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Surface Modification of Paper-Based Analytical Devices Using Polymer Inclusion Films as Optical Sensors for The Detection of Cu(II) Ions in Water

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ABSTRACT

This study presents the development of a microfluidic paper-based analytical device (μPAD) modified with a polymer inclusion film (PIF) for the detection of Cu(II) ions in aqueous samples. The PIF formulation comprised of polyvinyl chloride (PVC), bis(2-ethylhexyl)phosphoric acid (D2EHPA), and Aliquat-336, while sodium zincon salt served as the colorimetric reagent. The optimization was conducted by systematically varying several key parameters such as PIF composition and volume, reaction time, sample volume, and sample pH. The resulting color intensity was digitally quantified using smartphone, and the results were validated against UV–Vis spectrophotometry as the reference method. The optimized conditions were established at a composition of 50% PVC, 30% D2EHPA, 20% Aliquat-336 and 0.1% zincon, with a PIF volume of 20 μL, a reaction time of 40 minutes, a sample volume of 30 μL, and an optimal pH of 5. Under these conditions, the μPAD demonstrated excellent analytical performance, exhibiting strong linearity (R^2 = 0.9993), high precision (0.36%), good accuracy (0.368%), recovery rates between 98.18% and 102.44%, a limit of detection (LOD) of 0.143 mg/L, and a limit of quantification (LOQ) of 0.476 mg/L. Furthermore, selectivity assessments indicated that D2EHPA effectively reduced interference from Zn(II) ions, confirming the robustness of the developed sensing platform.

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INTRODUCTION

The significant increase of environmental pollution caused by both industrialization and domestic activities has appeared as a pressing global issue, largely due to its detrimental impact on the quality and availability of clean and safe water resources. Rapid expansion of industries, coupled with inadequate waste management practices, has resulted in the continuous discharge of heavy metals, organic pollutants, and other hazardous substances into aquatic environments. This condition not only disrupts the ecosystem balance but also presents serious health risks to human populations, relying on these water sources.

In Indonesian, the challenge of ensuring access to potable water remains substantial. According to the 2020 National Socio-Economic Survey (Susenas), approximately 58% of Indonesian households have not yet met the national standards for clean water access. Alarmingly, about 6.5% of these households continue to rely on recycled or reused water for daily activities, underscoring persistent disparities in water quality and infrastructure across regions [1]. These figures highlight the urgent need for sustainable monitoring and remediation strategies to safeguard water quality and support public health, particularly in areas experiencing rapid urbanization and industrial expansion.

Copper (Cu) is an essential trace element necessary for maintaining human physiological functions, particularly in facilitating red blood cell synthesis and supporting the proper functioning of the nervous and immune systems. However, when present in concentrations exceeding permissible limits, copper poses significant environmental and health hazards due to its non-biodegradable nature and tendency to bioaccumulate in aquatic organisms [2]. The progressive buildup of copper within aquatic ecosystems can disrupt ecological stability and, through biomagnification, adversely affect human health by impairing vital organs such as the brain, kidneys, and liver [3]. In recognition of these risks, the Indonesian Ministry of Health, through Regulation No. 2 of 2023, has established a maximum allowable copper concentration of 2 mg/L in wastewater intended for domestic or industrial discharge [4]. Hence, regular monitoring and evaluation of copper concentrations in water are essential to mitigate its adverse impacts.

Various analytical techniques have been widely employed for the quantification of copper ions in aqueous samples, including atomic absorption spectrophotometry (AAS), ultraviolet–visible (UV–Vis) spectrophotometry, inductively coupled plasma–optical emission spectrophotometry (ICP–OES), and classical volumetric analysis. While these methods exhibit excellent analytical performance in terms of sensitivity, precision, and low detection limits, their implementation is often constrained by labor-intensive procedures, the need for highly trained personnel, and the lack of portability for in-field applications [5]. Alternatively, microfluidic paper-based analytical devices (μPADs) have emerged as a promising analytical platform offering simplicity, affordability, and environmental compatibility for direct and on-site detection of heavy metal ions in environmental water. These devices can be seamlessly integrated with portable imaging systems such as digital cameras or smartphones, enabling rapid and on-site analysis. Structurally, μPADs are composed of hydrophilic zones that facilitate fluid transport and hydrophobic barriers that define fluid pathways [6]. Their detection mechanism relies on the immobilization of specific reagents within defined zones, where interaction with the target analyte induces a colorimetric response proportional to analyte concentration. Despite these advantages, several developed μPADs face challenges related to reagent stability, as the colorimetric reagents deposited on the paper substrate may diffuse and leach out from the paper matrix over time, potentially affecting measurement accuracy and reproducibility [7].

This study aims to enhance the stability of colorimetric reagents on μPADs and to minimize their leaching from the paper substrate through surface modification using polymer inclusion films (PIFs). In this approach, PIFs serve as immobilization matrices that entrap and stabilize the embedded reagents, minimizing its potential leaching out from the paper substrate. The PIFs are composite thin film consisting of poly(vinyl chloride) (PVC) as the polymeric backbone, providing mechanical strength and structural integrity [8], and Aliquat 336 as a lipophilizing and interfacial mediator. Aliquat 336, an amphiphilic quaternary ammonium compound, forms ion pairs with the anionic form of Zincon, thereby increasing its compatibility and homogeneous distribution within the PVC phase [9]. Owing to its dual affinity, electrostatic interaction through its positively charged head group with polar molecules and solubility of its long alkyl chains in the hydrophobic matrix, Aliquat 336 effectively promotes dispersion and stabilization of Zincon within the PIF layer. This configuration enhances reagent retention, uniformity, and the overall analytical performance of the μPAD-based sensing platform [10].

