Development of Paper-based Devices for Diagnostics and Biosensing

By
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Abstract

Research in paper-based analytical devices has been increasing rapidly in recent years. Many of these devices are used as low-cost alternatives for diagnostics and biosensing. In this work, two novel paper-based technologies were developed.

The first paper-based technology achieved was measuring streaming potential on paper-based microfluidic devices. The streaming potential measurements were able to detect the presence of adsorbed polyvinylamine or potassium polyvinylsulfate in paper-based microfluidic channels. The measured streaming potential ranged from -80 mV to 80 mV and the polarity was sensitive to the adsorbed polymer. Furthermore, the measured streaming potential on paper treated with BSA showed a polarity switch when the pH was changed from below the pKa to above the pKa of BSA. Lastly, streaming potential measurements may provide an electronic interface for paper-based sensors.

The second technology developed was a paper-based chromatographic pre-concentration device for biological and chemical applications. The device successfully concentrated a protein, streptavidin, via biotinylated microgels immobilized onto a selected area of the filter paper. The device was able to process a large volume of fluid with the incorporation of a passive pump made of superabsorbent polymer. The concentration factor achieved by the device was over 3000-fold. The flow dynamics through the paper was modeled using Darcy's law. This technology could be an excellent low-cost alternative for biochemical analysis for samples that require preconcentration, especially for the analysis of trace compounds in wastewater and drinking water.

Preface

This thesis was prepared as a "sandwich thesis". Chapter 2 is a publication in Colloid and Surface A: Physicochemical and Engineering Aspects, volume 364, issue 1, pages 16-18, July 2010. The data presented in the chapter was gathered by Abdul Shehata and myself. The compilation and analysis of the data was completed by me. Lastly, I wrote the paper under the guidance of Dr. Robert Pelton and Dr. Carlos Filipe.

Chapter 3 is a manuscript prepared for submission to a journal. The data presented was gathered by Sean Johnstone and myself. Yaqin Xu provided the microgels for the experiments. The analysis of the data and the compilation of the paper were completed by me with the help of Dr. Carlos Filipe and Dr. Robert Pelton.

Acknowledgments

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I would also like to thank Doug Keller and Sally Watson for all the administrative work that made my research possible. Furthermore, much of the experiments would not have been possible without Dan Wright and his technical expertise in instrumentation.

Moreover, I am extremely grateful for my summer students, Abdul Shehata and Sean Johnstone, for their hard work. Special thanks to all my colleagues in the Interfacial Science and Technologies Group at McMaster for fruitful discussions and making the research environment enjoyable.

I would like to thank, the Sentinel Bioactive Paper Network, NSERC and OGS for their financial support.

Lastly, I wish to express my love and gratitude to my parents and my wife for their endless support, and to God who gave me the opportunity and the ability to finish this thesis work.

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Chapter 1 Introduction

1.1 Paper-based Analytical Devices

Since its invention, paper has been widely used in a multitude of applications ranging from publishing and packaging to hygiene products. In the laboratory, paper filters are commonly used for chromatography and filtration purposes. In recent years, there had been much research activities in developing paper-based devices for biochemical analysis, medical diagnostics and bio-detection [1]. There are many advantages in using paper as the platform for diagnostic technologies.

1) Paper is widely manufactured from renewable resources and is inexpensive
2) It is combustible and biodegradable
3) The porous structure of paper enables wicking of liquid which is important for lateral-flow assays and chromatography applications
4) Paper is also suitable for biological applications since cellulose is compatible with biological samples
5) Paper surface can be easily manipulated through printing, coating and impregnation and can be fabricated in large quantities
6) It can be easily stored, transported and disposed
7) Existing use in analytical chemistry allow for easy transfer of techniques for new applications
8) Paper properties can be easily altered to suit different applications

One major area of research in this field is the development of low-cost paper-based medical diagnostic devices. The research is driven by the need for reliable diagnostic tools in the developing world where there is poor infrastructure, and the people cannot afford expensive

medical tests and have limited access to power and trained personnel [2].

One of the first paper-based diagnostic device created was for urinalysis [3, 4]. This research was led by Whitesides' group in Harvard University. These paper-based urinalysis devices utilize colorimetric assays to measure glucose and protein concentration in urine. Urinalysis using a paper-based diagnostic device is shown in Figure 1.1. The white color of the paper provides a strong contrast, which enhances the results of the colorimetric assays.

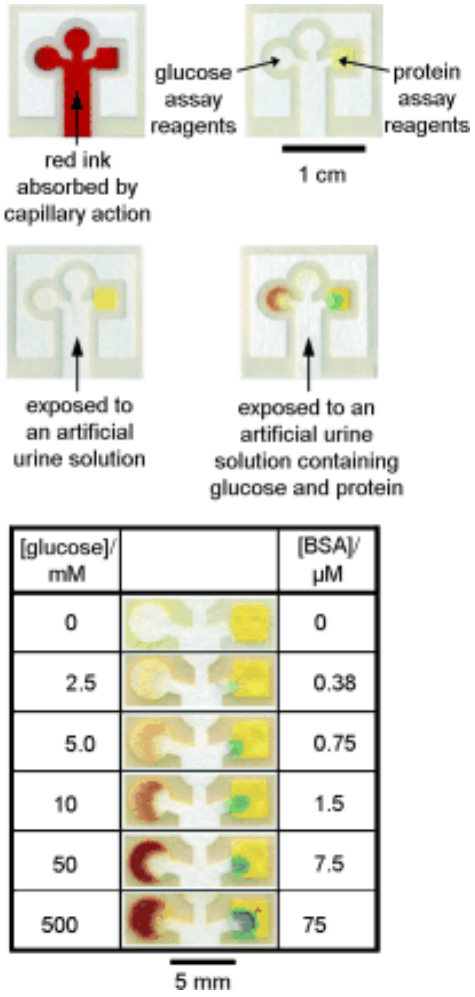

Figure 1.1 Example of a paper-based urinalysis device [3]. 5μL of artificial urine flowed into two detection areas, where it reacted with assay reagents. The colorimetric assay was scanned and the color intensity was processed by a computer to determine the concentration.

Furthermore, the concentration of protein and glucose in the urine sample was

determined by analyzing the color intensity in the detection zone. A later publication from the same group showed that an image of the device can be taken by a camera on a mobile phone and then sent to a remote location for analysis [4]. These studies are just beginning to show the potential for paper-based diagnostic devices for developing countries.

Another area of research in paper-based diagnostic is the development of paper microzone plates [5]. These paper plates are designed as a low-cost alternative to the conventional plastic microliter plates. Moreover, the ability to wick liquid can provide mixing of different analytes for different assays that might not be possible in a plastic plate. These paper-based devices provide a solid platform for low-cost high-throughput biochemical analysis [6]. Figure 1.2 shows an example of the paper microzone plates.

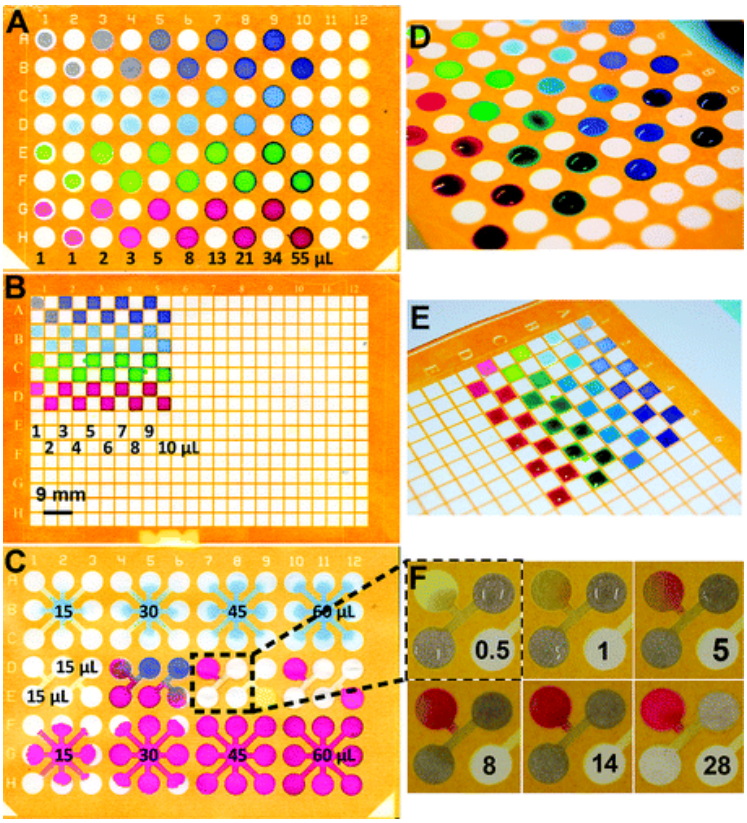

Figure 1.2 Examples of paper microzone plates, 96 wells (A,D), 384 wells (B,E) and plates with connected wells (C,F) [5]

Another important application for paper-based devices is in the area of pathogen and toxin detection. The issues of food and water contamination, spread of disease, and drug-resistant bacteria, which are concerns for the developing world as well as the developed world, are some of the major factors driving this research [1].

In general, these paper-based pathogen sensors are created by immobilizing a biosensor onto the paper-surface. Moreover, there are different types of biosensors that can be immobilized onto paper depending on the desired target. Antibodies [7], enzymes [8], DNA aptamers [9], phages [10] and cells [11] have all been shown in literature as viable biosensors in paper-based devices [1].

One of the first functioning paper-based detection devices had been developed by Brennan's research group, which is part of the Sentinel Bioactive Paper Network, in McMaster University [12]. In this work, sol-gel and enzyme were developed into a bioink which was printed using a piezoelectric inkjet printer onto filter paper. The bioink serves as an Ellman assay reagent which is a colorimetric assay for the rapid detection of acetylcholinesterase (AChE) inhibitors. The paper-based device was able to detect neurotoxins paraoxon and aflatoxin B1 within five minutes at low concentrations, ~100 nM and ~30 nM respectively [12]. Figure 1.3 shows the filter paper strip with enzyme doped sol-gel bioink printed onto its surface. It was shown that printing polyvinylamine (PVAm), a cationic polymer, onto the paper increases the effectiveness of the device by capturing the yellow assay product, 5-thio-2-nitrobenzoate (TNB), which is anionic. Lastly, the device was demonstrated to function as a lateral flow and a dipstick biosensor [12].

