EXPANDING THE NEUROANALYTICAL TOOLKIT: ELECTROCHEMICAL MEASUREMENTS OF NEUROTRANSMITTERS USING POLY(3,4-ETHYLENEDIOXYTHIOPHENE) CONDUCTING POLYMER MATERIALS

by

Richard F. Vreeland

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As members of the Dissertation Committee, we certify that we have read the dissertation prepared by Richard F. Vreeland entitled ENHANCED ELECTROCHEMICAL MEASUREMENTS OF NEUROTRANSMITTERS USING POLY(3,4-ETHYLENEDIOXYTHIOPHENE) CONDUCTING POLYMER MATERIALS and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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SIGNED: Richard F. Vreeland
I dedicate this work to
my mother, Nancy, and
my father, Dick
for their endless love, encouragement, and support.

I also dedicate this work to my friend Stephanie Stanley,
who was an incredible friend and mentor.
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<td>5-HT</td>
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<td>5-hydroxyindole acetic acid</td>
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<td>aCSF</td>
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<td>HEPES</td>
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<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HVA</td>
<td>Homovanillic acid</td>
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<td>IACUC</td>
<td>Institutional Animal Care and Use Committees</td>
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<td>i.d.</td>
<td>Inner diameter</td>
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<td>i.p.</td>
<td>Interperitoneal</td>
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<td>IPA</td>
<td>Isopropanol</td>
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<td>ITO</td>
<td>Indium tin oxide</td>
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<td>i.v.</td>
<td>Intravenous</td>
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<tr>
<td>L-DOPA</td>
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<td>MFB</td>
<td>Medial forebrain bundle</td>
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<td>Medial-lateral</td>
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<td>Nucleus accumbens</td>
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<td>NE</td>
<td>Norepinephrine</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
<td>-----------</td>
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<tr>
<td>NT</td>
<td>Neurotransmitter</td>
</tr>
<tr>
<td>OLED</td>
<td>Organic light-emitting diode</td>
</tr>
<tr>
<td>PD</td>
<td>Parkinson's disease</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
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<td>PFC</td>
<td>Prefrontal cortex</td>
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<td>PEDOT</td>
<td>Poly(3,4-ethylenedioxythiophene)</td>
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<td>ProDOT</td>
<td>Poly(3,4-propylenedioxythiophene)</td>
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<td>P3-HT</td>
<td>Poly(3-hexylthiophene)</td>
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<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
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<tr>
<td>PC12</td>
<td>Rat adrenal pheochromocytoma</td>
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<tr>
<td>PSS</td>
<td>Polystyrenesulfonate</td>
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<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
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<tr>
<td>RIE</td>
<td>Reactive ion etching</td>
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<tr>
<td>RMS</td>
<td>Root mean square</td>
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<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<td>S/N</td>
<td>Signal-to-noise</td>
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<td>Transmission electron microscopy</td>
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<tr>
<td>TH</td>
<td>Tyrosine hydroxylase</td>
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<tr>
<td>TRIS</td>
<td>Tris(hydroxymethyl)aminomethane</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral tagmental area</td>
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ABSTRACT

The human brain is an extraordinarily complex organ. The process of neurotransmission gives rise to sensory experience, cognition, and decision-making. Many common diseases of the brain are incurable and their symptoms are poorly treated. To better understand the underlying molecular problems in disease states, sensitive, selective, and rapid measurements of biomolecules are needed. Given the complexity of making biological measurements in vitro or in vivo, inquiring scientists must choose measurement tools wisely. While traditional electrode materials have been used to great success, conducting polymers such as PEDOT are an excellent way to modify or improve existing measurement tools. The chemical, spatial, and temporal resolution of in vivo and in vitro measurements can be improved, all while increasing the longevity of the sensor. Compared to existing electrode materials, PEDOT is amenable to a larger variety of substrates, easier to process, inexpensive, and has excellent electrochemical behavior for the detection of neurotransmitters. We have demonstrated the utility of PEDOT by fabricating and characterizing the first device for the separation of biogenic amines, and the first device for high-throughput measurements of exocytosis from single PC12 cells. These devices will allow scientists to inexpensively and rapidly study the effects of pharmacological challenges to model systems in disease states. A PEDOT and Nafion composite polymer coating has been developed for microelectrodes, granting increased sensitivity and selectivity towards dopamine. These improvements resulted in the first in vivo electrochemical measurements of dopamine transients without administration of a reuptake inhibitor. Lastly, we have expanded the chemistry of polythiophenes by developing the synthesis of oligo-EDOT:Nafion nanoparticles. These nanoparticles are easily prepared, inexpensive, and enable quantitative spectroscopic interrogations of water content in organic solvents.
CHAPTER 1

INTRODUCTION
1.1 ABSTRACT

The human brain is an extraordinarily complex organ. The process of neurotransmission gives rise to sensory experience, cognition, and decision-making. Many common diseases of the brain are incurable and their symptoms are poorly treated. To better understand the underlying molecular problems in disease states, sensitive, selective, and rapid measurements of biomolecules are needed. While the past three decades in particular have been a time of explosive growth in the ability to make biological measurements in a complex matrix such as the brain, new and improved tools are needed to make higher throughput, more sensitive, and longer timescale measurements both in vitro and in vivo. In this work, an intrinsically conducting polymer, poly(3,4-ethylenedioxythiophene) (PEDOT) is broadly applied, generating new tools for better measurements from biogenic solutions, single cells, and in the brain. PEDOT offers a variety of attractive features to each of the measurement systems explored, including optical transparency, biocompatibility, tunability, low cost, and easy processability. The field of biosensors is expanded by developing a microwave etching system for the inexpensive fabrication of PEDOT microelectrodes. Additionally, we characterize and demonstrate the utility of the first ever stand-alone conducting polymer electrodes for neurotransmitter detection. These electrodes are employed for the first separations at a polymer and glass microchip with electrochemical detection. We also used novel microfabrication technology to build the first injection-molded microchip for high throughput measurements of exocytosis from PC12 cells. Lastly, we demonstrate a new optical sensing paradigm utilizing conducting polymer nanoparticles. Utilizing the tunability of conducting polymers, inexpensive and rapid quantitative spectroscopic determination of trace water in organic solvents is made possible.
1.2 THE BRAIN AND NEUROTRANSMISSION

The human brain is a remarkable cognitive instrument made up of about 100 billion neurons making 100 trillion connections.\(^1\) This organ evolved over the course of 500 million years and the earliest examples of organisms with a nervous system include tunicates and jawless fish.\(^2\) The purpose of their early brain was to maintain homeostasis including the medulla and pons (hindbrain), two structures that control respiration, reflexive motor movements, bladder control, swallowing, eye movement, facial control, breathing, heart rate, and blood pressure.\(^3\) In the simplest terms, the early brain evolved to respond to external stimulation and extend the survival of the organism. Interestingly, genes coding for enzymes in metabolic pathway to dopamine synthesis including tyrosine hydroxylase (TH) and aromatic amino acid decarboxylase (AADC) have been observed in protochordates, indicating that dopamine has been involved with nervous processes for at least 400-500 million years.\(^4,5\) This genetic conservation reflects the importance of chemical communication in virtually all vertebrate and invertebrate life. If the reflexive ability of an organism is a limiting factor in its survival, the faster it can process, integrate, and respond to information should extend the life of the organism. Thus, over the course of the next 250 million years the amygdala and hippocampus emerged (midbrain), enabling more complex behavior including emotion, primitive memory, decision making, and fear. In pre-humans, the forebrain including the cortex then evolved, allowing for higher order decision making and planning.

At the root of each step up in complexity, the underlying structure of the most basic fundamental unit of neurotransmission was preserved.\(^6\) This structure is the synapse, where two neurons meet and cell-to-cell communication occurs (Figure 1-1). A fast electrical stimulus (the action potential) is translated into a chemical signal with the release of neurotransmitter molecules. These neurotransmitters diffuse a short length
Figure 1-1: Schematic of where two neurons meet – at the synapse. Here, the action potential is converted from an electrical signal to a chemical signal with the fusion of vesicles to the pre-synaptic cell membrane and release of neurotransmitters into the synaptic cleft. After the neurotransmitters are released, they diffuse a short distance (20-100 nm) and bind to receptors on the postsynaptic neuron.
across the synapse (20-100 nm) and activate target receptors on the postsynaptic cell. The activation of these receptors results in the depolarization of post-synaptic neuron integrating this signal and propagating the action potential further.\(^7\)

Neurotransmitter release is an elaborate multi-step process including biosynthesis, packaging into vesicles, trafficking, docking, priming, and finally fusion.\(^8\)–\(^{12}\) Each step in this process relies on the delicate symphony of a large number of proteins. Given the number of genes and proteins involved in these processes, there are many opportunities for failure. In particular, systematic dysfunction in monoamine (such as dopamine) neurotransmission is observed Alzheimer's disease (AD),\(^{13}\)–\(^{17}\) Parkinson's disease (PD),\(^{18}\)–\(^{21}\) Huntington's disease (HD),\(^{22}\)–\(^{28}\) amyotrophic lateral sclerosis (ALS),\(^{29}\)–\(^{32}\) schizophrenia,\(^{33}\)–\(^{37}\) depression,\(^{38}\)–\(^{41}\) chronic pain,\(^{42}\)–\(^{46}\) and addiction.\(^{47}\)–\(^{51}\) These diseases are widely prevalent, affecting tens of millions of Americans yearly and have devastating symptoms which manifest in enormous economic and emotional costs.\(^{52}\)–\(^{56}\) In addition, some of these diseases are incurable, poorly understood at a mechanistic level, and the symptoms are generally poorly treated or managed.\(^{57}\)–\(^{63}\) In order to develop more effective treatments for these neurological disorders, a better understanding of these complex biological systems must first be established. To achieve this goal, the development of better measurement tools for neurotransmitter systems in healthy and disease states are required.

