Linköping Studies in Science and Technology

Dissertation No 1923

Electroosmotic pumps with electrochemically active electrodes

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Linköping 2018

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While conducting the research for this thesis Per Erlandsson was enrolled in Forum Scientium, a multidisciplinary doctoral program at Linköping University.

Linköping Studies in Science and Technology. Dissertation No. 1923

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Printed by LiU-Tryck, Linköping 2018

ISBN: 978-91-7685-335-1 ISSN: 0345-7524

Electronic publication: <http://www.ep.liu.se>

Abstract

Electrokinetic phenomena, motion caused by an applied electric field, can be used to separate molecules based on charge as in capillary electrophoresis, or pump liquids with electroosmosis. As microfluidic devices are becoming more advanced, involving multiple stages (sequential reactions) and requiring smaller amounts of reagent, the demand for precise fluid control and integrated electrodes increases. One of the main reasons for developing lab-on-a-chip (LoC) devices is the realization of decentralized diagnostics, allowing patients to be monitored without going to a hospital or diagnosed in situations where healthcare infrastructure is not available.

The first paper of this thesis investigates the differences in characteristics between an electroosmotic pump with metal electrodes and one using electrochemically active polymer electrodes. Continuous electroosmotic flow requires an electric field, which is maintained by electrochemistry at the electrodes. With metal electrodes, such as platinum, reactions must take place at the metal/electrolyte interface where the electrolyte or species therein are either reduced or oxidized to maintain an electric current. For water-based electrolytes the electrolysis of water produces pH altering species and gas, the former can interfere with the chemistry of the system while the latter can cause blockages in microfluidic devices due to bubbles. As electrochemically active electrodes can themselves be reduced or oxidized, the amount of reactions at the polymer/electrolyte interface can be significantly decreased (less electrolysis of water). With Poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS) electrodes driving a simple electroosmotic pump (EOP) the charge transported before pH changes were observed was related to electrode PEDOT content. PEDOT:PSS electrodes showed pH changes after 10 Coulomb per gram of PEDOT while platinum electrodes started changing pH immediately upon operation.

The second and third papers investigate the use of potassium silicate frits or monoliths in microfluidic devices using electrochemically active electrodes. In most real world applications, an electroosmotic pump must work against a pressure gradient. An open-channel fused silica capillary, 100 μm inner diameter, can function (in combination with appropriate electrodes) as an electroosmotic pump (EOP) but its ability to pump against a pressure gradient is very poor. Utilizing the fact that pressure driven flow per area scales as radius squared, whereas electroosmotic flow per area is independent of radius (if above the diffuse layer thickness and

below 100 μm) a 100 μm channel could theoretically be replaced with one hundred 10 μm channels giving the same cross-sectional area (and thus the same electroosmotic flow) but with 100 times higher resistance to pressure driven flow. In order to convert a 100 μm capillary into a system resembling a large number of thin parallel fluid paths porous potassium silicate monoliths were created inside fused silica capillaries. The capillaries with porous silica monoliths showed a 15 000-fold increase in hydrodynamic resistance, with only a 7% decrease in electroosmotic mobility while retaining 70% effective cross-sectional area of the untreated 100 μm fused silica capillaries. In order to improve EOP compatibility with 3D printing, the next step was to produce potassium silicate (KSi) structures without a fused silica capillary as a scaffold. Filling cylindrical molds of polydimethylsiloxane (PDMS) with potassium silicate mixture resulted in KSi monoliths cylinders with diameters 50% of their mold's diameter while only shrinking to 85% of its length. Scanning electron microscope (SEM) images of the monolith cross sections revealed a porous core and what appeared to be a solid outer region, which could allow the monoliths to be directly inserted in a 3D printed template and covered with, e.g., PDMS mixture without blocking the monolith. During characterization of the standalone KSi monoliths a few showed asymmetric pumping properties which was interesting as this may be used for AC-driven EOPs. In an attempt to increase the asymmetric behavior, tapered monoliths were produced. The tapered monoliths were inserted as EOPs into simple microfluidic devices with integrated PEDOT:PSS electrodes. The most interesting EOP characteristic was the directional difference in volume transported per charge, as it determines the net flow after driving the same amount of charge back and forth. The tapered monoliths showed 33% higher volume transported per charge in the base-to-tip direction with 100 V applied in both directions. The volume transported per charge difference was increased to 55% with different potentials for base-to-tip (50 V) and tip-to-base (200 V). In combination with electrochemically active electrodes, a rectifying EOP could pump in the same direction indefinitely, albeit "two steps forward, one step back", with minimal electrochemistry on the electrolyte or species therein.

The last paper describes a microfluidic device utilizing lectin-carbohydrate interaction to detect fucose, a biomarker in urine for cancer and liver disease. Lectins are proteins specialized in binding to carbohydrates and most do not have enzymatic activity, which allows lectins to be used like antibodies in assays. However, lectin-carbohydrate interactions are generally low affinity

compared to antibody-antigen interactions. To increase fucose retention we used a recombinant form of the mushroom *Aleuria aurantia* lectin (AAL), with one binding site called S2-AAL. S2-AAL was immobilized inside a simple microfluidic chip and exposed to a solution containing fluorescently labeled lactoferrin, a globular protein with two fucosylated N-linked glycans. As sample enters the microfluidic chip the fluorescently labeled lactoferrin, with its glycans bound to S2-AAL, becomes displaced if the sample contains free L-fucose, which can be detected by a change in fluorescent intensity. The dynamic range of the detection was 0.1 to 2 mM fucose, to be compared with normal fucose levels in urine at around 0.1 mM.

Populärvetenskaplig sammanfattning

Elektrokinetik, att generera rörelse med hjälp av ett elektriskt fält, kan användas för att separera så små objekt som molekyler med till exempel kapillärelektrofores eller för att flytta betydligt större massor som vid pumpning av vätskor med elektroosmotiska pumpar. Utveckling inom mikrofluidik, hanteringen av vätskor i mikrosystem, och lab-on-a-chip teknik, kreditkortsstora chip som kan utföra flera reaktioner och mätningar, har ökat efterfrågan på förbättrad kontroll av vätskor och integrerade elektroder för att utnyttja elektrokinetiska effekter. En av de främsta orsakerna till att utveckla lab-on-a-chip tekniken är göra decentraliserande diagnoser tillgängligare, vilket skulle gör det möjligt för fler patienter att testa sig hemma och slippa åka till sjukhus. På samma sätt som blodsockermätare låter diabetiker kontrollera sina nivåer i hemmet förväntas framtidens ”hem nära-diagnostik” spara tid för patienter och resurser för samhället. Engångschip för decentraliserad diagnos kan även underlätta humanitär hjälp i situationer där sjukvårdsinfrastruktur inte är tillgänglig på grund av krig eller naturkatastrofer.

Den första delen av denna avhandling undersöker skillnaderna mellan en elektroosmotisk pump med metallektroder och elektrokemiskt aktiva polymerelektroder. Elektroosmotisk pumpning bygger på att laddade partiklar i en vätska lägger sig nära en vägg med stationära laddningar, t.ex. en glasyta blir negativt laddad i vatten varvid positiva laddningar ansamlas nära väggarna. Om glasytan är insidan på en tillräckligt fin cylindrisk kapillär (under 200 μm i diameter) och ett elektriskt fält appliceras längs kapillären kommer de positivt laddade partiklarna att flytta sig i riktning med det elektriska fältet och kommer som en innerslang dra med sig kapillärens innehåll. För kontinuerlig elektroosmotisk pumpning krävs det att dessa laddade partiklar transporteras genom pumpen hela tiden vilket ger upphov till en ström av joner genom elektrolyten. Med metallektroder som platina, måste reaktioner ske vid metall/vätske-gränssnittet där antingen lösningsmedlet eller molekylerna däri måste ta emot elektroner (reduceras) eller ge bort elektroner (oxideras) för att upprätthålla en elektrisk ström. Om lösningen är vattenbaserad så producerar elektrolys av vatten pH-förändrande produkter samt gas, den förstnämnda delen av processen kan störa kemin hos ett miniaturiserat system, särskilt innehållande biomolekyler, medan gasbildning kan orsaka blockeringar i systemet. Vår grupp är intresserad av att utveckla elektroder som minimerar dessa problem i biologiska system. Elektrokemiskt aktiva elektroder har förmågan att

själva ta emot eller lämna ifrån sig elektroner (reduceras eller oxideras), en mer gynnsam reaktion än elektrolys av vatten, vilket minskar mängden oönskade reaktioner vid polymer/elektrolyt-gränssnittet (mindre elektrolys av vatten). Poly(3,4-etylendioktiofen)-poly(styrensulfonat) (PEDOT:PSS) är en vanligt förekommande elektrokemiskt aktiv polymer som både leder ström och kan acceptera/donera elektroner. PEDOT:PSS-elektroder användes i en elektroosmotisk pump (EOP) där mängden laddning som transporterats genom pumpen jämfördes med när pH-förändringar dekreterades i elektrolyten vid elektroderna. PEDOT:PSS-elektroder visade på pH-förändringar efter 10 Coulomb per gram PEDOT i elektroderna medan platinaelektroder började ändra pH direkt. Denna kunskap är väsentlig för design och dimensionering av framtida mikrofluidiksystem med PEDOT elektroder.

Det andra och det tredje manuskripten undersöker användningen av kaliumsilikat (KSi) monoliter i mikrofluidiska system med elektrokemiskt aktiva elektroder (PEDOT). I de flesta applikationer måste en elektroosmotisk pump arbeta mot ett tryck. En öppen kanal med glaskapillär, 100 μm inre diameter, kan fungera som en elektroosmotisk pump men dess förmåga att pumpa mot en tryckgradient är undermålig. Genom att utnyttja att tryckdriven flödes hastighet är proportionell mot radien i kvadrat medan elektroosmotiskt flöde är oberoende av radie (för radier mellan 100 nm och 100 μm). Om en 100 mikrometer kanalen ersätts med hundra 10 μm kanaler fås samma area men motstånd mot tryckdrivet flöde ökar med en faktor 100. Porösa KSi-monoliter skapades inuti glaskapillärer och integrerades i mikrofluidiskasystem. Kapillärerna med KSi-monoliter visade en 15 000-faldig ökning i motstånd mot tryckdrivet flöde med en effektiva tvärsnittsarea över 70% och elektroosmotisk flödes hastighet 93% av den obehandlade 100 μm glaskapillären. För att förbättra monoliternas kompatibilitet med mikrofluidik-system tillverkade med hjälp av 3D-skrivare var nästa steg att producera KSi-monoliter utan glaskapillärer. Genom att fylla cylindriska former av gummi materialet polydimetylpolysiloxan (PDMS) med kaliumsilikatblandning erhöles KSi-monolit cylindrar med diametrar omkring 50% av sin forms diameter medan de krympte till 85% av dess längd. Svepelektronmikroskop (SEM) bilder av monoliternas tvärsnitt avslöjade en porös kärna och vad som verkade vara en solid yttre region. Detta solida ytterlager skulle kunna göra det möjligt att införa monoliterna direkt i en 3D-tryckt struktur som sedan täcks med exempelvis PDMS-blandning utan att monoliten blockeras av PDMS. Vid undersökningen av de fristående KSi-monoliterna visade ett fåtal monoliter asymmetriska pump egenskaper. I ett försök

att öka denna asymmetri producerades avsmalnande (koniska) monoliter. De koniska monoliterna användes som EO pumpar i mikrofluidiska system med integrerade PEDOT:PSS-elektroder. Den mest intressanta pump egenskapen var skillnaden i transporterad volym per laddning mellan riktningarna, eftersom det avgör nettoflödet efter att ha transporterat samma mängd laddning fram och tillbaka. De avsmalnande monoliterna visade 33% mer volym transporterad per laddning i bas-till-spets riktningen för 100 V applicerat i båda riktningarna. Skillnaden i volym transporterad per laddning kunde höjas till 55% med olika potentialer för bas-till-spets (50 V) och spets-till-bas (200 V). Kombinationen av elektrokemiskt aktiva elektroder och asymmetriska monoliter tillåter en EOP att pumpa i samma riktning med minimal elektrokemisk påverkan av lösningsmedlet eller lösta ämnen.

