ELECTRICALLY ACTUATED MICROFLUIDICS IN FABRIC

A Dissertation Presented

by

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To my mother, who taught me to persevere
ACKNOWLEDGEMENT

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ABSTRACT

Microfluidics, or the science behind the manipulation of small volumes of fluid, presents a viable solution to the rising global need for low cost and reliable medical testing. This dissertation utilizes existing textile weaving technology to pattern reagent-functionalized yarns and sensing components into fabric microfluidic devices in a single, scalable manufacturing step. Fabrics constitute a pump-free microfluidic medium, where liquids propagate along the fibers through capillary forces. In the primary goal, electrical functionality was incorporated into the device for integrated sample preparation and analysis, using a conceptually simple device, wherein the electrical sensing components were integrated entirely through the single-step weaving approach.

A systematic characterization of the ability of the device to dissipate Joule heat under a range of applied potentials, electrolyte buffers, and device dimensions, was performed. Next, the electrophoretic separation and concentration of sample constituents was demonstrated. In order to mitigate dispersion of the migrating sample bands through the larger pores in fabric, a mathematical models of the transport of a charged tracer through a porous medium, accounting for the hierarchical porosity of fabric, were developed. The models helped identify the packing density and the surface properties of the porous medium as key tuning parameters influencing electrophoretic separations.

Adding further to the functionality of the platform, yarns coated with a reversibly-wettable, conductive polymer -polypyrrole- were incorporated in close contact with an
electrode, forming active valves for reproducible, temporal flow control in natural fiber based chromatographic substrates. A chloride counterion chemistry was utilized to induce the reversible electrochemical reduction of a charged, hydrophobic polypyrrole barrier, into a hydrophilic material. This property was used in the development of an ON/OFF valve, and its utility in imparting precise sample reagent incubations was demonstrated for a time-sensitive total protein assay.

In the final segment of this work, the conductive polymer was functionalized with antibody receptors. The receptor-mediated capture of a target was measured as a function of the charge transfer resistance of the polypyrrole electrode via electrochemical impedance spectroscopy. Proof-of-concept assays for a broad spectrum cancer marker, Epidermal Growth Factor Receptor 2 (HER2) were demonstrated and detection sensitivities down to 0.5 ng of HER2 were obtained. The aforementioned techniques constitute an enhanced, tunable toolkit towards ultra low-cost (< $1.00) medical sensing.
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Figure 24  Electrophoresis and separations of charged dyes in fabric. (a) The electrophoresis of a negatively charged dye (Yellow 5) occurs away from the negative electrode and towards the positive terminal at applied field strengths ranging from 25-50 V/cm. A diffusion control provides the basis for comparison of the unique shape of the band migrating under an electric field. (b) A mixture of blue and yellow dyes (initially green) evolving into separate bands in order of charge to size ratio. (Applied field strength = 35 V/cm in 1x Gly-NaOH buffer). A diffusion control is included to show that separations did not occur spontaneously, within a 5 minute window. Reused with
Figure 25  Results from Table 3 have been plotted here. A correlation between SR and coverage is observed within fabric types. The two polyesters of low and high coverage have low and high SR respectively while the two polyurethanes of similar coverage have similar SR. The cupro nylon did not show any separations despite higher coverage, indicating that the material also has a role to play in separations. The material properties of polyester and nylon are similar, but better separations were obtained in polyester. The effects of these variables were decoupled and explored further using COMSOL models of electrophoresis in fabric.

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Figure 32  Protein focusing in fabric and associated pixel intensity distributions. A sample of naphthol-blue-black bovine albumin (blue BA) is electrophoresed in Glycine-NaOH, pH 8.5. The blue BA is negatively charged and migrates from the cathode to the anode as the band continues to focus. The band is imaged and spatial intensity distributions are plotted at the start versus the end of the run. Lower pixel intensities are obtained in darker regions. A sharp dip in intensity is therefore obtained corresponding to the position of the focused band.

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Washburn form, and the velocity dips in proportion to the amount of iron chloride in the polymer segment. The position of the valve has been indicated here by the shaded box. On/off valves to stop and restart liquid flow were developed from 0.1 M iron chloride, while partially on valves to slow the flow of liquid were developed using smaller amounts of iron chloride. We note that the velocity increases once the liquid front has crossed the valve. This is because flow is driven by the interfacial pressure gradient, which increases once the liquid encounters the wetting region of the fabric. (b) Plots of velocity against time for two strips of identical length and width, one with a mid-channel on/off valve, and one with no valve. The plot illustrates prolonged residence of a liquid upstream of the valve (25 s in this case), before flow is activated, as opposed to the non-valved strip.

Figure 43 Calculating the pore size of plain uncoated fabric versus partially wettable ppy-coated fabric, made using 0.001 M iron chloride. (a) Plots of velocity against (1/L) can typically be fitted to a straight line as they obey the Washburn Law. The slope of the line may be used to calculate the pore diameter of the strip. Overall, the coated strip has lower velocity, for the same flow rate. Note that the entire length of the strip was coated, and since 0.001 M ppy is partially wettable, no electrical activation was required. (b) The effective pore diameter calculated from (a) has been plotted. Uncoated strips have pore diameters of 2.98 µm, while 0.001 ppy coated strips have smaller pore diameters of 0.89 µm. The coated strips are not only less wettable, but also have smaller effective pore diameters.

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1.0 INTRODUCTION

Microfluidics is the science and the instrumentation behind the manipulation of small volumes of fluid through sub-millimetric channels. The primary motivation for the development of microfluidic technologies is the ability to perform rapid chemical and biological analyses on a small volume of sample. Another major driving force is the ability to analyze multiple samples through multiple ports within a single channel network\textsuperscript{1,2}. These advantages have led to an exponential growth in microfluidic research and development over the past decade (Fig. 1), particularly for such applications as cell and tissue culture, high throughput microparticle synthesis, and rapid medical diagnostic testing, to name a few. The challenges and motivations associated with developing commercial microfluidic platforms have been described below.
Figure 1: Annual trend of journal publications in microfluidics since 2005. Microfluidics research and publication rates have nearly quadrupled in the last decade, illustrating an increasing interest and awareness in the field. The data was obtained by counting the number of PubMed hits for the search term ‘microfluidics’.

1.1 Microfluidics and Miniaturization

The concept of manufacturing small devices originated in the semiconductor industry\(^3\). Micrometric relief structures patterned onto silicon wafers by existing chemical etching or photo-lithographic approaches, were then sealed and used as channels for liquid flow\(^4\). Flow was typically actuated by a pressure applied across the channel using pneumatic pumps, connected to the channel via airtight pneumatic fittings. The microchannel offers a high surface to volume ratio for chemical reactions to proceed rapidly and efficiently. In addition, flows typically occur under viscous conditions at low Reynold’s numbers, where particle separations can easily be carried out. The physics of fluid flow at the micrometric scale are considerably different from the macroscale, and were exploited for the development of a variety of unit operations, including mixing, separations, and the generation of chemical gradients, mainly by altering the geometry and
feature sizes in the channel. Since silicon wafers were difficult to interrogate optically, alternative fabrication techniques were developed. The relief pattern on the silicon wafer could be transferred onto softer elastomeric polymers from a single reusable master wafer, using a casting process known as soft lithography, thus leading to the advent of rapid prototyping approaches for the fabrication of flexible, optically transparent, polymer based microfluidic devices. These devices are cheaper, and their porosity and elasticity allow for the exchange of gases, which is especially useful for biology and cell culture applications. Elastomers are however not ideally suited to large scale manufacture and have been replaced by hard plastics, which are more scalable, and tend to perform more reproducibly under pneumatic actuation. However, despite the many advances in microfluidics, significant challenges to automating and commercializing these technologies have been identified over the past decade. These include: 1) the high cost of fabrication of microfluidic devices, and the use of stringent cleanroom-based processes which limit the scalability of manufacture, and 2) The issues associated with pneumatic actuation, including a lack of reproducible flow control, and the need for heavy instrumentation which tether pneumatic systems to the laboratory. Addressing these challenges can have a large positive impact on many commercial applications. One such application area is medical diagnostic testing, where low-cost, automated platforms are an urgent unmet need.

1.2 The unmet needs for low cost medical diagnostic testing

Infectious diseases account for over 15 million deaths annually in developing countries, while non-communicable diseases such as cancer, heart disease, and diabetes, are responsible for over 80% of deaths globally. The disability adjusted life years
(DALY)$^{1*}$ for certain groups of infectious diseases was estimated at 325 million per year in 2006$^{12}$, and is rising since. The major cause for the high mortality rates, despite advancements in medicine and pharmaceuticals, is that a disturbing majority of the global population have little or no access to timely health care. In the United States, a large percentage of this population fall into the lower income bracket. Among other factors that obstruct timely diagnosis and treatment is the fear and psychological stress associated with waiting in an emergency room$^{13}$. The manufacture of accessible medical diagnostic tests is therefore an important goal. Automated tests for disease diagnostics and therapeutic monitoring can help alleviate disease burden in many parts of the world. However, the development of medical diagnostic tests for ultra-low resource settings, such as rural and low income clinics, pose additional challenges due to the limited amount of laboratory infrastructure and equipment, fewer trained personnel, and high patient loads that such clinics are forced to handle. The development of low cost and equipment free testing platforms that require minimal maintenance is a significant need. In addition, medical samples often require treatment in order to remove interfering substances (such as red blood cells) which interfere with the optical readout or occlude the assay of the target molecules due to non-specific adsorption or steric hindrance effects. Since sample processing equipment is often unavailable or limited, a major criterion for a good on-site diagnostic test is to be able to integrate sample processing and separations with analysis.

$^{1*}$ DALY or disability-adjusted life years, are the number of years lost due to ill health, disability and early death.
1.3 Need for electrically actuated microfluidic devices

Microfluidic devices possess all of the advantages required for rapid and sensitive chemical and biological analyses. However, as mentioned earlier, the major challenges to commercialization are the high cost of manufacture of clean-room fabricated devices, and the heavy instrumentation and tethering associated with pneumatic actuation. Pregnancy testing was one of the first target areas to have overcome some of these challenges, with the help of devices that are completely pump-free\footnote{14}. Over the counter pregnancy tests consist of a series of reagent functionalized membranes, designed to allow a urine sample to wick through the device at controlled flow rates. A drop of sample deposited on one end of the device releases a nanoparticle-labeled detection reagent into solution. The target molecules in the sample complex with the detection reagent and the analyte-reagent complex is drawn further downstream, where it is captured by a complementary ligand immobilized in the device, leading to the formation of two or more colored lines. The result is therefore interpreted visually, and flow takes place entirely through capillary forces. An absorbent pad at the end of the device serves as a ‘passive’ actuator, drawing the unused reagent and fluid away from the test regions.

The affinity-mediated capture and estimation of analytes is known as an immunoassay, and the reagents are typically immune proteins known as antibodies, which possess complementary sites for analyte binding. Immunoassays can be performed at very high throughputs at the bench scale, but may require from 6 to 24 hours from start to finish with multiple intermediate washing and pipetting steps. Such assays are typically performed under stagnant conditions where the antibody reagents are immobilized to a solid phase, and the analyte is present in a stationary liquid phase layered over the solid
phase. The capture of the analyte is therefore diffusion-limited, and requires regular mixing and long incubation periods.

The speed at which the assay is completed in the pregnancy test is mainly credited to the fact that it is performed under **flowing conditions**, and that porous membranes offer a very high surface for efficient analyte capture. Membrane based tests have now evolved to encompass tests for infectious diseases (HIV, malaria, dengue)\(^{15}\), drugs of abuse\(^{16}\), environmental testing, and non-communicable diseases (cardiac troponin). However, LFTs are typically straight channel devices, and are difficult to multiplex. Further, the cost of a single Lateral Flow Test (LFT)-based pregnancy test is ~ $10-20, which is by far greater than the cost of a single meal.

In order to address these issues, analogs to membrane based tests were recently developed in paper, using cleanroom-based lithography, screen printing and wax printing procedures to pattern networks of wetting pathways onto absorbent paper\(^{17}\). As mentioned earlier, the benefit to patterning is that channel networks allow for multiplexing\(^2\*\). However, passive, capillary actuated flow control possess the following limitations: 1) Cellulosic materials are **hygroscopic** and flow rates can vary depending on the ambient humidity and temperature, **variations in fiber morphology and orientation** which typically occur at the scale of capillary flow, as well as edge defects during the cutting and shaping processes\(^{18,19}\). 2) Due to the sensitivity limits of the human eye, visual detection is **limited to high abundance analytes**, and 3) although paper is an inexpensive material,

\(^2\*\) In microfluidics, multiplexing typically refers to the distribution of the sample into multiple channels where it can be tested for the presence of different analytes. Multiplexing can also mean that many samples are tested within the same device.
cleanroom based fabrication is expensive, while printing processes have limited feature resolution\textsuperscript{20}.

A possible solution to the issues concerning flow control, reproducibility and sensitivity, is to augment capillary flow control with external actuation. A number of interesting \textbf{external actuation methods, including electrical, optical, magnetic, acoustic and electro-thermal} control, have been described for low cost platforms\textsuperscript{18,19,21-27}. Some of these activation methods have been used in conjunction with stimulus responsive coatings, which can alter the wetting behavior of the channel in response to external activation\textsuperscript{22,28-30}. Of the methods used above, electrical actuation is especially versatile and easy to integrate into microfluidic format. First, fluids can be pumped through capillary channels \textbf{by applying an electrical potential difference across the ends of a channel filled with a conductive liquid}\textsuperscript{31-36}. This actuation is achieved by the bulk flow of ions in the channel. Electrically actuated flows are continuous, plug like (or non-viscous) flows, and are not subject to pulsation like pneumatic flows. It is therefore the preferred method for sample injection in many high performance analytical instruments, such as capillary electrophoresis and flow injection analyzers. Second, \textbf{electrical contact is achieved simply by placing an electrode in a buffer reservoir, and no specialized hardware is required to make a pump-to-device connection}. Electrical circuitry and components are easily incorporated into the device by existing fabrication methods, and may be operated using simple portable hardware such as a battery pack.

Third, in addition to pumping, electrical actuation is utilized for particle separations and mixing of biological samples\textsuperscript{34,37-45}. Polar and ionizable particles and molecules can be made to migrate through a liquid matrix at a velocity that is proportional
to the intrinsic charge and hydrodynamic radius of the molecule. Mixtures of molecules are therefore resolved into their individual molecular components. This class of techniques, is termed **electrophoresis and is widely used in proteomic and genomic analyses.** Electrophoresis is a multidimensional approach that helps not only to separate components, but also to remove interfering high abundance components, preconcentrate the targets of interest, and identify and assay the targets of interest$^{34-42}$. Compared to other thermal, magnetic, or pH mediated actuation and separations, electrical methods are also gentle and biocompatible, and do not affect the pH, temperature or salt concentration of the sample. **Electrical actuation would therefore be a powerful and versatile approach to performing pump-free separations, analysis and flow control in low cost devices.**

1.4 Why use fabric microfluidic devices?

Weaving in an art-form that has been developed to a high degree of skill in several parts of the world, including India and China. Complex geometries can be patterned into fabric at single fiber resolution. In recent work, we used existing southern Indian weaving infrastructure to manufacture fabric-based microfluidic devices at ultra-low costs$^{46}$. Woven fabrics are composed of orthogonally intersecting threads, termed as yarns, which in turn are made from twisted bundles of silk, cotton, polyester, and other textile filaments, and can imbibe liquids through capillary forces (Fig. 2a). Fabrics therefore consist of a hierarchic porous structure where liquids are drawn through the yarns as well as through the spaces between yarns, whose diameters are typically sub-millimetric, on the order of a few microns in size, and can be controlled. At the very fundamental level, hydrophilic silk yarns were patterned against a hydrophobic metallized yarn background to weave wetting pathways in the device. Liquid flow was confined to these pathways, potentially allowing
us to multiplex the device (Fig. 2b-c). In addition, the weaving loom\(^3\)* was programmed to repeat the pattern across the length and breadth of the fabric (Fig. 2c). Programming may be accomplished using punched cards\(^4\)* or a computer aided interface.

\(^3\)* A weaving loom is a wooden or metallic frame on which yarns are affixed and interlaced to produce a fabric. A loom may be attached to a programming apparatus known as a ‘jacquard’ which controls a set of punched cards.

\(^4\)* Punched cards are cards with holes, through which a harness and strings can pass. The holes correspond to the design or pattern which is being woven. The harness raises or lowers different sets of yarns depending on whether there is a hole in the card, and this allows the different yarn components to be incorporated or excluded from specific areas of the pattern.
The process can therefore be scaled up to produce large sheets of fabric containing thousands of individual devices, per day (Fig. 2e). Yarns were then selected based on their wetting characteristics, coated with immunoassay reagents, and woven into lateral flow
devices (Fig. 3) in a single assembly step. Flow rates within the device were controlled by varying the twist frequency, surface properties, fiber morphology and thickness (ply) of individual yarns, as well as the packing density of yarns in the fabric, in a seamless manner along the length of the device. Since immunoassays are highly kinetics dependent, this tuning ability facilitates detection reagent release and analyte capture at the optimal rates, and sensitive tests for a variety of target analytes were manufactured (the theory of capillary flow control has been provided in detail in Section 3.0). However, silk and cotton are hygroscopic materials and a certain amount of variability was observed in the long term wetting behavior of the device. In addition, there are currently very few accounts of active (non-passive) valving for flow control in low cost lateral flow devices. We therefore believe that there is ample scope for improvement in the areas of flow control, sample processing, and sensitive, non-optical detection in low cost devices.

![Image of fabric lateral flow device](image)

**Figure 3:** A fabric lateral flow device where the components (sample pad, detection reagent pad, absorbent pad and analytical region) are integrated to form a seamless device with no overlapping junctions, and controlled flow rates through each segment.

In this dissertation, we use the weaving approach to integrate electrodes into fabric devices. We utilize this integrated platform for three major purposes: Electrophoretic protein separations were demonstrated in Aims 1 and 2. Electrophoretic separations and pre-concentrations potentially allow us to target low abundance, rare protein analytes, as well to enhance the sensitivity of detection in the device. An electroactive, surface active
polymer was then incorporated in close proximity to the electrodes in order to activate and deactivate liquid flow at fixed time points (Aim 3). The electroactive valve was used to enhance the sensitivity of optical readouts in the device. In the final aim (Aim 4), we functionalize the electroactive polymer coated fabric with antibodies for analyte capture, and utilize the functionalized textile to obtain a sensitive electrochemical impedance signal in place of an optical readout for the immunoassay.

1.5 Dissertation Outline

The four major goals associated with this dissertation are: i) To design an integrated fabric platform with electrodes, and to examine its behavior under the influence of an applied electric field. ii) To perform protein sample separations in the fabric, and to integrate separations, assay and analysis into a single step. iii) To develop a electroactive chemical valve that can be activated or deactivated at determined time points for precise and reproducible flow control, and iv) To examine the use of the electroactive coatings for electrochemical sensing, particularly as working electrodes in an electrochemical impedance-based immunoassay.

The dissertation is organized as follows:

Section 1 serves as an introduction and brief review of the literature.

Section 2: Literature review: A detailed review of the concepts associated with each of these goals have been provided in Section 2.

Section 3: Theoretical considerations: An overview of the theory, governing equations and mathematical modeling approaches used in all four aims of this dissertation have been provided in Section 3, in the order in which they are used.
**Section 4:** Experimental methods: A description of the experimental protocols, materials and methods has been provided here.

**Section 5:** Results and discussion: The results from all four sub-aims of the dissertation have been described in separate chapters in Section 5. Supporting data and additional information have been separated into a supplementary section in the Appendices in section 9.

**Sections 6:** Conclusions and recommendations: This section includes the outlook for this project, as well as a bill of materials cost analysis for the platform.

**Section 7:** Appendices: The appendices contain supplementary data (Appendix A) and exploratory work regarding the development of a fabric-based power source for integration with an electrically enabled device (Appendix B).
2.0 CRITICAL LITERATURE REVIEW

This section reviews pertinent literature in the field of low-cost device manufacture and development. A brief introduction to each of the concepts explored in this dissertation, especially in regards to the necessity of flow control and the active and passive techniques used for fluid actuation and flow control in porous, wicking media has been provided in Section 2.2. Due to the richness of the literature in this field, a condensed, comparative analysis of the existing flow control techniques has been provided in Section 2.2.3. Flow control is inherently tied to separations in terms of the ability to modulate the Peclét number in a device, which a performance metric used to express whether the flow conditions in the device are conducive to mixing (high advection), or to separations (low diffusion). A review of the techniques utilized in the separation and analysis of protein sample mixtures have been provided in Section 2.3, along with an introduction and review of electrophoretic separations techniques. Last in line after sample separations and sorting, is the detection, quantitation and analysis of the analytes of interest. Immunoassays have been introduced as a means to capture ligands of interest using an affinity-mediated approach in Section 2.4. Immunoassays may be coupled with two modes of detection, namely optical and electrochemical modes, and a comparison and review of the techniques used therein have been provided in Section 2.4.2.
2.1 Approaches to the large-scale manufacture of microfluidic devices

The goal of miniaturization is to be able to pattern networks of fluid bearing channels in devices with very small footprint, allowing us to perform multiplexed analyses using small volumes of sample and reagent\(^1,^2\). Due to its origins in the semiconductor industry, microfluidic channels were originally lithographed or etched into silicon\(^9\) and glass\(^4\) wafers, producing highly resolved physical relief features. However, in order to aid with the iterative design process, a rapid prototyping or ‘replica molding’ approach was developed in which elastomeric polymers such as PDMS (polydimethyl-siloxane) were cast from a silicon master wafer, allowing us to remake device prototypes at lower costs\(^6\). The deformability of PDMS and the challenges associated with the scalability of cleanroom fabrication process led to the advent of hard plastic devices. Hard plastics such as PMMA (polymethylmethacrylate)\(^7\) and COC (Cyclic Olefin Copolymer)\(^8,^47\), may be hot embossed or injection molded into a variety of shapes with high feature resolution. Rapid prototyping approaches such as laser or ion-beam milling\(^48-^50\), 3D printing, lithography, layering, and welding may be used to manufacture hard plastic devices in fewer than two steps. Despite the diversity of scalable manufacturing, techniques, channel-based devices are also associated with a tremendous amount of external hardware for pneumatic actuation and control, adding to the cost of the platform.

In contrast, inexpensive porous devices such as membrane-based LFTs, patterned paper, and fabric, are entirely self-actuated\(^10,^51-^54\), and imbibe liquids through capillary forces. The ability of a porous material to draw fluids through capillary forces is termed ‘wicking’. Membrane-based lateral flow tests, such as the home pregnancy test, represent the current gold standard for low cost testing. LFTs consist of a series of overlapping non-
woven membranes that are designed to wick samples at controlled flow rates\textsuperscript{14,55}. Flow is activated when a urine sample is deposited onto the device, releasing a loosely bound, colored detection reagent which specifically binds the analyte in the sample. The analyte-detection complex flows downstream where it is captured by antibody ligands to form two or more colored lines that can be interpreted visually. The integration and automation of an analytical assay in an inexpensive, user friendly format is a useful feature for onsite testing\textsuperscript{56,57}. The ever-expanding repertoire of LFTs include tests for infectious diseases\textsuperscript{15,58}, clinical chemistry (glucose, albumin etc.), drugs of abuse\textsuperscript{16}, and the detection of biowarfare agents\textsuperscript{59}. However, LFTs are difficult to multiplex, are known to have reproducibility issues, and are limited to colorimetric tests that are read visually\textsuperscript{60,61}.

Patterned paper-based devices first developed by Martinez. \textit{et. al} \textsuperscript{17}, have revolutionized the field of low cost testing. The paper substrate is typically impregnated with hydrophobic materials that can be used to restrict liquid flow to wetting pathways, allowing tests to be multiplexed. The original patterning approach followed the soft lithography process\textsuperscript{17,62-6917}, but was soon replaced by wax printing\textsuperscript{62-64}, plasma treatment\textsuperscript{65}, and screen printing approaches, which are more scalable and less expensive compared to cleanroom based lithography. Despite advances in the use of screen and inkjet-printed paper-based sensors, printed devices suffer from low feature resolution\textsuperscript{20}, due mainly to the fact that the inks tend to spread through the material prior to drying. In addition, conventional printheads are not suited to handling viscous, conductive inks that are required for certain specialized applications, leading to high printer turnover. As a potential solution to this problem, Yager \textit{et. al.} have developed shaped paper devices where channel features were cut out of the paper, circumventing the need for printing altogether\textsuperscript{60-}. 
However, paper based materials are easy to tear, crease, or deform, requiring customized manufacturing equipment and careful lamination and packaging.

