Research article

A Double-layered Paper-based Analytical Device for Determination of Iron in Water Samples based on Standard Addition Method

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Keywords

paper-based analytical device;

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Abstract

A simple method for the determination of iron involved a novel paper-based analytical device (PAD) was developed. The PAD was composed of two layers. Each layer contained a circular hydrophilic reservoir (10 mm Ø) that was situated in a rectangular filter paper (25 × 25 mm^2). The hydrophobic area was created by painting the paper with a "waterproof" glue. The top and the bottom layers were assigned as "the filtration" and "the detection" platforms, respectively. The procedure was started by pipetting an aliquot of bathophenanthroline (Bphen) onto the hydrophilic zone of the bottom layer followed by spiking of standard solutions (0.1-0.5 mgL^{-1} Fe^{2+}). The red complex was developed. Then, the top and the bottom layers were assembled by two-sided mounting tape. Later, a water sample was dropped onto the top layer, which removed (filtered) any suspended particles in the water sample. When the filtrate was exposed to the bottom layer, a further colored product formed. The bottom layer was removed and placed in a light-controlled box, and the optical image of the product was captured using a smartphone. Its intensity was evaluated through ImageJ™. Linear standard addition plots were obtained (r^2 > 0.99). The PAD provided high precision (RSD < 6%) with good recovery (92.6-102%). It was applied to the analysis of drinking, tap, canal and river water samples without any prior filtration. The iron amounts were compared to the results obtained by the spectrophotometric method, and there was not significantly difference at 95% confidence (Paired-*t* test, n = 5 samples, t_{stat} = 2.68, t_{cri} = 2.78).

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1. Introduction

Iron contamination in natural water and wastewater can have harmful effects on living beings and on the environment [1]. High concentrations of iron are associated with an increased risk of cancer, heart disease, and other illnesses including endocrine problems, diabetes, and liver diseases [2]. Thus, the contaminated iron amount must be below the regulation limit. In Thailand, Department of Health, Ministry of Public Health, and Industrial Estate Authority of Thailand specified concentration limits of 0.3 mgL^{-1} and 10 mgL^{-1} iron for drinking water [3] and wastewater [4], respectively, and the accurate determination of iron in such samples is important.

Several analytical methods for the quantitative analysis of iron have been reported, i.e., atomic absorption spectrometry [5], inductively coupled plasma spectrometry [6], mass spectrometry [7], electrochemistry [8, 9] and fluorometry [10-13]. Although these methods offer high accuracy and precision, they are not practical for on-site analysis as they often require the use of bulky equipment. Since a paper-based analytical device (PAD) was introduced by Martinez *et al.* in 2007 [14], the device has received much attention for exploiting as the analytical methods for various applications. The benefits of the PAD are that it is easy-to-use, cost-effective, and portable. It is thus qualified for on-site environmental monitoring.

This work demonstrates a PAD device for the determination of iron in water samples. To avoid the matrix effect, the standard addition approach was carried out on the PAD. The PAD was designed as a double-layered platform. The top layer was assigned as the "sample filtration" platform, and the bottom layer was designed as the "detection platform". Each layer was made of laboratory filter paper and included a circular-shaped hydrophilic zone, situated in middle of a rectangular-shaped paper sheet. The hydrophobic area was patterned by painting the paper with a modified waterproof glue solution. The top and the bottom parts of the device were combined using two-sided mounting tape. With this configuration, a sample can be directly aliquoted onto the PAD without any filtration before the measurement. This is very useful when applying the developed PAD to on-site analysis where equipment such as vacuum pumps, filtration flasks and funnels are not available or bulky to be used.