Det sista papperet beskriver ett mikrofluidisk chip som utnyttjar lektin-kolhydratinteraktion för att detektera fukos, en biomarkör för cancer och leversjukdom som finns i urinen. Lektiner är en typ av protein som är specialiserade på att binda till kolhydrater, och de flesta har inte enzymatisk aktivitet, vilket betyder att de inte förändrar det bundna ämnet. Vanligtvis binder inte antikroppar till kolhydrater vilket gör lektiner intressanta för att detektera kolhydrater i de detektionssystem som utvecklats för antikropp-antigen interaktioner. Dock har lektin-kolhydratinteraktioner i allmänhet lägre affinitet jämfört med antikropp-antigen-interaktioner, vilket betyder att de binder till lägre grad. För mätningen immobiliserades S2-AAL, en rekombinant form av lektin från svampen *Aleuria aurantia* (AAL), inuti ett mikrofluidiskt chip. Sedan utsattes lektin-ytan för en lösning innehållande fluorescensmärkt laktoferrin, ett globulärt protein med två sockerkedjor innehållande fukos som binder till lektinet. Om provet som introduceras in i det mikrofluidiska chipet innehåller fri fukos konkurreras det fluorescensmärkta laktoferrinet bort vilket kan detekteras genom att fluorescensintensiteten minskar. Det dynamiska området för chippet var 0.1 till 2 mM fukos där ett normalt fukos värde i urinen är 0.1 mM.

Included papers

Paper 1

Electrolysis-reducing electrodes for electrokinetic devices

Erlandsson, P. G., Robinson, N. D.

Electrophoresis 2011, 32, 784-790 (2011)

Author contribution: Planned and performed all experimental work. Wrote the manuscript together with the co-author.

Paper 2

Electroosmotic Pumps with Frits Synthesized from Potassium Silicate

Nilsson, S., Erlandsson, P. G., Robinson, N. D.

Plos One 2015, 10 (2015)

Author contribution: The first two authors contributed equally to system design and experimental work. Wrote the manuscript together with the co-authors.

Paper 3

Rectification of electroosmotic flow via macroscopic tapered monoliths

Erlandsson, P. G., Robinson, N. D.

Manuscript, submitted

Author contribution: Planned and performed all experimental work. Wrote the manuscript together with the co-author.

Paper 4

Determination of fucose concentration in a lectin-based displacement microfluidic assay

Erlandsson, P. G., Åström, E., Pålsson, P., Robinson, N. D.

Manuscript

Author contribution: Planned and performed the majority of all experimental work. Wrote the manuscript together with the co-authors.

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Abbreviations

CE	Capillary electrophoresis
CV	Cyclic voltammetry
EAP	Electrochemically active polymer
EDL	Electrical double layer
EOF	Electroosmotic flow
EOP	Electroosmotic pump
KSi	Potassium silicate
LoC	Lab-on-a-chip
μTAS	Micro total analysis systems
PDMS	Polydimethylsiloxane
PAGE	Polyacrylamide gel electrophoresis
PEDOT	Poly(3,4-ethylenedioxythiophene)
PoC	Point-of-care
PSS	Polystyrene sulfonate
Re	Reynolds number
SAV	Surface area to volume ratio
SHE	Standard hydrogen electrode

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1 Introduction

In the early 1800s, Ferdinand Frederic Reuss at Moscow State University discovered that applying an electric field across a porous clay plug separating two containers of solution caused fluid to be pumped through channels of the clay plug, an electrokinetic phenomenon named electroosmosis.¹ This type of fluid transport, called electroosmotic flow (EOF), requires charged walls around channels with a radius below $100\text{ }\mu\text{m}$,² and is fundamentally different from traditional pressure driven flow. For example, the average flow velocity for electroosmotic flow does not change as the channel radius is decreased, until it approaches 100 nm , unlike the case for pressure driven flow, where the average velocity is proportional to the radius squared (r^2) and decreases rapidly as a system is scaled down. Another difference is that the flow velocity flow profile of EOF is flat — fluid moves as a plug, while pressure driven flow has a parabolic profile shape. This variation in velocity means that a section of fluid that starts in one place will be “smeared out” as it is transported along the channel. At the time of Reuss’s discovery there was a limited number of systems where the advantages of electroosmosis could be realized. Today whole research fields are dedicated to microfluidic devices and how to design systems to best utilize electrokinetic phenomena such as electroosmosis. Lab-on-a-chip (LoC) devices are one of the most well-known of these systems, which, as indicated by the name, aim to replicate multi-step laboratory protocols in a microfluidic credit card-sized chip.

Lab-on-a-chip devices are ideally fully autonomous without the need for external pumps, detection or electronics, which requires integrated pumps, something electroosmotic pumps (EOPs) could provide. One of the most straightforward applications for autonomous (or semi-autonomous) LoC devices is decentralized healthcare — point-of-care (PoC) testing allowing patients to monitor themselves which ideally saves time for the user and resources for the caregivers.³ The most famous microfluidic point-of-care test is the glucose test, used daily by millions of people with diabetes in the comfort of their homes. With an ageing population and rising costs of health care in most countries, some hope that breakthroughs of new LoC devices will be the silver bullet to decrease costs and increase the quality of life for patients. Point-of-care testing not only has the potential to decrease costs and make tests more convenient, but can allow diagnosis to be carried out where previously impossible, e.g., in conflict zones or regions without sufficient medical facilities. A

container filled with medicine transported into a conflict zone has limited value without diagnostic tools to determine who needs what, highlighting the value of readily available autonomous diagnostic devices.

Microfluidic systems can also be designed for drug screening, exposing cell and tissue cultures to molecules in order to determine if a candidate drug has potential and should be tested in an animal model. Better microfluidic systems for drug screening should lead to fewer animal tests, which would be preferred by everyone. Microfluidic systems are also frequently used in research to study biomolecules, cells, tissues and blood, and their reactions to different physical and chemical microenvironments. The extra condition or drawback of electrokinetic phenomena such as EOF in microfluidic systems is the need to introduce electrodes into the system, meaning that electrochemistry needs to take place at the electrodes in order to maintain an electric field between electrodes. With traditional inert electrode materials, such as platinum, the electrochemistry is performed on the solvent, which in the case of water results in gas generation and pH altering species that can disrupt the system's microenvironments. With electrodes made out of electrochemically active polymer materials, most of the undesirable reactions at the electrodes can be delayed or completely avoided.

1.1 Aim

The aim of this thesis was to investigate the use of electroosmotic pumps with electrochemically active polymer electrodes for microfluidic systems. More specifically, we were initially interested in quantifying and minimizing the undesired electrochemical reactions (electrolysis) that occur with electrochemically active polymer electrodes compared to inert metal electrodes. In order to produce electroosmotic pumps able to operate against a pressure gradient (necessary for most applications), we produced and characterized electroosmotic pumps with a high resistance to pressure driven flow. Finally, we attempted to produce rectifying pumps, with directionally dependent fluid transported per charge, by changing the pumping regions' characteristics along the flow path.

1.2 Outline

After this general introduction, this thesis continues with a chapter on microfluidic devices. Then, electrophoresis and electroosmosis, two types of electrokinetic phenomena, are described. Chapter three covers electroosmotic pumps, a practical application of electroosmosis. The following chapter describes the electrochemistry required to maintain the current which generates the electroosmotic flow. A summary of the included papers and the context in which the projects were chosen concludes this section of the thesis.

2 Microfluidic devices

2.1 Definition

Microfluidics is the field which deals with design, production and development of devices and protocols for miniaturized fluidic systems. The potential applications for microfluidic systems are widespread but have so far mainly been used for analysis of biomolecules and cell assays. In some applications microfluidics aim to replicate and improve an existing technique, but there are several cases where these systems create new tools for researchers, such as suitable 3D microenvironments for cells or assays with minimal reagent requirements. Microfluidics are intimately linked to lab-on-a-chip (LoC) devices, micro total analysis systems (μ TAS) and point-of-care (PoC) diagnostics, the latter being systems specialized in monitoring the health of people in their own homes or for diagnostics in clinics without a lab. Just like glucose tests and pregnancy tests can be taken at home, PoC tests promise a future where more diagnoses can be carried out at home, called decentralizing care, which could reduce healthcare costs and save patients time. With an ageing population and healthcare costs rising, governments seem concerned yet optimistic that scientific development will mitigate these problems, and perhaps microfluidics will be the “silver bullet” they hope for.

2.1.1 Length-scales in microfluidics

Microfluidic devices are systems most commonly used in molecular analysis and molecular biology that manipulate small volumes of fluid, from nanoliters down to attoliters (10^{-9} L to 10^{-18} L). The channels and structures in microfluidic devices are typically on the micrometer scale and below, but can be interfaced with systems handling larger volumes.⁴ In figure 1, an illustration of a simple microfluidic chip on a standard microscope slide (75 x 25 mm) is shown with an open well to the left and the opposite side connected via tubing to an external system, e.g., a pump outside the picture. The open well and the tube interface are connected by a microfluidic channel with a diameter below 100 μ m, comparable to the diameter of a human hair. The total volume of the channel in figure 1 is approximately 80 nanoliters.

Figure 1 Schematic of a simple microfluidic chip on a microscope slide (75 x 25 mm) with an open well at the left end of the channel and a tube interface at the right end. To the right the channel has been magnified by a factor 100.

2.1.2 Biomimetic structures

The structures in microfluidic systems can be similar in size to animal cells (10-30 μm), bacteria (0.5-5 μm) and blood capillaries (5-10 μm). This allows the design of systems able to manipulate and capture cells, e.g., by transporting fluid containing cells through a “fence structure” where cells of interest can be sieved out of the fluid. Microfluidics can also be used to create 3D microenvironments suitable for different cell or tissue types, allowing *in vitro* studies and drug tests under conditions that more closely resemble those found *in vivo*.⁵ With careful design, these systems can also mimic the *in vivo* shear stress of blood and cells in contact with blood flow, which allows them to function normally.⁶⁻⁸ In the same way, care must be taken to avoid exposing cells to unwanted shear stress as both cell morphology and physiology can be affected.⁹ Microfluidics offer a number of new opportunities for creating assays for tests previously only possible inside animal models, and also allows us to mimic the elegant solutions found in biology to improve our own technology. For example, by copying the structures found in fish gills, microfluidic devices were developed to transfer oxygen from water into the blood transported through them. Both mass and heat transfer in microfluidic devices can be improved.¹⁰

2.2 Effects of miniaturization

2.2.1 Surface area to volume ratio

Decreasing the size of structures and channels increases the surface area to volume ratio (SAV), in other words, the amount of surface/fluid interfaces increases relative to the amount of bulk fluid. In biological systems, capillary beds, tissues and the inside of cells all have high SAVs and many interactions of interest occur at fluid/membrane interfaces. For assays with biomolecules and cells, it can often be desirable to mimic the SAV of biological systems if possible. Microfluidic systems with a high SAV can result in highly responsive temperature control or increased gas exchange rates.¹¹ However, for a microfluidic system, the increased SAV also increases the adsorption of proteins and other biomolecules to the walls. Such adsorption can be reduced by pre-treating the walls, e.g., with bovine serum albumin, but the unwanted adsorption and potential denaturation at the walls will always increase with SAV and must be taken into consideration when designing the system. Traditional *in vitro* cell culture systems, such as petri-dishes, have a relatively small SAV and a large medium volume to cell volume ratio. These properties make it nearly impossible for traditional cell culture systems to create a microenvironment which resembles those found *in vivo*.^{12,13}