### 1.3 MEASUREMENTS IN BIOLOGICAL SYSTEMS

Progress in the understanding of any system often involves leaps in the ability to perform quantitative measurements. Neurochemical measurements are no exception to this, where the concept of quantal release of dopamine was proposed in 1967 by Bernard Katz not to be validated by direct measurement until the early 1990s by Mark Wightman.
in chromaffin cells.\textsuperscript{64–67} Whereas prior measurement technology was not capable of temporally resolving quantal release and making measurements at the millisecond timescale, the advancement of analog electronics and the development of the ultra-small electrodes led to an explosion of knowledge in the field of neurochemical dynamics.

Historically, fast neurotransmitter recordings have been made with the carbon-fiber microelectrode, a 5 µm by 50 µm (D x L) diameter carbon cylinder electrode. These microelectrodes are implanted into the brain, briefly left to heal, and then used for measurements. At the electrode, dopamine or other neurotransmitters are rapidly oxidized and reduced, and measurements can be made on the millisecond timescale with a spatial resolution appropriately small enough to measure sub-regions of animal model brains. Carbon’s electron transfer rate coefficients are suitably fast, the material is tolerated by biological systems, and the capacitance is low, permitting scan rates exceeding 1000 V/s. The carbon fiber microelectrode is an excellent tool, capable of differentiating redox-active species from one another \textit{i.e.} ascorbic acid, dopamine, or norepinephrine.\textsuperscript{68,69} These measurements have advanced the understanding of basic neurotransmission,\textsuperscript{70} the brain’s reward pathways,\textsuperscript{71–76} addiction and movement disorders,\textsuperscript{77–86} and a number of other important neurological problems. As the development of these tools is progressed further, carbon fiber microelectrodes have elucidated the subtleties of neurotransmission, revealing differentiating neurotransmitter dynamics in sub-regions of the same brain structure,\textsuperscript{87,88} the neuromodulatory functions of dopamine,\textsuperscript{89} and the co-action of tonic and phasic dopamine dynamics.\textsuperscript{90} However like any measurement tool, these electrodes must interface with the environment around the analyte of interest.
In the simplest terms, useful measurements from or in the brain must be performed 1) by removing a biological sample from the system \((\textit{in vitro})\) or 2) by making the measurement in a whole living organism \((\textit{in vivo})\). Both of these measurement paradigms pose unique challenges for a measurement scientist. For \textit{in vitro} measurements, the sensor must respond to the analyte of interest with a signal of appropriate magnitude on an appropriate timescale, and must not respond (or must respond in a differentiating manner) to interfering molecules. The sensor also must continue to be functional for some period of time for replicate measurements.

Many \textit{in vitro} measurements from single cells or clumps of cells are made in microfluidic devices. These microfluidic devices have a highly controlled geometry, are inexpensive to mass-produce, and are well suited to the analysis of mass-limited samples\(^91\text{--}94\). This last feature is critically important, because single exocytotic vesicles from model systems such as PC12 cells contain just tens to hundreds of zeptomoles of analyte\(^95\text{,}96\). The low total fluid volume of microfluidic devices allows for high local concentrations of analytes. Because electrochemical detection relies on the diffusion of material to the electrode, a high local concentration corresponds to a higher signal\(^97\). Microfluidic devices are also amenable to automation, parallel operation, and high throughput measurements\(^98\text{--}100\). With the advent of high-throughput synthesis methods, these three characteristics make microfluidic devices excellent tools for rapidly screening the efficacy of drug candidates to treat neurological diseases\(^101\).

Because microfluidic devices are inherently two-dimensional, and the carbon fiber microelectrode relies on the three-dimensional diffusion of analyte to the electrode, it is not an ideal detector for these systems. Furthermore, carbon fibers are not easily incorporated into the fabrication process of these devices. Traditionally, carbon- or
metal-based (such as pyrolized photoresist or gold) electrode materials have been photolithographically patterned into two-dimensional band electrodes and utilized as detectors in these microfluidic systems. These electrode materials have the advantages of an established microfabrication procedures and a fairly well described chemistry. While microfluidic devices have been fabricated with these materials as electrochemical detectors, there are several major hurdles preventing their widespread adoption. First, the heterogeneous electron transfer rate coefficient for several neurotransmitters at gold electrodes are poor, prompting additional surface modifications, such as by mercaptopropanoic acid. These modifications add undesirable additional fabrication steps, and are not robust. Additionally, the deposition of gold requires expensive equipment such as an electron beam evaporator with gold targets, or a sputter coater. The electrochemistry of pyrolized photoresist film electrodes is much closer to carbon fibers, resulting in better electron transfer kinetics. Unfortunately, the fabrication of pyrolized photoresist requires an expensive quartz tube-furnace. This furnace pyrolizes a film of lithographically-defined photoresist in a reducing atmosphere at temperatures exceeding 1000 °C. This process severely restricts the options for substrate material for these electrodes, as only quartz and silicon can withstand these temperatures. Silicon is not transparent, preventing optical microscopy of the device, and quartz is expensive, prone to shattering, and not available in many geometries. Ideally, the choice of substrate is not restricted by the fabrication procedure, and an ideal electrode material does not require these onerous steps, is inexpensive to manufacture, and maintains good electrochemical kinetics and behavior.

For in vivo measurements, the demands for a sensor material are even greater. The most important difference is that the sensor must interface directly with the biological environment. Given the complexity of the brain, a hugely variable distribution of
molecules are present at any given location. These molecules include gases, enzymes, cholesterol, steroid hormones, gangliosides, fatty acids, proteins, protein complexes, peptides, amino acids, and small molecules. While each of these types of molecules are essential to homeostatic brain function in different capacities, for a sensor to be useful, it must be able to tease apart the signal from these various molecules. Not only do these potential analyte molecules span several orders of magnitude in molecular weight, but they are present in concentration ranges from the femtomolar to milimolar range. Additionally, neurons, glial cells, and astrocytes both whole and fractured will be near the sensor during the measurement. With the disruption of tissue, the release of intracellular molecules such as enzymes into the extracellular space will change the microenvironment around the sensor, so the sensor must be able to overcome these challenges.

The brain also has a specific set of responses to the implantation of a foreign body such as a sensor. While previous work indicated that the direct and immediate response to the implantation of a foreign body is relatively minimal, recent work utilizing two-photon microscopy has demonstrated that even in the first 30 minutes of implantation, microglia will move at ~2 µm/minute towards the implant site to begin protecting and healing the brain (Figure 1-2 and Figure 1-3). These biological responses are complex and can be highly detrimental to sensor function. These responses include the recruitment of microglia which kill pathogenic organisms and secrete proteolytic enzymes to degrade cell debris, the secretion of neurotrophic factors including brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), inflammatory cytokines, and reactive oxygen intermediates (ROIs). The presence of these molecules can cause the inflammation, encapsulation of the foreign object in scar tissue.
Figure 1-2: Experimental setup for in vivo multiphoton imaging of microenvironment surrounding implanted microelectrodes in mouse cortex. (a) A small dental acrylic well is made on the skull over the cortex region of interest. A craniotomy is made in the center of the well, and the probe is inserted through the dural tissue at a 30–35° angle. (b) Light microscope image of the probe implanted into cortex at a 30° angle. (c) Four-shank Michigan probe drawing overlaid on an in vivo multiphoton image of transgenic GFP labeled microglial cells ∼100 μm below the surface of the cortex. The white square indicates the image domain of all following figures. (d) Normal ramified microglia morphology from an unimplanted brain. Scale bars indicate 100 μm. Adapted with permission.¹⁴⁷
Figure 1-3: Microglia processes advance toward the electrode immediately after insertion. Blue transparencies indicate location of implanted probes. Earlier time points (red) are overlaid with later time points (green). Time points are indicated in red (before) and green (after) above the top right corner of each panel. Green processes closer to the probe indicate microglial processes advancing toward the probe. Yellow cell bodies indicate that virtually no cell body movement occurs in the first hour. Scale bar is 100 μm. Adapted with permission.¹⁴⁷
or glial cells,\textsuperscript{154} or the death of nearby neuronal cells.\textsuperscript{155} In addition to this, the brain is one of the most highly perfused organs in the body due to the high demand for nutrients and oxygen.\textsuperscript{156,157} Upon implantation, some of these blood vessels are invariably punctured, releasing blood into the brain. The presence of each of these biological responses will inherently change the system being measured with an implanted probe.

It is therefore desirable to minimize these responses to measure the most native biological system possible. To this end, a variety of methods have been developed to mitigate the so-called foreign body response. These methods include controlled release of anti-inflammatory molecules like dexamethasone,\textsuperscript{158–160} biological or bioinspired coatings such as peptides or chitosan,\textsuperscript{161–168} surface modifications such as nanotubes or nanoparticles,\textsuperscript{169–176} and minimization of the physical size of the sensor.\textsuperscript{177–179} Each of these methods have distinct advantages over using unmodified interfaces, but usually suffer from one or more drawbacks such as a decreased sensitivity, slower temporal response, elevated cost, or fragility. In the past several years, an attractive alternative that addresses many of these problems has emerged: conductive polymers.

