

Fabrication of a Paper-Based Microfluidic Device To Readily Determine Nitrite Ion Concentration by Simple Colorimetric Assay

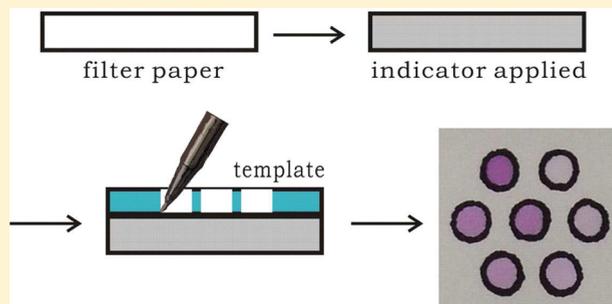
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S Supporting Information

ABSTRACT: Paper-based microfluidic devices (μ PAD) are a burgeoning platform of microfluidic analysis technology. The method described herein is for use in undergraduate and high school chemistry laboratories. A simple and convenient μ PAD was fabricated by easy patterning of filter paper using a permanent marker pen. The usefulness of the device was demonstrated by determination of nitrite ion concentration employing a colorimetric assay. The results for real sample detection using this μ PAD were satisfactory and show promise for use in other applications.

KEYWORDS: High School/Introductory Chemistry, First-Year Undergraduate/General, Second-Year Undergraduate, Analytical Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Laboratory Equipment/Apparatus, Microscale Lab, Quantitative Analysis



Microfluidics is the science and technology of systems that process or manipulate small (10^{-9} – 10^{-18} liters) amounts of fluids using channels with dimensions of tens to hundreds of micrometres.¹ After two decades of development, microfluidics has shown great potential as an emerging multidisciplinary field intersecting engineering, physics, chemistry and biochemistry, nanotechnology, and biotechnology. It has been widely used in genetics,^{2,3} cell analysis,^{4,5} clinical diagnosis,^{6,7} protein analysis,^{8,9} chemical synthesis,¹⁰ environmental monitoring,¹¹ and food analysis.¹²

While microfluidics is more and more frequently used by scientific researchers, it is still not very well-known among undergraduate students. An introduction of microfluidics into laboratory teaching is a good way for students to get familiar with this new technology, and it may be helpful in their later research as well. However, traditional microfluidic devices are quite sophisticated, and the chip fabrication process is both labor-intensive and time-consuming, which limits its entry into the teaching laboratory. The paper-based microfluidic device (μ PAD), a microfluidic device first introduced by the Whitesides group,¹³ has become a burgeoning platform for microfluidic chemistry.^{14,15} These devices have many unique advantages over traditional microfluidic chips including low cost, ease of use, and general convenience. Relatively expensive materials such as quartz, glass, silicon, or specialty polymers are avoided, and fabrication costs are minimal. The capillary action of paper substitutes for expensive pumps or other reagent delivery devices, and the overall chip design is readily replicated using commonly available technologies such as wax¹⁶ or inkjet¹⁷ printing, drawing,¹⁸ or other methods.^{19,20}

The cost-effective substrate and simple fabrication process makes paper-based devices promising mediums to bring

microfluidic concepts to high school^{21,22} and college students.²³ There have been many studies on the applications of μ PAD for forensic testing,²² food safety testing,²³ and medical diagnostics.²⁴ Here, a μ PAD for nitrite ion determination is developed and demonstrated. Nitrite exists naturally throughout the environment but is hazardous to human health. Excess nitrite ion can be fatal to infants by causing a condition known as methemoglobinemia,²⁵ and it also has a significant carcinogenic effect.²⁶ Hence, it is meaningful to introduce a simple nitrite ion detection method to students. In this 3-h experiment, a simple and convenient μ PAD is fabricated by easy patterning of filter paper using a permanent marker pen. On the prepared μ PAD, a colorimetric assay is employed to determine nitrite ion concentration in the samples. Finally, a camera-phone is used as the detector for the assay.