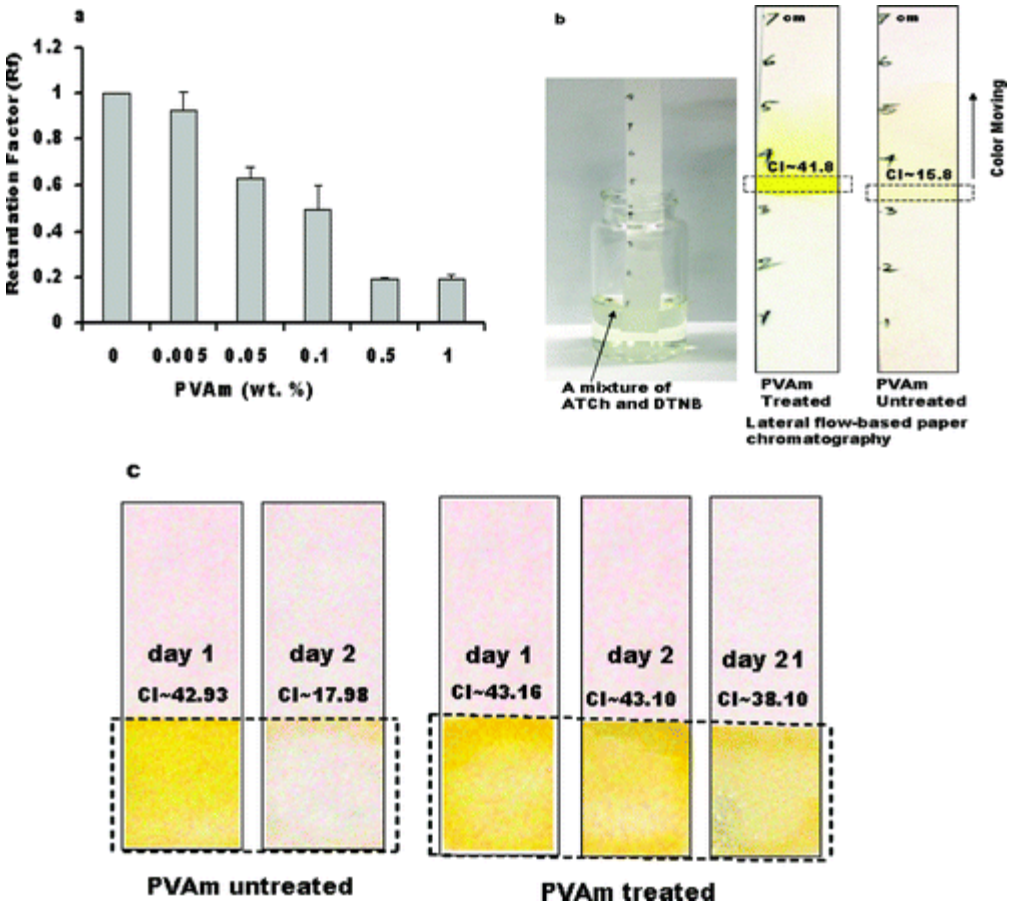

Figure 1.3 Example of paper-based biosensors for AChE inhibitors. The device function as a lateral flow biosensor (b) as well as a dipstick biosensor (c). PVAm treated devices had better detection efficiency due to better capture the assay product TNB by PVAm [12]

1.2 Paper-based Microfluidics

Most of the technologies discussed in the previous section exploit the wicking ability of paper to provide passive transport of fluids. Many research groups around the world have been developing paper-based microfluidic devices by patterning hydrophilic channels and hydrophobic barriers onto paper. These microfluidic devices can provide a low-cost alternative to the conventional glass, PDMS or other polymer based microfluidic devices. There are many approaches to fabricate paper-based microfluidic devices, such as, cutting [13], where a knife plotter is used to cut paper into designated patterns, however, these devices must be encased in tape . Another method is plasma etching [14] which was proposed by Wei Shen's group in

Australia. In this process the paper is first hydrophobized and then a patterned metal mask is placed on the paper and finally it is treated with plasma. The exposed portion that is treated with plasma becomes the hydrophilic channels. Paper exposure to solvents and polymers as well as the use of metal masks and plasma are major drawbacks of this method. A similar technique, inkjet etching [15] , was reported by Abe et al. in Japan. Their method involves first coating the paper in polystyrene and then using a modified inkjet printer to print an ethanol/water mixture to etch the polystyrene and create hydrophilic channels. This method, however, also exposes the hydrophilic channels to solvents and polymer, thus it is not an optimal procedure.

In comparison with the methods discussed above, photolithography [3, 4, 16, 17] and wax printing [18, 19] have received significantly more serious consideration as the procedure of choice. Whitesides' group was the first to demonstrate the use of photolithography to fabricate microfluidic channels on paper [3, 17]. The first step of the process is to impregnate the filter paper with photoresist, then a transparency film is placed on top of the paper and a black paper is placed under the filter paper. Then the desired pattern is printed onto the transparency film and exposed to UV light. The photoresist will polymerize forming a hydrophobic barrier in the paper. The paper is then removed from the film and baked to crosslink the exposed portion. Finally the paper is washed with solvent to remove the unexposed photoresist from the paper [17]. A diagram of the process and a picture of the microfluidic device are shown in Figure 1.4. Furthermore, using photolithography the group was able to create 3-dimensional microfluidic devices by layering paper and tape [16]. These 3-D paper-based devices can provide a low-cost alternative for more complex assays. However, there are a few drawbacks in photolithography; first, it is a multistep process and time consuming. Moreover, the paper channels are exposed to polymers and solvents which might affect the fluids that flow through the channels and

ultimately the assays being performed.

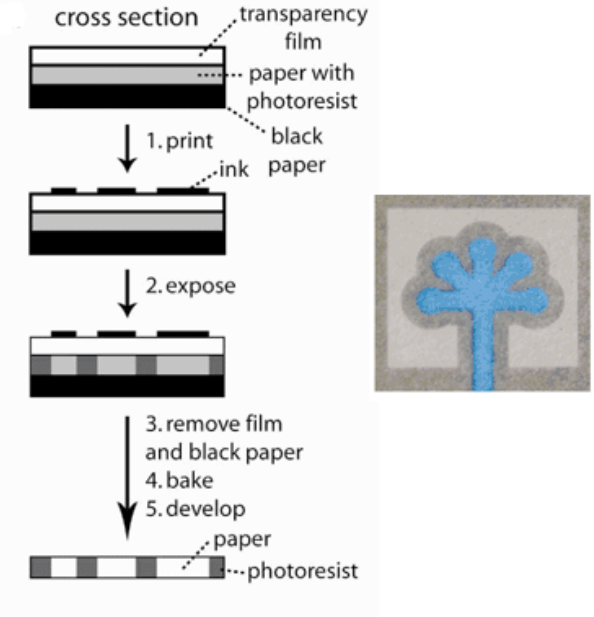

Figure 1.4 Photolithography process and paper-based microfluidic device fabricated via photolitography

The fabrication of paper-based microfluidic devices by wax printing was first introduced by Lu et al. [18]. Lu demonstrated the creation of microfluidic channels on paper with wax in three different experiments. The principle in the three experiments is the same they only differed in the method of patterning. A wax crayon was used to create the pattern in the first experiment, while in the second experiment the pattern was printed with ink and a wax crayon was used to color over the printed pattern, lastly the third experiment used a wax printer to print the pattern onto paper [18]. In each of the experiment, the paper was placed in an oven after the wax was applied. The heat from the oven melted the wax into the paper, thus creating hydrophobic barriers and hydrophilic channels. An example of paper-based microfluidics fabricated by wax printing is shown in Figure 1.5.

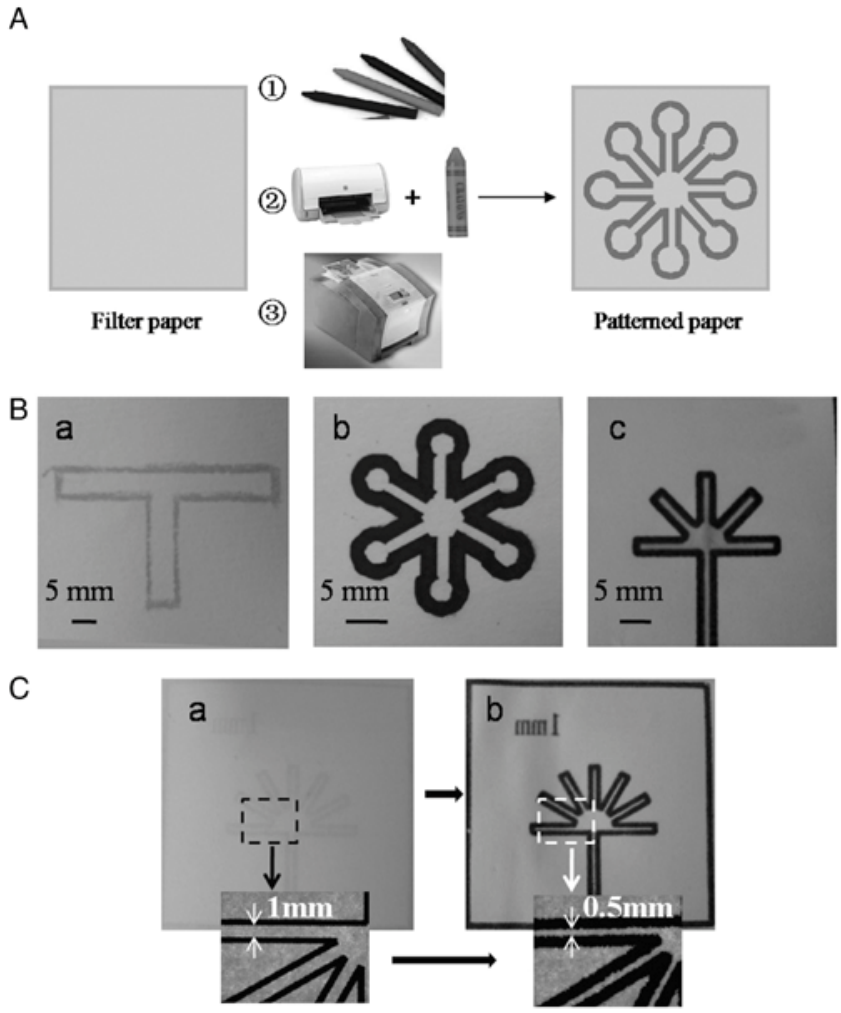

Figure 1.5 Paper-based microfluidics fabricated by wax printing, A) Three methods in creating wax patterned paper: A.1) wax crayons, A.2) wax crayons over printed lines, A.3) wax printer, B) The patterned paper from each method B.a) wax crayons, B.b) wax crayons over printed lines, B.c) wax printer, C) the dimension of the channels before (C.a) and after (C.b) melting of the wax.

