Modification of Microfluidic Paper-Based Devices with an Oxidant Layer for Distance Readout of Reducing Substances

Chunxiu Xu, Guoxing Zhou, Huihui Cai, Yicong Chen, Ling Huang, Longfei Cai, Jiaye Gong, and Zankai Yan*

ABSTRACT: We developed a novel strategy for modification of paper cellulose with water-insoluble oxidants for distance readout of reducing substances on microfluidic paper-based analytical devices (μPADs). Water-insoluble oxidants were formed and modified onto paper cellulose through the redox reaction that occurred between paper cellulose and potassium permanganate deposited on the paper channel, developing a yellowish-brown color on the channel. As aqueous solutions containing reducing substances flowed along the channel, reducing substances were consumed owing to the redox reaction that occurred between oxidants and reducing substances until the reducing substances were depleted, forming a discolored zone on the yellowish-brown channel. The redox reaction between insoluble oxidants and reducing substances on the paper cellulose could be used for distance-based detection of a wide variety of reducing substances, which is similar to the classical potassium permanganate titration that employs the redox reaction that occurred between potassium permanganate and reducing substances. We believe that this method will broaden the analytical applications of distance-based detection on μPADs. This method was applied to ascorbic acid assay and captopril assay in real samples with analytical results comparing well with the labeled values, demonstrating its great potential in real sample analysis.

INTRODUCTION

Since the concept of microfluidic paper-based analytical devices (μPADs) was first introduced by Whitesides et al., μPADs were widely used for environmental testing, medical diagnostics, and food analysis. μPADs have features of portability, disposability, low cost, easy fabrication, and operation; however, external equipment and trained personnel are usually required to obtain instrumental signals for quantitative analysis. Colorimetric assay is a relatively convenient and straightforward detection method compared with electrochemistry, fluorimetry, and chemiluminescence detection techniques. Unfortunately, a digital camera and image processing software are required to obtain the color intensity of detection zones on μPADs for colorimetric assay. This issue could be addressed by coupling titration with μPADs because titration is free of any instrumental signal and software. In a typical titration method (also known as volumetric analysis), a reagent of known concentration (titrant) was prepared to react with the identified analyte, and the consumed volume of the titrant was used to calculate the concentration of the analyte after the end point was determined by the color change with the naked eye. Karita and Kaneta accomplished acid–base titration using μPADs for the analysis of acid in hot spring water by visually detecting the end point, thus requiring neither any electronic instrument nor software. However, the volumes of the titrant and sample deposited onto the paper channel should be calculated and strictly controlled. Moreover, selection of the appropriate concentration of the titrant is also a challenge. These limitations pose difficulty for real sample analysis. Later, Taprab and Sameenoi demonstrated a paper-based titration method for rapid screening of formaldehyde in food. In their work, the color intensity was detected by the naked eye and compared with those produced by formaldehyde standard solutions of known concentrations for semiquantitative analysis. Nogueira et al. developed a redox titration on a foldable paper device for determination of alcohol in whiskey samples. They employed a classical permanganometry reaction on paper zones to detect alcohol based on the consumed volume of oxalic acid reacting with the excess of permanganate. However, permanganate may react with paper cellulose owing...
to the reducing property of cellulose, posing difficulties for accurate measurement of the analyte.

Another alternative is the use of a distance-based detection technique, in which the analyte was detected by measuring the length of color developed in the paper channel, allowing for quantitative analysis with the naked eye. However, the reagents (for example, the chromogenic reagents) deposited on the channel should be water-insoluble or adsorbed by paper cellulose; otherwise, the reagents may be eluted and flow to the end of the paper channel, disabling an analyte concentration-dependent length-based signaling. To fix the chromogenic reagents into the paper cellulose, Yamada et al.21 modified the paper cellulose with sulfonated polysaccharide to enhance the electrostatic interaction between the modified group and chromogenic reagents for distance-based detection of lactoferrin in tear samples. Rahbar et al.’s strategy22 is the use of ion-exchange filter paper. The ion-exchange filter paper demonstrated strong ion-exchange interactions between the oppositely charged chromogenic reagents and ion-exchange paper cellulose, which enable strong retention of chromogenic reagents. Although electrostatic or ion-exchange interaction on filter paper could adsorb or fix the reagents into the paper cellulose, selection of proper modification reagents and modification of the ion-exchange group onto cellulose are challenging. Therefore, a distance-based detection motif suitable for more species and a wider range of analytical applications are highly desirable.