In contrast, the weaving based approach is versatile in terms of the variety of fibers and materials which can be integrated into the platform, while feature resolutions down to the thickness of a single fiber may be achieved with ease. Woven fabrics are also mechanically strong, and robust to the application of high voltages, as opposed to nitrocellulosic membrane or paper materials that are flammable. Woven fabrics consist of two orthogonal intersecting yarns. The axial yarns which are arranged lengthwise along the weaving loom are termed the ‘warp’, while the secondary yarns that are interlaced with the warp, are termed the ‘weft’. Warp yarns are lengthy and serve as the structural framework for the fabric, while weft yarns are shorter and more economical to functionalize with reagents, conductive inks, or other device components, and integrate into a pre-arranged warp yarn sheet in a single step. Device components are therefore integrated seamlessly along the length of the fabric, while warp and weft yarn wettability are tuned in accordance with the kinetics of the assay (see Figs. 2 and 3)\(^{69-71}\).

The single step approach utilized to fabricate electrically integrated devices in this dissertation have been described in the methods section. To summarize, an appropriately wetting warp yarn sheet was chosen and preset along a sampling handloom. The sampling handloom is typically utilized for prototyping purposes, as it is smaller in size, and has a throughput of 10-25 devices per half hour weaving session. However, production can be scaled up to throughputs of > 5000 devices per day on industrial looms. The electrodes or electrically and chemically functionalized fibers were typically introduced into the device along the weft direction, while the wetting properties of the warp and weft were controlled.
in relation to one another, in order to tune fluid transport in these devices. A detailed account of these methods is provided in the theory, methods and results sections.

2.2 The significance of flow control in microfluidics

Flow control is a key requirement in microfluidics, driving sample separations as well as analysis. Kinetics-dependent assays are dictated by reaction rates, i.e. a predetermined rate at which binding and dissociation between a ligand and a receptor occur. Meanwhile clinical chemistry assays require sufficient periods to allow chemical oxidation or reduction to occur. Assays therefore benefit from maximizing the exposure of the sample to the reagents, either by mixing under high advection conditions, or by allowing sufficient time for the sample to interact with the reagents. In LFTs, high flow rates lead to a decrease in the amount and efficiency of analyte capture.

On the other hand, separations typically benefit from suppressing diffusion and allowing particles to focus into sharp zones. In this section, we focus on methods that have been utilized to control capillary actuation through porous wicking media. In particular, we are concerned with being able to mimic the complex fluidic architecture of conventional pneumatic microfluidic platforms in low cost media. A metric to express fluid transport that is used frequently in this dissertation is the Peclét number, which is essentially the ratio of advection to diffusion in the device.

2.2.1 Passive approaches to flow control in porous, wicking materials

Flow control is typically achieved by manipulating the capillary forces acting on the fluid. The use of selectively wetting patterned surfaces with hydrophobic ‘walls’ to confine fluid flow has been covered in section 2.1. Patterning confers the ability to
distribute fluid to different channels for multiplexed detection. However, in addition to confining fluid flow, flow rates through individual channels must be controlled, as they directly influence the kinetics of the assay being performed in the device. The Washburn Law for capillary flow dictates that the velocity of flow decreases with wet length. Traditionally, this property is used to control wicking rates through individual channels in LFTs and paper based devices. In lateral flow devices in particular, coating the receptor molecules further away from the inlet end is a strategy used to ensure that the sample flows through the reagent zone at a slower rate, and ample time is allowed for binding to occur. Likewise, the use of channel constrictions and expansions have also been exploited to time fluid wicking.\textsuperscript{72,73} The surface properties of the channel may also be controlled by impregnating the material to different degrees with a hydrophobic substance such as wax, altering the fluid permeability of the material.\textsuperscript{74} However, these techniques cannot be used to stop or restart fluid flow.

A passive valve was recently devised for paper via the use of dissolvable bridges between successive segments of a channel.\textsuperscript{68,75} (Fig. 4). In contrast to channel geometry which serves to slow down or speed up flow, valves confer the ability to stop or divert flow, which is useful in multiple step assays. Shown in Fig. 4 is a dissolvable bridge system that serves as a permanent shut-off valve. The bridge is composed of a water-soluble polymer, typically Poly-Vinyl Acetate (PVA). The quantity and solubility of the PVA in the bridge determines the time after which the bridge disintegrates. This technique is very useful at the point of care where fixed volumes of liquid can be metered into the device without the need for pipetting equipment, while flooding and backflow are prevented.
Figure 4: An illustration of the permanent shut off valve, adapted with permission from ‘Houghtaling et al., Dissolvable bridges for manipulating fluid volumes in paper. Anal. Chem. 85(23) 11201-11204 (2013)’. Two segments of a device are connected by a bridge (top) made of a water soluble material. The bridge is engineered to dissolve after a fixed volume of liquid has passed, thereby forming a permanent shut off valve (bottom). ©American Chemical Society 2013

Reversible valves were recently developed using bridgeable gaps that were closed by pushing different channels into contact\textsuperscript{75}. Unlike permanent valves, reversible valves may be used to meter multiple sample and reagent volumes into the device, and allow reuse. However, devices that rely entirely on passive approaches to flow control are at risk for high variability due to inconsistencies in fiber morphology and orientation\textsuperscript{14,25}, as well as the hygroscopic tendencies of certain materials such as silk, cotton and cellulosic paper\textsuperscript{25}.

2.2.2 Active approaches to flow control, and the phenomenon of reversible wettability switching

Active approaches make use of an external applied force to influence capillary wicking, and are potentially more precise and robust than passive approaches. Active control approaches have included the use of thermally responsive, swelling polymers for mechanical actuation in paper\textsuperscript{22-24}, the use of acoustic vibrations to atomize and draw liquid through paper\textsuperscript{25}, the use of magnetic forces to move paper structures impregnated with ferromagnetic particles\textsuperscript{21,76}. While the use of sound for fluid actuation, mixing and particle segregation has been explored widely in the literature\textsuperscript{18,19,27,77}, and consumes very little power\textsuperscript{78,79}, the effects of high frequency acoustic waves on the viability of biological
reagents is as yet unclear. In general, a gentler approach is recommended in order to preserve the structure and function of biomolecules. Magnetically controlled paper cantilevers\textsuperscript{76} and forceps\textsuperscript{21} are manufactured by impregnating shaped paper with magnetic particles suspended in a light oil, known as a ferrofluid\textsuperscript{80}, and can potentially be used as a magnetically controlled mechanical bridge between two segments of the device. However, the light oil coating hydrophobizes the surface of the paper. Since medical samples are aqueous, this method is not directly suited for our purposes.

Stimulus responsive, surface active coatings are an interesting approach to flow control, and are yet to be implemented for fluid actuation in microfluidics. An interesting characteristic that most such materials exhibit, is reversible wettability switching. **Reversible wettability switching is a phenomenon where a material temporarily changes its surface properties in response to an external stimulus.** Materials have been known to switch between a wide range of water contact angles depending on their surface roughness and porosity. Zinc oxide\textsuperscript{67,68} and spiropyran\textsuperscript{26} are examples of materials which undergo reversible changes in wettability in response to light. In one study, spiropyran underwent a change from superhydrophobic, where it rejects a drop of water (water contact angle $> 150^\circ$) to superhydrophilic (water contact angle $< 5^\circ$), where it readily absorbs a drop of water (Fig. 5).

**Figure 5:** A drop of aqueous liquid assumes a spherical shape when placed on a hydrophobic surface (left). The angle made between the liquid air interface and the solid-liquid interface, also known as the contact angle, is large. In comparison, a wetting surface allows the liquid to spread over its surface as the contact angle is relatively small. (Right). A reversibly wettable surface switches between these two states upon the application of external force.
These changes corresponded to a change in the conformation of the molecule from a polar, open form with charged ends exposed, to a non-polar, closed form with no charge, in response to exposure with UV light or visible light respectively. **The range of contact angle switching was enhanced in rougher surfaces**, a phenomenon known as the ‘lotus effect’ where the leaves of a lotus are known to reject water due to their rough texture. This is an interesting phenomenon that has widely been exploited for self-cleaning and smart surfaces. However, the use of light activation requires the integration of a UV light source, and is not robust to ambient light. Likewise, electrically-activated, reversibly switchable surface coatings have also been developed. **In contrast to optical methods, electrical hardware is integrated into microfluidic format with relative ease.**

**2.2.3 Electrically driven flow control, and reversible wettability switching in electroactive polymers**

Electrically driven fluid actuation is a prominent method of flow control in microfluidics. There is an abundance of techniques pertaining to the electrically driven flow of ions due to charge separation at the walls of the channel. These techniques include electroosmotic flow (EOF), a concept which has been described in later parts of this dissertation, as well as ‘electrowetting’\(^{32,33,35}\). Electrowetting involves the use of high applied voltages (~ several kV) to induce charge separation and wetting on non-conductive (dielectric) surfaces. We therefore focus on the use of electrically activated, biocompatible materials and gentler and less expensive alternative.

Applied voltages can cause multiple physical effects. First, the **application of an electrical potential may be used to induce a change a temperature through a resistive material**. This effect was exploited recently to manufacture switches and mechanical
actuators from paper, using thermally-responsive coatings. Thermally responsive conductive polymers such as Poly-3,4-ethylenedioxythiophene (PEDOT)\textsuperscript{22} and poly-N-isopropylacrylamide (pNIPAM)\textsuperscript{23-24} are characterized by a characteristic temperature, known as the Lower Critical Solution Temperature (LCST), below which they are hygroscopic, and possess the ability to imbibe atmospheric moisture and swell. PEDOT was coated onto electrically activated paper switches which expand and contract in response to electrical heating\textsuperscript{22}. However, this technique was found to be limited by the typical drawbacks of using hygroscopic materials, including its dependence on the presence of humid air, the lack of reproducibility outside of a controlled environment, and marginal changes in the dimensions of the actuator as a result of these drawbacks.

Electrical activation may also be utilized to oxidize or reduce an electroactive material, without causing the device to heat up\textsuperscript{59,60,74-78}. Lahann \textit{et. al.}\textsuperscript{28} were the first to describe reversible wettability switching on gold surfaces functionalized with a sparse monolayer of alkanethiolates. Alkanethiolates are amphiphilic, long chain organic molecules attaching to the gold via a thiol head group, while the tail end of the molecule terminating in a COO- was exposed to the surface. The surface was hydrophilic in this state. However, upon the application of a positive potential to the gold substrate, the molecules synergistically re-orient themselves to expose their hydrophobic backbone. Similar effects have been observed in nanoporous gold\textsuperscript{30}. The advantages of these techniques are that they are gentle, and no changes to sample pH, temperature or solvents were required. Both techniques may be implemented in porous media. However, they are expensive, best suited to metallic surfaces such as gold.
On the other hand, electroactive conductive polymers such as polypyrrole and polyaniline (PANI) are porous, bear a high surface area for functionalization, and are easily synthesized on a variety of flexible substrates including textile fabric. Polypyrrole is hydrophilic when neutral or reduced, and hydrophobic when oxidized. In a previous report, electrodeposited polypyrrole films doped with an oxidizing agent such as a perfluorinated polymer, underwent reversible changes in wettability from superhydrophobic to superhydrophilic, via the application of an electrical stimulus. The application of a negative potential drives the perfluorinated ions out of the polymer, reducing it, and vice versa. Switching was sustained over multiple doping and dedoping cycles by confining the dopant ions to a dry electrolyte gel.

The drawback of this technique is that perfluorinated ions are highly toxic and cannot be disposed easily. We address this problem by developing an alternative switching chemistry for polypyrrole using benign dopants, while retaining the electrically driven element of this switch. We therefore propose the use of polypyrrole as an electrically activated valve in a porous wicking material such as fabric.

In addition, the electrical conductivity of polypyrrole and the ability to functionalize its surface with biological reagents has been utilized to develop an electrochemical sensing surface for the capture and assay of analyte molecules.

2.3 Protein sample separations

Proteins are the end products of gene expression and disease- be it genetic, infectious, or non-infectious- is typically manifested in the structure, function, properties and relative amounts of the corresponding proteins. The goal of the separations processes
is to prepare the protein sample for analysis by isolating the target fraction from complex clinical samples such as serum, urine, cerebrospinal fluid or cell lysate, and improve the limit of detection of analysis. Shown in Fig. 6 is an example of the importance of albumin depletion from blood samples prior to analysis, where albumin being a high abundance blood protein, occludes the appearance of the other key protein bands in an electrophoretically separated sample.

![Electrophoretogram of blood plasma](image)

**Figure 6:** The importance of sample preparations prior to analysis conveyed through the electrophoretograms of blood plasma, in a range of dilutions. The electrophoretogram of the undiluted (neat) sample on the far right is overwhelmed by the albumin band which appears at approximately 67 kDa. This effect decreases with the dilution of the plasma sample. However, dilution also thins out the low molecular weight, low abundance protein fraction. Fractions smaller than 45 kDa seem to disappear with dilution. It is therefore important to be able to separate the high abundance and low abundance fractions before attempting analysis. Image obtained from in-house polyacrylamide gel electrophoresis performed on real plasma samples.

### 2.3.1 Choosing a separations modality

Proteins are separated based on differences in solubility, size, selective binding affinity, hydrophobicity, and charge. The ideal technique is rapid, well resolved, and
does not alter or denature the analyte. Proteins may either be recovered for further analysis, or in some cases, simultaneously characterized and quantified. ‘Precipitation’ and ‘differential solubilization’ techniques involve the addition of organic solvents and salting agents that alter the chemical potential of the aqueous environment, causing proteins to flocculate out, with larger proteins precipitating first\textsuperscript{91,96,97}. However, these techniques may require post-processing steps to desalt the eluate prior to analysis, and are typically bulk scale.

Affinity-based separations involve the interaction of the proteins with a complementary ligand, most often an antibody, either in free solution or bound to a porous solid support such as a packed column. The immuno-affinity based precipitation of albumin from a urine sample in free solution was recently described, where a centrifugation step is utilized to pellet the heavier protein-ligand complex\textsuperscript{45}. Affinity based separations can be reduced to a single processing step by immobilizing the ligands onto a solid support\textsuperscript{93}, by a set of techniques known as ‘immuno-chromatography’. The pore-mediated approach is therefore preferable as bound proteins may be eluted directly from the column without the need to use heavy equipment (although microfluidic analogs to the free solution approach have been developed in the recent literature, and utilize portable rotors to accomplish this). Most notably however, affinity-based separations result in better protein recovery as they are highly specific to the analyte(s) of interest. The antibodies can be chemically labeled with chromophores, and this approach is utilized in LFTs in order to quantify the bound protein. However, antibody-protein binding can also be inhibited by steric effects due to the presence of other larger molecules, non-specific binding, or cell debris.
Filtration techniques force the material through a porous sieve using centrifugal forces (Filter Aided Sample Preparation or FASP)\textsuperscript{98}, differences in osmotic pressure (dialysis)\textsuperscript{99} or a negative pressure differential (vacuum filtration). However, filtration based on a passive property such as diffusivity has also been demonstrated in paper-based analogs of H-filters\textsuperscript{73}. Two co-flowing streams consisting of a mixture of proteins and small molecules in one stream, and a buffer or water in the other, are allowed to traverse the length of the channel. The small molecules diffuse into the buffer stream owing to a concentration gradient and the fact that they diffuse faster than the heavier protein molecule. This technique evidently relies on a difference between the diffusivities of the mixture components. The minimum channel length required to achieve separation is calculated from the Peclét number, (Pe=Uw/D, where U is the velocity of liquid flow, w is a characteristic length of the channel, and D is particle diffusivity). Quick separations in a small channel are therefore contingent upon a large difference in diffusivity between the component molecules, and would be challenging to apply to macromolecular analytes that are generally less diffusive.

Chromatographic techniques rely on the selective distribution of sample components between two immiscible phases- most commonly a solid phase and a liquid phase. Porous supports and columns of packed beads -typically resins and gels- offer large binding surfaces to allow for a number of protein-resin interaction modalities including adsorption, hydrophobic interactions\textsuperscript{94}, affinity-based ligand-receptor interactions\textsuperscript{100}, pH and charge dependent ion exchange\textsuperscript{95} or even size-based exclusion or inclusion of a protein within the pores of the resin (gel filtrations)\textsuperscript{101}. Chromatographic techniques have utility in protein depletion and purification and are implemented commercially. An example is the
AgilentAffinity™ separations system which offers high throughputs of up to 16 mL of sample per run, and can be refreshed for multiple use. Chromatographic approaches may also be ‘multiplexed’, as bound-proteins can be eluted temporally in separate fractions102.

However, packed columns can be susceptible to fouling and expanded beds that are agitated under flow were developed to address this problem103. Columns consisting of a bed of magnetic adsorbent nanoparticles that bind proteins through electrostatic interactions were developed, and the magnetization was used to: 1. Trap and prevent loss of the nanoparticles during the elution step103, and 2. Manipulate and separate the particles from other suspended solids104. An optical counterpart of this technique was reported in which the phenomenon of optical trapping of particles at a focal point in a laser beam may potentially be exploited for molecular separations105. Though these methods effectively separate proteins, they yield very little information about the physicochemical attributes of the protein.

On the other hand, electrically-activated separations may be utilized to simultaneously separate, identify and characterize the protein in terms of its physicochemical attributes. Electrokinetic techniques are relatively mature and in a fundamental mode known as electrophoresis, molecules are separated in order of the ratio of the molecules’ charge/hydrodynamic size106. Proteins of similar size may be separated along a second dimension by combining this technique with pH gradients, porous sieving and affinity-based modalities described above. The following section reviews these modes of electrophoresis for separations and analysis.
2.3.2 Electrokinetic protein separations and analysis

Analyte mixtures migrate under the influence of an electric field and may be focused into narrow zones or separated by virtue of differences in migration velocity. Capillary zone electrophoresis (CZE)\textsuperscript{107} involves the injection of a sample mixture as a short plug into a capillary or microchannel filled with a background electrolyte of a concentration significantly higher than those of the analytes\textsuperscript{108}. When a potential is applied across the channel, analyte zones are separated based on a parameter known as electrophoretic mobility, formulated as, $\mu = \frac{v}{E}$, where $v$ is the migration velocity and $E$ is electric field strength (Volts/length). The electrophoretic mobility is typically an additive effect of electroosmotic flow (EOF) caused due to bulk flow of electrolyte ions and electrophoretic migration of the analyte. The theoretical treatment has been provided in Section 3.2. Good separation resolution requires individual analytes to be focused into narrow and distinct zones under low Peclét number conditions. However, in free solution CZE, advective zone dispersion may occur due to resistive heating, molecular diffusion, and co- and counter-flowing electroosmotic streams.

In order to address these challenges, analytes may be focused and enriched prior to being subjected to typical CZE. The ubiquitous cross-channel injection scheme was developed by Bharadwaj \textit{et. al.}\textsuperscript{43} for the purpose of tuning the shape of a sample injection plug in a microfluidic CE device. The device utilizes a cross channel architecture where the north-south channel is used for sample injection and the east west channel for separation. Sample is first electrophoresed from the sample source reservoir (North) to a sample sink reservoir (South). The sample stream is simultaneously pinched at the intersection by flowing two buffer streams in from the east and the west sides. In the second
step, sample flow is reversed by reversing the polarity of applied voltages, further pinching
the sample stream so that the width of the sample stream is uniform across the intersection.
In the final step, a potential is applied along the East-West channel so the pinched analyte
is now injected into the separation channel as a narrow plug. Although effective, this
multiple step procedure requires the automation of a rather complex voltage control
scheme.

In contrast, prefocusing may also be accomplished in fewer steps by exploiting the
bulk mobility of the buffer ions. A 2-electrolyte system with one electrolyte of greater
mobility than the analytes (Leading Electrolyte-LE) and one of lower mobility than the
analytes (Trailing Electrolyte-TE) allows the sample to be sandwiched into a narrow
intermediate zone in single or multiple injection steps. This method is known as
Isotachophoresis (ITP)\textsuperscript{44}. ITP is typically coupled with CE for sample preconcentration
and separation respectively and the coupled technique yields better signal to noise ratio and
excellent separation resolution. In addition, however, free solution methods require the
application of high electric field strengths on the order of several kV to overcome diffusion.
Another method is dielectrophoresis (DEP) in which non-uniform electric fields are used
to polarize and drive the migration of analytes\textsuperscript{39}. This method is easily applied to larger
systems such as cells and droplets that contain a reserve of ions that facilitate polarization,
but is difficult to achieve in molecules unless a strong electric field is applied\textsuperscript{40}, leading to
other negative side effects such as resistive heating, and mandating the use of cooling
systems.

A more energy efficient way to minimize diffusion is to electrophorese samples
through a porous molecular sieve. Ornstein\textsuperscript{41} and Davis\textsuperscript{38} described the first instance of
coupled ITP preconcentration and CE separation of serum proteins in a polyacrylamide gel with a spatial gradient in pore size. The gel acts as a molecular sieve and can be used to prevent diffusion, while encouraging the protein complement to line up in order of size. Up to 20 serum proteins were separated in a short span of time at far lower field strengths (~10V/cm). This technique, now termed Polyacrylamide Gel Electrophoresis (PAGE), has evolved to be the gold standard in protein pre-concentration and separation, preceding several research and diagnostics procedures. LE and TE are typically composed of chloride and glycinate ions respectively. PAGE has applications in novel biomarker discovery, medical diagnostics and proteomics, and in the study of the kinetics of protein-protein, protein-DNA, protein-drug, protein-carbohydrate, protein-dye and antibody-antigen interactions. Microfluidic, pore-mediated ITP/CE meet the criteria for point-of-care use as they require smaller applied field strengths and can efficiently dissipate heat from their surface. Polyacrylamide gels are however carcinogenic, yet their pore size can be tuned very accurately.

Continuous spatial gradients in pore size are utilized for sample preconcentrations and separations, also known as ‘stacking’ and ‘sieving’ respectively. Stacking is the method used to focus the analytes into a single compact zone by ITP, prior to separation, and is facilitated by a larger pore size gel. Sieving, which is the electrophoretic separation of analytes by CZE, is facilitated by the use of smaller pore size gels which act as molecular size exclusion sieves. It is important that the stacking to sieving transition be seamless, lest large disturbances are caused in the analyte. Paper-based systems for electrophoresis have been proposed that are safer for field use, but suffer from the a few fabrication drawbacks, including the fact that seamless pore size transitions cannot be achieved, and
the printing methods for the deposition of electrodes on 2D surfaces have limited resolution. We propose to use fabric as an alternative porous medium for electrophoresis, where some of the challenges of fabrication may be overcome while reducing cost and toxicity.

2.4 Assay and Analysis

Most diagnostic tests require analytes to be detected and quantified, and this step typically follows the separation and preconcentration of the analyte of interest from the larger sample. Assays are required to be sensitive, with minimal cross-specificity. A widely used approach is the affinity-based capture of analyte molecules by a ligand-functionalized detection reagent designed to produce an optical or electrochemical signal in response to capture.

2.4.1 Immunoassays

Immunoassays involve the non-covalent binding of an antigen by complementary sites on immune proteins known as antibodies. Immunoassays can measure antigens or antibodies from the medical sample. Antibodies are typically branched proteins, >150 kDa in size, and are produced by the host’s immune cells in response to the entry of a foreign object into the blood or lymphatic systems. The binding sites on an antibody, also known as the paratope or hypervariable region- are complementary to the binding sites on an antigen- also known as the epitopes. The format of an immunoassay is therefore chosen depending on the goal of the diagnostic test. A ‘primary’ antibody immobilized on a solid substrate form a solid phase which can capture an antigen from a sample deposited on the substrate. The captured antigens are then probed with a second antibody labeled with a fluorophore or a chromophore, allowing the complexed molecules to be detected optically.
This format is typically known as the ‘sandwich’ assay, and allows the detection of an antigen via two different antibodies functionalized against two separate epitopes, an example being the pregnancy test, which is an assay for human Chorionic Gonadotropin (hCG). In contrast, ‘indirect’ assays capture the antibodies from the patient’s sample, serving as the measure of an immune response due to a latent or active infection, or of vaccination. An example is the HIV diagnostic test, where one of the markers in the panel is the anti-HIV antibody produced by the patient upon infection. A combination of antigen and antibody markers may be therefore be assayed in a single panel in order to differentiate between a latent infection, a full blown infection, immunization, and vaccination.

