

Paper-based Microfluidics as an Alternative to Spectrophotometry

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One of the evolving fronts in chemical measurements is the development of low-cost, disposable devices that can provide extensive analytical information in a small footprint. A driving force for these efforts is the desire to make useful measurements in a resource-poor area, such as a “third-world” country or in a military environment where access to laboratory instrumentation is nonexistent.¹

Within this area, paper based devices hold particular appeal because they can be made inexpensively and are quite easy to dispose of. Paper-based devices are also capable of directing fluid flow using wicking and capillary action to produce microfluidic platforms that allow mixing of liquids and chemical reactions. This combination opens the door for a wide variety of analyses, with applications ranging from medical diagnostics to environmental monitoring and beyond.

In this experiment, you will create a paper-based microfluidic device and compare its analytical performance with that of a traditional bench-top instrument. You will prepare the device on filter paper by first generating a wax-based pattern to define microfluidic channels for reagent. A colorimetric reagent will then be introduced into the microfluidic channels, followed by standards and sample. After reaction, you will have a single device that contains colorimetric response for your standards and analyte that need to be converted to quantitative data for analysis. The transducer that will allow us to convert the chemical information held in the paper device to quantitative data will be the camera in your cell phone. Analysis of a photograph of your device using freely available image processing software will you to generate a calibration curve and determine the concentration of an unknown analyte. You will run a parallel analysis using traditional bench-top instruments and compare the performance characteristics of the two approaches.

Device Fabrication:

The devices you consist of a center well connected to six detection zones and will allow the analysis of a total of six solutions, typically one analyte and up to five standards.

1. Obtain a piece of Whatman #1 filter paper. *Be sure to handle the paper by the edges to avoid transferring finger oils to the paper's surface.*
2. Using the polystyrene template provided by your instructor and a pencil, lightly trace four copies of the template onto the filter paper, leaving ~5 mm between copies.
3. Use a wax pencil and trace over the pencil lines each device. Work to make dark, narrow wax lines and be sure that all line intersections are closed, otherwise your device will “leak”.
4. Place your filter paper in a 150°C oven for 5-10 minutes. This step allows the wax to flow through the paper and create a hydrophobic barrier through the thickness of the paper.
5. Remove the paper from the oven and allow it to cool to room temperature before using.

Device Use (follow these steps for each device on your filter paper):

1. Place the paper across the open end of a beaker or other container so that the devices are suspended. This will prevent unwanted fluid flow due to contact with the benchtop.
2. Using a micropipette, spot 40 μL of your color-forming reagent in the center well of each device. The solution will flow down the “arms” of the device and ultimately deposit the same amount of the color forming reagent in each arm. Allow at least 10 minutes for the fluid to flow and the device to dry.

3. Using a micropipette, deposit 3 μL of each standard, blank, or sample into each detection zone (one solution per detection zone). Allow the solutions to dry for at least 5 minutes
4. If follow-up steps are required (heating, etc.), perform them now.
5. Once all development steps are complete and the device is dry, use a camera phone, digital camera or flatbed scanner to capture an image of the device (jpg mode is preferred). Adjust camera or scanner settings to optimize contrast.
6. Follow the instructions in the "Using ImageJ for Data Analysis" document to quantify the response in each detection zone. Briefly, you will convert your photo to greyscale, invert the image, and determine the average grey values in the detection zones.
7. Using the results of your image analysis, prepare a calibration curve that relates concentration of your standards to average grey value in the detection zone. From this calibration curve, determine the concentration (with associated uncertainty) of the analyte in your sample solution.
NOTE: Ideally, the calibration curve will be linear. What do you do if it isn't?
8. Evaluate your results to estimate a detection limit and sensitivity for the paper-based method.

Traditional Spectrophotometric Measurement

1. Mix appropriate amounts of analyte, color-forming reagent and any other chemicals to quantitatively prepare standards in the same concentration range you used for the paper devices. Prepare an analyte "unknown" and reagent blank as well.
2. Using a spectrophotometer, measure the absorbance of each standard, analyte, and blank solution at an appropriate wavelength. Perform at least three replicate measurements of the analyte solution. (Do this by removing, rinsing, and refilling the cuvette for each replicate measurement).
3. Prepare a Beer's Law plot for your data, relating absorbance as a function of concentration. From this plot, determine the concentration (with associated uncertainty) of the analyte in your sample solution.
4. Evaluate your results to estimate a detection limit and sensitivity of the instrumental method.

Discussion and Conclusions

In the evaluation of your data, be sure to consider the following:

1. How do the figures of merit for the paper-based measurement compare to the bench-top instrument?
2. What are the key limitations of the paper-based method? What limits accuracy and precision?
3. What are the key limitations of the instrumental method? What limits accuracy and precision?