In terms of the detection chemistry of this work, we aimed to employ a colorimetric reaction because of its simplicity. Additionally, the colored product can be easily monitored by capturing through the mobile smartphone. This enhances the capability of the PAD for on-site measurement. Some chromogenic reagents were reported for the detection of iron [15, 16]. Although the reagents mentioned above were selective, they were synthesized under complicated procedures. The most widely used and commercially available reagents are orthophenanthroline (o-phen) [17, 18] and bathophenanthroline (Bphen) [19, 20]. Bphen provides higher sensitivity for the iron (II) detection [13]. Therefore, we selected Bphen as the chromogenic reagent for the trace analysis of iron in water. The complex formation between the iron (II) ion and Bphen ligand, in which a red-colored product is developed, is shown in Figure 1. Some researchers studied the use of Bphen for the analysis of iron by PAD [20], and their work involved simple external calibration assay using PAD for synthetic urine samples. To our knowledge, this work is the first time that a PAD with a two-layer design for the determination of iron in water using the standard addition approach.

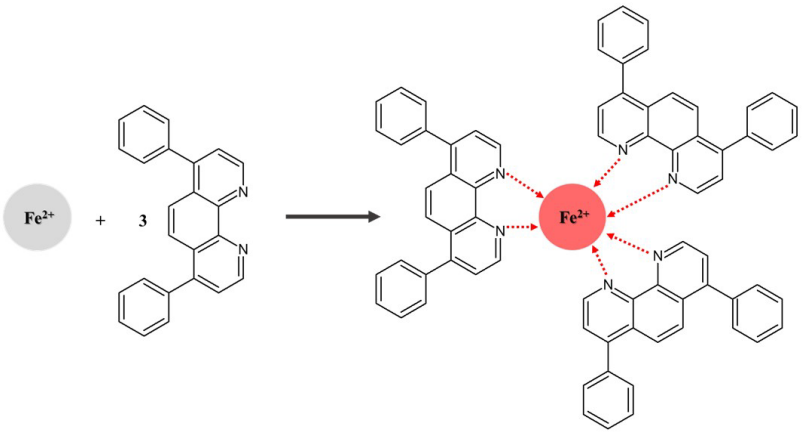

Figure 1. Colorimetric reaction between the iron (II) ion and Bphen

2. Materials and Methods

2.1 Preparation of standard, reagents, and sample solutions

All chemicals used in this work were of analytical reagent grade and used as purchased. Deionized-distilled water (18 MΩ·cm) purified with a Zeneer UP 900 water purification unit (Human Corporation, USA) was used throughout this study. Stock standard Fe^{2+} solution (3 mgL^{-1}) was prepared by dissolving 0.0351 g of $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ (Sigma-Aldrich, USA) with a small volume of water (~ 5.0 mL) and 0.05 mL conc.H_2SO_4. Finally, this solution was diluted to 50.00 mL with water. The working standard solutions (0.1 to 0.5 mgL^{-1}) were prepared daily by appropriate dilution of the stock standard solution with water. The chromogenic reagent (1 gL^{-1}) was prepared by dissolving 0.01 g bathophenanthroline (Bphen) powder (Sigma-Aldrich, USA) with 99.9% (v/v) ethanol (Thermo fisher scientific, Thailand) to obtain a final volume of 10.00 mL. Hydroxylamine hydrochloride (10% (w/v)) was prepared by dissolving 5 g of $NH_2OH \cdot HCl$ (Sigma-Aldrich, USA) with 50 mL water.

River and canal water samples were collected from Kok River in Chiang Rai, and the Prawet and Saensaeb canals in Bangkok. Tap water was collected from the Department of Chemistry, School of Science, King Mongkut's Institute of Technology Ladkrabang in Bangkok. Drinking water samples were brought from a convenience store in Bangkok. All samples were pretreated with hydroxylamine to convert Fe^{3+} to Fe^{2+} before detection by the following protocol. An aliquot of 0.3 mL sample was transferred into a 10.00 mL volumetric flask followed by 0.2 mL 10% (w/v) hydroxylamine. The solution was adjusted to the mark using water. The pretreated sample was then analyzed as described in Section 2.3. All samples were directly pretreated without any prior filtration.