When performing an immunoassay in the lab, each of these steps is interspersed with a ‘wash’ step, in which non-specific or cross-specific binding due to weaker Van Der Waals interactions are eliminated by a vigorous wash with a buffer such as PBS. Further, non-specific binding with the solid substrate is eliminated by blocking the empty sites on the solid substrate by a filler protein such as BSA, or casein. Immunoassays are typically performed in microwells present in plastic well plates, or after SDS PAGE, in which the electrophoreogram is transferred onto a 2-dimensional surface such as a nitrocellulose membrane, and then probed with labeled antibodies. In most cases the intensity of the bound labelled antibodies is proportional to the amount of analyte in the sample. 96 well plate immunoassays are high throughput and efficient, but are labor intensive, and may require from 2-24 hours to perform.

In contrast, assays that are miniaturized on chip are often performed in a single step while consuming small volumes of sample and reagent. Wash steps are often not required or automated. For instance, in a membrane-based lateral flow test, the wash step is
eliminated by performing the assay under flowing conditions rather than in stagnant, diffusion-limited conditions. A sample is deposited on one end of the strip where it releases the labeled secondary antibodies into solution. The antigen, if present, complexes with the secondary antibodies, and flows downstream where the antigen-antibody complex is captured by a set of primary antibodies immobilized on the membrane, leading to the formation of a colored line. The antigen-antibody complex is captured at the test line while the labeled antibodies are captured at the control line. Two lines therefore indicate a positive test, while a single line indicates a negative test. Excess, unbound reagent is drawn into the absorbent end of the strip, thus automating the wash step and helping to improve contrast on the strip. While LFTs provide binary qualitative readouts, wash steps have been automated in plastic chips using a solid phase composed of suspended microparticles or beads. In such cases the antibody-functionalized beads capture the antigen and are simply separated based on their density using centrifugal forces\textsuperscript{116}, or on their magnetic properties using magnetic forces\textsuperscript{117}. Likewise, electrophoresis-mediated immunoassays employ the electrophoretic separation of antibody-antigen complexes from unbound antibodies\textsuperscript{37}. Since the antibodies are labeled by a visible detection reagent, the two separate bands formed by the bound complex and the unbound antibodies light up, and are detected optically. The complex band is differentiated from the unbound antibodies by the fact that it is heavier and has lower mobility. This single step assay format is known as the Electrophoretic Mobility Shift Assay or EMSA. The unique advantage of the EMSA is that only a single labeled antibody is required for detection, and the assay combines pre-concentration and analysis in the same step. In addition, the ease
of integration of a pair of electrodes into a device make this an attractive low cost option. We demonstrate a proof-of-concept EMSA in fabric.

2.4.2 Optical and electrochemical detection methods

Ligand-receptor binding may be quantified either optically using a labeled detection reagent, or electrochemically, by measuring a change in voltage, current or impedance in response to analyte capture at a functionalized electrode. These techniques have been reviewed in this section.

2.4.2.1 Optical detection of separated analytes

Optical signals are generated using fluorescent, phosphorescent or colorimetric labels. Fluorescence is the emission of higher wavelength visible radiation by a fluorescent dye, in response to excitation by incident light at lower wavelengths. Phosphorescence is the longer lasting and more durable counterpart to fluorescence and is achieved using labels such as quantum dots. While both methods are highly sensitive and serve to amplify weak signals, they require an excitation light source such as a UV lamp or a laser, and light-tight housing for the optics. Colorimetric signals, on the other hand, may be detected visually. Colorimetric tests such as LFTs make use of gold and silver nanoparticles conjugated to antibodies for visual detection, and can be adapted to newer, more sensitive detection methods such as Surface Plasmon Resonance (SPR)\textsuperscript{118}. Metallic nanoparticles are easily synthesized using chemical reduction or seed-mediated growth processes, and bear a large surface area for the attachment of multiple antibody ligands. Although these methods are robust to ambient light, they are limited in sensitivity. In order to ameliorate this problem, we combine nanoparticle based immunoassays with electrophoretic focusing and pre-concentration by the EMSA method described above.
2.4.2.2 Electrochemical detection and electrochemical impedance analysis

In order to develop a comprehensive toolbox of detection methods in fabric, this dissertation also examines electrochemical detection as a separate. In contrast to optical methods, electrochemical methods are generally label-free, and may be performed using portable, low power instrumentation that can be miniaturized into chip format. The power supply, also known as the potentiostat, is an instrument which controls the potential difference between the electrodes by monitoring the current generated in the system\textsuperscript{119}. A three electrode electrochemical cell is the preferred configuration used for most sensing applications, and consists of a working electrode, a reference electrode and an auxiliary or counter electrode. The three electrode system measures half-cell potentials, as opposed to full cell potentials, allowing us to discount extraneous currents generated within the cell, and measuring the changes due to the reaction of interest only.

The reaction of interest occurs at the working electrode, typically due to the reduction or oxidation of an electroactive species present at the electrode, and is driven by the potential of the electrode with respect to the reference electrode. A positive potential drives oxidation, while a negative potential drives reduction. The potential difference is therefore maintained at a low, constant value by a feedback circuit in the potentiostat. The reference electrode is typically an electrochemically stable redox cell such as a silver-silver chloride system, whose net potential is zero. Changes in the current, voltage or resistance at the working electrode, are measured against the reference electrode. The working and reference electrodes are placed in close proximity in order to ensure that the electrolyte contributes very minimally to the voltage drop between the two electrodes. An auxiliary or
counter electrode consisting of an inert material of high surface area, such as a platinum wire or gauze, ensures that the system is stable by absorbing extraneous currents.

Different types of electroanalytical techniques may be used depending on the application being developed. Voltammetry is a technique where the potential is systematically varied either linearly or as a pulse train, and the current response at the working electrode is measured. A change in the current response is observed at the characteristic potential at which oxidation and reduction occur at the electrode. Voltammetry, amperometry and potentiometry, are a set of controlled potential methods where a current or voltage response to a redox reaction is observed, and are especially useful where enzyme-mediated oxidation and reduction reactions occur: a popular example is the point-of-care glucose sensor for diabetes management.

Antigen-antibody binding is a reversible, kinetically-controlled reaction. Put very simply, binding reactions at a functionalized electrode can cause a change in the current flowing through the electrode and can be measured in terms of electrochemical impedance. In impedance spectroscopy, a small AC perturbation is supplied between the working and reference electrodes, and the system’s return to equilibrium is measured in terms of the reactions, ionic diffusion, charge transfer, and capacitance effects associated with the electrode surface. Since impedance analysis characterizes the electrode at the solution-electrode interface, it is a useful method to measure the kinetics of antigen-antibody binding as well as to quantify the amount of antigen (analyte). The potential benefits to performing impedance versus optical measurements are that a single, label-free antibody is used for detection with minimal sample processing, and a quantitative readout is obtained.
The theory of impedance measurement relevant to this dissertation, has been provided in the following section. Impedance-based immunoassays have previously been developed using antibody-functionalized gold surfaces\textsuperscript{130} and micro-patterned glass chips\textsuperscript{124}, as well as semiconducting porous polymers such as polypyrrole\textsuperscript{125}. Low cost electrochemical sensors have been developed in paper, where conductive inks are ink-jet or screen-printed onto a paper-based substrate. However, some commonly encountered issues with printing are the low feature resolution, due to the fact that the inks tend to spread in the paper prior to drying\textsuperscript{20}, as well as high printer turnover, since print-heads are not traditionally built to handle viscous conductive inks.

Weaving presents an effective means to patterning coated conductive yarns into chips at single fiber resolution, while retaining the ultralow cost feature and scalability of the manufacturing process. In prior work by our collaborators, multiplexed woven electrochemical sensors for glucose and hemoglobin were developed in fabric, combining simple fluidic control with voltammetric sensing to achieve high sensitivities with minimal interference from multiplexing\textsuperscript{126}. In this dissertation, we hope to briefly examine the use of polypyrrole as a working electrode material for impedance-based immuno sensing in fabric.

2.5 Summary

Microfluidic sensors made from low cost, porous materials offer several advantages for point-of-care testing: they are self-actuated, have high surface to volume ratios for rapid capture and detection, and are made from inexpensive materials. The various approaches to rapid and low cost manufacture and prototyping were reviewed. Weaving presents an ultralow cost, scalable approach to manufacturing 2-D (and potentially 3D) microfluidic
devices by allowing us to pattern microfluidic channels in fabric using existing weaving infrastructure. Yarns are imparted various treatments in order to functionalize them as dry-reagent reservoirs, or as electrical components, and seamlessly integrated into a fabric device in a quasi-single weaving step. Fabric is therefore a viable platform for rapid sample separations and analysis. Sample analysis is a key element of diagnostic testing, and a widespread approach to diagnostic testing is the affinity-mediated capture of specific analytes from the sample using complementary ligands attached to the device. One such ligand is an immune protein produced naturally in an organism, known as an antibody, which is highly specific and selective to various analytes. Affinity-based capture and quantification procedures which make use of antibodies, are typically termed immunoassays. Antibody-antigen (analyte) binding reactions are kinetically-controlled, and in most microfluidic platforms immunoassays are performed under flowing conditions which help to speed up the reaction process and automate the intermediate steps in the assay, as opposed to the static conditions used in the laboratory. Flow control is therefore a critical component of an immuno-sensing device. In porous media, capillary mediated flow control is prone to irreproducibility due to the fact that all porous materials, including fabric, bear microscopic structural variations at the same scale as fluid flow. These variations have been known to cause drastic reductions in sensitivity and reproducibility. Hence, to overcome the effects of capillary flow, an activation component must be incorporated within these devices. Of the available magnetic, electrical, optic and acoustic approaches, electrical approaches are conceptually simple to integrate into a device by means of a pair of conductive electrodes and a portable, battery-operated power source for activation.
A variety of operations may be performed using electrical activation: first, sample preparations prior to analysis - which involves separation and preconcentration of the target fractions and the removal of interfering substances - may be performed using an all-inclusive electrophoretic approach. When integrated with a porous medium, effective electrophoretic separations may be achieved using small applied potentials by making use of the sieving effect. This approach can also be used to characterize the analyte molecule in terms of its size, charge and physicochemical attributes. Next, electroactive materials with tunable wettability have been described in the literature, and can be integrated within an electrically active fabric device to achieve flow control and overpower capillary defects. These including conductive polymeric coatings that can be synthesized on a fabric using scalable chemical and electrodeposition processes. These coatings can be used as valves, switches and fluid metering components in the device that are also seamlessly integrated by means of the weaving approach described. Conductive polymers are porous, biocompatible and tunable in terms of their electrical conductivity and wettability. While polymers such as pNIPAM and PEDOT are hygroscopic and susceptible to variations in atmospheric humidity, polypyrrole and PANI are robust and tunable. We therefore elect to use polypyrrole coatings for electrically-activated valving in fabric. An additional benefit to using polypyrrole is that it can be functionalized with antibody ligands and used to capture the analytes of interest from a sample. Since polypyrrole is electrically conductive, analyte capture can result in a change in the charge transfer resistance at the surface of the electrode, and most aptly measured using impedance analysis techniques. Further, weaving offers a means to overcoming some of the constraints of printed sensors, by allowing us to pattern electrodes at single fiber resolution. Since lateral flow immunoassays currently
provide binary, qualitative estimates of the analyte using optical approaches to detection, electrochemical detection may prove to be a more sensitive, quantitative means to estimating the amount of analyte.
3.0 THEORETICAL APPROACHES

This section introduces the computational approaches used in this dissertation. In particular, two main mechanisms of sample transport are considered: capillary wicking driven by interfacial tension, and electroosmotic and electrophoretic flow, driven by an applied potential difference. In porous media, electroosmotic transport often occurs in conjunction with a minimal amount of capillary wicking. Since the topology of fabric differs from that of paper and other homogeneous wicking media, the principle of weaving-based manufacture and the unique tuning ability of fabric are described first (Section 3.1). The equations governing capillary flow in porous media, have been provided in Section 3.2, along with some basic principles of flow control. The equations governing electroosmotic and electrophoretic transport have been provided in Section 3.3 and 3.4. These equations may be used in conjunction with capillary wicking in order to model the migration of a charged tracer in a porous medium (Section 3.3). For the final goal of this dissertation, the theory of impedance analysis, including the methods used to represent and analyze the raw data have been provided in Section 3.5.

3.1 Models for capillary wicking through fabric

Porous media are known to spontaneously imbibe liquids, typically through capillary forces. Shown below is an experimental setup to observe the macroscale wetting behavior of a piece of fabric. A drop of dye placed on one end of the fabric spontaneously wicks to the other end.
Figure 7: An aqueous dye being drawn through a strip of fabric through capillary forces. Wicking behavior can be observed by placing a drop of liquid on one end of a strip of fabric fastened to a flat surface, and recording the position of the fluid front with time.

Wicking is driven by an effective pressure differential, which is the sum of the capillary or interfacial pressure differential, the hydrostatic pressure, and the atmospheric pressure. \( \Delta P = \Delta p_c + P_a + P_h \), where \( \Delta p_c \) is the capillary pressure differential, \( P_a \) is the atmospheric pressure and \( P_h \) is the hydrostatic pressure. \( P_a \) and \( P_h \) are neglected in horizontal capillaries that are open to the atmosphere on both ends. Capillary pressure is expressed by the Young-Laplace equation:

\[
\Delta p_c = \frac{4 \gamma \cos \theta}{D} \quad (1)
\]

Where \( \gamma \) is surface tension (N.m\(^{-1}\)), \( D \) is the average pore diameter (m), and \( \theta \) is the contact angle at the solid-liquid interface. The Young-Laplace equation can be substituted in the Hagen-Poiseuille equation for pipe flow, in order to yield the Washburn equation for capillary flow\(^{133} \):

\[
L^2 = \frac{\gamma Dt}{4 \eta} (\cos \theta) \quad (2)
\]

Where \( L \) is the displacement of the fluid on the strip, \( \gamma \) is surface tension (N.m\(^{-1}\)), \( D \) is the average pore diameter (m), \( t \) is instantaneous time (s), \( \eta \) is fluid viscosity (Pa.s), and \( \theta \) is
the water contact angle. Differentiating equation (1) with respect to time gives us an expression for wicking velocity:

\[
\frac{dL}{dt} = \frac{\gamma D}{8\eta L} (\cos \theta) = v \quad (3)
\]

The Washburn equation **dictates that wicking velocity decreases with wicked length L, and increases with increasing pore size (D) and wettability (cos\(\theta\)).** In prior work\(^ {46}\), the Washburn equation was found to be a satisfactory model of the macroscale wicking behavior of yarn and fabric. The exact mechanism of capillary wicking in yarns and fabrics can be explained by a few different models. Capillary wicking is known to occur within the yarns, as well as in the interstitial spaces between the yarns (Fig. 8). Spaces within yarns are typically \(\sim 20 \mu m\) wide\(^ {127,128}\), while inter-yarn spaces are much larger. This hierarchical porosity differentiates fabric from other porous media such as paper. The other differentiating factor is the ability to tune liquid flow separately along the warp and weft axes.

![A bundle of fibers constituting a single yarn](image)

Figure 8: An SEM micrograph of knit cotton fabric. A fabric is composed of several yarns, and each yarn is composed of a bundle of microscopic filaments or fibers. Fabrics therefore exhibit a hierarchical porosity. Flow is believed to take place in the intra-yarn spaces, as well as in the spaces between yarns. Spaces within yarns are typically \(\sim 20 \mu m\) wide, while the interstitial spaces between yarns are much larger. This macro-and micro-porosity must be represented by any model of fabric. Image obtained with help from Bill Fowle at Northeastern University.
As a liquid moves through the fabric, it is distributed between the warp and weft yarns\textsuperscript{127}. The total volumetric flow rate is therefore:

\[ Q_{\text{total}} = Q_{\text{warp}} + Q_{\text{weft}} \quad (4) \]

Since flow rates are conserved, wicking velocity can be controlled to an extent, by tuning the wetting properties of the yarns. The wetting properties of the yarns can vary depending on the type of material used: cotton yarns are typically composed of short staple fibers and have high liquid retention, while silk and other extruded filament yarns such as polyester and nylon, have lower retention properties. Yarn parameters such as the material, thickness, wettability and packing density, have been used to tune flow rates in prior work. For instance, packing in a greater number of weft yarns per unit length of fabric allows us to increase \( Q_{\text{weft}} \). Since \( Q_{\text{total}} \) is conserved, this allows us to decrease the rate of flow along the warp direction. A moderate level of flow control is achievable by this means. Further, by packing the weft yarns in close contact to one another against a hydrophobic warp yarn background, flow can be restricted entirely to the weft. This type of flow control is beneficial, since the weft yarns are most often reagent-functionalized, and helps to maximize the amount of sample interacting with the reagent. In the flow control experiments that were performed in prior work, we demonstrated the ability to slow down or speed up the flow by altering the weft yarn packing density in a seamless manner along the length of the fabric\textsuperscript{5*}. Shown in Fig. 9 is the velocity profile in a strip of fabric with homogeneous, non-varying packing density. The profile is in agreement with the predicted Washburn model where velocity decreases with wet length. However, if the weft yarn

\textsuperscript{5*} Dendukuri \textit{et. al.}, unpublished work, Achira Labs Pvt. Ltd. (2012). Data reproduced with permission.
packing density is varied along the wicking pathway, we must note a deviation from the smooth Washburn trend (Fig. 10). We see that a decrease in packing density results in an increase in wicking velocity and vice versa. This is attributed to the flow rate model discussed earlier. The net velocity of flow \((Q/A)\) along the warp direction is controlled by adjusting \(Q_{\text{weft}}\), wherein a larger number of weft yarns per unit length result in greater \(Q_{\text{weft}}\).

![Image](image.png)

Figure 9: Predicted versus actual velocity profiles in an ordinary fabric strip with homogeneous yarn packing density. The predicted profile is estimated from the Washburn Law using an arbitrary pore size scaling factor assigned to the fabric. In this case the scaling factor is uniform along the length of the fabric. Actual velocity profiles were obtained by tracking the movement of a colored solution as it wicks through the strip of fabric. The resulting profile matches the Washburn profile, where velocity decreases smoothly with wicked length. The absolute value of velocity is however influenced by the wetting properties of the yarns.
Figure 10: A fabric with step changes in weft yarn packing density was woven (inset - top) and was used to demonstrate passive flow control in the device. Packing densities were denoted as high (90 yarns per inch), medium (60 yarns per inch), and low (30 yarns per inch). Fluid flow along the warp direction is recorded. (a)-(b) The velocity profiles were first predicted using the Washburn Law, by assigning a pore size scaling factor to each region, with smaller pore sizes assigned to higher packing densities. As per the Washburn equation, the velocity should therefore increase with each step decrease in packing density (a), and vice versa (b). The actual wicking velocity obtained experimentally are plotted in (c) and (d). We see overall, that the experimental data is in agreement with the predicted data. However, the step changes in velocity are not as sharp. The noise in the plots is caused due to the fact that the data was obtained manually. Overall, these data show that Q-total is indeed conserved and that velocity can be tuned to an extent, by tuning Q-weft. It also shows the Washburn Law is a good representative model for wicking in fabric, where a change in Q-weft is represented by a change in effective pore size (D).
The models were generated using Washburn’s Law, where changes in $Q_{\text{wef}}$ are represented by assigning a pore size scaling factor to each region- we assume that a higher packing density can be modeled analogously with a decrease in inter-yarn pore size, and vice versa. Therefore, high packing density (90 ypi) was assigned a pore size scaling factor of 3, medium (60 ppi) was assigned a pore size scaling factor of 2, and low (30 ppi) was used as the baseline and assigned a scaling factor of 1. The experimental data (Fig. 11c-d) obtained from recording the movement of an aqueous dye by visual analysis, prove that this is in fact true, and that the Washburn Law is a satisfactory governing law for flow in fabric.

However, the noise in the experimental data illustrates the need for a more precise means of flow control. In particular, we desire a means to impart the precise sample-reagent incubations required of immunoassay and clinical chemistry procedures. Precise incubations may be achieved using valves that can stop and restart liquid flow, and an active valve has thus far never been demonstrated in membrane and paper-based LFTs. This provides added impetus to develop an active chemical valve for flow control in porous media (Section 4.3 of this dissertation).

3.2 Equations to describe electrophoresis and electroosmotic flow (EOF) in fabric

The migration of a charged molecule through a conductive medium is driven by an applied potential difference $V$. The attractive forces driving the movement of the molecule towards the oppositely charged terminal are countered by the viscous drag forces acting on the molecule. The balance of opposing forces determine the rate of migration of the molecule through the medium, and are expressed as the electrophoretic mobility, which is the ratio of the charge on the molecule ($q$) to its hydrodynamic size ($r$) in an electrolyte medium of viscosity $\eta$. In addition, the bulk movement of the buffer ions also contributes
to the mobility of the molecule. The velocity of migration of the charged molecule is therefore expressed as a product of the effective mobility and applied electric field (E):

$$v = (\mu_{eo} + \mu_{ep})E \quad (5)$$

where $\mu_{eo}$ and $\mu_{ep}$ are electroosmotic and electrophoretic mobility respectively (m$^2$.s$^{-1}$.V$^{-1}$). $E$ is applied electric field strength (V.m$^{-1}$). $E$ is governed by the voltage drop across the channel (V/L) where L is the length of the channel. The electrophoretic and electroosmotic mobilities are individually expressed as:

$$\mu_{ep} = \frac{q}{6\pi\eta r} \quad (6),$$

and

$$\mu_{eo} = \frac{\varepsilon z}{\eta} \quad (7),$$

Where $\varepsilon$ is the dielectric constant of the buffer and $z$ is the zeta potential due to charge separation at the walls. The electroosmotic mobility in particular is a property of the device and is caused mainly due to the formation of a charge double layer at the walls. For instance, a glass wall ionizes into negatively charged SiO- groups in an aqueous environment, attracting positive ions from the buffer to the walls. These ions in turn attract a layer of negative ions to the walls, and so forth, leading to the formation of charge double layers. An applied potential difference across the ends of the channel draws the positive ions towards the negative terminal, in turn drawing the bulk of the ions towards the negative terminal as a continuous plug. This non-laminar flow phenomenon is known as EOF and may be exploited for valveless fluid pumping, sample injection and mixing$^{31,34,129}$, and is also a giveaway of the characteristic properties of the channel wall. EOF is measured by
observing the migration of a neutral tracer, whose movement is a result of electroosmotic mobility alone. EOF may also need to be suppressed in order to discourage analyte dispersion, and this aspect has been treated in greater detail in the results and discussion.

Since a DC potential is applied to perform electrophoresis, the overall behavior of a stable electrophoretic system is governed by Ohm’s and Kirchoff’s Laws. Ohm’s Law is most applicable to straight channels with no junctions, and dictates that the current flowing through the system (I, amperes) varies linearly with applied potential (V) and the resistance of the electrolyte buffer and the electrodes (R, Ohm).

\[ V = IR \]  \hspace{1cm} (8),

where \( V \) is the potential difference (Volt), \( I \) is current (A) and \( R \) is resistance (Ω).

And,

\[ R = \frac{\rho L}{A} \]  \hspace{1cm} (9),

where \( \rho \) is resistivity (Ω.m), \( L \) is channel length and \( A \) is cross sectional area.

As is the case with most linear electrical systems resistance increases when the length of the electrolyte filled channel is increased. Resistivity also causes Joule heating, which is expressed as:

\[ H \sim I^2Rt \]  \hspace{1cm} (10),

where \( H \) is the heat energy generated due the resistance in the channel (J). All systems must be characterized for their ability to dissipate Joule heat. The point at which the system is unable to dissipate heat at the rate at which it is generated is the point at which Ohm’s Law is no longer valid and current rises exponentially with \( V \). The intuitive parameters
influencing this threshold are the applied potential, buffer resistance, buffer ionic strength and composition and channel length. The limits of operation of any newly designed electrophoretic system are therefore determined by ramping up the voltage and recording the current generated in the system, and the system is henceforth operated below the point where linearity was lost. These plots are known as Ohm’s Law Plots, and were generated for fabric, as described in the results section.