Table 1 Pros and cons of miniaturization

Advantages of miniaturization	Challenges of miniaturization
SAV resembles <i>in vivo</i> conditions	Increased adsorption to walls
Non-turbulent flow	Lower pressure driven flow velocities
Lower sample and reagent consumption	More complex device and protocol design

2.2.2 Quantities and transport of sample and reagents

Microstructured systems are three-dimensional structures where the inner dimensions are under one millimeter, but most often between ten and a hundred micrometers. The chip in figure 1 has dimensions that are typical in microfluidic devices; it can be completely filled with less than 100 nanoliters of fluid. Compared to the volumes handled in traditional laboratory work, the volume requirements for microfluidic systems can be several orders of magnitude lower, which

can be invaluable for analysis of species that are very expensive to produce in large quantities, e.g., for screening pharmaceutical candidates, or working with samples that are challenging to purify. The sample and reagent consumption per assay can be decreased further by only introducing a small volume, or band, of the sample surrounded by another solution into the device, instead of filling the whole system with sample. However, in order to move a band of sample between different locations on a microfluidic chip a system for precise fluid transport is required. Fortunately, typical microfluidic devices have laminar flow, with Reynolds numbers below 100, whereas turbulent flow generally occurs at Reynolds numbers above 2000, which makes the transport of fluids in microfluidics more predictable than in turbulent systems.¹⁴

2.2.3 Fluid transport and miniaturization

Figure 2 Flow velocity profile for pressure driven laminar flow in channels with different radius under the same pressure gradient. Decreasing the radius by a factor 2 decreases the rate of fluid transported by a factor 16.

One consequence of an increase in the surface area to volume ratio is that greater pressure is required to maintain the same average fluid flow velocity through the system. As seen in figure 2, the local flow velocity for pressure driven laminar flow has a maximum at the center of a channel and the flow velocity decreases towards the stationary walls. The Poiseuille law states that pressure driven flow through a channel is proportional to the forth power of the radius (r^4) and since the

cross-sectional area is proportional to the radius squared (r^2), this gives that the average flow velocity also is proportional to the radius squared (r^2). So if a device with channel diameters of 1 mm is scaled down to 10 μm channels, the pressure gradient applied across the device needs to be increased by a factor 10 000 in order to maintain the same average flow velocity, this is discussed further in chapter 4. An alternative method for generating flow in a microfluidic system is with electroosmotic flow (EOF), which will be covered more in depth in chapter 3. Unlike pressure-driven flow, EOF maintains the same average flow velocity even when microfluidic channels are scaled down, however, this statement only holds true if the radius remains between 100 nm and 100 μm .¹⁵

2.3 Lab-on-a-chip devices

2.3.1 Transport of sample and reagents to multiple sites

Lab-on-a-chip (LoC) devices are in most cases microfluidic systems able to move a sample into multiple sites where interactions with reagents, separation or detection can occur. In other words, the chips are designed to carry out a series of steps that correspond to the steps of a protocol in traditional laboratory work.¹⁶ The microenvironment can be different at each site on the chip thanks to the high surface area to volume ratio, which allows local surface functionalization and temperature regulation to govern the environment.¹⁷ A lab-on-a-chip design with multiple sites for sample interactions able to mimic the steps of a traditional protocol, is a good start. However, to execute the protocol, the chip requires a system to transport the sample between sites, just like a sample needs to be transferred between various instruments and solutions in a laboratory. In many designs, it can be desirable to transport both sample and reagent to the interaction sites, as it can be impractical to contain the reagents at the site for an extended period of time. For other microfluidic devices a large pressure driven flow, e.g., medium flowing over an on-chip cell culture, passes through the device continuously and is maintained by an external pump. In such a system, e.g., designed for toxicity studies or drug screening, a specific amount of reagent (drug) is ideally transported into the larger pressure driven flow close to the cells by on-chip pumps for better control of reagent exposure; however, such transport will normally go against a significant pressure gradient.¹⁸

2.3.2 Pumps

The term lab-on-a-chip sets high expectations on the credit card sized devices, with ambitions to challenge the golden standard tests using an independent system with pumps, electronics and detection on-chip. Today, LoC devices are often found inside labs connected to multiple large instruments giving rise to the wisecrack term chip-in-a-lab,¹⁹ which indicates that there still is a lot of improvement needed before most LoC devices become autonomous. However, this term can paint an unfair picture, as many research groups working with LoC devices specialize in integrating and improving one aspect of the system while using external equipment for the other aspects, e.g., in this thesis the focus is on integrated fluid transport while using external instruments for electronics and detection. The reality that a majority of researchers have focused on improving an individual aspect of LoC systems instead of developing complete LoC devices has been stated as the reason for the lack of a wide commercial breakthrough of LoC devices, according to one of the field's founders, G. M. Whitesides.⁴

The use of external pumps comes with several intrinsic drawbacks, the main ones being an increased distance of transporting sample and reagents, and that the pumps only provide pressure-driven flow. As a band of sample (or reagent) in a buffer solution is transported with Poiseuille flow, the band will stretch out, due to the parabolic flow velocity profile, as seen in figure 2. As the band of sample is stretched out the concentrations in the front and the back of the band will be lower than in the introduced sample. Eventually not a single cross section of the band will have the concentration of the original sample introduced into the system. To counter this dilution, an alternative is to continuously pump only sample or reagent into the microfluidic device, instead of introducing a band containing the minimum amount needed for the LoC protocol. The most obvious drawback in such an approach is the drastic increase in sample and reagent consumption, which counteracts one of the main advantages of using microfluidic devices. Also, continuous pressure driven flow does not remove the dilution at the forward front between what is being pumped in and the solution initially inside the device. Sample and reagents can be pumped continuously through a section of a microfluidic chip to make sure the concentrations at the pump and in the device are equal. However, a system with a continuous flow through a section of a device

in which no fluid is allowed to reach the interaction sites of the LoC device until undiluted sample enters the device adds significant restrictions to the system design.

2.3.3 Detection

Since LoC devices are so varied in shape and function, there are several possible detection methods available. The most common alternatives for detection in polymer/glass devices are based on optical methods or electrochemistry.^{20,21} Optical detection has been one of the preferred methods in microfluidic designs, partly because ligand binding assays such as enzyme-linked immunosorbent assay (ELISA) from traditional bioanalysis were promising protocols to translate into early LoC devices.²² The predominant optical methods are fluorescence-based, which require a source to generate electromagnetic (EM) radiation of specific wavelengths, a fluorescently marked species to excite and emit EM radiation of different wavelengths, and finally a detector to measure the emitted radiation. The source, detector and even light guides can be on-chip and/or external. EM radiation close to the visual spectrum has low absorption through the components commonly used in microfluidic systems, with glass, water and polymers such as polydimethylsiloxane (PDMS) being transparent in the visual spectrum.

Electrolysis of water, half reactions at anode and cathode

Electrochemical detection methods are based on measuring the current through, and/or potential between, multiple electrodes in a fluidic system. One of the most well-known electrochemical detections systems is the glucose meter, used by millions of diabetics around the world to monitor their blood sugar at home. Leland Clark and Ann Lyons developed the biosensor of the first glucose meter, which used a two-step process with an electrode covered with a thin film containing the enzyme glucose oxidase that turns glucose and oxygen molecules into gluconolactone and hydrogen peroxide. With a low potential applied across the electrode/fluid interface the hydrogen peroxide can quickly be reduced to form hydroxide and the current caused by this reduction corresponds to the concentration of glucose in the sample.²³ Simpler electrochemical methods directly detect the species of interest as it undergoes electrochemistry at an electrode. However, the

species must be reduced or oxidized at a potential within the solvent's (typically water's) or any other high-concentration molecule's electrochemical stability window, to avoid the current from the species of interest being drowned out by electrochemistry performed on the rest of the solution. For this reason, the potentials at which electrolysis of water occurs is of particular interest. The electrolysis of water requires a 1.23 V potential difference, at 25 °C and pH 7 the half reactions for reduction requires -0.8 V vs a standard hydrogen electrode (SHE) and the oxidation requires +0.4 V vs SHE, see equation (2.1) and (2.2). A type of electrochemical detection that can minimize the amount of undesired electrochemistry is based on sensing the local conductivity. By applying a constant potential for a short duration or an alternating potential the conductivity between the electrodes can be measured and information regarding the state of the species between the electrodes can be deduced.²⁴ Finally, the microfluidic systems can be used to deliver processed or separated fluid directly into an external detector by placing a LoC device in series with another system. A natural candidate to combine with a microfluidic devices are mass analyzers, using either electrospray ionization (ESI) or matrix-assisted laser desorption/ionization (MALDI) to ionize the sample and detect the mass per charge distribution of the species of the sample inside the mass spectrometer.²⁵

3 Electrokinetics

The first observations of electrokinetic phenomena were published by F.F. Reuss in 1809, when he described pumping water through a porous clay plug by placing electrodes on opposite sides of the plug and applying an electric potential, observing what we today call electroosmosis. Reuss was also the first to report electrophoresis when he observed that clay particles in water migrate in an electric field.¹ Electrokinetic phenomena is a group of effects that occur either in a solution containing particles or fluid filled systems e.g., capillaries or porous structures. A common component in all these effects is the electrical double layer (EDL) that forms at the solid/fluid interface, caused by charges on the solid's surface and the attracted ions in the fluid, see figure 3. Since the exact location of where the EDL ends and bulk fluid starts is hard to define, the characteristic thickness of the EDL, called the Debye length (λ_D), is defined as the distance outside the Stern plane at which the potential has changed by a factor $1/e$ (~ 0.368).²⁶ Equation (3.1) describes the electric potential at a distance x from the Stern plane (in the direction away from the charged surface), Ψ_0 is the potential at the Stern plane, the electric potentials and λ_D are visualized in figure 3. The inverse of the Debye length represents the solution's ability to shield the bulk from the surface charge. Debye lengths decrease with ionic strength and are approximately 1 nm and 10 nm thick for aqueous solutions with monovalent ion concentrations of 100 mM and 1 mM respectively.²⁷ The EDL consists of the solid surface plus any ions adsorbed as one layer, and the free ions attracted to the solid surface from the liquid making up the second layer, which unlike the adsorbed ions are more loosely associated with the surface. This is why the second layer is called the diffuse layer, which starts at the Stern plane and ends in the plane where the EDL ends and the bulk fluid starts. There is also a slip plane where fluid is no longer attached to the surface and can move more freely, the potential at this plane is called the zeta-potential (ζ).²⁸ If an electric field is applied along the solid liquid interface the layers in the EDL will experience net Coulomb forces due to the difference in the amount of charges from cations and anions. The ability to set the diffuse layer in motion with an applied electric field, which in turn sets the bulk fluid in motion, is what makes electroosmotic pumping possible. Similarly if fluid is forced through a capillary, with an EDL at the fluid/solid interface, a current is transported through the capillary and a streaming potential can be detected between the capillary ends.²⁹ The focus of this chapter is on two electrokinetic effects, electrophoretic separation and electroosmotic flow.

$$\psi(x) = \psi_0 e^{-\frac{x}{\lambda_D}} \quad (3.1)$$

Figure 3 Schematic of the electric double layer where ions between the charged surface and the slip plane, or shear plane, adhere to the surface while the ions in the diffuse layer are free to move yet still have a charge imbalance. The solution's ability to shield the bulk from the charged wall is described by the Debye length (λ_D), the distance outside the Stern plane at which the potential decreases by a factor e (2.718). The potential at the slip plane is the zeta-potential (ζ). Dimensions of the layers are not to scale.