1.4 CONDUCTING POLYMERS

The vast majority of organic plastic materials are outstanding insulators. In a practical sense, these plastics largely find utility by virtue of their excellent physical properties. These properties include a high strength-to-weight ratio, shatter-resistance, optical transparency, flexibility, low-cost production, food safety, and insulating behavior. Starting in the 1960s, the field of conducting polymers began to emerge as a product of the work of R. Buvet and M. Jozefowicz. The field slowly matured, focusing on increasing the conductivity of polyaniline. This was until 1977 in the laboratory of Hideki Shirakawa, when a graduate student was polymerizing one of the simplest linear
conjugated molecules, acetylene, via a Ziegler-Natta catalyzed polymerization. He accidentally added over 1000 times the normal molar quantity of an AlEt₃ catalyst (Figure 1-4). Instead of forming the usual black powder, the reaction produced a thin, flexible, porous silvery plastic film, as the reaction proceeded only at the surface. These films were much more amenable to traditional analysis techniques than powders were, and were also more easily doped in the vapor phase by iodine or other electron-withdrawing elements. After more characterization, it was realized that the double bond configuration of these polymer films was highly dependent on the temperature during synthesis (Figure 1-5). These isomers were readily differentiated by infrared spectroscopy, and higher temperatures produced a trans-polyacetylene, with conductivities in the $10^{-5}$ to $10^{-4}$ S/cm range, while lower temperatures produced a cis-polyacetylene with conductivities in the $10^{-9}$ to $10^{-8}$ S/cm range. This work sparked a significant growth in the field of conductive polymers, and development continued into the next several decades.

Unfortunately, polyacetylene’s utility as a conductive material was not realized in a practical or commercial sense, mostly due to its insolubility, weak mechanical strength, and undesirable reactivity with ambient oxygen. Concurrently in the 1970s and 1980s, other groups worked on elucidating the various available monomer backbones for conducting polymers, and Bayer AG began to examine a particular monomer called 3,4-ethylenedioxythiophene (EDOT). When EDOT polymerized into poly(3,4-ethylenedioxythiophene) (PEDOT) (Figure 1-6), and was doped, it exhibited some exciting properties: stability against humidity, high temperatures, and oxygen. Importantly, by the 1990s it was also developed to be processed in water after being prepared with a polystyrenesulfonate (PSS) counter ion.
Figure 1-4: Ziegler-Natta catalyzed polymerization of acetylene.
**Figure 1-5:** Temperature dependence of polyacetylene polymerization.
Figure 1-6: Structure of oxidized poly(3,4-ethylenedioxythiophene) with a tosylate counter ion.
Conducting polymers are not inherently conductive. The introduction of charge carriers via p-doping, where the polymer becomes oxidized, or or n-doping, where the polymer is reduced, renders the material conductive.\textsuperscript{183} This doping process usually occurs during polymer synthesis and is more often n-doped than p-doped. The range of conductivities in conductive polymers extends from $10^{-9}$ (in the undoped state) up to $10^3$ or $10^4$ S/cm (Figure 1-7). Other work has demonstrated that a proportional relationship between doping level and conductivity is apparent, though an increase in conductivity by several orders of magnitude can often be achieved with as little as 1\% dopant by weight.\textsuperscript{184}

Once doped, electrons can move within and between chains of the polymer via delocalized $\pi$-conjugated systems including polarons or bipolarons, giving rise to the conductivity of these polymers.\textsuperscript{185–187}

Conducting polymers also offer several distinct advantages over traditional conductive materials such as carbon or metallic conductors. First, they are optically transparent. This optical transparency has allowed scientists to develop new sensor geometries that allow for simultaneous optical and electrochemical measurements. Second, they are easily processed, leading to the incorporation of PEDOT in devices with a variety of geometries. With the availability of spin coating, doctor blading, inkjet printing, and electrochemical deposition, PEDOT can be coated on nearly every 2 or 3 dimensional geometry. Third, they are flexible. This flexibility allows for the fabrication of flexible PEDOT-containing devices such as organic light-emitting diodes (OLEDs), solar cells, or the incorporation of PEDOT onto polydimethylsiloxane (PDMS) surfaces. Fourth, they are inexpensive, allowing the incorporation of PEDOT into disposable devices. Fifth, many of the properties of conducting polymers are tunable. This tunability spans an enormous that influence the electrical properties, solubility, morphology, optical properties, wettability, porosity, conductivity, average molecular weight ($M_\text{w}$), and
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<td></td>
</tr>
<tr>
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</tr>
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<td>10^-4</td>
<td>Silicon</td>
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<td>10^-18</td>
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<tr>
<td>10^-20</td>
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</table>

**Figure 1-7:** Electric conductivity of isolators, semiconductors, and conductive materials. Adapted with permission. 182
flexibility of the conducting polymer. Additional changes can be induced by changing the identity of the dopant ion or counter ion.

While conducting polymers emerged in the 1960s and 1970s, the first example of a conducting polymer used as an electrode was in 1980 at IBM, where a polyaniline film on a platinum wire was characterized by cyclic voltammetry. The redox reaction of the film cycled in 0.1 M sulfuric acid caused a noticeable color change, accompanied with three distinct oxidation and two distinct reduction peaks (Figure 1-8). The authors also oxidized ferrocene in acetonitrile, and noted that the film behaved well as an electrode.

PEDOT in particular has been shown to increase the adhesion of primary neurons to substrates. With respect to measuring a less perturbed system, this is a tangible advantage. Surface modifications of PEDOT by changing the dopant, doping level, roughness, or redox state can lessen the foreign body response, direct cell motility, promote growth, or liberate attached cells. PEDOT has also been used to covalently attach and physically immobilize enzymes or biomolecules of interest to the surface of the PEDOT. These change the function or surface properties of the electrode. In the past decade, materials scientists have employed post-synthesis treatment of PEDOT by weak organic acids. This has increased the record conductivities of PEDOT to 3300 S/cm - just a factor of 4 below the ubiquitous but inflexible indium tin oxide.

Much work has been done to engineer these conducting polymers into practical materials for a variety of uses including coatings for electrodes, but the use of conducting polymers as stand-alone electrochemical detectors has been comparably limited in scope. Examples of conducting polymer electrochemical detectors have been applied to gases such as nitric oxide or methanol vapor, creatinine in blood...
Figure 1-8: Cyclic voltammogram of a polyaniline film on a Pt surface in 0.1 M Et$_4$NBF$_4$-CH$_3$CN solution using a sodium chloride calomel reference electrode. Adapted with permission.$^{188}$
serum, herbicides such as atrazine, DNA, and a variety of other biomolecules. The use of conducting polymers as neurochemical detectors was first reported in 1986 by Ewing et. al. at polypyrrole-coated electrodes, and has since been incorporated into an enormous variety of detection schemes. Despite a 30-year history of conducting polymers being used for neurochemical detection, very few examples of stand-alone polymer electrodes have been published. Given the well-documented benefits of polymeric materials, this provides an excellent opportunity to exploit these benefits to enhance neurotransmitter measurement tools in a variety of systems.

1.5 USING CONDUCTIVE POLYMERS TO IMPROVE MEASUREMENTS

In this work, conducting polymers are broadly used to enhance the existing detection schemes of neurotransmitters in a variety of systems, including from single cells, sub-regions of the brain, and from whole-brain analysis systems. The field of biosensors is expanded by introducing and characterizing stand-alone PEDOT electrodes for the detection of neurotransmitters. The fabrication and processing of these conducting polymer electrodes is made more accessible to the average laboratory by introducing microwave-generated plasma etching. Furthermore, we introduce the first polymer and glass microchip for the rapid sensitive separation and detection of biogenic amines. The field of in vivo electrochemical measurements of dopamine is pushed forward with a novel conductive polymer-enhanced surface chemistry. These improvements increase the lifetime and sensitivity of the implanted electrode without sacrificing the temporal resolution of the measurement. This increased sensitivity allowed for the first ever measurement of transient dopamine release in the nucleus accumbens without the administration of a dopamine reuptake inhibitor. We have also expanded the field of microfluidics and microsensor fabrication by assembling and characterizing the first
microchip capable of capture, stimulation, and measurement of exocytosis from single PC12 cells. This microchip allows for high-throughput screening of drug candidates against disease model systems. Lastly, we have exploited the physical properties of conducting polymers to develop a new sensor platform based on easily synthesized polymer nanoparticles, and demonstrate their utility in the rapid, inexpensive optical detection of solvation environment. A novel synthetic route to polythiophenes was discovered in the preparation of these nanoparticles which appears to proceed through a disproportionation reaction. This shows great promise towards the development of a rapid high-yield catalytic synthesis scheme for polythiophenes.

Given the complexity of making biological measurements in vitro or in vivo, inquiring scientists must choose measurement tools wisely. While traditional electrode materials have been used to great success, conducting polymers such as PEDOT are an excellent way to modify or improve existing measurement tools. The chemical, spatial, and temporal resolution of in vivo and in vitro measurements can be improved, all while increasing the longevity of the sensor. Lastly, the diverse chemistry of conducting polymers provides ample opportunity for developing tools uniquely suited to each measurement challenge.
1.6 REFERENCES


CHAPTER 2

CHARACTERIZATION OF POLY(3,4-ETHYLENEDIOXYTHIOPHENE):TOSYLATE

CONDUCTIVE POLYMER ELECTRODES FOR TRANSMITTER DETECTION
2.1 ABSTRACT

Here, we investigate the physical and electrochemical properties of micropatterned Poly(3,4-ethylenedioxythiophene):tosylate (PEDOT:tosylate) microelectrodes for neurochemical detection. PEDOT:tosylate is a promising conductive polymer electrode material for chip-based bioanalytical applications such as capillary electrophoresis, high-performance liquid chromatography, and constant potential amperometry at living cells. Band electrodes with widths down to 3 µm were fabricated on polymer substrates using UV lithographic methods. The electrodes are electrochemically stable in a range between -200 mV and 700 mV vs. Ag/AgCl and show a relatively low resistance. A wide range of transmitters are shown to oxidize readily on the electrodes. Kinetic rate constants and half wave potentials are reported. The capacitance pr area was found to be high 1670 ± 130 µF/cm² compared to other thin film microelectrode materials. Finally, we use constant potential amperometry to measure the release of transmitters from a group of PC12 cells. The results show how the current response decreases for a series of stimulations with high K⁺ buffer.
2.2 INTRODUCTION