Furthermore, Whiteside's group studied the melting of wax into paper [19]. It was shown that dimensions of the hydrophilic channels were harder to control due to the spreading of wax during the melting process. However, if the properties of the wax are known, an estimate of the spreading can be made. Despite a small loss in resolution, there are many advantages to wax printing. The hydrophilic channels are never exposed to solvents or polymers, existing commercial wax printers are readily available at a relatively low cost, and the process is rapid (~5 min) and simple. Given the benefits, the study concluded that wax printing is the optimal

method for large scale production of paper-based microfluidic devices [19]. The paper-based microfluidic devices presented in this thesis are all fabricated by wax printing with Xerox Phaser 8560N wax printer.

1.3 Streaming Potential in a Paper-based Device

Currently, most paper-based devices utilize colorimetric assays, although there have been reports of electrochemical sensing in paper-based devices for the blood and urine analysis [20] and for heavy metal detection in water [21]. In this thesis, a novel method of electrokinetic sensing in a paper-based microfluidic device is proposed.

Streaming potential is an electrokinetic phenomenon that is induced by the flow of an electrolyte across a channel or a porous medium with charged surfaces. Due to the charge on the solid surface, there is a potential difference at the solid/liquid interface which creates an uneven distribution of ions in the electrolyte solution near the surface. Figure 1.6 is a diagram of an electrolyte flowing across a positively charged surface. The positively charged surface attracts the negative counter ions from the bulk solution, causing a higher density of negative ions near the surface. This area near the charged surface is called the diffused electric double-layer. The flow of the bulk solution causes the negative ions to move across the charged surface and creates a measurable potential difference in the flow direction, which is the streaming potential.

Furthermore, there have been extensive electrokinetics studies of cellulose fibers in the area of papermaking [22]. More importantly, streaming potential measurements can be used in biosensing for different biospecifc interactions [23]. Thus, the integration of streaming potential measurement and paper-based microfluidics may provide a novel method for biosensing on paper.

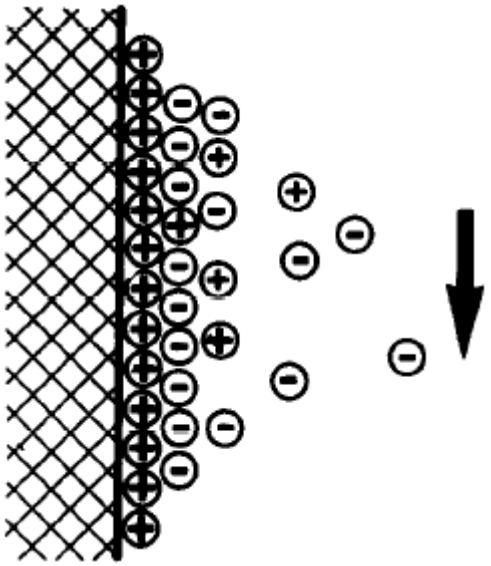

Figure 1.6 Flow of an electrolyte across a charged surface and the formation of an electric double-layer

1.4 Preconcentration

The research in paper-based biochemical detection had made great advances in the recent years, however, in many applications the concentration of a toxin or health hazardous materials are extremely low. One prominent example is the detection of trace amounts of pharmaceuticals and pesticides in wastewater and drinking water [24-26]. In many cases, the concentration is much lower than the lower detecting limit of the analytical device and preconcentration of the samples is needed. Moreover, chromatographic techniques have been used extensively in preconcentration of various analytes [24, 27-32]. Solid-phase extraction (SPE) coupled with either GC/MS or LC/MS is the most common strategy for the analysis of these trace compounds. The possibility of using paper in preconcentration is obvious, since it is already widely used in chromatographic applications and is much cheaper than conventional materials used in SPE. Furthermore, paper spray ionization, has been recently reported as a direct sampling ionization method for mass spectrometric analysis [33]. Thus, the use of paper as a platform for preconcentration and mass spectrometric analysis can be an excellent low-cost alternative to the

conventional analytical methods for trace compounds.

1.5 Objectives

The main objective of this work is to add to the corpus of research in paper-based technology, by developing devices for biosensing and diagnostic applications. The first approach to accomplish the goal is to explore the integration of paper-based microfluidics with streaming potential sensing. Since streaming potential measurements are sensitive to the surface chemistry of the channels, the proposed technology provides a simple approach to quantitatively measure the chemical or biological interactions at the interface of the hydrophilic channels, which can be translated to concentration measurements of target analytes.

The second approach is to increase the sensitivity of paper-based detection by the development of a paper-based preconcentration device. Furthermore, with the combination of paper-based preconcentration and detection technology, the range of toxins and biochemical hazards that can be detected could be increased. Lastly, combined with paper spray ionization and mass spectrometry, this technology can be ideal in the area of wastewater and drinking water analysis.

Chapter 2 Streaming Potential Sensing in Paper-based Microfluidic Channels

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2.1 Abstract

Streaming potential measurements detected the presence of adsorbed polyvinylamine or potassium polyvinylsulfate in paper-based microfluidic devices. Capillary driven flow in filter paper induced measurable potentials in the range -80 to +80 mV. The magnitude and polarity of the measured potentials were sensitive to the presence of adsorbed polymer. In a second example, paper treated with BSA gave a positive streaming potential at pH 3.7, below the isoelectric point of BSA, and a negative potential at high pH. We propose that streaming potential may provide an electrical interface for paper-based biosensors.

2.2 Introduction

The last few years have seen significant efforts from a number of laboratories to produce low cost biological and chemical sensors printed on paper [1]. In some applications, microfluidic devices formed by hydrophobic patterns on filter paper have been used to split fluid samples and transport them to biological or chemical sensing zones on paper [3, 15, 34]. Herein we report measurements of streaming potentials on paper-based microfluidics devices. We will show that the polarity and magnitude of the measured potentials are sensitive to the presence of charged

polymers adsorbed on the surfaces of the cellulose channels. Thus streaming potential measurements could provide an electrical interface for paper-based sensors, perhaps providing a simpler alternative to paper-supported redox based sensors recently reported [35].

2.3 Experimental

The polyvinylamine was a gift from BASF. Polyvinylsulfate, bovine serum albumen (BSA), and all buffers and filter paper were purchased from Sigma Canada. The microfluidic patterns were created using Microsoft PowerPoint and were printed onto Whatman #1 filter paper with a Xerox Phaser 8560N wax printer [19]. The patterns were printed with black ink with a printed line thickness of 3.5 pt. The patterns consisted of channels 0.5 cm in width and 1.5 cm in length, with semi-circular pads, the loading pad and the end pad, at each end of the channel. The filter paper sheets were cut into 21.59 cm x 27.94 cm (8.5 inch x 11 inch) sheets and printed so that the axes of the channels were aligned with the cross-machine direction of the filter paper. This choice was arbitrary and probably not critical as most filter paper is made with little directional variation.

The printed papers were heated at 120°C for 10 minutes to melt the wax into the paper. Two conducting leads were prepared by manually painting lines of conducting silver paste (E1660 Ercon Inc.) across the wax barrier 0.5 cm apart, half way along the channel. After room temperature drying, the leads were attached to a Fluke 12 digital multimeter with the upstream connection to the positive terminal. Figure 2.1 shows a photograph of the device.

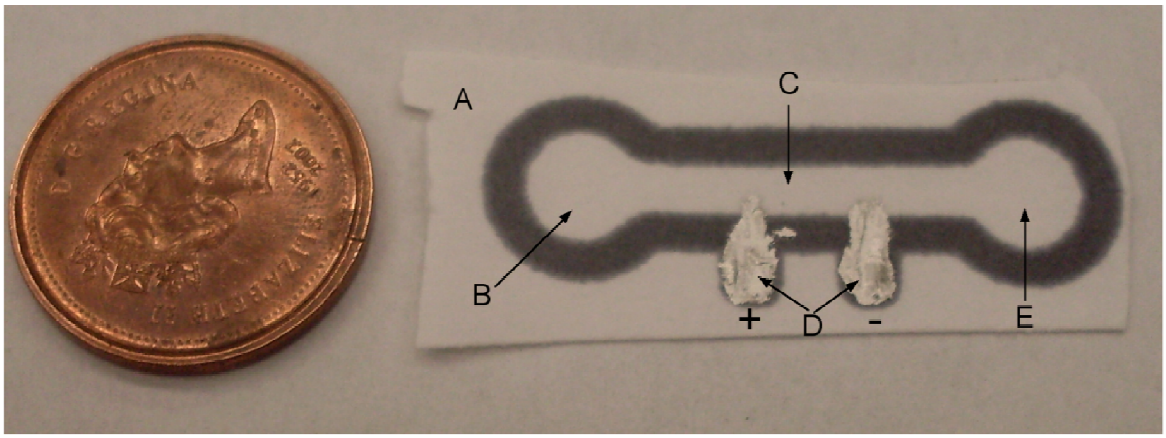

Figure 2.1 A. WHATMAN 1 filter paper, B. Loading pad, C. Channel, D. Silver conducting lead, E. End pad

In some cases, the channels were treated with 950 kDa polyvinylamine (PVAm) or with 150 kDa potassium polyvinylsulfate (PVSK). For this, 20 µL of 0.1 g/L polymer solution, pH 3.1, was distributed along the surface of the channel by spotting from a pipette in an effort to get a uniform distribution of polymer. Polymer distribution was not independently measured. The treated strips were air-dried. The treated paper had an average polymer content of 0.34 mg per gram of dry paper, which is close to ~1 mg/g required to saturated cellulose fibers [36].

For streaming potential measurements, 20 µL of 1 mM NaCl (pH 6.4) was spotted onto the loading pad. The electric potential across the leads was recorded at 10 second intervals for 10 minutes.

In a second series of experiments, the streaming potential of BSA coated paper was measured below and above the isoelectric point. Each of the triplicate experiments consists of the following three steps:

Step 1: 40 µL of 1 mM NaCl, pH 3.7 was placed on the loading pad of an unmodified channel via a pipette. Streaming potential was measured for 10 minutes.

Step 2: The unmodified channel was immersed in 1 mL solution of 1 g/L BSA (Sigma), pH

3.7, for 5 minutes and then air dried. 40 µL of 1 mM NaCl, pH 3.7 was placed on the loading pad of the channel via a pipette. Streaming potential was measured for 10 minutes.

