Determination of phosphorus in water and chemical fertilizer samples using a simple drawing microfluidic paper-based analytical device

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Abstract
A simple one-step drawing for the cost-effective fabrication of microfluidic paper-based analytical devices (µPADs) for the determination of phosphate content in water and fertilizer samples is presented in this paper. The hydrophobic barrier of µPAD was patterned using a 2-mm tip marker pen using a transparent acrylic sheet template. The molybdenum blue reaction using ascorbic acid as a reducing agent was used. A pre-concentration step of samples is proposed to improve the sensitivity of the measurement. The blue complex produced on the µPADs was recorded using a smartphone camera. The color intensities (red, green, blue and gray) were analyzed using ImageJ program. The proposed µPAD method provides a linear calibration range from 0 to 100 mg L⁻¹ P. The limit of detection (LOD) was found to be 0.7 mg L⁻¹ P with a precision of 3.1% RSD for 50 mg L⁻¹ P (n = 10). The proposed method was successfully applied to the determination of phosphorus contents in water and liquid chemical fertilizer samples. The results obtained from µPAD agreed with a spectrophotometric method using paired t test at a 95% confidence level.

Keywords
Microfluidic paper-based analytical device · Phosphate · Water · Fertilizer · Drawing

Introduction
Microfluidic paper-based analytical devices (µPAD) are green chemistry techniques which widely used [1–3]. There are many advantages of µPAD, including micro-liquid consumption, low waste production, low cost, portable, and simple methods [4]. Therefore, µPADs are applied in many fields, such as diagnostic, food, forensic science, and environmental analysis [1–3, 5, 6].

There are several techniques reported in the literature to produce paper-based microfluidic devices, such as photolithography [7], inkjet etching [8], plasma treatment [9], wax printing [4], inkjet printing [10], flexography printing, screen printing [6] and drawing [11, 12]. These fabrication techniques were developed to create a hydrophobic barrier on paper substrate. The drawing fabrication technique is a simple, low cost and instrument-free technique which can use a permanent marker [11–13] or a wax pen drawing on a filter paper [14].

Phosphorus is an essential nutrient for plant and animal growth. Phosphorus in orthophosphate form (PO₄³⁻) is the available form used by plants usually found in natural waters. Furthermore, phosphates stimulate the growth of plankton and water plants that provide food for fish [15]. This may increase fish population and improve the waterway’s quality of life. However, at high phosphate level, algae and water weeds grow rapidly (algal bloom) [16], choking the waterway, and using up large amounts of oxygen. Many
Fish and aquatic organisms can die. The recommended maximum phosphate concentration in ground and surface waters is 0.1 mg L\(^{-1}\) P [17].

Moreover, phosphorus is one of the main nutrients in fertilizers. Chemical fertilizers are widely used because of their exact amount of minerals. Normally, chemical fertilizers contain the main plant nutrients such as nitrogen, phosphorus, and potassium [18, 19]. Therefore, it is necessary to analyze the amount of nutrients in fertilizers to control their quality. In Thailand, the chemical fertilizers must have a certified amount of nutrients not less than 10% and have to be specified on the label. The phosphorus content in fertilizers is determined as total phosphorus (total phosphorus, P\(_2\)O\(_5\)), which is the value indicated on the fertilizer label [20]. Therefore, the determination of phosphorus in water and fertilizers are an essential factor for quality control.

Several analytical methods have been employed for the determination of phosphorus in water samples such as chemiluminescence [21, 22], amperometric [23], and spectrophotometric detection [24–26]. The most used method for the analysis of phosphate is molybdenum blue (MB) method. It involves the reaction of the orthophosphate with ammonium molybdate under acidic conditions to form 12-molybdo phosphophosphate, a yellow-colored complex. This complex is reduced by either ascorbic acid or stannous chloride in the presence of antimony to give a phosphor-MB complex, which is spectrophotometrically determined at 660–880 nm, depending on reaction conditions [27].

In this research, a microfluidic paper-based analytical device (µPAD) for the determination of phosphate in water and fertilizer samples has been developed. The µPADs were fabricated using a simple writing method. The molybdenum blue color complexes on the µPADs were recorded using a smartphone and analyzed with the ImageJ program.

### Experimental

#### Reagents and standards

All chemicals used were analytical reagent grade. Deionized water (Sartorius, Arium Advance EDI, Germany) was employed for standard and reagent preparations.