Neurotransmitters are an important class of molecules, enabling neurons to communicate with target cells using chemical signals. These signals are responsible for controlling and integrating sensory inputs into behavioral outputs. Direct measurement of these neurotransmitters and other signaling molecules provides insight into their role in biological systems. Indeed, many studies target a group of biogenic amines including dopamine, epinephrine, norepinephrine, histamine, and serotonin; all of these are electroactive and can be easily oxidized at an electrode. Due to the electroactive nature of these biogenic amines, electrochemical methods have been developed to measure them. Electrochemical methods are sensitive, quantitative, dynamic, and therefore widely used in bioanalytical approaches targeting transmitter detection.1–3 Electrochemical methods can be used in situ or they can be coupled to an off-line separation technique. An attractive detection scheme for mass-limited samples is capillary electrophoresis (CE) coupled to electrochemical detection. Electrochemical detection is an alternative to laser-induced-fluorescence detection due to the fact that many compounds can be detected without derivatization with a fluorophore. In addition, both the detector and instrumentation can be miniaturized.4 CE can be used to study transmitter distributions in small biological samples such as cells or even single vesicles.5 Coupling electrochemical detection to high-performance liquid chromatography (HPLC) delivers comparable benefits (see above).6 HPLC coupled with electrochemical detection can be used to rapidly measure transmitter concentrations in biological fluids.7 In other applications, the transmitter is measured as it released in vivo or in vitro. The release of transmitters from individual vesicles (a process called exocytosis) can be detected using constant potential amperometry performed at single cells.8 In this technique, a microelectrode is held close to the cell membrane and the
released transmitter is oxidized at the electrode surface giving rise to peaks on the resultant current versus time trace.

Chip-based devices offer advantages such as high throughput, automation, and the ability to integrate different analytical features into one device. In contrast, manual laboratory techniques, while they have yielded stable and successful tools for researchers, they require serial measurements and are difficult to automate. In the last decade a strong effort has been undertaken to develop chip-based devices to overcome these limitations. Micropatterned thin-film electrodes made of platinum, gold, carbon fibers, palladium, and pyrolyzed photoresist have been used in microfabricated devices for microchip CE applications. Chip-based devices for measuring exocytosis have also been developed using platinum, gold, mercapto-propeonic acid modified gold, indium tin oxide, and nitrogen-doped diamond-like-carbon as electrode materials. These electrodes are typically fabricated on a glass or silicon substrate which can be further modified by bonding a PDMS counterpart.

More recently, conductive polymers have emerged as an alternative to traditional electrode materials. Polymer electrodes combine the electrical properties of metals and semiconductors with the light weight and processing properties of common polymers. Among conductive polymers PEDOT is a promising material for biosensor devices focusing on neurotransmitter detection. It is biocompatible with a variety of different cells, conductive, transparent, and stable over long time period. Dopamine and other transmitters can be selectively detected on PEDOT-modified metal and glassy carbon electrodes in the presence of ascorbic acid and uric acid. Recently, Yang et al. demonstrated that transmitter release from single chromaffin cells can be detected at PEDOT:PSS microelectrodes.
The synthesis of PEDOT can be carried out by chemical or electrochemical polymerization of the monomer 3,4-ethylenedioxythiophene (EDOT). An easy and robust chemical polymerization method uses the monomer EDOT and iron tosylate (iron(III) p-toluenesulfonate) catalyst (commercially available from Clevios™) as dopant. By adding a small amount of base (pyridine) to the monomer and dopant, polymerization can be retarded, which allows the solution to be spincoated onto substrates. After baking and rinsing, a film with a positively charged polymer backbone balanced by negatively charged tosylate ions is formed.

PEDOT:tosylate electrodes can be fabricated by spin-coating a solution directly onto a variety of substrates. Electrodes can then be easily patterned and integrated in chips by bonding the substrates to counterparts containing a microfluidic channel system. Due to the relatively low resistivity of PEDOT:tosylate compared to other conductive polymers, no metal layer is needed for supporting the electrode. This allows for inexpensive and mass-producible all-polymer devices. In this work we characterize the physical and electrochemical properties of PEDOT:tosylate microelectrodes to examine their capacity for sensing transmitters. A variety of transmitters are shown to oxidize at the electrode surface, and heterogeneous rate constants and half wave potentials are reported. In addition, transmitter release from cells is measured. This opens the way for cheap and easy-to-fabricate all polymer devices for electrochemical detection of transmitters in various systems.
2.3 MATERIALS AND METHODS

2.3.1 ELECTRODE FABRICATION AND CHARACTERIZATION

Ø 50 mm polymer substrates were prepared by injection molding of TOPAS® 5013 Cyclic Olefin Copolymer (Topas Advanced Polymers GmbH). PEDOT:tosylate films were fabricated by spin-coating a solution of 6.5 mL Clevios™ CB 40 V2 (H.C.Starck), 2 mL butanol, 150 µL pyridine (Fluka) and 220 µL Clevios™ M V2 (H.C.Starck) onto the TOPAS substrates at 1000 rpm for 30 seconds. The substrates were baked on a hot plate at 70 °C to remove the remaining solvent and then washed in deionized water. Conductivity was measured with a four-point probe (Jandel Engineering Ltd) connected to a Keithley 2400 SourceMeter (Keithley Instruments Inc) using currents in the range 1 – 10 µA. The height of the PEDOT:tosylate layer was measured using a Dektak 8 profilometer (Veeco Instruments).

Electrodes were patterned by photolithography. AZ5214E photoresist was spin-coated on the PEDOT:tosylate-coated substrates at 4500 rpm for 30 s. The samples were soft baked on a hot plate at 95 °C for 5 minutes before being exposed in a Karl Suss MA6/BA6 Mask Aligner for 3 seconds (intensity 7.0 mW/cm²) and developed with AZ351B Developer. The photomask was ordered at Delta Mask B.V. After photolithographic patterning, the exposed PEDOT:tosylate was removed by reactive ion etching. The remaining resist was flood exposed in the Mask Aligner for 35 seconds and stripped off in an acetone bath for 5 minutes. The electrodes were rinsed thoroughly with deionized water and dried using compressed nitrogen. Band electrodes between 3 µm and 50 µm wide were fabricated.
2.3.2 ELECTROCHEMICAL MEASUREMENTS

Electrochemical measurements were made on a device constructed by placing a PDMS mold on a substrate (Figure 2-1). The potentiostat (Dagan Chem Clamp, Dagan Corporation) was connected to the electrode either by a Ag wire immersed in KCl or by using conductive epoxy glue (Conductive Epoxy, Chemtronics). The electroactive area of the PEDOT:tosylate film was defined by one of two methods: 1. The electrode protruded into a channel (125 µm x 110 µm, width x height) to create a band electrode (Figure 2-1) whose electroactive area was defined by the width of the channel or 2. the band electrode protruded into a second well which was filled with solution. The electrode length depended on the penetration depth into the well and it was measured using a microscope (Nikon TE2000U, Nikon Inc.). The two geometries enabled different boundary conditions to be probed; semi-infinite or finite conditions. Data was collected using custom programs written in LabVIEW (National Instruments, Austin, Texas). These were used to apply voltages and measure the resultant current. A Ag/AgCl reference electrode (RE-5B, BASi) was placed with the tip in the PDMS well and buffer solution was added. A pneumatically actuated six port HPLC valve (Vici, Austin, TX) was connected to the solution inlet (Figure 2-1). The valve controlled the injection of a bolus onto the electroactive area. A Harvard Ph.D. 2000 pump (Harvard Apparatus, Holliston, MA) controlled the flow rate of solutions (20 – 70 µL per minute). For rate constant determination, the background collected prior to the bolus reaching the electrode was subtracted from the cyclic voltammogram.

2.3.3 CHEMICALS

Electrochemical measurements were performed in phosphate-buffered saline (Lonza). Ferrocene methanol, carboxy-ferrocene, dopamine, epinephrine, norepinephrine,
**Figure 2-1**: Diagram of a microfluidic device for electrochemical measurements at Pedot:tosylate electrodes. Electrical contact to the Pedot:tosylate electrode is made through a Ag wire in a KCl solution. The electroactive area is defined by the geometry of the PDMS mold (not to scale). An optical image (inset) is the PDMS mold defining the electroactive area.
homovanilic acid, L-DOPA, 5-hydroxyindole acetic acid, serotonin, and histamine were purchased from Sigma-Aldrich and dissolved in PBS buffer.

### 2.3.4 CELL EXPERIMENTS

Passage 12 rat pheochromocytoma (PC12) cells were cultured on Collagen (type 1, Sigma-Aldrich) coated Nunclon T25 flasks (Nunc A/S). All cell medium was carefully removed by flushing the cells with a physiological buffer (150 mM NaCl, 5 mM KCl, 1.2 mM MgCl$_2$, 5 mM glucose, 10 mM HEPES and 2 mM CaCl$_2$, from SigmaAldrich). Electrodes were coated with Poly-L-Lysine (incubation in room temperature for 1 hour, 0.01 mg/mL) before being used for cell experiments. Fresh physiological buffer was added to the flask and the cells were loosened by gently agitating the flask. The cell suspension was added to the PDMS well containing the PEDOT:tosylate electrode and cells were allowed to sediment on the surface for 10 minutes. Release of transmitters was triggered by exchanging the physiological buffer with an isotonic K$^+$-rich buffer (KCl increased to 105 mM).