3.1 Electrophoretic separation

Electrophoresis is the movement of charged particles in a fluid with an applied electric field. The Coulombic force given by the product of the electric field strength and the particle's charge, the particle's size/shape and the medium all determine the migration rate of the particles. Gel electrophoresis methods specialize in separating large macromolecules by size and/or charge inside a hydrogel. A common technique is polyacrylamide gel electrophoresis (PAGE) which has the

option to either separate proteins in their native state (native-PAGE) or proteins denatured with sodium dodecyl sulfate (SDS), which binds to proteins at a known ratio (one SDS for every two amino acids) and has a negative charge during separation (SDS-PAGE).³⁰ A gel-free separation alternative is capillary electrophoresis (CE), where an electric field is applied across a capillary instead of a gel. The electric field applied both separates the charged species in the small band of sample at one end of the capillary, and simultaneously generates an electroosmotic flow towards the other end where a detector can analyze the species. Without an electroosmotic flow, the species that were neutral or migrated away from the detector would never reach the detector. CE is a good example of a system where both sample separation and sample transport occur. Compared to gel electrophoresis, capillary electrophoresis is more suitable for small molecules and can be paired with a mass spectrometer (CE-MS) to analyze complex samples.³¹

Figure 4 Illustration of a four-way intersection used to isolate a band of sample prior to electrophoretic separation.

In a microfluidic system, the options to implement both gel electrophoresis and CE are viable. For a macroscopic system, a band of sample can be injected directly into the separation region. In a Lab-on-a-chip (LoC) device, the introduced sample may require additional steps prior to separation. One way to select a small amount of sample for separation inside a microfluidic system is with a four way intersect shown in figure 4. The sample is introduced at W and is electroosmotically driven towards E until the intersection is filled with sample. Then, as an electric

field is applied between N and S, a smaller band of sample from the middle of the intersection is electroosmotically transported towards S. By also applying a potential between N and the other two reservoirs, fluid can be moved from N into all other channels, resulting in a small amount of sample isolated in the separation region as shown in figure 4. The separation region can either be gel-filled or contain fluid, depending on the desired separation. Applying an electric field between N and S (optionally a field between N and E/W to minimize sample in the E/W arms entering the separation region) the sample can be electrophoretically separated.

3.2 Electroosmotic flow

3.2.1 Theory of electroosmotic flow

Even though F.F. Reuss discovered electroosmosis and electrophoresis in the early 19th century it was not until 1853 that Hermann von Helmholtz presented the first description of the electrical double layer (EDL). The model was improved by Louis Georges Gouy and David Leonard Chapman during the 1910s in a theory which added the diffuse layer and distributed ions according to Maxwell-Boltzmann statistics. While the Gouy–Chapman theory was an improvement over the Helmholtz theory, it assumes ions are point charges and often underestimate the EDL thickness. 1924 Otto Stern suggested adding the Gouy–Chapman and Helmholtz theories together and use the models in series, see figure 3. Since 1924 the Gouy–Chapman–Stern model has been modified and further improved, most notably by Grahame³² and Bockris et al,³³ but for describing EOF under normal conditions the Stern model will suffice. In the Gouy–Chapman–Stern model the potential as a function of distance from the charged surface drops linearly inside the Stern layer, with ions adsorbed to the surface, followed by an exponential potential decrease inside the diffuse layer. A part of the diffuse layer, the region closest to the adsorbed ions in the Stern layer, is considered attached to the surface and a slip plane can be introduced to separate the mobile fluid in the diffuse layer from the attached fluid. The potential at the slip plane is called the zeta-potential (ζ), the potential between the bulk fluid and the first stationary layer of fluid adhering to the surface at the slip plane.^{34,35} The value of the zeta-potential is important for the calculations of many electrokinetic phenomena. The zeta-potential is also an indicator of how likely particles in a solution are to aggregate, colloidal dispersions containing particles with higher zeta-potentials are more resistant to aggregation.²⁹

Figure 5 A. Illustration of the electric double layer in a capillary with negatively charged walls. The cations at the walls represent the mobile diffuse layer and are not to scale. B. Microfluidic capillary with an applied electric field setting the diffuse layer in motion. The movement of the diffuse layer sets the bulk fluid in motion through viscous interactions generating the characteristic plug-like velocity flow profile of electroosmotic flow.

Electroosmotic flow utilizes the mobile part of the diffuse layer to set the bulk fluid inside a capillary in motion. Previously, the charged surfaces described have been in the form of a flat sheet, but if the surface is the inside of a thin cylindrical capillary, the diffuse layer outside the slip plane becomes a tube, see figure 5. If an electric field is applied along the capillary, the ions in this “tube”, or diffuse layer, begin moving and pull the bulk fluid with them through viscous interactions, while the charges on the solid surface stay in place, as shown in figure 5B. Due to movement at the walls generating flow, the velocity profile for electroosmotic flow is plug-like, where the flow speed reaches its maximum value at the end of the diffuse layer a short distance from the walls³⁶ — as opposed to pressure driven flow through a capillary where fluid moves fastest in the middle and gets progressively slower towards the stationary channel walls, creating a parabolic velocity flow profile. For a microfluidic channel with no pressure drop, uniform cross sectional area and electroosmotic flow and a radius significantly larger than the EDL thickness, the system can be described as follows:

$$u_{EO} = \mu_{EO}E = \frac{\varepsilon\zeta}{\eta}E \quad (3.2)$$

$$Q_{EO} = u_{EO}A = \mu_{EO}EA \quad (3.3)$$

$$\zeta = \frac{\mu_{EO}\eta_{bulk}}{\varepsilon_{bulk}} \quad (3.4)$$

Where u_{EO} is average EO flow speed, E the electric field strength, μ_{EO} is electroosmotic mobility [m^2/Vs], Q_{EO} is flow rate, A is the cross-sectional area, ζ is the zeta-potential, η is fluid viscosity and ε is the electric permittivity of the bulk solution.¹⁵ From an EO pumping perspective, equation (3.2) shows how μ_{EO} is a figure of merit of a pump, as it represents the flow speed per unit of applied field. Under the assumption that the channel radius stays well above the thickness of the diffuse layer and below $100 \text{ }\mu\text{m}$,² the average EO flow speed is largely independent of A . Equation (3.3) states that total flow rate scales linearly with A for electroosmotic flow, which is not the case for pressure driven flow. Finally, in equation (3.4), both viscosity and electric permittivity are properties of the fluid used, so for a given fluid the zeta-potential is proportional to the electroosmotic mobility. In other words, the magnitude of the potential at the slip-plane has an impact on EO flow speed, and the sign of the potential determines the direction of the EO flow in relation to the applied electric field.

3.2.2 Electroosmotic flow in microfluidics

In lab-on-a-chip systems, the pumps can be either external or internal to the chip. After the previous section one might ask why we don't use an EO pump outside of the device to generate that desirable plug-like velocity flow profile. The problem is that the constant velocity profile only forms in the pumping region, e.g., the capillary with charged walls between the electrodes. As with all pumps, the EO pump builds up a pressure, which can be used to drive the flow in the rest of a microfluidic system (without an applied field), but a short distance after the EOP the flow takes on a parabolic velocity profile. Even when integrated into the chip, the sample transport only gains the benefits of plug-like flow if the whole sample transport occurs between electrodes and if the pumping region has an almost constant zeta-potential (within the EO pump).^{37,38} A well-designed microfluidic EO system can offer precise flow control, but during transport the sample will be exposed to an electric

field so it is desirable to maximize the electroosmotic mobility in order to minimize unwanted electrophoretic separation of the sample during transport.

Figure 6 Illustration of an electroosmotic flow rate measurement in a microfluidic device. A. Schematic of two reservoirs containing solution of different ionic strength (low to the left and high to the right) connected via a capillary functioning as an EOP. B. Voltage and ideal ionic resistance vs time plot for a channel filled with solution of high ionic concentration at $t = 0$ with EO flow from high concentration to low concentration (0-500 sec), then flow from low to high (500-1500 sec) and then high to low again (between 1500 and 2500 sec).

Measurement of the EO flow speed in a microfluidic system can be carried out in a number of ways, e.g., by adding particles or colored dye to trace with optical imaging. A method based on current monitoring utilizes the same electrodes used to generate the EOF and has a small impact on the system. For a microfluidic system with an EOP operating continuously at a set potential, kept at the same temperature and containing the same ion-containing solution, the current should quickly stabilize to a constant value. Replacing the solution with an identical solution of slightly higher ionic strength (a higher concentration of charged particles) should yield a new, slightly higher steady-state current. By filling reservoirs at opposite ends of the pumping region with solutions of slightly different ionic strengths, as seen in figure 6A, the current will reach one value if pumping continues in one direction and another value for fluid transport in the opposite direction as the solutions completely displace each other in the pumping region. While one solution is being displaced by EOF, a high/low ionic strength front moves through the pumping region; the ionic

resistance (voltage divided by current) across the channel corresponds to the position of the front. If the front moves at a constant velocity, the ionic resistance across the pumping region will change at a constant rate toward the new value, see 6B. If the dimensions of the pumping region are known, then the flow rate can be calculated by measuring this current as a function of time.³⁹

4 Electroosmotic pumps

4.1 Electroosmotic pumping regions

4.1.1 Capillary EOPs

Electroosmotic pumps (EOPs) can be designed in a number of ways, depending on the desired pump characteristics. This chapter will mainly focus on pumping regions that are cylindrical channels and porous monoliths. The simplest form of an electroosmotic pump is a fused silica capillary (typically 2 μm to 200 μm inner diameter) connecting two wells, each containing an electrode. If the system is filled with a water based solution above pH 5.5,⁴⁰ the surface of the silica is negatively charged (pKa for silanol groups is typically 5-6) and an electric double layer (EDL) forms (see section 3.2.1). Applying an electric potential between the electrodes creates an electric field in the capillary, which creates an electric force on the positively charged diffuse layer. This, in turn, generates movement of the ions and, through viscous interaction, the bulk fluid, assuming the radius of the capillary is below $\sim 100 \mu\text{m}$.^{41,42} For the fused silica capillary, the only pump property that changes when the diameter decreases is the resistance to pressure driven flow, until the capillary shrinks to a size where the electric double layers takes up a significant part of the cross sectional area, at which point the presented models and equations for EDL and EOF no longer apply.^{43,44} However, both the thickness and charge of the EDL, and therefore zeta-potential, change with the charge density on the surface. With chemically modified surfaces, the zeta potential can be tailored to yield different EOF directions in relation to the applied field in different regions of a microfluidic device. It is also possible to generate gradients of zeta-potential along channels; however, for efficient and plug-like EOF the zeta-potentials in the pumping region should be kept as similar as possible.⁴⁵ For a channel to be able to generate EOF, the channels should be produced in a material such as glass that becomes charged in contact with water. If the bulk material in the microfluidic device does not, by itself, become charged when in contact with water, EOF can be achieved by incorporating a capillary, e.g., fused silica, into the device or by functionalizing the surfaces in the pumping regions with molecules that provide the desired surface charge.

