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

Myra T. Koesdjojo, Sumate Pengpunkit, Yuanyuan Wu, Anukul Boonloed, Daniel Huynh, Thomas P. Remcho, and Vincent T. Remcho*

Department of Chemistry, Oregon State University, Corvallis, Oregon 97331, United States

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 Fe²⁺ and Cu²⁺ 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,⁶⁻⁹ 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 devices⁶⁻⁷,¹⁰ 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.

Published: March 17, 2015
Unknown samples containing both Fe²⁺ and Cu²⁺ 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). FeCl₂·4H₂O was purchased from Fluka (Buchs, Germany). CuSO₄·5H₂O was purchased from Fisher Scientific (NJ, USA). KI and K₃[Fe(CN)₆] (III) were purchased from Sigma-Aldrich (MO, USA) and Flinn Scientific, Inc. (IL, USA), respectively. Paraflm (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 (Fe²⁺) Assay

A 1000 μg/mL Fe²⁺ standard stock solution was prepared by dissolving 0.1779 g of FeCl₂·4H₂O (FW 198.81) in 50.0 mL of 0.5 M HCl solution. A 5 mM K₃[Fe(CN)₆] solution was prepared by dissolving 0.08231 g of K₃[Fe(CN)₆] (FW 329.26) in a 50 mL volumetric flask with Milli-Q water (ultrapure) water.

Copper (Cu²⁺) Assay

A 1000 μg/mL Cu²⁺ standard stock solution was prepared by dissolving 0.1964 g of CuSO₄·5H₂O (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 Fe²⁺ and Cu²⁺ was prepared by diluting the standard stock solutions with Milli-Q water as shown in Table 1.

<table>
<thead>
<tr>
<th>Samples</th>
<th>[Fe²⁺] (μg mL⁻¹)</th>
<th>Volume of Fe²⁺ Stock Added (μL)</th>
<th>Volume of Cu²⁺ Stock Added (μL)</th>
<th>Volume of Milli-Q Water Added (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1000</td>
</tr>
<tr>
<td>Standard 1</td>
<td>100</td>
<td>100</td>
<td>800</td>
<td>1000</td>
</tr>
<tr>
<td>Standard 2</td>
<td>200</td>
<td>200</td>
<td>600</td>
<td></td>
</tr>
<tr>
<td>Standard 3</td>
<td>300</td>
<td>300</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>Standard 4</td>
<td>400</td>
<td>400</td>
<td>200</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Dilution Scheme for Preparation of Fe²⁺ and Cu²⁺ Standards

■ ASSAY DETERMINATION

A volume of 6 μL of the four standard solutions was added to the finished paper chip to demonstrate the utility of the device. The Fe²⁺ and Cu²⁺ 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,
we worked with an iPhone 5 and ColorAssist, as described previously. Students could instead work with other smart-phones, 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$_3$Fe(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 quantitative 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
Laboratory Experiment

The authors gratefully acknowledge Peng Wei for his support and technical assistance in the design and fabrication of the paper assays.

**ACKNOWLEDGMENTS**

The authors gratefully acknowledge Peng Wei for his support and technical assistance in the design and fabrication of the paper assays.

**REFERENCES**


