TRENDS IN SCIENCES 2022; 19(16): 5616 RESEARCH ARTICLE
https://doi.org/10.48048/tis.2022.5616

Thread-Based Analytical Device for Determination of Phosphorus Content in Fertilizers

Kamonthip Sereenonchai[1,2,*], Chawarit Inla[1] and Siriwit Buajarern[1]

[1]Department of Chemistry, Faculty of Science and Technology, Thammasat University, Pathumthani 12120, Thailand
[2]Flow Innovation-Research for Science and Technology Laboratories (FIRST Labs.), Thammasat University, Pathumthani 12120, Thailand

([*]Corresponding author's e-mail: ksereenonchai@hotmail.com)

Received: 22 April 2021, Revised: 4 July 2021, Accepted: 11 July 2021

Abstract

A simple microfluidic thread-based analytical device (μTAD) for determination of phosphorus content in fertilizers based on the formation of phosphomolybdenum blue (PMB) complex was proposed. The proposed μTAD was simply fabricated by stitching the 100 % cotton thread to the polyethylene (PE) mesh canvas. A color developing reagent, which are the mixture of sulfuric acid, ammonium molybdate, potassium antimony tartrate, and ascorbic acid, was firstly loaded into the middle of the thread. A sample solution was subsequently introduced into the same position to form PMB. A digital camera and image processing program were employed for evaluating color intensity of PMB. Variable parameters affecting the μTAD efficiencies were optimized *i.e.,* solution volumes, chemical concentrations, and reaction time. The applicability of the proposed μTAD method was evaluated by analyzing fertilizer samples. The accuracy as expressed by percentages of recovery ranged between 83 and 96 %. The method was highly reproducible with the relative standard deviation (RSD) of 0.84 % (1 mgP/L), 0.92 % (3 mgP/L) and 1.04 % (10 mgP/L) for 10 replicates. The main features of the system are the used of commonly available materials, simple and rapid fabrication, small scale, reduced chemical waste, less sample consumption, and low-cost.

Keywords: Thread-based device, Phosphorus, Colorimetry, Molybdenum blue, Cotton

Introduction

Phosphorus is one of the essential major nutrients for plants. Typically, phosphorus in dissolved inorganic forms of phosphate ion ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) are vital for plant. Not only it helps promote root growth but also stimulate flower development and seed maturity. However, from the fixation process on the surface of soil particles resulting the phosphorus to plants in soil solution become deficit [1,2]. Thus, a superphosphate fertilizer is often added to supply phosphorus in the soils to overcome phosphorus deficiency. Nonetheless leaching fertilizer through natural water can lead to algal blooms and the creation of aquatic dead-zones.

Colorimetric method based on the molybdenum blue reaction has been most utilized for determination of phosphorus in reactive orthophosphate (o-PO_4^{3-}) form due to its high sensitivity and fast color development [3]. It involves reaction of o-PO_4^{3-} with molybdate ions under acidic condition to form 12-molybdophosphoric acid (12-MPA) which is subsequently reduced to a blue-product, phosphomolybdenum blue (PMB) complex, by a mixture of stannous chloride and hydrazine or ascorbic acid and antimony(III) tartrate as catalyst [3,4]. The color change of PMB can be related to concentration of phosphorus in sample solution.

In various publications, the determination of phosphorus in water [4], sediment or soil extracts [2,5] and fertilizer samples [1,7], can be performed by a wide choice of instrumental approaches for example colorimetric batch and automated flow-based systems [4,6,8]. However, these conventional instrumentation methods must operate in the laboratory, require electricity and maintenance not to mention consume large amount of sample and reagent solution hence generate large amount of waste. This leads to development of microfluidic paper-based analytical device (μPAD) for quantification of phosphorus [9-12]. The advantages of μPAD are low-cost, disposability, ease-of-use, and power-free fluid transport via capillary action. However, to fabrication μPAD we have to pattern the paper with hydrophobic agent by using such methods (*i.e.,* inkjet printer, wax printer, rubber stamp) [13,14] to create hydrophilic pathway of sample and reagent solutions. Paper will also have lower physical and mechanical strength when it wet. Moreover, after modification, paper might lose some properties such as mechanical strength and wrinkle.

To overcome some limitations of paper-based device, in 2010, Li *et al.* [15] and Reches *et al.* [16] proposed microfluidic devices based-on multifilament threads as a versatile substrate, namely thread-based analytical device (μTAD) with analogous concept of paper-based device. In recent year, the μTAD plays a significant part in the development of diagnostical and analytical methodologies [15-20] because threads are low-cost, general availability and rather chemically and mechanically resistant. In addition, threads can be patterned freely to achieve suitable hydrophilic-hydrophobic contrast without mold fabrication, creating hydrophilic channels which allow the control of liquid flow by capillary action without external pumps. There are several thread materials availably used as the substrate of μTAD such as acrylic, cotton, wool, hemp, silk, and polyester [17,20]. However, cotton was found to employ a range of applications since its inexpensive and general availability, high physical strength, wettability, and excellent color display property [17].