Step 3: 40 µL of 1 mM NaCl, pH 6.5 was placed on the loading pad of the channel via a pipette. Streaming potential was measured for 10 minutes

2.4 Results and Discussion.

Figure 2.1 shows an example of the microfluidics devices used in this work. The devices were fabricated using Carrilho's wax printing approach in which a line of black wax is printed onto Whatman 1 filter paper [19, 37]. After printing, the paper was heated to melt the wax into the paper structure. The wax bands can confine the aqueous solutions in the device by two mechanisms – the wax can simply fill the pores blocking water migration or it could coat surfaces, increasing the contact angle and thus preventing capillary flow. The filter paper pore volume of 69% was determined by comparing the bulk density of the filter paper with that of pure cellulose. The ink density from our wax printer was measured (9.2 g/m^2) and the wax ink specific gravity was estimated to be 0.93. Assuming no lateral spreading during the melting process, only 8 % of the paper pore volume underneath the printed lines was filled with wax after heating. Thus the wax prevents fluid transport by lowering the cellulose surface energy and not by completely blocking the pores.

Figure 2.2 summarizes the results of a three-step experiment. In the first step, electrolyte solution was eluted along an untreated microfluidic device. There was no signal until the liquid front reached the second silver lead, which took about 100 seconds. The streaming potential was negative and showed poor reproducibility over triplicate experiments.

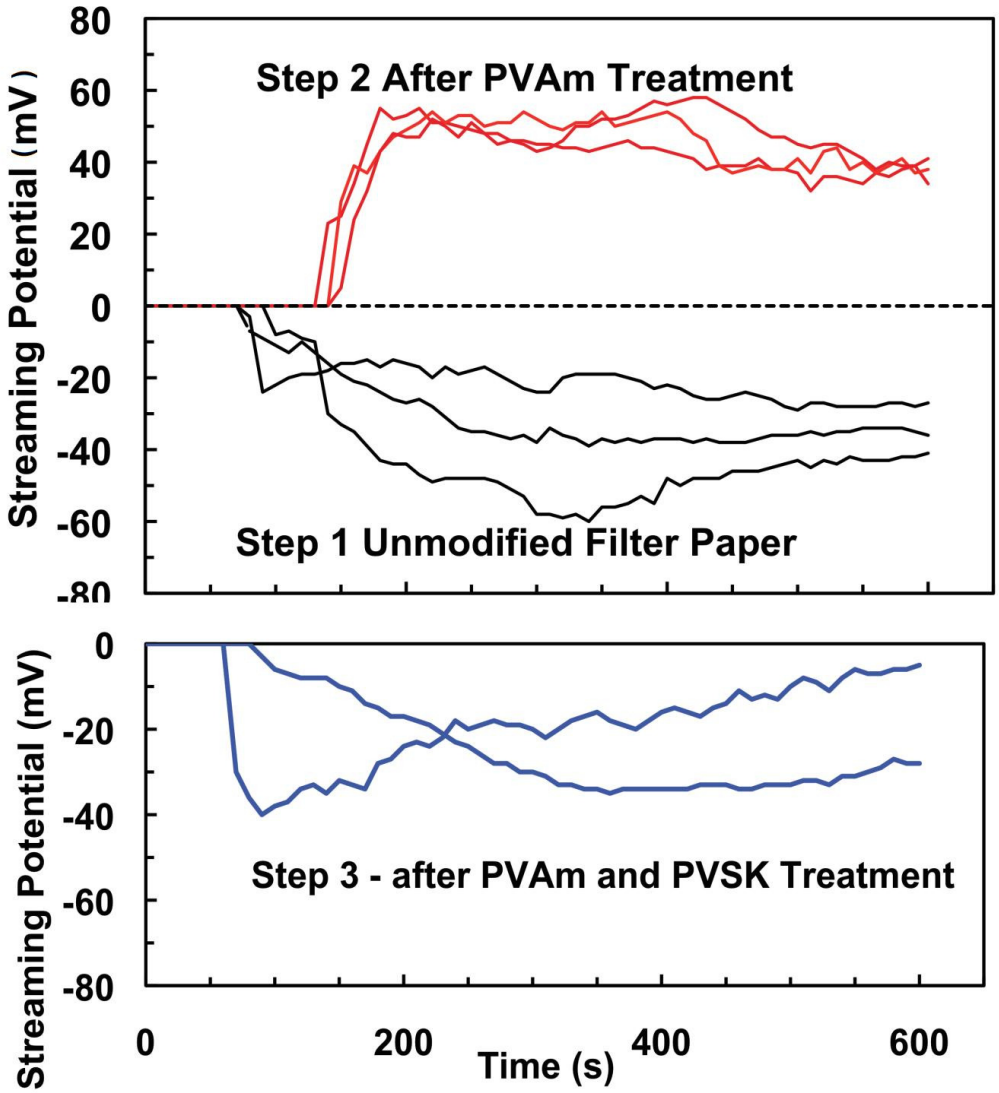

Figure 2.2 Triplicate streaming potential measurements after: 1 – no treatment of the paper-supported microfluidic device; 2 –PVAm treatment of paper; and, 3 – subsequent treatment with PVSK.

In a second step, the microfluidic devices were coated with polyvinylamine (PVAm), a very cationic polymer, and the streaming potential measurements were repeated. The polarity of the measured streaming potentials switched from negative to positive, reflecting the presence of PVAm at the cellulose/water interface. The PVAm treated surfaces gave more reproducible results than the untreated cellulose. The times required for the solution front to reach the second

lead were substantially longer for the PVAm treated paper compared with the untreated paper, suggesting the polymer inhibited fluid flow (see Figure 2.2).

In the third step, the paper PVAm coated paper was further treated with negatively charged potassium polyvinylsulfate (PVSK). The streaming current results indicated that the net charge of the cellulose/water interface was again reversed. Only two curves are shown because one of the microfluidic devices failed (leaked) during Step 3 experiments.

An indicator of the average fluid velocity was the time required for the liquid front to reach the second electrode, initiating a finite streaming potential. Although noisy, these times for Step 1 and Step 3 were nearly half the values for PVAm treated paper in Step 2. Presumably polyelectrolyte complex formation with PVSK caused the PVAm layer to collapse and thus interfere less with flow.

Figure 2.3 summarized the results of a second series of experiments demonstrating the utility of streaming potential measurements. In this case the paper was treated with BSA and the streaming potentials were recorded below and above the isoelectric point of the protein. At low pH the protein-coated paper was positively charged whereas at high pH it was negative, which is consistent with BSA's isoelectric point of 4.7 [38].

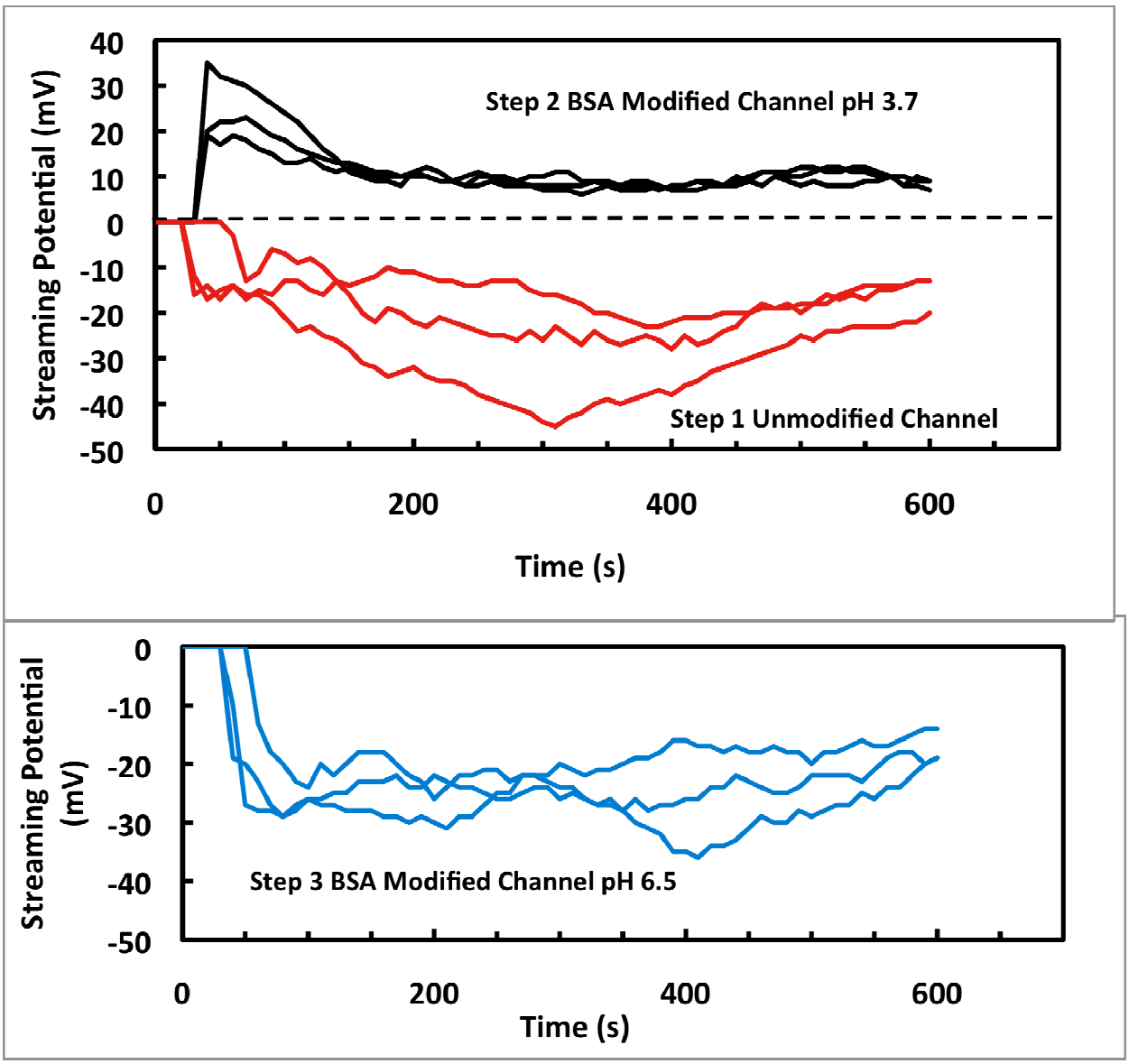

Figure 2.3 Streaming potential of BSA treated paper below and above the isoelectric point of BSA.

