# Simple Microfluidic Paper-Based Analytical Device (µ-PAD) Coupled with Smartphone for Mn(II) Detection Using Tannin as a Green Reagent

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*Received: February 22, 2023*
*Accepted: July 14, 2023*

**DOI:** 10.22146/ijc.82511

***Abstract:*** *The development of a simple yet greener microfluidic paper-based analytical device (µ-PAD) for on-site detection of Mn(II) in various types of waters using tannin as a natural reagent was described. The µ-PAD consists of twelve detection zones, created on a Whatman Number 1 filter paper by a simple drawing technique using an acrylic watercolor. The detection of Mn(II) was based on the color change on the reaction zone due to the reaction between Mn(II) and the pre-deposited tannin. The µ-PAD image was captured by a portable smartphone detector, and the blue intensity was digitized using a color picker application to generate the reflectance as the analytical response. The proposed method was characterized by a linear dynamic range of 0.05–0.25 mg L$^{-1}$ with the limit of detection (LOD) for the determination of Mn(II) of 0.026 mg L$^{-1}$. The other analytical merits of the proposed method, such as precision (RSD, 1.107%), accuracy (E, 6.697%), and recovery (104–112%), were all comparable to the existing spectrophotometric methods. The method's successful application to natural water samples from manganese mining sites aligns with the reference spectrophotometric method, indicating its good selectivity and accuracy without significant influence of commonly associated interfering ions.*

***Keywords:*** *microfluidic paper based analytical device (µ-PAD); Mn(II) detection; tannin; green reagent*

## ■ INTRODUCTION

As an essential trace element in humans, manganese (Mn) is required in small quantities for both growth and development as well as the maintenance of human health. Manganese has been identified as part of the chemical composition of several essential enzymes (i.e., arginase, pyruvate carboxylase, superoxide dismutase, and glycosyltransferase), which directly involves in several important processes in the human body, including bone system development, carbohydrate and lipid metabolism, immunity, nervous system, and reproductive hormone function [1]. Despite its significant roles, long-term excessive exposure to manganese in larger quantities may be detrimental to human health. It has been reported that excessive exposure to manganese can primarily lead to the disruption in neurological function and trigger the development of manganism, an occupational disease resembling Parkinson's disease [2-3]. In addition, excessive exposure to manganese has also been associated with several early chronic symptoms such as pneumonitis, cough and bronchitis, metal fume fever, decrease in lung function and chronic obstructive lung disease [4-5].

As the 12th most abundant element in the earth's crust, manganese can be naturally found not only in rocks and soil but also in water and a variety of food. While manganese can exist in natural waters in several oxidation states, it is most frequently found as its ionic Mn(II) in water with a pH lower than 7 and/or low dissolved oxygen content [6-7]. Mn(II) contamination could occur through natural processes, anthropogenic activities, and agricultural or household products.

Natural processes such as volcanic eruptions, rock weathering and erosion of the earth's crust are among the main sources of Mn(II) in aquatic systems. An elevated Mn(II) concentration in water could also be the result of anthropogenic activities such as from iron and steel plants, production of matches, fireworks, dry-cell batteries, and porcelain and other manganese compounds. Moreover, many agricultural and household products, including fertilizers, pesticides, fungicides, livestock supplements, antialgal agents, disinfectants, metal cleaners, tanning, and bleaching agents containing either manganese sulfate or potassium permanganate that also contribute to the high level of Mn(II) contamination in water systems. Due to its wide range of use and its high solubility characteristics in water, manganese can ubiquitously be present in environmental water because of the release or discharge from industrial and domestic waste or as leachate from landfills and agricultural runoff. Thus, considering its abundance along with its adverse health effects, a regular determination of Mn(II) ions in a variety of environmental waters, wastewater, and industrial effluents is of great significance, especially in ensuring a sustainable public health system.

To date, numerous analytical techniques have been commonly applied for the regular measurement of Mn(II) ions in various environmental waters. These include graphite furnace atomic absorption spectroscopy (GF-AAS), atomic absorption spectroscopy (AAS), and inductively coupled plasma mass spectrometry (ICP-MS) [8-11]. Despite their high accuracy, precision, and sensitivity; these techniques require the use of sophisticated instrumentations and involve laborious operational procedures, which contributes to the high analytical cost and hampers their use for the regular on-site analysis of Mn(II) ions. In order to comply with the standards established by the national and international environmental agencies and by considering the need for regular on-site monitoring of Mn(II) concentration, it is therefore vital to develop a simple yet cost-effective technique for the detection of the Mn(II) ions which can be easily applied for regular on-site monitoring in a variety of environmental samples.