With a lot of advantages characteristics of μTAD, in this work, a colorimetric microfluidic device based on cotton thread (a colorimetric μTAD) for analysis of phosphorus using molybdenum blue reaction was described to achieve a simple, low-cost, and environmentally friendly method. Color intensity of the phosphomolybenum blue complex (PMB) was monitored by digital camera then, evaluated by image processing program. The application of the proposed method was demonstrated by determining phosphorus content in fertilizers.

Materials and methods

Chemicals and reagents

All chemicals used were of analytical reagent (AR) grade. All solutions were prepared in deionized-distilled water (ELGASTATUHQPS, ELGA, England).

A stock standard solution of phosphorus (100 mgP/L) was prepared from potassium dihydrogen phosphate (KH_2PO_4, Merck, Germany), and the working standard solutions were prepared by stepwise dilution of the stock solution at concentration of 1.0 - 12.0 mgP/L.

A color developing reagent was daily prepared by mixing 5 mL of 2.5 M sulfuric acid (H_2SO_4, RCI Labscan, Thailand), 0.5 mL of 8.2 mM potassium antimony tartrate ($C_4H_4KO_7Sb{\cdot}1/2H_2O$, Carlo Erba, Italy), 1.5 mL of 80 g/L ammonium molybdate tetrahydrate ($(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, Carlo Erba, Italy) and 3 mL of 0.1 M ascorbic acid ($C_6H_8O_6$, Carlo Erba, Italy).

Sample preparation

Four fertilizer samples with different percentages of phosphorus (5, 7, 16 and 24 % P_2O_5) were purchased from a gardening equipment store in Pathumthani, Thailand. Each sample, 0.1 g was accurately weighed and dissolved in 30 mL of deionized-distilled (DI) water. It was then filtered through a filter paper (Whatman No.1) and made up to mark in a 50 mL of volumetric flask with DI water. Further dilution with DI water into 10 mL volumetric flask was required before analysis (1 mL for the sample solution of 5 and 7 % P_2O_5, and 0.25 mL for 16 and 24 % P_2O_5). The sample solutions were analyzed by 1) the proposed μTAD method and 2) the standard spectrophotometric batch method [21] with a spectrophotometer (V1200, Mapada, China), equipped with a 1 cm cuvette.

Thread treatment method

100 % Cotton thread (i.d. 0.61 ± 0.07 mm, n = 10 by Olympus BX 50 microscope) was purchased from a handicraft store in Pathumthani, Thailand. Removal of possible impurities of the thread that could have been interferences was necessary, 1 g of thread (ca. 360 cm) was soaked in 100 mL of 1 % (w/v) sodium carbonate solution (Na_2CO_3, UNIVAR, Australia) then put into the mechanical stirrer (Color Squid, IKA, Germany) at 300 rpm for 30 min before changing the carbonate solution. After 4 cycles of soaking in Na_2CO_3 solution (total volume of 400 mL), followed by washing the thread with DI water to remove excess Na_2CO_3 until pH in the range of 6 - 7. Then, it was dried at room temperature for 24 h. The 1 g of the treated cotton thread could be fabricated the μTAD approximately 80 devices.

Fabrication and operating procedure of µTAD

Fabricating the µTAD was done by stitching method as shown in **Figure 1**. A polyethylene (PE) mesh canvas (7 cm × 7 cm) was cut into small pieces of 0.8 cm ×5 cm by knife cutter. Then, vertical grids of the small PE mesh canvas were removed to create a narrow long space. The thread was stitched onto the edges of PE mesh canvas and established the hydrophobic ends with nail polisher which the originally idea plan to use the thread end as the starting and finishing points. However, in the proposed method, with/without barrier will not affect the results. The effective length of the thread was 4.5 cm, and the capacity of solutions absorption was 12 µL.

The experimental procedure was conducted at room temperature. The µTAD was placed on a small piece of white paper. For each measurement, 4 µL of the color developing reagent and 8 µL of a sample solution was sequentially loaded into the thread using a micropipette at the middle of the effective length. Then the µTAD was covered with transparent adhesive tape (3 M, Thailand) for 2 reasons 1) to prevent evaporation of the solutions during reaction process [9,22] and 2) to intensify the color of the photos. The reaction time of 6 min after the addition of the sample solution, the µTAD was then photographed inside a studio box by a 10M pixel digital camera (DSC-S5000 Cyber-shot, Sony) with no flash and 35 mm focal length. The distance between the camera to the µTAD was fixed at 26 cm to obtain reproducible image. The color profile of the pictures was evaluated by Pipette®, a freeware image processing and analysis software (https://www.sttmedia.com/pipette). The blue colored profile of PMB complex was easily examined just by pointing cursor at the reagent spotting area for detecting 3 adjacent spots per device (This size of the pointed area is 1 pixel). The mean value was then inverted to a recognition analytical response in RGB mode using the red value channel due to its highest sensitivity. The analytical responses corresponding to phosphorus concentrations were corrected by subtracting the mean intensity of PMB on thread with reagent blank. In addition, standard deviation (SD) of this work was obtained from at least triplicated measurements of 3 µTADs.

