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Design considerations for reducing sample loss in microfluidic paper-based analytical devices

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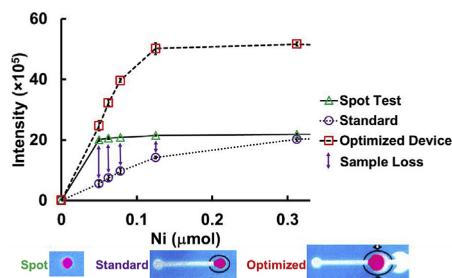
HIGHLIGHTS

- Sample retention modes in μPADs were determined and minimized.
- Analysis shows ≤50% of Ni(II) deposited reaches the detection zone.
- Simple design considerations can improve sensitivity and colorimetric signal by 28% and 78%, respectively.
- The developed approach can be utilized with other analytes and detection motifs.

GRAPHICAL ABSTRACT



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ABSTRACT

The field of microfluidic paper-based analytical devices (μPADs) is most notably characterized by portable and low-cost analysis; however, struggles to achieve the high sensitivity and low detection limits needs required for many environmental applications hinder widespread adoption of this technology. Loss of analyte to the device material represents an important problem impacting sensitivity. Critically, we found that at least 50% of a Ni(II) sample is lost when being transported down a 30 mm paper channel that is representative of structures commonly found in μPADs. In this work, we report simple strategies such as adding a waste zone, enlarging the detection zone, and using an elution step to increase device performance. A μPAD combining the best performing functionalities led to a 78% increase in maximum signal and a 28% increase in sensitivity when transporting Ni(II) samples. Using the optimized μPAD also led to a 94% increase in maximum signal for Mn(II) samples showing these modifications can be applied more generally.

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1. Introduction

Endeavors, such as the Human Exposome Project, demonstrate the growing interest in understanding the relationship between chronic exposure to environmental pollutants and changes in human health [1]. To understand complex exposures, efficient and selective methods are needed that can both identify and quantify chemical exposures from a variety of sources. Microfluidic paper-based analytical devices (μPADs) provide a tool that brings this level of analysis from the laboratory to the point-of-need due to their ease of use, portability, and low cost. Interest in μPADs has grown rapidly, and a number of applications have been developed in areas of both biological and environmental interest [2–9]. μPADs have used electrochemical and colorimetric detection motifs to measure concentration, with electrochemical detection frequently

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providing improved detection limits relative to colorimetric methods [10]. While the number and variety of applications continue to increase, an understanding of the paper properties that affect sensitivity has been lacking. Exploring the properties of paper as well as the interactions of samples and reagents with the cellulose substrate will enable further optimization of μPADs and related sensors.

Flow in μPADS is due to capillary action (imbibition) within the hydrophilic porous network; this flow may be modelled by Darcy's law and the Lucas-Washburn equation as described in multiple articles and reviews [11–13]. However, Li et al. identified a major limitation of current μPAD research as inefficient sample delivery to the detection zone through a combination of sample retention by the cellulose network and sample evaporation [14]. Due to sample losses during transport, limits of detection (LODs) on μPADs are frequently higher than traditional techniques or larger sample volumes are required to achieve comparable LODs. Recent work has focused on modifying flow rates and enhancing sample delivery by modifying the paper substrate using dissolvable bridges, creating flow channels with polymer films by applying coverings with varying contact angles, varying the channel geometries, or selecting substrates with different pore sizes [15–20]. While these studies have shown improvement, they have neither evaluated the fundamental impacts of the unmodified cellulose on sensitivity and detection limit nor provided simple solutions to aid in addressing this problem.

The work reported here expands on previous studies focusing on reducing sample retention within the cellulose network. To test strategies for reducing sample loss, colorimetric detection was chosen for its simple quantification. Using $Ni(dmg)_2$ as a model colorimetric system, sample retention in a lateral flow μPAD was indirectly determined by comparing the intensity of the detection zone against a spot test [21]. The spot test acts as a "zero-loss" test for which the sampling zone and detection zone are the same and there is no channel for sample loss. Sample loss to the cellulose was also determined by extracting the retained fraction into solution and quantifying with electronic absorption (UV-Vis) spectroscopy. The results suggested 50% or more of the initial sample never reached the detection zone. The extent of sample retention was investigated as a function of distance of travel, detection zone geometry, the inclusion of a flow-through waste region beyond the detection zone, fiber compression, and addition of a subsequent elution step. These investigations led to an optimized device, which resulted in a maximum signal increase of 78% and sensitivity increase of 28%.