**Reagents and standards for µPAD method**

0.02 mol L\(^{-1}\) ammonium molybdate reagent (R1) was prepared by dissolving 0.6206 g of ammonium heptamolybdate tetrahydrate ((NH\(_4\))\(_6\)Mo\(_7\)O\(_{24}\)·4H\(_2\)O) (Ajax Finechem, Australia) and 0.061 g tartaric acid ((CHOCH\(_2\)COOH)\(_2\)) (Ajax Finechem, Australia) were dissolved in 0.6 mol L\(^{-1}\) of sulfuric acid (H\(_2\)SO\(_4\)) (LabScan, Ireland), and then adjust volume to 25 mL [28]. R1 solution was stored in a brown glass-stopped bottle and kept in the refrigerator. These solutions are allowed to reach room temperature before use in the phosphate analysis. The 0.01 mol L\(^{-1}\) ammonium molybdate reagent was prepared by dilution of the 0.02 mol L\(^{-1}\) ammonium molybdate reagent.

Ascorbic acid reagent (R2) was daily prepared by dissolving 0.4403 g of L-ascorbic acid (C\(_6\)H\(_8\)O\(_6\), Chem-supply, Australia) and adjusted to a volume of 5.0 mL with deionized water.

Crystals of potassium dihydrogen phosphate (KH\(_2\)PO\(_4\)) was oven dried at 120 °C for 2 h and then stored in the desiccator until used. Stock solution of 100 mg L\(^{-1}\) P was prepared by dissolving 0.0439 g of potassium dihydrogen phosphate (Ajax Finechem, Australia) in water and made up to 100.0 mL.

#### Reagents and standards for the spectrophotometric method

A 0.0699 g weight of potassium antimony tartrate (Ajax Finechem, Australia) was dissolved in water and adjusted to volume in a 25-mL volumetric flask. A 2.2092 g amount of ammonium molybdate (Ajax Finechem, Australia) was dissolved in water and adjusted to volume in a 50-mL volumetric flask. A 0.8815 g weight of L-ascorbic acid (C\(_6\)H\(_8\)O\(_6\), Chem-supply, Australia) was dissolved in water and adjusted to volume in a 50-mL volumetric flask.

The combined reagent was prepared by mixing 5 mL of 2.5 mol L\(^{-1}\) H\(_2\)SO\(_4\), potassium antimony tartrate solution, 15 mL of ammonium molybdate, and 30 mL of ascorbic acid.

Vanadiumolybdate reagent was prepared in two steps. A solution of 2.0018 g of ammonium molybdate in 20 mL of water was first prepared. Then 0.1038 g of ammonium metavanadate (Ajax Finechem, Australia) in 20 mL of 70% HClO\(_4\) (LabScan, Ireland). Finally, the ammonium molybdate and ammonium metavanadate solutions were mixed and adjusted to volume with water in a 100 mL volumetric flask.

#### Sample collection and preparation

Natural water samples were collected from the Lopburi River and canals in the area of Mueang Lop Buri District, Lopburi Province, Thailand. The samples were filtered with a 0.45 μm porous membrane (Cellulose acetate membrane, Satorious stedium biotech, Germany). The samples were stored in a refrigerator at 4 °C and left at room temperature before analysis. All collected samples were analyzed within 2 days [29, 30].

Liquid chemical fertilizers were purchased from local agricultural suppliers in Lopburi, Thailand. The liquid
fertilizers were diluted with deionized water to be within the linear working range of the µPAD and spectrophotometric methods [30].

**Design and fabrication of the device**

The microfluidic paper-based analytical devices (µPADs) were fabricated by drawing the hydrophobic pattern with a permanent marker pen using a transparent acrylic sheet as a template. The template was designed to have multiple circular holes, each with a diameter of 12 mm (Sciware Systems SL, Bunyola, Spain), as shown in Fig. S1. The template was placed on filter paper (125 mm diameter, Whatman No.1, UK). The pattern was drawn with a black permanent marker pen (Horse, twin-pen, Thailand). The permanent marker pen has 2 types of tip, a pointed tip and a square tip. The pointed tip was used in this work because it gave a consistent line thickness, as shown in Fig. 1. After drawing, the filter paper was left at room temperature for about 5 min or until the ink became dry. The µPADs were kept in a plastic zip bag and placed in a dry container. The circular paper was cut into squares for use. The average diameter of the detection zone of µPADs, obtained by measuring 10 µPADs with a digital vernier caliper, was 8.0 ± 0.3 mm (3.9%RSD, n = 10). The thickness of the hydrophobic part was 2.00 ± 0.12 mm (6.0%RSD, n = 10).