3.3 COMSOL™ modeling of electrophoresis in fabric

Further, in order to model the parameters influencing analyte dispersion and focusing, COMSOL models of the electroosmotic transport of a charged tracer in fabric were generated. The models take into account the three modes of transport: electroosmosis, electrophoresis, as well as capillary wicking due to non-uniform evaporation from the strip. For the COMSOL model, we used the inbuilt Darcy Law module. Darcy’s Law presents a generalized, volume averaged approach to modeling capillary wicking through a homogeneous porous medium such as paper, where the orientation of fibers is random. It is derived from the Stoke’s form of the Navier-Stoke’s equations, where wicking takes place under the laminar regime, and the non-linear inertial terms disappear. Velocity is as expressed as follows:

\[ v = -\frac{K}{\eta} (\nabla p - \rho g) \quad (11) \]

Where \( v \) is the volume averaged velocity (m.s\(^{-1}\)), \( K \) is medium permeability (m\(^2\)), \( \eta \) is fluid viscosity (Pa.s), \( \nabla p \) is the pressure drop (Pa/m), \( \rho \) is fluid density (kg.m\(^{-3}\)) and \( g \) is gravitational acceleration (m.s\(^{-2}\)). The volume averaging method is used in cases where
the material has a complex irregular topology\textsuperscript{134}, and is very similar in form to the Washburn equation.

Unlike paper however, fabric exhibits hierarchical porosity. The simplified geometry utilized to represent this hierarchical porosity is provided in Fig. 11. The macropores (spaces between yarns) are represented as circular gaps in the medium. The parameters associated with the micropores (pore spaces within the yarns) are input into the Darcy equation. The use of circular features to represent the inter-yarn gaps has adapted from a geometry described previously\textsuperscript{135}. However, the simplified hierarchical aspect of this model is original, and was found to be effective when modeling a complex medium such as fabric.

![COMSOL geometry representing the hierarchical porosity of fabric](image)

Figure 11: COMSOL geometry representing the hierarchical porosity of fabric. The inter-yarn spaces are represented by the circular spaces in the geometry. The diameter, spacing and frequency of the circles are related to the thickness of the yarns and the number of yarns per unit length, or the packing density. The remainder of this 2D surface (in grey) represents the yarns themselves. The parameters associated with the intra-yarn spaces such as the porosity, permeability and pore size, are input into the mathematical component of the model.

The total pressure differential is expressed as the sum of electroosmotic and capillary pressure, adapted from a COMSOL model developed previously\textsuperscript{136}.

\[
\nu = \nu_p + \nu_{el} \quad (12)
\]
Where $v_p$ is the capillary wicking velocity obtained from Darcy’s law, and $v_{el}$ is the electroosmotic velocity (m/s). The following steps were followed to calculate the concentration of the tracer plug as it migrates through the fabric. First, the electric field distribution across the channel was calculated using the AC/DC module, by specifying the anode, cathode, and applied potential difference (50 V). The velocity $v$ (Equation 12) was calculated next. Finally, the electric field distribution, applied potential, diffusion coefficient, and tracer charge number, were then used as inputs in the ‘Transport of Diluted Species’ module in COMSOL to calculate the concentration distribution of the tracer as it migrates under the influence of an applied electric field. The model therefore accounts for tracer migration due to the capillary wicking, the bulk EOF, and electrophoresis. The model developed in this dissertation is the first of its kind used to study the effects of yarn packing density on the electrophoretic focusing of the analyte.

### 3.4 Analysis of electrochemical impedance

The final aim of this dissertation is to develop a polypyrrole-coated, fabric-based working electrode for electrochemical impedance analysis, applied specifically to immunoassays. Electrochemical impedance is the AC counterpart of DC resistance, and is expressed as:

$$Z = \frac{E}{I} = \frac{E_0 \exp(j \omega t)}{I_0 \exp(j \omega t - \phi)} \quad (13)^{137}$$

Where $E$ is the applied AC potential (V/m), $I$ is the current (A), $E_0$ and $I_0$ are the amplitude of the applied potential and current respectively, $\phi$ is the phase angle, and $\omega$ is the angular frequency (rad/s), also expressed as $2\pi f$, where $f$ is the frequency (s$^{-1}$). Equation (13) results in the following expression for impedance:
\[ Z = Z_0 \exp(j\phi) = Z_0(\cos \phi + j \sin \phi) \quad (14) \]

which is essentially a complex number term consisting of a real component, \( Z' = Z_0 \cos \phi \) and an imaginary component, \( Z'' = j Z_0 \sin \phi \). Impedance is represented using a Nyquist plot, made by plotting the real component, \( Z' \), on the x-axis, against the imaginary component, \( Z'' \), on the y-axis. The impedance is calculated from its x and y components as per the formulaic method:

\[ Z = \sqrt{Z'^2 + Z''^2} \quad (15). \]

A more direct visual representation of the changes in impedance are provided by the Bode plots, where the impedance \( Z \) is plotted against the scanning frequencies and phase angles. Nyquist and Bode plots are used together for data analysis.

As we know from the literature review section, the experimental setup for electrochemical measurements consists of a three electrode cell with a working, reference and counter electrode. Put very simply, the reaction of interest, which is the affinity driven capture of analytes by an antibody-functionalized polypyrrole working electrode, causes a change in electrochemical impedance at the working electrode. An RC circuit representation of the system is provided in Fig. 12, where each of the capacitive and resistive effects contributing to impedance have been provided. The accumulation of charges near the electrode causes a double layer capacitive buildup, denoted as \( C_d \) (Farad). Capacitance is the storage of charge in a non-conductive or dielectric medium, and is potentially enhanced in a semiconducting electrode. Capacitors are typically discharged over a period of time, and the rate of discharge is determined by the resistance of the system. The electrolyte resistance is denoted as \( R_\Omega \) (Ohm), and is ideally minimized by
placing the working and reference electrodes in close proximity. In order to obtain consistent readings, $R\Omega$ must be consistent between experiments. The resistance to electron transfer and polarization at the electrodes is represented by the charge-transfer resistance $R_{ct}$ (Ohm), and is known to be directly influenced by binding events at the surface. $R_{ct}$ may either increase or decrease depending on the charge transfer properties of the antigen and antibody molecules. **Our goal is to distil the $R_{ct}$ values from the impedance data.**

![Randles equivalent circuit model and Nyquist plot](image)

**Figure 12:** Randles equivalent circuit model (upper) and Nyquist plot (lower) which were used to analyze the impedance data

From the circuit model above, and from equation (14), we obtain the following expression for impedance$^{122}$ (expression adapted from reference 129).

$$(Z' - \left( R_\Omega + \frac{R_{ct}}{2} \right))^2 + Z'' = \frac{R_{ct}^2}{4} \quad (15)$$

Equation 15 is of the same form as the equation of a circle of radius $R_{ct}/2$ with its center at $(R_\Omega + R_{ct}/2, 0)$. Therefore, the charge transfer and solution resistances are readily
obtained from the Nyquist plot (Fig. 13). The Nyquist plot is generated from right to left, scanning from lower to higher frequencies. A perfect semicircle may not always be obtained, and in such cases the Nyquist plot is extrapolated using Zplot™ software associated with the Solartron potentiostat, and fitted to an equivalent circuit model such as the one provided in Equation 15. The software then utilizes the fit to output an Rct value. An examination of the Nyquist plots, and the Rct values is conducted in order to derive meaningful information from this data.
4.0 MATERIALS AND METHODS

This section describes the experimental methods associated with the goals of this dissertation. The approaches to fabricating, characterizing, modeling and performing the proof-of-concept demonstrations for each goal have been described here.

4.1 Design and optimization of the fabric-based electrophoresis device*6

Electrically enabled fabrics were first developed and characterized in terms of their ability to dissipate Joule heat and process a charged sample. Devices were designed on the basis of classic CE systems (Fig. 13) using a simple concept in which the separation channel is flanked on either side by a buffer reservoirs. A voltage is applied across the capillary tube by means of electrodes immersed in either buffer reservoir. Separations occur as the component molecules of an injected sample migrate through the capillary at a velocity that is proportional to the ratio of charge to size. The spectral signature that helps to identify and characterize each analyte is in part formed by the time at which it arrives at a detector located downstream. The analyte may also be interrogated by a laser in order to obtain a fluorescence signature.

Polyacrylamide gel systems function on the same principle, except that the gel serves as the capillary conduit and a molecular sieve, and the band pattern serves as visual proof of separation.

Figure 13: Illustration of the capillary electrophoresis process. The sample injection and optical detection systems have been excluded for the sake of simplicity. In a glass capillary with exposed SiO₂ groups, electroosmotic (bulk buffer) flow is expected to occur towards the negative terminal.
4.1.1 Assembly and operation of fabric devices for characterization studies

Device prototypes used for the optimization of design and operating parameters, were assembled manually (Fig. 14) below.

Figure 14: Manual assembly of device components for initial characterization and optimization experiments

These devices were used for the generation of Ohm’s Law Plots, which are a study of the stability of the system under various operating conditions. A synthetic (nylon or polyester) fabric separation channel was layered over two cotton buffer reservoirs on either side. The fabric layers were laminated onto a rigid adhesive backing card (Lohmann Corporation, Orange, VA) in order to secure the components in place. A pair of inert platinum wire electrodes (99.99%, 0.5mm diameter, Goodfellow Corporation, Coraopolis, PA) were secured between the fabric and the adhesive backing at either end of the separation channel. As the buffer evaporates from the separation channel, it is replenished by fresh liquid from the buffer reservoirs. A partial overlap was provided between reservoirs and the separation channel in order to ensure fluidic contact. Nylon and polyester were used here as they have
a narrow pore size distribution and higher tolerance to heat as compared to natural materials such as silk and cotton. The schematic for the operation of the device has been provided in Fig. 15 below.

**Figure 15: Schematic of electrophoretic device operation.** Buffer is added to the separation channel and the buffer reservoirs. The wetting of the fabric closes the circuit between the two electrodes. A sample is then placed on a cotton sample manifold on the left end, and a potential is applied such that the sample migrates towards the right at a speed that is proportional to its charge to size ratio.

The addition of buffer to the fabric establishes electrical contact between the electrodes. A potential difference was applied across the separation channel using an FB 3000Q electrophoresis power supply (Fisher Biotech). Connections were made using SureGrip™ right angle test leads and Transcat™ alligator clips. The risk to the user is minimized by the fact that the power supply automatically shuts off when low currents are detected, typically due to the fact that the channel has run dry or no buffer has been added. In order to perform electrophoresis, 2-4 µL of sample is pipetted onto a cotton sample manifold located on one end of the separation channel. The manifold prevents premature dispersion of the sample. Characterization experiments to check for Joule heating under different operating conditions were performed using buffer alone. Tracer focusing under different optimization parameters were performed using food coloring dyes. Food coloring dyes
FD&C Yellow 5 (Tartrazine, MW 0.534 kDa) and FD&C Blue 1 (Brilliant Blue FCF, MW 0.792 kDa) from Durkee™ were used in the separations and optimization experiments.

4.1.2 Computational modeling of the effects of yarn packing density on electrophoretic focusing

COMSOL™ models were developed alongside experimental models to investigate the performance parameters associated with electrophoresis in fabric. Since pore-mediated electrophoresis is known to reduce dispersion, this model provided necessary input on the effects of fabric porosity (in terms of weave density) on electrophoretic focusing and band dispersion. The model consisted of a small segment of fabric with two embedded electrodes, and the effect of the material on the electroosmotic transport of a charged tracer, were investigated. The geometry was adapted from previous work\textsuperscript{135}, where the microporous spaces within yarns, as well as the mesoporous spaces between yarns were represented in 2D. Guidance for COMSOL modeling was taken from a previously developed COMSOL module for electroosmotic transport through a porous sintered material\textsuperscript{136}. The software allows the user to combine different physics modalities in order to estimate the combined effect on the movement of the tracer molecule. A 2D geometry was created. The ‘macropore’ spaces between yarns were built into the geometry and represented by the circular discontinuities in the medium. The thickness of the yarns is represented by the distance between the circles. The micropores were built into the equations used to calculate electroosmotic transport through the geometry, where the terms porosity and pore size refer to the porosity and pore size of the micropores in the continuous region. The modeling was performed as follows: First, the electric potential gradient across the fabric was modeled using the ‘electric currents’ physics module by inputting the applied
potential difference and the polarity of the applied potential at each electrode. Next, the electroosmotic velocity field (or bulk flow velocity) was calculated by factoring the porosity of the medium into the standard equations for electroosmotic mobility. Finally, the electrophoretic transport of the charged tracer was modeled using the standard approach where the velocity of migration is a combined effect of the electroosmotic flow and the electrophoretic transport of the tracer as a result of its charge, hydrodynamic size, and diffusion coefficient. The model was used to test a variety of different macroporous structures and provided insight into the effects of fabric weaving, knitting and yarn thickness on the sharpness of the tracer band.

The computational models were used alongside experimental work to show the combined effects of the surface properties of the fabric medium, and the macroporosity, on separation resolution. These models were then used to tune the electrophoretic device to the size and surface properties of the analyte.

### 4.1.3 Weaving-based manufacture of fabric electrophoresis devices

Fabric devices with the optimized specifications were woven at Achira Labs Private Limited, Bangalore, India using yarns obtained from Silk Touch™, Bangalore. The schematic for weaving is provided in Fig. 16. Appropriate warp yarns were first arranged on the loom using an evenly spaced reed. In order to weave the devices, thick cotton weft yarns were woven into the leftmost end to form the buffer reservoirs, followed by the electrode (either metal-coated “zari” yarn or Pt wire), followed by the separation channel yarns at the appropriate packing density, and the electrode and buffer reservoir on the right. Each weaving session produces a “sheet” consisting of 10–12 devices which were then cut
into individual strips, 5 mm in width, using a fabric strip cutter. Each region of the device transitions seamlessly into the next, thereby maintaining good fluidic contact.

Figure 16: Weaving based manufacture of fabric devices. Appropriate yarns are first obtained from the manufacturers, and may be surface treated in order to modify their wicking properties. In this case, surface treated polyester was readily available from the manufacturer and was used without any further modification. A polyester ‘warp’ yarn set is first preset on the loom by the weaver. The second set of yarns known as the ‘weft’ is interlaced orthogonal to the warp. The device components, which include cotton yarns for buffer reservoirs, polyester yarns for the separation channel, and metallized yarn (or metal wires), for the electrodes, are introduced in the weft direction to produce a sheet of monolithic devices, in which each component is integrated seamlessly (inset). The sheet is then cut into individual devices. Reprinted with permission from T. Narahari, D. Dendukuri, S.K Murthy, ‘Tunable Electrophoretic Separations Using a Scalable Fabric-Based Platform, Anal. Chem. 2015, 87, 2480–2487. © (2015) American Chemical Society.
4.2 Electrophoresis experiments

To demonstrate practical utility, the electrophoresis device was utilized to characterize a protein in terms of its mobility, separate a binary mixture of proteins into its component fractions and, assay and analyze proteins using a combination of electrophoretic and immunoassay methods. The reagents and protocols used in this work are described in this section.

4.2.1 Protein separations and characterization

Protein samples were electrophoresed and analyzed in woven devices. Although samples are typically denatured prior to size sorting, we used a protocol for ‘native’ electrophoresis where the folded structure and binding affinity of the proteins are preserved to facilitate additional affinity-based probing of the separated bands. For protein characterization experiments, bovine albumin, MW ≈ 67,000, pI~4.6 pre-conjugated with a naphthol blue black dye, MW 616.49, purchased from Sigma Aldrich. The mobility of the albumin was then calculated from the observed migration velocity. The blue dye helps to visually monitor the progress of the run. The conditions for protein electrophoresis were optimized using the protocol for blue native polyacrylamide gel electrophoresis (BN-PAGE). For the protein separation experiments, human albumin (hAlbumin, MW 67,000, pI~5.3) and human immunoglobulin G (hIgG, MW 130,000, pI~8.2) were purchased from Sigma. Samples were prepared by dissolving the protein to a final concentration of 4 µg/µL in single strength (1x) glycine-NaOH-NaCl, pH 8.58 as the

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electrophoresis run buffer (selected via an optimization process). For protein separations a 1:1 (w/w) mixture of hAlbumin and hIgG was prepared in the run buffer.

4.2.2 Visualization and staining of protein bands

**Coomassie staining** was performed after the completion of the run, in order to fix and visualize the protein bands. For this step, the device was flooded with a staining solution consisting of 0.1 g/L Coomassie Brilliant Blue R250 (Biorad) in a solution of 40% methanol (Fisher) and 10% glacial acetic acid (Fisher) in distilled water. The stain was incubated with the protein bands for ~10 minutes. De-staining was performed in order to remove excess background stain, by flooding the device with solution of 40% methanol and 10% glacial acetic acid in distilled water, and carefully blotting the excess liquid using a Kim wipe.

4.2.3 Electrophoretic mobility shift assays (EMSAs)

The principle for the mobility shift assay was adapted from a previous protocol\textsuperscript{139}, and utilizes gold nanoparticles to help visualize the protein. An illustration of gold-nanoparticle mediated immunoassay is provided in Fig. 17 below.

![Gold nanoparticle-mediated immunoassay](image)

**Figure 17: Gold nanoparticle-based capture of target antigen leading to the formation of a heavier antigen-antibody complex**

A labeled antibody is allowed to capture the target analyte from a sample. The mixture is then electrophoresed to separate the bound complex from the unbound antigen and
antibody. Nanoparticles and beads functionalized with antibodies are preferable as the change in electrophoretic mobility due to antigen capture is directly correlated with the amount of antigen bound to a single particle. Assuming that there are no steric effects inhibiting binding, the weight of the complex is expected to increase linearly with antigen concentration, until all the antibody binding sites on the particle are occupied. The concentration of antigen can then be measured as a function of the electrophoretic displacement (or mobility) of the complex. The analyte consisted of different concentrations of mouse IgG antibodies (mIgG) purchased from Sigma Aldrich. The detection reagent consisted of Goat Anti-Mouse antibodies (GAM) that can specifically bind the mIgGs. A suspension of GAM pre-conjugated to 40 nm gold nanoparticles (BBI solutions, Cardiff, U.K) by an adsorption process was used for convenient visual detection. A 1:1 v/v ratio of GAM-GNP conjugate (Optical Density 10) was incubated with mIgG in a series of concentrations (0.631 mg/mL, 0.210 mg/mL, 0.126 mg/mL and 0 mg/mL) for 1 h. The dilutions were performed in the electrophoresis run buffer.

4.3 Electroactive polymer-mediated flow control in fabric

In the second goal, the electrically-enabled fabric platform is augmented with a flow control element, intended for use in lateral flow devices. The electroactive properties of polypyrrole were investigated for the purpose of building a chemical valve for fabric (or any porous medium). The polymer changes its wettability in response to an electrical signal, typically through reversible oxidation or reduction. First, a novel, non-toxic chemical switching mechanism was developed for the polymer in order to make it point-

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*Portions of this work have been submitted for publication: T.Narahari, D. Dendukuri and S.K Murthy, Development of electrically actuated valves for woven fabric lateral flow tests, Lab On a Chip 2016.
of-care friendly. Next, the polymer was coated onto yarns and patterned into a fabric lateral flow device, where it was used to stop and restart liquid flow at fixed time points. The practical utility of this valve was demonstrated in a clinical assay called the Lowry protein assay, where it was used to automate the incubation of the sample with reagents to demonstrate enhanced detection sensitivities.

4.3.1 Synthesis of polypyrrole by the chemical catalytic method

Figure 18: Schematic diagram of the procedure used for polypyrrole coating on fabric. The polymer is synthesized in situ by drop coating the fabric with equal volumes of iron (iii) chloride catalyst and pyrrole monomer. The coating mixture is let to stand in a covered petridish for 30 min, after which the fabrics are rinsed and dried.

Polypyrrole was synthesized by the chemical catalytic oxidation of pyrrole monomer (Fig. 18). The protocol was adapted from chemical synthetic protocols described previously in which the monomer is mixed with an oxidative catalyst to induce polymerization on non-conductive flexible substrates\(^\text{84}\). *In-situ* synthesis confers control over the properties of the polymer. The protocol was therefore modified to optimize the conductivity and wetting behavior of the polypyrrole. Synthesis was initially aimed at generating a conductive polymer coat and the catalyst was selected from among a few different catalyst and dopant combinations including ammonium persulfate, iron (iii) chloride, and combinations of ammonium persulfate and sodium chloride. In order to coat the textile, equal volumes of
catalyst (in concentrations ranging from 0.001 M to 0.1 M) and 0.1 M pyrrole monomer were drop coated on the textile, and polymerization was allowed to proceed for 30 min (Fig. 18). The liquids were allowed to penetrate the thickness of the fabric. A change in the color of the coated surface from pale yellow to deep black appears to accompany oxidative polymerization, and the depth of the color was found to vary with the extent of oxidation, where darker coats resulted from higher concentrations of catalyst. The textiles were then rinsed in DI water, and oven dried at 40 °C. Polymerization was observed to occur throughout the thickness of the fabric, and was confirmed by observing the coat color and assessing the conductivity of the fabric using a multimeter.

4.3.2 Assembly and operation of wettability switching setup (for polymer characterization)

Reversible wettability switching experiments were first performed under static conditions. A systematic investigation of the effects of operating voltages, activation times and catalyst concentration were carried out in the manually assembled setup shown in Fig. 19 below.

![Figure 19: Construction of fabric-based switching setup used to induce wettability changes in polypyrrole coated fabrics for the initial characterization studies. The setup aims is performed in fabric under static (non-flow) parameters in order to investigate the major tuning parameters that effect wettability changes in polypyrrole.](image-url)
Polymer films deposited on metallic substrates are typically oxidized or in an electrochemical bath containing perfluorinated polymer ions\textsuperscript{29}. In order to circumvent the need for perfluorinated ions, we induce redox changes in the presence of biocompatible buffers containing oxidizing and reducing species such as Cl\textsuperscript{-} and Na\textsuperscript{+}. Saline containing buffers such as PBS, and biological samples such as plasma and urine, meet this criteria. The devices were assembled by placing a piece of coated fabric (1 cm x 1 cm) in conformal contact with aluminum foil to ensure uniform electrical contact, and layering the foil over an electrode. A strip of absorbent cotton was layered over the polymer coated fabric to act as a buffer reservoir. The stack is laminated onto a rigid adhesive backing (Lohmann Corp.) to secure the components in place. The cotton was then drop coated with buffer in order to establish contact between the electrodes, and a potential was applied using a Fisher Biotech FB3000 Q power supply.
Figure 20: Illustration of the switching setup operation and sequence of switching treatments provided. The polymer coated fabric is sandwiched between the buffer reservoir and an electrode to allow for the electrically-driven exchange of oxidizing and/or reducing species between the fabric and the buffer. ‘Cathode treatments’ are provided by applying a negative potential to the polymer coated fabric. This step is expected to drive any electronegative species out of the polymer, while ‘anode treatments’ are imparted by reversing the polarity of the applied potential and are expected to attract electronegative species from the electrolyte and back into the polymer.

Fig. 20 describes the switching treatments that were imparted to the fabric. ‘Cathode treatment’ was performed by placing the fabric at the negative electrode for time durations ranging from 0.5-2 min, and voltage drops ranging from 5-20 V/cm. Wettability was measured, after which the anode treatment was imparted wherein the polarity of the applied voltages was reversed.
4.3.3 Contact angle measurements and elemental analysis

Changes in the wettability and composition of the polymer were measured at every step of the switching process. The chemical mechanisms responsible for the wettability switches were investigated here. Wettability changes were assessed via water contact angle measurements, the principle being that a drop of water spreads on a wettable surface, and the angle made by the solid-liquid interface with the liquid-air interface is small. In contrast, a non-wettable surface is expected to cause the drop to bead up as a means to minimizing surface energy, and the corresponding angle is larger. The fabric was fastened to a horizontal surface, and a 100 µL droplet of aqueous liquid was placed on the dry fabric. The droplet profile was imaged using a Proscope HR2 camera, and contact angles were measured using Image J software. Changes to the elemental composition of the polymer after wettability switching were investigated using Electron Dispersive x-Ray Spectroscopy (EDS). EDS yields both qualitative and quantitative information in terms of the elemental composition of the sample as well the quantity of analytes. EDS measures the energy of the x-Rays produced as a byproduct of sample excitation by an electron beam. An EDAX system associated with a Hitachi X 4800 Scanning Electron Microscope was used for this purpose. Samples were sputter coated with platinum prior to imaging in order to enhance conductivity and facilitate SEM-EDS. SEM images were obtained at acceleration voltages of 3kV appropriate to imaging the surface, while EDS was performed at 12 kV.
4.3.4 Modeling the hydraulic resistance through a polymer valve in a lateral flow device.