Sodium zincon salt is utilized as a colorimetric reagent owing to its high sensitivity toward divalent metal ions such as Cu(II) and Zn(II); however, its selectivity toward Cu(II) remains relatively low due to potential interference from Zn(II). To address this limitation, bis(2-ethylhexyl)phosphoric acid (D2EHPA) is incorporated in the PIF as an effective masking or complexing agent, particularly within the pH range of 3–4, where it exhibits a stronger binding affinity for Zn(II) ions [11]. Through preferential complexation with Zn(II), D2EHPA effectively minimizes interference, thereby allowing zincon salt to selectively interact with Cu(II) and produce a distinct and reliable colorimetric signal. This approach significantly enhances the analytical specificity of the μPAD system for copper ion detection [12].

RESEARCH METHODS

Chemicals, Reagent, and instruments

The reagents and materials utilized in this study comprised copper(II) sulfate pentahydrate ($CuSO_4{\cdot}7H_2O$), acetic acid (CH_3COOH), sodium acetate (CH_3COONa), sodium thiosulfate ($Na_2S_2O_3$), polyvinyl chloride (PVC), bis(2-ethylhexyl)phosphoric acid (D2EHPA), Aliquat-336, sodium zincon salt, tetrahydrofuran (THF), calcium chloride ($CaCl_2$), zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$), potassium sulfate (K_2SO_4), magnesium sulfate ($MgSO_4$), and sodium sulfate (Na_2SO_4), all obtained from Sigma-Aldrich, USA. Additional materials included Whatman No. 1 filter paper (Whatman/GE Healthcare) as the substrate for μPAD fabrication, distilled water (OneMed) for solution preparation and rinsing, acrylic paint for hydrophobic barrier formation (Hepi Iop1), glossy photo paper for device support, and tissue paper for cleaning during the experimental procedures.

The laboratory tools included volumetric flasks, Erlenmeyer flasks, beakers, droppers, micropipettes, test tubes, brush for hydrophobic zone patterning, spatulas, pipette tips, aluminum foil, tweezers, aluminum trays, sealing plastic bags (13 × 20 cm), and a photo box, which were primarily utilized for reagent preparation, device fabrication, and sample handling. The analytical instruments comprised a UV–Vis spectrophotometer, employed as the reference method for absorbance measurements; a Redmi 12C smartphone (50MP main rear camera), used for digital image capture and color intensity analysis; a magnetic stirrer, applied for solution homogenization; and an analytical balance, used for precise weighing of reagents.

Research Procedures

This research was conducted through several sequential stages, including the design and fabrication of the μPAD, optimization of analytical parameters, validation of the analytical method, determination of Cu(II) ion concentrations in water samples, selectivity evaluation, stability testing, and data analysis.

Design and fabrication of μPAD

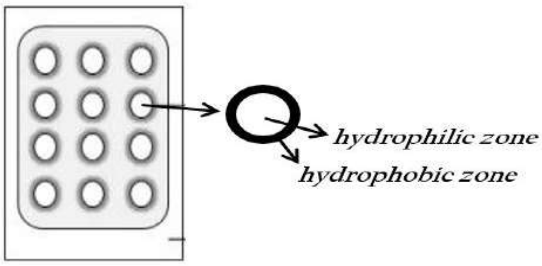

Fig 1. Schematic design of μPAD

The μPAD was fabricated using Whatman No. 1 filter paper measuring 9 × 8 cm, featuring 12 reaction zones, each with an internal diameter of 0.5 cm. Whatman No. 1 filter paper was selected due to its fine pore structure that facilitates slower yet more uniform capillary flow, ensuring consistent liquid distribution and improved analytical reliability. This characteristic offers a significant advantage for paper-based diagnostic sensors by enhancing precision and reproducibility, unlike substrates such as Whatman No. 4 with larger pores that promote faster fluid migration but often produce irregular flow patterns, thereby reducing measurement accuracy [24]. Acrylic paint was used as the hydrophobic material because of its strong reagent retention capability. The hydrophobic zone was created by manually applying the acrylic paint to the filter paper with a brush to define the circular hydrophilic reaction area. A plastic film template was used to maintain uniform shape and size. The coated paper was then ironed from the opposite side to enhance penetration of the acrylic into the cellulose fibers, ensuring a well-defined hydrophobic barrier. Finally, the µPAD was laminated with ID-card-size plastic to minimize evaporation during analysis. Prior to the lamination, a 2 mm hole was punched at the center of each reaction zone for sample and reagent introduction [13]. The schematic design of the µPAD is illustrated in Figure 1.

Optimization and analytical procedures

The analytical procedure for the optimization of the µPAD and measurement of Cu(II) ions using the µPAD modified with PIF was conducted by first depositing a specific volume of PIF solution containing the zincon reagent onto the hydrophilic detection zones. The PIF solution was prepared according to the procedure for fabricating the polymer inclusion membrane described by Nitti et al (2025) [11]. The base polymer PVC, masking agent D2EHPA, extractant Aliquat 336, and chromogenic reactant zincon, with a combined total mass of 320 mg, were dissolved in 5.0 mL of THF under constant agitation. The solution was stirred continuously for approximately 8 hours to ensure complete dissolution of all components, yielding a clear and homogeneous solution, which was subsequently used as the PIF solution deposited on the surface of µPAD. The optimized parameters of PIF components examined in this research are summarized in Table 1. Following the deposition of PIF solution, the µPAD was then air-dried for several minutes to allow uniform film formation before a certain volume of Cu(II) standard solution or water sample containing Cu(II) ions was added to each detection zone. The device was left at room temperature for a pre-optimized duration to ensure complete colour development. Upon reaching the optimal reaction time, the µPAD was placed in a custom-designed imaging box to minimize the influence of ambient light, and the resulting colour change was recorded using a smartphone camera. The RGB values of each detection zone were extracted using the freely available "*Color Picker*" software from the central region of each spot, and the absorbance values were calculated following the Birch and Stickle method (Eq. 1), where A represents the absorbance, I is the mean colour (red or green or blue) intensity for the sample, and I_0 is the mean colour (red or green or blue) intensity of the blank obtained using deionized water.

$$A = \log\frac{I_0}{I} \qquad \text{Eq. 1}$$

Prior to sample analysis, key operational parameters, including volume of PIF, reaction time, sample pH and sample volume, were systematically optimized using a univariate approach, where one variable was varied while others were held constant. The conditions yielding the highest absorbance were selected as the optimum parameters for subsequent Cu(II) detection in real water samples.