The performance of the hydrophobic barrier was tested by dropping a red color solution (Winner’s strawberry red food additive, Thailand) into the detection zone of the µPAD. The images at 10× and 20× magnification were obtained with a stereo microscope (Stereo Microscope MDX503, Nikon, Japan). The images show that the red solution was confined to the measurement portion defined by the black circular ring (Fig. S2). Therefore, the black marker pen could be used for drawing hydrophobic barrier.

**Analytical procedures**

**µPAD microfluidic device method**

The operating procedure for phosphorus analysis is shown in Fig. 2. In step 1, 2.5 μL of standard solution or sample is added to the detection zone of µPAD. This aliquot is blow-dried for 15 s at room temperature (step 2) [30]. An aliquot of 2.5 μL ammonium molybdate (R1) is added and left to dry at room temperature for 1 min (step 3). Then 2.5 μL of ascorbic acid reagent (R2) is added to the reservoir (step 4), leading to a blue color complex. The µPAD is placed in the light control box at a distance of 20 cm between the µPAD and the camera to obtain the entire image of the µPAD. The image of the colored detection zone is recorded with a smartphone camera (Samsung, galaxy A6 plus, Korea).

![Fig. 1 Schematic diagrams showing the fabrication method](image)
exactly 5 min after the addition of ascorbic acid solution (step 5) (Fig. 2a). The pre-concentration step of standard or sample solution is carried out by repeating steps 1 and 2, followed by steps 3–5 (Fig. 2b). The intensities of the pictures were analyzed using the ImageJ program. Analyzed area of the detection zone of the µPAD was defined by the circular line set in the software.

**Spectrophotometric method**

A spectrophotometric method for the determination of phosphate was used as the reference method [31]. The molybdenum blue reaction was used as the reference method for phosphorus measurement of water samples. For this purpose, 0, 0.1, 0.25, 0.50, 0.75 and 1.00 mg L\(^{-1}\) phosphate

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Fig. 2 Operating procedure of µPADs for phosphorus analysis a without a preconcentration step, and b with a pre-concentration step
standard solutions were prepared. Volumes of 0, 100, 250, 500, 750 and 1000 µL of 10 mg L\(^{-1}\) P standard were pipetted into 10-mL volumetric flasks. A few drops of phenolphthalein indicator were added to these flasks. Then 1.0 mol L\(^{-1}\) NaOH was added dropwise to just discharge a pink color and the solutions restored to colorless with one drop of 2.5 mol L\(^{-1}\) H\(_2\)SO\(_4\) solution. A 2.00 mL aliquot of the combined reagent was added to each flask, followed by dilution to 10.00 mL using deionized water. After 30 min, the absorbances of these solutions were measured at 880 nm with a spectrophotometer (Thermo Scientific, Evolution 201 UV–visible Spectrophotometer, USA). A calibration curve was plotted between absorbance and concentration of phosphorus.

The vanadomolybdate method was used for the analysis of phosphorus in fertilizer samples. Standard solutions of phosphorus, 1–7 mg L\(^{-1}\) P, were pipetted from a stock of 100 mg L\(^{-1}\) P into 10-mL volumetric flasks, 1 mL of vanadomolybdate reagent was added and adjusting to volume with deionized water. After 30 min the absorbance of the yellow solutions was measured at 420 nm.

**Results and discussion**

**Optimization of the experimental conditions**

**Effect of the number of addition of standard solution on to µPAD**

To apply the pre-concentration technique, the number of addition of a standard substance to the µPAD was studied. The procedure without the pre-concentration step is shown in Fig. 2a, where 25, 50, 75 mg L\(^{-1}\) of phosphorus was used. After that, a similar experiment was performed, but the standard was added two or three times (steps 1–2), respectively. Ammonium molybdate (R1) at 0.01 mol L\(^{-1}\) in 0.6 mol L\(^{-1}\) H\(_2\)SO\(_4\), and 0.50 mol L\(^{-1}\) ascorbic acid (R2) with reaction time of 5 min were used for this study. The sensitivity (slope) of the red intensity of the calibration plot was determined. The results, as shown in Fig. 3, indicate that the sensitivities obtained using pre-concentration steps were higher than without a pre-concentration step. The sensitivities obtained from 2 and 3 additions were not different. Furthermore, the coefficient of determination (\(r^2\)) of multiple additions was greater than 0.99. Therefore, the procedure with 2 additions was selected in further works (Fig. 2b).