4.1.2 Porous media EOPs

As mentioned in previous chapters, there are both advantages and drawbacks to scaling down a microfluidic system. One design alternative is to use frits, porous monoliths, or pack spheres to

increase the surface to volume ratio (SAV) of the pumping region. Packing glass spheres inside a region of a microfluidic device requires that the spheres are either held in place by two porous plugs or that the system is designed in a way that the spheres never experience forces that would displace them. A more robust alternative is to create a frit, a structure of closely packed μm sized glass spheres sintered together.⁴⁶ In many designs, it would be desirable to pack glass spheres inside the device and sinter them together once in place. Unfortunately in most cases, the temperatures required to sinter the spheres together would be too high for the materials in the device, with the liquid temperature for normal bottle glass at 1 000 °C and fused silica at 1 700 °C.⁴⁷ However, just like the previously mentioned fused silica capillaries, frits can be integrated into the device during production, with the rest of the microfluidic device built around the frits pumping region. A low-temperature and bottom-up alternative to sintered frits is the sol-gel process for creating porous monoliths. The term sol-gel refers to a solution (sol) that builds an integrated network (gel).^{48,49} In the papers of this thesis, potassium silicate (KSi) monoliths have been used, but the sol-gel process is used in many research fields to create a variety of structures of different chemical composition. In Paper 2, porous potassium silicate monoliths were produced inside fused silica capillaries, which later were used as EOPs in microfluidic devices. The temperatures required for this sol-gel process were all below 100 °C, making it possible to carry out the curing process without damaging the rest of the PDMS/glass devices. However, the time required for a sol-gel process to form monoliths generally decreases as temperature increases. It should be noted, as shown in Paper 3, that the monoliths produced can be significantly smaller than the structures they were produced in if monolith material does not form on the structure's walls during the sol-gel process, compare KSi monoliths in fused silica and PDMS structures. Monoliths with significantly smaller diameters than the diameter of the channels in which they were formed would be of limited use in most devices, so the type of monoliths in Paper 3 need to be produced first and then integrated into microfluidic systems, whereas the monoliths in Paper 2, in theory, could be produced directly inside a device. It is clear that all the options for porous EOPs with higher SAV increase the complexity of production compared to the single capillary. Unfortunately, the single "large diameter" capillary EOP has limited use in real world applications, as will be covered in the next section.

4.2 Hydrodynamic resistance of EOPs

4.2.1 Flow profiles of EOF and pressure driven flow

An EOP operating in a system with pressure gradients will create flow that has components from both elektrokinetic and pressure forces. This usually means that the pressure driven component counteracts the EOF and is undesirable in most microfluidic designs. In chapter 2, the main impacts of miniaturization in fluidic systems were discussed, with a focus on the surface area to volume ratio (SAV). Reducing the diameter of a channel or using porous media, e.g., silicate monoliths or glass frits, can reduce the average channel radius several orders of magnitude, e.g., from 100 μm to 1 μm . The Poiseuille law, equation (4.1), describes the pressure driven laminar flow of incompressible and Newtonian fluids through cylindrical channels.

$$\Delta P = \frac{8\eta L Q}{\pi r^4} \quad (4.1)$$

Here the flow rate is Q , fluid viscosity η , the pressure drop along the channel ΔP , the channel length L , radius r and cross-sectional area A (πr^2). As seen in equation (4.1), the pressure difference required to achieve a certain flow rate is proportional to the fluid viscosity η and channel length L , but inversely proportional to the fourth power of the radius (r^4). The change in cross-sectional area of the channel contributes r^2 , which means the average flow velocity for a given pressure difference is proportional to the remaining r^2 .

In microfluidic systems, especially using EOPs, where samples are to be transported in a precise manner, working with the flow velocity can often be more intuitive than the total flow rate. Equation (4.1) can be rearranged as in equation (4.2) to show the average flow velocity (flow rate divided by cross sectional area).

$$\bar{u} = \frac{Q}{A} = \frac{\Delta P r^2}{8\eta L} \quad (4.2)$$

The Poiseuille law has been rearranged to show how the other values impact average flow velocity (Q/A), with $\Delta P/L$ being the pressure gradient along the channel, viscosity η related to the fluid's resistance to laminar flow and radius r . Figure 2 in chapter 2 showed how flow velocity profiles change with radius, without going into further details about laminar flow. In this section,

the aim is to both describe the flow profiles of pressure driven laminar flow and electroosmotic flow, as well as the impact on flow in regions where both types of flow are significant.

The first step in estimating the velocity flow profile of a system is to determine which effects are of greatest importance during operation. The Reynolds number (Re) is a quantity in fluid mechanics describing the relationship between inertial forces and viscous forces that helps predict if flow in a region will be laminar or turbulent, with Re values below 2100 considered laminar.^{50,51} The equation for the Reynolds number in a cylindrical pipe is shown in equation (4.3), with ρ as the density of fluid and u the mean velocity of the fluid. A cylinder with 100 μm inner diameter filled with water reaches a Re of 2100 when the average flow velocity is 21 m/s, which is significantly faster than the flowrates used in most microfluidic systems. Equation (4.3) indicates that Re increases with radius, and 100 μm is a relatively large channel diameter, so laminar flow can be expected in the vast majority of microfluidic devices.

$$Re = \frac{\rho r Q}{2\eta A} = \frac{\rho r}{2\eta} u \quad (4.3)$$

Pressure driven laminar flow in cylindrical channels has a parabolic flow velocity profile, with the maximum velocity in the center. Equation (4.4) describes the axial flow velocity u_R as a function of distance R from the center, with a maximum value at the channel center and velocity of zero for $R = r$ (at the wall).^{52,53} Integrating the flow velocity over the circular cross-section reveals that the average flow velocity is half of the maximum velocity, recovering equation (4.2). Both maximum and average flow velocity are proportional to the radius squared (r^2), presuming that the pressure gradient remains the same.

$$u_R = \frac{1}{4\eta} \frac{\Delta P}{L} (r^2 - R^2) \quad (4.4)$$

The electroosmotic flow in a cylindrical channel is fundamentally different from pressure driven laminar flow, where an external force on the fluid is counteracted by the friction forces experienced by fluid layers at different distance from the stationary walls. The forces generating EOF appear as an electric field is applied along the channel and Coulomb forces act upon the charged particles in the diffuse double layer, the unbound outer region of the electric double layer. Unlike the neutral

bulk solution, the diffuse layer has a net charge with polarity opposite that of the wall, causing the majority of ions in the diffuse layer, plus the solvent molecules they are solvated by, to move in the direction of the Coulomb forces. Inside the diffuse layer the movement of these solvated ions causes the fluid to move through viscous interaction. In addition to the forces from ions moving in an electric field, the diffuse layer experiences shear forces from (and imposes shear forces on) the surrounding fluid layers if they move at a different velocity. Inside the diffuse layer, the flow velocity increases with distance from the stationary wall. The theory describing the flow velocity profile inside the diffuse layer is beyond the scope of this text, but there are theoretical papers on electroosmotic flow for the interested reader.⁵⁴⁻⁵⁶ Where the diffuse layer ends and bulk fluid starts, the solution is effectively charge neutral and the net Coulomb force is zero. The bulk of the solution only experiences forces from the adjacent diffuse fluid layers. In a cylindrical channel with a radius below 100 μm these shear forces eventually cause the entire bulk (everywhere but near the wall) to move at the same velocity as the edge of the diffuse layer, since fluid layers in the bulk fluid will experience net forces (accelerate) until all layers inside the EDL have the same flow velocity. For such channels, with water based electrolyte and no pressure gradients, the steady state velocity flow profile is flat, with the same value from the edge of the diffuse layer as for the center of the channel. As the Debye length of the EDL normally is several orders of magnitude smaller than the channel radius, the average EO flow velocity can be considered equal to the maximum flow velocity.

Figure 7 A. Velocity flow profile in the pumping region of an electroosmotic pump with a radius much larger than the Debye length. B. Velocity flow profile of pressure driven laminar flow in the opposite direction. C. Velocity flow profile inside an EOP working against a significant pressure gradient, in the center of the channel the direction of the flow is opposite to the pumping direction.

In real world applications, electroosmotic pumps are often expected to operate against pressure gradients. As seen in figure 7 the desired plug-like flow profile can be severely deformed by pressure flow unless the EOP was designed to handle such pressures. Flow profiles for systems with EO flow in a pressure gradient are described by equation (4.5), where Φ is the potential in the channel, which is a function of the distance from the center R divided by the Debye length λ_D , also known as the Debye shielding distance. The first term in equation (4.5) is familiar as the pressure driven flow velocity, but the second term is the maximum EO flow velocity multiplied by a term that decreases inside the diffuse layer and reaches zero at the slip plane. For channels where the radius is much larger than the Debye length, the impact of the term containing the potential Φ becomes negligible for the majority of the fluid layers and equation (3.2) can be used for the EOF term. However, already at radius ten times the Debye shielding distance ($r/\lambda_D = 10$), the flow profile loses its plug-like shape, which for EDLs on silica surfaces in deionized water can occur for channels on the μm scale.^{57,58} The Debye length decreases with ionic strength and is approximately 10 nm for an aqueous solution with 1 mM NaCl. While the electrolytes in most biological systems have ionic strengths closer to 100 mM NaCl which corresponds to a Debye length of 1 nm.⁵⁹

$$u_R = \frac{\Delta P}{4\eta L}(r^2 - R^2) - \frac{\varepsilon E \zeta}{\eta} \left(1 - \frac{\phi(R/\lambda_D)}{\zeta}\right) \quad (4.5)$$

4.2.2 Measurement of hydrodynamic resistance

Hydrodynamic resistance R_H relates the pressure difference to the flow rate through a given geometry as seen in equation (4.6). This formulation is analogous with electronic resistance and the relationship between potential and current. From an experimentalist's perspective, measuring the hydrodynamic resistance is straightforward; either apply a constant pressure and measure the flow rate, or run a constant flow and measure the pressure difference. There are many equations describing R_H for different geometries, but here the focus remains on cylindrical channels. By rearranging equation (4.1), the hydrodynamic resistance of a cylindrical channel can be written as equation (4.7). Of all the values of in equation (4.7), the radius of the microstructures will often be the only value that is challenging to measure without special equipment, but, since r is the only unknown, it can be calculated from the rest, assuming the radius is constant.

$$R_H = \frac{\Delta P}{Q} \quad (4.6)$$

$$\Delta P = \frac{8\eta L Q}{\pi r^4} \rightarrow R_H = \frac{8\eta L}{\pi r^4} \quad (4.7)$$

In order to measure the hydrodynamic resistance of a microfluidic device, or a component thereof, a reliable pump and a pressure sensor with an appropriate range is required. The fluidic system between the pump and the part to be measured should be as short as possible and contain as few connections or interfaces as possible, both to decrease the risk of leakages and to minimize radial expansion of tubing due to internal pressure. Ideally, a syringe pump with a max capacity slightly higher than the volume pushed through the part during the measurement is used. The main advantage of a syringe pump is that, when using an incompressible fluid and the system does not leak, the volume of fluid pushed out of the syringe pump and the volume entering the part to be measured are equal, at least after the initial pressure buildup. A microfluidic pressure sensor located inside the fluidic system on the pump side of the part being characterized monitors the pressure difference during constant flow. Thanks to the hydrodynamic resistance of cylindrical channels being defined in equation (4.7), the accuracy of R_H measurement systems can easily be verified using a known fused silica capillary of the appropriate length and inner diameter.

Pumping against pressure is one of the main challenges of EOP design, and the term maximum backpressure is often stated among pump characteristics. However, it may not be completely clear what the maximum backpressure is for practical purposes. From figure 7, it is apparent that the flow in the center of a channel can oppose the EO flow while still resulting in a net flow in the direction of the EO flow, which would result in significant band broadening if transporting a band of sample. An alternative “maximum backpressure” could be the pressure where the whole flow profile is in the direction of the EOF or zero, with the fluid in the center of the channel not moving. The most demanding and highly subjective definition would be the maximum pressure where the velocity flow-profile still is “plug-like”. Using equation 4.5 to find the pressure gradient that yields no flow in the center of the channel for our previously-used 100 μm inner diameter capillary, filled with 10 mM NaCl in water and with an electric field of 5 000 V/m, yields a pressure gradient of 19 kPa/m, or slightly below 0.2 bar per meter. The pressure resistance scales with the inverse of

the radius squared so a 10 μm channel could handle 20 bar/m under the same conditions and a system with 1 μm channels could theoretically handle 2000 bar/m before pressure driven flow would decrease the EOF velocity flow profile at the center to zero.