### 2.4 RESULTS AND DISCUSSION

#### 2.4.1 PHYSICAL PROPERTIES OF PEDOT:TOSYLATE FILMS

PEDOT:tosylate electrodes were fabricated on Topas substrates. The oxidized structure of the film can be seen in Scheme 1. Then, the physical and electrical properties of the PEDOT:tosylate electrodes were characterized (Table 2-1). The PEDOT:tosylate layer was measured to be 190 ± 10 nm high after the deposition of one layer. The height can be increased by adding multiple PEDOT:tosylate layers. Four point probe measurements revealed a sheet resistance of 113 ± 7 Ω (n =10) for newly prepared PEDOT:tosylate films. The measured sheet resistance corresponds to a bulk conductivity of 470 ± 30
S/m which is in concordance with conductivities reported in the literature for PEDOT:tosylate films. In addition, the resistance was observed to increase over time. After 3 weeks of exposure to atmospheric conditions at room temperature, the sheet resistance of the films was 151 ± 11 Ω.

<table>
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<th>Sheet resistance</th>
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<td>Capacitance per unit area</td>
<td>1700 ± 100 µF/cm²</td>
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<tr>
<td>Potential-limits (vs. Ag/AgCl)</td>
<td>-200 mV, 700 mV</td>
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Table 2-1. Physical properties of PEDOT:tosylate film electrodes (thickness = 190 nm).

Error is the standard deviation (n = 10).

The apparent capacitance of the electrodes was measured by collecting background cyclic voltammograms at the electrodes immersed in PBS buffer. A Ag/AgCl reference electrode was used. Figure 2-2 shows background scans of a PEDOT:tosylate band electrode with dimensions of 12 µm X 6000 µm. Between the potential limits -200 mV and 700 mV the charging current is approximately constant. The apparent capacitance was calculated by dividing the charging current by the scan rate. The capacitance was measured to be 1670 ± 120 µF/cm² (n=10). In contrast to the resistance, the capacitance was stable over time and did not change significantly over a period of 8 months. These capacitance values are quite high when compared to traditional electrode materials used for transmitter detection (20 – 100 µF/cm² typical). This high capacitance may limit the performance of PEDOT:tosylate films in some applications. The root mean square noise has been reported to scale with the electrode capacitance in low-noise amperometric experiments performed at carbon-fiber microelectrodes. This means that PEDOT:tosylate electrodes have to be patterned on a few micrometer scale for low-noise amperometric experiments. The noise properties of PEDOT:tosylate
Figure 2-2: Cyclic voltammograms showing the background current towards an Ag/AgCl reference electrode for a Pedot:tosylate electrode in PBS buffer. Electrode area 12 µm x 6000 µm. Scan rate 100 mV/s.
electrodes is a follow up for future studies. The increased capacitance will also affect the RC time constant of the electrode, ultimately limiting the scan rates attainable at PEDOT:tosylate electrodes. These measurements indicate that stable films can be mass produced and easily stored for use.

2.4.2 ELECTROCHEMICAL PROPERTIES OF PEDOT:TOSYLATE ELECTRODES

The performance of the PEDOT:tosylate films was evaluated. Ferrocene methanol, a well-characterized molecule was oxidized at the electrode and the electrochemical properties of the PEDOT:tosylate films were evaluated. Figure 2-3A shows the oxidation of ferrocene methanol at different concentrations at a PEDOT:tosylate electrode. A band electrode was placed in bulk solution and as expected, the current approached a quasi-steady state value at voltages above the oxidation potential (measured to be 210 mV vs. Ag/AgCl). The quasi-steady state current at a band electrode is given by the following equation:

\[ i_{\text{qss}} = \frac{2\pi n F D C}{\ln \left( \frac{64 D t}{w^2} \right)} \]

Equation 2-1

where C is the bulk concentration, n is the number of electrons in the reaction, F is Faraday’s constant, D is the diffusion coefficient, and the dimensions of the band electrode are given by the width w and length l.\(^{39}\) Quasi-steady state current values were measured on each voltammogram by calculating the difference between the current before the peak and the current 100 mV past the half wave potential \(E^{1/2}\). These data were compared to calculated theoretical values (Equation 2-1), using \(t = 20 \text{ s}\), \(F = 96485 \text{ C/mol}\) and \(D = 6 \times 10^{-6} \text{ cm}^2/\text{s}\). As seen in Figure 2-3B, the measured values are in good agreement with the theoretical values. This suggests that the PEDOT:tosylate film is
Figure 2-3: a) Slow scan cyclic voltammograms showing the oxidation of Ferrocene Methanol at different concentrations at a 13 µm X 5600 µm Pedot:tosylate electrode. Scan rate 5 mV/s. A Ag/AgCl reference electrode was used. b) Quasi steady state oxidation currents for different concentrations of ferrocene methanol compared to Equation 2-1.
uniform and that the measured electroactive area is in congruence with the geometric area. This allows for estimation of concentration or geometric area if one of the two is known.

Next, we investigated the oxidation of biogenic amines at the PEDOT:tosylate electrode. The oxidation of dopamine, epinephrine, and norepinephrine at a 12 µm x 6000 µm PEDOT:tosylate microelectrode is shown in Figure 2-4. The transmitters were diluted to 20 µM in PBS buffer and a Ag/AgCl reference electrode was used. The same electrode was used for all three scans. Between experiments the electrode was cleaned by rinsing in ethanol and water and flat background scans were measured in PBS buffer in order to assure that all oxidized material was removed before addition of a new solution. The voltammograms show comparable characteristics, reflecting the similar chemical structure and oxidation potentials of catecholamines. The current reaches an approximate steady state value. By using Equation 2-1, the theoretical quasi-steady state current at 50 mV above $E^{\frac{1}{2}}$ can be calculated to be $i_{\text{qss}} = 9.2 \text{nA}$ ($C = 20 \mu M$, $D = 6 \times 10^{-6} \text{cm}^2/\text{s}$ and $t = 50 \text{s}$, $w = 12 \mu m$, $l = 6000 \mu m$), which is comparable to the data in Figure 2-4.

The electrochemical and heterogeneous electron transfer kinetics for different transmitters and their metabolites were evaluated at PEDOT:tosylate electrodes. Results are listed in Table 2-2. PBS buffer (100 mM, pH = 7.4) was flowed over the electrode. A six-port HPLC valve was used to introduce a bolus solution containing the analyte to the electrode. The method of Nicholson was then used to determine the heterogeneous electron transfer rate constants. These electrodes show typical rate constants for the selected transmitters. This suggests that they could be used to measure these transmitters in a variety of applications. However, the anodic potential limit of 0.7 V vs.
Figure 2-4: Cyclic voltammograms showing the oxidation of dopamine (DA), norepinephrine (NE) and epinephrine (EPI) at a 12 µm X 6000 µm Pedot:tosylate electrode. Scan rate 1 mV/s. Concentration 20 µM.
Ag/AgCl should be taken into account. For example, histamine could not be oxidized at this potential, and thus could not be detected.

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<th>NE</th>
<th>E</th>
<th>L-DOPA</th>
<th>5-HIAA</th>
<th>5-HT</th>
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<td>0.7</td>
<td>0.9</td>
<td>-</td>
<td>0.6</td>
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<tr>
<td>(E_{1/2})</td>
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<td>2</td>
<td>7</td>
<td>4</td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 2-2. Heterogeneous electron transfer rate constants for selected molecules. Rate constants (cm/s x 10\(^{-3}\)) were determined by the method of Nicholson.\(^{40}\) Error is the standard deviation (n = 2 -3). Half wave potentials (mV) referenced to Ag/AgCl (n = 3-4).

2.4.3 MEASUREMENT OF NEUROTRANSMITTER RELEASE FROM CELLS

To test the materials ability to measure release from cells, we used a PEDOT:tosylate electrode to measure the release of transmitters from a population of PC12 cells using constant potential amperometry. Prior to measurement, the electrode was coated with Poly-L-Lysine to promote cell adhesion. PC12 cells are known to release catecholamines upon stimulation with a K\(^+\)-rich buffer. In this experiment, the cells were rinsed thoroughly with a physiological buffer prior to harvesting, to ensure that any oxidizing species in the growth buffer was removed. The same buffer was used to transport the suspended cells to the electrodes where they were plated on the substrate. A microscope image of a large group of PC12 cells on a PEDOT:tosylate electrode is shown in Figure 2-5. After applying a 350 mV potential, the current decayed to a value below 100 pA and the recording was started. Without removing the cells from the surface, the physiological buffer was exchanged with an isotonic buffer containing elevated K\(^+\) (105 mM). The current was recorded for 3 minutes before the buffer was
Figure 2-5: PC 12 cells sedimented on a 7 µm wide Pedot:tosylate electrode.
exchanged to physiological buffer. A rest time of 4 minutes was used between subsequent stimulations. The electrode was visually inspected to ensure that the same group of cells was present between each stimulation. Figure 2-6 shows the current traces obtained by stimulating a group of PC12 cells five times. The current response clearly decreases after each successive stimulation. This could be due to depletion of vesicles by the long-lasting stimulation used to evoke exocytosis.