**Effect of reaction time**

Reaction time in this study is the minimum period of time for the reaction to be considered essentially complete and the intensity of the color of the blue complex constant. The reaction time starts after the addition of ascorbic acid solution (R2) to the paper. The reaction was followed from 0 to 30 min using 50 mg L\(^{-1}\) phosphorus standard as the sample. From the experimental results, it was found that the blue product increased with increasing reaction time from 1 to 5 min, and then remaining constant from 5 to 25 min, as shown in Fig. 4a. After 25 min the blue color of the reaction product changed because the paper became dry. Therefore, reaction time of 5 min was chosen for this work.

**Effect of sulfuric acid concentration in the preparation of ammonium molybdate solution.**

The effect of sulfuric acid concentration used in the preparation of the ammonium molybdate solution ranged from 0.3 to 8 mol L\(^{-1}\), as shown in Fig. 4b. When the concentration of sulfuric acid increased, the intensity of the blue color complex was reduced (red intensity increased). Although a sulfuric acid concentration of 0.3 mol L\(^{-1}\) produced the highest blue color, the blue product can undergo self-reduction from Mo(VI) to Mo(V) [32]. Therefore, 0.6 mol L\(^{-1}\) was chosen for this study because the color of the product was sufficiently intense and did not undergo self-reduction when the ammonium molybdate solution is left for a long time.
Effect of ammonium molybdate concentration (R1)

Ammonium molybdate solutions ranging from 0.001 to 0.020 mol L\(^{-1}\) in 0.6 mol L\(^{-1}\) H\(_2\)SO\(_4\), were studied. The experimental data (Fig. 4c) showed that the measured red intensity decreased when the ammonium molybdate concentration increased from 0.001 to 0.010 mol L\(^{-1}\). However, the color intensity remained almost constant for 0.010 to 0.020 mol L\(^{-1}\) ammonium molybdate. Therefore, 0.010 mol L\(^{-1}\) ammonium molybdate was chosen for this work.

Effect of ascorbic acid concentration (R2)

In this experiment, ascorbic acid was used as the reducing agent to change ammonium molybdophosphate which is a yellow complex to molybdenum blue which is a blue complex. In this work, ascorbic concentrations ranging from 0.010 to 0.600 mol L\(^{-1}\) mol/L were studied. A 0.010 mol L\(^{-1}\) ammonium molybdate (R1) prepared in 0.6 mol/L sulfuric acid was used. In Fig. 4d, the red color intensities are constant from 0.250 to 0.600 mol L\(^{-1}\) ascorbic acid, showing that these concentrations are sufficient to give a complete chemical reaction. To take into account the instability of the ascorbic acid during the measurement period of one day, the 0.500 mol L\(^{-1}\) concentration was chosen to ensure sufficient ascorbic acid throughout the experiment.

Analytical features

The calibration curve of phosphorus analysis by the proposed µPADs method was produced using the optimal conditions as described above, viz. 0.01 mol L\(^{-1}\) ammonium molybdate in 0.6 mol L\(^{-1}\) H\(_2\)SO\(_4\), 0.50 mol L\(^{-1}\) ascorbic acid, with reaction time 5 min. The image of the blue complex of the reaction product recorded with a smartphone camera was analyzed for intensity values (red, green, blue and gray intensity scale) using ImageJ program. The results of the analysis revealed that all color intensity scales gave good linear plots (see Fig. S3). However, the red intensity provides the highest sensitivity (slope of calibration) since red is the complementary color of the blue color of the product. In this work, the red intensity was chosen for the phosphorus analysis. The linear calibration range from 0 to 100 mg L\(^{-1}\) P (Fig. 5). The measurement limit or LOD was 0.7 mg L\(^{-1}\) P (LOD = [(mean value of blank-3SD of blank) − intercept]/(slope)), and the relative standard deviation of 3.06% for 50 mg L\(^{-1}\) P (n = 10).

Fig. 4 Effect of a reaction time, b sulfuric acid concentration, c ammonium molybdate concentration, and d ascorbic acid concentration on the measured red intensity values
Interference study

Silicate and arsenate are the common ions that can affect the formation of molybdenum blue complex [33, 34]. To study these interference ions, various concentrations of either Si(IV) or As(V) were added to a 25 mg L\(^{-1}\) standard phosphate. The concentration of Si or As which gave an alteration of the signal of ± 3SD of the signal of the phosphate standard was considered as the maximum interference level for this work.