Over the past decade, microfluidic paper-based analytical devices (μ-PADs) have garnered significant attention as a powerful analytical platform due to several attractive features, including portability, cost-effectiveness, user-friendliness, and less reagent consumption [12]. The main advantage of using the paper sheet as the support is that it is cheap, readily available, and easy to handle and streamline the chemical reaction through the capillary channel without external energy sources [13]. There has been a variety of fabrication techniques for creating the hydrophobic barrier and hydrophilic zone on μ-PADs, including screen printing, inkjet printing, flexography printing, wax printing, photolithography, inkjet etching, drawing, and plasma treatment [14]. Of all the fabrication techniques, the drawing technique is preferable due to its simplicity, and cost-effectiveness, as it needs only a permanent marker, a wax pen or an acrylic watercolor to manually hand draw the hydrophobic barrier on a filter paper [15].

Since the first report by the Whitesides group from Harvard University [16], there has been a vast development of μ-PADs as analytical tools for applications in food safety [17], health care [18-19] and environmental science [20-21], with some μ-PADs particularly devoted to the detection of Mn(II) in water samples [22-24]. Meredith et al. [22] reported the development of paper-based microfluidics for the selective determination of Mn(II) in the presence of other cations using 4-(2-pyridylazo)resorcinol (PAR) as the non-specific colorimetric ligand. It was reported that when using triethylenetetramine hydrate (0.1 M) and dimercaptosuccinic acid (0.1 M) in borate buffer (0.125 M) pH 10 as the masking agent, Mn(II) could be selectively detected with 87% masking of all interfering metals including Cu, Zn, Cd, Pb, Co, Ni, and Fe [22]. Lee et al. [24] developed a colorimetric chemosensor filter paper to distinguish between Mn(III) and Mn(II) in the aqueous solution. The research undertaken indicated that the differentiation between Mn(II) and Mn(III) was based on the reaction time of each ion with a colorimetric chemosensor which was synthesized from the combination of 2-(aminomethyl) aniline and

4-(diethylamino)-2-hydroxybenzaldehyde. It was revealed that Mn(II) could be detected via the complex formation with a colorimetric chemosensor with a shorter reaction time in comparison to that of the Mn(III) ion [24]. In a more recent study, Kamnoet et al. [23] proposed a µ-PAD for the selective and simultaneous detection of Cu(II), Co(II), Ni(II), Hg(II), and Mn(II) in water samples. They reported that Mn(II) could be selectively detected by using a µ-PAD containing a 2.5 µL pre-deposited reagent containing 5 mM PAR and 5% w/w poly(diallyldimethylammonium chloride) in a 0.1 M borate buffer pH 9.3. Under the optimum conditions, it was revealed that a linear range of 0.0020–0.0100 mM Mn(II) with a limit of detection (LOD) of 0.0020 mM could be achieved. Thiourea (1 M) and ethylenediamine (8 M) were previously added to the pre-treatment zone to obtain a selective detection of Mn(II) by masking other interfering ions such as Co(II), Cu(II), Ni(II), Zn(II), Cd(II), and Pb(II) [23].

While the above-mentioned µ-PADs have been successful for the detection of Mn(II) in a variety of water samples, their continuous use in the regular detection of Mn(II) is not in line with green chemistry due to the use of synthetic reagents which are expensive and mostly toxic [22-25]. Moreover, the existing µ-PADs for Mn(II) detection were not fully suitable for on-site applications to monitor environmental Mn(II) pollution due to the use of desktop equipment such as the scanner and the use of laptop-based application in processing and digitizing the response image such as image J [22-23]. Therefore, to further extend the greenness, simplicity, and portability of the current µ-PAD technique, herein we report the development of a simple µ-PAD technique coupled with a smartphone for on-site detection of Mn(II) in water using a natural reagent, tannin. Tannin is a high molecular weight phenolic compound that can be easily extracted from a variety of plant tissues using a variety of solvents. Due to its abundant presence in plant tissues and low toxicity, tannin is an excellent greener alternative compound for a variety of applications, including coagulants, food additives, flotation agents, antioxidants, dyeing agents, and adsorbents [26-28]. Additionally, its numerous hydroxyl group content provides tannin with high-water solubility and excellent metal chelating properties, which is promising for other analytical and environmental applications [29-30]. Thus, the present research explores the use of tannin as the complexing agent for Mn(II) in µ-PAD for the analytical determination of Mn(II) in water. The µ-PAD was fabricated using a simple drawing technique [31-33] using the commercially available acrylic watercolor, and the resulting color on µ-PAD due to the reaction between Mn(II) and tannin was captured using a smartphone as a portable detector [34-36]. A free-download "color picker" application which has been successfully utilized and reported elsewhere [37] as an alternative method to the existing desktop computer-based application (i.e., image J) was used for digitizing the color change of the µ-PAD which was further used as the analytical response.

## ■ EXPERIMENTAL SECTION

### Materials

All chemicals used in this research were of analytical grade and were used as received without any further purification. $MnSO_4$ was obtained from Merck and was used in the preparation of Mn(II) solutions. Other chemicals used in this research, such as $CH_3COOH$, $CH_3COONa$, $H_2SO_4$, $NaIO_4$, and $CH_3OH$ were also obtained from Merck and used as received. Tannin powder was commercially available and was bought from Kempan®. The µ-PADs were fabricated using Whatman No.1 filter paper (Whatman/GE Healthcare) using commercially available acrylic color set (Hepi Iop1), crayon (Heppi pop 1), and acrylic spray paint (Nippon paint) as the hydrophobization agents. All the aqueous solutions were prepared in deionized water obtained from OneMed. Mn(II) standard solutions used throughout the experiment were prepared in acetic acid buffer pH 3.