Streaming potential is an electrokinetic effect induced by the flow of liquid across an interface [22, 39]. The classic model, shown in Eq. (2-1), shows that the measured potential, ΔE, is a linear function of the zeta potential of the cellulose surface, ζ, and the pressure drop driving flow, ΔP. The other terms in equation (2-1) are solution properties - viscosity, η, equivalent conductivity, λ_o, and dielectric properties, $\varepsilon_o\varepsilon_r$.

$$\Delta E = \frac{\Delta P \zeta \varepsilon_o \varepsilon_r}{\eta \lambda_o} \qquad (2\text{-}1)$$

Equation (2-2) can be applied to interpret our results. The driving force for flow, ΔP, can be estimated by the classical capillarity equation for zero contact angle, where r is the effective pore radius of the paper and γ is the surface tension of water.

$$\Delta P = \frac{2\gamma}{r} \qquad (2\text{-}2)$$

An interesting feature illustrated in both Figure 2.2 and Figure 2.3 is that experiment-to experiment variation in the streaming potentials for untreated paper was much greater than for the polymer or protein coated samples. Since streaming potential reflects both the density of surface charge groups and the fluid flow rate near the surface, variability could either be due to variation in charge density or in the capillary driven flow. We propose that the variance on untreated paper might be due to variations in the extent to which wax ink spread into the measurement channel. The presence of ink contaminants could increase the contact angle, lowering the rate of capillary driven flow near the electrode. Polymer or BSA adsorption could overcome wettability loss due to trace wax components. Alternatively, an adsorbed layer of polymer or protein could give a more uniform charge density compared to the original wax contaminated paper. More work is required to understand the cause of the variability.

Applying the above equations to our experiments, and assuming an effective pore size of Whatman 1 is 2.54 μm [40], the measured streaming potentials were converted to zeta potentials by using $\zeta = 0.315 \Delta E$. Thus the zeta potentials of the untreated paper from the results in Figure 2.2 were -6 to -19 mV, which is consistent with published zeta potentials for cellulose from a variety of sources [41].

In summary, we used polyelectrolytes to illustrate that streaming potential measurements can

be used to monitor the adsorption of charged molecules onto cellulose surfaces in microfluidics devices. We propose that the binding of biological targets to receptors conjugated to the cellulose surface could be detected by changes in streaming potential.

2.5 Acknowledgements

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Chapter 3 Paper-based Device for Pre-concentration of Target Analytes

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3.1 Abstract

A novel, low cost paper-based chromatographic pre-concentration device for biological and chemical applications was developed. By immobilizing biotinylated microgels onto a selected area of the filter paper, the device was able to bind specifically to a target analyte, the protein, streptavidin. With the incorporation of a superabsorbent passive pump, created using Cross-linked poly(acrylic acid), partial sodium salt-*graft*-poly(ethylene oxide) the paper-based device was able to process a significant volume of dilute solution. Using confocal laser scanning microscopy (LSM), the concentration factor achieved by the paper-bed device was determined to be over 3000x. The flow dynamics through the paper was modeled using Darcy's law.

3.2 Introduction

In the past few years significant research activity had been focused on the development of biological and chemical analytical devices on paper [3, 9, 21, 35, 42-44]. The attraction to paper-based products is due to its ease of fabrication and low cost. A major application for these paper based devices is for the detection of different bioactive molecules. However, many analytical devices require pre-concentration, since most samples have very low concentrations which are below the detection limit of the analytical system. Some examples of where

preconcentration is needed are, preconcentration of food volatiles in the food industry[45], detection of lead in food and the environment [46], and for analysis of dilute biological and environmental samples [30].

Currently, pre-concentration can be performed using a few different techniques, such as electrophoretic and chromatographic techniques [30]. The advantage of the electrophoretic approach is in its simplicity, however, only small volumes can be processed at one time. Furthermore, complex solutions might lead to poor separations. Conversely, chromatographic techniques are able to process larger volumes and provide better separation for complex solutions [30]. In the past, paper has been used in chromatographic applications [47-49], therefore paper can be a suitable platform for a pre-concentration device. The advantage to a paper-based concentration device is that it will be low in cost, biodegradable, combustible and simple to manufacture.

Herein we report the development of a paper-based chromatographic pre-concentration unit. We will show specific binding to the protein streptavidin was achieved by immobilizing biotinylated poly(*N*-isopropylacrylamide-*co*-vinylacetic acid), PNIPAM-VAA, microgels onto a designated area on the paper. Streptavidin has a very high affinity to biotin, thus the incorporation of the biotinylated microgels can dramatically increase the concentrating power on the paper-based device. We demonstrate the relationship between concentration factor and time. Concentration factor is defined as the ratio between the apparent concentration of the filter paper and the concentration of the dilute solution. Moreover, we proposed a simple method to calculate the concentration of an unknown solution, given the volume uptake and the apparent concentration measured. We also developed a simple passive pump to increase volume uptake by the device. Furthermore, the paper-based device can achieve over 3000x concentration of the

protein from dilute solution. Finally, the paper-based concentration unit can provide a cost effective solution to lower the detection limits of the paper-based biochemical analytical devices.

3.3 Methods

Materials. Atto 610 Streptavidin (SA), a fluorescently labeled protein, and polyethylene glycol (PEG) (20kDa) were obtained from Fluka. Cross-linked poly(acrylic acid), partial sodium salt-*graft*-poly(ethylene oxide), a super absorbent, was received from Aldrich. Whatman No. 1 filter paper was purchased from Fisher. Biotinylated PNIPAM-VAA microgel (MG) was prepared by Yaqin Xu [50].

Biotinylated Microgel Preparation. PNIPAM-VAA microgel was prepared according to Hoare's method [51]. A 500-mL three-necked flask fitted with a condenser and a glass-stirring rod was charged with 12.4 mmol NIPAM (1.403 g), 0.65 mmol MBA (0.100 g), 0.17 mmol sodium dodecyl sulfate (0.049 g), and 1.2 mmol vinyl acetic acid (0.10 g) and 150 mL water. The mixture was heated to 70 °C under a nitrogen blanket and 0.52 mmol (0.100 g) of ammonium persulfate initiator (dissolved in 10 mL) water was added. The mixture was stirred overnight at 250 rpm with a glass/Teflon paddle stirrer and then cooled to room temperature. Several cycles of ultracentrifugation (Beckman model Optima L-80 XP, 50 min at 50,000 rpm) were used to wash the microgel dispersion. The washed microgel was then redispersed in water. Microgels were lyophilized and stored at room temperature. The average diameter of the microgel particles was approximately 300 nm and was determined using dynamic light scattering. Biotinylation of the microgel was accomplished by adding 156 mg biotin hydrazide in 10 mL dimethyl sulfoxide to 150 mg of microgel at room temperature. N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and sulfo-NHS were added after 5 minutes and the pH was adjusted to 7.4 with 0.01 mol/L NaOH. The pH was maintained

at 7.4 by checking it every 30 minutes and adjusting it using 0.01 mol/L HCl while the mixture was stirred for 10 hours. Several cycles of ultracentrifugation (50,000 rpm, 50 min) and redispersion in water were used to purify the biotinylated microgel. The biotinylated microgels used where found to have a biotin content of 24.39 µg/mg MG, using the procedure described by Xu *et al.* [50].

Preparation of Concentration Device. Whatman No. 1 filter paper was cut into 0.5 x 10 cm ribbons along the machine direction. The concentration zone was created by applying 10 µL of 1 g/L biotinylated PNIPAM-VAA microgel 3 cm from the bottom of the paper ribbon and then air-dried. This ensured that the SA solution did not immediately contact the concentration zone. Once microgel is dried on the filter paper, the ribbon is submerged in a 25 g/L PEG solution for 5 minutes and then air-dried. PEG is used to deter unspecific binding of streptavidin to the filter paper. The high concentration of PEG was used to ensure good coverage on the filter paper and excess PEG was washed off the surface.

Passive Pump. A passive pump was created to increase the liquid flow through the concentration zone. First, a pouch was created from Whatman 1 filter paper. Then 200 mg of cross-linked poly(acrylic acid), sodium salt was added to the pouch. Finally, the pouch was attached to the paper ribbon 3 cm above the concentration zone. This made for a super absorbent, passive pumping system. The super absorbent increased the liquid uptake capacity, thus allowing more liquid to flow through the paper ribbon.

Experimental Setup. In a 200 mL beaker, 2 µL of Atto 610 Streptavidin stock solution (1 g/L) was diluted with 1x PBS solution, pH 7.4, to a volume of 20 mL, resulting in a 0.1 mg/L SA solution. Then the paper ribbon with the super absorbent passive pump was suspended inside the beaker by tape so that the bottom of the ribbon is in contact with the bottom of the beaker and

about 1 cm of the ribbon was submerged in the SA solution, thus, the solution was never in direct contact with the biotinylated PNIPAM-VAA microgel. Finally, the beaker was sealed by paraffin wax to prevent evaporation. The experiment was performed under constant temperature and humidity, at 25ºC and 100% RH.

Figure 3.1 is a diagram of the experimental setup. Eight individual beakers were prepared to investigate the concentration factor as a function of time. Each ribbon was removed from its beaker at a different time and the ribbon analyzed. The final ribbon was removed after 160 hours. The final volume in each beaker was determined by weighing the remaining solution in each of the beakers on a balance.

Confocal Microscopy. Each filter paper ribbon was analyzed using Zeiss LSM 510 laser scanning confocal microscope. The imaging of Atto 610 Streptavidin was done using a HeNe laser with excitation wavelength of 543 nm and emission wavelength of 560 nm. The ribbons were imaged at 1cm intervals at 5x and 20x magnification.

Data Analysis. The images were analyzed relative to the control samples. 1 µL of 1 g/L, 0.5 g/L and 0.1 g/L Atto 610 streptavidin solutions were spotted on three Whatman 1 paper strips respectively to be used as controls.

From the LSM images, the fluorescence intensity of SA was analyzed using the CMYK colour scheme, specifically the intensity of magenta was used to measure the presence of SA. Using the histogram function in Adobe Photoshop 10.0, the intensity data was recorded. A calibration curve was constructed using the intensity data from the control samples. This calibration led to calculated values of SA concentration in each of the samples.