For the silicate interference study, concentrations of Si standard ranging from 25 to 120 mg L\(^{-1}\) Si were studied. The signal obtained from these solutions was compared to the signal of the control solution (25 mg L\(^{-1}\) standard phosphate). The results indicated that the µPAD method has a tolerance limit of 75 mg L\(^{-1}\) Si (Fig. S4a). Silicate concentrations in natural waters are in the range of 0–28 mg L\(^{-1}\) Si [28]. Therefore, silicate would not significantly interfere with the phosphate determination in our system.

For the arsenate interference study, experiments were performed similar to the silicate interference study, but various concentrations of arsenate from 0.10 to 3.0 mg L\(^{-1}\) As were employed. The µPAD method has the tolerance limit of more than 3.0 mg L\(^{-1}\) As (Fig. S4b). However, the maximum concentration of arsenic in unpolluted natural water should not exceed 0.005 mg L\(^{-1}\) As [34]. Therefore, the proposed method also provides a high tolerance limit for arsenate in natural water samples.

Application and validation

The µPADs method was applied to the determination of phosphorus in surface water samples (water samples 1–5) from Muang District, Lopburi Province, Thailand, and wastewater samples from laboratories. The chemical liquid fertilizer samples were purchased from local distributors in Lopburi Province.

For validation, the µPAD method was compared with the spectrophotometric method. Statistical data were compared using the paired \(t\)-test two samples for means in Microsoft Excel. No significant differences were found between these methods at 95% confidence level of paired \(t\) test, \(t_{\text{stat}} = 0.74 < t_{\text{crit}} = 4.30\) of water samples, and \(t_{\text{stat}} = 0.12 < t_{\text{crit}} = 12.71\) for fertilizer samples. The results of the study on the percent recovery (% recovery) obtained from adding 10 mg L\(^{-1}\) P of standard solution into each sample were in the range of 96–105%. The results of the study on the percent recovery (% recovery) obtained from adding 10 mg L\(^{-1}\) P of standard solution into each sample were in the range of 96–105%. The molybdenum blue reaction was used for water samples (Table 1). The vanadomolybdate reaction was used for fertilizer samples (Fig. S5, and Table S1).

The surface water samples collected from locations 1–3 had phosphate levels that were less than 0.7 mg L\(^{-1}\) P, the LOD of the system. The surface water samples 4 and 5 had phosphate levels higher than the surface water standard due to their locations being near houses and restaurants. Household effluents may be discharged into water sources. We found satisfactory recoveries of the water samples, even though our LOD is higher than the maximum permissible in natural water (0.1 mg L\(^{-1}\) P). Our developed method is still applicable as the screening test for a water source that is contaminated with phosphate above 0.7 mg L\(^{-1}\) P.

For liquid fertilizer samples, the phosphorus content was calculated in the form of P\(_2\)O\(_5\) according to the fertilizer product label. The formula 3-4-17 liquid fertilizer (Fertilizer 1) is recommended for fruits, the 12-9-5 (Fertilizer 3) for fruits and vegetables and formula 10-18-4 (Fertilizer 2) for field crops, such as corn, which is an important economy crop of Lopburi Province, Thailand. The phosphorus values (P\(_2\)O\(_5\)) in the fertilizer samples agreed with the standard method and are also close to the label values.

Currently, many studies have proposed methods for convenient phosphate analysis to reduce the chemical components when compared to the traditional standard method (see Table 2). For example, the automatic analyzer was used for the determination of orthophosphate in river water samples [35]. The automated analyzer is a flow system which consists of a proportional pump, colorimeter, and flow cell. This method can be used to determine concentrations of phosphorus in the range from 0.002 to 0.200 mg L\(^{-1}\) using molybdenum blue reaction. The concentration range may be extended to 2.00 mg L\(^{-1}\) by utilizing a dilution loop. Sample throughput is approximately 30 samples per hour [35, 36]. Nagul et al. proposed a highly sensitive flow analysis method for
rapid analysis of dissolved reactive phosphate using ethanol and UV light to reduce phosphomolybdic acid to molybdenum blue [37]. A wide linear range of 0.005–1.000 mg L⁻¹ P was obtained. Other flow system such as sequential injection analysis (SIA) is also widely used for automated measurements. Deng et al. proposed a fully automated integrated syringe pump-based environmental-water analyzer (iSEA) for the determination of phosphorus in riverine-estuarine-coastal waters [38]. The system could be used for automated pre-concentration of samples. The advantages of using SIA with selection valves are robustness, high precision and low reagent consumption. It can be seen that an automated phosphate analysis system reduces experimental waste because it uses less amount of chemicals. Furthermore, it can be controlled with a computer from the introduction of the sample to the final absorbance detection. However, these systems are considered expensive systems and the analyst must be proficient in using the instrument. Also, it may not be possible for on-site measurements.