Reducing substances play important roles in physiological processes for human beings and animals. For example, ascorbic acid (vitamin C) could promote the growth, formation, and maintenance of bones and teeth and the repair of tissues and vessels and increase resistance to infections. Captopril, a drug with reducing property, could prevent conversion of angiotensin I to angiotensin II, which leads to decreased vasoconstriction and, ultimately, to lowered blood pressure. Thus, monitoring of reducing substances in food and drugs is important for the health of human beings. Classical redox titration is commonly used for detection of reducing substances. However, it suffers from the drawbacks of large volume consumptions of reagents and sample, time-consuming operations, requirement of trained personnel, and a large amount of glassware. Herein, we developed a novel and simple strategy for distance-based detection of reducing substances by the redox reaction that occurred on μPAD. In an easy way, a water-insoluble oxidant (MnO2) was formed and modified onto paper cellulose through the reaction that occurred between paper cellulose and potassium permanganate, generating a water-insoluble yellowish-brown layer of oxidants on the paper cellulose. As solutions containing reducing substances flowed along the channel, a redox reaction occurred between MnO2 and reducing substances, generating a discolored zone whose length is dependent on the concentration of reducing substances. This presented method was applied to the determination of ascorbic acid and captopril in commercial drugs with analytical results comparing well with the labeled value, demonstrating the great potential of this method in real sample analysis.

RESULTS AND DISCUSSION

Principle. Our objective is to employ the redox reaction that occurred between KMnO4 and paper cellulose to modify the channel with a water-insoluble oxidant layer, developing a universal strategy for distance-based detection of reducing substances using redox reaction on μPAD.

Potassium permanganate, a strong oxidant, could react with a variety of reducing substances and organic compounds. The reduced products of potassium permanganate varied with acidity during reaction. In strongly acidic and alkaline media, potassium permanganate would be reduced to Mn2+ and K2MnO4, respectively, allowing the color to turn from pink to colorless and blackish-green, respectively. In a weakly acidic medium, however, potassium permanganate could be reduced to MnO2, making the color turn from pink to yellowish-brown. Since filter paper is made of paper cellulose, a polysaccharide rich in hydroxyl groups, a redox reaction may occur between potassium permanganate and paper cellulose. To verify our hypothesis, KMnO4 solutions prepared in 1.0 mol L−1 H2SO4 (strongly acidic medium), 0.1 mol L−1 H2SO4 (weakly acidic medium), and 2.0 mol L−1 NaOH solution (strongly alkaline medium) were deposited onto paper channels, respectively. Figure 1A,B shows that the color of the paper channel deposited with KMnO4 in 1.0 mol L−1 H2SO4 medium turned from pink (color of KMnO4) to colorless; this may be due to the consumption of KMnO4 and formation of Mn2+ in the strongly acidic medium owing to the reaction that occurred between potassium permanganate and paper cellulose. In 2.0 mol L−1 NaOH medium, the paper channel turned blackish-green immediately after KMnO4 solution was deposited onto the channel (Figure 1C), which may be attributed to the consumption of KMnO4 and formation of K2MnO4 owing to the redox reaction that happened between KMnO4 and paper cellulose in the strongly alkaline medium. The blackish-green channel then turned yellowish-brown (Figure 1D), which may
be due to the formation of MnO₂, resulting from the disproportionation reaction of K₂MnO₄. In the weakly acidic medium, the paper channel modified with KMnO₄ turned yellowish-brown (Figure 1E,F), which could be attributed to the formation of MnO₂ and depletion of KMnO₄ in the weakly acidic medium.

The MnO₂ layer on the paper channel could not be dissolved and eluted by water flowing on the channel since MnO₂ is water-insoluble. Figure 1G demonstrates that the paper channel modified with MnO₂ remains yellowish-brown even when water is added onto the circular zone to flow along the channel. Manganese dioxide is an oxidant that could react with reducing substances and organic compounds. Thus, reducing substances and organic compounds could consume manganese dioxides to form a discolored zone on the paper channel, which may enable distance-based detection of reducing substances and organic compounds. To test our hypothesis, ascorbic acid solution was added onto the circular zone. As ascorbic acid solution flowed along the channel modified with MnO₂ owing to the capillary action, a discolored zone on the channel was generated (Figure 1H). A preliminary experiment indicated that the length of the discolored band was dependent on the ascorbic acid concentration. Thus, the length of the discolored band could be used for the quantitative analysis of reducing substances.