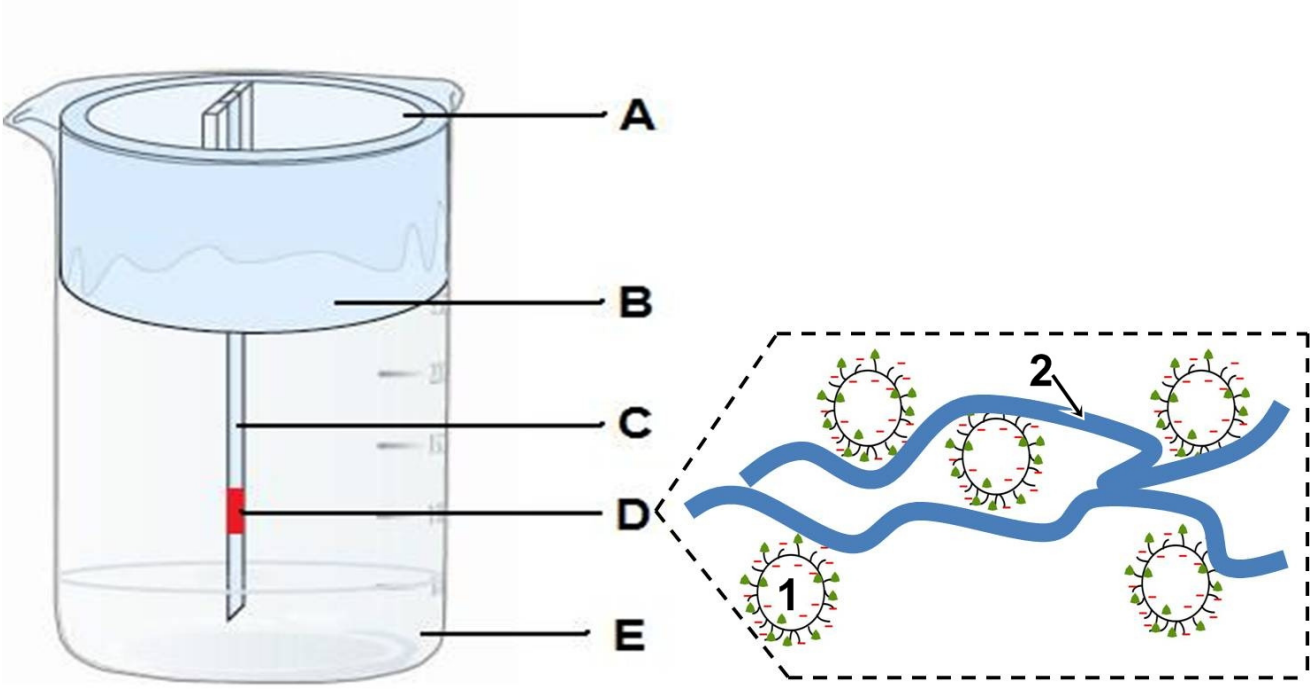

Figure 3.1 Diagram of experimental setup. A. Paraffin Wax to seal the beaker, B. Pouch with cross-linked poly(acrylic acid) as a passive pump, C. Whatman 1 filter paper ribbon, D. Biotinylated PNIPAM-VAA microgel concentration zone and magnified view of microgel (D.1) immobilized onto paper fibers (D.2), E. Dilute streptavidin solution

At 5x magnification, 2 mm x 2 mm frame images are taken by the LSM. In order to grasp the complete image of total captured SA, a 6 mm x 6 mm frame composite image was created using Adobe Photoshop from LSM images of the concentration zone. Furthermore, each 6 mm x 6 mm image was divided into three sections and the intensity of each section was measured. These three measurements were used to determine the standard deviation of concentration between the three sections in the concentration zone.

3.4 Results

The paper-based device was effective in providing significant concentration of SA. Figure 3.2 compares the LSM images of a control sample, a filter paper with 0.1 g/L SA applied on the surface (Figure 3.2A) and the paper-based concentrator at the end of the experiment (Figure 3.2B). It is clear that a substantial amount of SA was captured by the paper-based device. Figure

3.3 shows the LSM images of the concentration zone on the filter paper ribbon over time. The images show that the intensity and the area of captured SA increases as the time of the experiment increases. The eight images were of eight distinct paper-based devices with identical set-up, each was removed from the protein solution at a different time. The calculated concentration factor for each paper ribbon as a function of time is shown in Figure 3.4. From each image, the apparent concentration of SA was calculated using a calibration curve generated from the control samples, which took into account the fluorescence intensity and the coverage area of SA. The apparent concentration is calculated relative to the protein concentration of the control solution sample and is not a representation of the concentration on the filter paper itself. For example, an apparent concentration of 0.5 g/L SA represents a filter paper that has the same fluorescence intensity as a filter paper with a solution of 0.5 g/L SA applied on it. Concentration factor was then calculated by dividing the apparent concentration by the solution concentration, 0.1 mg/L. Figure 3.4 summarizes the relationship between concentration factor and time elapsed. Each image was divided into three sections and the error bars indicate the standard deviation of the intensity between the sections within each image.

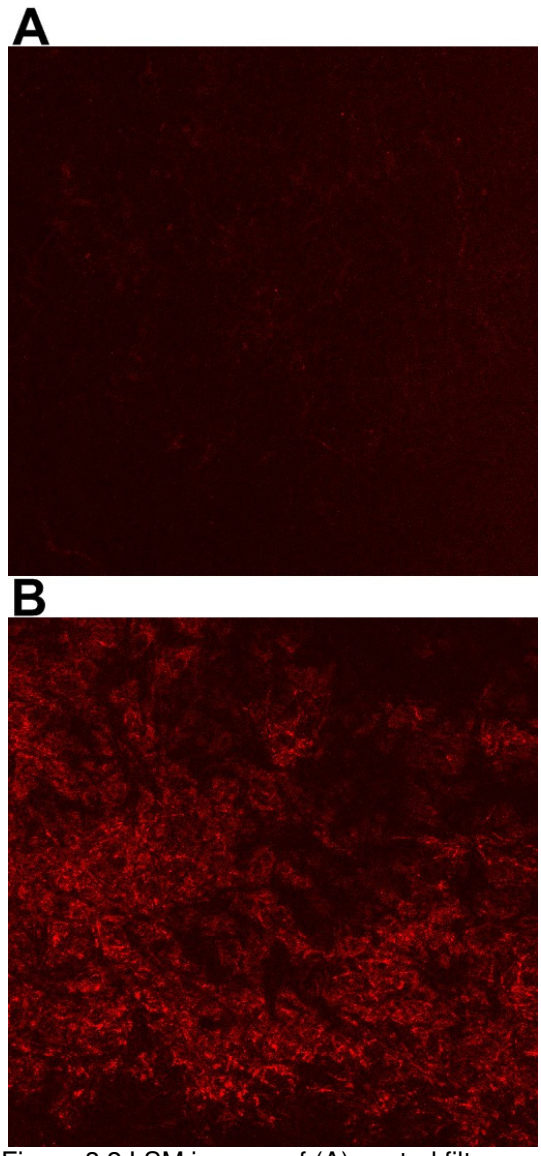

Figure 3.2 LSM images of (A) control filter paper with 0.1g/L SA applied and (B) Concentration area of the paper-based device after 160h