2.2 Fabrication of the PAD

The PAD was fabricated according to the schematic drawing presented in Figure 2. The PAD was designed with Microsoft Powerpoint™ and was printed onto A4-sized Whatman™ No.1 filter paper for mass production. The hydrophobic area was created by painting a waterproof glue solution onto both front and back side of the paper around the circles (Figure 2A). The glue was prepared by

dissolving approximately 5 g of transparent waterproof glue additive (Galen^{TM}, China) in 5.0 mL of 50% (v/v) ethanol. The solution was homogeneously mixed and kept overnight before use. After painting, the fabricated paper was dried at ambient temperature for one night. Then, it was cut into small pieces (Figure 2B). A single PAD consisted of the circular-shaped hydrophilic area (10 mm Ø) located in the middle part of the rectangular-shaped (25 × 25 mm^2) paper sheet. Two pieces of the PAD were assembled for the determination of iron in water using the standard addition method (see Section 2.3).

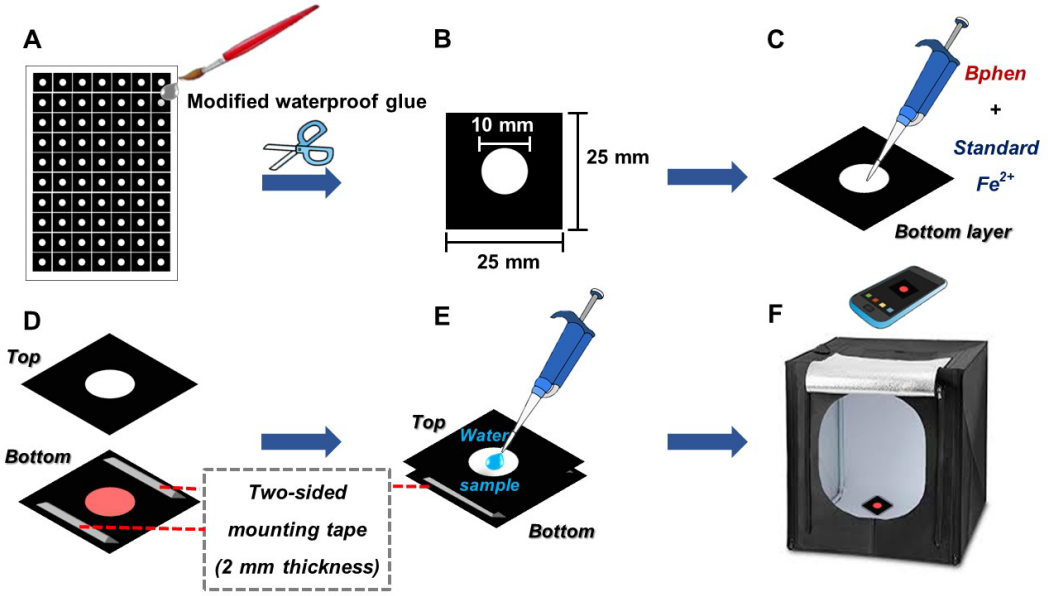

Figure 2. Schematic drawings represent the fabrication of the double-layered PAD and the analytical workflow for the standard addition assay for the iron determination in water sample.

2.3 Standard addition assay on the double-layered PAD

A schematic diagram of the analytical workflow for the analysis of the iron content in water sample by the double-layered PAD based on the standard addition assay is illustrated in Figures 2C to 2F. Ten microliters (10 µL) aliquots of 1 g L^{-1} Bphen and 100 µL aliquots of standard Fe^{2+} solutions (0.0 to 0.5 mgL^{-1}) were pipetted onto the hydrophilic areas of the individual bottom layers (Figure 2C). The top and the bottom layers were then attached to each other (Figure 2D) using a 3M^{TM} (Nanmee Co. Ltd., Thailand) two-sided mounting tape (2 mm thickness). Later,120 µL of the as-pretreated water sample (see Section 2.1) was pipetted onto the top layer (Figure 2E). At exactly 15 min, the bottom layer was then unpacked and was accommodated inside the small light-controlled studio (width×length×height: 400×400×400 mm^3). An optical image of each product was captured using a smart phone (iPhone14^{TM} Pro Max, USA) as shown in Figure 2F. The intensity of the red colored that developed was read using ImageJ^{TM} software. The standard addition plot of the intensities against the concentrations of the spiked Fe^{2+} standards was constructed for the quantification of the iron amount. The amounts were compared to the results obtained by the spectrophotometric method.