**Concentration of KMnO₄ and H₂SO₄.** The effect of the concentration of potassium permanganate in the range of 0.007–0.10 mol L⁻¹ on the length of the discolored band and color intensity was studied by keeping the concentration of H₂SO₄ and the volume and concentration of ascorbic acid at 0.10 mol L⁻¹, 20 μL, and 0.004 mol L⁻¹, respectively. As shown in Figure 2, the length of the discolored band decreased with the concentration of KMnO₄, indicating that higher detection sensitivity may be obtained using KMnO₄ solution at a lower concentration. On the other hand, the color intensity of the MnO₂ layer on the channel increased with the concentration of KMnO₄ solution deposited. Accurate measurement of the length of the discolored band is challenging at a low concentration of KMnO₄ solution due to the decreased intensity. Potassium permanganate solution with a concentration of 0.047 mol L⁻¹ was selected and deposited onto the channel for modification of paper cellulose by compromising the detection sensitivity and reliability of length measurement.

The effect of H₂SO₄ concentration in KMnO₄ solution on determination of ascorbic acid was studied in the range of 0–0.50 mol L⁻¹. The length of the discolored band increased with the concentration of H₂SO₄ (Figure 3). On the other hand, the color intensity of the channel modified with MnO₂ decreased with the concentration of H₂SO₄. Moreover, the error of length measurement was large as the concentration of H₂SO₄ was larger than 0.33 mol L⁻¹, posing a negative effect on the accuracy of analytical results. Additionally, a discolored zone was obtained at the end of the channel when the concentration of H₂SO₄ was larger than 0.1 mol L⁻¹ (Figure 3). As aqueous solution was added onto the circular zone and flowed along the channel owing to the capillary action, the previously deposited H₂SO₄ on the channel may be dissolved and eluted, which would concentrate H₂SO₄ at the end of the channel. Thus, the oxidant on the channel was consumed and reduced to colorless Mn²⁺ at a high concentration of H₂SO₄ solution. A longer discolored zone was observed at the end of the channel with the increased concentration of H₂SO₄, making length measurement difficult at a high concentration of ascorbic acid. H₂SO₄ (0.1 mol L⁻¹) was selected as the medium of KMnO₄ solution by compromising the detection sensitivity and detection error.

**Ascorbic Acid Assay.** To quantify ascorbic acid in real samples, ascorbic acid standard solutions in the range of 1.0 × 10⁻³ to 1.2 × 10⁻² mol L⁻¹ were prepared to plot a calibration curve between the length of the discolored band and the ascorbic acid concentration. After KMnO₄ solution was deposited onto paper channels, ascorbic acid standard solutions were added onto circular zones, producing discolored zones on channels. Figure 4A indicates that the length of discolored zones increased with the ascorbic acid concentration. The length was measured to plot a calibration curve between the length and the ascorbic acid concentration. As shown in Figure 4B, the linear correlation between the length of the discolored band (L, mm) and the concentration of ascorbic acid (C_AA mol L⁻¹) is

\[
L (\text{mm}) = 1.79 \times 10^3 C_{\text{AA}} \text{(mol L}^{-1}) + 3.82
\]

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Effect of KMnO₄ concentration on the length of the discolored band in the range of 0.02–0.10 mol L⁻¹ (data were obtained from three repetitive runs). Concentration of ascorbic acid: 0.004 mol L⁻¹; concentration of H₂SO₄: 0.10 mol L⁻¹; volume of ascorbic acid solution: 20 μL; volume of KMnO₄ solution: 7 μL. Data were obtained from three repetitive runs. 

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Effect of H₂SO₄ concentration on the length of the discolored band in the range of 0–0.50 mol L⁻¹ (data were obtained from three repetitive runs). Concentration of KMnO₄: 0.047 mol L⁻¹; other conditions were the same as those in Figure 2 (photograph courtesy of Y.C. and L.H.).
with a correlation coefficient of 0.978. A detection limit of 8.4 \times 10^{-4} \text{ mol L}^{-1} ascorbic acid was obtained based on the 3S/K method (S is the standard deviation by analyzing 0.001 mol L^{-1} ascorbic acid solution 11 times, and K is the slope of the standard curve).