Fabric lateral flow devices with midstream polymer segments were created in order to characterize fluid flow in the device. The concentration of catalyst used for the synthesis of the polymer was varied as follows: 0.001 M, 0.01 M and 0.1 M, and 0 M- no polymer segment), and the corresponding Washburn velocity profiles were obtained in order to investigate the dependence of flow rate on polymer strength. The term ‘strength’ is used to denote the amount of catalyst used for synthesis, as larger amounts yielded heavily polymerized polypyrrole matrices. Two of the four cases were modeled mathematically. First, an average effective pore diameter of the polymer and the uncoated segments were estimated from the slope of the velocity profiles using the Washburn equation. Pore diameters were directly correlated with the polymer strength and the hydraulic resistance to flow through the polymer. However, since the Washburn law dictates also that the velocity varies inversely with wet length (v ~ 1/L), additional parameters such as the position of the polymer segment on the strip (distance from the inlet) and the length of the polymer segment, were modeled. Mathematical modeling enabled investigation of a large parameter space. The models also provide insight into the effects of sudden increases and decreases in hydraulic resistance on the wicking rates along a strip, effectively creating a toolbox for the design of a variety of valves, including on/off valves to stop and restart flow, and partially on valves. The on/off valve was implemented in an assay application.
4.3.5 Manufacture and operation of woven lateral flow devices with polymer valves

**Figure 21:** Manufacture of woven lateral flow devices with polymer valves. (a) Hydrophilic viscose (rayon) yarns were first coated with the polymer by the wet chemical synthesis procedure shown. (b) Devices were woven by inserting the coated yarns along the weft direction into a hydrophobic warp yarn set composed of raw silk yarns. (c) The device components are integrated into a unified sheet and are downsized into individual strips as shown in (d). (d) Individual strips are operated by placing the liquid (sample) on the left end. The sample flows from left to right where it encounters the polymer valve. The valve is in off mode by default. So liquid flow is stopped. The valve can be turned on at any point, by applying a potential between the two wire electrodes. Depending on the magnitude and polarity of the potential, and the intrinsic properties of the valve, flow may have been stopped completely, and can now be restarted, or flow may simply be slowed down and sped up.

The schematic for the manufacture of woven lateral flow devices with polymer valves is provided in Fig. 21. The polymer was first synthesized on viscose (rayon), weft yarns (Fig. 21a) using the wet chemical synthesis technique described previously. The coated yarns...
and metal wire electrodes were integrated into a device by weaving. A hydrophobic warp yarn set was used in combination with a hydrophilic weft, in order to restrict the flow of liquid to the weft and prevent leakages across the polymer segment (Fig. 21b and 21c). The sheet of integrated devices was then cut into strips (Fig. 21d). A liquid that is placed on one end of this strip is expected to flow unhindered, until it encounters the polymer coated region. As described in Fig. 22, the valve is in off mode by default. So liquid flow is stopped. The valve can be turned on at a fixed time point by applying a potential between the two wire electrodes. Depending on the magnitude and polarity of the potential, and the intrinsic properties of the valve (the tuning parameters which were characterized earlier), flow may have been stopped completely, and can now be restarted, or flow may simply be slowed down and sped up.

4.3.6 Protein estimation by the modified Lowry method

The utility of the valve was demonstrated via a Lowry Protein Assay performed in the woven device. The modified Lowry method\textsuperscript{140} was followed in which the protein is first reacted with an alkaline copper solution (Lowry Reagent or LR) followed by a chromogenic reagent (Folin-Ciocalteu or FC reagent) to produce a visible blue compound whose intensity is proportional to the amount of protein in the sample. Under static conditions this procedure typically requires 45 minutes to 1 h to complete. The chemical reactions must proceed to completion for the prescribed period of time, else a reduction in sensitivity is noted. A conceptually simple reaction schematic was used in which the Lowry reagent was prespotted on the device upstream of the valve, and the FC reagent was pre-spotted downstream of the valve. Sample is then flowed through the device and flow is timed using the valve. The results were imaged and intensity analysis was performed using
a Proscope HR2 camera and Image J software. Intensity of the blue result was plotted against protein concentration to obtain a standard curve. The assay was repeated in control devices where no valves were used and a standard, geometry-based means of flow control was employed. The assay was used to demonstrate the usefulness of the valve in enhancing the sensitivity of an LFT-based Lowry Assay by imparting precise sample-reagent incubations.

**4.4 Impedance-based immunoassays in fabric**

An alternative means of detection, as opposed to visual assessment, is obtainable through impedance analysis. Under this study, the conductivity of the polypyrrole coated material was exploited in order to construct a polypyrrole-based working electrode for the impedance analysis of protein analytes. The goal was to check the feasibility of this system and to obtain proof-of-concept. Polypyrrole coated fabrics were first functionalized with antibodies using a simple adsorption process, and a change in the resistance at the electrode due to antigen binding was recorded. The change in resistance is expected to correlate with Ag-Ab binding. Comparisons with a control electrode that was functionalized with a non-specific antibody were used to confirm whether the change in resistance was indeed due to antigen binding. This setup offers an alternative avenue for analysis in an electrically enabled device.
4.4.1 Design and operation of the impedance chip\textsuperscript{9*}

Impedance analysis was performed in a three electrode electrochemical cell consisting of a polymer-functionalized working electrode, a silver-silver chloride reference electrode, and a platinum wire counter or auxiliary electrode. Device prototypes were assembled as shown. Feasibility experiments were conducted either in a beaker or in on a manually assembled chip (Fig. 22). To manufacture the chip setup, a small piece of antibody coated fabric (4 x 4 mm) is then laminated to a glass slide and connected to the cathodic leads of a Solartron impedance analyzer and potentiostat using conductive copper tape. A circular masking tape is applied to the fabric in order to confine the liquid to a small and consistent surface. A droplet of electrolyte buffer, typically 100-300 µL, large enough to establish contact between the WE, CE and RE, is placed on the circular window, and the tips of all three electrodes are now in contact with the drop. The drop acts as the reservoir providing electrical contact between the electrodes, and supplying analytes for capture. After making the appropriate connections to the potentiostat, the working electrode was given a small

\textsuperscript{9*} This work was conducted in collaboration with Hunter Sismaet and Avinash Kola under the supervision of Professors Edgar Goluch, Elizabeth Podlaha-Murphy and Shashi Murthy.
negative DC bias relative to open circuit potential to ensure current flow. A frequency scan from 0.1 Hz to 100,000 Hz is performed, and the resulting data is acquired and analyzed.

Figure 22: Design and operation of prototype impedance sensors. (Top) The materials are laminated onto a glass slide. The conductive copper tape helps immobilize the fabric on the slide as well as make the electrical connection with the WE lead of the potentiostat. (Bottom) A droplet of sample is placed over the WE and the RE and CE are immersed in the droplet prior to making the measurements.

4.4.2 Preparing the polymer-coated working electrodes

The working electrode, where the reaction of interest takes place, was manufactured as follows: the fabric was coated with polypyrrole using the chemical synthetic method described before, with 0.1 M pyrrole monomer and 0.1 M iron (iii) chloride. The polypyrrole is allowed to form over 30 min, after which the material is baked and dried to
a moist state at 40 °C. The moisture facilitates the absorption of the antibody reagent dissolved in 1x PBS buffer. Antibody surface concentrations ranged from 2 to 0.5 µg/cm². The antibody is drop coated onto the fabric, allowed to absorb for 30 min, after which the material is dried again at 40 °C. Test electrodes were coated with Polyclonal Anti-HER2 antibodies (Life Technologies, #280004), while control electrodes were coated with Goat Anti-Mouse antibodies which do not carry complimentary sites to HER2 protein. Polyclonal antibodies have broader specificities as compared to monoclonal antibodies, and were used to maximize the chances of HER2 capture. Post antibody addition, a solution of 1% BSA is added to ‘block’ the empty sites on the polypyrrole electrode and prevent the sample analyte from adsorbing onto those sites. The fabrics are then rinsed gently with PBS and oven dried.

4.4.3 Analysis of impedance data

The methods used to analyze the impedance data and obtain a charge transfer resistance value have been described in the theory and results sections. To summarize, impedance data is obtained in the following formats: Nyquist plots, or the complex plane plots of the real versus imaginary components of impedance which are semicircular and centered on the x axis; and Bode plots of the magnitude of impedance and phase shift against frequency. The data from the two plots is fitted to a predefined CPE-Rp equivalent circuit model, where CPE (constant phase element) is the real world analogy to the capacitance, and is used in non-ideal systems where the Nyquist plot appears as a depressed semicircle with

10* ‘Monoclonal’ antibodies are a set of antibodies produced by a specific line (or clone) of white blood B cells at the latter stages of immunization, as opposed to polyclonals which have a broader specificity to multiple binding sites on the antigen.
its center below the x-axis. The fit outputs the values of Rp, which is the charge transfer or polarization resistance. The charge transfer resistance is the resistance of charge transfer at the surface of the working electrode and is directly influenced by binding events occurring on this surface.
5.0 RESULTS AND DISCUSSION

This section details the experimental and computational results associated with the three primary goals of this dissertation. An electrically enabled fabric platform was first built, tested for its operating limits, and utilized for electrophoretic protein separations and assay. The platform was then repurposed with an electroactive polymer valve, for flow control. The use of the polymer as a functionalized electrode for impedance sensing was demonstrated last.

5.1 Designing an electrically enabled fabric platform

Sample separations are often the first step to a number of analyses. An integrated system for sample preparation and analysis is a useful tool in biology. As described earlier, capillary electrophoretic separations and analyses may be performed nearly simultaneously, yielding information on the identity as well as the physicochemical properties of the analyte. Further, the development of a low cost integrated system is a need both in the laboratory as well as in the field. In this section we discuss the design of the low cost and scalable fabric-based electrophoretic platform and the optimization of its performance.

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5.1.1 Optimization and characterization of the operating limits of the system

The overall goal here is to not only to optimize the construction of the device, but to establish that fabric behaves in a predictable manner concurrent with the theory of electrophoresis. In electrophoresis, analytes migrate as narrow zones or focused bands moving at a constant velocity under the influence of a uniform electric field. The migration velocity is a result of a balance between the hydrodynamic drag experienced by the molecule and the applied potential difference which is the driving force acting on the molecule: \( v = \mu E \), where \( E \) is applied field strength (Vm\(^{-1}\)), \( v \) is migration velocity (m.s\(^{-1}\)), \( \mu \) is the effective electrophoretic mobility (m\(^2\)V\(^{-1}\)s\(^{-1}\)). Electrophoretic mobility is typically defined as the ratio of charge to hydrodynamic size of the molecule.

However, there are a number of additional factors that intricately affect electrophoresis. These factors are often influenced by the design and operation of the device. One of these factors, is Joule heating, which develops as a result of the generation of the flow of current in the device (a simple approximation for Joule heat is \( H \approx I^2 R t \), where \( H \) is the heat generated (J), \( I \) is generated current (A), \( R \) is resistance (ohm), and \( t \) is time (s). Joule heating above a threshold value can set up thermal gradients and convective flows that lead to band dispersion and a reduction in separation resolution. The electric currents passing through the channel must therefore be modulated. The effects of variables such as electrolyte buffer composition, ionic strength, applied voltages and device dimensions were studied. Plots of applied voltage against the current were systematically generated for each of the tuning parameters above. The behavior of most electrophoretic devices follows Ohm’s law where the resistance varies linearly with applied potential difference \( (V = IR) \)\(^{31,34,129}\). Therefore, the point at which the plot loses its linearity is
typically the threshold at which the system is no longer able to dissipate Joule heat. This limit exists in most CE systems and its magnitude varies depending on the applied voltage, and the ionic strength and conductivity of the electrolyte buffer. The associated parameter space pertaining to the buffer types tested and their respective applications is summarized in Table 1. The Ohm’s Law plots generated for the different buffer types are provided in Fig. 22. Optimization plots were generated in devices that were manually assembled.

Table 1: Electrophoresis buffer types that were tested for Joule heating in the fabric strip

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Total ionic strength</th>
<th>Buffer conductivity (mS)</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate Buffered Saline, pH 7.4</td>
<td>154 mM (1x PBS)</td>
<td>16.8 ± 1.06</td>
<td>Nucleic Acid Electrophoresis</td>
</tr>
<tr>
<td>Tris-Glycine, pH 8.3</td>
<td>16.8 ± 1.06 (1x TG)</td>
<td>0.0445±0.00</td>
<td>Protein Isotachophoresis (Native Polyacrylamide Gel Electrophoresis)</td>
</tr>
<tr>
<td>Glycine-NaOH-NaCl, pH 8.58</td>
<td>147.5 mM (1x Glycine-NaOH)</td>
<td>10.7 ± 0.03</td>
<td>Protein Isotachophoresis and Capillary Electrophoresis (Microchip free solution electrophoresis)</td>
</tr>
</tbody>
</table>

We see from Fig. 23 that high ionic strength buffers such as PBS lead to the generation of larger currents, and the plot loses its linearity at a lower applied field strength, while low ionic strength buffers such as TG generate very small currents in the device (due to their high resistances) that were not sustainable using the FB 3000Q power supply.

Buffers that contain glycinate and chloride ions are especially suited to the isotachophoretic focusing and separation of proteins, as the glycinate and chloride ions
form a leading electrolyte/trailing electrolyte pair. Based on the Ohm’s Law plots, 1x Glycine-NaOH-NaCl, pH 8.58, was therefore selected for future use. As we see from the Ohm’s Law plot, the viable range of applied field strengths is 5-40 V/cm. Based on Ohm’s Law plotting of generated currents when the separation distance between electrodes is varied, the dimensions of the device were finalized at 10 cm x 0.5 cm. This size also allows for convenient handling and visual inspection. However, in order to effectively apply a stronger electric field without consuming additional power, the separation distance between electrodes may be reduced while leaving the voltage unchanged, providing the minimum effective length required for separations is maintained (see Appendix A, Fig. A1).
Figure 23: Ohm’s Law plots of generated current against applied field strength (V/cm) for different electrolyte buffers in a range of ionic strengths and conductivity (refer Table 1). PBS has the highest ionic strength and conductivity, and the plot loses its linearity at a lower applied voltage as compared to the other buffers. 1x TG and 0.5x Glycine-NaOH, on the other hand, have low conductivities and generate currents that are too low to establish proper connectivity between the electrodes. Buffers containing glycinate and chloride ions are desirable due to their usefulness for isotachophoretic focusing, and on this basis, 1x Gly-NaOH-NaCl was selected for future use. Plots were generated in the manually assembled devices. Reproduced with permission from Anal. Chem. 2015, 87 (4), 2480–2487 ©2015 American Chemical Society.

5.1.2 Proof-of-concept of dye electrophoresis and separations in fabric

Proof of principle of electrophoresis of small molecule dyes was demonstrated using the optimized operating conditions. The electrophoretic migration of anionic food coloring dye, Yellow 5 (tartrazine, MW 0.534 kDa) against a diffusion control was successfully demonstrated (Fig. 24a). The dye migrates away from the negative electrode and towards the positive electrode.
Figure 24: Electrophoresis and separations of charged dyes in fabric. (a) The electrophoresis of a negatively charged dye (Yellow 5) occurs away from the negative electrode and towards the positive terminal at applied field strengths ranging from 25-50 V/cm. A diffusion control provides the basis for comparison of the unique shape of the band migrating under an electric field. (b) A mixture of blue and yellow dyes (initially green) evolving into separate bands in order of charge to size ratio. (Applied field strength = 35 V/cm in 1x Gly-NaOH buffer). A diffusion control is included to show that separations did not occur spontaneously, within a 5 minute window. Reused with permission from Anal. Chem. 2015, 87 (4), 2480–2487 ©2015 American Chemical Society.

The effects of applied voltages on analyte band focusing were then tested (Appendix A, Fig. A2). Analyte bands were narrower and more consistent throughout the run at applied field strengths ranging from 30-35 V/cm. Inconsistencies in band width were observed at higher potentials approaching the system limit (40-50 V/cm), presumably due to heating, while the same inconsistencies occur at lower potentials where the field is typically not strong enough to overcome hydrodynamic drag. Subsequently, a 1:1 v/v mixture of Yellow
5: Blue 1 (Brilliant Blue FCF, MW 0.792 kDa) was electrophoresed at 35 V/cm. The sample appears green when first deposited in the device, and evolves into yellow and blue bands within a 5 minute window (Fig. 24b). A diffusion control has been provided for comparison, where no separations were observed within this time frame (Fig. 24b). Further, since the dyes bear identical charge, they are resolved in order of size, with the smaller Yellow 5 tracer migrating faster than the Blue 1 tracer. This confirms that separations were the result of electrophoresis. Most notably, separations were achieved at low field strengths, between two analytes with a small difference in molecular weight (< 0.2 kDa). However, diffusion and spreading was observed between bands even with the use of submicroliter volumes of dye sample, leaving scope for the improvement of separation resolution in this system. The effects of additional factors that influence separation resolution were therefore examined.

5.1.3 Models to identify the factors affecting electrophoretic separation resolution in fabric.

As discussed earlier, separation resolution is a key performance metric that can be improved by mitigating analyte dispersion. Some techniques to suppress band dispersion include the use of high electric field strengths to overpower the hydrodynamic drag on the molecule\textsuperscript{141}, the use of high ionic strength buffers\textsuperscript{142}, wall coatings to suppress charge separation and electroosmotic flow\textsuperscript{141,143}, and fine gels which restrict diffusion\textsuperscript{41}. Here, the goal is to tune separation resolution without being limited by the operating parameters, such as the applied potential difference. We also envision developing the ability to weave predefined regions in the fabric for ‘stacking’ and ‘sieving’ by making use of intrinsic fabric-based tuning parameters.
In electrophoresis, particles continue to diffuse as they migrate down the channel. The Peclét number for electrophoresis, that is the dimensionless ratio of advection to diffusion, is expressed as $Pe = \frac{vL}{D}$, where $L$ is channel length and $D$ is particle diffusivity. High Peclét numbers are viewed as desirable and as per this requirement, separation resolution may be improved by providing a longer migration path length to the electrophoresing analyte.

Our preliminary hypothesis was that tortuous ‘knits’ and high fabric packing densities may provide longer migration paths at the microfluidic scale (inset in Table 2 below). Fabric packing density, also termed ‘coverage area’, refers to the total number of yarns per unit area of fabric. It is usually calculated as the number of warp yarns x number of weft yarns per square inch of fabric. In order to test this hypothesis, five types of commercially available fabrics were assembled into devices for dye separations. The fabrics and their packing densities have been provided in Table 2 below. The mixture of tracer dyes consisting of 1:1 v/v, yellow : blue were electrophoresed in these fabrics and separation resolution was calculated as follows after a run time of 2.5 min:

$$SR = \frac{\Delta L}{w_{avg}}$$

where $SR$ is dimensionless separation resolution, $\Delta L$ is the center-to-center distance between the analyte bands (pixels), and $w_{avg}$ is the average width of an analyte band (pixels). The measurements were performed digitally by image analysis (a detailed description of the technique is provided in Appendix A, Fig. A3).
Table 2: The selection of fabrics used to test the hypothesis that separation resolution increases when knit fabrics consisting of curving interlaced yarns, are used in place of woven fabrics, which consist of straight orthogonal yarns. Knit fabrics are presumed to offer a longer migration path per unit length of fabric, as opposed to woven fabrics. The other theory is that migration path, and separation resolution, may be increased by increasing the packing density of yarns. The packing densities are expressed in terms of a ratio of image pixels obtained using an in-house image analysis algorithm developed by Estelle Beguin and Adedayo Catlett. Images were acquired at 200x magnification using a Proscope HR2 camera. Data reused with permission from Anal. Chem. 2015, 87 (4), 2480–2487 ©2015 American Chemical Society.

<table>
<thead>
<tr>
<th>Fabric</th>
<th>Composition</th>
<th>Structure</th>
<th>Measured coverage Area* (yarn-occupied image pixels/total image pixels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cupro-Nylon</td>
<td>20% Nylon, 80% Cotton</td>
<td>Weft knit</td>
<td>0.805 (Medium)</td>
</tr>
<tr>
<td>Nylon-1</td>
<td>100% Nylon</td>
<td>Warp knit</td>
<td>0.882 (Medium)</td>
</tr>
<tr>
<td>Nylon-2</td>
<td>100% Nylon</td>
<td>Weft knit</td>
<td>0.881 (Medium)</td>
</tr>
<tr>
<td>Polyester-1</td>
<td>100% Polyester</td>
<td>Weft knit</td>
<td>0.514 (Low)</td>
</tr>
<tr>
<td>Polyester-2</td>
<td>100% Polyester</td>
<td>Plain weave</td>
<td>1.000 (High)</td>
</tr>
</tbody>
</table>
The results from the experiments are provided in Table 3.

Table 3: The results of the separations experiments in fabrics of different packing densities and weaves. The results show that a correlation was obtainable between packing density and SR within fabric types, i.e., nylon or polyester. ‘Cupro’ nylon refers to a hydrophilic blend of nylon and cotton. The cupro-nylon is an outlier where separations did not occur despite the higher packing density. SR was measured as the ratio of center to center distance between bands divided by average width of the bands, and the technique is detailed in Appendix A, Fig. A3. The results show that further investigation of the effects of the material, yarn properties and porosity is warranted.

<table>
<thead>
<tr>
<th>Fabric</th>
<th>Coverage Area</th>
<th>Electrophoresed dye mixture at t=2.5 min (n=5)</th>
<th>SR*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cupro-Nylon</td>
<td>Mediu</td>
<td><img src="image1.png" alt="Image" /></td>
<td>0</td>
</tr>
<tr>
<td>Nylon-1</td>
<td>Mediu</td>
<td><img src="image2.png" alt="Image" /></td>
<td>0.40±0.02</td>
</tr>
<tr>
<td>Nylon-2</td>
<td>Mediu</td>
<td><img src="image3.png" alt="Image" /></td>
<td>0.35±0.01</td>
</tr>
<tr>
<td>Polyester-1</td>
<td>Low</td>
<td><img src="image4.png" alt="Image" /></td>
<td>0.52±0.04</td>
</tr>
<tr>
<td>Polyester-2</td>
<td>High</td>
<td><img src="image5.png" alt="Image" /></td>
<td>0.69±0.03</td>
</tr>
</tbody>
</table>
Figure 25: Results from Table 3 have been plotted here. A correlation between SR and coverage is observed within fabric types. The two polyesters of low and high coverage have low and high SR respectively while the two nyons of similar coverage have similar SR. The cupro nylon did not show any separations despite higher coverage, indicating that the material also has a role to play in separations. The material properties of polyester and nylon are similar, but better separations were obtained in polyester. The effects of these variables were decoupled and explored further using COMSOL models of electrophoresis in fabric.

The data from Table 3 have been plotted in Fig. 25. Within fabric types, SR improves with increased packing density. However, the properties of the material appear to have a marked effect on separations. Further, since it is difficult to vary these parameters in a systematic manner, we sought to model dye migration using COMSOL.

The COMSOL geometry is provided in Fig. 26. A small section of fabric is represented here. The circles represent the larger pore spaces between yarns, while the smaller pore spaces within yarns (due to each yarn being composed of bundles of microfilaments), have been input into the parameter space in the model. The COMSOL model was adapted from a previously developed model of electroosmotic transport through a porous sintered material\(^{136}\).
However, this model is an original representation of the hierarchic porosity in fabric. The movement of the tracer is driven by electroosmosis, electrophoresis, and potentially also due to capillary flow driven by pressure gradients which are created due to evaporation from the strip. Snapshots of the migrating band under different conditions of fabric packing density have been provided in Figs. 27-30. **We see that higher fabric packing densities and the use of thicker yarns help to mitigate band distortion.** In these cases the movement of the band is uninterrupted. Knit structures have been represented by staggered pore spaces. **We also observe that the tracer molecules are evenly distributed across the band in the case of knit fabrics.** These results may be attributed to the fact that larger inter-yarn spaces in woven fabrics lead to the formation of non-uniform electroosmotic pressure gradients around the pores (the surface distribution of pressure has been provided Appendix A, Fig. A4). **The model** allows us to systematically vary the applied voltages, channel geometries, and inter- and intra-yarn pore sizes without having to physically experiment with a large parameter space.