### Instrumentation

A laminating machine (GK-Tech) was used in the fabrication of the µ-PADs to prevent the evaporation of the reagent and avoid any possible contamination. The image of the µ-PADs was captured using a smartphone

Iphone 6S Plus (camera resolution of 12 megapixels). Spectrophotometer UV-vis 100DA-X (B-one) was used as the reference method in the μ-PADs validity test.

## Procedure

### *Fabrication of the μ-PADs*

The proposed μ-PAD (Fig. 1) consisted of 12 reaction zones (0.5 cm internal diameter) with the size of a credit card (9 cm × 8 cm). Several commercial products were assessed for their use as hydrophobization agents to create the hydrophobic barrier on the μ-PAD, including an acrylic color set, crayon, and acrylic spray. When an acrylic color set or crayon was used as the hydrophobization agent, the hydrophobic barrier was manually drawn on the filter paper to create a hydrophilic reaction zone using a paintbrush. When the acrylic spray was used as the hydrophobization agent, the manual spraying technique was used to create the hydrophobic barrier. In both cases, the hydrophobization agents were only applied on one side of the filter paper and a plastic film as the template was used to create the circular hydrophobic barrier. The resulting filter paper was then ironed from the other side to facilitate the penetration of the hydrophobization agent into the cellulose fiber of the filter paper. After that, the μ-PAD was laminated using an ID-card size laminating plastic to prevent the evaporation of the sample and tannin solutions during the detection process. Prior to the laminating, a screw punch was used to punch a sample insertion hole of 2 mm in diameter in the laminating plastic over the center of each sampling zone which will be used for introducing the reagent and sample.

The resulting μ-PADs produced using the above-mentioned hydrophobization agents were assessed for the ability of the fabricated hydrophobic zone to contain an aqueous solution (25 μL of deionized water), which was deposited on the created reaction zone (hydrophilic zone) [14]. For the optimization and the analysis, the selected hydrophobization agent was used to fabricate the hydrophobic barrier and the preparation of μ-PAD involves the addition of the pre-optimized tannin solution as the reagent. In each case, the μ-PAD with pre-deposited tannin reagent was air-dried for 1–2 min prior to the addition of the water sample.

### *Analytical procedures and optimization of the μ-PADs*

As shown in Fig. S1, the measurement of Mn(II) using the μ-PAD was carried out by initially depositing a certain volume of tannin solution as the chromogenic natural reagent to the hydrophilic zones. The μ-PAD was air-dried for several minutes before a certain volume of Mn(II) standard solution or real sample solution containing Mn(II) was dropped onto each detection zone. The μ-PAD was further kept for a pre-optimized reaction time at room temperature. Immediately after the optimum reaction time was achieved, the μ-PAD was

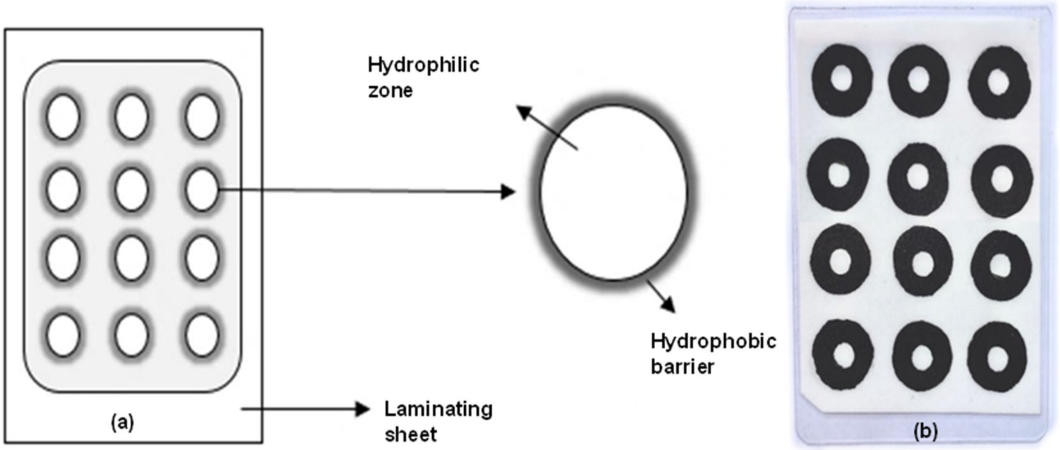

**Fig 1.** (a) Schematic representation of the proposed μ-PAD. The diameter of the hydrophilic zone was 0.5 cm (b) photographic of the fabricated μ-PAD

placed in a homemade box before the µ-PAD image was captured using the smartphone. In this way, the effect of external radiation by the laboratory lighting conditions could be minimized. Finally, the RGB intensity of each detection zone was determined using the free download software "color picker" from the center of each zone. The optimum RGB intensity was then used to calculate the reflectance of each detection zone using the Birch and Stickle method (Eq. 1) [38].

$$R = -\log\frac{I}{I_0} \qquad (1)$$

The R variable indicates the reflectance of each detection zone, I is the mean blue color intensity of each standard or real sample solution, and $I_0$ shows the mean blue color intensity for the blank. The blank intensity was obtained by performing the measurement using the µ-PAD using deionized water as the replacement.