MATERIALS

The nitrite indicator was prepared by dissolving 0.86 g of *p*-amino benzenesulfonamide (J&K Scientific Ltd., Shanghai), 0.19 g of *N*-(1-naphthyl)ethylenediamine (J&K Scientific Ltd., Shanghai), and 6.34 g of citric acid (Wuxi Minfeng Reagent Factory, Jiangsu) in 20% (V:V) water in methanol solution to give a total volume of 100 mL. The indicator solution was then kept in the dark. Standard sodium nitrite solutions with concentrations of 0.0780, 0.156, 0.312, 0.625, 1.25, and 2.50 mmol L⁻¹ were obtained by diluting a 0.250 mol L⁻¹ sodium nitrite stock solution. Deionized water from a Milli-Q water purification system (Millipore, Bedford, MA) was used

throughout. All reagents were analytical grade. The real sample was pond water collected from a local pond source.

The templates (shown in Figure 1) made of poly(methyl methacrylate) (PMMA, 5 cm \times 5 cm; thickness, 2.0 mm) were

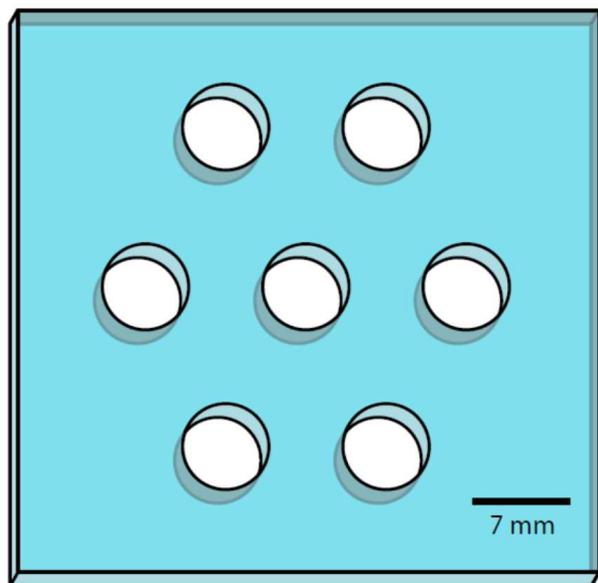


Figure 1. Schematic of the PMMA template.

custom-made by Nanchang Yinte Industrial Co., Ltd. A permanent marker pen (IENTI-PEN, STA) was used to transfer the pattern of the template onto a filter paper (Whatman No. 1). A camera-phone was used to capture images of the μ PADs.

RATIONALE

The filter paper's high water absorbing capacity provides a good medium for the colorimetric reaction to take place, which makes filter paper an ideal candidate for μ PAD. For a reaction on the paper, the reagents and samples can be driven and mixed by the paper capillary force. In this experiment, students use permanent marker pens to form hydrophobic barriers on a piece of filter paper with a patterned PMMA template. The hydrophobic barriers constrain the reaction between the indicator and nitrite solution in defined areas. N-(1-naphthyl)ethylenediamine and *p*-amino benzenesulfonamide are used as a mixed indicator. The nitrite ion reacts with sulfonamide by diazotization, followed by the coupling reaction between the

intermediate and N-(1-naphthyl)ethylenediamine. This causes a color change of the solution from colorless to red (Scheme 1). The resulting images on the μ PADs are then captured by a camera-phone. Adobe Photoshop CS5 software is employed to get the gray values of the color in the images. A calibration curve is built based on concentrations of the standard solutions and their corresponding gray values. The nitrite ion concentration of an unknown may be calculated according to the calibration curve.

FABRICATION OF THE PAPER-BASED DEVICE

Three pieces of filter paper (5 cm \times 5 cm) were placed into a dry glass Petri dish. Fresh indicator solution was poured into the dish to completely soak the paper. The presoaked filter papers were then taken out of the indicator solution and kept in the dark for about 20 min to dry. The PMMA template consisting of a 7-circle array was put onto the dried paper, and seven circles were drawn onto the paper with a permanent marker pen. The diameter of each circle was 7 mm, and the ink of the marker pen was left on the edge of the circles to create hydrophobic barriers around hydrophilic circular regions. Noteworthy, the presoaked μ PADs should be protected from light because the indicator is sensitive to light. Also, it is important that the hydrophobic barriers are only created after the indicator solvent was completely evaporated since the ink is soluble in methanol.