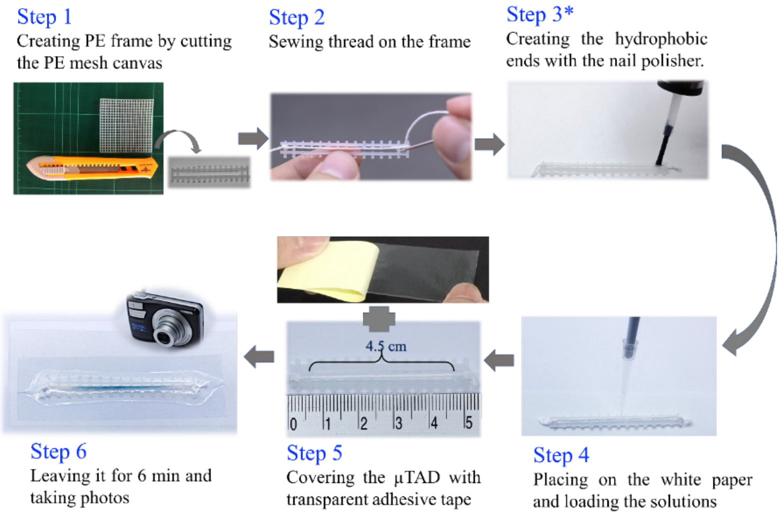

Figure 1 Photographs of the µ-TAD fabrication and operating procedure for phosphate detection; *However, in the proposed method, with/without barrier will not affect the results.

Results and discussion

The proposed colorimetric µTAD method relies on the well-known molybdenum blue reaction between orthophosphate (*o*-PO_4^{3-}) and a mixture solution of color developing reagent to determine phosphorus contents. Here *o*-PO_4^{3-} was reacted with ammonium molybdate in acidic medium to produce 12-MPA complex that is subsequently reduced to PMB complex by ascorbic acid with antimony (Sb(III)) as a catalyst.

Thread treatment method

Preliminary investigation was first made on the color change of untreated cotton thread after loading only the color developing reagent. The blue color was immediately appeared even the absent of *o*-PO_4^{3-}

solution. Therefore, the cotton threads required some treatments to remove the remaining possible interferences (*i.e.,* sodium hydrosulphite ($Na_2S_2O_4$) as reductive bleaching agents or residual phosphate-detergents used in textile industrial process [23]) prior o-PO_4^{3-}analysis.

1 % w/v Na_2CO_3 solution and DI water was tested as treated solutions. The dilute Na_2CO_3 solution, which are commonly used for removal the fiber surface wax in the thread treatment method [17,19], was examined as the treated solution based on the purpose of this work whereas DI water was examined for observation based on non-chemical treatment method. Untreated cotton threads 1 g were submerged in the tested solutions (a 100 mL aliquot of Na_2CO_3 (1 % w/v) or a 1,000 mL aliquot of DI water) with mechanical stirring 300 rpm at ambient temperature by varying numbers of scouring time (30 min per aliquot for 1 % w/v of Na_2CO_3 and 60 min per aliquot for DI water). Then 2 cm of thread samples (untreated and treated threads) were cut and rinsed with DI water, followed by dipping in aliquots of 500 µL of the color developing reagent for investigation of the color change on the thread within 10 min.

The results in **Figure 2** revealed that the suspected interferences can be eliminated by using only DI water or dilute Na_2CO_3 solution. However, Na_2CO_3 (1 % w/v) offered significantly higher efficiency of interferences removal than DI water since the treatment process was much faster than using DI water.