Prior to the application of the µ-PAD, several important parameters were optimized to obtain the optimum conditions of the measurement using the µ-PAD. The parameters include reagent volume, reaction time, and sample volume. The optimization process was carried out using the univariate technique, where one variable was varied when all the others were kept constant. The highest reflectance obtained for each optimized parameter value was chosen as the optimum combination for further analysis of Mn(II) in real water samples.

The analytical performance of the µ-PAD was assessed by evaluating the analytical figures of merit of the proposed method, including the precision, accuracy, % recovery, LOD and limit of quantitation (LOQ). The precision, accuracy and % recovery of the µ-PAD based method were characterized by measuring the reflectance for 0.02 mg $L^{-1}$ Mn(II) standards (n = 6). The precision of the µ-PAD based method, characterized as the repeatability of the measurement, was described as the relative standard deviation (RSD) and was determined using Eq. (2):

$$\%RSD = \frac{SD}{\overline{x}} \times 100\% \qquad (2)$$

where SD is the standard deviation of the measurement and $\overline{x}$ is the average concentration of Mn(II) determined using µ-PAD based method.

The accuracy of the µ-PAD based method was characterized as the % error of the measurement and was determined using Eq. (3):

$$\%E = \left(\frac{\overline{x} - \mu}{\mu}\right) \times 100\% \qquad (3)$$

The recovery of the µ-PAD based method was determined using Eq. (4):

$$\%R = \frac{\overline{x}}{\mu} \times 100\% \qquad (4)$$

where $\overline{x}$ is the average concentration of Mn(II) determined using µ-PAD based method, and µ is the true concentration of Mn(II).

The LOD was defined as the minimum analyte concentration that can be detected with confidence using the proposed method. It was determined by using Eq. (5).

$$LOD = \frac{3S_Y}{S} \qquad (5)$$

The LOQ was characterized as the lowest concentration of the analyte that can be reliably quantified using the proposed method. It was determined by applying Eq. (6):

$$LOQ = \frac{10S_Y}{S} \qquad (6)$$

where $S_Y$ is the standard error of the intercept, and S is the slope of the calibration curve of the proposed method.

### *Selectivity of the µ-PADs*

The effect of several interfering cations on the determination of Mn(II) using the proposed µ-PADs was investigated individually by introducing different amounts of each interfering cation in the determination of a 0.2 mg $L^{-1}$ Mn(II). These include the most encountered cations in environmental waters, such as Na(I), K(I), Ca(II), Mg(II), and Fe(III). The response of the µ-PADs indicated as reflectance was measured both in the presence and absence of the interfering ions. The tolerable limit was defined as the amount of interfering ions causing ±5% error in the determination of 0.2 mg $L^{-1}$ Mn(II).

### *Validation of the µ-PADs*

The applicability of the proposed µ-PAD was demonstrated by the measurement of Mn(II) concentration in environmental samples. The natural water samples used in this research were collected from the surrounding manganese mining sites in Kupang Regency (East Nusa Tenggara Province, Indonesia). Validation was performed by comparing the measurement results using the µ-PAD to the corresponding measurement results obtained using the standard spectrophotometric UV-Vis. The standard spectrophotometric UV-Vis was based on the measurement of colored permanganate absorbance due to the oxidation of Mn(II) ions in the natural water samples by $NaIO_4$ [39]. The quantification of Mn(II) in the natural water samples was performed by comparing the absorbance of the unknown signal with the pre-constructed standard calibration curve. Each sample was measured in triplicates, and the average concentration of Mn(II) was determined.

#### *Stability of the µ-PADs*

The stability of the optimized µ-PAD was assessed under two different storage conditions. First, the µ-PAD was stored under laboratory conditions and was kept in an open air at room temperature; second, the µ-PAD was vacuum sealed in a vacuum sealing bag. In both conditions, the µ-PADs were stored in the dark to avoid the degradation of the tannin reagent due to the light effect. The stability of µ-PAD under both storage conditions was studied by measuring the reflectance of their detection zones after the addition of a pre-optimized volume of the Mn(II) standard containing 1 mg $L^{-1}$ Mn(II). For the determination of Mn(II), the devices were removed from storage bags, and left them at room temperature for at least 15 min prior to the addition of the standard solution.