References

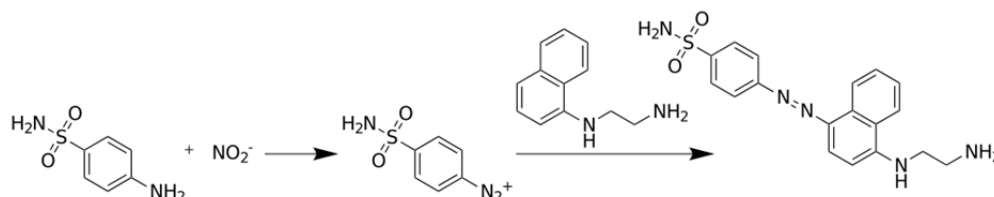
1. Martinez, A.W.; Phillips, S.T.; Whitesides, G.M.; Carrilho, E. "Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices", *Anal. Chem.* **2010**, *82*, 3–10.
2. Cai, L.; Wu, Y.; Xu, C.; Chen, Z "A Simple Paper-Based Microfluidic Device for the Determination of the Total Amino Acid Content in a Tea Leaf Extract", *J. Chem. Educ.*, Article ASAP

Analyte/Reagent Combinations

Nitrite Determination

Color Forming Reagent

Nitrite will be detected and quantified by treatment of a nitrite-containing sample with the Griess reagent, which is an aqueous solution of 0.1% naphthylenediamine dihydrochloride, and 1% sulfanilamide in 5% phosphoric acid. Reaction of nitrite with sulfanilamide produces a diazonium compound that further reacts to form a pink-colored azo compound, as shown in the reaction below.



A 0.1% naphthylenediamine dihydrochloride solution, and a 1% sulfanilamide in 5% phosphoric acid solution have been prepared for you. Prepare the Griess reagent by mixing equal volumes of these solutions. The naphthylethylenediamine compound is somewhat expensive, so care should be used not to waste the reagent. Ten milliliters of the Griess reagent should be sufficient for your analysis.

Standard Preparation (use 18 MΩ water for all solutions and dilutions)

1. From solid sodium nitrite, prepare 50 mL of 0.100 M NaNO₂.
2. Perform 1:100 volumetric dilution of your 0.100 M NaNO₂ to prepare a 1000 μM nitrite standard. From this solution, you will prepare two sets of standards, one for microfluidic analysis and one for spectrophotometric analysis.
3. Using dilution by mass in plastic vials, prepare 5 standards in the range of 10-300 μM nitrite. Prepare no more than 20 g of any single solution. A table such as that shown below might be useful in organizing your data.

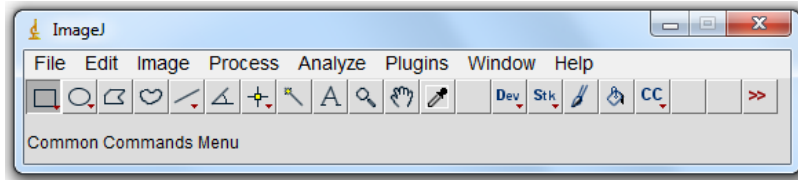
Solution	Conc. of Stock Solution	Microfluidic Solutions		
		Original Mass (g)	Final Mass (g)	Final Concentration (μM)
A				
B				
C				
D				
E				
Unknown				

4. Once your standards have been made, obtain an “unknown” from your instructor and prepare a 1:10 dilution by mass.
5. Solutions for the spectroscopic investigation will be prepared in cuvettes. You will need to obtain a total of seven cuvettes (for 1 blank, 1 unknown, and 5 standard solutions). Into each cuvette, pipet 300 μL of the solution to be tested, 100 μL of the Griess reagent and 2000 μL of water and mix well. Allow the solutions to sit for at least 10 minutes prior to collecting spectra.

Using *ImageJ* for Data Analysis

If *ImageJ* is not installed on your computer, you may download it at: <http://rsbweb.nih.gov/ij/>. The software is made freely available by the NIH and runs on all platforms that support Java (which is pretty much everything). Substantial documentation for ImageJ appears on the NIH website.


Once it is installed, locate the *ImageJ* program file and start the program. You should be greeted with the floating window below:

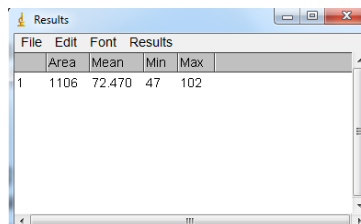


To open a file select **File** from the menu and select **Open** (or press Ctrl+O) and use the dialog box that appears to locate your file. The file will open in a new floating window.

To make quantitative measurements of "color" on your image, it is best to convert the image from color to grayscale so that the contribution of all colors to the depth of the image can be measured. Grayscale conversion is accomplished by selecting **Image** → **Type** from the menu and selecting **32-bit**. The result will be a 32-bit grayscale version of your image.

For increasing concentrations to produce a positive slope in our data, we must also invert the colors in the image by selecting **Edit** → **Invert** from the menu (or press Ctrl + Shift + I). We are now ready to make measurements.

Select the region of the image to analyze using one of the selection tools  in the *ImageJ* menu. Once a region has been selected, chose **Analyze** → **Measure** from the menu (or press Ctrl + M). A results window will appear with the area of the selection and the Mean, Max, and Min greyscale values of the selection (you can change the data that is displayed by selecting **Results** → **Set Measurements** from the menu in the results window.



Repeat selecting areas and analyzing until you have analyzed all pertinent areas of the image. When you are finished, the data can be transferred into a spreadsheet or other program by selecting **Edit** → **Select All** from the results window (or pressing Ctrl + A) and selecting **Edit** → **Copy** (or pressing Ctrl + C) and pasting the data into the new program.