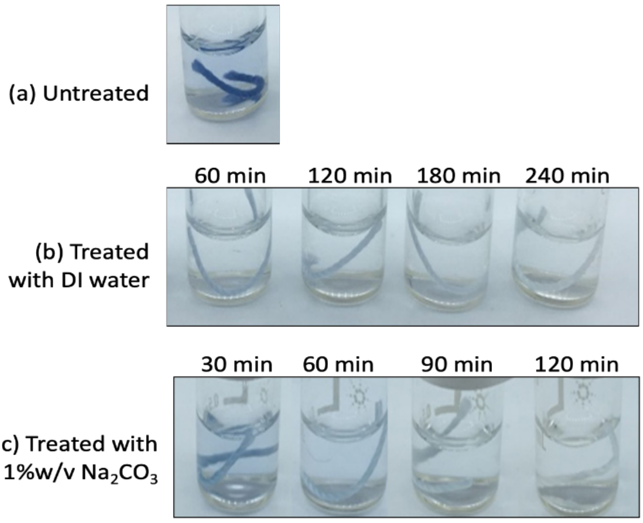

Figure 2 Investigation of treatment methods. (a) untreated thread, (b) treated with 1,000 mL of DI water, and (c) treated with 100 mL of 1 % w/v Na_2CO_3.

Optimization of chemical composition on color developing

The reagent conditions described by the APHA in 4500-P E. Ascorbic Acid Method [21] were used as an approximate starting point for optimization of chemical composition on the color intensity of PMB complex. The PMB formation by using ascorbic acid ($C_6H_8O_6$) as reducing agent and antimony (Sb(III)) as catalyst are 2 steps reactions as follow [3].

$$PO_4^{3-} + 12MoO_4^{2-} + 27H^+ \rightarrow \underset{\text{12-MPA}}{H_3PO_4(MoO_3)_{12}} + 12H_2O \qquad (1)$$

$$H_3PO_4(MoO_3)_{12} + C_6H_8O_6 \quad \overrightarrow{Sb} \quad \underset{\text{PMB}}{[H_4PSb_2Mo_{12}O_{40}]^{3-}} \qquad (2)$$

Effect of volume ratio of color developing reagent and sample solution

The volume capacity of the cotton thread in this study is 12 µL thus the limit volume of reagents. To accomplish the highest color intensity of PMB complex, the volume of color developing reagent and the volume of sample need to be considered based on the following criteria, not only the color developing reagent must be excessed but also the sample volume should not too little and become non-detectable or too much and become non-stoichiometrically. Also using too much of color developing reagent means that only small amount of sample can apply hence produce low analytical signal. Optimization of volume ratio between color developing reagent and sample (5 mg P/L solution) had been investigated at the combination of 6:6, 4:8 and 3:9 (reagent:sample with total volume of 12 µL). The ratio that produced highest color intensity will be chosen as the optimized ratio. **Figure 3** shows that the sample volume had effect on the color intensity of PMB complex. Increasing the sample volume from 6 µL to 8 µL resulting the increasing of color intensity but when the combination was 3:9 the intensity decreasing because of insufficient amount of color developing reagent. So, 4:8 was applied as the optimal volume ratio throughout this work.

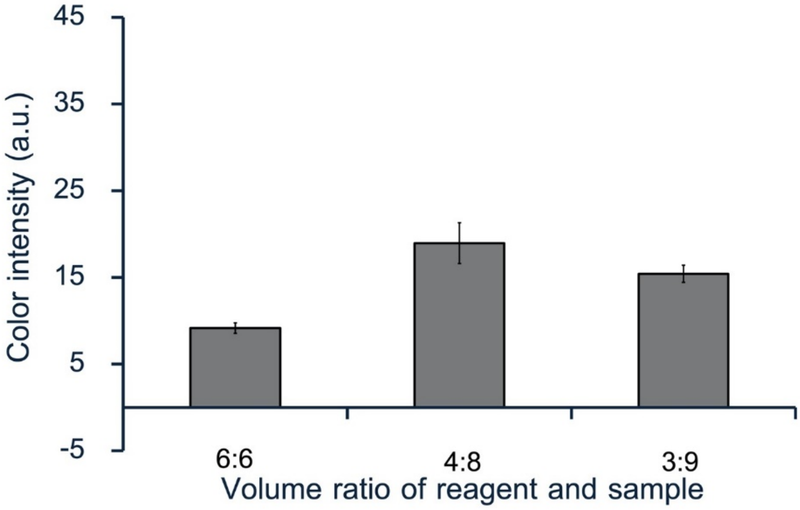

Figure 3 Effect of volume ratio of color developing reagent and sample solutions.

Effect of sulfuric acid concentration

Concentration of acid is one of the important parameters in the PMB formation because the reaction takes place in acidic medium as shown in the Eq. (1). There are reports on suitable pH for PMB formation reaction that the pH in the final reaction should not greater than 0.9 [9]. In this work, sulfuric acid (H_2SO_4) concentration was examined from 0.1 to 2.5 M by mixing 5 mL of each acid concentration in the color developing reagent (total volume of 100 mL). We found that the reaction was not carried out when the acid concentration was 1.5 M or lower (pH in final reaction around 1 at 4:8 volume ration) because self-reduction of molybdate ion occurs [9]. However, at high concentration of H_2SO_4 could diminish sensitivity because reaction rate of PMB formation was slow at any given time [3,4,9]. Therefore, 2.5 M H_2SO_4, which is equivalent to acid concentration of used in standard spectrophotometric batch method [21] and the pH of final reaction was approximately 0.8 at 4:8 volume ration, was considered as the optimum acid concentration.