## ■ RESULTS AND DISCUSSION

### Selection of the Commercial Hydrophobic Products

A preliminary study was conducted to assess the suitability of using several commercial products in the fabrication of the hydrophobic barrier on the µ-PADs, including crayons, acrylic spray paint, and acrylic watercolor. The successful µ-PAD was indicated by the ability to contain the aqueous solution within the hydrophobic barrier. The results indicated that hydrophobic barriers produced using acrylic spray paint were unevenly distributed, and the resulting µ-PAD was softened when compared to the original filter paper, which made it tear easily when handling. Moreover, the hydrophobicity test indicated that water dropped into the hydrophilic zone and seeped through the hydrophobic barrier. When a crayon was used as the hydrophobization agent, it was found that the hydrophobic barrier was able to contain the water inside the hydrophilic zone and no water seepage through the hydrophobic barrier was observed. However, the hydrophobic surface of the µ-PADs was found to peel off after the drawing and heating process, and the solidified crayon was clumped on the surface of the paper substrate. On the contrary, the hydrophobic barrier on the µ-PADs created using the acrylic color set results in an evenly distributed hydrophobic barrier which could contain the aqueous solution within the hydrophobic barrier. Based on these results, it was decided that the acrylic color set was selected to create the hydrophobic barrier on the µ-PADs for the further optimization process.

### Optimization Results

An initial analysis was performed on the successfully produced µ-PADs using the acrylic color set to select the highest analytical response among the red, green, and blue intensity. The analysis was based on the reaction between the pre-deposited tannin solution with Mn(II) present in the standards/samples. Tannins can form coordination complexes with Mn(II) ions due to their phenolic groups, which can act as ligands. The phenolic groups within tannin molecules can coordinate with the Mn(II) ions through oxygen atoms, forming chelates. This coordination gives rise to the formation of stable complexes Mn(II)-tannin complex with distinctive structures and properties, as shown in Fig. S2. The resulting color change of the tannin indicator on the µ-PADs was captured and then converted to the reflectance based on Eq. (1). Fig. S3 presents the individual reflectance calculated using the red, green, or blue intensity. As seen, the highest analytical response expressed as the reflectance was obtained when calculated using the blue intensity. Therefore, the blue intensity was then chosen and applied as the analytical response for further analytical experiments.

The effect of reagent volume on the reflectance of the μ-PADs was examined within the range of 10–50 μL. It was shown that the reflectance increased following the increase in the volume of the reagent which could be attributed to the increase in the total amount of tannin present in the reagent solution (Fig. 2). However, the reflectance tends to significantly decrease when the volume of the reagent used exceeds 40 μL. This could be due to the excessive volume of water, which fades off the color of the complex formed between tannin and Mn(II). Thus, 40 μL reagent was considered optimum and was used for further experiments.

As an analytical device intended for field analysis, the time required to conduct a measurement is considered a crucial factor. It is expected that the time required per analysis is faster or at least within the range of 5–30 min, which is equal to that of the commercially available Mn(II) test kits. Thus, the interval time required for the analysis was examined within the interval of 10–50 min. The color development time was determined after the introduction of a known volume of Mn(II) standard solution into the hydrophilic zone containing a pre-deposited tannin reagent. The results presented in Fig. 3 indicated that a gradual increase in the color development time was achieved from 10–30 min, after which the reflectance leveled between 30 to 40 min. After 40 min of reaction, a significant decrease in the reflectance was observed, which could be attributed to the degradation of the complex initially formed between Mn(II) and the tannin reagent. Consequently, 30 min of reaction was selected as the optimum color development time for further analytical experiments.

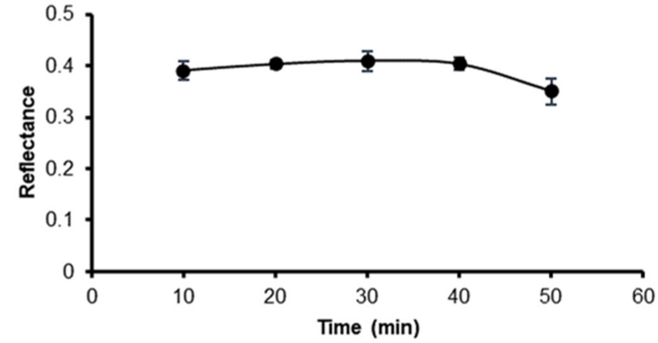

**Fig 3.** The effect of reaction time on the reflectance of the μ-PADs

The effect of sample volume on the reflectance was examined between 10–30 μL. The results, presented in Fig. 4. indicated that the reflectance was gradually decreased as the volume of sample was increased from 10 to 30 μL. This was due to the excessive volume of water, which may fade the pre-formed color developed due to the reaction between tannin and Mn(II). Consequently, based on the presented results, a sample volume of 10 μL was selected for further experiment as it yielded the highest reflectance within the range of sample volume examined.