2.4 The validating spectrophotometric method

In this work, the spectrophotometric method was employed as the validating method. The iron amount was determined based on the external calibration approach. A standard curve was constructed of absorbance readings at 510 nm versus standard Fe^{2+} concentrations (0 to 5 mgL^{-1}). It was necessary to filter each sample to eliminate the suspended particles before the measurement. An aliquot of 5.0 mL of the filtrate was pipetted into 25.00 mL volumetric flask. Then, 0.25 mL of 10% (w/v) $NH_2OH{\cdot}HCl$ (Sigma-Aldrich, USA) and 0.5 mL of 0.5% (w/v) orthophenanthroline monohydrate (Sigma-Aldrich, USA) were added into the flask. This was followed by the addition of 2 drops of 25% (w/v) sodium citrate (Sigma-Aldrich, USA). The final volume was adjusted to mark with distilled water. The absorbance of the solution was finally measured at 510 nm using an UV-visible spectrophotometer (JASCO V630, Japan).

3. Results and Discussion

3.1 Design of the double-layered PAD

Firstly, we aimed to employ a single-layered PAD for the determination of iron. Although it was easy to use, prior sample filtration was required to remove the suspended or colloidal particles that were present in water samples. This process was time consuming and tedious because bulky devices such as a filtration flask (with its funnel) and a vacuum pump were necessary. These devices obstructed the use of the PAD for on-site analysis. We, therefore, adopted a double-layered PAD platform, as shown in Figure 2D. The top and the bottom layers were assigned as the "sample filtration" and the "detection" layers, respectively. When both layers were attached using two-sided mounting tape, a space above the bottom layer was created. After loading a small volume of sample onto the top platform, the liquid flowed vertically through the paper into the space while the suspended matter was trapped (Figures 3B and 3C). The filtrate was then exposed to the immobilized chromogenic reagent in the hydrophilic area of the detection layer, and a color of the product developed (Figures 3D to 3F). This design was very useful for on-site determination as the sample solution could be dropped directly onto the PAD without any prior filtration. However, we found in our preliminary results that poor recoveries were observed (< 80%) when the PADs were used for canal and river water samples. This might be because of the sample matrix effect. To eliminate this drawback, standard addition was used with the paper platform. A series of concentrations of standard Fe^{2+} from 0.0 to 0.5 mgL^{-1} were spiked onto the bottom layer detection zones after the addition of the chromogenic reagent (Bphen). For each device, the upper and lower layers were then mounted together before dropping the sample onto the top one. Good recoveries (92.6 to 102%) were obtained. The advantages of the standard addition assay lay not only in the elimination of the matrix effect but also in the paper assay that was more convenient than the conventional procedure. Therefore, the standard addition approach with the PAD was selected as the quantification method in this work.

It is noted that we tried to capture the optical image at the backside of the bottom layer by flipping the device without unpacking the double-layered platform. Unfortunately, the intensities of the red color products on the PADs in terms of "without" and "with" the standard addition are not different as presented in Figure 4. This may have been because sufficient volume of the product solution could not penetrate from the front side to the back side of the bottom layer. Therefore, it was necessary to capture the optical image of the front side of the bottom platform of the PAD.

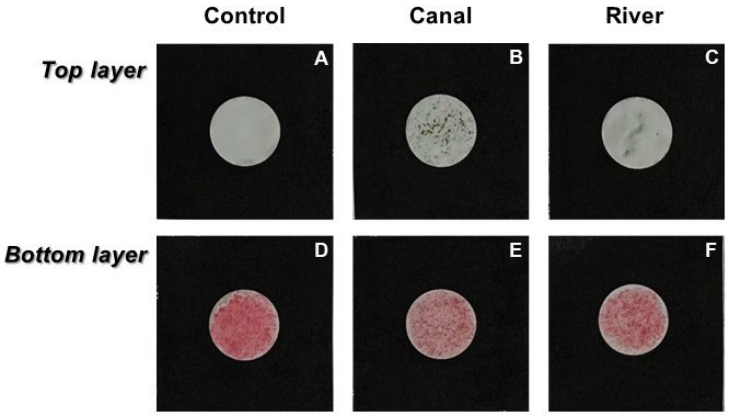

Figure 3. Optical images of the exploded views of the double-layered PAD (Top and Bottom layers) after applying: A; 2.0 mg L^{-1} of standard Fe^{2+} solution (Control), B; Sansaeb canal and C; Kok River onto the top layer.