Effect of ammonium molybdate and ascorbic acid concentrations

The effect of the concentration of ammonium molybdate on the color intensity value was studied in the range from 40 to 120 g/L, using 1, 6 and 12 mgP/L standards (**Figure4(a)**). The color intensity values increased when increasing the concentration of the ammonium molybdate up to 80 g/L and leveled off thereafter. Increasing ammonium molybdate concentration up to 120 g/L, resulted in the considerably increase the blank signal. Therefore, 80 g/L of ammonium molybdate was chosen as the optimum concentration for the color developing reagent.

Effect of ascorbic acid concentration

Ascorbic acid was functioned to reduce the 12-MPA thereby changing to PMB complex. Hence the concentration of ascorbic acid was optimized in range of 0.1 - 0.5 M for the 1, 6 and 12 mg P/L standards (**Figure 4(b)**). Results show that no significant difference in the color intensity of PMB complex. This indicated that 0.1 M ascorbic acid was enough for oxidation of 12-MPA to form PMB complex.

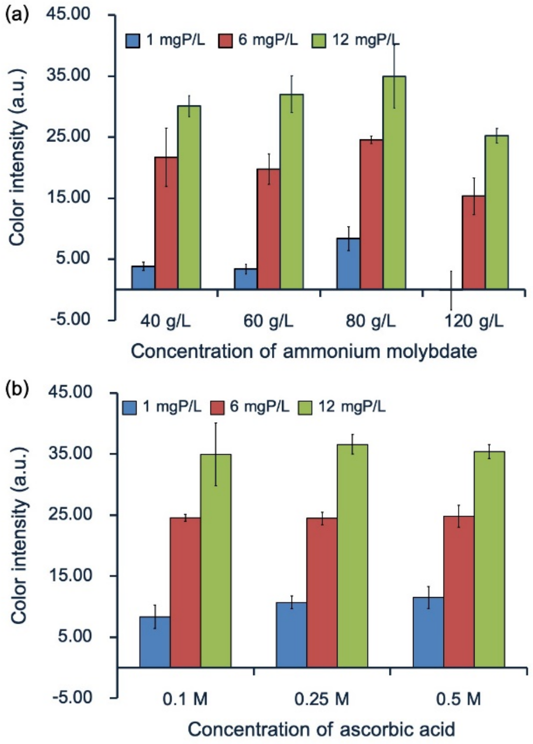

Figure 4 Effect of chemical concentrations in the color developing reagent; (a) concentrations of ammonium molybdate, and (b) ascorbic acid.

Effect of reaction time

Reaction time optimization was aimed to obtain sufficient sensitivity and swiftly analysis. Reaction time in this work is defined as the period between loading the sample solution into the cotton thread and the picture of μTAD device being taken. The reaction time of standards 3 and 12 mgP/L were examined between 6 to 10 min at room temperature (24 - 26 °C). **Figure 5** shows the relatively steadiness of color intensities over reaction times between 6 to 10 min. This indicates that the reaction had been completed under the optimal conditions of this work. Therefore, the reaction time of 6 min was selected as the sufficient time to achieve highest color intensity and sample throughput.

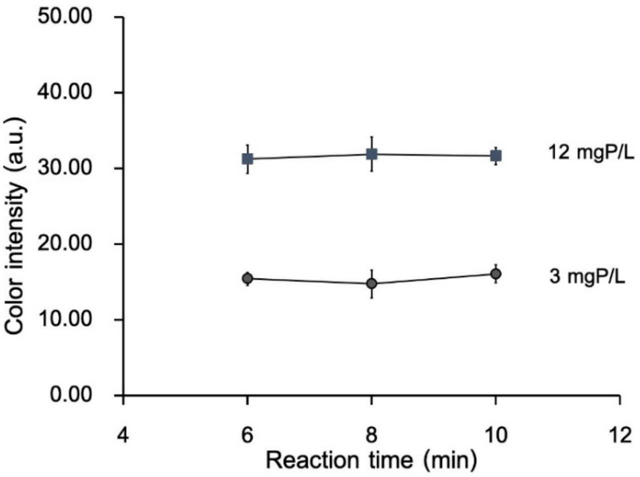

Figure 5 Effect of reaction time on color intensity of PMB.

Effect of transparent adhesive tape covering on μTAD

Since the formation reaction of the PMB complex is pH dependent, and the disappearance of solutions on μTAD through evaporation, especially sulfuric acid which could increase the mean intensity of reagent blank due to self-reduction of the molybdate (if pH in the final reaction above 0.9) [9]. Thus, in this work, influence transparent adhesive tape covering on the μTAD was examined by comparing the sensitivities of 3 - 12 mgP/L calibration curves (with/without adhesive tape).