Table 1. Optimized parameters of the proposed µPAD

Number	Optimized parameters	Range	Unit
1.	PVC in PIF	40-80	%wt
2.	D2EHPA in PIF	10-30	%wt
3.	Aliquat 336 in PIF	10-30	%wt
4.	Zincon reagent	0.1-0.5	%wt
5.	PIF Volume	10-50	µL
6.	Reaction time	10-50	minutes
7.	Sample volume	10-50	µL
8.	Sample pH	4-9	

Preparation of Calibration Curve for both μPAD and UV-Vis Spectrophotometer

The preparation of the calibration curve was conducted to establish a quantitative relationship between Cu(II) ion concentration and the corresponding absorbance values, serving as the analytical basis for sample quantification. Standard Cu(II) solutions with concentrations ranging from 0.01 to 2.0 mg/L, reflecting the permissible limits for Cu(II) in water, were prepared and subsequently applied to the reaction zones of the μPAD pretreated with the PIF solution. Following the drying process, the resulting color change was digitally analyzed using the *Color Picker* application on a smartphone to obtain the color intensity, which was then converted into absorbance values. The absorbance data for each standard concentration were utilized to construct the calibration curve. For comparative purposes, an additional calibration curve was generated using a UV-Vis spectrophotometer by measuring the absorbance of the Cu(II) standard solutions with the wavelength of 600 nm. The calibration curves derived from both methods were subsequently employed to determine the Cu(II) concentration in unknown water samples through interpolation of their respective absorbance values.

Validation of the proposed μPAD

Validation of the μPAD method was performed based on several analytical parameters, including linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ), detail in Table 2, with the results compared to those obtained using the standard UV-Vis spectrophotometric method [13]. Precision, accuracy, and recovery tests were conducted by analyzing a Cu(II) solution with a concentration of 0.50 mg/L in five replicates using both the μPAD and the reference method. Precision, defined as the reproducibility of measurements among repeated analyses of the same sample, was assessed through the calculation of relative standard deviation (%RSD) using Eq. 2, where a %RSD value ≤ 2% is considered acceptable. Accuracy represents the closeness of the measured value obtained by the developed method to the true analyte concentration and is expressed as percent error (%E), calculated using Eq. 3, where acceptable accuracy falls within a %E range of 4–8% at a 95% confidence level. Recovery (%R) indicates the proportion of analyte successfully recovered from the sample relative to the known concentration, expressed as %R and calculated using Eq. 4, with an acceptable range between 80–110%. The limit of detection (LOD), which represents the lowest analyte concentration that can be reliably distinguished from the blank, was determined using the Eq. 5, whereas the limit of quantification (LOQ), denoting the lowest concentration that can be quantified with acceptable precision and accuracy, was calculated using Eq. 6.

Table 2. Analytical parameters calculation for the proposed μPAD and the reference method

Analytical	Equation	Eq. Number	Description
Precision	$\% \, RSD = \frac{SD}{\bar{x}} \times 100\%$	2	*SD* is the standard deviation of the measurement and $\bar{x}$ is the average concentration of Cu(II) determined using μ-PAD
Accuration	$\% \, \mathrm{E} = \left(\frac{\bar{x}-\mu}{\mu}\right) \times 100\%$	3	$\bar{x}$ is the average concentration of Cu(II) determined using μ-PAD based method, and μ is the true concentration of Cu(II).
Recovery	$\% \, R = \frac{\bar{x}}{\mu} \times 100\,\%$	4	$\bar{x}$ is the average concentration of Cu(II) determined using μ-PAD based method, and μ is the true concentration of Cu(II).
Limit of detection (LoD)	$\mathrm{LOD} = \frac{3\,S_Y}{\mathrm{S}}$	5	S_Y is the standard error of the intercept, and S is the slope of the calibration curve of the proposed method.

Limit of Quantification (LoQ)	$LOQ = \frac{10\, S_Y}{S}$	6	S_Y is the standard error of the intercept, and S is the slope of the calibration curve of the proposed method.

Selectivity Test

The selectivity of the µPAD was evaluated to assess its ability to detect Cu(II) ions in the presence of potentially interfering cations commonly found in natural water systems. The interfering species tested included Na(I), K(I), Ca(II), Mg(II), Zn(II), and Fe(II), all of which frequently coexist with copper in aquatic environments. Each ion was introduced at relevant environmental concentrations, and measurements were performed for Cu(II) at a fixed concentration of 0.2 mg/L using the PIF-modified µPAD. Absorbance readings were recorded both in the absence and presence of these interfering ions to determine their effect on Cu(II) quantification. A deviation greater than ±5% from the standard Cu(II) response was considered indicative of significant interference. This evaluation was essential to confirm the analytical specificity of the µPAD system and to ensure its reliability for Cu(II) detection in complex water matrices.

Stability test and validation of the µPAD

The evaluation of µPADs' stability was carried out to find their prolonged performance under different storage conditions. The devices were stored under two distinct environments: (i) at ambient room temperature and (ii) vacuum-sealed and protected from light. After predetermined storage intervals, absorbance measurements were performed using a 1 mg/L Cu(II) standard solution to assess any degradation in analytical response. Prior to testing, the µPADs were equilibrated at room temperature for 15 minutes to maintain consistent conditions and ensure accurate performance evaluation. Validation of the developed µPAD was performed by comparing the analytical performance of the PIF-modified µPAD with that of a standard UV-Vis spectrophotometric method. Water samples collected from various locations within Kupang City and Regency were analyzed using both techniques to verify the accuracy and reliability of Cu(II) determination. The Cu(II) concentrations were obtained by interpolation from the respective calibration curves, and each measurement was conducted in triplicate to ensure reproducibility and minimize random errors.