**Figure 26:** Fabric geometry and boundary conditions specified in the COMSOL model. The inter-yarn pores, or packing density, is represented by the circular spaces in the geometry while the smaller intra-yarn pores are specified in the parameter space for the model.
Figure 27: Snapshot of the migrating tracer (charge number -1, tracer moves from left to right) at a run time of 1 minute, an applied potential difference of 50 V, intra-yarn porosity of 0.6, and inter-yarn pore size of 25 micron. The presence of large inter-yarn spaces and the use of thinner yarns seems to cause some band distortion. The tracer molecules are unevenly distributed within the band.

Figure 28: Snapshot of tracer migration under the same conditions as Fig. 26, and a reduced inter-yarn pore size of 10 micron. Thicker yarns cause less distortion, and the edges of the tracer band appear to be more even.
Figure 29: Snapshot of tracer migration under the same conditions as Fig. 26, and negligible inter-yarn pore size, showing a much improved band with uniform edges and a uniform concentration distribution of the tracer.

Figure 30: Tracer migration in a knit fabric represented by staggered inter-yarn spaces of 25 micron diameter, showing great improvement over woven fabrics in terms of the concentration distribution of tracer molecules. Rather than allowing a longer migration path length, knit fabrics potentially allow us to stagger the inter-yarn spaces and allow the tracer to distribute uniformly throughout the band.
The model confirmed that higher yarn packing densities, aid in focusing. Cupro-nylon fabrics were the only exception, where separations did not occur despite the high packing density of this fabric. Cupro nylon is a blend of cotton and nylon, and is more hydrophilic than the other materials. We believe that this hydrophilicity may have caused the dye to disperse through the fabric. **The choice of material significantly influences SR.**

A simple and widely applied approach to measuring the hydrophilicity of a fabric is to measure its wettability using an aqueous liquid\(^{144}\). An example of this measurement is provided in Fig. 31a (inset). A drop of the mixed dye is placed onto each fabric and is allowed to spread through the fabric by means of capillary wicking. The size of the drop is then measured, and correlated with wettability. Fabric wettability was plotted against the SR obtained from the separations experiments (Fig. 31a), and it is clear from the results that increasing wettability leads to greater dispersion and lower SR. **However, since protein analytes require passivated channel surfaces that are often hydrophilic, it is important to use an optimal combination of coverage area and wettability that allows protein mixtures to successfully separate in fabric.**
Figure 31: Tuning SR using a combination of fabric packing density and wettability

(a) The effects of wettability on SR have been plotted to show that better separations are achieved in relatively hydrophobic materials. However, protein analytes interact with hydrophobic surfaces. Therefore, packing density and hydrophilicity may be used in combination. (b) This combined effect is captured by a parameter that we term the ‘tuning factor’ which is the ratio of packing density (coverage area) to wettability (drop area). This is an analogy to the Peclét number, where larger values of the tuning parameter imply better focusing versus dispersion. Adapted with permission from Anal. Chem. 2015, 87 (4), 2480–2487 ©2015 American Chemical Society.

In order to obtain the ideal combination of wettability and packing density, we coined an analogy to the Peclét number for electrophoresis in fabric, where:

\[ SR \sim \frac{\text{Focusing effects}}{\text{Dispersive effects}} \sim f\left(\frac{\text{Fabric packing density}}{\text{Wettability}}\right) \]

Packing density and wettability are measured in terms of the coverage area and drop size, both in units of image pixels. The dimensionless ratio of coverage and drop size was therefore termed the tuning factor, which is our equivalent of the Peclét number:

\[ Pe_{\text{fabric electrophoresis}}(\text{tuning factor}) = \frac{\text{Fabric coverage area}}{\text{Drop size}} \]

The fabric-based Pe may be tailored to the physicochemical properties and hydrodynamic size of the analyte to obtain the ideal tuning parameters. Please see Appendix A on tailoring the properties of the fabric medium to analytes ranging in size from small molecules (< 1 kDa), to proteins (50-150 kDa), to larger nanoparticle conjugates. As mentioned before,
protein analytes require passivated, hydrophilic surfaces and therefore, a relatively hydrophilic fabric with high coverage area was selected for protein electrophoresis.

5.2 Electrophoresis-mediated separations, analysis and assay of proteins

Demonstrations of the practical utility of the device in the separation of binary mixtures of proteins and their characterization and quantification have been described below.

5.2.1 Protein separations in fabric

Two major physiological protein analytes, human IgG and human albumin, were electrophoresed in fabric as proof of principle. Devices for this purpose were woven using the protocol described in the methods section, from polyester that was surface treated by the manufacturers to be more hydrophilic, and thereby discourage non-specific interactions with protein analytes. The maximum possible coverage area was used for weaving, in order to discourage dispersion. The buffer reservoirs were composed of polyester x cotton (warp x weft, 3 cm), followed by reusable Pt wire electrodes (99.99%, 0.5 mm diameter) and the separation channel consisted of polyester x polyester (warp x weft, 10 cm). A sample manifold consisting of polyester x cotton (0.2 cm) was included to control the shape of the injection plug. The manifold holds the sample and restricts its diffusion prior to the start of the run. The ‘smile effect’, which is the curving of the migration front of the analyte band, is also minimal in devices with the manifold. A sheet of 10-12 devices is woven in a single batch and cut into individual strips. Proteins were electrophoresed in their native or non-denatured state using Glycine-NaOH buffer, pH 8.58. Proteins consist of amino acids, which are essentially zwitterionic in solution with a positively charged amine group (NH3+) balanced by a negatively charged carboxylate group (COO−). The pH at which both groups are ionized equally and the molecule bears a net neutral charge is known as the
isolectric pH (or pI). Since different proteins differ in their amino acid composition, they have different characteristic pI. The net charge on the protein is therefore influenced by the pH of its environment, where pH < pI causes the protein to bear a net positive charge, and pH > pI causes the protein to bear a net negative charge. The pH of the glycine-NaOH is slightly higher than the isoelectric point of both proteins and ensures that the protein molecules bear a net negative charge. Separations can therefore occur in order of charge to size ratio. However, the difference in pI between the two proteins may also be exploited to elicit separations. First, napthol blue black BA was focused into sharp bands, and a spatial intensity distribution of was generated in order to demonstrate focusing (Fig. 32).

Figure 32: Protein focusing in fabric and associated pixel intensity distributions. A sample of naphthol-blue-black bovine albumin (blue BA) is electrophoresed in Glycine-NaOH, pH 8.5. The blue BA is negatively charged and migrates from the cathode to the anode as the band continues to focus. The band is imaged and spatial intensity distributions are plotted at the start versus the end of the run. Lower pixel intensities are obtained in darker regions. A sharp dip in intensity is therefore obtained corresponding to the position of the focused band.
A 1:1 mixture of hAlbumin and hIgG is then electrophoresed in fabric using 1x Glycine-NaOH at 35 V/cm. This mixture needed to be stained after the run, as per the Coomassie staining procedure described in the methods section. Two bands were detected after staining as opposed to a single band in the blue BA runs, indicating that separations did in fact occur. In order to verify that the two bands were not an artifact of staining, the separations were performed using a set of Glycine-NaOH buffers at different ionic strengths. As is expected, separations were obtained at lower potentials in higher ionic strength buffers (Fig. 33). Further, these results also confirm separation by electrophoresis.

Figure 33: Electrophoretic separations of hAlbumin (MW ~67 kDa) and hIgG (MW ~150 kDa) at 35 V/cm using Glycine-NaOH at a series of ionic strengths. The bands were visualized by staining them using the Coomassie procedure where all proteins are stained blue. As is expected, separations were observed at the higher ionic strengths, with the bands becoming sharper and clearer with increasing ionic strength. The appearance of two distinct bands can therefore be attributed to electrophoretic separations.
5.2.2 Estimating the characteristic mobility of an analyte

Electrophoretic mobility is a characteristic constant for an analyte molecule at a certain buffer pH, and is determined from the physicochemical properties of the analyte as:

\[ \mu_{ep} = \frac{q}{6\pi \eta r} \]

Where \( q \) is the charge on the molecule that drives its movement towards the oppositely charged terminal, \( \eta \) is electrolyte viscosity and \( r \) is hydrodynamic radius. The values of mobility and migration distance can help to identify unknown bands in an electrophoretogram. Blue BA may be used as a visual tracer in an electrophoresis experiment, and obtaining its mobility is important to designing and verifying an experiment. Mobility may be estimated from the velocity of migration using the following expression:

\[ \mu_{ep} = \frac{v}{E} = \frac{(l_d)L}{(t)V} \]

where \( \mu_{el} \) is the apparent electrophoretic mobility (m\(^2\)V\(^{-1}\)s\(^{-1}\)), \( v \) is band migration velocity in m/s\(^{-1}\), and \( E \) is applied electric field strength in V.m\(^{-1}\). \( V \) and \( L \) are the applied voltage (V) and separation channel length (m) respectively, \( l_d \) is the distance migrated by the bovine albumin band (m) and \( t \) is the run time (s). The average apparent mobility for the blue BA molecule was obtained at a series of voltages and the results were plotted (Fig. 34). The experimental mobility values averaged at \( \sim -2.5 \times 10^{-8} \) m\(^2\)V\(^{-1}\)s\(^{-1}\). A negative sign was attributed to the calculated value since BSA is negatively charged in an alkaline environment.
Figure 34: Apparent mobility of the leading edge of a blue BA band electrophoresed in nylon fabric, at field strengths of 10, 25, 35, and 50 V/cm in 1x Glycine-NaOH, pH 8.58, n =5. The mobility is a characteristic constant of the protein and remains the same across all applied field strengths, conforming to the theory that $\mu = v/E$.

5.2.3 Electrophoresis-mediated immunoassay of protein analytes

In a separate application, an electrophoresis mediated immunoassay was performed in the fabric device. The immunoassay, also known as a mobility shift assay, makes use of the decrease in electrophoretic mobility of an antibody when an antigen is captured and complexed with the antibody. This is due to the fact that mobility is a function of the size of the analyte. As discussed earlier, this form of immunoassay does not require a wash step, as the un-bound reactants simply separate into a different band. We demonstrate a mobility shift assay of mouse IgG using gold nanoparticle conjugated-anti mouse antibodies as detection reagents. The results are provided below in Fig. 35. A trend in relative migration velocity with mIgG concentration was observed.

An attempt was made to duplicate this experiment in a vertical polyacrylamide gel electrophoresis setup. These gels are typically used for protein electrophoresis, and their molecular sieving function is accomplished via the use of a chemical cross linking agent, in order to create fine sieving gels. Since GNPs are much larger, course gels were made
using very small amounts of cross linker. However, the course gels were found to be soft, mechanically unstable and not very well suited to vertical GE. Fabric is ideally suited to the electrophoresis of GNP-based analytes due to the larger average pore diameters in fabric. As compared to the conventional gel-based system, the major advantages of this system are the lower cost (the GE setup cost ~ $100 while the fabric setup minus the power supply cost << $0.5- see bill of materials cost analysis in the conclusions), the use of small volumes of sample and buffer (1-2 mL of buffer in the fabric, and a 2 micrograms of sample) and lower power consumption.

Figure 35: An electrophoretic mobility shift assay was performed in fabric. GNP labeled anti-mouse antibodies were incubated with mouse IgG, after which they were electrophoresed at 35 V/cm in non-denaturing 1x Glycine-NaOH, pH 9.6, with 0.1% Tween-20. The image on the top shows the decrease in migration distance with increasing amounts of antigen. The relative migration distances with respect to the migration distance of the negative control (detection reagent only) were plotted to generate a calibration curve, n=3.
The other potential use of the fabric system is single step Western Blotting, in which the lossy and labor intensive transfer step from a gel to a membrane is eliminated. The separated band pattern may be probed directly in fabric using labeled antibodies. However, the fabric system is as yet limited in its ability to properly resolve a complex mixture of macromolecular analytes. The pore sizes required of molecular sieving are not realizable in commercial textiles. In order to remedy this issue, we developed an alternative tuning parameter where dispersion may be countered by increasing fabric packing density and reducing wettability. Although this method is effective for simple mixtures of analytes, complex mixtures consisting of three or more analytes will require pore-mediated sieving, and porous conductive fibers or other small pore size yarn alternatives must be identified. The portability of the system is an important consideration when developing devices for field use, or for use in peripheral diagnostic laboratories. The development of batteries in fabric was briefly explored for this purpose and has been detailed in the Appendices. Such battery-operated power supplies can be easily incorporated in the housing for the device for field use.

5.3 Electroactive polymer-mediated flow control in fabric lateral flow devices

Flow control is a key aspect in all microfluidic platforms, and there has been an increasing interest in developing newer and more precise ways to actuate and control liquid transport in LFT devices. Capillary wicking is an inbuilt and self-sustained mechanism of pressure-driven actuation in porous media. However, capillary wicking is susceptible to variation due to microstructural defects in the material, non-uniform edges, and manufacturing artifacts. Further, hygroscopic materials such as cellulose and silk alter their

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12 Portions of this work have been submitted for publication: T.Narahari, D. Dendukuri and S.K Murthy, Development of electrically actuated valves for woven fabric lateral flow tests, Lab On a Chip 2016.
wetting behavior due to variations in ambient humidity. These issues ultimately result in poor sensitivity and reproducibility in these platforms. Active techniques involving the control of flow using optic, acoustic, magnetic and electro-thermal stimuli have been proposed in the literature, and could potentially overcome these issues. A critical review of these techniques has been provided in Section 2. In particular, the use of electrically-activated pumping (electroosmotic flow) and electrokinetic sample separations and analysis, are well characterized phenomena, and electrical components are integrated into microfluidic devices with relative ease.

**Stimulus-responsive surface coatings are gaining in popularity as surface coatings mimic the porosity and high surface area of wicking media.** Previous electrically activated techniques have involved the use of hygroscopic, thermally responsive polymers such as pNIPAM, which change their shape in response to resistive heating, and can act as mechanical actuators, as well as self-assembled monolayers of amphiphilic molecules that change their conformation in response to electric fields, and can switch reversibly between wetting and non-wetting states. However, the former was found to be susceptible to variations in atmospheric humidity. The latter, though highly effective, is suited mainly to conductive substrates such as gold surfaces. In contrast, conductive polymers such as polypyrrole and PANI are reversibly wettable, and can be chemically synthesized on a variety of non-conductive, flexible substrates including textile fabric. Electroactive conductive polymers do not require changes to pH, temperature or solubility for activation, and preserve the environment in which the medical sample is analyzed. In this section, we develop a reversibly wettable, polypyrrole valve which is integrated into the electrically enabled fabric device developed in the previous section.
The idea is to have a segment of polymer within a fabric strip with electrodes. The polymer segment is in close electrical contact with an electrode, and in the presence of buffer, an electrical potential may be applied to tune the wettability of the polymer. Chemical valves made in this manner may be used to stop and restart sample flow, as well as to slow down and speed up sample flow, at predetermined time points, thereby allowing us to automate and enhance the sensitivity of a lateral flow clinical assay for proteins.

5.3.1 Synthesis of flexible, conductive surface coatings of polypyrrole

Polypyrrole is a biocompatible, semi-conductive polymer with tunable porosity, formed by the oxidation of pyrrole monomer into linear chains. The oxidative polymerization of polypyrrole may be carried out either electrochemically, where it is deposited onto a conductive surface, or by direct oxidation using a chemical catalyst. Some major considerations during synthesis of polypyrrole on fabric were: 1) the generation of highly conductive polymer deposits that are easily integrated into an electrochemical circuit, such as an electrically enabled fabric device developed previously, as well as 2) the scalability of synthesis and 3) the safety of the switching mechanism and its suitability for use in a disposable point of care sensor. In addition, the polymer must tether to the fabric and not flake or peel away.

The polymer was synthesized in-situ in fabric wherein the polymerization reactions take place through the thickness of the material and within the pores. Polymer surface coatings synthesized in this manner were robust to mechanical manipulation, and mimic the flexibility and porosity of the fabric. Yarns coated with polypyrrole could therefore be wrapped on spools and used in the weaving process without risking damage to the coating. In order to generate a conductive coat by the chemical
catalytic method, polypyrrole was synthesized from a selection of catalysts reported previously, which included ammonium persulfate, combinations of ammonium persulfate and metallic dopants, and iron (iii) chloride. The conductivity of a square inch of fabric was measured and is plotted in Fig. 36. We found that iron (iii) chloride yielded the most conductive coat, indicating that the polymer matrix becomes doped with ferrous iron during synthesis. Further, since iron (iii) chloride is an oxidative catalyst, the amount of iron (iii) chloride used for synthesis influences the extent of oxidation and the surface properties of the polymer.

Figure 36: The dependence of polymer coat conductivity on the catalyst used for synthesis (ammonium persulfate or ferric chloride), as well as doping with conductive materials (graphite, zinc, and sodium chloride). Polymer synthesized using iron (iii) chloride is highly conductive as the ferrous ions are incorporated into the polymer matrix during synthesis. Conductivity was measured on a square inch of fabric using a multi-meter.
5.3.2 Reversible wettability switching in polypyrrole

Polypyrrole is composed of a positively charged backbone, surrounded by negatively charged counterions\(^{29,145}\). The counterions may be doped into the polymer matrix during synthesis. In its doped state, the polymer typically continues to carry a delocalized positive charge, and the resulting polymer is hydrophobic. Electrochemical reduction of the polymer results in the neutralization of the charge and the dopant ions are freed from the matrix. In its neutralized state, the polymer is hydrophilic. The dopant may be driven into or out of the polymer via the application of oxidizing or reducing potentials respectively, and the chemical scheme depicting the reversible doping and dedoping of the matrix has been depicted below\(^{29,145}\).

![Chemical scheme for reversible oxidation and reduction of polypyrrole](image)

- **Figure 37**: Scheme for the reversible oxidation and reduction of polypyrrole adapted from Xu et. al.(26). The polymer carries a delocalized positive charge in its oxidized state, and negatively charged ions (anions) exist as counterions in association with the polymer matrix. Upon reduction, the polymer is neutralized, and the counterions are released into solution. This counterion flux facilitates the reversible oxidation and reduction of polypyrrole.

Polypyrrole coats synthesized using equimolar quantities of iron (iii) chloride catalyst and pyrrole monomer were subjected to electrochemical treatments. The coated fabrics were found to transition from non-wetting to wetting states in a reversible manner (Fig. 37b): As-synthesized polymer coats are non-wetting at the outset, indicating an oxidized state. These fabrics transitioned to a wetting state upon cathodic treatment, and reverted to a non-wetting state upon subsequent anodic treatment. In order to investigate the underlying switching mechanism in greater depth, the amounts of iron (iii) chloride used
for synthesis were varied. Three grades of polypyrrole synthesized using three concentrations of iron chloride (0.1 M, 0.01M and 0.001 M) and 0.1 M pyrrole monomer, were studied. The wettability of these fabrics have been plotted in terms of the observed water contact angle (Fig. 37c).

The following may be inferred from the results: First, all as-synthesized polymer surfaces are in the low-wetting state initially (90° ≤ θ ≤ 150°), indicating an oxidized state. However, the contact angle was found to increase with increasing iron (iii) chloride. One reason for the correlation between iron chloride concentration and the wetting properties of the polymer is that the addition of iron chloride results in the formation of textured or rough polymer deposits. The larger surface area resulting from the surface roughness of the polymer deposit leads to the amplification of the surface properties of the deposit, often resulting in superhydrophobic or superhydrophilic behavior. This phenomenon is termed the Lotus Effect\(^{26}\), named for the fact that the leaves of aquatic plants such as the lotus, have microtextured surfaces that allow them to reject water and remain clean and dry in an aqueous environment.

Second, when subjected to a cathodic potential (-5 to -20 V/cm), all coated fabrics were observed to switch from a low-wetting state to a highly wettable state (0° ≤ θ ≤ 90°). However, the wettability was reversible only in fabrics with higher amounts of iron chloride. The aforementioned observations indicate that iron chloride has a key role to play in the switching process. An elemental analysis of the composition of the fabrics was performed at each switching step using Electron Dispersive X-Ray Spectroscopy (EDS). The results from EDS analysis have been plotted in Fig. 38 (the original spectra are provided in the appendix, Fig. A7). A notable decrease in chloride species content from ~
3 wt% in the as-synthesized polymer to ~1.5 wt% in the cathode treated polymer was observed, followed by an increase in chloride species content to ~4 wt % upon subsequent anodic treatment. The large increase in chloride species content after anodic treatment may be attributed to the saline-containing electrolyte buffer, 1x PBS.

Figure 38: Wettability switching in polypyrrole. Polypyrrole coated fabrics are cycled from non-wetting to wetting and back to non-wetting states by applying an electrical potential to the polymer. (a) Reversible changes in the wettability of the polymer matrix. The polymer switches from hydrophobic state (higher contact angle) in the as-synthesized state to a hydrophilic state when subjected to a cathodic potential, and reverts to a non-wetting state when subjected to an anodic potential. (b) The wettability of the polymer has been plotted in terms of its contact angle. The plot shows that . The initial contact angle (0) decreases with decreasing iron (iii) chloride concentration, suggesting that the extent of oxidation is directly influenced by iron (iii) chloride. Further, polymer coats synthesized with 0.1 M iron chloride undergo near-complete reversal in contact angle, while those synthesized with < 0.1 M iron chloride seem to remain in the wetting state, suggesting that the presence of excess iron chloride in matrix helps with reversible switching.

From these results, it is postulated that chloride ions are incorporated into the polymer matrix during synthesis with iron (iii) chloride, and exist as loosely bound
counterions attracted to the positively charged polymer backbone. Upon reduction at the cathode, which typically involves the addition of electrons to the polymer, the charge on the polymer is neutralized, and the chloride species leave the polymer to exist as ions in free solution. A similar mechanism has been described in the work by Xu et. al.\textsuperscript{26}, except that a low surface energy counterion dopant- Perfluorooctane sulfonate (PFOS), was deliberately incorporated into the polymer during synthesis, and wettability changes were mediated by reversible oxidation and reduction accompanied by a PFOS- ion flux. Our mechanism however makes use of a benign, non-toxic dopant (iron chloride) which can be safely incorporated into a disposable sensor.

The chloride counterion theory is also supported by the fact that lower strength polymers synthesized using smaller quantities of iron chloride have a smaller chloride counterion reserve, and are less wettable. These fabrics are therefore unable to revert to the oxidized state upon anode treatment, due to the heavy depletion of chloride counterions from the matrix. This poses a minor limitation on the lifespan of the reversibly wettable polymer, and the number of switching treatments that can be imparted before the reversible property is lost. Evidence of this limitation was obtained from the fact that even the strongest grade of polymer (containing 0.1 M iron chloride) is unable to fully revert to its initial superhydrophobic state at the end of one switching cycle. However, this limitation is offset by the fact that multiple switching cycles may not be necessary within a single-use disposable device.

Another important factor influencing the switching process is the composition of the electrolyte buffer. The electrolyte buffer must contain a sufficiently large chloride species reserve to guarantee re-oxidation upon anodic treatment. The characterization
experiments were performed using 1x Phosphate Buffered Saline as the electrolyte. When the PBS is replaced with a synthetic urine sample, the same reversibility is observed (Fig. 39). Similar behavior is expected with any biological sample, serving as evidence that the sample can serve as the electrolyte, and that a dedicated electrolyte reservoir or gel is in fact not required. **The chloride-mediated switching chemistry is therefore sustainable and simple enough to incorporate into a low cost point of care sensor.**

Additional tuning parameters such as the switching potential and treatment duration were also studied. Relatively low applied potentials ranging from 5-20 V/cm, and short treatment durations ranging from 30-120 s were required in order to tune the percent change in wettability. The use of these operating parameters to tune the switching process has been illustrated in the appendices (Figs. A6, A7).

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**Figure 39:** Investigation of the doping and dedoping mechanism of polypyrrole. (a) SEM-EDS analyses show a change in chloride species content at each step of the switching process. Two fabric samples (samples 1 and 2), 4 mm x 4mm in size, were tested. The error bars were generated from measurements obtained from different spots on the fabric sample. The results show that electronegative chloride species are driven out of the polymer during cathode treatment and reenter the polymer during anode treatment. (b) Based on the observations above, a proposed mechanism for the reversible reduction and oxidation of the polymer matrix and the resulting doping and de-doping of the matrix with anionic chloride species has been provided, where it is postulated that chloride species exist as counterions on the positively charged polymer backbone.
Figure 40: Reversible wettability switching using synthetic urine as the electrolyte in place of 1x PBS. The saline (NaCl) reserve in biological samples can elicit reversible wetting behavior in the polymer valve.