With covering the adhesive tape on the μTAD, the sensitivity was increased. The slope obtained from adhesive tape covering, color intensity = (2.27 ± 0.05) [P] + (6.35 ± 1.01), was greater than the slope obtained from without covering, color intensity = (1.81 ± 0.09) [P] + (5.39 ± 1.51). These results indicated that use of the adhesive tape could improve sensitivity due to prevention of solution evaporation lose. In addition, it also provided focus and sharpness of the photos which enhance detection capability.

Recommended condition of the μTAD and analytical features

Table 1 presents a list of the investigated μTAD parameters. Each optimal parameter value corresponds to the highest color intensity of PMB, and fast analysis obtained by varying these parameters.

Under the optimal conditions, the proposed μTAD was characterized by linear working ranges of 1 - 12 mgP/L but the regression analysis suggested 2 separate regression lines in concentration range of 1 - 3 and 3 - 12 mgP/L. Linear equations fitted to the regression line represented the relationship of color intensity and mgP/L as formulae color intensity = (4.97 ± 0.01) [P] + (2.39 ± 0.01) with r^2 0.999 and color intensity = (2.27 ± 0.05) [P] + (6.35 ± 1.01) with r^2 0.995, for range 1 - 3 and range 3 - 12 mgP/L, respectively.

Reproducibility of the method, expressed as the relative standard deviation (RSD) of 10 measurements was 0.84 % (at 1 mgP/L), 0.92 % (at 3 mgP/L), and 1.04 % (at 10 mgP/L) which demonstrates high precision. The limit of detection (LOD), calculated using 3 times the standard deviation of the mean reagent blank using the regression method [9,24] was 0.24 mgP/L. The limit of quantitation (LOQ) was determined by the analysis of minimum concentration of standard solution that can be reported as a quantitative result. LOQ obtained from the proposed method was 1 mgP/L using the low-volume consumption of the reagent (8 μL) and standard (4 μL) solutions with reaction time of 6 min.

Fertilizers contain many nutrients to promote plant growth including primary macronutrients (nitrogen (N), phosphorus (P), and potassium (K)), lower levels of secondary macronutrient (calcium (Ca), magnesium (Mg), and sulfur (S)), and trace levels of minerals (iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), boron (B), and molybdenum (Mo)). In addition, silicate and arsenate are known to interfere in some condition of the analysis of phosphate in the molybdenum blue method. If the sample contains both species, the method is subjected to have positive inference because they can form similar blue complexes with molybdate. For these reasons, recovery studies were employed for evaluation of accuracy of the proposed μTAD method in real sample matrices. By spiking of standard phosphorus solution (3 mgP/L) into the sample solution. The proposed method presented good recoveries between 83 and 96 %. It proved that our method was suitable for analysis of phosphorus in fertilizers without interferences from other nutrients and sample matrices.

Table 1 Summary of the optimized µTAD parameters.

Parameter	**Range tested**	**Optimum value**
Cotton thread treatment method	DI water[a] or 1 % w/v Na_2CO_3[b]	1 % w/v Na_2CO_3
Volume ratio of reagent (µL): sample (µL)	6:6, 4:8, 3:9	4:8
Sulfuric acid concentration (M)	0.1 - 2.5	2.5
Ammonium molybdate concentration (g/L)	40 - 120	80
Ascorbic acid concentration (M)	0.1 - 0.5	0.1
Reaction time (min)	6 - 10	6
Transparent adhesive tape encapsulation	Yes or No	Yes

[a] 1,000 mL of DI water (4 times) at 300 rpm.

[b] 100 mL of 1 %w/v Na_2CO_3 (4 times) at 300 rpm.

A comparison of the proposed colorimetric µTAD method with previously reported colorimetric µPAD methodologies for analysis of phosphate [9-11] was shown in **Table 2**. Design and fabrication of microfluidic device from cotton thread are the special relevance in this work. When compare with µPADs, our µTAD fabrication method is much simpler and a lot less requirement. Stitching and tightening the thread on a PE mesh canvas is all there is, without the use of hydrophobic materials and expensive/specific equipment (*i.e.* oven, printer or custom mold). Comparison of the analytical features of the proposed µTAD with 3 reported on µPADs were given in **Table 2**.

Table 2 Comparison of the proposed µTAD with some recent studies on colorimetric µPADs reported in the literature.