4.3 Porous monoliths

4.3.1 Monoliths in microfluidics

Monoliths are structures consisting of an interconnected network of cross-linked particles, produced through a bottom up sol-gel process. In microfluidics, monoliths are typically porous and silica- or polymer-based, both with a wide variety of options for functionalization depending on the application. An advantage of monoliths comes from their ease of production, in which three-dimensional microstructures are created (self-assembled) without requiring advanced equipment. Since monoliths are created when a solution turns into a gel via cross-linking, the solution can be introduced into a microchannel, allowing the monolith to form “*in situ*”; a common method is to utilize photo-initiated radical polymerization where UV-light is used to create monolith structures where desired. An alternative to starting the polymerization with light is thermally-initiated polymerization, which requires less equipment but often lacks the spatial control. The most straightforward alternatives are the sol-gel solutions that start polymerization at room temperature, e.g., the aqueous potassium silicate/formamide mixtures used in paper 2 and paper 3, which only requires vortexing and centrifugation followed by injection into the desired location.⁶⁰

4.3.2 Monoliths produced from potassium silicate and formamide mixtures

Paper 2 describes the fabrication of potassium silicate monoliths inside cylindrical fused silica capillaries. In summary, 100 μm i.d. fused silica capillaries were rinsed with 1 M NaOH, 1 M HCl and deionized water prior to being filled with mixtures containing water, potassium silicate and formamide in various ratios. The capillary ends were capped and then the capillaries were kept at 90 °C for one hour, converting the mixtures into monoliths. A cross-section of a fused silica capillary with a monolith network is shown as the bright structure in figure 8A; the darker regions outside are the fused silica capillary. It should be noted that during monolith formation, the silica grew from the capillary wall to create a porous structure. In contrast, the potassium silicate monoliths in paper 3 were formed inside of PDMS molds, seen in figure 8B, and did not grow from the PDMS walls but instead formed free-standing monoliths with half the diameter of their molds.

The monoliths formed in PDMS had a less porous, apparently solid, outer layer and a more porous core. If the outer layer is solid, these monoliths would come with their own significantly thinner “fused silica capillary” facilitating integration into 3D printed devices and other forms of prototyping.

Figure 8 Potassium silicate monoliths, inside a fused silica capillary, A, and free-standing, B.

4.4 Asymmetric electroosmotic properties

4.4.1 Nanofluidics and flow rectification

The term rectification comes from the electrical device called a rectifier which converts alternating current (AC) into direct current (DC). For EOPs rectification refers to the difference between flow rates, or ion currents, through an object depending on which direction an electric field is applied, the rectification ratio is the ratio between operating the pump forward and backwards. The classic example of electrical rectification is the diode, where the current ratio for a small positive and negative potential can be several orders of magnitude. In microfluidics, rectification typically occurs in channels below 100 nm in diameter and normally rectification ratios are significantly smaller than for diodes. The most widely studied structures where EOF and ionic current rectification occurs have been thin membranes with conical nanopores of well-defined dimensions. As seen in figure 9, one end of the nanopore contains bulk fluid while all the fluid at the narrow end is part of an EDL, and these regions will have different transference numbers for cations and anions. Transference number, or ion transport number, is the fraction of the total electric current transported through an electrolyte by each type of ion. In this conical geometry, applying an electric

field of one polarity across a channel with differences in ion transport numbers results in charge accumulation, whereas the opposite polarity causes charge depletion, resulting in current rectification.⁶¹ For most microfluidic structures, the current rectification might not be important, but for the pumping regions of EOPs the rectification opens up new possibilities. An electroosmotic pump transports fluid by applying a potential across a pumping region, resulting in both transport of a volume of fluid and charge. Operating an EOP continually in the same direction will eventually generate undesired reactions at the electrodes, as discussed in chapter 5. Applying a square potential waveform across a non-rectifying EOP would result in the same transport of charge and fluid in both directions, resulting in zero net transport. So, if an EOP could be designed to rectify flow and current so that the volume transported per charge was different when operated in the forward and reverse directions, then the pump could move the same charge back and forth while still generating a net flow, albeit in a “two steps forward, one step back” manner.

Figure 9 Schematic of a conical nanopore with a negative surface charge and a positively charged diffuse layer due to an excess of cations. A cross-section at the left side of the nanopore contains mostly bulk solution whereas a cross section at the right side only contains solution inside the electric double layer.

4.4.2 Rectification in membranes and porous monoliths

As previously mentioned, the ionic current rectification through μm -thick membranes with conical pores of varying geometries have been studied extensively, and equations for electroosmotic flow velocities have been proposed.⁶¹ Rectification through porous monoliths is less well-understood due to their larger, more complex, and less uniform networks of channels than the well-defined conical nanopores. Sol-gel processes allow for bottom-up production of porous monoliths with sub

micrometer structures. Paper 3 investigates structures generating EO flow rectification created by producing potassium silicate monoliths with a tapered macroscopic geometry. The ability to mold porous monoliths into tapered conical geometries to produce rectifying structures, as shown in Paper 3, offers a cheaper and more accessible option than the nanopores created by anisotropic track-etching methods.⁶¹ It should also be noted that, for integration into microfluidic devices, the nanopore membranes must be integrated during device production and have limited thickness, while porous monoliths can be produced “*in situ*” and should be able take on a variety of geometries while maintaining rectifying properties.

5 Electrochemistry

The electrokinetic phenomena described in the previous chapters all require an electric field. By applying a potential between two electrodes in an electrolyte an electric field will initially form, but as charged species move towards their respective electrodes due to Coulomb forces the electric field strength in the bulk electrolyte will decrease. In order to maintain an electric field electrochemical reactions need to take place at the electrodes, reducing and oxidizing species at the same rate as charge is transported through the system. This chapter covers electrochemical cells, inert and electrochemically active electrode materials, and electrolysis of water together with its impact on microfluidic systems.

5.1 Faradaic processes

5.1.1 Galvanic and electrolytic cells

Faradaic current refers to the part of the current which requires species to undergo oxidation or reduction at an electrode/electrolyte interface. Electrochemical cells, systems with faradaic currents at their electrode/electrolyte interfaces, can be sorted into two categories based on their operation, electrolytic and galvanic cells. The simplest possible design of an electrochemical cell can be seen in figure 10, two electrodes in the same electrolyte connected with conducting wire via either a voltage source (like a battery) or an electrical load (like a lightbulb) depending on the type of cell. In electrochemical cells charge must be transported both as electrons in electrical conductors and as ions in an ionic conductor (an electrolyte). Since the electrons are unable to travel freely through the electrolyte between the electrodes, and ions are unable to move through electrical wiring, reactions need to take place at the electrode/electrolyte interfaces to convert the charges of ions to free electrons and vice versa. In galvanic cells the faradaic reactions (reactions causing a faradaic current) occur spontaneously as soon as the wire is connected to close the circuit. Galvanic cells can be designed with the purpose of energy storage, converting chemical energy to electrical energy, the most widely used examples being batteries and fuel cells. The electrolytic cell, on the other hand, does not generate a current when the electrodes are connected; electrolytic cells require an applied external potential for faradaic reactions to occur and charge to move through the system. Where galvanic cells store energy, electrolytic cells are used in processes where electrical energy is spent to achieve the desired chemical reaction (e.g., electrosynthesis). Examples where

electrolytic cells are used in industry include electroplating conductive objects and extracting aluminum from bauxite. Some devices can function as both galvanic and electrolytic cells, e.g., a rechargeable battery is a galvanic cell while used as a power source but an electrolytic cell while recharging. A microfluidic system with an EOP consisting of a pumping region and two electrodes requires an applied potential to function, and can be described as an electrolytic cell.⁶² However, for an EOP, the potential is applied to create an electric field which generates an ionic current through the system which causes the transport of fluid, and in most cases the faradaic reactions at the electrodes are simply a byproduct required to maintain the electric field. For typical microfluidic EOP designs, the faradaic reactions at the electrodes have a negative impact on the system.

Figure 10 An electrochemical cell with two electrodes connected via a voltage source. M and A represent the species in the electrolyte and the electrodes undergoing oxidation and reduction respectively.

5.1.2 Electrode/electrolyte interfaces

The two types of reactions which change the oxidation state of species are called reduction and oxidation, when one species is reduced and one is oxidized it is called a redox reaction. When a species is reduced, it receives electrons and its oxidation state is reduced. In oxidation the opposite occurs, a species loses electrons and its oxidation number increases, named after species being exposed to the highly electronegative oxygen atoms that steal electrons from most other species during reactions. In an electrolytic cell, the reduction reactions occur at the cathode, with the

electrode providing electrons for to the reaction, while oxidation occurs at the anode, with the electrode claiming the electrons freed by the reaction. In an electrolytic cell, an applied potential is required to create the faradaic current at the electrode/electrolyte interfaces. However, unlike the current through a simple electronic resistor, the relationship between current and applied potential is usually not linear for electrolytic cell. Several factors influence the relationship between the potential drop across the electrode/electrolyte interfaces and the faradaic currents, for instance the mass transfer of the species which undergoes reduction or oxidation at the lowest negative or positive potential. Knowing the relationship between current and potential drop at the electrode/electrolyte interface is of great interest in most electrochemical cells. Unfortunately knowing the applied potential and resulting current in a system like the one shown in figure 10 is not enough, as only the sum of the potential drops at the electrode/electrolyte interface are known, while the individual potential drops are not. This makes techniques for measuring the potential drops at electrodes, which govern the species able to undergo electrochemical reactions, an important part of electrochemistry.

In order to better understand the reactions at an interface and isolate an electrode from the rest of the system, measurement-wise, a passive reference electrode together with a potentiostat for controlling the applied potential can be used. A reference electrode has a very stable and well known potential difference between the electrode and electrolyte, under the condition that current is not driven through it. Unlike the previously discussed electrode/electrolyte interfaces, the reference electrode is often immersed in a specific electrolyte instead of being surrounded by the system's electrolyte. However, the reference electrode bathing in its electrolyte will not function unless it has an ionic connection with the electrolyte of the rest of the measurement, so porous junctions are used to allow ionic exchange with the bulk electrolyte, see figure 11. This will minimize fluid exchange and dilution of the unique reference electrolyte and prevent changes in the composition of the bulk electrolyte. In a three electrode setup the reference electrode is placed in the electrolyte and connected to an electrode where the electrode/electrolyte potential drop is to be measured (working electrode) via a high impedance voltmeter so that a minimal amount of current passes through the reference electrode. The other electrode, which is not being measured upon, is called the counter electrode, as seen in figure 11. In a normal three electrode measurement, a potential between the working and counter electrode is applied and then adjusted until the

voltmeter, connected between the reference and working electrode, measures the desired potential while monitoring the current, alternatively a constant current is maintained while the potential between reference and working electrode is recorded. Cyclic voltammetry (CV) is a measurement where a computer controlled potential is applied between working and counter electrodes and adjusted to change the potential between working and reference electrodes at a certain rate, effectively scanning current vs. voltage of the electrode/electrolyte interface. However, it should be noted that the voltage from such a measurement is the difference between the two electrode/electrolyte interfaces (working electrode/electrolyte and reference/unique-electrolyte) and not the absolute potential drop at the interface. Using a different reference electrode shifts the cyclic voltammogram along the potential-axis, but the relative positions of any other features remain unchanged.

Figure 11 Three electrode setup, with working, reference and counter electrodes. All electrodes are connected to a potentiostat working to maintain the desired potential between the working and the reference electrode during measurements.