To demonstrate the applicability of this method to real sample analysis, several vitamin C tablets were ground into powder. After 0.1410 g of sample powder was accurately weighed into a beaker and dissolved with water, this solution was filtrated and diluted to 100 mL. Ascorbic acid in sample solution was assayed as described below. Briefly, 20 \mu L of sample solution was added onto the circular zone and flowed along the modified channel, forming a discolored zone on the yellowish-brown channel. The length of the discolored band is \(13.3 \pm 0.6 \text{ mm} (n = 3)\), and the concentration of ascorbic acid in sample solution is calculated as \(5.3 \times 10^{-3} \pm 3.2 \times 10^{-4} \text{ mol L}^{-1}\). The contents of ascorbic acid in vitamin C tablets (\(\omega, \%\)) were calculated by eq 2:

\[
\omega(\%) = \frac{C_{AA} \times V_S \times M_{AA}}{m_S} \times 100\%
\]

(2)

where \(C_{AA}, V_S, M_{AA}, m_S\) are the concentration of ascorbic acid in sample solution (mol L^{-1}), the volume of sample solution (L), the molecular weight of ascorbic acid (g mol^{-1}), and the sample mass (g), respectively. The averaged content of ascorbic acid in vitamin C tablets is 66.2\% \pm 4.0\%, which agrees well with the labeled value (70.9\%).

**Captopril Assay.** To demonstrate the applicability of this presented method to the analysis of captopril in commercial captopril tablets, a calibration curve between the length of the discolored band and the captopril concentration was plotted. The captopril standard solutions in the range of 0.001–0.01 mol L^{-1} were added onto sample zones to flow along the channel previously deposited with potassium permanganate, forming discolored bands on the channels (Figure 5A). The length of discolored bands was measured with a ruler. The linear correlation between the length (\(L, \text{ mm}\)) and the concentration of captopril (\(C_C, \text{ mol L}^{-1}\)) is

\[
L (\text{mm}) = 1.11 \times 10^{3} C_C (\text{mol L}^{-1}) + 3.1
\]

(3)

with a correlation coefficient of 0.995 (Figure 5B). A detection limit of 1.1 \times 10^{-3} \text{ mol L}^{-1} captopril was obtained based on the 3S/K method (S is the standard deviation by analyzing 0.001 mol L^{-1} captopril solution 11 times, and K is the slope of the standard curve).

To quantify captopril in captopril tablets, 0.2500 g of sample powder was accurately weighed after several captopril tablets were ground into powder. The sample was then dissolved with water followed by filtration. The filtrates were diluted to 50 mL. The sample solution was analyzed as described below. The
length of the discolored band is 10.1 ± 0.4 mm (n = 3), and the concentration of captopril in sample solution is calculated as 6.3 × 10⁻³ ± 3.6 × 10⁻⁴ mol L⁻¹. The content of captopril in captopril tablets (ω, %) is calculated as 27.4% ± 1.6% (n = 3) according to the equation

\[ \omega(\%) = \frac{C_C \times V_S \times M_C}{m_S} \times 100\% \]  

(4)

where \( C_C, V_S, M_C, \) and \( m_S \) are the concentration of captopril sample solution (mol L⁻¹), the volume of sample solution (L), the molecular weight of captopril (g mol⁻¹), and the sample mass (g), respectively. This measured value compared well with the labeled value (25.0%), demonstrating the applicability of this presented method to captopril assay.

■ CONCLUSIONS

We developed a new strategy to modify paper channels through the redox reaction that occurred between potassium permanganate and paper cellulose, which may broaden the applications of the distance-based detection motif. Similar to classical redox titration such as potassium permanganate titration, this method employs the reaction that occurred between the oxidant and reducing substances to accomplish quantitative analysis. This method has advantages of reduced sample/reagent volume, easy operation, portability, and disposability over classical redox titrations. We applied this method to ascorbic acid and captopril assays in real samples with analytical results agreeing well with labeled values, demonstrating the great potential of this method in real sample analysis. Moreover, we believe that this modification strategy could be used to modify thread cotton to accomplish the distance-based assay of reducing substances using thread-based microfluidic analytical devices, which may further broaden the applications of the distance-based detection technique. The limitation of this presented strategy is the interference since a variety of reducing substances and organic compounds could react with manganese dioxides, which may pose challenges for the analysis of complicated samples. This limitation could be addressed by using appropriate masking reagents or separation.

■ EXPERIMENTAL SECTION

Chemicals and Apparatus. All chemicals used were of analytical grade, unless stated otherwise. Ultrapure water (18.25 MΩ cm) used throughout was produced by an ultrapure water purification system (EPED-EQ-10T, Nanjing

Figure 6. Schematic diagram depicting \( \mu \)PADs fixed by sandwiching with glass plates.

