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Laboratory Experiment

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Cost Effective Paper-Based Colorimetric Microfluidic Devices and Mobile Phone Camera Readers for the Classroom

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Supporting Information

ABSTRACT: We have developed a simple and direct method to fabricate paper-based microfluidic devices that can be used for a wide range of colorimetric assay applications. With these devices, assays can be performed within minutes to allow for quantitative colorimetric analysis by use of a widely accessible iPhone camera and an RGB color reader application (app) to measure color intensity. In the described laboratory experiment, students design and create their own microfluidic devices with common laboratory supplies such as Kimwipes, Parafilm, and a thermal laminator, and gain hands-on experience in the analysis of Fe^{2+} and Cu^{2+} by colorimetric determination.

KEYWORDS: *High School/Introductory Chemistry, First-Year Undergraduate/General, Analytical Chemistry, Hands-On Learning/Manipulatives, Microscale Lab*

INTRODUCTION

During the past decade the emerging science of microfluidics has moved to the forefront of science, particularly in the field of analytical chemistry. Recent developments in microfluidic technologies have demonstrated that fluidic components can be miniaturized and joined together, forming a single integrated chip capable of performing important chemical and biological processes from beginning to end. There has been tremendous interest in exploiting the full potential of this technology that consequently has led to the development of numerous fabrication methods. In microfabrication, rapid prototyping ability is highly desirable as it allows for design modifications to be made quickly and inexpensively. Conventional machining, in contrast, is often time-consuming, complicated and expensive. Currently, many methods are used to produce microfluidic chips, including etching of glass, casting of soft polymers such as poly(dimethylsiloxane) (PDMS), micromachining, and injection molding.[1−5] Recently, in a move to cut costs and simplify manufacturing, many researchers have combined microfluidic concepts with the use of paper substrates to promote sample movement and reagent stabilization.[6,7] These new fluidic platforms, called microfluidic paper-based analytical devices (μPADs), provide a novel system for fluid handling and analysis for a variety of applications. They require only a fraction of the time, resources, and infrastructure necessary to produce conventional devices while still providing a compact, inexpensive and simple-to-use platform for quantitative assays that are well suited for a variety of applications including health diagnostics, environmental monitoring, as well as food quality testing. These systems offer many of the capabilities of the conventional microfluidic methods, but with the simplicity of diagnostic strip tests. Unlike traditional microfluidics, which often require pumps to move fluids through the microfluidic channels, paper microfluidic devices can be operated without such instrumentation. Fluid flow is driven by capillary action through the paper. As such, these devices require only small volumes of samples, reagents and solvents.

Microfluidics has become an important commercial technology, with extensive applications in biotechnology, medicine, chemistry, and materials science,[8,9] and can provide an excellent means to educate and expose students to the basic principles of chemistry and the broad applicability of highly integrated microscale systems. The best means of accomplishing this is by engaging students in the process of designing and building systems; this is possible owing to the accessibility of materials and methods that lend themselves to prototyping, even in the hands of newcomers. While conventional microfluidic glass and silicon foundry tools and fabrication processes are costly and generally unavailable to high schools and undergraduate institutions, with paper-microfluidic devices[6,7,10] students can gain hands-on experience in microfluidics.

Here, we describe an experiment focused on building and applying microfluidic devices that involved high school and freshmen undergraduate students in chemistry laboratories. The experiment can be performed in a 3 h period (a single laboratory session) and is easily carried out by students in groups of three. Students learn a simple and direct method for creating paper-based microfluidic devices by utilizing a common laboratory hydrophobic film material, Parafilm, to create channels on a piece of paper. These paper chips can be applied to the identification and measurements of metal ion in samples using colorimetric reactions as the detection methods.

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Unknown samples containing both Fe^{2+} and Cu^{2+} are evaluated by each group, and each group is asked to determine the concentration of each analyte by colorimetric analysis using a smartphone—which many students possess—as a reader. Previous inspirational work that utilizes phones or tablet cameras for colorimetric quantitation has been reported elsewhere,[10−17] and these references will be of interest and use to instructors and students alike. These efforts showed the value of smartphones and paper microfluidics for demonstrating analytical principles to students. Here, we seek to build on that by adding a cost-effective, simple, complete experiment that can be conducted by newcomers to chemical analysis.