RESULTS AND DISCUSSION

µPAD Design and Fabrication

The proposed µPAD functions as a paper-based microfluidic analytical platform composed of hydrophilic (water-absorbing) and hydrophobic (water-repellent) regions. In this work, acrylic paint was utilized to form the hydrophobic barriers owing to its capacity to produce well-defined boundaries, fast drying behavior, and chemical stability toward analytical reagents [8]. The hydrophilic detection zone was designed with a small diameter of 0.5 cm to reduce reagent consumption and enhance analytical efficiency. In the µPAD configuration, the wider hydrophobic boundaries serve to stabilize capillary-driven fluid flow, prevent sample leakage, and preserve the precision of the microchannel structure, ensuring that liquid movement occurs exclusively within the hydrophilic regions and thereby improving the accuracy and reliability of detection. The ironing step was conducted on the reverse side of the paper to promote deeper infiltration of the acrylic paint, while lamination was applied to shield the detection zone from possible environmental contamination and physical degradation. The schematic layout of the µPAD is illustrated in Figure 2.

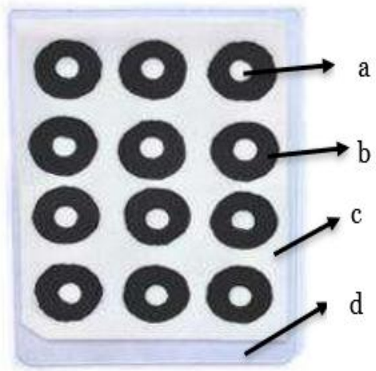

Fig 2. The fabricated µPAD: (a) hydrophilic zone; (b) hydrophobic zone; (c) Whatman No. 1 filter paper; (d) laminating pouch film

Optimum Composition of *Polymer Inclusion Film* (PIF)

Optimization of the PIF composition was conducted to determine the most effective formulation for subsequent analytical optimization, ensuring enhanced reagent stability and retention on the µPAD surface. The PIF was composed of three primary components: polyvinyl chloride (PVC) as the supporting polymer matrix, Aliquat-336 as the lipophilizing and interfacial mediator, D2EHPA as the masking agent, and zincon sodium salt as the colorimetric reagent. All components were dissolved in a polar THF solvent to ensure homogeneity and effective film formation. The optimization results indicated that a PIF composition consisting of 50% PVC, 30% D2EHPA, and 20% Aliquat-336 provided the most stable and responsive film characteristics [17]. After obtaining the optimum PIF composition, further tests were conducted to optimize the zincon concentration, which was varied from 0.1% to 0.5%. However, the results showed that zincon concentrations above 0.1% produced an excessively intense color on the µPAD, potentially affecting the sensitivity of the proposed µPAD. Therefore, the PIF with a composition of 50% PVC, 30% D2EHPA, and 20% Aliquat-336 containing 0.1% zincon was selected for subsequent analysis. The detailed composition ratios and their corresponding analytical performances are summarized in Table 3.

Table 3. The description of PIF composition optimization

PIF Composition	Optimization Color	Result Description
100% PVC		Only a faint light-purple layer was observed, resulting from the passive adsorption of zincon onto the surface. This composition exhibited no visible colour change upon the addition of Cu(II) ions, as the sample failed to remain within the reaction zone. The absence of colour response is attributed to the inert and highly hydrophobic nature of PVC, which restricts the diffusion and interaction of metal ions with the µPAD surface [11].
80% PVC + 10% D2EHPA + 10% Aliquat-336		This composition exhibited a weak response toward Cu(II), as indicated by the appearance of a faint blue spot in the detection zone. The limited colour development is attributed to the high proportion of PVC, which hinders the uniform dispersion of the extractant and promotes phase separation within the film matrix [18].

50% PVC + 20% D2EHPA + 20% Aliquat-336		This formulation produced an insignificant colour change with uneven colour distribution, primarily due to the excessive proportion of PVC, which restricted the proper dispersion of the zincon reagent. Furthermore, phase separation between the polymer matrix and the extractant reduced the overall reactivity and hindered the complexation efficiency with Cu(II) ions [19].
50% PVC + 30% D2EHPA + 20% Aliquat-336		This composition demonstrated the best analytical performance, characterized by an open and uniform film structure with evenly distributed blue coloration. The formulation enabled effective dispersion of the zincon reagent, thereby facilitating optimal interaction and complex formation between Cu(II) ions and the reagent on the µPAD surface.
40% PVC + 30% D2EHPA + 30% Aliquat-336		The irregular colour development was attributed to the low PVC content, which rendered the film brittle and overly elastic, as well as the excessive amounts of D2EHPA and Aliquat-336 that produced an oily surface layer. This condition impeded the interaction between Cu(II) ions and the zincon reagent within the polymer matrix, thereby reducing the efficiency of the colorimetric response.

Complementary Colour Selection

The complementary colour selection was employed to facilitate data processing, with colour selection based on the RGB component exhibiting the highest absorbance value. Previous studies have demonstrated that the red channel provides the strongest absorbance response for the zincon–Cu(II) colorimetric complex compared to the blue and green components [20]. Consequently, subsequent measurements were conducted using the red absorbance value, as illustrated in Figure 3.