5.3.3 Implementing the polypyrrole valve in a lateral flow device

The next objective was to implement polypyrrole valves in fabric lateral flow devices. Weft yarns were coated with polypyrrole and incorporated into lateral flow devices (Fig. 39a). However, liquid flow takes place along both warp and weft yarns. In order to prevent seepage along the warp, the device was manufactured entirely using hydrophilic weft yarns and a hydrophobic raw silk warp (Fig. 40a). Flow takes place along the weft yarns, while the warp serves only as the structural framework for the fabric. An electrode is woven in close contact with the polymer barrier, such that the polymer and the electrode form a unified conductive terminal connected to the power supply leads. Fig. 40b illustrates the operation of the valve in a test device, alongside a control device in which no potential is applied. The barrier is expected to wet when a potential is applied at the electrode, and reduction occurs gradually at the solid-liquid interface, as the liquid front moves through the barrier. In the demonstration provided, the liquid enters the polymer at approximately \( \sim 60 \) s upon applying a potential of \(-20\) V/cm. In contrast, no flow takes place when the potential is not applied. In theory, the control strip may remain impervious to flow for extended periods of time (\(> 10\) minutes). The time at which a potential is applied, and the magnitude and duration of the applied potential will determine the time of activation, which may be decided by the user, or programmed into a potentiostat. The
barriers in Fig. 39 are made from 0.1 M iron chloride, and operate by going from fully closed, to fully open. A 2 mm wide polymer segment was chosen for the purposes of this demonstration. However, the segment is extensible to a ½ inch length as per the characterization experiments that were done previously using the manually assembled devices. Longer segments will however require longer treatment times and higher applied field strengths within the limits of the system, which, according to our previous work on electrophoresis, can be as high 50 V/cm for certain materials. Lengths beyond ½ inch were not characterized in this work as they are not typically relevant to microfluidic systems.
Figure 41: Demonstration of polymer-mediated on/off flow control in a fabric lateral flow device. (a) The fabric device with the mid-channel polymer barrier in close contact with an electrode. (b) Snapshots of polymer-mediated valving in fabric. In snapshot (1) liquid flow from one end of the strip to the other is stopped by a non-wetting polypyrrole barrier. A cathodic potential (~20 V/cm) is then applied to the test strip on the left, and the barrier begins to wet (snapshot 2), while the control strip remains non-wetting. The liquid front crosses the barrier on the left after ~90 s of continuous reduction (snapshot 3), while the control barrier remains non-wetting, and may remain non-wetting for an indefinite period of time.

5.3.4 Modeling the effects of catalyst concentration and valve geometry on flow

In this section, we observe that the wetting behavior of a fabric LFT conforms to the Washburn equation for capillary flow, and mathematical models based on the Washburn
equation were implemented in order to illustrate the effects of key tuning variables such as the iron chloride concentration, length and position of the polymer segment. The models allow us to explore a large parameter space, and design a system that is appropriate to the application being developed.

The macroscale wetting behavior of fabric is accurately described by the Washburn equation for capillary flow, which dictates that the velocity of the fluid front decreases with wet length:

\[ L^2 = \frac{\gamma D t}{4 \eta} (\cos \theta) \]

Where \( L \) is the distance moved by the fluid front (m), \( t \) is the time elapsed (s), \( D \) is the average effective pore diameter of the fabric/polymer coat (m), \( \theta \) is the liquid contact angle (°), and \( \eta \) and \( \gamma \) are liquid viscosity (Pa.s) and surface tension (N/m) respectively. The Washburn equation is differentiated with respect to time to yield an expression for velocity:

\[ \frac{dL}{dt} = \frac{\gamma D}{8 \eta L} (\cos \theta) = v \]

The wettability of the material is a major factor influencing the velocity of flow. Iron chloride concentration was used to tune the wettability of the valve and hence the velocity (Fig. 41a).

Two modes of flow control were therefore developed. Valves made from larger amounts of iron chloride are initially very low-wetting, and are impervious to flow until a switching potential is applied. These valves therefore operate in an on/off mode, where flow can be stopped and restarted (Fig. 39). On/off valves are useful when long incubations are desired. Fig. 40b shows a plot of velocity against time. The plot illustrates that the sample may reside in the strip for the desired time before flow is activated, and the time of activation is determined by the user.
The on/off valve is also reversible, in theory. As the reader may recall, we demonstrated reversible wettability changes in higher strength polymers, using the manually assembled switching setup, where we had the opportunity to rinse and dry the treated fabric before proceeding to the reversing step. However, in a lateral flow device that has already been wetted, reversing the valve is not guaranteed to stop fluid flow. Instead, reversing the valve may help to reduce flooding and backflow, or to meter fixed volumes of liquid into the strip. In rare cases where strips must be reused, the valve may be renewed by reversing it to a non-wetting state using a wash buffer such as 1x PBS.

On the other hand, valves made from lower amounts of iron chloride (≤ 0.01 M) are partially wettable, and will cause the velocity to drop by an amount that is proportional to the amount of iron chloride used (Fig. 40a). Partially wetting valves do not require the application of a switching potential. Rather, these valves become more wettable as they come in contact with the liquid.
Figure 42: Velocity profiles illustrating the various modes of flow control developed by tuning the iron chloride concentration in the polypyrrole (ppy) valve. (a) Plots of velocity against wicked length. The velocity profile of a plain, uncoated strip is in agreement with the Washburn Law, and decreases smoothly with wet length. In contrast, when a polymer barrier is incorporated in the strip, the profile deviates from the Washburn form, and the velocity dips in proportion to the amount of iron chloride in the polymer segment. The position of the valve has been indicated here by the shaded box. On/off valves to stop and restart liquid flow were developed from 0.1 M iron chloride, while partially on valves to slow the flow of liquid were developed using smaller amounts of iron chloride. We note that the velocity increases once the liquid front has crossed the valve. This is because flow is driven by the interfacial pressure gradient, which increases once the liquid encounters the wetting region of the fabric. (b) Plots of velocity against time for two strips of identical length and width, one with a mid-channel on/off valve, and one with no valve. The plot illustrates prolonged residence of a liquid upstream of the valve (25 s in this case), before flow is activated, as opposed to the non-valved strip.
Capillary wicking is driven by the capillary pressure gradient ($\Delta p_c$), which is a function of liquid contact angle (wettability) and the average pore diameter of the material:

$$\Delta p_c = \frac{4\gamma \cos \theta}{D}$$

From the Washburn equations, velocity ($v_x$) may be related to pressure gradient as:

$$\Delta p_c = \frac{32\eta v}{D^2}$$

This equation is of the same form as the Hagen-Poiseuille equation for pipe flow, where the volumetric flow rate, $Q$ (m$^3$/s) is proportional to an effective pressure gradient $\Delta P/L$ (Pa/m) and is related to the pressure gradient as:

$$Q = \frac{\Delta P}{32\eta L} = \frac{\Delta P}{A.D^2}$$

Therefore,

$$\frac{Q}{A} = \frac{\Delta P}{32\eta L} = \frac{\Delta P}{R} = v$$

Where $\Delta P = \Delta p_c + p_{\text{atmospheric}}$, $A$ is channel cross sectional area (m$^2$), velocity is $Q/A$ (m/s) and $R$ is a lumped term used to denote the hydraulic resistance to flow (Pa/s.m). This equation states that channels with smaller pore diameters, and lower wettability ($\cos \theta < 1$) offer a greater resistance to flow. Hydraulic resistance is a key parameter that can be used to model the effects of valve length and position. In order to estimate hydraulic resistance, we must calculate the pore sizes in the coated and uncoated regions.
Pore sizes were estimated from the Washburn velocity profile, where the plot of velocity versus 1/L is linear, and has a slope of:

$$\gamma D \cos \theta \over 8 \eta$$

The average effective pore diameter in 0.001 M polymer-coated fabric, as well as in plain, uncoated fabric was estimated as follows (Fig. 42):

**Figure 43:** Calculating the pore size of plain uncoated fabric versus partially wettable ppy-coated fabric, made using 0.001 M iron chloride. (a) Plots of velocity against (1/L) can typically be fitted to a straight line as they obey the Washburn Law. The slope of the line may be used to calculate the pore diameter of the strip. Overall, the coated strip has lower velocity, for the same flow rate. Note that the entire length of the strip was coated, and since 0.001 M ppy is partially wettable, no electrical activation was required. (b) The effective pore diameter calculated from (a) has been plotted. Uncoated strips have pore diameters of 2.98 µm, while 0.001 ppy coated strips have smaller pore diameters of 0.89 µm. The coated strips are not only less wettable, but also have smaller effective pore diameters.

The results indicate that in addition to being less wettable, coated fabrics have smaller pore diameters (2.98 µm in uncoated fabric, versus 0.89 µm in 0.001 M ppy-coated fabrics). This decrease in pore size is expected, and is due to the fact that the coated regions comprise a dense polymer matrix. The density of the polymer matrix is expected to increase further
in the high concentration polymers. Pore size data can be used to model the hydraulic resistance.

In keeping with the Washburn Law, the overall resistance to flow decreases with increasing strip length. The hydraulic resistance in the polymer segment is therefore a cumulative effect of wettability and distance from the inlet end. **Mathematical models were used in order to illustrate the effect of the length and position of the polymer segment.** The equation for Washburn velocity is differentiated with respect to wicked length \( L \), and integrated using the boundary conditions: \( v(L_0) = v_0 \), and \( v(L_x) = v_x \), to give:

\[
v_x = \frac{\gamma D \cos \theta}{8\eta} \left[ \frac{1}{L_x} - \frac{1}{L_0} \right] + v_0
\]

; \( (L_x, L_0 > 0) \)

Where \( L_0 \) and \( L_x \) are the starting and ending positions of the liquid plug. The data points obtained in the experimental velocity profiles above were used as boundary conditions to obtain more extensive wicking data for valves of identical wettability (0.001 M) located at different positions in a strip. Hydraulic resistance was estimated from the velocity and pore size data to generate resistance profiles for each of these valves (Fig. 43). We observe an increase in hydraulic resistance through the coated polymer segments. Further since \( v \sim 1/L \), as per the Washburn Law, **polymer segments placed farther away from the inlet end offer a greater resistance flow.** Therefore, valve wettability and position may both be utilized as parameters to tune flow rates in lateral flow strips.
Figure 44: Plots of hydraulic resistance versus wicked length. Hydraulic resistance was calculated from Washburn velocity data and pore size estimates. (a) A uniform, polymer coated strip has a higher overall resistance as compared to flow as compared on a uniform uncoated strip. In both cases, the resistance increases naturally with wicked length. This is in agreement with the Washburn equation. (b)-(d) A 1 cm long coated segment, is introduced at different distances from the inlet end. An increase in resistance is observed within all coated segments. However, segments positioned farther away from the inlet offer a larger resistance to flow. The flow rate can therefore be tuned using both the wettability of the segment, as well as its length and position.

However, the wicking front resumes its original velocity once the barrier has been crossed. This is due to the fact that capillary flow is driven by the interfacial tension between the fabric and the liquid. This post-barrier surge is undesirable, since the sample moves as a plug, and its velocity cannot be maintained once the barrier has been crossed. This issue may be remedied by using long valves, valves in series, or valves with
wettability gradients. However, a short valve with a mid-channel barrier was found to be ideally suited for the clinical assay that is demonstrated in the next section.

5.3.5 A clinical assay for protein estimation

Clinical assays are of wide relevance in infectious and non-infectious disease diagnostics. Protein assays, in particular, are applied in the assessment of liver and pancreatic function. Multiple step clinical assays with prolonged incubations can be difficult to execute in strip format, unless a valving system is used. The Lowry protein assay has been demonstrated as a simple example of the practical utility of the valve, and benchmarked against a control system without valves, as well the standard test tube-based procedure. The schematic for this procedure in our device has been provided in Fig. 44.

In the Lowry procedure, the sample is first mixed and incubated with an alkaline copper reagent (the Lowry reagent, denoted as LR), for 10 minutes. A chromogen (Folin-Ciocalteu reagent, denoted as FC) is then added to the mix and incubated for a further 45 minutes, leading to the formation of a visible blue complex whose intensity varies in proportion to the amount of protein present in the sample. Each of these incubations is distinct, and requires a fixed period of time to complete. A strip with a mid-channel on/off barrier was used for this demonstration. The schematic for the strip-based procedure is provided in Fig. 43.

The devices are prepared by pre-spotting the LR upstream of the valve, while the FC is pre-spotted downstream of the valve. BSA samples in concentrations of 0, 1, 250 and 1500 µg/mL were tested. The sample is deposited at the upstream end and is held with the LR for an 8 minute period, after which a reducing potential of -20 V/cm is applied for 2 min in order to open the valve. The copper protein complexes flow downstream where they
are allowed to contact the FC reagent, and the resulting blue compound is allowed to
develop for a full 45 minutes. Back flow is prevented by continued wicking into the dry
space downstream. Backflow may also be prevented by reversing the applied potential and
closing the valve. **The valve therefore helps to segregate the two steps of the assay and
prevent premature contact between the two reagents.**

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**Figure 45:** Schematic for the Lowry protein assay using a valved device. LR is dried
onto the strip, upstream of the valve, while FC is dried onto the strip downstream of
the valve. The sample is deposited upstream, releasing the LR into solution (Step 1).
The reaction is allowed to proceed for 8 minutes (Step 2), after which a two minute
switching potential is applied in order to open the valve and release the mixture
downstream, where it contacts the FC reagent (Step 3). A wash buffer may be added
to ensure that the entire volume of sample is washed downstream. Backflow is
prevented either by ensuring continued wicking into a dry space downstream, or by
reversing the valve. The intensity of the blue complex is read either visually or
digitally using an image analysis algorithm (Step 4).
The significance of this system was demonstrated by replicating the protein assay in **strips without valves**. The results have been plotted in Figs. 45-46, and benchmarked against the standard test-tube based procedure. In **valved strips**, color development was proportional to the amount of protein in the sample. A quadratic correlation between protein concentration and color intensity was observed, and the limit of detection was found to be 1 µg/mL protein (Fig. 45a). The limit of detection is comparable with that of the standard test-tube based procedure.

![Figure 46: Results from the Lowry protein assay in strips with on/off control. (a) The inset image shows the end point of the Lowry assay for BSA, in devices with valves. The end user can make a semi-quantitative assessment of the results using a color card for reference. Quantitative assessments were made by imaging the strips under uniform illumination and analyzing the intensity of color over the latter half of the strip using Image J software. Pixel intensities ranging from 0 to 255 are indicative of brighter to fainter blue, and were plotted against protein concentration to yield a calibration curve. (b) The standard test tube procedure was performed for benchmarking purposes. The inset images show the gradient in blue intensity in solution. Image analysis was performed on the solutions and plotted, and a quadratic curve similar to the strip based assay was obtained. Further, an LOD of 1 µg/mL was obtained using the valved system, which is comparable to the LOD obtainable using the test tube based procedure. These results serve to validate the strip based assay procedure.](image-url)
Valve-less systems employing standard, geometry-based flow control were tested for comparison purposes (Fig. 45). Color development was observed to be erratic in these systems.

Figure 47: The strip based Lowry assay was repeated in devices employing passive flow control or ordinary flow control. These devices did however use larger and thicker fabric, as compared to the valve devices, so as to slow liquid flow without complete stoppage. The assay completes in less than 2 minutes, and there is no correlation between the color development and protein concentration. Further, color development is faint, as compared to the valved devices. These comparison studies further illustrate the importance of being able to segregate and time the steps of the assay using valving methods.

The usefulness of the valve in segregating the two reaction steps is made apparent from this comparison. In addition, the straight channel geometry and small size (4 x 40 mm$^2$) of an LFT is retained, and no complex modifications to the shape or size of the device were required. Pre-coating of reagents helps to minimize the operational steps for an end user, and the wettability cycling steps may be programmed into a portable chip-based potentiostat. For a broader range of tests including immunoassays, we envision using reversible control to release metered volumes of liquid into the device. For instance, wash buffers are often required to eliminate excess background in lateral flow immunoassays,
sample lysis buffers may be used to lyse and release intracellular analytes, and additional volumes of sample may need to be added to augment a faint signal. Additional volumes of fluid may be gradually released into the reacting zone by cycling the valve.

**5.3.6 Electrically activated flow control in lateral flow devices: Conclusions**

In Section 5.3, we describe the development of a versatile chemical valve for low cost chromatographic sensors. The valve makes use of the electrically tunable wetting properties of conductive polymer, polypyrrole. In this work, polypyrrole was synthesized on textile fabrics and yarns using a scalable, chemical catalytic procedure employing iron (iii) chloride, and subjected to small switching potentials of 5-20 V/cm for short durations ranging from 30-120 s, in order to effect reversible wettability changes. A non-toxic switching chemistry was devised as an improvement over a method reported previously, where perfluorinated dopants were replaced with the intrinsic iron (iii) chloride content in the polymer. In addition, the weaving method can potentially be used to pattern polymer-coated yarns into fabric devices, in a variety of programmable geometries with single fiber resolution. The method developed by us is therefore amenable to be used in a disposable point of care sensor. The tuning parameters influencing electrical actuation, including iron chloride concentration, applied voltage, switching times, and polymer valve length, and position were identified and modeled in detail. Multiple modes of valving including an on/off mode to stop and restart flow, as well as a partially on mode to slow the flow were demonstrated. This toolkit of tuning variables may be utilized in order to develop valves for a variety of sensing applications. As an example, we have demonstrated a strip-based procedure to automate a clinical chemistry assay for total protein, where the valve allows us to segregate and time the assay steps in order to obtain the best possible sensitivity.
Benchmarking studies were made with strips employing traditional, valve-less flow control, and show a clear benefit to using the valving system.

5.4 Development of a polymer-coated, fabric-based working electrode for electrochemical impedance spectroscopy\textsuperscript{13*}

Electrochemical detection offers a rapid and sensitive means to quantifying an analyte. The impedance of an electrochemical circuit is a function of the resistance of the solution and the resistive and capacitive effects due to charge transfer and double layer formation at the surface of the electrode. It is measured by supplying a small AC perturbation between the working and reference electrodes. Impedance is therefore a useful tool to measure antigen-antibody binding at the surface of a functionalized electrode using only a potentiostat, with no additional optical imaging equipment. In this section, we make use of the semiconducting properties of polypyrrole to devise an antibody-functionalized working electrode for analyte capture and electrochemical impedance measurement. In order to differentiate between specific and non-specific antigen capture, we utilize a control electrode functionalized with the non-specific antibody, and compare the differences in antigen capture between the two. The system setup consisted of three electrodes: i) a polymer-functionalized fabric working electrode where the reaction of interest takes place, ii) a stable, zero potential silver-silver chloride reference electrode, against which all changes at the working electrode are measured, and iii) a platinum wire counter electrode which absorbs any currents generated and helps to maintain a constant cell potential between the working and the reference. In the optimization studies, we use impedance data

\textsuperscript{13*}Unpublished work, done in collaboration with Hunter Sismaet, Avinash Kola, Dr. Edgar Goluch and Dr. Elizabeth Podlaha-Murphy
to determine if the antibody adsorption coating process has indeed been effective, and proceed to optimize the composition of the surface coat based on the impedance data. An impedance based immunoassay for cancer marker HER2 was then demonstrated using the optimized system. HER2, or Human Epidermal Growth Factor Receptor-2, is a transmembrane protein that is overproduced in certain types of breast cancer, and is one of the markers used to diagnose HER2 positive cancer. The levels of HER2 also determine the course of treatment targeted towards HER2 + breast cancers, and the HER2 diagnostic test may also be used to monitor the patient during the course of therapy.

5.4.1 Impedance assays in beaker-based setup: target added to the solution phase

The protocol for coating polypyrrole described earlier, in Section 5.3, was retained here. Polypyrrole was synthesized by the chemical catalytic procedure, using 0.1 M pyrrole monomer and 0.1 M iron (iii) chloride catalyst. Our first objective was to develop a physical understanding of the behavior of the polypyrrole-coated fabric working electrode. Initial experiments were performed in a bulk, beaker-based setup using 5 mL of 1x PBS electrolyte. The electrodes were immersed in the electrolyte. A small DC potential (-10 mV) relative to open circuit potential is applied to the WE to ensure that a current is flowing. The system is then scanned at frequencies of 0.1 to 100,000 Hz, and the impedance response of the system is measured. The response of the system to the addition of a charged analyte- pyocyanin\textsuperscript{14*}- to the beaker was also recorded. The beaker concentrations of pyocyanin were 2, 4 and 6 µM.

\textsuperscript{14*} Pyocyanin is a redox-active, bacterial toxin produced by the bacterium \textit{Pseudomonas aeruginosa}. For the initial experiments involving bare polypyrrole electrodes, pyocyanin was chosen arbitrarily as an analyte of interest. Pyocyanin typically carries a charge in solution.
The plot of frequency versus phase shift of the bare electrode, prior to the addition of pyocyanin is provided in Fig. 47a. We observe that the phase angle is negative (≤ 90°) across the spectrum of scanned frequencies, indicating that the system behaves as a capacitor. This behavior can be attributed to the semiconducting nature of polypyrrole and the formation of an electrical double layer on the surface of the electrode. At frequencies higher than 10^4, the phase shift begins to increase towards zero indicating a shift from capacitive to resistive (ohmic) behavior. Further, an upward shift in phase angle is observed with the addition of pyocyanin. The Bode plot of the magnitude of impedance (|Z|) versus frequency (Fig. 47b) indicates that the magnitude of impedance increases with the addition of pyocyanin at frequencies < 10^4. The impedance spectra or Nyquist plots have been provided in Fig. 48a. The low frequency end of the impedance spectrum (on the right side of the plots) typically corresponds to the polarization or charge transfer resistance, where, the real component of impedance Z’ equals the Ohmic charge transfer resistance Rct at the surface of electrode. The increase in charge transfer resistance is indicative of redox processes or interactions at the surface of the electrode, consistent with the fact that polypyrrole is electroactive. Meanwhile, a decrease in impedance with increasing pyocyanin was observed at higher frequencies (10^4-10^5). The high frequency end of the impedance spectrum corresponds to the solution resistance, RΩ, and is located on the left side of the plots. Since pyocyanin is a charged molecule, this result confirms that the addition of pyocyanin to the electrolyte leads to a decrease in electrolyte resistance. The data was fitted to the simplified Randle’s equivalent circuit model, and extrapolated to find the x intercepts at which RΩ (solution resistance) and Rct (charge transfer resistance) may be obtained (Fig. 48b). Extrapolation was deemed necessary due to the fact that the system
is capacitive and the Nyquist plots do not actually touch the x axis. A theoretical correlation between pyocyanin concentration and the two resistances was observed from the extrapolated data (Fig 48b), leading us to believe that the polypyrrole-coated fabric WE holds promise as a functioning working electrode capable of detecting changes in solution resistance and binding at the surface of the electrode.

Figure 48: Bode plots of the impedance response of the bare polypyrrole electrode immersed in a beaker containing 10 mM PBS, before and after the addition of pyocyanin analyte. The final concentrations of pyocyanin that were tested are 2 µM, 4 µM and 6 µM. (a) A negative phase angle throughout the scan indicates a large capacitive effect, mainly due to the semiconducting electrode and the formation of a double layer. Phase shift also increases with increasing analyte concentration. (b) The impedance of the system (|Z|) decreases with the addition of analyte at high frequency side, where the resistive behavior of the system predominates. This is indicative of a decrease in solution resistance with the addition of pyocyanin.
Figure 49: Complex plane impedance and resistance data obtained from the equivalent circuit model for bare polypyrrole working electrodes, before and after the addition of pyocyanin. (a) Nyquist plots have been zoomed in to show the shift in data at the higher frequency end of the impedance spectrum. The shift appears to correlate to the addition of increasing amounts of pyocyanin to the electrolyte. (b) The data in these plots were fit to the simplified Randle’s equivalent circuit model to obtain solution resistance $R_Q$, and electrode polarization (or charge transfer) resistance $R_{ct}$ under ideal conditions. A correlation between pyocyanin concentration and the two resistance components is observed, leading us to believe that the polypyrrole-coated fabric does in fact function as a WE, and is capable of detecting changes in the solution and the electrode surface.
The behavior of antibody-functionalized polypyrrole was studied next. Two types of antibody were used in this study: a test antibody (Anti-HER2) sensitized to capture cancer marker HER2, and a non-specific control antibody, Goat Anti-Mouse (GAM) IgG. An optimization was performed in order to determine if the antibody should be incorporated in the polymer matrix during polymer synthesis, or adsorbed onto the surface after synthesis. The best response was obtained when the antibody was adsorbed onto the fabrics after polymerization, potentially due to the fact that the binding sites are readily accessible near the surface. Antibody was pre-adsorbed to the coated fabrics at a surface concentration of 1 µg/cm². A baseline frequency scan was performed on both electrodes prior to the addition of HER2 analyte to the beaker (final beaker concentration of HER2 analyte = 0.5 ng/mL).