MATERIALS

All reagents were of analytical grade and distilled water was used for solution preparation. Kimwipes were purchased from Kimberly-Clark Professional (Roswell, GA, USA), Whatman filter paper No. 1 was purchased from Whatman International Ltd. (Maidstone, England). $FeCl_2{\cdot}4H_2O$ was purchased from Fluka (Buchs, Germany). $CuSO_4{\cdot}5H_2O$ was purchased from Fisher Scientific (NJ, USA). KI and $K_3[Fe(CN)_6]$ (III) were purchased from Sigma-Aldrich (MO, USA) and Flinn Scientific, Inc. (IL, USA), respectively. Parafilm (Pechiney Plastic Packaging Company, Chicago, IL, USA) was purchased from Sigma-Aldrich, while aluminum foil was purchased from a local grocery store. A Quickutz Silhouette SD (Silhouette America, West Orem, UT, USA) or similar cutting plotter can be purchased from a local crafting store for ∼$150.

SAMPLE PREPARATION

Iron (Fe^{2+}) Assay

A 1000 μg/mL Fe^{2+} standard stock solution was prepared by dissolving 0.1779 g of $FeCl_2{\cdot}4H_2O$ (FW 198.81) in 50.0 mL of 0.5 M HCl solution. A 5 mM $K_3[Fe(CN)_6]$ solution was prepared by dissolving 0.08231 g of $K_3[Fe(CN)_6]$ (FW 329.26) in a 50 mL volumetric flask with Milli-Q (ultrapure) water.

Copper (Cu^{2+}) Assay

A 1000 μg/mL Cu^{2+} standard stock solution was prepared by dissolving 0.1964 g of $CuSO_4{\cdot}5H_2O$ (FW 249.68) in 50 mL of Milli-Q water. A 0.4 M of KI solution was prepared by dissolving 3.32 g of KI (FW 166.01) in a 50 mL volumetric flask with Milli-Q water.

The series of four standard solutions with differing concentrations of Fe^{2+} and Cu^{2+} was prepared by diluting the standard stock solutions with Milli-Q water as shown in Table 1.

Table 1. Dilution Scheme for Preparation of Fe^{2+} and Cu^{2+} Standards

Samples	$[Fe^{2+}]$ $[Cu^{2+}]$ (μg mL^{-1})	Volume of Fe^{2+} Stock Added (μL)	Volume of Cu^{2+} Stock Added (μL)	Volume of Mill-Q Water Added (μL)
Blank	0	0	0	1000
Standard 1	100	100	100	800
Standard 2	200	200	200	600
Standard 3	300	300	300	400
Standard 4	400	400	400	200

FABRICATION OF PAPER MICROFLUIDIC CHIPS

Paper microfluidic devices were fabricated by cutting the microfluidic designs on a Parafilm sheet using a Quickutz Silhouette SD cutting plotter or using an X-Acto knife. A similar cutting process has been demonstrated using a cutting plotter: xurography is a method widely used in the sign industry for cutting graphics in adhesive vinyl films. This process uses a knife blade to directly cut the microfluidic designs into various film or plastic substrates. Gale et al. reported a DNA melting analysis performed on a microchip fabricated using a cutting plotter to manufacture tape bonded microchannels with dimensional accuracy of 10 μm. To achieve the highest resolution possible, the knife plotter was set to its lowest speed and acceleration.[18]

The microchannels were produced by stacking alternating layers of paper and Parafilm cutouts according to the channel patterns provided in Figure 1. The chip assembly was placed between two layers of aluminum foil and was passed through a thermal laminator purchased at a local office supply store. The heat from the laminator melted the wax in the Parafilm, which was absorbed into the paper layer, creating hydrophobic channel boundaries. For fabrication of chips that require multilayer patterns with more complicated designs, the fabrication process can be performed by stacking the paper/Parafilm layers one at a time and passing them through the laminator individually. The stacked paper/Parafilm layers can then be passed through a second time, and will adhere to one other by virtue of the residual wax from that fraction of the Parafilm that did not penetrate the paper when preparing the original, individual hydrophobic barriers.

