiocarbazone), a selective ligand that binds Pb2+. The sulfide groups of dithizone enable covalent interactions with AgNPs, thereby enhancing the stability and selectivity of the sensing system [9].

In this study, a combination of silver nanoparticles (AgNPs) and dithizone nanoparticles (DTZ-NPs) was employed to enhance the sensitivity and selectivity of PADs for Pb2+ detection. AgNPs were synthesized via a green synthesis route using red dragon fruit *(Hylocereus costaricensis)* peel extract as both the reducing and capping agent, and subsequently combined with DTZ-NPs prepared through the reprecipitation method. Unlike previous PAD-nanoparticle systems that typically employed a single nanoparticle type, our work integrates biogenic AgNPs with dithizone nanocrystals to enhance both sensitivity and selectivity toward Pb2+ ions.

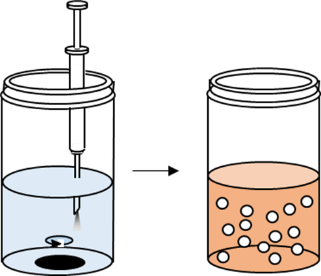
# MATERIALS AND METHODS

This research was carried out in a laboratory setting over approximately four months at the Laboratory of Instrumentation and Analytical Science, Department of Chemistry, Institut Teknologi Sepuluh Nopember. The equipment employed included laboratory glassware, micropipettes, an analytical balance, Whatman qualitative filter paper No. 93, a glass vacuum suction filtration kit with a 250 mL Büchner funnel, a hot plate, a magnetic stirrer, a UV–Vis spectrophotometer (Genesys 10S UV–Vis), a particle size analyzer (Malvern Panalytical–Zetasizer Pro), and a Puluz portable photo studio lightbox. The materials used were dragon fruit peel waste, distilled water, acetone, AgNO3, TAPS buffer, dithizone (1,5-diphenylthiocarbazone), NaOH, and Pb(NO3)2.

The preparation of PADs began with cutting Whatman No. 93 filter paper, followed by printing 2.5 mm-diameter circles using a Fuji Xerox DocuCentre-V C3375 laser printer with Fuji Xerox toner. The printed papers were then oven-dried at 165 °C for one hour. The results of PAD fabrication are shown in **FIGURE 2(a)**.

For the synthesis of AgNPs, dragon fruit peel extract was prepared by blending 20 g of peel with 220 mL of distilled water and heating the mixture to 100 °C for 15 minutes. The extract was cooled and filtered to obtain the filtrate. A total of 220 mL of the filtrate was then mixed with 110 mL of 10 mM AgNO3 solution and stirred at room temperature for 24 hours using a magnetic stirrer. The synthesized AgNPs were stored in a refrigerator for further use.

DTZ-NPs were synthesized using the reprecipitation method, as illustrated in **FIGURE 1** [10]. A 10 mM dithizone solution was first prepared in acetone. Subsequently, 100 µL of this solution was injected into 10 mL of 0.1 M TAPS buffer (pH 7) at room temperature using a syringe while stirring vigorously. After injection, the stirring was stopped, and the resulting DTZ-NPs were allowed to stand at room temperature.



**FIGURE 1.** Schematic of the Reprecipitation Method

The AgNPs and DTZ-NPs obtained from the synthesis stages were characterized using a UV-Vis spectrophotometer (Genesys 10S UV-Vis) to confirm the surface plasmon resonance (SPR) absorption band and to estimate the nanoparticle bandgap width. The particle size distribution and zeta potential were determined using a Particle Size Analyzer (Malvern Panalytical Zetasizer Pro). These characterizations were performed at room temperature using aqueous dispersions of the nanoparticles.

For nanoparticle immobilization, AgNPs and DTZ-NPs were mixed in a 5:1 ratio in a vial, and the pH was adjusted to 7. The Ag-DTZ NPs suspension was then immobilized on PADs by placing the PADs in a glass filtration funnel connected to a vacuum suction bottle. The suspension was applied dropwise using a micropipette and allowed to stand for 30 minutes in a petri dish. The immobilization procedure is illustrated in **FIGURE 2(b)**. For Pb2+ detection, a Pb(NO3)2 solution was immobilized on the PADs already loaded with Ag-DTZ NPs, as shown in **FIGURE 2(c)**.

Images of the PADs before and after Pb2+ application were captured using a smartphone camera (Vivo Y02t, 8 MP) placed at a fixed distance of 12 cm in a Puluz portable photo lightbox under standardized LED illumination to minimize lighting variation. The change in color intensity (ΔE) was analyzed using ImageJ software by extracting and comparing the L, a\*, and b\* values from the images.