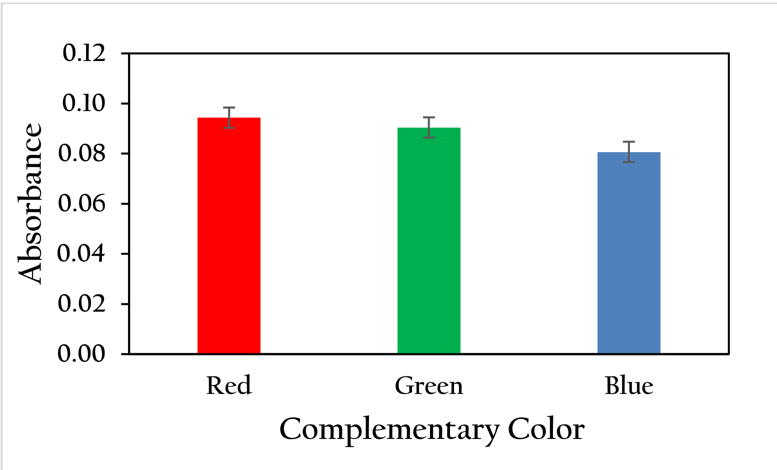

Fig 3. The absorbance after the addition of Cu(II) standard in the µPADs calculated using R, G and B intensity

Optimum volume of PIF

The optimization of the PIF volume was performed to determine the amount of PIF solution that produced the highest absorbance response. The volume of the applied PIF directly affects the resulting film thickness, which plays a crucial role in controlling the diffusion of metal ions within the µPAD matrix. Experimental observations revealed that at higher volumes (30-50 µL), the absorbance decreased due to the formation of an excessively thick film that hindered the diffusion of Cu(II) ions. In contrast, when a lower volume (10 µL) was used, the film became too thin, leading to poor and uneven dispersion of the zincon reagent, which in turn reduced both the sensitivity and stability of the analytical response. At a PIF volume of 20 µL, the film exhibited an optimal thickness and uniform zincon distribution, promoting efficient Cu(II) ion diffusion and enabling the stable formation of the blue zincon-Cu(II) complex [21]. Therefore, a PIF volume of 20 µL was identified as the optimal condition for effective complex formation, as illustrated in Figure 3.

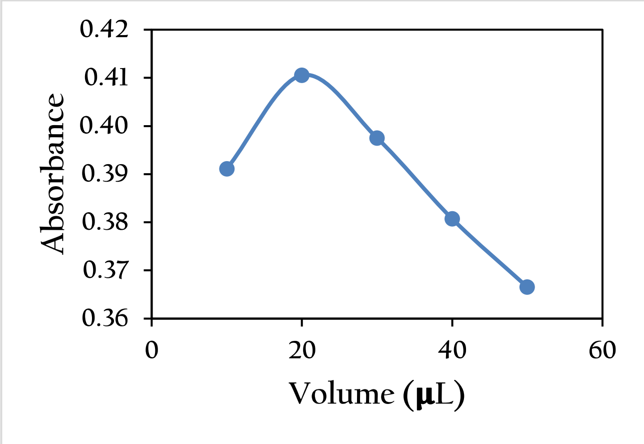

Fig 3. The effect of PIF volume on the absorbance of the µ-PAD

Optimum Reaction Time

Optimization of the reaction time was conducted to determine the duration necessary to achieve the highest absorbance response during the formation of the zincon-Cu(II) complex within the PIF-modified µPAD. Reaction time is a critical parameter that governs the extent of interaction and diffusion between Cu(II) ions and the immobilized zincon reagent [22]. Experimental findings showed that at prolonged reaction times of around 50 minutes, the absorbance values decreased, suggesting instability of the zincon-Cu(II) complex, likely resulting from partial degradation or reduced homogeneity within the reaction zone. Although longer contact times allow extended diffusion, they may also promote complex decomposition, leading to a less stable colour response. In contrast, shorter reaction times of 10, 20, and 30 minutes yielded relatively low absorbance, indicating that the complexation process was incomplete due to insufficient interaction and diffusion between Cu(II) ions and zincon. Inadequate reaction time can thus produce weak analytical signals, whereas excessive reaction time may decrease accuracy through over-diffusion or degradation effects [23]. The optimal response was observed at a reaction time of 40 minutes, where the maximum absorbance was obtained, reflecting the establishment of a stable and intense blue zincon-Cu(II) complex. Therefore, a reaction time of 40 minutes was identified as the optimal condition to ensure reliable analytical performance of the µPAD for Cu(II) detection. The results of the reaction time optimization are illustrated in Figure 4.

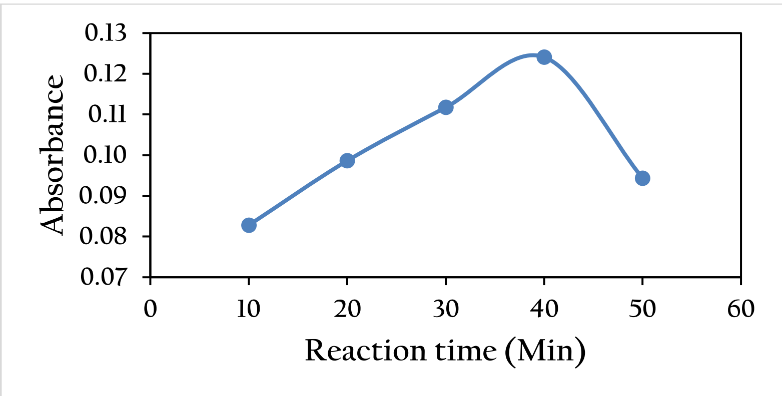

Fig 4. The effect of reaction time on the absorbance of the µ-PAD

Optimum Volume of Sample

The optimization of the sample volume was performed to determine the ideal amount of solution necessary to produce a stable and reproducible absorbance response for Cu(II) detection using the PIF-modified μPAD. The sample volume plays a pivotal role in influencing both the degree of interaction between the analyte and the immobilized reagent and the efficiency of ion diffusion within the detection zone. At smaller volumes (10 and 20 μL), the absorbance values were relatively low, indicating that the limited amount of Cu(II) ions in the reaction zone was insufficient to achieve complete complexation with the zincon reagent, resulting in weak and inconsistent colour development. Conversely, when larger sample volumes (40 and 50 μL) were applied, a decrease in absorbance was also observed, likely due to oversaturation of the detection zone, which led to non-uniform colour formation and diminished reaction efficiency. The optimal analytical response was obtained at a sample volume of 30 μL, where the analyte concentration and diffusion dynamics were balanced, allowing efficient Cu(II) ion penetration into the PIF matrix and the formation of a stable, intense blue zincon-Cu(II) complex. Accordingly, a sample volume of 30 μL was identified as the optimal condition to ensure high sensitivity, accuracy, and reproducibility of the μPAD for Cu(II) analysis [21]. The results of this optimization are presented in Figure 5.