Whatman paper No. 1 and Kimwipes were used in this experiment, but other types of paper can be used as well. Small circular cut-outs were generated from the Whatman paper No. 1 using a hole punch.[19] The paper dots were spotted with the Fe^{2+} and Cu^{2+} assay reagents and allowed to dry at room temperature for 2 h prior to final device assembly. The drying step is easily accommodated while the other device components are produced and preassembly is conducted. Following assembly of the device shown here, it was possible to rely on capillary action to drive fluid flow to transport the sample through the test zones containing the assay reagents. Whatman paper No. 1 is thick relative to the Kimwipes used for the preponderance of the chip, and can hold a substantial quantity of reagent when dried. We found that the paper cut-outs helped to ensure consistent, more uniform color production, simplifying detection. Figure 1 depicts the fabrication procedure. The chip consists of four assay zones, with each zone branching out into two channels, each containing either the Fe^{2+} or the Cu^{2+} assay (Figure 2). Thus, each individual paper chip can be used to evaluate four different samples generating eight data points.

ASSAY DETERMINATION

A volume of 6 μL of each of the four standard solutions was added to the finished paper chip to demonstrate the utility of the device. The Fe^{2+} and Cu^{2+} in the standard solutions reacted with the assay reagents and formed blue and red-brown colored complexes in the detection zones, respectively. Images (digital photos) of the colored products in the detection zones were taken with an iPhone and their RGB intensity values were measured with the ColorAssist application ("app"). To measure the color intensities of the color products with a smartphone,

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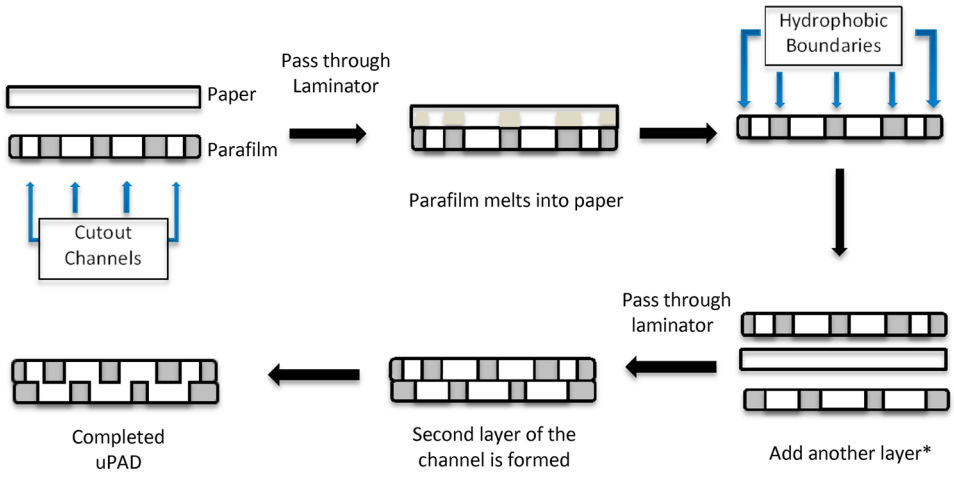

Figure 1. Schematic diagram of the process of microfluidic device fabrication to be followed using patterned, pre-cut Kimwipes and Parafilm as materials. At the layering-up step (indicated by asterisk (*)), filter paper reagent-laden components may be added as desired.