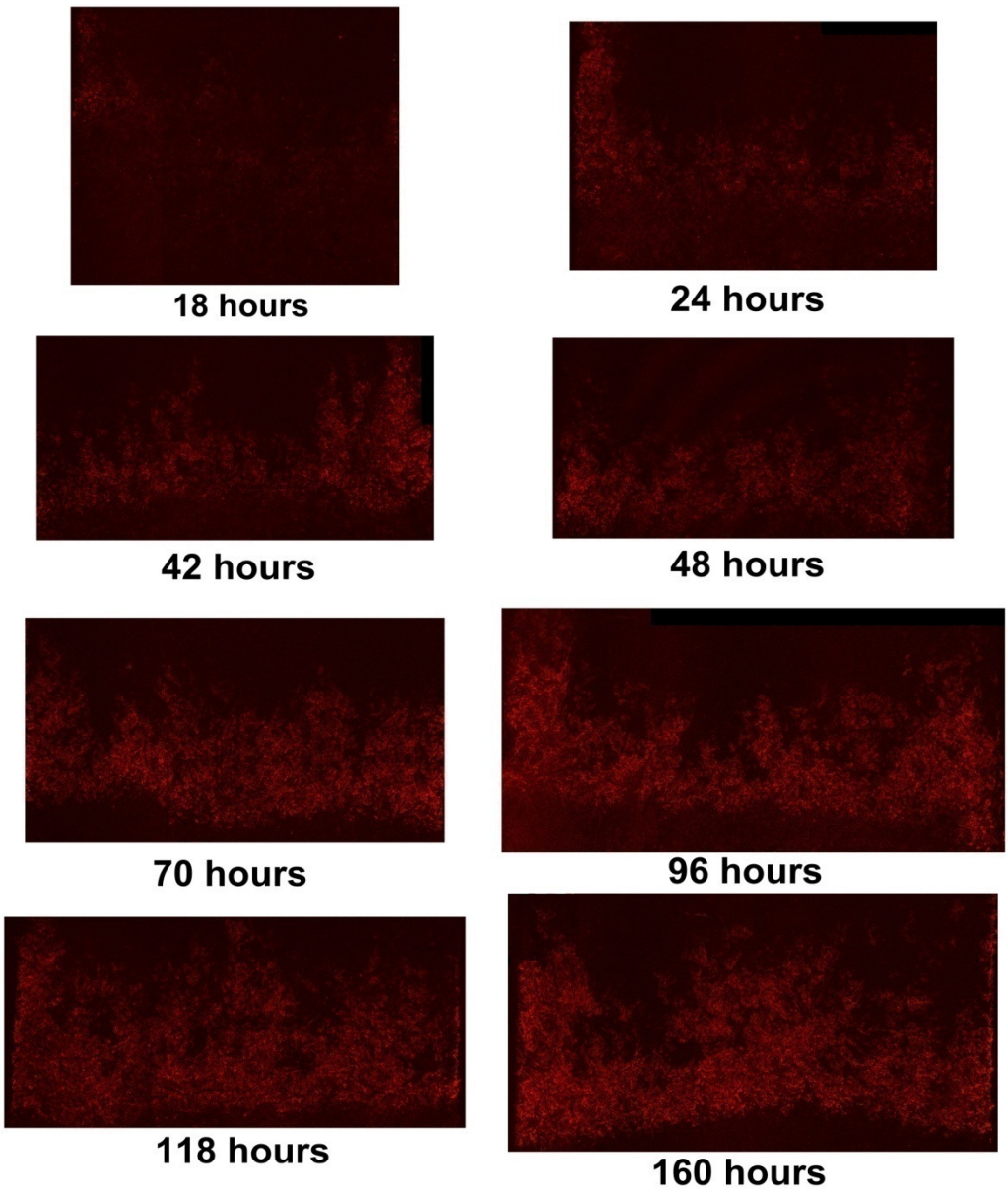

Figure 3.3 LSM images of the concentration area of paper-based device over time

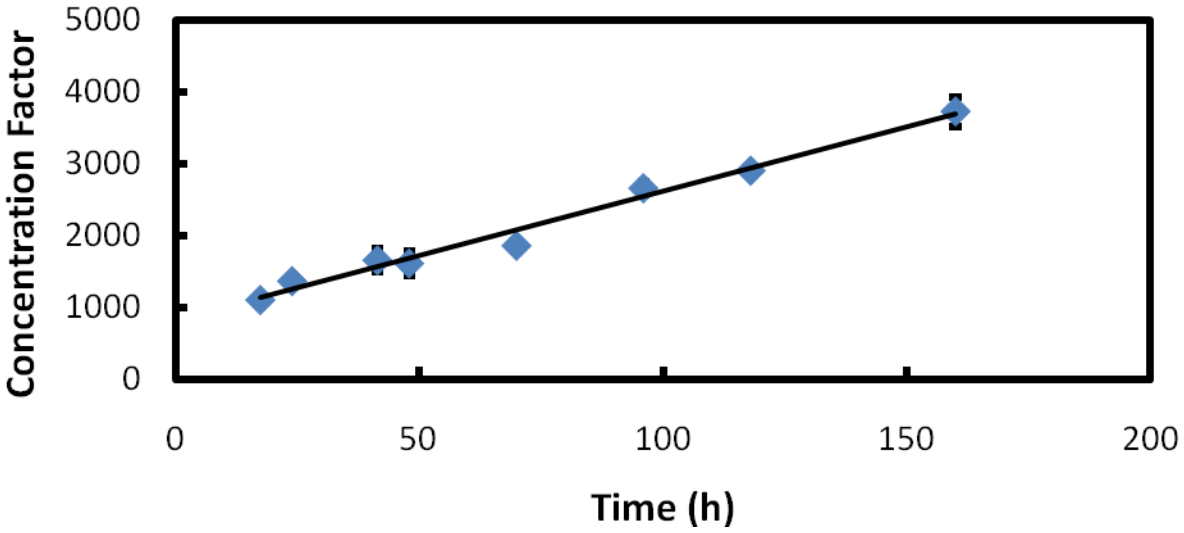

Figure 3.4 Concentration factor achieved as a function of time, the standard deviation indicated by the error bars demonstrates the concentration deviation within the capture area on the paper strip and was calculated using intensity measurements from three different sections of each LSM image. The linear fit is indicated by the solid line.

In this study, the effectiveness of the superabsorbent passive pump was also of interest. The initial volume in each beaker was 20 mL, thus the initial solution mass was 20 g. After the removal of the paper ribbon from the beaker, the remaining solution is weighed and the uptake volume was calculated as the difference between the final mass and initial mass. Figure 3.5 shows the relationship between solution uptake and the time elapsed. Without the passive pump the device was only able to take up approximately 5 ml of solution after 160 hours, thus, the use of the superabsorbent increased the solution uptake and the effectiveness of the device considerably.

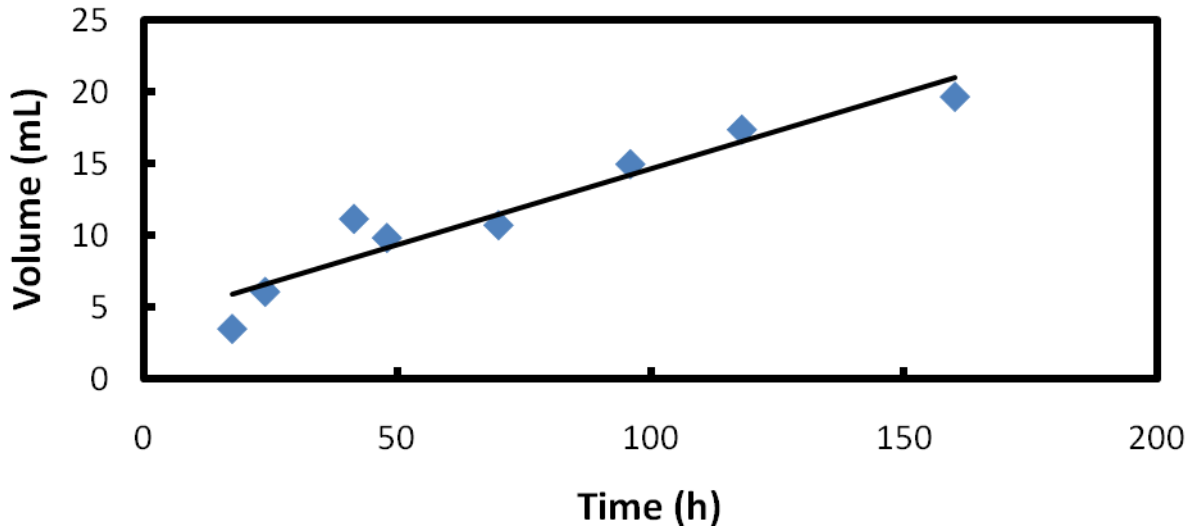

Figure 3.5 Volume uptake by the paper-based device with superabsorbent passive pump as a function of time

3.5 Discussion

In this work, the protein solution is wicked up the filter paper as a result of capillary action. The lateral flow of the solution in the filter paper can be modeled by Darcy's law [52].

$$Q = \frac{KA\Delta P}{\eta L} \qquad (3\text{-}1)$$

where Q is the volumetric flow rate, K is the permeability of the paper, A is the cross-sectional area perpendicular to flow, ΔP is the pressure gradient, η is the viscosity of the solution and L is the height of the liquid. Assuming that there are no applied pressures and neglecting gravitational terms, ΔP can be calculated using the Laplace equation, where γ is the surface tension of the solution, θ is the contact angle and r is the effective pore radius of the filter paper.

$$\Delta P = \frac{2\gamma\cos\theta}{r} \qquad (3\text{-}2)$$

For the system of interest, initially the solution penetrates through the filter-paper ribbon and the concentration zone, and then it comes into contact with the superabsorbent. Thus, we can model the process in two phases. In the first phase the liquid front is moving up the filter paper

and can be modeled using a modification of the Darcy's law shown in Eq. (3-3) [53],

$$L = \sqrt{\frac{2K\Delta P}{\phi\eta}t} \qquad (3\text{-}3)$$

where ϕ is the porosity of the paper and t is time.

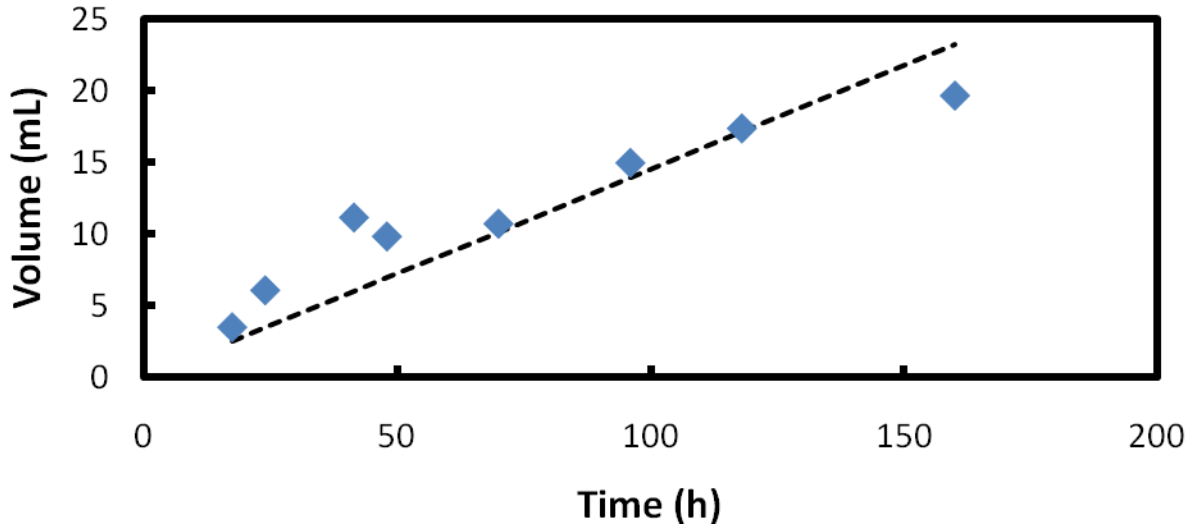

Figure 3.6 Volume uptake data compared with Darcy's law prediction (dashed line) with K= 0.093 x 10^{-12} m^2

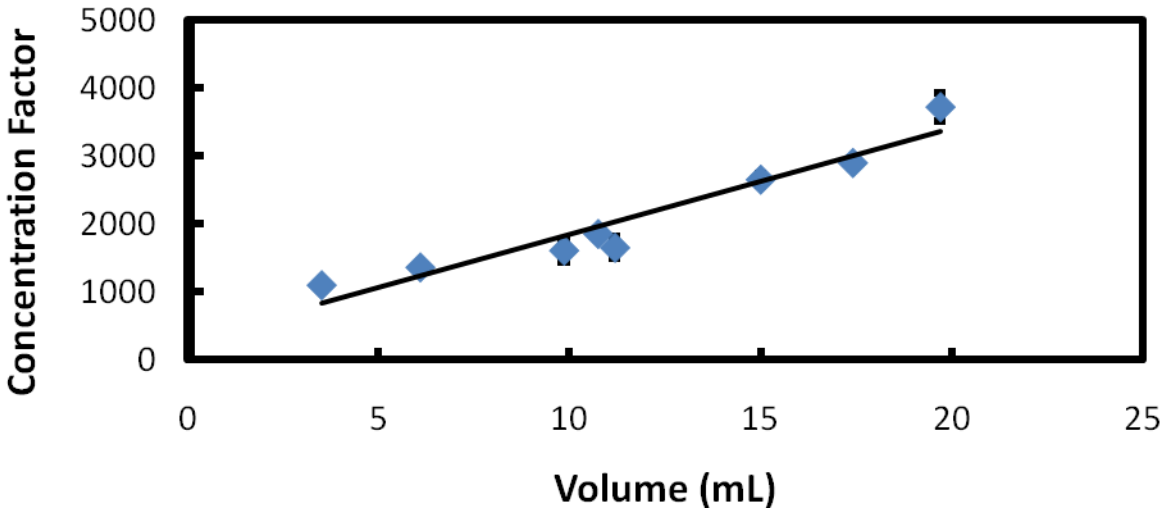

Figure 3.7 Concentration factor achieved by paper-based device as a function of volume uptake, the standard deviation indicated by the error bars demonstrates the deviation in concentration factor within the capture area on the paper strip and was calculated using intensity measurements from three different sections of each LSM image.

Once the solution comes into contact with the superabsorbent the height of solution in the filter paper is assumed to be constant. Therefore, when employing Eq. (3-1), Q is constant in this second phase.

From the calculations, the solution reaches the superabsorbent within 30 minutes. Therefore given the length of the experiment Eq.(3-1) is adequate to model the system. Using the concentrations calculated from LSM images, the average permeability, K, of the paper-based device is 0.093×10^{-12} m^2, which is consistent with published permeabilities of paper [54]. Figure 3.6 shows the experimental data with the model based on Darcy's law (dashed line).