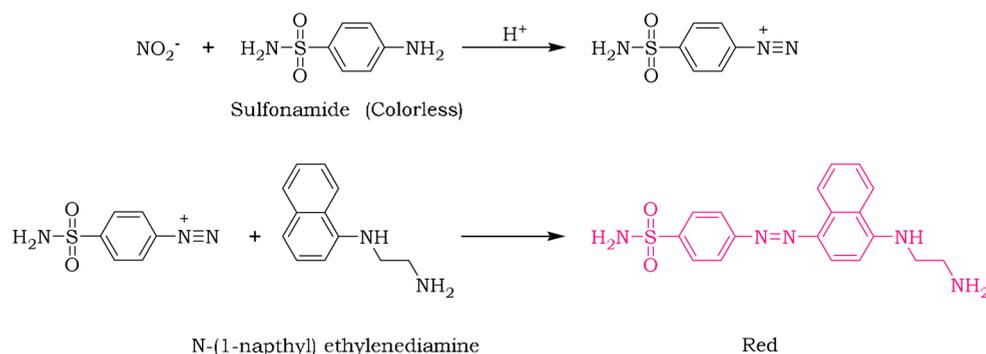
SAMPLE PREPARATION

Standard solutions with concentrations of 0.0780, 0.156, 0.312, 0.625, 1.25, and 2.50 mmol L⁻¹ were prepared from a 2.50 mol L⁻¹ sodium nitrite stock solution supplied by the instructor. Students were required to dilute 1.00 mL of the stock solution 100-fold, which gave 2.50 mmol L⁻¹ NaNO₂ solution. Two-fold serial dilution of this solution provided the reference standards mentioned above. The unknown sample solution, S₁, was prepared by 100-fold dilution of a stock solution provided by the instructor. In practice, 1.00 mL of the stock solution was delivered to a 100 mL volumetric flask and then diluted to volume with deionized water.

PROCEDURES FOR THE DETERMINATION OF NITRITE IONS

Three sheets of the prepared μ PADs were spread out in a glass Petri dish, and 1.40 μ L each of the standard sodium nitrite solutions (with concentrations of 0.0780, 0.156, 0.312, 0.625, 1.25, and 2.50 mmol L⁻¹) was dropped onto the six outer circles, respectively. The same volume of S₁ was dropped onto

Scheme 1. Mechanism of the Determination of Nitrite Ion



the central circle of each μ PAD (Figure 2A). The samples were allowed to develop in the dark for about 2 min. With the

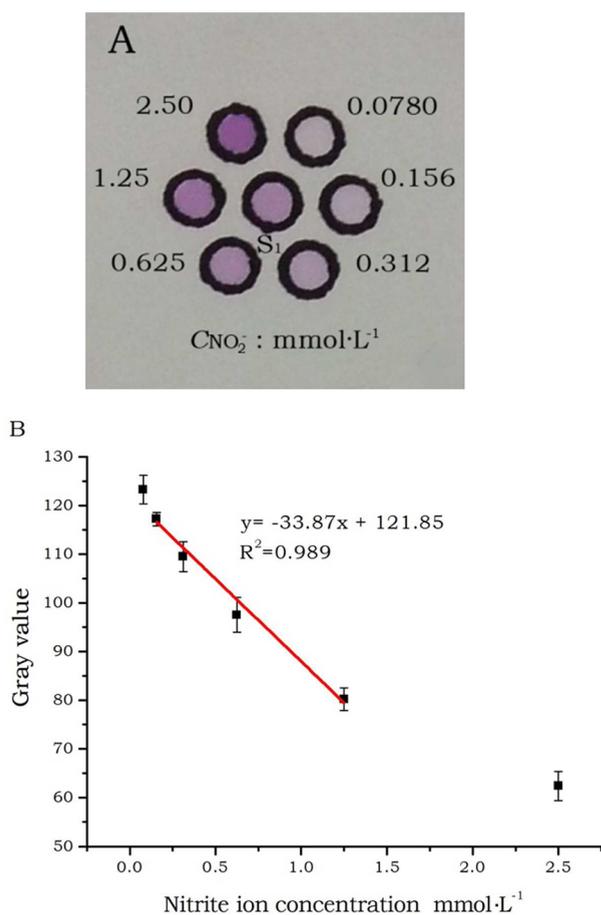


Figure 2. (A) An image of the μ PAD after added with different concentrations of nitrite ion (the unknown sample S_1 was dropped in the central circle). (B) The relationship between gray value and concentration of nitrite ion.

addition of nitrite, the colors of the hydrophilic areas surrounded by ink gradually turned from colorless to red. After the reaction, a camera-phone was used to capture the images of the μ PADs immediately. Adobe Photoshop CSS software was employed to get the gray values of the detection areas from the collected images.