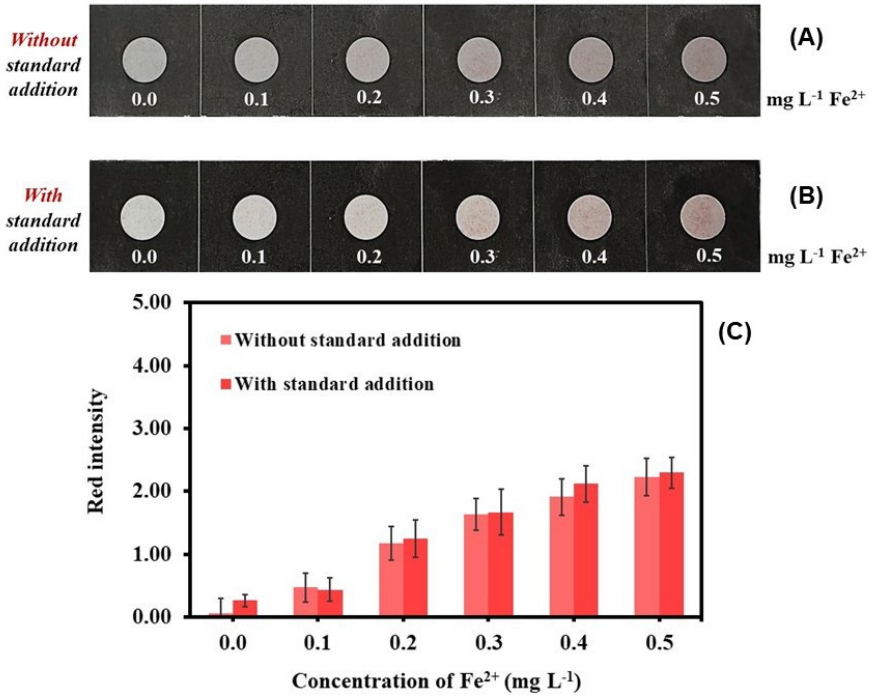

Figure 4. The optical images of the back side of the bottom layer of the PAD: A; without and, B; with standard addition process. C; comparison of the corresponding red color intensities

3.2 Optimization study

We started the optimization study by investigating the appropriate concentration of ethanol, to be employed as the solvent for the waterproof glue. The glue was cheap and commercially available on-line. Unfortunately, it was highly viscous, and it was not possible to use it as produced by the manufacture to paint directly onto the filter paper. Modification of the glue by dissolving it in ethanol was therefore necessary. The concentrations of ethanol varied from 5 to 99.9% (v/v) while the weight of the glue and the volume of the solvent were fixed at 5 g and 5.0 mL, consecutively. The as-prepared glue solution was manually painted onto the hydrophobic zone of the single-layered PAD (in Figure 2B). Then, 100 µL of red dye solution was pipetted onto the hydrophilic area and the optical image of each PAD was captured. The results in Figure 5 show that leakage of the solution out of the hydrophilic zone did not occur for all studied concentrations of ethanol. This meant that the hydrophobic zones had been successfully created. However, the glue solutions were still slightly viscous when the concentrations of ethanol from 5 to 25% (v/v) were used. When the glue was softened with 99.9% (v/v) ethanol, the solution was too clear to control its flow on the paper while painting. Moreover, the solvent was easily volatilized even at ambient temperature. Therefore, a concentration of 50% (v/v) was considered suitable.