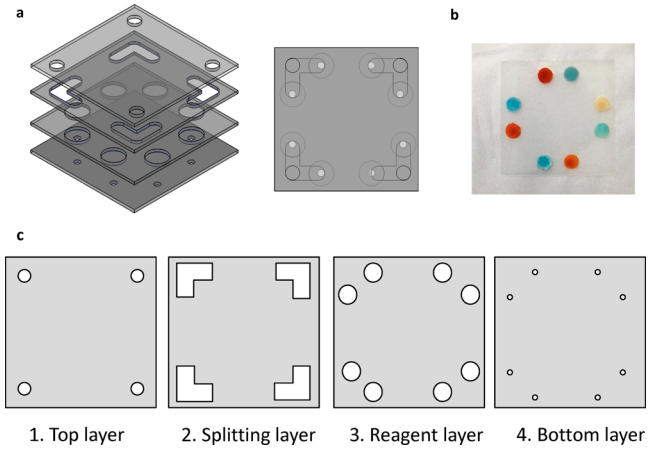

Figure 2. (a) Schematic diagram of the paper chip design. All layers shown in the diagram are produced in Parafilm. Kimwipes are inserted in between the parafilm layers, and the Whatman filter paper reagent pads are inserted prior to the final lamination step, which yields a complete assembly. (b) Image of the paper test device showing results for Fe^{2+} and Cu^{2+} assays at varied sample concentrations. (c) Schematic diagrams of the four layers suitable for use in building a device.

we worked with an iPhone 5 and ColorAssist, as described previously. Students could instead work with other smartphones, iPods equipped with cameras, etc. Likewise, color-matching app's other than ColorAssist could also be used to capture RGB values in real time. A plot of the RGB color intensity versus substrate concentration was generated from the raw RGB values using Microsoft Excel to generate a standard curve from which unknowns can be determined at the discretion of the laboratory instructor.

HAZARDS

$K_3Fe(CN)_6$ and KI can cause skin and eye irritation. Students should handle these chemicals with care. The transition metals are considered toxic to aquatic life. Leftover solutions—in very small quantities—were therefore collected in a waste container for disposal. Standard laboratory safety protocols must be followed. Students should wear protective goggles, gloves, and long sleeve lab coats while conducting the experiment. That being said, risks associated with this lab exercise are minimal because of the small quantities, low toxicities, and low chemical concentrations used throughout.

DISCUSSION

The use of colorimetric assays for analysis is attractive because these assays produce visual results and are usually simple to perform, stable, and inexpensive. However, visual quantification of a colored product can be difficult; consequently the interpretation of the results can be a challenge. To obtain more accurate quantitative data, we utilized a color analyzer on a camera phone to measure the red, green and blue (RGB) color channel values of the color products developed on the test paper. The sample concentrations were determined by the use of a smartphone, in this case an iPhone, with the RGB color reader app ColorAssist, to read and correlate the color intensities of the color products to the sample concentrations. Reactions leading to the color changes for each of the chemistries are discussed in the Supporting Information. The digital image was analyzed by measuring the color intensity of the generated color in each respective detection region. The ColorAssist app used to obtain red, green, and blue component data for the red-brown (Cu^{2+}) and blue (Fe^{2+}) color produced is representative of a variety of similar applications available at very low cost to smartphone users on multiple platforms, enabling students to compare apps and devices of many kinds. Acceptable alternatives to ColorAssist include ColorSmart, ColorLife, and others; a key requirement is that the app provide a quantiative readout in some color space (RGB, HSV, and LAB are all acceptable options). The iPod touch, iPad, Android phones and tablets, etc., are all viable readers.

Color intensities of the detection zones were recorded and analyzed and the RGB values of the four standard solutions were used to create calibration curves for the Fe^{2+} and Cu^{2+} assays by plotting the color intensities against the analyte concentrations using Microsoft Excel. An app such as ColorAssist provides a quick and easy way to obtain RGB values for the target of interest, facilitating quantitative analysis based on color intensity.