2.5 CONCLUSIONS

In this paper we demonstrated the use of conductive polymer PEDOT:tosylate microelectrodes for electrochemical transmitter detection. A wide range of transmitters were shown to oxidize readily on the electrodes and kinetic rate constants and half wave potentials were reported. Out of 10 tested transmitters, only histamine had its oxidation potential outside the potential-limits of PEDOT:tosylate, and could not be detected. The capacitance per area was found to be high compared to other thin film microelectrode materials, which could be a limitation for use in low-noise amperometric measurements. However, this limitation can be overcome by making the electrodes sufficiently small. Further, we used constant potential amperometry to measure the transmitter release from a group of PC12 cells following a series of stimulations with high K⁺ buffer. Since the cells stay fixed during the exchange of buffers, current responses from the same group of cells can be compared and used for pharmacological screening applications. The study shows that PEDOT:tosylate is a promising electrode material in chip-based devices for transmitter detection. This opens the way for cheap and easy-to-fabricate all polymer devices for several bioanalytical applications such as HPLC, capillary electrophoresis, and drug screening.
Figure 2-6: Amperometric responses resulting from transmitter release from a group of PC 12 cells at a Pedot:tosylate electrode. The cells were alternately exposed to a K⁺-rich buffer for 3 minutes and a low K⁺ buffer for 4 minutes. The highest response resulted from the first stimulation by a K⁺-rich buffer. Subsequent stimulations resulted in decreasing current responses.
2.6 ACKNOWLEDGEMENTS

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2.7 REFERENCES


CHAPTER 3

A MICROWAVE-PLASMA DRY-ETCH FOR FABRICATION OF CONDUCTING POLYMER MICROELECTRODES
3.1 ABSTRACT

An inexpensive dry etch technology based on a low-pressure microwave plasma generated in a countertop microwave oven is characterized for the patterning of a conductive polymer microelectrode. The etch process is described, and the microwave-generated plasma is characterized by emission spectroscopy. The plasma is generated with an atmospheric mixture of mostly nitrogen and oxygen. A 10 µm wide band microelectrode composed of PEDOT:Tosylate, an optically transparent conductive polymer, is fabricated on a plastic substrate. Conductive polymer etch rates are approximately 280 - 300 nm/minute. A patterned microelectrode is characterized by atomic force microscopy. The horizontal distance of a 10-90 % height of a plasma-etched 150 nm thick electrode was measured to be 360 ± 200 nm (n=5). Electrodes are further characterized using steady-state cyclic voltammetry, and they have an electroactive area congruent with their geometric area. Finally, a complete device is assembled and used as a separation platform for biogenic amines. A microwave-etched 250 µm PEDOT:PSS electrode is employed for end-channel electrochemical detection on this microchip, where an electrophoretic separation of dopamine and catechol and a micellar electrokinetic chromatography separation of dopamine and serotonin are performed. Both mass and concentration LODs are comparable to other electrochemical detectors in an end-channel configuration. With the added advantages of easy processing, robustness, optical transparency, and a low cost, we expect microwave-etched polymer films to be a viable alternative to traditional electrodes.
3.2 INTRODUCTION

Conducting polymer electrodes have recently emerged as an attractive alternative to metal and carbon-based electrodes in a variety of applications. They are inexpensive, easy to process, and robust. Additionally, these electrodes have electron transfer kinetics appropriate to measure the redox properties of biologically relevant molecules. The poly(3, 4-ethylenedioxythiophene), PEDOT family of conducting polymers offers the additional benefits of high optical transparency, electrochromism, thermal stability up to 260 °C, and demonstrated biocompatibility. The optical transparency and biocompatibility of PEDOT electrodes has prompted uses in optogenetic and electrophysiological measurements. PEDOT is most commonly dip-coated or electropolymerized onto metal or carbon electrodes before being employed for electrochemical measurements. PEDOT-only band electrodes (i.e. those without a conducting metal under-layer) have emerged only recently and seen use as an electrochemical sensor for biogenic amines. Other applications of patterned conducting polymer films include antistatic coatings, ITO-free and ITO-containing photovoltaic cells, capacitive touch screens in mobile devices, capacitors, lead-free solder, electrochemical actuators, and flexible displays in organic light emitting devices. Patterned PEDOT films are found in PEDOT:PSS-containing solar cell arrays, large-area organic diodes, PEDOT nanotube arrays in dye sensitized solar cells, and electrochemical sensor platforms with both bare PEDOT and enzyme-modified PEDOT.

Polymer films are commonly deposited on substrates by gravure printing, inkjet printing, vacuum/vapor deposition, or spin coating. Gravure and inkjet printing allow for the simultaneous deposition and patterning of polymer films. However, the technique also suffers from problems with film thickness control and droplet aggregation,
though substantial effort has been put forth to minimize these issues. Film etching offers advantages over gravure and inkjet printing. When patterning vacuum or vapor deposited films, the film is deposited on a substrate and then subsequently etched to define structures. Modern etch methods include laser ablation, wet etches (e.g. KOH, hydrofluoroether solvents), and dry etches. Common dry etches for polymers include sputter etching, vapor-phase etching, and reactive ion etching (RIE). The latter technique is well established for defining polymer structures from PEDOT films in a predictable manner; however, RIE can be prohibitively expensive. Gas usage, turbomolecular pumps, and vacuum control systems drive the cost even higher. Additionally, many reactive ion etchers are made from largely proprietary parts, making them difficult or expensive to service.

Here, we introduce an inexpensive, facile alternative to reactive ion etching for defining the geometry of thin-layer conducting polymer electrodes using a microwave-plasma generated in a countertop microwave oven. Microwave plasmas have previously been established as a viable method for etching polymers. Additionally, microwave plasmas have been utilized to modify the surface of polydimethylsiloxane (PDMS) for permanent bonding of these devices to glass substrates. Microwave etching allows for the fabrication of simple or complex polymer patterns on common substrates, and can facilitate many structures on one substrate. The other required components are a sealed glass chamber with a spigot and an inexpensive rotary vane pump. The possible feature sizes are amenable to fabricating microelectrodes as well as larger structures – with a theoretical lower limit of hundreds of nanometers to an upper limit of several inches. Additionally, microwave etching is less expensive than most other methods of dry etching and can be executed on a bench top. It requires no exotic gases or regulators, no reactive ion etcher, and no turbomolecular pumps, lowering the cost to the
user by several thousand dollars. Here, we use the etch process to fabricate PEDOT microelectrodes and characterize their geometry and electrochemical performance. We demonstrate the use of microwave-etched microelectrodes as an amperometric sensor for biological molecules in a microchip electrophoresis platform for the separation and detection of transmitter molecules.

3.3 MATERIALS AND METHODS

3.3.1 PEDOT:TOSYLATE ELECTRODE FABRICATION

PEDOT:Tosylate films were deposited by spin coating 1 mL of 20% w/v iron (III) tosylate in n-butanol (Sigma Aldrich, St. Louis, MO) and 25 µL anhydrous pyridine (Sigma Aldrich, St. Louis, MO) at 1500 rpm for 15 seconds with an acceleration of 1500 rpm/s onto TOPAS substrates. Pyridine concentration was varied to control the rate of polymerization and the film thickness. The coated substrates were placed on a mesh rack (elevated ~ 3 cm) inside a nitrogen-purged desiccator held at atmospheric pressure. Ethylenedioxythiophene (EDOT) monomer (~ 200 µL) (Sigma Aldrich, St. Louis, MO) was deposited at the bottom of the desiccator, and the desiccator was placed on a hot plate with a surface temperature of 100 °C to evaporate the monomer. The chamber was sealed for 20-30 minutes until a faint green tint in the films was observed. PEDOT:Tosylate-coated substrates were removed from the chamber then baked in an oven at 70 °C for 20 minutes. Substrates were rinsed in ethanol and water to remove residual iron (III) tosylate and then dried using N₂. After drying, the edge bead was removed using a Kim-wipe dipped in ethanol.

3.3.2 UV LITHOGRAPHY DETAILS

One milliliter of AZ3312 (AZ Electronic Materials, Branchburg, NJ, USA) photoresist was spin coated on the PEDOT:Tosylate-coated substrates at 3000 rpm for 10 s with an
acceleration of 1000 rpm/s. The substrates were then soft baked on a hot plate at 95 °C for 2 minutes before a 15 second (9.0 mW cm$^{-2}$, 365 nm) contact exposure on a Karl Suss MJB3 mask aligner (SÜSS MicroTec AG, Garching, Germany) through a positive-tone printed plastic photomask (CAD/Art Services, Inc.) containing several electrodes. The substrates were then baked on a hotplate at 100 °C for 2 minutes and cooled on a benchtop before two sequential 30 second submersions in AZ351 MIF developer with agitation. The substrates were then rinsed twice in ultrapure 18.2 MΩ water (Barnstead Diamond UV/TOC) and dried using N$_2$.

A microwave etch chamber was custom fabricated at the University of Arizona glassblowing facility (Figure 3-1B). The two-piece Pyrex® chamber was fabricated into a cylinder 30 cm in length and 10 cm in diameter with a Teflon stopcock and o-ring. A 14 cm x 6 cm x 1 mm piece of sheet aluminum was kept inside the chamber to spark the plasma, but was not in contact with the substrates during the etch process. Substrates were then placed in the etch chamber and the pressure was pumped down using a rotary vane pump. The pressure of the microwave etch chamber was measured to be approximately 1 Torr using a thermocouple gauge. The etch chamber was sealed and placed in a 1200 watt microwave oven (General Electric Co, Fairfield, Connecticut, USA, Model Number JES2051DN2WW, $f = 2.45$ GHz) and the microwave was turned on for 10 - 15 seconds and the sample was allowed to cool for 2 minutes. This process was repeated three times. Plasma sparked approximately three seconds after the microwave was turned on. Substrates were removed from the chamber and rinsed with semiconductor-grade acetone for 1 minute to dissolve the photoresist structures, and rinsed with ethanol and water prior to use. A microwave oven from one other brand, a 700 Watt Sunbeam SGN30701W, was successfully used to etch PEDOT electrodes as well, indicating the insensitivity of the technique to the particular microwave oven.
**Figure 3-1:** a) UV/visible emission spectrum of the microwave-generated plasma. b) Photograph of the glass chamber used to contain the plasma and samples. Chamber is 30 cm in length and 10 cm in diameter.
3.3.3 SU-8 3050 MASTER MOLD FABRICATION

Approximately 2 mL of SU-8 3050 was dispensed on piranha-cleaned silicon and spin coated at 500 rpm for 10 seconds at an acceleration of 100 rpm/s and then 5000 rpm for 30 seconds at an acceleration of 1000 rpm/s. The wafer was then placed on a hot plate and softbaked for 10 minutes at 95 °C. The wafer was then exposed for 40 s (9.0 mW cm\(^2\)) on a Karl Suss MJB3 mask aligner through a negative-tone printed plastic photomask (CAD/Art Services, Inc.). The wafer was then baked for 1 minute at 65 °C, and the temperature was ramped up to 95 °C where it was held for 5 minutes. The wafers were cooled to room temperature and developed in SU-8 developer (1-methoxy-2-propyl acetate) for 5 minutes with agitation. Wafers were then rinsed with isopropyl alcohol and dried under N\(_2\). Wafers were then silanized in the vapor phase with chlorotrimethylsilane in the vapor phase overnight in a dessicator.