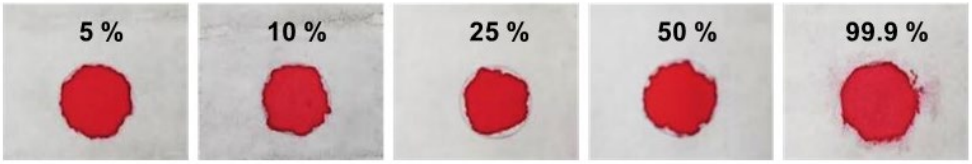

Figure 5. The optical images of the dropped red dye solutions on the single PADs where their hydrophobic areas were created by painting with 5 g waterproof glue in 5.0 mL of various concentrations of ethanol (from 5 to 99.9% (v/v)).

The other optimized parameters were the aliquoted volumes of sample and Bphen. The effect of sample volume was studied by pipetting a red dye solution from 100 to 140 µL onto the filtration platform (the top layer, see Figure 2E). As the PAD was designed as a double-layered platform, the suitable sample volume was taken to be the minimum volume that could allow the filtrate to be exposed to the bottom layer. It was observed that the droplets of the dye had contacted the bottom layer when a minimum volume of 120 µL had been added. Therefore, this volume was selected as the suitable sample volume. The effect of Bphen volume was examined by transferring 7 to 15 µL of 1.0 gL^{-1} of Bphen in 99.9% (v/v) ethanol onto the circular-shaped hydrophilic zone of the single PAD. We observed that the solution of Bphen in ethanol moved rapidly, compared to the red dye solution. This may be because the viscosity of Bphen in ethanol was less than that of the red dye in water. It was also found that a volume lower than 10 µL was not enough to completely fill the zone. Nevertheless, a greater volume meant that too much of the chromogenic reagent was consumed. The volume of 10 µL was therefore considered as appropriate (results are not shown).

It should be noted that in this work the pH effect on the colorimetric detection of Fe^{2+} was not investigated. However, the pH values of the reaction solutions were measured using universal pH papers and pH values of 5.0 were observed when the concentrations of the standard Fe^{2+} solutions ranging from 0.0 to 1.5 mgL^{-1} were studied. This pH value was close to the results of Senanayake *et al*. [13] in which the optimal pH value of 6.6 was considered suitable for the complex formation between iron (II) ion and Bphen.

3.3 Selectivity study

To examine the selectivity of the PAD for the colorimetric detection of Fe^{2+} using Bphen as the chromogenic reagent, the effects of metal ions, i.e., Fe^{3+}, Bi^{3+}, Zn^{2+}, Mn^{2+}, Cd^{2+}, Hg^{2+}, Ba^{2+}, Ag^{+}, Na^{+} and K^{+} were investigated. Solutions of the individual ions (4.0 mM) were spiked onto the Bphen-immobilized hydrophilic zone of the single PAD. Figure 6A clearly demonstrates that only Fe^{2+} caused the red-colored product. Further study on selectivity was also carried out by dropping the standard Fe^{2+} solutions with concentrations ranging from 0.15 to 1.2 mgL^{-1}onto the PAD. Each standard Fe^{2+} solution contained a fixed concentration of Fe^{3+} (1.2 mgL^{-1}). The results in Figures 6B and 6C reveal that the slopes of the standard linear equations in the presence and in the absence of Fe^{3+} were not different. These results imply that Fe^{3+} did not interfere with the colorimetric reaction between Fe^{2+} and Bphen. This was because Bphen selectively forms the stable red complex with Fe^{2+} ions but not with Fe^{3+} ions [13, 19]. Reduction of Fe^{3+} to Fe^{2+} by hydroxylamine as explained in Section 2.1 is therefore an essential step to be performed before the colorimetric detection.