The data showed that the intensity of the color developed was consistent with and proportional to the amount of analyte present in the sample. With sample concentrations ranging from 100 to 400 μg/mL, linear correlations between the concentrations of Fe^{2+} and Cu^{2+} in the samples and the color

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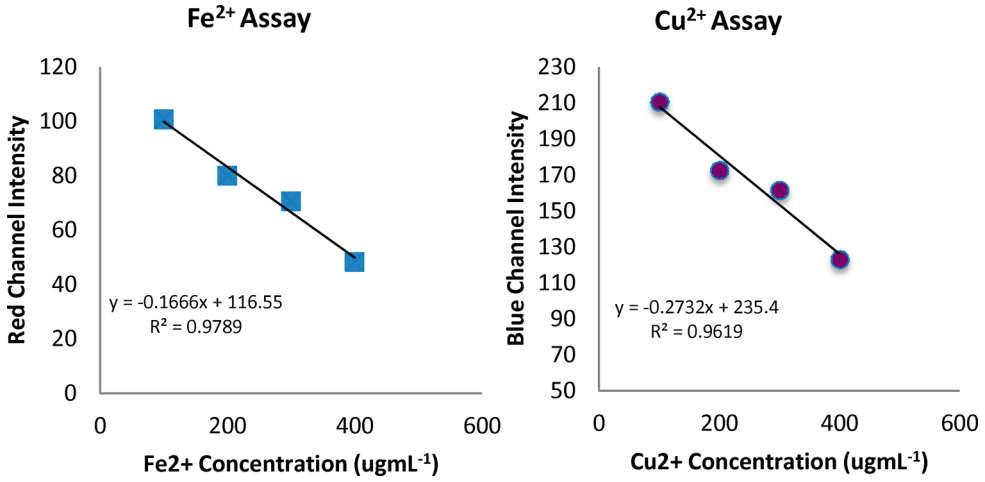

Figure 3. Plots of the color intensities versus the Fe^{2+} and Cu^{2+} standard concentrations determined using the ColorAssist app on an iPhone 5.

intensities from the RGB values were obtained, with correlation coefficients of 0.96 or greater for both the Fe^{2+} and Cu^{2+} assays.

Additional exercises for the students might include preparing and running an unknown mixture of Fe^{2+} and Cu^{2+}, studies on cross-reactivity or synergistic effects, and running true unknowns. Possible samples include tap water, rainwater, laboratory DI water, etc. The students can calculate the concentration of Fe^{2+} and Cu^{2+} in the unknown sample using the linear equations obtained for each of the assay calibration curves as shown in Figure 3.

CONCLUSION

We have described a simple and straightforward method to produce paper-based microfluidic devices utilizing common laboratory supplies: Kimwipes and Parafilm. This laboratory activity engages students in designing and building paper-based microfluidic devices and to the wide range of applications that these kinds of devices can serve. Our students enjoyed the hands-on experience of fabricating microfluidic devices and the challenge of using their own hand-held devices to perform colorimetric analysis using a smartphone camera as a reader. The paper-based assay is inexpensive and easy to fabricate, making it an ideal exercise for high school and freshmen undergraduate teaching laboratories. Paper-based microfluidic assays have tremendous potential in the field of healthcare and environmental monitoring, and can be utilized to address a wide range of other applications.

ASSOCIATED CONTENT

Supporting Information

Instructor notes. 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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REFERENCES

(1) Fenton, E. M.; Mascarenas, M. R.; Lopez, G. P.; Sibbett, S. S. Multiplex lateral-flow test strips fabricated by two-dimensional shaping. *ACS Appl. Mater. Interfaces* **2009**, *1*, 124−129 DOI: 10.1021/am800043z.

(2) Koesdjojo, M. T.; Tennico, Y. H.; Rundel, J. T.; Remcho, V. T. Two-stage polymer embossing of co-planar microfluidic features for microfluidic devices. *Sens. Actuators, B* **2008**, *131*, 692−697 DOI: 10.1016/j.snb.2008.01.008.

(3) Koesdjojo, M. T.; Tennico, Y. H.; Reincho, V. T. Fabrication of a microfluidic system for capillary electrophoresis using a two-stage embossing technique and solvent welding on poly(methyl methacrylate) with water as a sacrificial layer. *Anal. Chem.* **2008**, *80*, 2311−2318 DOI: 10.1021/Ac7021647.