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**FIGURE 2.** (a) Example of PADs, (b) Nanoparticle Immobilization, and (c) Sample Immobilization [11]

# RESULT AND DISCUSSION

Those are Silver Nanoparticles (AgNPs) Synthesis, Dithizone Nanoparticles (DTZ-NPs) Synthesis, UV-Vis Spectrophotometry Measurements, Particle Size Analyzer (PSA) and Zeta Potential Measurements, Optimization of PADs Condition, and Detection Test for Pb2+ Ions. It should be noted that the analytical performance parameters such as limit of detection (LOD), sensitivity, and reproducibility were not evaluated in the present work. This study was mainly aimed at demonstrating the feasibility of the proposed PAD–nanoparticle system. Future investigations will therefore focus on assessing these analytical performance aspects in detail.

# Silver Nanoparticles (AgNPs) Synthesis

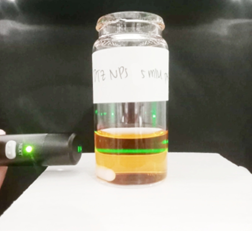
Mixing the dragon fruit peel extract with a 10 mM AgNO3 solution produced a pale brown, transparent dispersion, indicating the formation of AgNPs. The presence of AgNPs was further confirmed by the Tyndall effect test, which detects colloidal particles. As shown in **FIGURE 3(a)** and **3(b)**, a bright green laser beam was observed passing through the dispersion, confirming the existence of colloidal particles. In contrast, no beam was observed in the AgNO3 solution **(FIGURE 3(c))**, indicating a true solution with particle sizes below 1 nm that are unable to scatter light. Meanwhile, colloidal particles typically range from 1 to 1000 nm and are capable of scattering light, which explains the observed Tyndall effect in the AgNPs dispersion.

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**FIGURE 3.** (a) AgNPs Dispersion, (b) Dragon Fruit Peel Extract, and (c) AgNO3 Solution (c)

# Dithizone Nanoparticles (DTZ-NPs) Synthesis

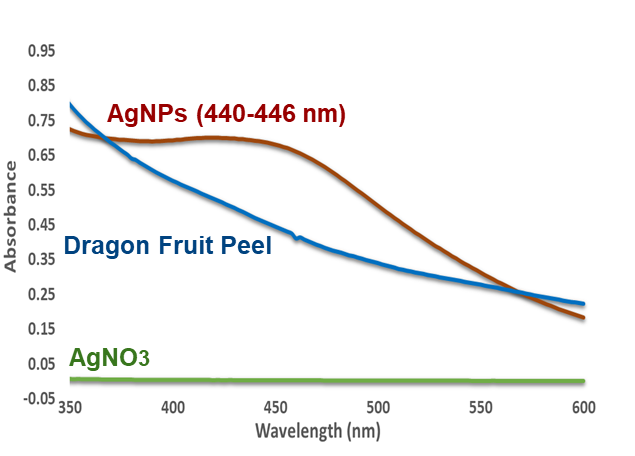
The synthesis of dithizone nanoparticles (DTZ-NPs) was performed by preparing a 10 mM dithizone solution in acetone, followed by injecting 100 µL of this solution into 10 mL of 0.1 M TAPS buffer (pH 7) under high-speed stirring at room temperature. The resulting dispersion appeared pale orange, as shown in **FIGURE 4**. The presence of colloidal particles was further confirmed by the Tyndall effect test, in which a bright green laser beam was observed passing through the dispersion, indicating the successful formation of DTZ-NPs.



**FIGURE 4.** DTZ-NPs Dispersion

# UV-Vis Spectrophotometry Measurements

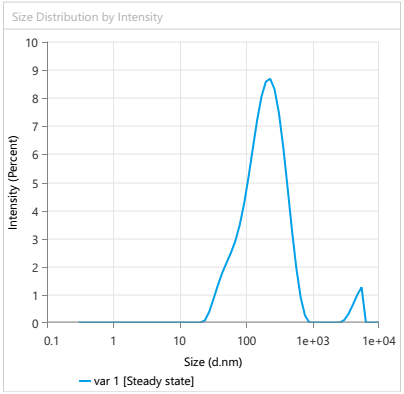
The AgNPs dispersion was characterized using UV-Vis spectrophotometry. As shown in **FIGURE 5**, the AgNPs exhibited a distinct absorption peak in the range of 420–450 nm. The appearance of this peak within the characteristic surface plasmon resonance (SPR) region of AgNPs (400–500 nm) confirms the formation of silver nanoparticles [12]. In contrast, no absorption peaks were observed in the same region for either the AgNO3 solution or the dragon fruit peel extract, indicating that nanoparticle formation did not occur in these solutions. These findings demonstrate the successful green synthesis of AgNPs using dragon fruit peel extract.



**FIGURE 5.** UV-Vis Spectrophotometer Measurement Results

# Particle Size Analyzer (PSA) and Zeta Potential Measurement

In this study, characterization was also performed using a Particle Size Analyzer (PSA) for AgNPs and DTZ-NPs to determine the size of the synthesized particles. The PSA results, as shown in **FIGURE (6)**, revealed an average particle size distribution for silver nanoparticles (AgNPs) of 150 nm. The main peak with the highest intensity around 150 nm indicates that the most dominant particle size in the sample is approximately 150 nm. The secondary peak around 1000 nm suggests the presence of larger particles, albeit in smaller quantities.