## Analytical Figures of Merit

The analytical performance of the μ-PAD for the determination of Mn(II) was evaluated under the pre-optimized conditions, and the corresponding analytical figures of merit are summarized in Table 1. The analytical

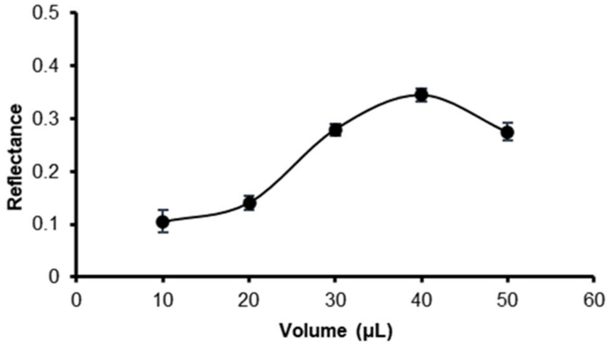

**Fig 2.** The effect of tannin reagent volume on the reflectance of the μ-PADs

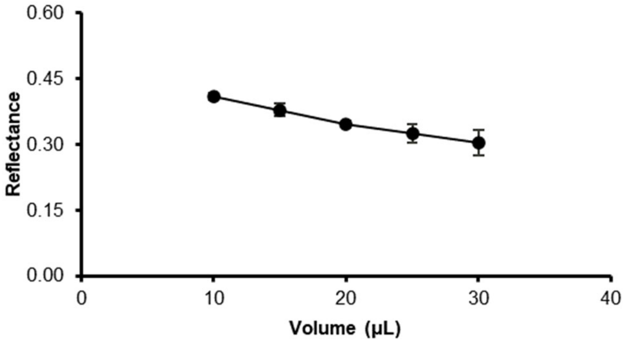

**Fig 4.** The effect of sample volume on the reflectance of the μ-PADs

**Table 1.** Analytical figures of merit of the newly developed μ-PAD method for the determination of Mn(II) and the standard reference spectrophotometric UV-vis method

| Parameters | Methods | |
|---|---|---|
| | Spectrophotometer UV-vis | μ-PAD |
| Linear range (mg L$^{-1}$) | 0–0.25 | 0.05–0.25 |
| Accuracy (%E) | 1.509% | 6.697% |
| Precision (%RSD) | 0.311% | 1.107% |
| Recovery (%) | 97–105 % | 104–112% |
| Limit of detection (LOD) | 0.0068 mg L$^{-1}$ | 0.0260 mg L$^{-1}$ |
| Limit of quantification (LOQ) | 0.0227 mg L$^{-1}$ | 0.0860 mg L$^{-1}$ |

merits of the μ-PAD for the determination of Mn(II), such as linear dynamic range, calibration curve equation, correlation coefficient, accuracy, precision and limit of detection, were studied and compared to that of the standard reference method of spectrophotometric UV-Vis based on the use of $NaIO_4$ reagent. The proposed method offered a relatively satisfied linear dynamic range of 0.05–0.25 mg L$^{-1}$ (Fig. 5) with the regression coefficients for Mn(II) determination described by Eq. (7) where Y is the reflectance and X is the concentration of Mn(II) in mg L$^{-1}$, respectively. The linear relationship between the reflectance and the concentration of Mn(II) is expressed by coefficient correlation ($R^2$) of 0.9912, which confirms that the proposed method complied with the Lambert-Beer law of an applicable method (Eq. (7)).

$$Y = 0.360X - 0.0059 \tag{7}$$

The data presented in Table 1 indicated that the accuracy (%E), precision (%RSD) and recovery (%) were 6.697, 1.107, and 104–112 and were all comparable to the corresponding analytical merits obtained using the standard reference spectrophotometric method. The LOD and LOQ of the proposed method, determined by the linear regression method of Miller and Miller, for the determination of Mn(II) were 0.026 and 0.086 mg L$^{-1}$, respectively [40]. These values are four times higher than the LOD and LOQ of the standard reference method, which are 0.0068 and 0.0227 μg L$^{-1}$, respectively. However, the LOD and LOQ of the proposed μ-PAD method are lower than the tolerable concentration of Mn(II) in drinking water and clean water specified by the Indonesian Ministry of Health Regulation Number 492/MENKES/PER/IV/2010 of 0.4 mg L$^{-1}$. These results indicated that the newly developed μ-PAD is suitable for the determination of Mn(II) concentration in drinking water and clean water.

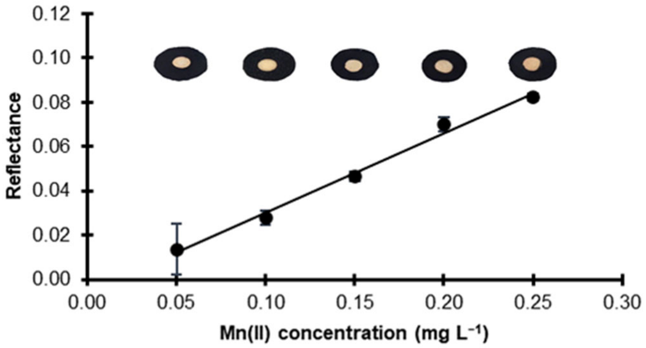

**Fig 5.** The calibration curve of the μ-PADs for the determination of Mn(II) concentration in water

## The Effect of Interfering Cations

Selectivity is obviously one of the most important characteristics of the μ-PAD based method. This property signifies the preference response of the proposed μ-PAD method to the Mn(II) ions with respect to the potential interference effects caused by various interferents. In this research, the primary focus was on the effect of the competing cations such as Na(I), K(I), Ca(II), Mg(II), and Fe(III), as these are the most common cations present in environmental waters and can form complexes with tannin and can thus affect the accurate determination of Mn(II). Under the optimum conditions, each of the interfering cations was added individually to a solution containing 0.2 mg L$^{-1}$ of Mn(II). The introduction of increasing amounts of the interfering ion continued until a tolerable ±5% error in the relative reflectance was observed. Table 2 summarizes the influence of these cations on the relative reflectance of the tannin-Mn(II) complex. The results