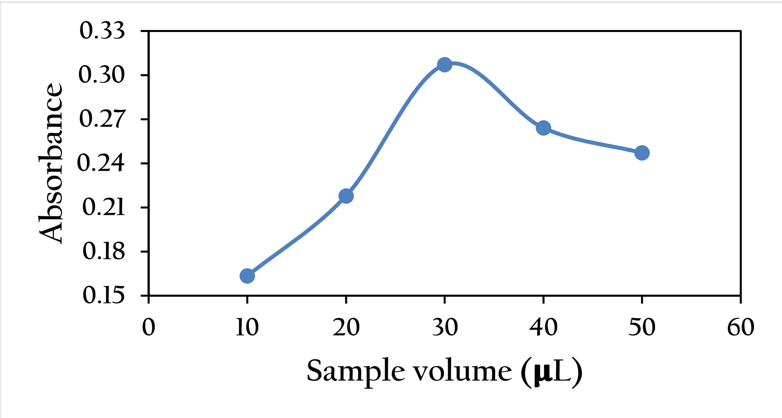

Fig 5. The effect of sample volume on the absorbance of the μ-PAD

Optimum pH of Sample

The optimum pH for the Cu(II) sample was determined based on the condition that produced the highest absorbance value. Since the Cu-zincon complexation occurs effectively within the pH range of 4–5, pH optimization was essential to ensure accurate and efficient detection using the PIF-modified μPAD. Experimental results demonstrated that pH had a significant effect on the analytical response. At pH 4, the absorbance was relatively low due to the competitive binding of protons (H^+) with Cu(II) ions, which hindered the formation of the Cu-zincon complex. In contrast, at higher pH values (6-9), a decline in absorbance was also observed, attributed to the hydrolysis of Cu(II) ions and the subsequent formation of insoluble hydroxides that reduced the availability of free Cu(II) ions for complexation. The maximum absorbance was recorded at pH 5, indicating that this condition provided an optimal balance between zincon activity and Cu(II) ion stability. Therefore, pH 5 was identified as the optimum condition to achieve reliable, sensitive, and stable colorimetric detection of Cu(II) ions [12]. The results of the pH optimization are illustrated in Figure 6.

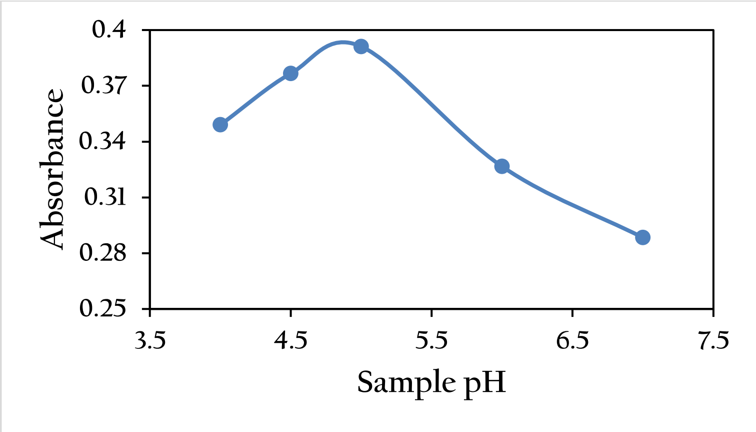

Fig 6. The effect of sample pH on the absorbance of the μ-PAD

Standard Calibration Curve for Cu(II) Analysis

A standard calibration curve was constructed to evaluate the linear relationship between Cu(II) concentration and absorbance using both μPAD and UV-Vis spectrophotometric methods. The results demonstrated that the absorbance for both methods increased proportionally with the increase of Cu(II) concentration, which confirms a direct linear correlation between these two parameters [10]. This linearity indicates the reliability of the μPAD system for quantitative analysis of Cu(II), as illustrated in Figure 7.

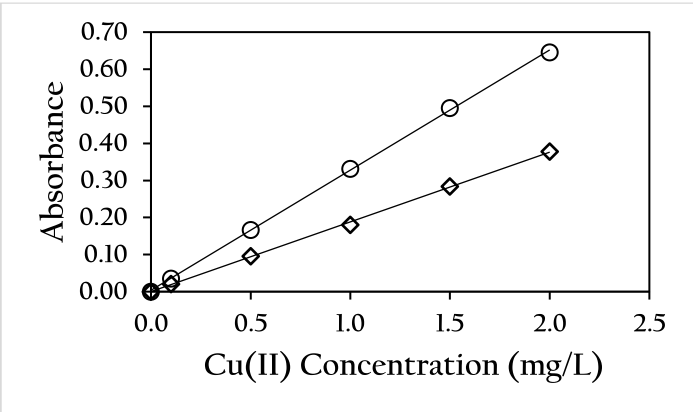

Fig 7. The calibration curve of Cu(II) using both (◊) μPAD and (o) UV-Vis spectrophotometry methods

In the analysis conducted using the UV-Vis spectrophotometric method as the standard reference, an acetate buffer of pH 5 was employed to prevent Cu(II) precipitation and to maintain the solution under mildly acidic conditions, which enhances the stability of the zincon-Cu(II) complex formation [3]. Based on the calibration data, the standard curve exhibited an excellent correlation between absorbance and Cu(II) concentration, with concentration levels of 0, 0.1, 0.5, 1.0, 1.5, and 2.0 mg/L corresponding to absorbance values of 0, 0.03581, 0.167, 0.33115, 0.49585, and 0.6455, respectively. The resulting linear regression equation was $y = 0.3241x + 0.0037$ with a correlation coefficient (R^2) of 0.9997, confirming that the calibration curve met the linearity standard (0.9-1.0). Similarly, the μPAD-based analysis demonstrated a strong linear correlation between absorbance and Cu(II) concentration for the same series of standard solutions, with absorbance values of 0, 0.02106, 0.09628, 0.18026, 0.28441, and 0.37793, yielding a linear equation of $y = 0.188x + 0.0002$ and an R^2 value of 0.9993. Although the μPAD exhibited slightly lower sensitivity than the UV-Vis spectrophotometric method, as indicated by its smaller slope (0.188), it demonstrated high accuracy and reliability for Cu(II) detection in water samples [15].