Figure 7. Schematic diagrams illustrating ascorbic acid and captopril assays with the distance-based detection technique on \( \mu \)PADs. (A–C) Schematic diagrams of \( \mu \)PAD before (A) and after depositing KMnO₄ solution onto the straight channel (B), followed by air drying (C). (D, E) Schematic diagrams of \( \mu \)PAD on which a solution containing reducing substances was added onto the circular zone (D), followed by air drying to form a discolored band on the channel (E).

Eped Technology Development Co., Ltd., Nanjing, China). A wax printer (Xerox ColorQube 8580) was used to print the pattern onto the filter paper (Hangzhou Fuyang Beimu Pulp Paper Limited, Hangzhou, China). Potassium permanganate was purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). Ascorbic acid and captopril (98%) used for preparing standard solutions were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China) and Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China), respectively. Vitamin C tablets and captopril tablets used as samples were purchased from Jiangmen Hengjian Pharmaceutical Co., Ltd. (Jiangmen, China), and Sinopharm Shantou Jinshi Pharmaceutical Co., Ltd. (Shantou, China), respectively. Concentrated sulfuric acid was purchased from Xilong Scientific Co., Ltd. (Shantou, China).

Fabrication of \( \mu \)PADs. The devices were fabricated as described elsewhere. Specifically, the pattern was designed using CorelDraw X3 software. The designed pattern consists of a circular zone (6 mm in diameter), a straight channel (3 x 40 mm), and a ruler (40 mm). The straight channel and circular zone were designed for deposition of KMnO₄ and sample solutions, respectively. After the patterns were printed onto filter paper with a wax printer, the printed devices were heated
at 120 °C for 2 min, allowing the wax to melt and penetrate into the thickness of the filter paper to generate a hydrophilic–hydrophobic contrast on the paper.

**Assays of Ascorbic Acid and Captopril.** The μPADs were fixed using glass plates as shown in Figure 6. Briefly, after two glass plates were put on the benchtop about 6 mm apart as a support for paper devices, another two glass plates were put on the devices. The paper devices were hence fixed by sandwiching with glass plates. To assay reducing substances such as ascorbic acid and captopril with μPADs, 7 μL of KMnO₄ solution containing 0.1 mol L⁻¹ H₂SO₄ was deposited onto the straight channel with a micropipette (Figure 7A,B). After the device was air-dried for 10 min, allowing the color of the channel to turn from purple to yellowish-brown (Figure 7C), 20 μL solution containing ascorbic acid or captopril was then added onto the circular zones (Figure 7D), allowing the solution to flow along the channel. The device was then air-dried for 15 min, forming a discolored zone on the yellowish-brown paper channel (Figure 7E). The length of the discolored band was measured with a ruler for the quantitative analysis of ascorbic acid and captopril.

![Image](https://pubs.acs.org/10.1021/acsomega.2c02537)

**ACKNOWLEDGMENTS**

Financial support from Guangdong Provincial Research and Reform Fund for Higher Education Learning and Teaching and Guangdong Provincial Education Research Innovation Fund (2018GJJK108) is gratefully acknowledged.