(4) Esch, M. B.; Kapur, S.; Irizarry, G.; Genova, V. Influence of master fabrication techniques on the characteristics of embossed microfluidic channels. *Lab Chip* **2003**, *3*, 121−127 DOI: 10.1039/B300730h.

(5) Becker, H.; Heim, U. Hot embossing as a method for the fabrication of polymer high aspect ratio structures. *Sens. Actuators, A* **2000**, *83*, 130−135 DOI: 10.1016/S0924-4247(00)00296-X.

(6) Dunfield E. M., W Y. Y., Remcho T. P., Koesdjojo M. T. Remcho V. T. Simple and rapid fabrication of paper microfluidic devices utilizing Parafilm®. *Chips and Tips* (10 April).

(7) Martinez, A. W.; Phillips, S. T.; Whitesides, G. M. Three-dimensional microfluidic devices fabricated in layered paper and tape. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 19606−19611 DOI: 10.1073/pnas.0810903105.

(8) Li, X.; Tian, J.; Nguyen, T.; Shen, W. Paper-based microfluidic devices by plasma treatment. *Anal. Chem.* **2008**, *80*, 9131−9134 DOI: 10.1021/ac801729t.

(9) Abe, K.; Suzuki, K.; Citterio, D. Inkjet-printed microfluidic multianalyte chemical sensing paper. *Anal. Chem.* **2008**, *80*, 6928−6934 DOI: 10.1021/ac800604v.

(10) Murdock, R. C.; et al. Optimization of a Paper-Based ELISA for a Human Performance Biomarker. *Anal. Chem.* **2013**, *85*, 11634−11642 DOI: 10.1021/Ac403040a.

(11) Kehoe, E.; Penn, R. L. Introducing Colorimetric Analysis with Camera Phones and Digital Cameras: An Activity for High School or General Chemistry. *J. Chem. Educ.* **2013**, *90*, 1191−1195 DOI: 10.1021/Ed300567p.

(12) Wang, S.; et al. Integration of cell phone imaging with microchip ELISA to detect ovarian cancer HE4 biomarker in urine at the point-of-care. *Lab Chip* **2011**, *11*, 3411−3418 DOI: 10.1039/c1lc20479c.

(13) Mudanyali, O.; et al. Integrated rapid-diagnostic-test reader platform on a cellphone. *Lab Chip* **2012**, *12*, 2678−2686 DOI: 10.1039/c2lc40235a.

DOI: 10.1021/ed500401d
J. Chem. Educ. 2015, 92, 737−741

(14) Coskun, A. F.; Nagi, R.; Sadeghi, K.; Phillips, S.; Ozcan, A. Albumin testing in urine using a smart-phone. *Lab Chip* **2013**, *13*, 4231−4238 DOI: 10.1039/C3lc50785h.
(15) Coskun, A. F.; et al. A personalized food allergen testing platform on a cellphone. *Lab Chip* **2013**, *13*, 636−640 DOI: 10.1039/c2lc41152k.
(16) Ozcan, A. Mobile phones democratize and cultivate next-generation imaging, diagnostics and measurement tools. *Lab Chip* **2014**, *14*, 3187−3194 DOI: 10.1039/c4lc00010b.
(17) Vashist, S. K.; Mudanyali, O.; Schneider, E. M.; Zengerle, R.; Ozcan, A. Cellphone-based devices for bioanalytical sciences. *Anal. Bioanal. Chem.* **2014**, *406*, 3263−3277 DOI: 10.1007/s00216-013-7473-1.
(18) Sundberg, S. O. Microfluidic techniques for DNA melting analysis and digital polymerase chain reaction. University of Utah, *101*, December 2010.
(19) Koesdjojo, M. T.; Wu, Y.; Boonloed, A.; Dunfield, E. M.; Remcho, V. T. Low-cost, high-speed identification of counterfeit antimalarial drugs on paper. *Talanta* **2014**, *130*, 122−127 DOI: 10.1016/j.talanta.2014.05.050.

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