Sylgard 184 (Dow Corning, Midland, Michigan, USA) was mixed with its curing agent in a 1:10 ratio, degassed, poured over the master mold in a Petri dish, and baked in an oven at 70 °C for three hours. The PDMS device was peeled back from the master mold, and five 6 mm wells were punched into the device with a biopsy punch. The device had a 2.9 cm (L) x 45 µm (H) x 30 µm (W) separation channel. The distance from well A to the junction was 0.1 cm, and the distance from wells B and C to the junction was 0.3 cm. The junction had an offset between channels B and C of 50 µm. The PDMS was then exposed to microwave plasma in the chamber for 8 seconds to activate the surface for permanent bonding to the substrate. The PDMS was then bonded to the glass substrates with patterned electrodes at the end of the separation channel. The electrode was aligned under a microscope to be perpendicular with the end of the separation channel at distance of 10 microns from the outlet.
3.3.4 ELECTROPHORESIS MICROCHIP FABRICATION

Electrodes for microchip electrophoresis were fabricated on 2 inch square glass slides. Glass slides were cleaned with Alconox® and ethanol, rinsed with 18 MΩ water, and dried under N₂. A thin adhesion layer composed of Poly-3,4-ethylenedioxythiophene:polystyrene sulfonate (PEDOT:PSS, Sigma Aldrich, St. Louis, MO) was spin-coated onto these substrates at 5000 rpm for 60 s at an acceleration of 5000 rpm/s, and baked on a hotplate at 200 °C for 10 minutes. This baking step removed the electrical conductivity of the adhesion layer. An additional layer of PEDOT:PSS was spin-coated onto the substrate at 3000 rpm for 30 s with an acceleration of 3000 rpm/s, and dried at 70 °C in an oven. Photolithography for PEDOT:PSS films was performed in the same manner as for the PEDOT:Tosylate electrodes (*vide supra*).

3.3.5 ELECTROCHEMICAL CHARACTERIZATION OF BAND ELECTRODES

Electrochemical experiments were performed at 10 µm band electrodes to determine whether microwave etched films behaved as predicted by electrochemical theory. A 2 cm x 10 µm electrode was fabricated and a 2165 µm x 10 µm visible electrode area was isolated using a PDMS mold. Cyclic voltammograms were collected in a 100 mM pH 7.4 phosphate buffered saline solution containing 10 mM KCl and varying concentrations of ferrocene carboxylic acid. Slow-scan cyclic voltammetry (5 mV/s) was performed (Figure 3-2) and compared to theoretical electrode responses. Theoretical quasi-steady state band electrode currents were calculated using Equation 3-1:

\[
qss = \frac{2\pi n F D C}{\ln (64Dt/w^2)}
\]
Figure 3-2: a) Quasi-steady state cyclic voltammograms showing the oxidation of ferrocene carboxylic acid at different concentrations on a microwave-etched PEDOT:Tosylate electrode. Scan rate is 5 mV/s. Voltammograms collected in 100 mM pH 7.4 phosphate buffered saline with 10 mM KCl. Potential is with reference to Ag QRE. b) Quasi-steady state oxidation currents for different concentrations as compared to theoretical values predicted by Equation 3-1.
where \( n \) = the number of electrons transferred, \( F \) is Faraday’s constant, \( l \) is the length of the electrode, \( D \) is the diffusion coefficient, \( C \) is the bulk concentration of the analyte, \( w \) is the width of the electrode, and \( t \) is the approximate time at steady state. Values of \( D = 8 \times 10^{-6} \text{ cm}^2\text{s}^{-1} \), \( t = 10 \text{ s} \), \( l = 2.165 \times 10^{-1} \text{ cm} \), \( w = 1 \times 10^{-3} \text{ cm} \), \( n = 1 \), and \( F = 96485 \text{ C mol}^{-1} \) were used. The measured currents are well-predicted by the calculated theoretical values. This affirms that the geometric area of the electrodes is in congruence with the electroactive area. The electrochemical performance of microwave-etched films is indistinguishable from those patterned with reactive ion etching.\(^1\) Furthermore, PEDOT:Tosylate and PEDOT:PSS electrodes showed no change in electrochemical behavior over the course of approximately five months when stored in sealed plastic bags.

TOPAS® 5013 Cyclic Olefin Copolymer (COC, Topas Advanced Polymers Inc, Florence, KY, USA) substrates (Ø 50 mm x 2 mm wafers) were generated via injection molding. PEDOT:Tosylate was deposited on these substrates via vapor deposition. Then, films were patterned using UV lithography (Figure 3-3). Film etching was performed in a microwave etch chamber was custom fabricated at the University of Arizona glassblowing facility (Figure 3-1B).

3.3.6 ELECTROPHORESIS

Electrophoretic separations were carried out in unmodified PDMS microchannels. High voltage was applied using a 4-channel custom power supply (University of Arizona Electronics Facility). Voltage was applied through platinum wires placed in wells A, B, and C, and controlled using in-house LabVIEW (National Instruments, Austin, TX) software. Two chloridized silver wires were used as the reference electrode and the ground in well D. For the separation of dopamine and catechol, the buffer system was
Figure 3-3: Schematic of the microwave-etch process.  

a) PEDOT:Tosylate film is generated on a TOPAS cyclic olefin copolymer substrate either by spin-coating or vapor deposition.  
b) AZ 3312 photoresist is deposited on the surface of the PEDOT:Tosylate and UV photolithography is used to define the geometry of protective structures.  
c) The wafer is rinsed and inserted in the microwave plasma chamber for etching.  
d) Once etched, the wafer is submerged in acetone for 5 minutes to dissolve the photoresist. We are left with PEDOT:Tosylate microstructures.
20 mM MES at pH 6.0. For the separation of dopamine and serotonin, micellar electrokinetic chromatography (MEKC) was used, and the buffer was 50 mM borate at pH 9.6 with 20 mM sodium dodecyl sulfate added. Standard solutions (100 μM) were prepared daily from fresh 2.7 mM stock solutions in 0.1 M perchloric acid, and diluted in the separation buffer. Separation was performed by placing the analyte in well A, buffer in wells B, C, and D, and applying the separation voltage (750 V in MES or 500 V in borate / SDS) to well C, and grounding in well A and D, while B floated. Injection was performed by floating the potential of well C and applying the separation voltage at well A for the duration of the injection. Injection times were 3 seconds for the dopamine and catechol separation, and 5 seconds for the MEKC dopamine and serotonin separation.

3.3.7 PLASMA CHARACTERIZATION
Microwave plasma emission spectra were collected using an Ocean Optics USB4000 fiber optic spectrometer (Ocean Optics, Dunedin, FL). The fiber optic cable was held against the microwave window and all light sources in the microwave were removed. The apparatus was covered in a blackout curtain and UV/Vis spectra were collected every 500 ms (20 spectra were averaged).

3.3.8 SURFACE CHARACTERIZATION
Atomic force microscopy was performed using a Veeco Dimension 3100 (Veeco, Plainview, NY) in tapping mode using a Mikro Masch NSC-15 n-type silicon tip (Mikromasch USA, San Jose, California). Measured topography was software low-pass filtered twice (Nanoscope V531r1). Optical micrographs were collected using a Nikon Ti-2000 microscope (Nikon Corporation, Tokyo, Japan).

3.3.9 ELECTROCHEMISTRY
Electrochemical data was collected using an Ensman EI-400 potentiostat (Ensman instruments, Bloomington, IN) and custom LabVIEW software (National Instruments, Austin, TX). Electrical contact to the electrode was established by placing a platinum wire in a solution of 3.0 M KCl above the contact pad. Custom LabVIEW software utilizing real-time oversampling filtering collected the data. Data was collected at 250 kHz (NI 6221) and stored at 100 Hz. A 50 point nearest-neighbor smooth was applied to the MEKC separation traces. Cyclic voltammograms were collected at 5 mV/s and swept from -0.6 V to 0.4 V vs. Ag QRE. All electrochemical data were hardware low-pass filtered at 500 Hz and software low-pass filtered at 2 Hz. For cyclic voltammetry experiments, electrode geometry was defined by placing a PDMS well on top of the electrode and filling it with solution. The wire provided electrical contact with the PEDOT:Tosylate electrode through this KCl solution.