Methods [ref]	**Materials**	**Fabrication methods**	**Design and Fabrication conditions**	**Sample matrices**	**Linearity range**	**LOD and LOQ**	**% Recovery**	**Reaction time for color development**
µPAD [9]	Whatman Grade 4 filter paper	AKD printing	Uses Microsoft Word™ to design the hydrophilic-hydrophobic zone. Requires heating (150 °C, 30 min) to melt alkenyl ketene dimer (AKD) to form hydrophobic barrier into paper.	CRM Soil water Natural water	0.1 - 1.0 mgP/L and 1.0 - 10 mgP/L	0.05 mgP/L and 0.16 mgP/L	99.6 - 101	10 min
µPAD [10]	Whatman Grade 1 filter paper	PDMS stamping	Uses custom design surface of the elastomeric stamp. Requires heating (80 °C, 30 min) to cure PDMS into paper	Standard phosphate solution	1 - 20 mgPO$_4^{3-}$ in 1 mL water (326 - 6,526 mgP/L)[a]	Not reported	Not reported	Not reported
µPAD [11]	Whatman Grade 4 filter paper	Wax printing	Uses Adobe Illustrator for pattern design. Requires wax printer and hot lamination (120 °C, 2 min) to melt the wax into paper.	Ultrapure water Synthetic fresh water Synthetic seawater Sargasso seawater	0.1 - 10 mgPO$_4^{3-}$/L (0.03 - 0.32 mgP/L)[a]	0.13 - 0.99 mgPO$_4^{3-}$/L (0.04 - 0.32 mgP/L)[a]	Not reported	4 min
µTAD (this work)	100 % cotton thread	Stitching	Simple design and simple fabrication (by stitching and tightening the thread	Fertilizer	1 - 3 mgP/L and 3 - 12 mgP/L	0.24 mgP/L and 1 mgP/L	83 - 96	6 min

Methods [ref]	Materials	Fabrication methods	Design and Fabrication conditions	Sample matrices	Linearity range	LOD and LOQ	% Recovery	Reaction time for color development
			on a PE mesh canvas) but can be applied for more complex design. Usually hydrophobic barrier was not necessary but if needed applied small drops of nail polisher on thread can create one.					

[a] calculated concentration

Application to sample and method validation

The proposed colorimetric µTAD was applied for analysis of phosphorus contents in fertilizer samples using the recommended condition. The results were compared with a standard spectrophotometric batch method [21] and the nominal values shown in **Table 3** including the images of real devices subjected to different concentrations of phosphorus samples. Statistical analysis at 95 % confidence level by linear regression method [24], showed no significant difference between the proposed µTAD and standard method. The output yielded the correlation coefficient (r) of 0.9996 which close to 1.00, and the intercept was −0.9217, with lower and upper confidence limits of −2.1149 and + 0.2714: This range included the ideal value of zero. In addition, the accuracy of the µTAD method was evaluated by fortifying a series of sample solutions, with known concentration of phosphate standard. All these results showed that the developed method was accurate and reliable.

Table 3 Determination of phosphorus contents in fertilizer samples by using the proposed µTAD method and standard spectrophotometric batch method.

Sample	Phosphorus content (% P_2O_5), (n = 3)			Device	Color profile	Recovery[a] (%)
	Nominal	Batch method	µTAD method			
A	5	5.53 ± 0.40	5.33 ± 0.74		■	96
B	7	5.45 ± 0.32	5.77 ± 0.76		■	95
C	16	10.35 ± 0.47	11.25 ± 1.55		■	94
D	24	22.19 ± 1.27	25.27 ± 2.75		■	83

[a] mean recovery of the proposed µTAD method

Conclusions

The proposed µTAD method relies on implementation of the well-known molybdenum blue reaction using commercially available 100 % cotton thread substrate for determination of phosphorus content in fertilizers. The µTAD was simply designed from a single cotton thread (4.5 cm) fastened on the PE mesh canvas. Simplicity of the design resulting in the ease of fabrication procedures allowed production of 80 devices from 1 g of the thread in very little time. The estimated cost of the proposed µTAD was only 1 baht/device ($0.032 USD/device). The proposed colorimetric µTAD method was fast analysis, high sensitivity (as low as nmol P level within 6 min), yield satisfactory precision and using very small amount of reagent and sample solutions. Thus, this µTAD method would be applicable as low-cost and portability. Moreover, good analytical performances of this µTAD assured reliability and potentially to apply for various environmental samples for analysis of phosphate.

Acknowledgement

The authors would like to acknowledge Department of Chemistry, Faculty of Science and Technology, Thammasat University, Thailand for facility supports.

References

[1] S Somnam, K Grudpan and J Jakmunee. Stopped-flow injection method for determination of phosphate in soils and fertilisers. *Maejo Int. J. Sci. Tech.* 2008; **2**, 17281.

[2] W Tiyapongpattana, P Pongsakul, J Shiowatana and D Nacapricha. Sequential extraction of phosphorus in soil and sediment using a continuous-flow system. *Talanta* 2004; **62**, 765-71.