**REFERENCES**

(1) Martinez, A. W.; Phillips, S. T.; Butte, M. J.; Whitesides, G. M. Patterned Paper as a Platform for Inexpensive, Low-Volume, Portable Bioassays. *Angew. Chem., Int. Ed.* 2007, 46, 1318–1320.
(2) Sameenoi, Y.; Panyimeesamer, P.; Supalakorn, N.; Koehler, K.; Chailapakul, O.; Henry, C. S.; Volckens, J. Microfluidic Paper-Based Analytical Device for Aerosol Oxidative Activity. *Environ. Sci. Technol.* 2013, 47, 932–940.
(3) Mentele, M. M.; Cunningham, J.; Koehler, K.; Volckens, J.; Henry, C. S. Microfluidic Paper-Based Analytical Device for Particulate Metals. *Anal. Chem.* 2012, 84, 4474–4480.
(4) Mu, X.; Zhang, L.; Chang, S.; Cui, W.; Zheng, Z. Multiplex Microfluidic Paper-based Immunoassay for the Diagnosis of Hepatitis C Virus Infection. *Anal. Chem.* 2014, 86, 5338–5344.
(5) Guo, X.; Zong, L.; Jiao, Y.; Han, Y.; Zhang, X.; Xu, J.; Li, L.; Zhang, C.-W.; Liu, Z.; Ju, Q.; Liu, J.; Xu, Z.; Yu, H.-D.; Huang, W. Signal-Enhanced Detection of Multiplexed Cardiac Biomarkers by a Paper-Based Fluorogenic Immunodot Developed with Zinc Oxide Nanowires. *Anal. Chem.* 2019, 91, 9300–9307.
(6) Nouanthavong, S.; Nacapricha, D.; Henry, C. S.; Sameenoi, Y. Pesticide analysis using nanoceria-coated paper-based devices as a detection platform. *Analyst* 2016, 141, 1837–1846.
(7) Puangbanlang, C.; Sirivibulkovit, K.; Nacapricha, D.; Sameenoi, Y. A paper-based device for simultaneous determination of antioxidant activity and total phenolic content in food samples. *Talanta* 2019, 198, 542–549.
(8) Karita, S.; Kaneta, T. Acid-Base Titrations Using Microfluidic Paper-based Analytical Devices. *Anal. Chem.* 2014, 86, 12108–12114.
(9) Nogueira, S.; Sousa, L.; Silva, N.; Rodrigues, P.; Coltro, W. Monitoring Acid–Base Titrations on Wax Printed Paper Microzones Using a Smartphone. *Micromachines* 2017, 8, 139.
(10) Taprab, N.; Sameenoi, Y. Rapid screening of formaldehyde in food using paper-based titration. *Anal. Chim. Acta* 2019, 1069, 66–72.
(11) Nogueira, S. A.; Lemes, A. D.; Chagas, A. C.; Vieira, M. L.; Talhavini, M.; Morais, P. A. O.; Coltro, W. K. T. Redox titration on foldable paper-based analytical devices for the visual determination of alcohol content in whiskey samples. *Talanta* 2019, 194, 363–369.
(12) Kaewchuny, N.; Jantra, J.; Khettalat, C.; Ketnok, S.; Peungrpas, N.; Teepoo, S. On-site microfluidic paper- based titration device for rapid semi-quantitative vitamin C content in beverages. *Microchem. J.* 2021, 164, 106504.
(13) Cate, D. M.; Dungchail, W.; Cunningham, J. C.; Volckens, J.; Henry, C. S. Simple, distance-based measurement for paper analytical devices. *Lab Chip* 2013, 13, 2397–2404.
(14) Cate, D. M.; Noblitt, S. D.; Volckens, J.; Henry, C. S. Multiplexed paper analytical device for quantification of metals using distance-based detection. *Lab Chip* 2015, 15, 2808–2818.
(15) Wei, X.; Tian, T.; Jia, S.; Zhu, Z.; Ma, Y.; Sun, J.; Lin, Z.; Yang, J. C. Microfluidic Distance Readout Sweet Hydrogel Integrated Paper-Based Analytical Device (µDiSH-PAD) for Visual Quantitative Point-of-Care Testing. *Anal. Chem.* 2016, 88, 2345–2352.
(16) Gerold, C. T.; Bakker, E.; Henry, C. S. Selective Distance-Based K⁺ Quantification on Paper-Based Microfluidics. *Anal. Chem.* 2018, 90, 4894–4900.
(17) Tian, T.; An, Y.; Wu, Y.; Song, Y.; Zhu, Z.; Yang, C. Integrated Distance-Based Origami Paper Analytical Device for One-Step Visualized Analysis. *ACS Appl. Mater. Interfaces* 2017, 9, 30480–30487.
(18) Rahbar, M.; Paull, B.; Macka, M. Instrument-free argentometric determination of chloride via trapezoidal distance-based microfluidic paper devices. *Anal. Chim. Acta* 2019, 1063, 1–8.
(19) Alsaeed, B.; Mansour, F. R. Distance-based paper microfluidics: principle, technical aspects and applications. Microchem. J. 2020, 155, 104664.

(20) Lai, H.; Li, Z.; Zhu, S.; Cai, L.; Xu, C.; Zhou, Q. Naked-Eye Detection of Aluminum in Gastric Drugs on a Paper-Based Analytical Device. J. Chem. Educ. 2020, 97, 295–299.

(21) Yamada, K.; Henares, T. G.; Suzuki, K.; Citterio, D. Distance-Based Tear Lactoferrin Assay on Microfluidic Paper Device Using Interfacial Interactions on Surface-Modified Cellulose. ACS Appl. Mater. Interfaces 2015, 7, 24864–24875.

(22) Rahbar, M.; Wheeler, A. R.; Paull, B.; Macka, M. Ion-Exchange Based Immobilization of Chromogenic Reagents on Microfluidic Paper Analytical Devices. Anal. Chem. 2019, 91, 8756–8761.