Validation of the proposed µPAD

Table 4. Validation of analysis method

No.	parameters of the analysis	Analysis Method		Standard Range
		µPAD	UV-Vis	
1.	Precision	1,36%	0,84%	≤ 2%
2.	Accuracy	0,368%	1,2%	1-8 %
3.	% recovery	98,18-102,44%	100,04-102,2%	80-110%
4.	LOD	0,143 mg/L	0,026 mg/L	-
5.	LOQ	0,476 mg/L	0,088 mg/L	-
6.	Linearity	0,9993	0,9997	0,99-1

Validation of the analytical method was performed to ensure the accuracy and reliability of measurements based on key analytical performance parameters, including precision, accuracy, recovery, LOD, LOQ, and linearity. The results of the validation tests are summarized in Table 4. Precision was evaluated using the %RSD (Relative Standard Deviation), with an acceptable limit of ≤2%. The µPAD method exhibited a %RSD of 1.36%, while the UV-Vis spectrophotometric method showed 0.84%, indicating that both methods demonstrated excellent repeatability and precision [14]. Accuracy was determined by calculating the %error, where the acceptable range is 4–8%. The µPAD and UV-Vis methods yielded %error values of 0.368% and 1.2%, respectively, both well within the acceptable limits, confirming good accuracy [15].

Statistical evaluations using the *t*-test and *F*-test were performed to compare the accuracy and precision of the µPAD method with the standard UV-Vis spectrophotometric method. The *t*-test results showed that the calculated *t*-value ($t_{(count)}$ = 1.24) was lower than the critical *t*-value ($t_{(table)}$ = 2.262), indicating no significant difference in accuracy between the two methods at the 95% confidence level. Similarly, the *F*-test results showed that the calculated *F*-value ($F_{(count)}$ = 2.008) was less than the critical *F*-value ($F_{(table)}$ = 6.26), suggesting no significant difference in precision. These results confirm that the analytical performance of the µPAD is statistically comparable to that of the UV-Vis spectrophotometer. Therefore, the µPAD satisfies the validation requirements and can be considered a reliable and accurate method for Cu(II) detection.

The %recovery parameter reflects the proportion of analyte recovered during measurement compared to the actual amount present, with acceptable recovery rates ranging from 80% to 110%. The µPAD showed recovery rates of 98.18–102.44%, while the UV-Vis spectrophotometer achieved 100.04–102.2%, both satisfying validation criteria and demonstrating good analytical recovery [13]. The LOD represents the lowest analyte concentration that can be reliably detected. The µPAD achieved an LOD of 0.143 mg/L, whereas the UV-Vis spectrophotometer exhibited a lower LOD of 0.026 mg/L, indicating higher detection sensitivity for the latter. Similarly, the LOQ, the lowest concentration measurable with acceptable precision and accuracy, was 0.476 mg/L for the µPAD and 0.088 mg/L for the UV-Vis method, confirming the superior quantification capability of the spectrophotometric approach [13].

Despite the lower sensitivity compared to UV-Vis, the µPAD remains a viable analytical tool due to its portability, simplicity, low operational cost, and suitability for on-site testing. Moreover, the µPAD developed in this study exhibited improved analytical performance compared to previously reported µPADs for Mn(II) detection, highlighting its potential as a promising alternative for heavy metal analysis. Linearity, which assesses the proportional relationship between analyte concentration and absorbance, was evaluated using the correlation coefficient (R^2), with acceptable values ranging from 0.99 to 1. The µPAD and UV-Vis spectrophotometer produced R^2 values of 0.9993 and 0.9997, respectively, confirming excellent linearity and strong positive correlation. Thus, the µPAD fulfilled the linearity criteria and demonstrated reliable analytical performance [13].

Selectivity Test

The selectivity test was conducted to verify the ability of the analytical method to specifically detect the target analyte without interference from other ions. The results demonstrated that the µPAD modified with PIF and the masking agent D2EHPA was able to selectively detect 0.2 mg/L Cu(II) in the presence of five potential interfering ions (Mg(II), Zn(II), K(I), Ca(II), and Na(I)), maintaining a maximum interference limit below 5%. Without D2EHPA, the zincon reagent also reacted with Zn(II) to form a blue complex; however, the incorporation of D2EHPA effectively suppressed this cross-reactivity, enhancing selectivity toward Cu(II). Among the tested ions, Zn(II) showed the highest interference (5.267% at 40 mg/L), followed by Mg(II) (4.87%) and Ca(II) (4.453%), while K(I) (2.86%) and Na(I) (3.8%) caused minimal interference. These results confirm the effectiveness of D2EHPA as a masking agent in improving the selectivity of the µPAD, as illustrated in Figure 8.