2. Materials and methods

2.1. Materials

All of the following commercially available reagents were analytical grade and used as received without further purification: $NiSO_4 \cdot 6H_2O$, dimethylglyoxime (dmg), $MnCl_2 \cdot H_2O$, isopropanol, sodium tetraborate, and 4-(2-pyridylazo)resorcinol (PAR). The 0.1 M dmg solution was made using isopropanol. The $NiSO_4 \cdot 6H_2O$, $MnCl_2 \cdot H_2O$ and 0.1 M PAR solutions were made with Ultrapure water (18.2 MΩ cm) from a Mill-Q system (Merck Millipore Darmstadt, Germany) and used throughout. Whatman Grade 4 Qualitative (W4Qual) filter paper was purchased from GE Healthcare Life Sciences. Scotch™ heavy duty packing tape and 3 mil Scotch™ thermal lamination pouches sealed devices. The 0.1 M borate buffer was made using sodium tetraborate (pH 9.35).

2.2. Equipment

CorelDraw X4 was used for device design and the devices were printed on a Xerox ColorQube 8870 wax printer. A Xerox DocuMate 3220 was used to scan the devices prior to image analysis. ImageJ 1.49 was used to analyze the red colored $Ni(dmg)_2$ complex. An Agilent 8453 UV-visible spectrophotometer was used for electronic absorption measurements of $Ni(PAR)_2$ and $Mn(PAR)_2$. A Sartorius PR-50 pH meter with a PY-P28-2S electrode was used for all pH measurements and calibrated daily prior to use. A 30W CO_2 Epilog Laser Engraver was used to fabricate the wax-free devices. An Apache AL 13P thermal laminator was used to seal devices in lamination pouches.

2.3. Device designs

Devices were constructed by first cutting the Whatman paper into 8.5″ x 11″ sheets to fit them into the printer. A "Sky Blue" (R = 0, G = 124, B = 195) colored wax was used for the barriers to provide good contrast to the red-pink colored $Ni(dmg)_2$ complex. The diameter of the sample and detection zones for the standard device were 5 mm and the channel was 2.5 mm wide. From one end to another, the device was 30 mm long. These printed rings helped align the image analysis tool with a regular size and position. After devices were wax printed, the wax was melted (150 °C, 90s) into the fibers to create a hydrophobic barrier. An aluminum plate was placed over the device during heating to uniformly distribute pressure and heat. After melting, the bottom layer of the devices was sealed with Scotch™ Heavy Duty packing tape to prevent leaking. A 0.5 μL portion of borate buffer was first pipetted to the detection region and allowed to dry prior to adding 1 μL of the dmg ligand dissolved in isopropanol. Once the reagents were completely dry (~15 min), devices were ready for sample addition (Fig. S1). Laser cut, wax-free devices were also made for comparison. The dimensions of laser cut devices were the same as the melted wax devices. For laser cut devices, a filter paper sheet with packing tape on the back was used to create an array of devices that used only the edge of the device as a barrier. This method provided a leak-free barrier for the volumes used. All reagent depositions were identical to those for the wax devices. Laminated devices followed the same procedure as the other devices, but after the buffer and ligand deposition, the device was placed in a 3 mil thermal lamination sheet and melted with two passes into a laminator at 350 °F. Then the sampling zone was punched out with a hole-punch and resealed on the back with packing tape. To carry-out the extraction in Section 3.3, the detection zone was cut off a used device and the remainder of the device (sampling zone and channel) was placed in 4 mL of deionized water for 24 h to extract residual Ni(II). The resulting Ni(II) sample was analyzed by using PAR as the colorimetric indicator.

2.4. Image analysis

Image analysis was done using ImageJ following the procedure of Mentele et al. [22]. Instead of using the 8-bit grey scale, the green color space was used. This was achieved by splitting the color channels ("Image" → "Color" → "Split Channels") and using the "green" channel data. After the image was processed, the "oval" tool was used to select the image data for the detection zone area. In some devices, a circular black ring was added to the device to aid the oval placement and improve the consistency of the image analysis. The "Raw Integrated Density" values were divided by

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