APPLICATION IN REAL SAMPLE DETERMINATION

To validate the feasibility of the μ PADs in practical applications, the detection of nitrite ion in pond water was carried out. The pond water was supplied by the instructor, and it should be filtered by the students to remove suspended solids before usage. Sample S_2 was obtained by the standard addition method described in the accompanying Supporting Information. The final added concentration of NaNO_2 in S_2 was 1.00 mmol L^{-1} . Three parallel experiments were conducted where sample S_2 was treated in a manner identical to that described for unknown sample S_1 .

HAZARDS

p-Amino benzenesulfonamide may cause inflammation on skin and eyes. Methanol is highly volatile, and it may cause blindness or death if ingested in large amounts. It also has an anesthetic

effect on the central nervous system and can cause serious erosion of the skin. Thus, students are required to wear a long-sleeve lab coat, compatible chemical-resistant gloves, and chemical safety goggles.

DISCUSSION

The diazotization reaction between nitrite ions and the indicator performed on the prepared μ PAD should be protected from light. Figure 2, panel A shows that the intensity of the color increases as the concentration of added nitrite ion increases from 0.0780 – 2.50 mmol L^{-1} . After the images were converted to their corresponding gray values by Adobe Photoshop CSS, a calibration curve was established with a linear range of 0.156 – 1.25 mmol L^{-1} (Figure 2B). The linear equation was expressed as

$$y = -33.87x + 121.85 \quad (1)$$

where x represents the nitrite ion concentration (mmol L^{-1}), and y represents the gray value. The correlation coefficient was 0.989 as shown in Figure 2, panel B.

This μ PAD-based colorimetric method gave a concentration of $0.87 \pm 0.05 \text{ mmol L}^{-1}$ for unknown sample S_1 . This correlates reasonably well with the known value of $0.081 \text{ mmol L}^{-1}$. The 7% difference between the known and experimentally determined nitrite ion concentration is readily rationalized given the approximations implicit to this methodology.

On the basis of the values obtained from Figure 3 (which is an image of the μ PAD following reaction with different

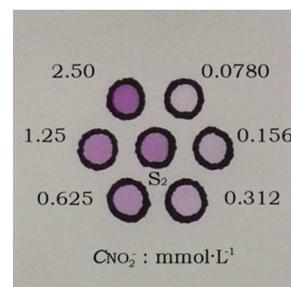


Figure 3. An image of the μ PAD after added with different concentrations of nitrite ion (the spiked sample S_2 was dropped in the central circle).

Table 1. Determination of Nitrite Ion in Pond Water Sample S_2

Sample ($n = 3$)	Added (mM)	Found (mM)	Recovery (%)	RSD (%)
S_2	1.00	0.976	97.6	4.83
		1.02	102	
		1.07	107	

concentrations of nitrite ion and the spiked sample S_2), Table 1 shows that the recoveries of the spiked sample S_2 were in the range of 97.6 – 107% with an RSD less than 5% . The desirable recoveries demonstrate the reliability of the proposed method for the detection of nitrite ion in practical applications.

CONCLUSION

In this experiment, a μ PAD was fabricated by patterning a filter paper with a permanent marker pen. A colorimetric assay was

performed on the μ PAD to determine nitrite ion concentrations of both a preprepared unknown sample and a sample of pond water. The color change was easily observed by the naked eye, recorded by a camera-phone, and quantified using computer software. The fabrication process of the μ PAD was simple, safe, fast, and could be easily carried out by students in laboratories with limited resources. Compared with the prepared value, the nitrite concentration obtained from the μ PAD was satisfactory. The results obtained from pond water samples were also acceptable. During this experiment, students not only learn fundamentals about the μ PAD, but also get the opportunity to use their mobile phones as tools for chemical analysis.

■ ASSOCIATED CONTENT

● Supporting Information

Notes for instructor and students and set of students' data. This material is available via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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