[3] EA Nagul, ID McKelvie, P Worsfold and SD Kolev. The molybdenum blue reaction for the determination of orthophosphate revisited: Opening the black box. *Anal. Chim. Acta* 2015; **890**, 60-82.

[4] P Worsfold, I McKelvie and P Monbet. Determination of phosphorus in natural waters: A historical review. *Anal. Chim. Acta* 2016; **918**, 8-20.

[5] FTSM Ferreira, LS Mesquita, RBR Mesquitaand and AOSS Rangel. Improved sequential injection method for phosphate quantification within a wide dynamic range with in-line pre-concentration to monitor soil leachates. *Talanta Open* 2020; **2**, 100015.

[6] NK Ibnul and CP Tripp. A solventless method for detecting trace level phosphate and arsenate in water using a transparent membrane and visible spectroscopy. *Talanta* 2021; **225**, 122023.

[7] S Pradhan and MR Pokhrel. Spectrophotometric determination of phosphate in sugarcane juice, fertilizer, detergent and water samples by molybdenum blue method. *Sci. World* 2013; **11**, 58-62.

[8] JM Estela and V Cerdà. Flow analysis techniques for phosphorus: An overview. *Talanta* 2005; **66**, 307-31.

[9] BM Jayawardane, ID McKelvie and SD Kolev. A paper-based device for measurement of reactive phosphate in water. *Talanta* 2012; **100**, 454-60.

[10] B Waghwani, S Balpande and J Kalambe. Development of microfluidic paper basedanalytical device for detection of phosphate in water. *Int. J. Innovat. Tech. Explor. Eng.* 2019; **8**, 592-5.

[11] JM Racicot, TL Mako, A Olivelli and M Levine. A paper-based device for ultrasensitive, colorimetric phosphate detection in seawater. *Sensors* 2020; **20**, 2766.

[12] H Heidari-Bafroui, B Ribeiro, A Charbaji, C Anagnostopoulos and M Faghri. Portable infrared lightbox for improving the detection limits of paper-based phosphate devices. *Measurement* 2021; **173**, 108607.

[13] G Sriam, MP Bhat, P Patil, UT Uthappa, HY Jung, TAltalhi, T Kuimeria, TM Aminabhavi, R Krishna, P Madhuprasad and MD Kurkuri. Paper-based microfluidic analytical devices for colorimetric detection of toxic ions: A review. *TrAC Trends Anal. Chem.* 2017; **93**, 212-27.

[14] CT Kung, CY Hou, YN Wang and LM Fu. Microfluidic paper-based analytical devices for environmental analysis of soil, air, ecology and river water. *Sensor. Actuator. B Chem.* 2019; **301**, 126855.

[15] X Li, J Tian and W Shen. Thread as a versatile material for low-cost microfluidic diagnostics. *ACS Appl. Mater. Interfac.* 2010; **2**, 1-6.

[16] M Reches, KA Mirica, R Dasgupta, MD Dickey, MJ Butte and GM Whitesides. Thread as a matrix for biomedical assays. *ACS Appl. Mater. Interfac.* 2010; **2**, 1722-8.

[17] A Nilghaz, DR Ballerini and W Shen. Exploration of microfluidic devices based on multi-filament threads and textiles: A review. *Biomicrofluidics* 2013; **7**, 051501.

[18] S Sateanchok, S Wangkarn, C Saenjum and K Grudpan. A cost-effective assay for antioxidant using simple cotton thread combining paper-based device with mobile phone detection. *Talanta* 2018; **177**, 171-5.

[19] P Jarujamrus, N Malahom, S Puchum, R Meelapsom, M Amatatongchai, A Siripinyanond, S Chairam and C Kulsing. Complexometric and argentometric titrations using thread-based analytical devices. *Talanta* 2018; **183**, 228-36.

[20] S Farajikhah, JM Cabot, PC Innis, B Paull and G Wallace. Life-saving threads: Advances in textile-based analytical devices. *ACS Comb. Sci.* 2019; **21**, 229-40.

[21] LS Clesceri, AE Greenberg and AD Eaton. *4500-P E. Ascorbic acid method. In*: APHA-AWWA-WEF (Ed.). Standard methods for the examination of water and wastewater analysis. 23th ed. American Public Health Association, Washington DC, 2017, p. 524-5.

[22] A Gonzalez, M Gaines and FA Gomez. Thread-based microfluidic chips as a platform to assess acetylcholinesterase activity. *Electrophoresis* 2017; **38**, 996-1001.

[23] A Walters, D Santillo and P Johnston. *An overview of textiles processing and related environmental concerns.* University of Exeter, Exeter, England, 2005.

[24] JN Miller and JC Miller. *Statistics and chemometrics for analytical chemistry*. Prentice Hall, New Jersey, 2010.