5.1.3 Electrolysis of solvent

Microfluidic devices containing EOPs normally have electrodes made of inert metals that do not undergo reduction or oxidation, and use water-based electrolytes containing low concentrations of species that easily undergo redox reactions. For this type of system, the reactions at the electrode/electrolyte interfaces are dominated by the electrolysis of water, half-reactions shown in

equations (2.1) and (2.2) in chapter 2. This makes the potentials at which the reduction and oxidation of water occur of particular interest, and is described as water's electrochemical stability window, as seen in the Pourbaix diagram in figure 12.⁶³ The potentials (vs. a standard hydrogen reference electrode) required for the redox reactions depend on pH, but the difference between the potential when reduction and oxidation start remains around 1.23 V. Unless the current per electrode area is high, the potential drops at the electrode/electrolyte interfaces in a water-based solution should be close to the potentials required to reduce or oxidize water. This means that species that only undergo electrochemistry at significantly larger potentials than water (either above or below water's stability window) are unlikely to undergo reduction or oxidation. Similarly, any species that react within the stability window of water will risk undergoing electrochemical reactions should they come in contact with the electrodes.

Figure 12 Pourbaix diagram showing the electrochemical stability window of water. Potential vs SHE required to reduce or oxidize water vs pH. Chemical equations show the preferential gas generating reactions at high and low pH.

5.2 Metal electrodes

5.2.1 Metals commonly used in electrochemistry

Electrodes have historically been made out of metal and, in many types of early electrochemical cells, the metals themselves were involved in the electrochemical reactions. The use of reactive metal electrodes in galvanic cells is useful for energy storage, e.g., lead batteries with lead and lead oxide electrodes in a sulfuric acid electrolyte. Certain electrolytic cells also utilize reactive metal electrodes to facilitate the reaction at the other electrode. In this function they are called sacrificial electrodes.⁶⁴ Some common reactive electrode materials are copper, zinc, lead and silver. On the opposite side of the reactive spectra are the inert electrodes that conduct electricity but do not partake in any reactions under normal conditions. The main advantage of inert electrodes is that they are never consumed and never need to be “recharged” to maintain their function. Popular inert electrode materials are platinum, gold, rhodium and graphite (technically not a metal)

5.2.2 Metal electrodes in microfluidic systems

In microfluidic systems, the reactions at typical metal electrodes either perform electrochemistry on the solvent or species therein, or risk releasing metal ions into the electrolyte. Inert electrodes are the natural choice if the release of metal-ions into the system is unacceptable, which often is the case in biomolecular and cell assays, or if both the reduction and oxidation of species in the electrolyte is desired. The drawback of inert metal electrodes is that the water-based electrolyte must undergo electrolysis instead, which is often undesirable as it can result in the generation of gas and pH-altering species, or species in the electrolyte can undergo undesired electrochemistry. In either case, the metal electrodes would ideally be placed far away from any sensitive samples or interaction sites; however, for microfluidic device designs this is seldom an option.

5.3 Electrochemically active polymer electrodes

5.3.1 Conductive polymers and electrochemically active electrodes

Conducting polymers are an alternative to metals as electrode materials. In conductive polymers, charges move along the carbon backbones of molecules with conjugated bonds, so-called conjugated polymers. These molecules have long chains with alternating single and double bonds with the overlapping p-orbitals which combine to form a molecule wide set of orbitals, called a π -conjugated system. The conjugated polymers can rather easily be oxidized or reduced (doped), a

state that can have high conductivity.⁶⁵ Just like reactive metal electrodes, some polymers can undergo reduction or oxidation within the stability window of water. These materials will be referred to as electrochemically active polymers (EAPs). A widely used EAP is poly(3,4-ethylenedioxythiophene)-poly(styrenesulfonate) (PEDOT:PSS), which was the electrode material used in Paper 1-3. The conjugated backbone of PEDOT is seen in figure 13A. Unlike the surface atoms of metal electrodes, which most of the time undergo reactions to become ions and released into the electrolyte, these polymer electrode materials consist of long molecules woven together to form a hydrogel in water based solutions. When an electron is added or removed from the conjugated molecule, no polymer material is released into the solution. However, with the new charge of the conjugated molecule induced by, e.g., oxidation, a counter ion (with opposite charge) needs to move into the polymer film or an ion with the same charge must move out, as seen in figure 13B. It should be noted that after continuous switching between an oxidized and neutral state, the counter ions inside a PEDOT hydrogel will correspond to the ions of the electrolyte, and if exposed to a different electrolyte, these ions will release once switched. One of the main differences between reduction and oxidation of EPAs like poly(3,4-ethylenedioxythiophene), or PEDOT, and electrolysis of water are the potential drops required to create a faradaic current. An electrode made with commercially available PEDOT:PSS solutions will contain PEDOT in both its neutral (PEDOT^0) and oxidized (PEDOT^+) state, making both reduction and oxidation possible. As seen in the voltammogram in figure 14, the inert platinum electrode in an aqueous 20 mM NaCl solution requires an electrode/electrolyte potential almost 1 V away from the potential of an Ag/AgCl reference electrode before current increases significantly. For the PEDOT electrode, even a small change in potential across the electrode/electrolyte interface gives rise to a current and the oxidation potential for PEDOT^0 and reduction of PEDOT^+ to PEDOT^0 , marked with arrows, occur much closer together than the previously discussed 1.23 V for electrolysis of water.

Figure 13 A. Conjugated backbone of poly(3,4-ethylenedioxythiophene) (PEDOT). B. Electrolytic cell with PEDOT:PSS electrodes. C. Faradaic reaction in PEDOT:PSS electrodes.

Figure 14 Cyclic Voltammetry of PEDOT and Pt electrodes in 20 mM NaCl aqueous solution with a sweep rate of 25 mV/s. Arrows mark the oxidation peak of PEDOT^0 and reduction peak of PEDOT^+ . Reproduced with permission.⁶⁶

5.3.2 Capacity limitation of electrochemically active electrodes

Electrochemically-active polymer electrodes have a limited capacity – the “number” of reactions that can be carried out to drive current in one direction is finite. While the polymer electrodes do not themselves transform into soluble species like most reactive metal electrodes, EAPs still have a finite number of molecules able to undergo reduction and oxidation. This is in contrast to a system that consumes the electrolyte (electrolysis), which is seemingly infinite in laboratory-scale devices.

After driving a significant amount of charge in one direction in an electrochemical cell with EAPs, when (almost) all of the polymer units in one or both electrodes have switched state to maintain the faradaic current, the polymer will stop undergoing electrochemistry itself. Assuming the polymer still conducts electrons in this state, the electrode will continue functioning as an inert electrode with electrochemistry on the electrolyte taking over. Reaching this stage is highly undesirable, as all the advantages of using an EAP electrode compared to an inert electrode are lost. There are several ways to design a system to operate the cell within the capacity limits of the electrodes, maintaining the advantages of electrochemically active polymer electrodes. The most straightforward option is to avoid constant current (DC) operation, but rather design the system so that the direction of the current can alternate, moving the same ionic charge back and forth between electrodes and avoiding the electrodes from becoming completely switched to one state or the other. In microfluidic systems the alternating current operation is typically achieved by applying a sine or square potential waveform. Unfortunately, it is not always possible to design microfluidic systems to allow for AC operation; however, for existing microfluidic systems with integrated metal electrodes and alternating current, e.g., dielectrophoresis capture, switching to EAP electrodes could be an interesting option. The brute force option to avoid depleting the EAP electrodes is to increase their size until the capacity of the electrodes exceeds the capacity required for operation. Scaling the EAP electrode to match the charge transport requirements of the measurement protocol can be a pragmatic solution for one-use disposable lab-on-a-chip devices. An alternative that lands somewhere between AC operation and scaling the electrodes is a protocol in which the current is driven in one direction during operation, but where the electrodes are “reset”, via a reverse current, between sessions of active operation. The resetting or switching back of the EAP electrodes would be the equivalent of recharging a battery.

5.3.3 Lifetime of electrochemically active electrodes

For many of the systems previously discussed, replacing traditional metal electrodes with electrochemically active polymer electrodes can add a level of uncertainty with regards to lifetime and operational performance. With an inert electrode, the reactions take place in the electrolyte which is replaced over time. Even with a reactive metal electrode, the loss of electrode material is predictable; however, the molecules in EAP electrodes are only effectively part of the electrode if there is conductance between the molecule and the main part of the electrode. Depending on the electrochemically active material, there can be several, more or less likely, reactions that remove or significantly decrease the conductance and ability to switch state of a polymer molecule, comparable to the decrease of capacity of rechargeable batteries. If a region of the electrode becomes isolated from the rest of the electrode, it and any molecules which were dependent on it for contact with the main electrode are no longer accessible. The commercially successful PEDOT has shown extraordinary resilience against both physical and chemical factors which can decrease performance over time compared to other EAPs. But even PEDOT shows a significant decrease in conductance if overoxidized.⁶⁷ In paper 1, a lifetime experiment was conducted on PEDOT:PSS electrodes used continuously for electroosmotic pumping during 37 days without any significant decreases of the electrodes' charge capacity. However, if the PEDOT electrodes had been exposed to higher temperatures, more extreme pH and more reactive species during operation, the electrode performance might have decreased significantly.

5.4 Integrated electrodes in microfluidic systems

5.4.1 Generation of gas and pH altering species

In large scale systems utilizing electrokinetic phenomena like capillary electrophoresis, where a sample is separated inside a microfluidic capillary, the electrodes can be placed in large volumes of electrolyte far away from the sample, as seen in figure 15A. The undesired reactions at the electrode/electrolyte interfaces, such as the electrolysis of water or reduction/oxidizing of species in samples or reagents, can easily be handled in this format. Excess gas generated due to solvent electrolysis can be dissolved in the solvent or exit via the electrolyte surface into the atmosphere. Any pH altering species can be neutralized by a buffer in the electrolyte, assuming that the electrolyte volume and buffer concentration are sufficient in relation to the charge being

transported through the system. Since the sample is injected into the capillary, separated in the capillary and detected in the capillary, the sample is at no risk of being exposed to the electrode or the nearby environment. For a microfluidic system with integrated electrodes, the microenvironment at the electrodes has a significantly larger impact on the rest of the system. The problems associated with gas generation inside microfluidic devices range from changes in the chemical environment at interaction sites to complete loss of function. While gas bubbles in a macroscopic system can form on the electrodes and float to the surface once large enough, the same gas bubbles in a microfluidic system, as seen in figure 15B, can either break the electrolyte connection between the electrodes or block off regions of the microfluidic system. The production of pH-altering species at integrated electrodes in microfluidic devices is often problematic, since many of these systems are used for biomolecular and cell assays. Most electrolytes used for biomolecules and cells are buffered, granting the solution some resistance to pH altering species. However, if enough pH altering species are produced, even the pH of the buffered solutions will change. Furthermore, for sensitive systems, even a partial depletion of the solutions buffering capacity can be problematic.