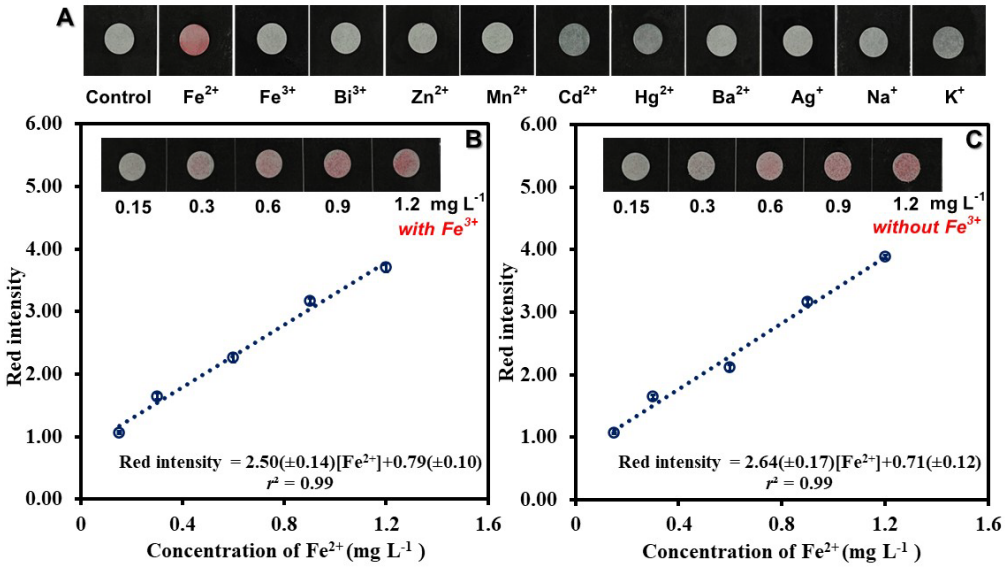

Figure 6. A: The optical images of the single PADs obtained when the individual metal ions (4.0 mM) were studied. B and C: The calibration plots of standard Fe^{2+} (0.15 to 1.2 mgL^{-1}) in the presence and in the absence of 1.2 mgL^{-1} Fe^{3+}, respectively. Note: The insets in Figures 6B and 6C are the corresponding optical images of the single PADs.

3.4 Analytical performances

The analytical performance of the developed PAD for the determination of iron was evaluated in terms of linear working range, minimum detectable level (MDL), precision (RSD) and analytical recovery. It was observed that the standard addition curve was linear in the concentrations range from 0.0 to 0.5 mgL^{-1} Fe^{2+} with good linearity (r^2 = 0.99), as presented in Figure 7. It should be noted that the red intensity values in the y-axis were obtained by subtracting the intensities of the reacted PAD with the blank PAD. The MDL was defined as the lowest concentration of standard

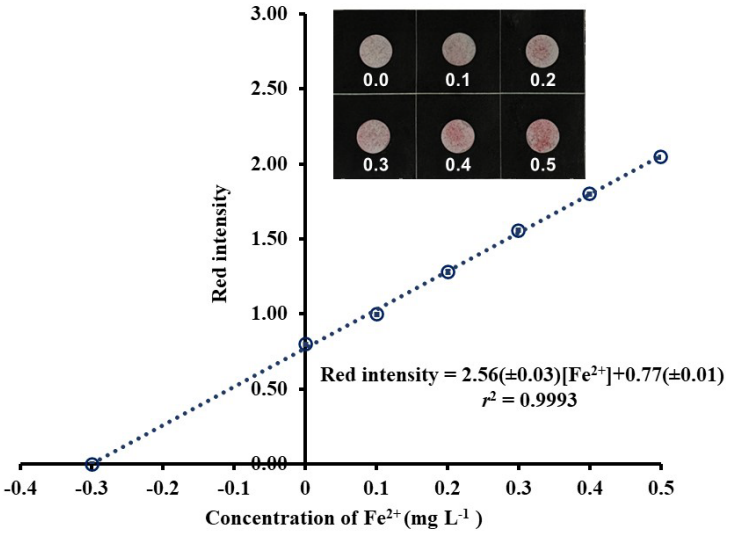

Figure 7. An example of the standard addition curve when standard Fe^{2+} from 0.0 to 0.5 mgL^{-1} were spiked into the water sample for the determination of iron using the double-layer PADs. The inset photo is the corresponding optical image of the combined six double-layer PADs.

