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Demonstration

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Demonstrating Electrophoretic Separation in a Straight Paper Channel Delimited by a Hydrophobic Wax Barrier

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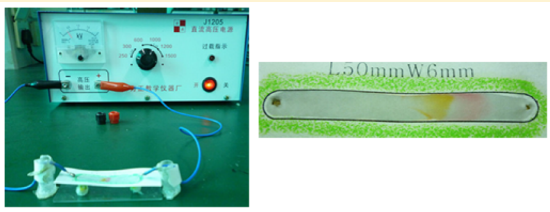
Supporting Information

ABSTRACT: A demonstration is described of electrophoretic separation of carmine and sunset yellow with a paper-based device. The channel in the paper device was fabricated by hand with a wax pen. Electrophoretic separation of carmine and sunset yellow was achieved within a few minutes by applying potential on the channel using a simple and inexpensive power supply. This demonstration could not only motivate the students' passion in learning analytical chemistry but also introduce two important analytical techniques (microfluidic paper-based analytical devices and electrophoresis) to the classroom. With further modification and improvement, this demonstration may be suitable for students to perform as a laboratory experiment in the teaching lab.



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A new trend in chemical education is the introduction of microfluidic paper-based analytical devices (μPADs) into classrooms at universities and high schools. Microfluidic paper-based analytical devices, proposed by Whitesides' group,[1] have a number of advantages, including ease of use, easy and fast fabrication, low cost, portability, and disposability. Rapid development of μPADs for use in university and high school education has been recently observed,[2−6] because of the significant potential in convenient and cost-effective analytical applications, including environmental monitoring,[7,8] food safety control,[9,10] and clinical diagnostics.[11−13] In 2013, we, for the first time, described an introductory experiment in the teaching laboratory which was performed by undergraduate students.[2] The students fabricated a flower-shaped paper-based analytical device by wax drawing and used it to determine the amino acid contents in tea leaf extracts. Wang et al.[3] and Koesdjojo et al.[4] subsequently described simple methods to fabricate μPADs for determination of nitrite and metal ions. Ravgiala et al.[5] designed an activity in which the high school students modeled forensic investigation and colorimetric assays using paper-based diagnostics.

One main limitation of μPADs, however, is the lack of separation function, making it unsuitable for complicated samples. Hence, development of separation function on μPADs is highly desirable. Carvalhal et al.[14] created an inexpensive paper-based separation device by combining amperometric detection with strips of paper or patterned strips of paper. Unfortunately, the fluid flow rate decreased with the distance migrated by the fluid, resulting in a progressively slower migration of mobile phase, thereby negatively influencing the separation efficiency. Electrophoretic separation techniques are based on the different migration rates of the charged compounds or particles in an electric field. This technique has been widely used for separation and analysis of complicated samples such as environmental samples, foods, biological samples, and cells. More recently, Ge et al.[15] for the first time successfully demonstrated the integration of an electrophoretic separation on a μPAD. In their work, electrokinetic flow was used to manipulate the flow of aqueous fluid in paper channel, which is promising for performing multiple-step or complicated assays on μPADs. More importantly, electrophoretic separation could be integrated on a μPAD, demonstrating significant potentials in assays for complicated samples. We believe that this concept will broaden the capabilities of μPADs for a wide range of analytical applications, including diagnostic testing, environmental monitoring, and food analysis. Student exposure to this concept is important considering that the paper-based electrophoretic separation devices have significant potential in convenient and cost-effective analytical applications. Furthermore, two important analytical techniques, μPADs and electrophoretic separation, were introduced to the students through this demonstration. However, the μPADs were fabricated by wax printing using an expensive wax printer, making it unsuitable for high schools and universities in undeveloped countries and resource-limited settings. Moreover, the integration of electrogenerated chemiluminescence onto μPADs seems difficult. Thus, a simple experiment that did not require any expensive instruments and time-consuming procedures is highly desirable to serve as a demonstration.

We demonstrated electrophoretic separation of mixed dyes in a straight paper channel which was fabricated by wax drawing using a wax pen. The negatively charged dyes (carmine and sunset yellow) were separated in the straight channel delimited

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by hydrophobic wax barrier due to their different migration rates in an electric field. This demonstration is free of any expensive instruments, reagents, and complicated detection apparatus. The demonstration was performed in analytical chemistry class for the undergraduates majoring in chemical education at the third semester. Through this demonstration, a promising, convenient, and cost-effective analytical platform, which can be used to perform assays for complicated samples, was introduced to the classroom. The main purposes of this demonstration are to

1. Make the students understand the mechanism of electrophoretic separation by using a simple, visible. and easy-to-perform experiment.
2. Introduce the concept of μPADs by using a paper channel delimited by the hydrophobic wax barrier.

This demonstration can be carried out within 10 min.

■ MATERIALS

All the chemicals used were of analytical grade unless otherwise noted, and demineralized water was used throughout. A mixed solution containing 1.23×10^{-1} g L^{-1} carmine and 1.13×10^{-1} g L^{-1} sunset yellow was prepared by combining 0.0123 g of carmine (Shanghai Maikun Chemical Co., LTD) and 0.0113 g of sunset yellow (Shanghai Maikun Chemical Co., LTD) in 40 mL of H_2O, followed by dilution to 100 mL. 10 mmol L^{-1} $Na_2B_4O_7$−10 mmol L^{-1} NaH_2PO_4 (pH 9.3) was used as the working electrolyte for electrophoretic separation of carmine and sunset yellow on paper-based analytical device. Detailed procedure for preparation of solutions is provided in the Supporting Information.