Figure 15 Gas generation at integrated electrodes, A. macroscopic system. B. Microfluidic system

5.4.2 Minimizing undesired electrochemical reactions in microfluidic devices

Even if the challenges of electrolysis of water are mitigated there is a possibility that other molecules in the electrolyte or sample can undergo electrochemistry at the electrodes, which, in most cases, is undesirable. As seen in figure 14 the inert electrodes have a larger potential across their electrode/electrolyte interfaces for a certain current compared to an electrochemically active

polymer electrodes like PEDOT. The smaller potential drop for PEDOT electrodes translates both to significantly less electrolysis of water but also less of any other possible electrochemical reactions with non-water species in the electrolyte. As previously described, the electrochemically active electrodes allow these unwanted oxidation and reduction processes to be minimized; however, EAP electrodes can transduce a limited quantity of charge before behaving like inert electrodes. A way to decrease the quantity of unwanted byproducts for fully switched EAP electrodes or inert electrodes is to alternate the applied polarity (low frequency square waveform), which will not reverse any gas generation of water electrolysis, but does limit the accumulation of H^+ and OH^- , decreasing the changes in pH. Ideally the applied electric field would be reversed before any of the EAP electrodes become fully switched, which would minimize the amount of these undesired reactions. Combining EAP electrodes, allowing for limited DC operation, with rectifying pumping regions (directional difference in EOF to current ratios), makes a new type of EOP operation possible. An EOP with EAP electrodes and a rectifying pumping region operating with a low frequency alternating current (square potential waveform modified to give zero net charge transfer) can create a net EOF in one direction indefinitely, albeit in a two steps forward, one step back manner, with minimum electrochemistry on the electrolyte.

6 Summary and background of papers

6.1 Paper 1: Electrolysis-reducing electrodes for electrokinetic devices

In the first paper, electrodes of drop cast PEDOT:PSS were used in an EOP setup with relatively large and open wells containing electrolyte connected via a fused silica capillary. The goal was to compare the generation of pH altering species during EOP operation between PEDOT and inert metal electrodes (platinum). Cyclic voltammetry employing the two electrode types indicates that electrolysis of water should be minimal for PEDOT electrodes until almost all of the conjugated polymer has switched state. Placing the platinum electrodes in the EOP setup with the same unbuffered electrolyte and intermittently measuring pH in both wells while pumping revealed that the pH changes significantly already at the first measurement point and pH keeps on increasing and decreasing in the anode and cathode wells, respectively. With PEDOT:PSS electrodes, the pH remained unchanged during EOP operation until approximately 10 Coulomb per gram of PEDOT (dry weight) had been transferred, after which the pH changed in a similar fashion to that observed with the platinum electrodes. The results showed that electrolysis of water was minimal as long as there was PEDOT readily available to undergo electrochemistry, and a relationship between the quantity of PEDOT in drop cast electrodes and the charge transported before electrolysis of water occurs was established. The former is important for the viability of integrated electrochemically active polymer electrodes, and the latter is important when designing devices with integrated PEDOT electrodes.

6.2 Paper 2: Electroosmotic Pumps with Frits Synthesized from Potassium Silicate

The second paper investigated a method aimed at creating an EOP with a high resistance to flow driven by external pressure gradients. If utilizing a 25-100 μm inner diameter fused silica capillary or a $>20\ \mu\text{m}$ wide channel produced with soft lithography as an EO pumping region is straightforward, and can pump at high flow velocities if the backpressure is low. However, in real world devices, the conditions are seldom ideal and an EOP can be required to operate against high pressure gradients, which the previously mentioned large diameter pumping regions are not suited for. In order to increase the surface area to volume ratio of the pumping area, a potassium silicate sol-gel process was used to create a network of μm -sized channels inside a fused silica capillary. In previously published papers, the porous potassium silicate material was most commonly used

as a thin “stopper” when packing capillaries with beads.⁶⁸ In one example, the potassium silicate (KSi) was used to produce a porous monolith for EO pumping; however, serious issues with reproducibility were reported.⁶⁹ In this paper, we report a process for producing porous KSi monoliths inside fused silica capillaries as well as identifying and removing the capillary sections that were incompletely filled with the monolith. Monoliths were produced in 100 μm i.d. capillaries with three different mixtures (varying water content) which all resulted in monoliths with similar electroosmotic mobility, and with pressure resistance equivalent to around 11 000 parallel capillaries with 0.85 μm inner diameter, increasing the hydrodynamic resistance over four orders or magnitude. Adding 10% more water to the KSi-mixture had a small impact on the monolith characteristics, indicating that the sol-gel process is robust with regards to variations in initial water content. The use of porous monoliths is an attractive fabrication method, as production is both rapid and μm structures can be created without the use of expensive equipment, e.g., track etch technology.

6.3 Paper 3: Electroosmotic flow rectification in macroscopically tapered monoliths

The third paper covers the production and characterization of stand-alone KSi monoliths with rectifying properties. The production of porous monoliths inside of fused silica capillaries described in the second paper is limited to the capillary geometry, the silica capillaries used by our group have had homogenous inner diameters and 360 μm outer diameter. For easier integration of EOPs based on KSi monoliths into microfluidic devices, we investigated ways to produce stand-alone porous monoliths, as opposed to those created inside a fused silica capillary. Injecting the KSi-mixture into cylindrical channels in PDMS and allowing the mixture to cure resulted in cylindrical monoliths with roughly half the diameter of the mold they were formed in. SEM images of the monolith cross-sections revealed porous center regions and denser edges, appearing to be solid. The possibility of creating porous monoliths with a solid outer layer with variable diameters was very attractive for integration into microfluidic devices based on 3D printed templates. During characterization of stand-alone monoliths, it was observed that a few monoliths had rectifying properties; for a certain applied electric field strength, the current was directionally dependent. We attempted to increase whatever mechanism created these random (uncontrolled) rectification properties by making the macroscopic geometry of the monoliths asymmetric. The natural choice

was to make molds that were conical or had conical regions, e.g., cast around needles or pipette tips. Porous KSi monoliths produced in conical molds were integrated into simple microfluidic devices with integrated PEDOT electrodes. Characterization of the devices showed a higher electroosmotic mobility, current and volume transport per charge in the base-to-tip (forward) direction than in the reverse direction. The ability to produce rectifying structures, used for, e.g., EO pumping, with a straightforward sol-gel process opens up several interesting possibilities for designing microfluidic systems.

6.4 Paper 4: Determination of fucose concentration in a lectin-based microfluidic displacement assay

The final paper describes a microfluidic assay designed to detect fucose, a biomarker for certain types of cancer and liver diseases found in urine. The polypeptide S2-AAL, based on the lectin from the fungus *Aleuria Aurantia*, was picked for its affinity towards fucose to make a displacement assay.⁷⁰ The detection region of the microfluidic device was functionalized with S2-AAL and then filled with a solution containing fluorescently marked lactoferrin, a globular protein with two polysaccharides that are fucosylated to varying degrees. Lactoferrin proteins can potentially have both polysaccharides bound to S2-AAL on the surface. As a flow of sample is introduced into the device, the concentration of free fucose in the sample changes the rate of release of lactoferrin from the surface, due to competition for the S2-AAL binding sites. As the fluorescently marked lactoferrin is displaced and transported from the detection region, the fluorescent intensity decreases, which can be detected with a fluorescence microscope. The dynamic range for this assay was 0.1 mM to 2 mM fucose, with the high end for fucose concentration in urine of a healthy person being 0.1 mM fucose. The change in fluorescence intensity vs. time for various sample fucose concentrations showed a clear concentration dependence, which was to be expected. However, exponential decay functions fit to the fluorescent intensity vs time data showed an unexpected concentration dependence on the steady-state value towards which the fits were approaching. For a simple model of a displacement assay, where only the interactions between lectin and fucose groups determine the removal of fluorescently marked species, two different samples containing fucose would both approach the same intensity, given enough time to displace the labeled species. According to our experiments, lower fucose concentrations appear unable to displace as much lactoferrin as the higher concentrations, as if the lower fucose concentration had no chance of

displacing a part of the bound lactoferrin population, while higher fucose concentrations could displace all of the lactoferrin. The use of a globular protein with varying numbers of fucosyl groups could be the reason for this unexpected increase in the complexity of the displacement over time for different fucose concentrations. The results from the microfluidic chip assay was compared to lectin-lactoferrin assays using a Biacore microfluidic surface plasmon resonance system and an ELISA platform. The use of “scaffolds” to attach multiple antigens for assays based on low affinity antigen-antibody interactions could allow for increased dynamic-range concentrations and make new assays viable.

The project that led to the paper on the lectin-based microfluidic displacement assay started with three research groups attempting to integrate their techniques into the same microfluidic platform. The modified lectin for detection of fucose was developed by Peter Pålsson et al, while on-chip excitation and optics, utilizing total internal reflection,⁷¹ with fluorescent detection via a cellphone camera was provided by Daniel Filippini et al., and finally our group would create the microfluidic platform and use EO pumps to transport some, or all, of the solutions into the detection area of the device. Unfortunately, the detection proved to be more challenging than expected in the early versions of the devices, which also had an unrefined fluid transport system that could have further undermined the detection setup. As a result, the detection via cellphone camera was abandoned in favor of a traditional microscope, and eventually the on-chip optics offering excitation via the interesting total internal reflection inside the glass substrate of the device was replaced with a fluorescent microscope in order to better detect fluorescent intensities. Similarly, while attempting to understand the fluorescence response, we used external syringe pumps. Once fucose detection reached an acceptable level, the project was long-overdue and the choice was made to focus on the best possible detection of fucose and do without integrating electroosmotic pumps.

6.5 Concluding remarks

Most of my practical work at Linköping University has been with soft lithography, designing, building and characterizing microfluidic glass/PDMS devices. Even though I do not feel that soft lithography justified a chapter of its own in this thesis I would like to take this opportunity to stress the importance of having such a flexible option for rapid prototyping. As for the future outlook for the work presented here, I feel that the most interesting option to pursue would be optimizing and

exploring new geometries for the stand-alone KSi porous monoliths. The use of channels inside PDMS as molds for KSi monoliths was suggested by my colleague Dr. Katarina Bengtsson, I was surprised to see it work the first time, and continue to be fascinated every time these miniature monolith cylinders form. The second finding of special interest is the option to attach multiple antigens to a “scaffold molecule” in order to change the dissociation dynamics for antigen-antibody-like interactions with low affinity. This could be used to modify the dynamic range of an assay, or make it easier to use “antigens-antibody” pairs that have so low affinity that an unacceptable amount of antigens would get removed during washing steps. Finally, I would like to see further progress in the use of integrated electrochemically active polymer electrodes in microfluidic devices for electrokinetic phenomena utilizing AC, such as dielectrophoretic capture, on which I did some initial, and unpublished, work.

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Acknowledgements

Supervisor **Nate Robinson** for giving me this opportunity and always encouraging me.

Co-supervisor **Mats Fahlman** for taking your time to talk with me and sharing your extensive knowledge of work in academia.

Past and present members of the **Transport and separations group**, especially Dr **Sara Nilsson** and Dr **Katarina Bengtsson** who added great value and enjoyment to my PhD studies.

Members of **Surface physics and chemistry division**, for many interesting discussions and giving me a good excuse to travel the world to meet you again.

All the **administrative staff at IFM**, especially **Kerstin Vestin** och **Karina Malmström**.

Skalman och **Ulf Frykman** and the rest of IT Support at IFM.

Both current and former members of my research school **Forum Scientium**. Special thanks to **Stefan Klintström**, the steward of the research school, for our discussions over the years.

The patrons of the rubber lab, which have shared joy and hardship while playing with soft lithography, blade coating and FL microscopy: **Anders Elfving**, **Fredrick Bäcklund**, **Jonas Christofferson**, **Jonas Bergqvist**, **Gunnar Bergström** (all doctors).

The **Coffee-club/Kaffeklubben** members for making fika a lot more fun.

The board game enthusiasts, **Adam**, **Daniel**, **Mark**, **Mats**, **Jesper**, **Joel**, **Simon**, **Troi** and **Ylva**, that help get my mind of work and instead relax with some resource optimization.

Dr **Jonatan Eriksson** for proofreading the thesis and being a great roommate during the early part of my PhD studies.

My parents, **Eva** and **Ragnar**, for everything.

Linnéa for your sage advice and all the hugs.

Finally a big thanks to **everyone** I forgot to acknowledge.

Papers

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