The impedance spectra and phase angle plots are provided in Fig. 49. The antibody functionalized system is more resistive as compared to the bare polypyrrole system tested previously, indicating adsorption of the antibodies to the surface. The phase angle approaches 0 indicating a predominantly resistive response. Additionally, the magnitude of impedance appears to increase after analyte addition to the test system, but remains unchanged from baseline value in the control system (Fig. 49b). These results indicate a unique change in resistance upon binding at the test electrode surface. Further since the change is observed at intermediate frequencies, there is a charge transfer contribution from the surface of the electrode, and can be interpreted as successful binding and analyte capture by the test electrode. The exact number obtained from the RC circuit fit are provided in Fig. 50. The noise at the higher frequency end corresponding to solution resistance may have arisen from the fact that the spacing between the working and
reference electrodes may not have been precisely consistent between measurements. In order to ameliorate this problem, we switched to the chip-based setup described in the methods section. The chip setup ensures consistent and spacing and a small distance of separation between the WE and RE, and is intended to minimize solution resistance and noise. The beaker based system is also diffusion limited, relying on the diffusive transport of the analyte molecules through the bulk of the solution onto the electrode surface. In contrast, the chip setup allows us to use smaller volumes of buffer and sample, and allows us to interrogate under LFT-like flow conditions without any diffusion limitations.
Figure 50: Impedance response of the antibody functionalized system in the beaker. (a) The plot shows that the behavior of the system is now predominantly resistive as opposed to the capacitive behavior observed previously with the bare polypyrrole electrodes. This indicates that the antibody adsorption process was successful. (b) Further evidence of successful adsorption is offered by the fact that a change in impedance magnitude is observed with the addition of HER2 target protein to the test system. This change is observed at the intermediate frequencies indicating contributions from electrode charge transfer, and is interpreted as evidence of analyte capture. The noisy readout at the higher frequency end may be due to minor inconsistencies in solution resistance due to inconsistencies in the spacing between electrodes, which can be remedied by switching to a chip-based system with consistent spacing between electrodes.
**Figure 51:** Charge transfer resistance at the electrode surface obtained before and after HER2 addition to the beaker. A large increase in $R_{ct}$ in the test system and no increase in $R_{ct}$ in the control system serves as evidence of analyte capture at the test electrode. The next step is to correlate the analyte concentration with $R_{ct}$. This work was carried over to a chip-based system due to the noise in solution resistance ($R_Ω$) observed in the beaker system.

At this point, we must also comment on the RC circuit models used to fit the data. Typically, for smooth electrodes, the complex plane impedance plot (Nyquist plot) appears semicircular with its center on the x-axis. However, in many real systems, a depressed semicircle is observed due to the surface roughness of the electrode and heterogeneous reaction kinetics across the electrode. Since all of the Nyquist plots resembled depressed semicircles, the data was fit to a Constant Phase Element- Polarization resistance (CPE-$R_p$), as opposed to the Capacitance- Polarization resistance (C-$R_p$) circuit model. The CPE constitutes a non-ideal capacitance element with an altered phase angle.

### 5.4.2 Impedance immunoassays on chip: target added to solid-phase

The chip-based systems were constructed layer by layer as described in the methods section. We expected changes to the solution resistance, charge transfer resistance and other system parameters owing to miniaturization. Measurements were made by placing the electrodes in a bubble containing 100-200 $\mu$L of buffer. Antibody surface
concentrations were re-optimized. The results provided in Fig. 51 attest to the fact that the antibody was successfully coated to the surfaces, as a trend in the polarization resistance with increasing capture antibody concentrations was noted prior to target addition.

Figure 52: Top- a schematic of the chip-based system where the WE consists of a piece of antibody functionalized polypyrrole fabric. The surface concentration of adsorbed antibody is varied from 2 to 0.5 µg/cm². A trend in charge transfer resistance is obtained with increasing antibody surface concentrations, which is expected since charge transfer occurs at surface of the electrode. When a target is added to the surface, incubated for 30 min, washed, and then the impedance spectra are obtained afresh, a marked increase in Rct is observed in all test systems. However, there is a considerable increase in Rct in the control systems as well. This is indicative of non-specific adsorption on the polypyrrole surface.
After obtaining the baseline reading, the electrolyte bubble is removed, and the antigen (10 µL of 50 ng/mL HER2 protein) is added to the solid phase polypyrrole surface. The surfaces were let to stand for 30 min to allow binding to occur. A gentle wash with PBS buffer is then imparted, in which a drop of PBS is placed on the active area of the WE, and then wicked away using an absorbent tissue, without the tissue contacting the electrode surface. This is to prevent any adsorbed antibodies from being plucked off the surface by the force of the wash. The experiments indicate a definite increase in Rct on all the test electrodes. However, a notable increase in Rct is also observed at the control electrodes, though not to the extent observed on the test surfaces. This is indicative of non-specific adsorption of the HER2 antigen to available spaces in the polymer.

The next set of experiments was performed using electrodes that were treated with a blocking agent (0.1 % BSA) after antibody coating. BSA is roughly half the molecular weight of an antibody, and will occupy the empty sites on the electrode, potentially preventing non-specific adsorption of the target. The results from this experiment have been plotted in Fig. 52. The results indicate an overall increase in charge transfer resistance after target addition to the test system functionalized with Anti-HER2 antibodies, while the changes in Rct at the control system were erratic and did not appear to correspond with the concentration of target. An impedance immunoassay for cancer marker, HER2 was therefore demonstrated using a polypyrrole coated, antibody functionalized electrode.
Figure 53: HER2 antigen assays performed on-chip after BSA blocking. An increase in Rct is observed after target addition to the test system. However, the changes in Rct in the control system appear to be erratic and unrelated to target addition.

The on-chip assays were performed using a small volume of buffer, and a low surface concentration of antibody. Antigen concentrations below 0.5 ng were not tested, and form the basis for future work in this area. Commercially available tests for HER2 measure from 0.008 to 2 ng/mL of HER2, while the physiological level of the serum component (or extracellular HER2) found in circulation in a HER2 positive breast cancer is > 15 ng/mL. Based on this data, there is scope for the development of the fabric-based HER2 test into a low cost diagnostic for breast cancer.

5.4.3 Impedance assays in fabric: conclusions

Impedance detection is an alternative, sensitive means to quantifying the results of an immunoassay. We tested the feasibility of using polypyrrole coated fabrics as working
electrodes, with the ultimate goal of integrating such a fabric into a woven electrochemical sensor. Our studies revealed that impedance is a comprehensive method that can help us characterize the many complex electrochemical phenomena that occur at the surface of the electrode. The capacitive behavior of the polypyrrole in accordance with its semiconducting nature were observed through this analysis. Further, surface phenomena led to a charge in charge transfer resistance and were used to identify binding events. The goal of this work was to establish the feasibility of using polypyrrole coated fabric for electrochemical sensing, and this goal was met via proof of concept experiments for pyocyanin and antibody assays. This work is still in its infancy, however, and further development into a fully functional electrode is underway. For instance, inconsistencies in the spacing and setup of the manually assembled chips may have led to the poor correlation between HER2 concentration and Rct. However, this problem may be remedied by fabricating woven sensors where the spacing between electrodes can then be kept precisely consistent. Yarns coated with the appropriate conductive inks for reference and counter electrodes would be utilized in conjunction with the polypyrrole working electrode.
6.0 CONCLUSIONS AND RECOMMENDATIONS

An electrically enabled fabric-based platform for microfluidic, protein sample separations and analysis was demonstrated. Weaving presents a versatile means to manufacturing biosensors and microfluidic platforms using existing, scalable manufacturing equipment. As demonstrated in prior work, manufacturing throughputs can be raised to several thousands of devices per day per weaver. Low cost systems are primarily passive systems that rely on the imbibition of liquids through capillary forces. Although this frees the system from any type of expensive hardware, it also limits the system to binary/qualitative tests typically performed on analytes present at high physiological concentrations. Wicking materials are prone to variations in their wetting characteristics, due to variations in ambient humidity, temperature and fiber morphology and orientation, which typically occur at the microfluidic scale. Indeed, to this date, actively controlled low cost platforms have not been implemented commercially, despite the widely recognized need for such platforms. The weaving approach to device manufacture was chosen due to the ease with which coated or functionalized fibers are integrated into fabric, using existing weaving infrastructure, and at no changes to the cost or portability of the platform. Proof of concept techniques were developed for electrophoretic protein separations and assay in the platform. Since the electrophoretic platform did not utilize any reagents, the cost of manufacture of a single device is \(<< \$1.00.\)
However, certain challenges were faced during the development of this platform. For instance, the pore sizes in yarns and commercially available textile materials are not comparable to the fine pore sizes in gels. Textile fibers are therefore challenging to develop into molecular sieves for electrophoretic separations. An attempt was made to address this problem by controlling the Peclet number in the system by increasing packing density (which reduces fluid dispersion) and by altering the surface properties of the material. A moderate level of focusing was achieved, and a rudimentary mobility shift assay on a simple sample was demonstrated. The use of a gold-nanoparticle based EMSA further reduces sample complexity by allowing only the complexed or colored bands to be visualized. The other bands are therefore not visualized and in this case separation resolution is an insignificant issue. The electrophoretic platform is therefore suitable for low cost EMSA type applications, as well as for Western Blotting, and holds promise in drastically reducing the cost and complexity of the assay procedure using gels or other chip based systems.

The primary advantage of using woven fabric for electrophoresis is the ability to use a versatile set of textile materials that can withstand high applied field strengths in comparison to nitrocellulosic paper based constructs which are easily flammable. The other benefits include seamless transitions as opposed to the overlapping junctions typically present in assembled devices, which can drastically alter the flow profile and disturb the shape of the analyte band, which is critical to achieving separations by electrophoresis. We therefore recommend the fabric platform for the development of EMSA systems.

An additional sample processing feature that may be added to assist with sorting real world biological samples is to include a module to filter out larger debris and
interfering cellular components from the sample. This is especially useful when analyzing cell lysates or tissue samples, including whole blood. Size based separations may be achieved by incorporating layers of filtering fabric upstream of the analytical regions, such that cells are filtered out while only the lysate/molecular components may pass through.

In prior work, woven lateral flow immunoassay devices were developed using silk yarns. Silk is a versatile and biocompatible material which can be used for a variety of molecular and cellular procedures. However, silk is also a hygroscopic material, alongside cotton and other natural materials, and day to day variations in the wicking rates were noted. This problem extends to other lateral flow systems composed of paper-based materials, which are also hygroscopic. In addition, variations in the random orientation of fibers and non-uniformities or manufacturing defects on the edges of the device have been known to cause variations in the flow, which can drastically affect the outcome of the test. An avenue to address these problems was developed by integrating electroactive polymer-functionalized yarns in proximity with an electrode, to construct an active chemical valve that can potentially overpower the shortcomings of passive control. The chemical valve is the first of its kind, and is reversible, i.e, it could potentially be turned on and off again. In addition, intermediate valving modes were also developed in which the valve could be made partially wetting, and to different degrees by tuning the concentration of iron (iii) chloride in the valve. This shows that the valve can be used not only to time flow, but also to precisely tune the flow. Since the polymer is not hygroscopic and its wetting properties were found to be stable over the course of several weeks, we believe that the wetting characteristics of polypyrrole will remain unaffected by variations in ambient temperature and humidity. The polymer valve therefore presents
a universal means to controlling flows in fabric, paper and membrane-based lateral flow systems, as it requires the application of weak electric fields and do not pose a flammability risk. The usefulness of the valve in performing a Lowry assay for proteins was also demonstrated, and was benchmarked against ordinary valve-less lateral flow systems to demonstrate the importance of timed flow in this case. We recommend that further work be performed in implementing valves for increasing the residence time of the sample in the vicinity of the reagent loaded regions of the device and potentially enhancing the sensitivity of LFTs.

In the final aim, we briefly examine the use of polypyrrole coated textile as a working electrode in an electrochemical impedance sensor. Using impedance spectroscopy, the behavior of the electrode was characterized in terms of the resistive and capacitive effects that were observed at its surface, and proof-of-concept assays of a variety of analytes including bacterial toxin pyocyanin and cancer protein HER2 were demonstrated in a large volume beaker based system, as well as in a chip. The electrode can now be woven into an electrochemical sensor for impedance spectroscopy. The advantage of using polypyrrole over conventional screen printed or metallic electrodes is that polypyrrole is inexpensive and easier to synthesize than most metallic systems. Since the polymer is porous it also lends a large surface area for antibody functionalization and antigen capture. As demonstrated in the flow control aim, the conductivity of the polymer can also be tuned. Future work may include the manufacture of flexible, woven impedance sensors, and wearable electronics. The reference and auxiliary electrodes may be made by coating yarns with silver-silver chloride and carbon based inks as demonstrated in prior work by our collaborators. We demonstrated an assay for a cancer
target in this dissertation. However, woven sensors may be incorporated into bandages and clothing and their best use is in infectious disease diagnostics and monitoring. In the appendix (Appendix B), we also describe some exploratory experiments towards the development of a fabric-based battery pack for very low power applications. A bill of materials cost analysis of the platform has been provided below (Table 4). The platform essentially consists of a fabric chip and the associated hardware. The fabric chip can be supplied as a low cost disposable cartridge, while any instrumentation would be a one-time investment. Cheaper iterations of the instrument may be developed. For instance, a battery pack may replace a power supply for the electrophoresis and electroactive valving devices, and a chip based potentiostat designed for the specific application may replace a fully functional potentiostat used in the lab. Such compact devices are made commercially (Ex: Biosense™) and can be incorporated into the portable housing for the fabric device. Each prototyping session can produce 20-30 devices per hour per weaver on a sampling loom, and production can be scaled up to several thousands of devices per day on an industrial loom.
Table 4: Bill of materials cost analysis of a disposable chip or cartridge. Note that the cost of reagents can be reduced even further during large scale manufacture, by purchasing the chemical reagents in bulk.

<table>
<thead>
<tr>
<th>Device function</th>
<th>Material</th>
<th>Cost per unit</th>
<th>Quantity used per device</th>
<th>Total cost per device ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrophoresis</td>
<td>Separation channel and buffer reservoir fabric: polyester warp and weft yarns</td>
<td>$3 per kg</td>
<td>1 g</td>
<td>$0.0035</td>
</tr>
<tr>
<td></td>
<td>Cotton weft yarns</td>
<td>$0.3 per unit</td>
<td>1 g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Electrodes: Gold-Metallized silk yarn</td>
<td>$50 per kg</td>
<td>1 mg</td>
<td></td>
</tr>
<tr>
<td>Electroactive valving</td>
<td>Raw silk warp and viscose weft yarns</td>
<td>$70 per kg</td>
<td>1 g</td>
<td>$0.07 (inclusive of electrodes)</td>
</tr>
<tr>
<td></td>
<td>Pyrrole monomer</td>
<td>$23 per 25 g</td>
<td>1.7 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ferric chloride catalyst</td>
<td>$37 per 100 g</td>
<td>4.0 mg</td>
<td></td>
</tr>
<tr>
<td>Antibody functionalized sensor</td>
<td>Antibody reagents</td>
<td>$442 per 1 mL</td>
<td>3.0 µL</td>
<td>$1.31 (inclusive of the fabric platform)</td>
</tr>
</tbody>
</table>
7.0 APPENDICES

Appendix A: Supporting Data

A1: Electrophoresis device characterization through Ohm’s Law plots

Figure A1: Ohm’s law plots for different separation distances between electrodes. (a) The applied voltage is kept constant and the distance between electrodes is varied. We see that wider separation lead to higher tolerances. (b) The data in (a) collapses when the voltages are divided by the separation distance (cm) to yield electric field strength. This shows that in order to effectively apply a stronger electric field, the separation distance between electrodes may be reduced while leaving the voltage unchanged, providing the minimum effective length required for separations is maintained. Reproduced with permission from the supporting information for publication: Anal. Chem. 2015, 87 (4), 2480–2487 ©2015 American Chemical Society.)
A2: Optimization of operating voltages for band focusing

The applied voltage influences the shape of the analyte band. A series of voltages were tested to study the shape of a tracer band as it is electrophoresed through the fabric.

Figure A2: Plots of the position of the leading and lagging edge of a dye band against time at fixed applied voltages. The difference between the two is illustrative of the width of the band as it migrates from one end of the device to the other. Lower voltages (25 V/cm) are not sufficient to overcome the hydrodynamic drag on the molecule, while very high voltages, approaching the system limit (45-50 V/cm) create inconsistencies in the shape of the band through the run. Applied fields of 30-40 V/cm are ideal in order to maintain a sharp band of consistent width.
A3: Estimation of separation resolution (SR) in fabrics by image analysis

![Image of fabric with bands and measurement notation]

$$\Delta L = \frac{[L2 + L0] - L1}{2}$$

$$W_{avg} = \frac{[L1 + (L2 - L0)]}{2}$$

Separation Resolution = $$\frac{\Delta L}{W_{avg}}$$

Figure A3: The procedure used to calculate separation resolution from an image of the analyte bands is provided here. Calculations were made towards the end of a 2-3 minute run.

A4: Estimation of drop size and the ‘tuning parameter factor’, the fabric equivalent of the Peclét number

<table>
<thead>
<tr>
<th>Fabric</th>
<th>Spot Area (spot pixels/total pixels)</th>
<th>Tuning Factor (coverage area/spot area)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nylon-Cupro</td>
<td>0.052</td>
<td>15.48</td>
</tr>
<tr>
<td>Nylon-1</td>
<td>0.02</td>
<td>44.1</td>
</tr>
<tr>
<td>Nylon-2</td>
<td>0.022</td>
<td>40.09</td>
</tr>
<tr>
<td>Polyester-1</td>
<td>0.007</td>
<td>73.43</td>
</tr>
<tr>
<td>Polyester-2</td>
<td>0.001</td>
<td>769.23</td>
</tr>
</tbody>
</table>

Figure A4: Coverage area was obtained using an in house matlab code, as detailed in the results section. Spot areas were obtained using a similar analysis where a threshold pixel intensity value determines the area of the fabric colored (darkened) with dye versus the area that remains unwet or white. Coverage area was then divided by spot area to yield the tuning parameter where higher tuning parameters are associated to lower dispersion and better focusing. The woven polyester material yielded the best resolution of the selection and was used in all further experimentation.
A5: Choosing the optimal substrate for a particular analyte type

While the tuning parameter factor (packing density/drop size) accurately determines the ability to focus food coloring dyes on the platform, protein and protein-nanoparticle conjugates require a surface that is inert, and choosing the optimal platform can often be counter-intuitive. While our knowledge of tuning parameters tells us that hydrophobic surfaces favor focusing, protein analytes typically stick to hydrophobic surfaces through hydrophobic interactions. Choosing an intermediate tuning parameter is therefore important. The following is a pictorial representation of the ability to focus an analyte band in fabric by tuning parameter value, and analyte size. Note that since proteins and nanoparticle conjugates are larger in size than small molecule dyes, they are less diffusive and are easier to focus using fabrics with smaller tuning parameter values. Likewise, larger sized analytes also require larger pore sizes to prevent clogging, fouling and high background during the run. The following tables illustrates these points. For instance, food coloring dyes form bands directly in correlation with the value of the tuning parameter, with hydrophobic surfaces giving the best results. Meanwhile, proteins benefit from using more hydrophilic surfaces, and intermediate-to-low tuning parameters are more suitable. Gold-nanoparticle conjugates become immobile on hydrophobic surfaces, and also benefit from the use of lower tuning parameters.
<table>
<thead>
<tr>
<th>Tuning Factor</th>
<th>Metabolites (&lt;1 kDa)</th>
<th>Proteins and peptides (10-500 kDa)</th>
<th>Protein-nanoparticle conjugates (~10^9 kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>73</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>44</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>40</td>
<td><img src="image7.png" alt="Image" /></td>
<td><img src="image8.png" alt="Image" /></td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td>15</td>
<td><img src="image10.png" alt="Image" /></td>
<td><img src="image11.png" alt="Image" /></td>
<td><img src="image12.png" alt="Image" /></td>
</tr>
</tbody>
</table>

Figure A5: Table of tuning parameters and band focusing depending on analyte type and surface properties. Dyes focus better on surfaces that are more hydrophobic and more densely packed. However, proteins and nanoparticle conjugates require passivated, less hydrophobic surfaces and benefit from carefully chosen substrates with intermediate tuning parameters. The arrows point to the position of the band at the end of the run. For the proteins, tuning parameters of 40-44 work well, but low tuning parameters lead to band spreading, and the band appeared not to migrate during the run. GNP conjugates behaved in a similar manner where the bands were stuck to the surface at very high tuning parameters and did not move or focus at very low tuning parameters. Intermediate values were therefore used in the EMSA described in the results.
A6: Reversible wettability switching in polypyrrole coated textiles: Optimization of applied voltages and treatment times

Figure A6: Effects of tuning parameters such as applied field strength and treatment time. (a) The effect of applied potentials show that wettability changes are greater when higher potentials are used and vice versa. (b) Likewise, larger changes in wettability are affected by longer treatment at the cathode (or anode).
A7: SEM-EDS spectra for polymer coated fabrics before and after wettability switching

Figure A7: EDS spectra of coated fabric samples. The large carbon and oxygen peaks correspond to the cotton fabric (a) EDS spectrum of coated fabric prior to treatment. A small chloride peak is visible (b) (Inset) Portion of EDS spectrum of coated fabric after cathodic treatment, chloride peak is diminished and (c) (Inset) Portion of EDS spectrum of cathode-treated, coated fabric after subsequent anodic treatment. The chloride content has increased dramatically.
Appendix B: Notes on the development of a fabric-based power source

A fabric-based battery was developed to power low voltage LEDs, and has been discussed in this section. In fact, similar technologies have been explored in the literature, in the interest of developing flexible power sources for printed circuits\textsuperscript{147} and point of care sensors. An array of zinc sulfate-copper sulfate cells, and alkaline manganese cells were made using a simple concept, in which a dumbbell shaped piece of fabric with metallic wire electrodes was used. The wide ends of the fabric were used as buffer reservoirs and were soaked with zinc sulfate and copper sulfate solution, while the stem was used as a salt bridge containing potassium chloride. A piece of metallic zinc was immersed in the zinc sulfate reservoir, while a piece of copper wire was immersed in the copper sulfate reservoir. The zinc terminal is oxidized, causing zinc ions to be released into solution. The electrons released from the zinc terminal flow through an external resistance to the copper terminal, where cupric ions from the electrolyte are reduced and deposit onto the copper wire. The salt bridge helps to maintain equilibrium in the reservoirs by releasing positive or negatively charged ions into either reservoir as they are consumed/produced. The open cell potential of a single galvanic cell should in theory be 1.1 V, and was measured as 1.07 V with a 0.03 V drop across the voltmeter. Two cells could also be connected in series to generate 2.06 V. However, the more resistances that are added to the cell in terms of connectors and jumpers, the greater the voltage loss. LEDs are rated as per the power required for them to glow. Green LEDs require roughly 2.1 V and were powered using two batteries in series (Figs. A8-A10).
Figure A8: Single zinc sulfate copper sulfate galvanic cell. Open circuit potential of 1.07 V read using multimeter.

Figure A9: Two cells connected in series with open circuit potential of 2.06 V.

Figure A10: The two cells discharge through a 2.1 V green LED causing the LED to light up.
8.0 REFERENCES


Amendola, V. *Surface plasmon resonance of silver and gold nanoparticles in the proximity of graphene studied using the discrete dipole approximation method*. Physical Chemistry Chemical Physics 18, 2230-2241 (2016).