Furthermore, Figure 3.7 shows the relationship between volume uptake and concentration factor. Based on regression analysis, the linear relationship between volume and concentration is statistically significant with a 95% confidence interval. As the solution passes through the filter paper, SA is captured in the concentration area, therefore as more solution is taken up the amount of SA on the filter paper increases proportionally. It is important to note that the apparent concentration on the paper-based device is a function of geometry; a smaller concentration area would increase the apparent concentration for the same volume uptake. However, a couple of issues need to be considered. First, a change in geometry affects the volumetric uptake rate since Q is a function of cross-sectional area, A, in Eq. (3-1). Secondly, there is an adsorption limit for the microgel to be immobilized on the paper surface.

From the LSM images, there were no signs of breakthrough of SA despite only 10 µg of biotinylated microgel was applied onto the paper, which would approximately bind 1µg of SA [50]. However, 2 µg of SA was in the original solution. There are a few explanations, first some of the SA might be on the surface of the beaker and was not taken up. Second, some SA might bind to the paper but was not significantly concentrated to be detected by LSM. This can occur

both prior to and after the concentration zone, thus breakthrough might have occurred without detection.

Non-specific binding of SA onto cellulose can be estimated assuming the adsorption coverage of a protein on cellulose to be approximately 1 mg/m^2, and the specific surface area of cellulose to be 9.76 m^2/g [55]. The estimated protein coverage of SA to Whatman 1 filter paper is 859 mg/m^2. However, the amount of SA bound to cellulose will be much lower because of the PEG on the surface.

Moreover, the concentration of an unknown solution can be estimated given the total volume uptake and the apparent concentration. In the calibration step, the intensity from LSM image is function of the mass of SA and the area of the control samples and can be expressed in Eq. (3-4),

$$I = k\frac{m}{A} \qquad (3\text{-}4)$$

Where I is the intensity, m is the mass of SA, A is the area of the filter paper, and k is a constant calculated from the calibration. Therefore, given the intensity from an LSM image of a concentration area and the size of the area, one can estimate the mass of SA present. Furthermore, if the volume uptake is known then the concentration of the unknown solution can be calculated. However, as previously discussed there might be unspecific binding and other factors that lower the efficiency of the device. Therefore, a calibration experiment using a known solution can be utilized to determine the overall efficiency of the device. Thus, given Eq. (3-4) the concentration of an unknown solution can be estimated by Eq. (3-5),

$$c = \varepsilon\frac{IA}{kV} \qquad (3\text{-}5)$$

Where c is the solution concentration, ε is the efficiency of the device and V is the volume taken up because m=cV. From LSM data gathered the efficiency of the device was calculated to be approximately 25%, thus, $\varepsilon = 0.25$. Moreover, for applications where volume uptake is

difficult to measure, such as sampling of a flowing stream, the volume uptake can be estimated using Eq. (3-1) given that the time elapsed is known.

One major drawback of this device is the time required to process a given volume of solution. Nevertheless, it is important to note that SA was concentrated 1000x and detectable with LSM within the first 24h. The time required could be reduced by using an active pump system to aid the solution through the filter paper. In the proposed system, ΔP would be the sum of the applied pressure and the capillary pressure. However, studies are needed to determine the effect of flow rate on the binding efficiency of the biotinylated microgel.

Lastly, the incorporation of the superabsorbent as a passive pump greatly increased the volumetric capacity of the device. This simple design might have applications for other paper-based technologies, especially for paper-based microfluidic devices [3, 17-19] to increase the amount of liquid that the device can process.

3.6 Conclusion

1. A novel low cost paper-based device was developed to concentrate a protein, streptavidin, from dilute solution by immobilizing biotinylated microgels on the paper surface.
2. The incorporation of a superabsorbent passive pump using cross-linked poly(acrylic acid), partial sodium salt-*graft*-poly(ethylene oxide) significantly increased the volumetric capacity of the device.
3. Concentration factors of more than 3000x were achieved by the paper-based device.
4. The flow dynamics of the device was modeled using Darcy's law.

3.7 Acknowledgements

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Chapter 4 Conclusions

4.1 Summary of Work

The field of paper-based diagnostics and biochemical detection has grown at a rapid rate in the past decade. The objective of this work was to add to the wide body of research in the field of paper-based technologies, and this was accomplished through the demonstration of electrokinetic sensing in paper microfluidics and the development of a paper-based preconcentration device.

From the experimental results, it was successfully demonstrated that streaming potential sensing can be achieved in wax printed paper-based microfluidic channels. The following can be concluded from this work,

(1) Capillary driven flow in filter paper channels induced a measurable streaming potential in the range of -80 to +80 mV

(2) The measured potentials were able to detect the presence of polymers adsorbed on the paper surface

(3) The polarity and the magnitude of the measured streaming potential were sensitive to the surface charge of adsorbed polyvinylamine and potassium polyvinylsulfate

(4) The measured potential were also sensitive to the change in surface charge of the protein, bovine serum albumin (BSA), at different pH

(5) The zeta-potential of the surface was estimated using simple classic electrokinetic equations

Another contribution this work has made to the field of paper-based diagnostics and detection is the development of a paper-based preconcentration device. The motivation for this work was to increase the detection power of paper-based sensors and to decrease the cost of biochemical

analysis for trace compounds. The following remarks can be made about the work on the paper-based preconcentration device:

(1) A novel paper-based preconcentration device was developed by immobilizing biotinylated microgels onto a designated area on the paper

(2) A dilute solution of the protein, streptavidin, was successfully concentrated on the paper strip

(3) Concentration factors of more than 3000x were achieved

(4) The volumetric capacity of the device was increased by using cross-linked poly(acrylic acid), partial sodium salt-*graft*-poly(ethylene oxide) as a superabsorbent passive pump

(5) Flow dynamics through the paper-based device can be modeled using Darcy's law.

4.2 Future Directions

Overall, the work in paper-based microfluidics and paper-based preconcentration was successful in achieving the objective specified in the beginning of the thesis. However, further research is needed to fully develop these technologies and to increase the contribution to this field as a whole.

For the work in streaming potential sensing in paper-based microfluidic channel, the study of fundamental principles is required. Since the streaming potential is a function of the surface charge and of the flow dynamics, more work is needed to understand the flow of fluid in the channels. Although the movement of the fluid through the paper had been studied in the past, it is much more difficult to model the flow of fluid through paper that has been modified with polymers or proteins. From the data gathered, there was a lot of fluctuation in the measured streaming potential and this causes the device to be less sensitive to subtle changes in surface charge. These fluctuations are likely due to the disturbance in fluid flow which is affected by the

presence of polymers or proteins on the paper surface. There are a couple of proposed methods in resolving this issue. Pressure can be applied to drive the fluid through the channel overcoming the disturbance caused by the polymer. This would provide a constant flow through the channel and a more consistent streaming potential measurement. However, this would minimize the benefit of passive flow that paper provides. Another possible solution is to lower the amount of polymer applied onto the surface. However, this may lead to non-uniform polymer coverage of the paper surface. Furthermore, for some biosensing application it might not be practical to lower the amount of biosensor on the paper surface.

Moreover, another possible cause for fluctuation in the data is the fabrication of the device. Although some research had been done to better understand wax printing and the patterning of microfluidic channels [19], there is little work done on the effect of wax printing on fluid flow in the channels. From the wax melting step, there are uneven edges in the hydrophobic barrier which can lead to different local flow rates. Furthermore, the printing process might have certain artifacts that affect the hydrophilic channels, thus a more detailed study of the effect of wax printing on fluid flow may improve streaming potential sensing in paper-based microfluidic channels. Moreover, the use of screen printing to fabricate silver electrodes might provide a better electrical contact on paper [21, 35].

Streaming potential sensing for biosensing systems on the paper-based channels should also be studied. Literature has shown that streaming potential measurements can be used to study biospecific interactions such as protein-sugar interactions and antigen-antibody interactions [23]. Furthermore, the antigen-antibody system had been shown to be viable for paper-based biosensing applications [42]. Thus, studying streaming potential sensing of antigen-antibody

interaction in paper-based microfluidic channels would be an excellent first step in integrating biosensors and electrokinetic sensing.

There are also a few areas that require more investigation for the work in paper-based preconcentration. One of the major drawbacks is the slow rate of fluid uptake by the device. The incorporation of an active pumping system or changing the passive flow by modifying paper can increase the flow rate through the device and decrease the time required for preconcentration. However, the increase in flow rate could decrease capture efficiency of biotinylated microgels, therefore it is necessary to investigate the relationship between flow rate and concentration factor.

Moreover, it is important to further the research by finding relevant biochemical targets to preconcentrate. One major area of application for this technology is water analysis. Many studies have been conducted on the presence of trace pharmaceuticals and endocrine disrupting compounds in drinking water and wastewater [25, 56-58] and most of the analysis techniques require solid phase extraction [29, 32, 59] or membrane separation [60-62]. Paper-based preconcentration can provide a low-cost alternative to these methods. Therefore, it is important to demonstrate the effectiveness of the paper-based device to concentrate the relevant pharmaceuticals and endocrine disruptors. From the literature, there are a few categories of compounds that are of special interest for example, hormones from contraceptives such as 17 beta-estradiol [63], a wide range of anti-inflammatory drugs, diclofenac and naproxen, and antidepressant such as fluoxetine [25]. These target compounds would be excellent choices for further research in paper-based preconcentration.

Furthermore, work could be done to exploit the chromatographic ability of paper. Separation of compounds using paper chromatography had been studied extensively in the past [48, 49, 64],

utilizing the ability to separate different compounds and combining it with concentration capabilities can greatly enhance the usefulness of the device. Since samples that require preconcentration such as wastewater, would have multiple target analytes, it would be beneficial to capture and concentrate different target compounds on one paper-based device. For example, multiple concentration areas can be applied on the paper strip, where each of the concentration area would target a different compound. Then these compounds can either be eluted or be directly analyzed using paper spray ionization and mass spectrometry.

Overall, paper-based diagnostic and biosensing technologies have great promise in providing low-cost alternatives for the field of medical diagnostics and analytical chemistry. This research work has contributed in this growing research area by the demonstration of two novel paper-based devices. Streaming potential sensing was achieved in paper-based microfluidic channels and this technology may provide an electronic interface for paper-based biosensors in the future. Furthermore, preconcentration of protein with concentration factor of more than 3000x was demonstrated in a paper-based device. This technology may provide an excellent low-cost alternative for chemical analysis for trace compounds and also increase the sensitivity of different paper-based biosensors.

Chapter 5 References

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