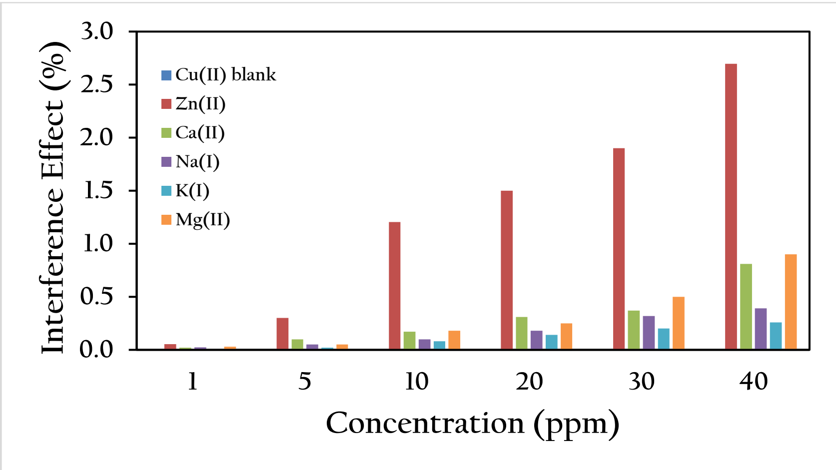

Fig 8. µPAD selectivity test with 5 types of interfering ions

Determination of Cu(II) in Natural Water

In this study, the determination of Cu(II) concentrations in well water samples collected from four sites, including Matani, Oesapa, Baumata, and Tanah Putih, was conducted using the PIF-modified µPAD incorporating D2EHPA, alongside a UV-Vis spectrophotometric method as the reference standard. The results demonstrated that both analytical approaches produced consistent concentration trends, with the highest Cu(II) levels observed in Oesapa, followed sequentially by Baumata, Tanah Putih, and Matani. Statistical comparison revealed no significant difference between the two methods, thereby confirming the analytical reliability and validity of the developed µPAD. These findings substantiate the potential of the PIF-modified µPAD as a credible and practical alternative for the quantitative determination of Cu(II) in environmental water samples, as detailed in Table 5.

Table 5. Water sample measurements

Sample Location	Measured Cu(II) (mg/L)	
	µPAD	UV-Vis spectrophotometer
Matani	0,0630	0,0634
Baumata	0,1063	0,1060
Oesapa	0,1990	0,2001
Tanah Putih	0,0965	0,0973

Stability Test

The stability of the µPAD is a critical parameter that determines its reliability for routine analytical applications. In this study, stability evaluation was performed by storing the µPAD devices under two distinct conditions: (i) exposed to ambient laboratory atmosphere and (ii) sealed within airtight plastic bag. The devices were monitored over a period of five days to assess changes in analytical performance, as indicated by absorbance measurements.

The results demonstrated a progressive decline in absorbance for µPADs stored under open laboratory conditions, attributed to environmental exposure leading to degradation of the immobilized zincon reagent. Conversely, µPADs stored in airtight plastic exhibited superior stability, with minimal absorbance reduction over time. This preservation effect indicates that limiting air and moisture exposure effectively retards reagent degradation, thereby prolonging the shelf life and maintaining the analytical reliability of the µPAD. The comparative stability performance under both storage conditions is presented in Figure 9.

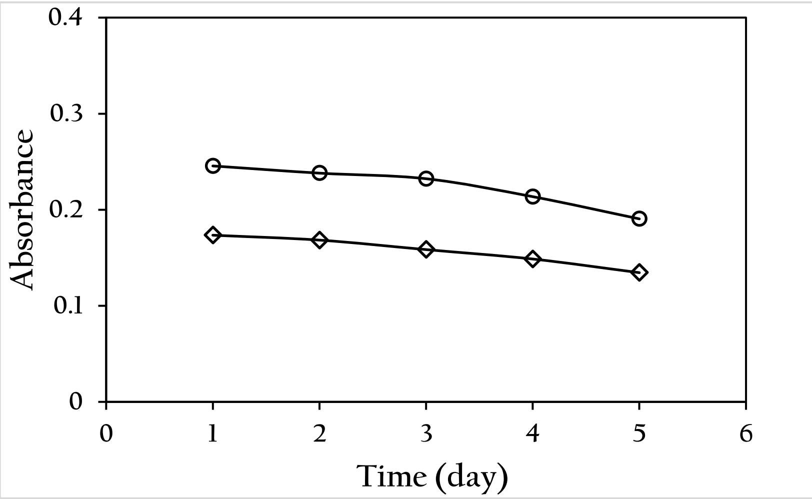

Fig 9. The stability test of µPAD for the determination of Cu(II) over a period of 5 days stored (◊) in ambient laboratory atmosphere and (o) sealed within airtight plastic bag

CONCLUSION

The findings of this study demonstrate that the Polymer Inclusion Film (PIF) can serve as an effective matrix for immobilizing zincon reagents in the colorimetric detection of Cu(II) ions using µPAD. Comprehensive optimization of analytical parameters established that the optimal PIF composition consisted of 50% polyvinyl chloride (PVC), 30% di-(2-ethylhexyl) phosphoric acid (D2EHPA), and 20% Aliquat-336, and 0.1% zincon with a PIF volume of 20 µL, a reaction time of 40 minutes, a sample volume of 30 µL, and a sample pH of 5. The selectivity assessment confirmed that the inclusion of D2EHPA significantly enhanced method specificity by effectively suppressing Zn(II) interference. Validation of the PIF-modified µPAD exhibited excellent analytical performance, characterized by high linearity (R^2 = 0.9993), precision (%RSD = 0.36), accuracy (%error = 0.368), recovery (98.18–102.44%), limit of detection (LOD = 0.143 mg/L), and limit of quantification (LOQ = 0.476 mg/L). Application of the optimized μPAD to real water samples demonstrated consistent and reliable quantification of Cu(II) ions, with concentrations of 0.0630 mg/L (Matani), 0.1063 mg/L (Baumata), 0.1990 mg/L (Oesapa), and 0.0965 mg/L (Tanah Putih). These results collectively affirm that the developed PIF-modified μPAD represents a robust, accurate, and cost-effective analytical platform for trace-level determination of Cu(II) in environmental water samples.

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