**Table 2.** The tolerable interference effects of Na(I), K(I), Ca(II), Mg(II), and Fe(III) ions on the determination of 0.2 mg $L^{-1}$ Mn(II) using the proposed μ-PAD method

| Interfering cations (salt) | Tolerable ratio |
|---|---|
| Na(I) (NaCl) | 50 |
| K(I) (KCl) | 50 |
| Ca(II) ($CaCl_2$) | 12.5 |
| Mg(II) ($MgSO_4$) | 25 |
| Fe(III) ($FeCl_3$) | 2.5 |

revealed that Fe(III) ions had the most severe interference effect when compared to the other investigated interfering cations. However, the co-existence of Fe(III) with Mn(II) in the sample was tolerable up to a ratio of 2.5. The interfering effect of $Fe^{3+}$ indicated a potential cross-sensitivity of the proposed μ-PAD method when determining Mn(II) concentration in a sample containing also Fe(III) ions. However, this unwanted cross-sensitivity caused by the presence of Fe(III) could be eliminated by the addition of 0.5 M orthophosphate as a suitable masking agent, which has been proposed by Zhang et al. [41]. Under this condition, Fe(III) could be precipitated as $Fe_3(PO_4)_2$ prior to the determination of Mn(II) using the proposed method leaving Mn(II) ions to complex with tannin.

While the effect of the other interfering cations was observed at higher ratios compared to that of the Fe(III), these ratios are not sufficiently significant for the determination of Mn(II) in water with high matrix complexity. Thereby, it is anticipated that the use of the proposed method for an accurate determination of Mn(II) was limited in water samples with less complex matrices.

## Analysis of Environmental Water Samples

The applicability of the proposed μ-PAD method to measure Mn(II) in water was examined by the determination of Mn(II) in several natural water samples. The results of Mn(II) concentration obtained using the μ-PAD method were compared to those obtained using the standard reference spectrophotometric method and presented in Table 3. A 2-tail Student's t-test was performed to assess the difference in the concentration of Mn(II) determined using the μ-PAD method and the standard reference method. The null hypothesis was accepted (i.e., no statistically significant difference between Mn(II) concentration determined by using both methods) if the calculated t-value was lower than the critical t-value at the selected confidence level (95%). It can be seen from the table that below the LOD of the μ-PAD (i.e., samples 1 and 2), a high deviation of the concentration of Mn(II) determined using μ-PAD method from those determined using the standard reference method was observed. The t-value calculated for both samples were respectively 181.26 and 39.60, while the critical t-value was 2.77 at the 95% confidence level (df = 4). The significantly higher calculated t-value compared to the critical t-value showed the presence of a statistically significant difference between the results of Mn(II) concentration determined by using the μ-PAD method and the standard reference method. Thus, this indicated the limitation of the proposed μ-PAD method to measure Mn(II) concentration in water below its limit of detection.

Above the limit of detection of the proposed μ-PAD method (Sample 3 and 4), the concentration of Mn(II) determined using the μ-PAD method were respectively 0.104 and 0.101 mg $L^{-1}$ while the corresponding Mn(II) concentration determined using standard reference method were 0.105 and 0.097 mg $L^{-1}$. The t-value calculated for samples 3 and 4 were respectively 0.40 and 1.03, while the critical t-value was 2.77 at the 95% confidence level (df = 4), which indicated

**Table 3.** Mn(II) concentration in natural water samples collected from the surrounding manganese mining sites (n = 3 per sample) was determined using μ-PAD method and the standard reference method (Spectrophotometric UV-vis)

| Sample | Mn(II) concentration (mg $L^{-1}$) | | Calculate t-value | Critical t-value (% confidence) |
|---|---|---|---|---|
| | Spectrophotometric UV-vis | μ-PAD | | |
| 1 | 0.014 | 0.029 | 181.26 | 2.77<br>(95%) |
| 2 | 0.086 | 0.186 | 39.60 | |
| 3 | 0.105 | 0.104 | 0.40 | |
| 4 | 0.097 | 0.101 | 1.03 | |