Fe^{2+} that could be detected on the PAD. In this work, an MDL of 0.09 mgL^{-1} was achieved. This observed level was lower than the regulation limits for iron in drinking water (0.3 mgL^{-1}) and wastewater (10 mgL^{-1}) as issued by Department of Health, Ministry of Public Health [3], and Industrial Estate Authority of Thailand [4]. The RSD value was determined by replicating measurements (n = 10) of the red color intensity when 0.1 mgL^{-1} Fe^{2+} was studied using ten pieces of the double-layered PAD. RSD < 5.81% was observed and this result showed that the PAD provided good precision. The recovery was investigated by fortifying standard Fe^{2+} into water samples to obtain a final concentration of 0.3 mgL^{-1}. From the results in Table 1, it can be concluded that the recovery values obtained were in the range of 92.6 to 102%. This indicated that under the standard addition approach, the sample matrices did not interfere with the quantitative analysis.

Table 1. Recovery of the method double-layered PAD for the determination of iron in water samples with standard addition on the double-layered PAD

Sample No.	Types of Samples	Iron Concentration (as mgL^{-1})[a]		Recovery (%)
		Fortified	Observed	
1	Drinking	0.30	0.301±0.01	100
2	Tap	0.30	0.305±0.02	102
3	Canal[b]	0.30	0.290±0.01	96.5
4	Canal[b]	0.30	0.278±0.01	92.6
5	River[b]	0.30	0.307±0.01	102

Note: [a] Reported as mean±SD (n=3). [b] Sample nos. 3 and 4. were collected from Prawet and Sansaeb canals in Bangkok while sample no. 5 was obtained from Kok River in Chiang Rai province.

3.5 Application to real sample and validation study

The fabricated double-layered PAD was used to determine the iron levels in various kinds of real samples of drinking, tap, canal and river water. The samples were spiked to obtain the final concentration of 10.0 mgL^{-1} Fe^{2+}. The samples were directly pretreated and measured without prior filtration. Instead of performing one PAD individually, we simultaneously carried out the determination using a combination of six PADs for construction of one standard addition plot (see inset photo of Figure 7). This was done to shorten the analysis time per sample. Following this strategy, the standard addition assay of one sample was completed within 15 min. This was very useful and convenient for routine work. The results were compared to the iron concentrations, determined by the UV-visible spectrophotometry (the validating method), and are presented in Table 2. The iron contents agree well, and there were no significant differences in the means according to the paired *t*-test [21] at 95% confidence (t_{stat} = 2.68, t_{cri} = 2.78, n = 5). These results guaranteed that the developed PAD offered high accuracy.

Table 2. Comparison of the iron concentrations determined by the developed PAD and by the UV-Visible spectrophotometry.

Sample Code[a]	Iron Concentration (as mg L^{-1}) [b]	
	PAD	Spectrophotometric Method
DW	10.04±0.20	10.38±0.03
TW	10.15±0.59	10.46±0.03
PW	9.65±0.18	9.82±0.01
SS	9.26±0.22	9.61±0.07
KK	10.24±0.40	10.16±0.00

Note: [a] DW: Drinking water, TW: Tap water, PW: Prawet canal water, SS: Sansaeb canal water, KK: Kok River water. [b] Presented as mean±SD (n=3).

4. Conclusions

In this work, a novel paper-based analytical device for the colorimetric determination of iron which involved the use of the standard addition method with the paper platform was developed. The PAD was easily fabricated and had a double-layered design that allowed filtration (in the top layer) with subsequent detection of iron in the samples (in the bottom layer). One major benefit of this design was that the sample could be directly transferred onto the PAD without any prior filtration step. The hydrophobic zone was simply patterned by painting the paper with low-cost waterproof glue solution. Based on the standard addition approach, elimination of the sample matrix effect was achieved. The PAD was successfully applied for the analysis of the iron concentrations in various kinds of water samples containing simple and complicated matrices. The PAD also offered high precision and accuracy. Furthermore, the PAD and its accessories were portable. Thus, the developed PAD can be employed as an effective alternative for the on-site determination of iron in water.

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