The µPADs method is thus an option that is cheaper, use less chemicals and can perform on-site screening analysis to select samples for further analysis at the laboratory. In this work, only 2.5 µL of sample and reagents were used for the molybdenum blue reaction. Although smartphone colorimetric determination can be employed for liquid samples, the volume of the sample solution must be sufficient (≥ 60 µL) to detect changes in the transmittance [39–41].

Table 2 compares our proposed µPAD method with other paper-based methods for phosphorus determination. It can be seen that the detection limit of our work is higher than that of other methods. However, this work has many other advantages, such as very simple one-step fabrication, low cost, simple to use, and can be applied to the determination of phosphorus in both water and fertilizer samples. Other works use more complicated fabrication methods for making their multiple-layer paper devices [32, 42]. The cost of the commercial marker is 10 Thai baht or approximately US$ 0.33 that is used for the fabrication of the µPAD in this work. The cost of a single µPAD device is therefore approximately US$ 0.003.

### Conclusions

This work has described very simple and low-cost paper-based analytical devices (µPADs) for the determination of phosphorus (approximately $0.003 per a µPAD). The fabrication method uses only an acrylic template, a marker pen, and filter paper. The acrylic template was designed to be able to draw many circles on the filter paper using a marker pen with a tip size of 2 mm. The µPAD was applied for phosphorus measurement based on molybdenum blue complex reaction, using ascorbic acid as the reducing agent. A pre-concentration step (by adding standard solution or sample twice) was employed. The proposed method required a reaction time of only 5 min. The µPAD method was successfully applied to the determination of phosphorus in natural surface water, wastewater, and liquid chemical fertilizers. The results obtained from our µPAD method agreed with the spectrophotometric method as shown by the paired t test at a 95% confidence level. The proposed µPAD method provided many advantages over the batch spectrophotometric method.
and automated flow-based systems, such as low reagent consumption, low cost, and low waste production. Therefore, it is a method that is environmentally friendly. The linear calibration range of phosphorus in this work is from 0 to 100 mg L$^{-1}$ P and is suitable for on-site screening analysis of phosphorus in samples with the possibly high phosphorus content.

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**Table 2** Comparison of μPAD method of this work with other current studies for phosphorus analysis

| References | Method | Sample | Chemical reaction | Linear range | LOD | Throughput (samples/hour), or reaction time (minutes) |
|------------|--------|--------|-------------------|--------------|-----|-----------------------------------------------|
| [35]       | Automatic analyzer (colorimeter detection at 880 nm) | River water | Molybdenum blue (reducing agent: ascorbic acid) | 0.002–0.200, 0.02–2.00 mg L$^{-1}$ P | – | 30 samples/hour |
| [37]       | Flow injection analysis | Natural waters | Ethanol and UV light were used to reduce phosphomolybdic acid to molybdenum blue | 0.005–1.000 mg L$^{-1}$ P | 0.0013 mg L$^{-1}$ P | 57 samples/hour |
| [38]       | Fully automated integrated syringe pump-based environmental-water analyzer (iSEA) | Natural waters | Molybdenum blue (reducing agent: ascorbic acid) | 0–10 µmol L$^{-1}$, 0–80 nmol L$^{-1}$ | 0.11 µmol L$^{-1}$ (without pre-concentration step) | > 20 h$^{-1}$, > 8 h$^{-1}$ (pre-concentration volume 25 mL) |
| [32]       | µPAD Fabrication: lamination (2 layers of filter paper) | Natural and soil waters | Molybdenum blue (reducing agent: ascorbic acid) | 0.2—10 mg L$^{-1}$ P | 0.05 mg L$^{-1}$ P | Reaction time: 40 min |
| [42]       | Fabrication: lamination (2 layers of filter paper) | Freshwater | Molybdenum blue (reducing agent: ascorbic acid) | 3–10 mg L$^{-1}$ PO$_4^{3-}$ or 0.98–3.26 mg L$^{-1}$ P | 3 mg L$^{-1}$ PO$_4^{3-}$ | Reaction time: 3 min |
| This work  | µPAD Fabrication: drawing (1 step) | Natural waters, wastewater, and liquid fertilizers | Molybdenum blue (reducing agent: ascorbic acid) | 0–100 mg L$^{-1}$ P | 0.7 mg L$^{-1}$ P | Reaction time: 5 min |

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