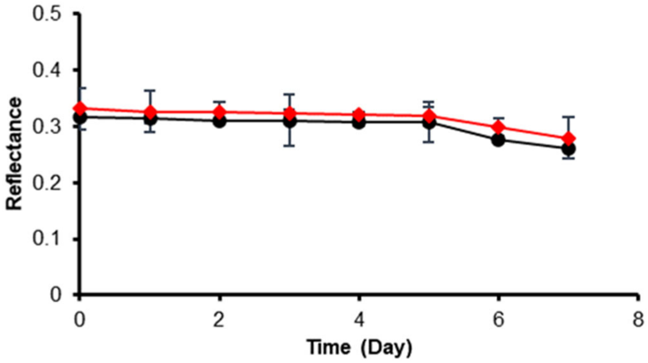

**Fig 6.** The stability of the μ-PADs for the determination of Mn(II) concentration in water over 7 day examination with the μ-PAD stored in vacuum sealed in a vacuum sealing bag (♦) and in open air at room temperature (●)

that there were no statistically significant differences in the average concentration of Mn(II) determined using the proposed μ-PAD method and the standard reference method. This specified a good agreement between the concentration of Mn(II) determined using the proposed μ-PAD method and the standard reference method, with %Error range from −0.952–4.124. The comparable concentration of Mn(II) in similar samples determined by using both the proposed method and the spectrophotometric method indicated that the proposed method was applicable for the measurement of Mn(II) in the contaminated environmental water. It was important to note that a good agreement between the proposed μ-PAD method and the standard reference method in terms of Mn(II) concentration also indicated no significant effect of interfering cations or anions. These results demonstrated the suitability of the proposed μ-PAD method for the measurement of Mn(II) concentration not only in drinking water or clean water with low interference species but also in natural environmental waters with relatively complex matrix.

### Stability Study

One of the most important advantages of the proposed μ-PAD method is its portability for the direct measurement of Mn(II) concentration in the field in remote areas, which requires longer transport time [20]. Therefore, it was an immense important to assess the stability of the μ-PAD as to meet the required transport time to the point of measurement. For this purpose, the μ-PAD was prepared with pre-deposited tannin solution as the green reagent and the stability expressed as reflectance was assessed using a known concentration of Mn(II) solution over a 7-day period under two different storage conditions. Fig. 6 indicated that the in both storage conditions, the reflectance of μ-PAD was stable up to day 5th when assessed using a known concentration of Mn(II). However, after 5 d, the reflectance of μ-PAD under both conditions tends to decrease significantly. These results indicated that, under both storage conditions, the maximum storage time of the μ-PAD prior to the field analysis was 5 d.

## ■ CONCLUSION

In summary, a new, simple and greener μ-PAD method for the determination of Mn(II) in water has been successfully developed based on the use of tannin as a natural reagent. The proposed μ-PADs were created on a Whatman Number 1 filter paper (9 cm × 8 cm) by a simple drawing technique using a commercially available acrylic watercolor. The detection of Mn(II) was based on the color change observed in the reaction zone of the μ-PAD due to the reaction between Mn(II) and the pre-deposited tannin in the detection zone. The red-green-blue intensity of the complex was captured by a smartphone that functioned as a portable detector, and the blue intensity of the complex digitized using a free-download color picker application was used to calculate the reflectance as the analytical response. It has been demonstrated that the analytical merits of the proposed μ-PAD method, including the accuracy (%E), precision (%RSD) and recovery (%), were 6.697, 1.107, and 104–112 and were all comparable to the corresponding analytical merits obtained using the standard reference spectrophotometric method. The proposed method offered a relatively satisfied linear dynamic range of 0.05–0.25 mg $L^{-1}$ with the limit of detection (LOD), and limit of quantification (LOQ) for the determination of Mn(II) were 0.026 and 0.086 mg $L^{-1}$, respectively. It was also demonstrated that the μ-PAD was stable for at least 5 days when stored under vacuum-sealed bags. These findings demonstrated that the μ-PAD prepared using a simple drawing technique using a commercially available acrylic watercolor as the hydrophobization agent and tannin as green reagent could potentially be used as an alternative to the corresponding spectrophotometric method for the on-site determination of Mn(II) in environmental and drinking water samples.

## ■ ACKNOWLEDGMENTS

This work was financially funded by a DIPA PNBP research grant 2023 No. 15/UN15.15.3.PPK/SPP/FST/IV/2023 of the Faculty of Science and Engineering, University of Nusa Cendana, Kupang, Indonesia. The authors thank Maria F.D. Noe and Yohana F. Dhema for their assistance in the examination of the selectivity of the μ-PAD.

## ■ AUTHOR CONTRIBUTIONS

Fidelis Nitti formulated the idea, secured funding, performed the experiments and data analysis, supervised the project, wrote the original draft, and revised the manuscript. Wendelina Archangela Ati performed the experiment and data analysis, wrote the original draft, and revised the manuscript. Fidelis Nitti and Wendelina Archangela Ati contributed equally to this work. Philiphi de Rozari, Pius Dore Ola, Luther Kadang formulated the idea, secured funding, supervised the project, and revised the manuscript. David Tambaru wrote the original draft and revised the manuscript. All authors agreed to the final version of this manuscript.

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