**FIGURE 6.** The Result of PSA Measurement of AgNPs

The synthesized dithizone nanoparticles (DTZ-NPs) were further characterized using Particle Size Analysis (PSA) and Zeta Potential measurements. As shown in **FIGURE 7(a)**, the PSA results revealed an average particle size distribution of 87.99 nm, confirming that the DTZ-NPs fall within the nanoscale range. In addition, the Zeta Potential measurement **(FIGURE 7(b))** yielded a value of –33.36 mV, which indicates good colloidal stability. The relatively high negative charge suggests strong electrostatic repulsion between particles, thereby preventing aggregation and ensuring the dispersion stability of DTZ-NPs.

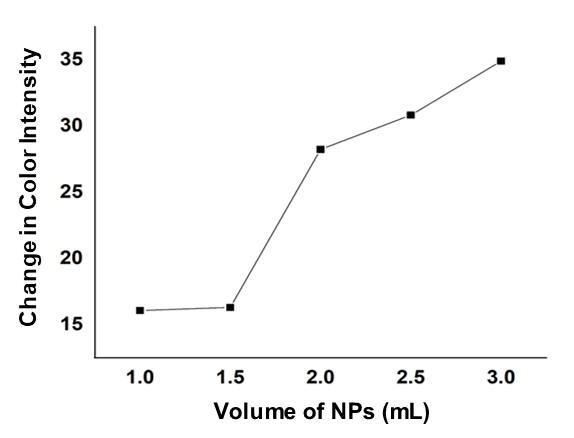
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**FIGURE 7.** (a) The Result of PSA Measurement and (b) Result of Zeta Potential Measurement of

AgNPs.

# Optimization of PADs Condition

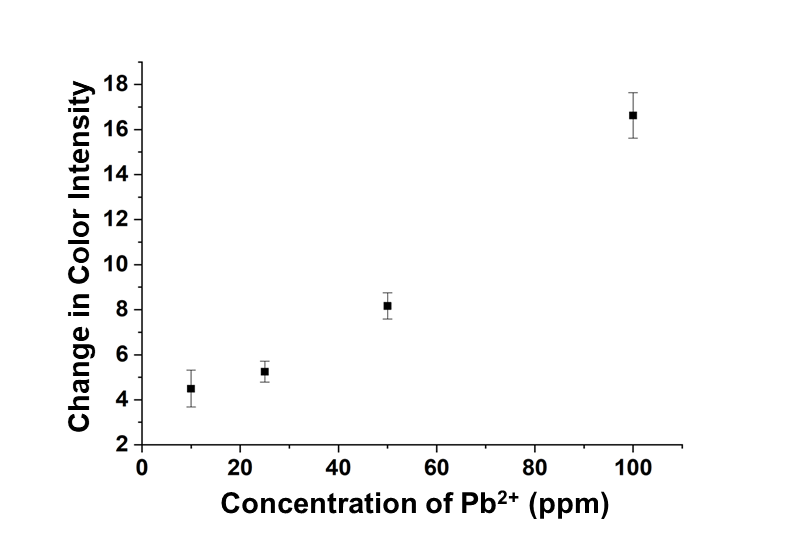
The optimization of PADs was carried out to determine the most effective conditions for producing measurable changes in color intensity (ΔE). In this study, AgNPs and DTZ-NPs were combined at a ratio of 1:2, and different total volumes (1–3 mL) were tested. To evaluate the optimum condition, PADs were exposed to Pb2+ ions at a concentration of 1000 ppm with a total volume of 3 mL. As shown in **FIGURE 8**, the greatest ΔE value was obtained when 3 mL of Ag–DTZ NPs were immobilized on the PADs. Therefore, this condition was selected for subsequent experiments to investigate the optical response of PADs toward Pb2+ at ppm concentration levels.



**FIGURE 8.** Graph of Variation in Ag-DTZ NPs Volume versus Change in Color Intensity (ΔE)

# Detection Test for Pb2+ Ions

The PADs under optimized conditions were subsequently employed to evaluate their optical response toward varying concentrations of Pb2+ at the ppm level. This screening was intended to observe the performance of the PADs under relatively high Pb2+ concentrations. In these experiments, a 1:2 ratio of AgNPs to DTZ-NPs was used, with a total immobilized nanoparticle volume of 3 mL and the addition of 2 mL Pb2+ solution. As shown in **FIGURE 9**, the PADs exhibited a distinct colorimetric response, where higher Pb2+ concentrations resulted in greater changes in color intensity (ΔE).



**FIGURE 9.** Graph of Variation in Pb2+ concentration versus Change in Color Intensity (ΔE)

# CONCLUSIONS

In this study, Paper-based Analytical Devices (PADs) integrated with dual nanoparticles were successfully developed for the detection of Pb²⁺ ions, as demonstrated by distinct changes in color intensity at ppm concentration levels.

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