■ PAPER-BASED SEPARATION SYSTEM

The paper-based separation system consists of a paper sheet with a channel and a simple power supply (Figure 1). A channel pattern (50 mm long and 6 mm wide) designed with CorelDRAW X3 software was printed onto a filter paper sheet (102, Hangzhou Xinhua Paper Limited, Hangzhou, China) with a LaserJet printer (HP LaserJet 1020 plus, USA) (Figure 1A), followed by drawing with a wax pen (Figure 1B). The device was then heated at 135 °C for 30 s, allowing the melted wax to completely penetrate through the filter paper to form a hydrophobic barrier. Thus, a hydrophilic channel delimited by the wax barrier was prepared on the paper sheet. Two plastic posts cut from a plastic tube were fixed onto a glass slide using double-sided tape, which were used to support the paper sheet and fix the platinum electrodes (Figure 1C, D). A simple and inexpensive power supply (J1205, Jiangdu Fangzheng Teaching Instrument Factory, Jiangsu, China) was used to perform electrophoretic separation on the paper channel. Construction of paper-based separation is available in the Supporting Information.

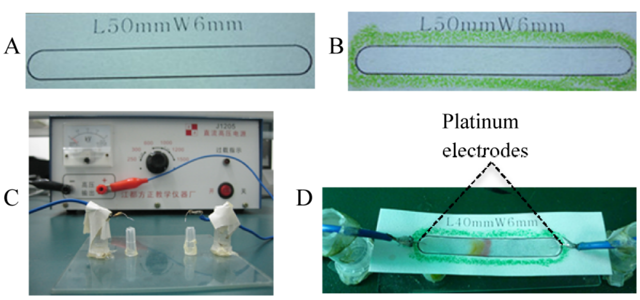

Figure 1. Paper-based separation system. Photograph of (A) native filter paper sheet printed with a straight channel pattern, (B) paper device with a straight channel fabricated by wax drawing by hand with a wax pen, (C) paper-based separation system before the paper was installed, and (D) a paper sheet on the plastic posts with platinum electrodes inserted into the terminals of paper channel.

■ HAZARDS

The chemicals used in this demonstration, including carmine, sunset yellow, $Na_2B_4O_7$, and NaH_2PO_4, may be harmful to the respiration system and skin; the operator should wear protective gloves and long-sleeve lab coat when dealing with these chemicals. Additionally, the apparatus should be handled with caution, and the operator should wear nonconductive gloves and the students should keep away from the electrodes and power supply during demonstration, owing to the relatively high voltage applied during the electrophoretic separation.

■ DEMONSTRATION

This demonstration was performed during the electrophoresis section of the analytical chemistry class. The mechanism of electrophoretic separation was introduced before this demonstration.

Prior to separation, the paper sheet with a paper channel was laid on the plastic posts on the glass slide, and two platinum electrodes were fixed onto the other two plastic posts followed by inserting into the terminals of paper channel. The separation was then performed with following procedures (also available in the Supporting Information):

1. 30 μL of buffer solution was added onto the terminals of the paper channel, the solution wicked through the paper channel by capillary action.
2. After the whole channel was completely wetted by the buffer solution, 5 μL of mixed solution (containing 1.23×10^{-1} g L^{-1} carmine and 1.13×10^{-1} g L^{-1} sunset yellow) was pipetted onto the left side of the channel (closer to the negative electrode than positive electrode).
3. 250 V was applied between two terminals of channel, allowing the separation of carmine and sunset yellow in the channel owing to the difference in mobility between carmine and sunset yellow.

As shown in Figure 2, separation efficiency varied with separation time in the range of 1−10 min. Carmine and sunset yellow were completely separated with a separation time greater than 6 min. This result shows that carmine and sunset yellow could be separated within a few minutes. Thus, the demonstration could motivate the passion of students for

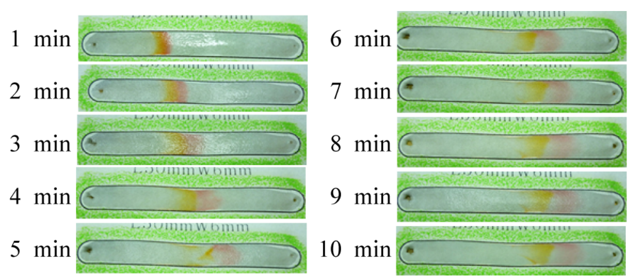

Figure 2. Time-sequential images captured 1−10 min after potential was applied between two terminals of the channel.

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learning analytical chemistry within a minimal class period. After the demonstration, teachers could describe the principle of the method in detail, including the fabrication of paper channel and electrophoretic separation, both of which are very important concepts in analytical chemistry.

QUESTIONS

After the demonstration, a teacher could elicit discussion with the students, starting with these two questions:

1. How were the dyes separated in the channel?
2. Why were the aqueous solutions (including buffer and sample solutions) confined within the area defined by the wax?

CONCLUSIONS

The electrophoretic separation of mixed dyes was demonstrated in a straight paper channel delimited by the hydrophobic wax barrier. This demonstration has the advantages of rapid separation (less than 10 min), easy fabrication, and easy operation without using expensive instruments and reagents. These features make it suitable for classroom demonstrations, especially in the undeveloped countries and resource-limited regions. With further modification and improvement, this experiment may be designed as a laboratory experiment in teaching lab. Feedback and questions from the students included these examples (translated from Chinese by the authors):

Could the separation be carried out using other substrates besides paper?

What is the role of the wax?

Very cool, but how do you quantify these separated compounds?

By responding to these questions, students' understanding of the electrophoresis mechanism was improved, and the concepts of μPADs were clearly introduced to the students.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00674.

Detailed procedure for preparations and step-by-step instructions (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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