3.4 RESULTS AND DISCUSSION

3.4.1 PEDOT ELECTRODE PATTERNING

The microelectrode fabrication process (Figure 3-3) started with the deposition of a conducting polymer film on a substrate (Figure 3-3A). Both PEDOT:PSS and PEDOT:Tosylate were used to make devices due to their ubiquity in organic electronics. The substrate material, TOPAS® cyclic olefin copolymer (COC), has excellent optical properties, chemical inertsness to ketones, and a relatively high glass transition temperature ($T_g = 115\degree C$). Additionally, PEDOT:Tosylate films adhere strongly to TOPAS. Glass slides were used as substrates for PEDOT:PSS electrode patterning as PEDOT:PSS does not adhere well to TOPAS. After PEDOT films were generated on substrates, UV photolithography was used to define protective structures (Figure 3-3B) prior to etching. A diazonaphthoquinone and novolac (DQN) based photoresist was chosen for (AZ-3312®), a high glass transition temperature (125 °C), fast processing,
high resolution (i-line 0.4 µm, g-line 0.6 µm), and high solubility in acetone for easy removal. A PEDOT-coated substrate with patterned photoresist structures was then placed in the microwave etch chamber (vide infra, Figure 3-1B), sealed in open atmosphere, and pumped down to ~1 Torr. The chamber was placed in the microwave and the plasma was sparked, etching the unprotected PEDOT off of the substrate (Figure 3-3C). The substrate was then removed and soaked in acetone to dissolve the photoresist (Figure 3-3D), revealing the patterned microstructures. Three alternative methods were also successfully used to mask the PEDOT and define structures: 1) Glass cover slip placed up against a portion of the PEDOT-coated substrate, 2) A 15-nm thick sputtered gold coating, and 3) a vinyl sticker with fluoropolymer coating (Bytac®) pressed against the PEDOT:Tosylate. The AZ-3312® photoresist was chosen for ease of use and excellent feature resolution.

3.4.2 PLASMA CHARACTERIZATION

A conventional countertop microwave oven was used to generate plasma for etching films. A sealed glass chamber with a valve was pumped down to ~1 Torr with a rotary vane pump. The chamber was placed in the microwave oven and a plasma was sparked in the chamber. A small piece of metal (e.g. aluminum plates, foil, or small metal screwdrivers) was sufficient to initiate the plasma. At pressures higher than approximately 100 Torr, a plasma is still generated, but arcing and excessive chamber heating occurs, along with a characteristically white plasma that does not etch films successfully. At pressures substantially lower than 1 Torr, a plasma is not initiated.

Figure 3-1A shows the emission spectrum of the microwave plasma during typical operation. The bright pink plasma (Figure 3-1B, inset) is characteristic of air plasmas. A schematic of the chamber and construction materials used is shown in Figure 3-4. The microwave plasma shares common emission wavelength peaks with other air plasmas.
Figure 3-4: A schematic for the construction of a Pyrex® glass vessel to be used for microwave etching of conducting polymers. The O-ring (black) and stopcock are composed of polytetrafluoroethylene (PTFE) to minimize reactivity with plasma or etchant gas. The steel sheet can be replaced with other scrap metals such as aluminum or copper. When the vessel is ready to be used, the substrate and metal piece sit inside the chamber, held apart by indentations made in the glass tube. The three components (cap, o-ring, and vessel) are assembled and vacuum is pulled on the device for approximately 1 minute to bring it to ~1 Torr.
Atomic hydrogen electronic emission lines are visible at 486.1 and 655.2 nm, while atomic oxygen emission lines are present at 777.2 and 844.7 nm. The atomic hydrogen emission lines are hypothesized to be due to the presence of water vapor in the chamber. Emission manifolds for $\text{N}_2^+$ and $\text{N}_2^{2+}$ are present at 560-780 and 350-480 nm respectively. While the $\text{N}_2^{2+}$ emission manifold reaches down to ~250 nm, it was not observed in the collected emission spectra. This is because UV-absorbing materials were present between the emission and the optical fiber probe for data collection.

### 3.4.3 ETCH CHARACTERIZATION

An etch process punctuated by short pauses in plasma exposure provided the most clearly defined structures when compared to long etch times (10 second plasma exposures separated by 2 minutes repeated three times for PEDOT:Tosylate films, and 15 second plasma exposures separated by 2 minutes repeated six times for PEDOT:PSS films). The advantages a time-modulated are described elsewhere, though here our etches are punctuated by longer times. During long plasma exposures (>20 seconds), the temperature of the chamber becomes sufficiently high to pass the glass transition temperature of the photoresist (125 °C), causing reflow in the structures. With the 2 minutes of cooling time between plasma exposures, several repetitions of short etch times allowed for more temperature control. The COC surface morphology was unchanged for plasma exposure times under 2 minutes as measured by atomic force microscopy before and after microwave etches. The surface RMS roughness of the COC substrate increased from $3.87 \pm 0.13$ nm RMS ($n = 3$) to $3.99 \pm 0.59$ nm RMS ($n = 3$), but this difference was not statistically significant (Student’s t test, two tailed, $P = 0.80$, Figure 3-5). The temperature of the chamber and substrate was 60-70 °C after 10 seconds of etching (as measured by an infrared thermometer). Etch rates of ~300 nm/minute were observed.
Figure 3-5: Tapping mode atomic force micrographs of a TOPAS® COC substrate a) before and b) after four sequential 15 second microwave etches with 2 minutes between each 15-second plasma exposure.
nm/minute are attained for PEDOT:Tosylate films, and ~150 nm/minute are attained for PEDOT:PSS films. This is on the same order as etch rates of polymer films attained with other oxygen plasmas.\textsuperscript{47} High power microwave ovens (2-3kW) should be explored further, though sample heating may be of concern.

Microwave-etched 10 µm-wide PEDOT:Tosylate electrodes were imaged using atomic force microscopy (Figure 3-6). The image shows clearly defined edges on an electrode cast out of a 150 nm thick vapor-generated PEDOT:Tosylate film on a TOPAS® substrate. The horizontal distance of a 10-90 % height step to from substrate to the top of a vapor-generated film was measured to be 360 ± 200 nm (n = 5). The atomic force micrograph and horizontal 10-90 % height step distance suggest that smaller feature sizes are possible.

3.4.4 SEPARATION AND DETECTION OF BIOGENIC AMINES WITH MICROCHIPS

Microwave-etched PEDOT:PSS microelectrodes (250 µm x 1 cm x 100 nm) were utilized as end-channel electrochemical detectors for chip electrophoresis experiments. Here, PEDOT:PSS was employed because it is more robust than PEDOT:Tosylate in the presence of strong electric fields. In the first application, dopamine and catechol were separated on a 5 cm x 5 cm microchip and detected amperometrically using a PEDOT:PSS electrode. The separation channel had dimensions of 2.9 cm (L) x 45 µm (H) x 30 µm (W), all separations were performed at a field strength of 262 V/cm, and the detection electrode was aligned to be approximately 10 µm outside the end of the separation channel. In the second application, dopamine and serotonin were separated in a microchip and detected amperometrically. Representative amperometric traces of these separations are shown in Figure 3-7. A summary of separation figures-of-merit including resolution ($R_s$), limit of detection (LOD, calculated from peak height), sensitivity, peak current, relative standard deviation (RSD), and separation efficiency
Figure 3-6: Atomic force micrograph of a section (50 μm²) of a 10 μm wide microwave-etched PEDOT:Tosylate band microelectrode on a TOPAS substrate illustrating the edge fidelity of the etch. Electrode width is approximately 10 μm. Inset: Optical micrograph of a 2165 μm x 10 μm PEDOT:Tosylate band microelectrode extending into a PDMS well.
Figure 3-7:  

a) Schematic of the microfluidic device used to separate and detect dopamine, catechol, and serotonin.  

b) Separation and detection of 100 µM dopamine and 100 µM catechol in 20 mM MES buffer at pH 6 with a field strength of 262 V/cm.  

c) MEKC Separation and detection of 100 µM dopamine and 100 µM serotonin in 50 mM borate buffer at pH 9.6 with 20 mM SDS with a field strength of 175 V/cm.
(TP) are quantified in Table 3-1 for one device with 19 consecutive injections. Baseline resolution of the two analytes was achieved in both separations. Sub-micromolar limits of detection typical of an end-channel electrochemical detection scheme were achieved, with limits of detection for dopamine and catechol comparable to carbon fiber, carbon ink, or palladium electrodes. Given the height of the chip separation channel (45 µm) and the end-channel geometry of the detector, a low coulometric detection efficiency was expected.

<table>
<thead>
<tr>
<th>Sep. Buffer</th>
<th>Analyte</th>
<th>Rs</th>
<th>TP</th>
<th>LOD (µM)</th>
<th>LOD (amol)</th>
<th>Sens. (pA/µM)</th>
<th>Io RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MES pH = 6.0 (n = 19)</td>
<td>Dopamine</td>
<td>2.0 ± 0.2</td>
<td>810 ± 30</td>
<td>0.24 ± 0.06</td>
<td>110 ± 30</td>
<td>24 ± 1</td>
<td>6.4</td>
</tr>
<tr>
<td>Borate MEKC pH = 9.6 (n = 3)</td>
<td>Dopamine</td>
<td>1.8 ± 0.2</td>
<td>380 ± 40</td>
<td>0.35 ± 0.08</td>
<td>160 ± 40</td>
<td>30 ± 1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>5-HT</td>
<td></td>
<td>510 ± 60</td>
<td>1.4 ± 0.2</td>
<td>620 ± 80</td>
<td>8 ± 1</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Table 3-1. Figures of merit from electrophoretic separations with amperometric detection at a 250 µm PEDOT:PSS electrode. (TP = theoretical plates)

The devices separated analytes continuously for approximately 1 hour without replenishment of separation buffer or additional operator intervention, with an average of 8 ± 5 % decrease (for the dopamine and catechol separation, n = 19 injections and separations) in sensitivity between the first and last runs, and a 22 ± 11 % increase in RMS noise (from 2.7 pA to 3.3 pA). At this point, devices were regenerated by pressure-flushing with 0.1 M NaOH for 3 minutes, followed by 3 minutes of nanopure water, finally followed by 5 minutes of separation buffer. The decrease in electrode sensitivity was accompanied with a slight broadening of peaks suggesting this is likely separation-related and not detector related. Given these comparable detector figures of merit, along with the optical transparency, low cost, facile electron transfer kinetics and easy
fabrication, PEDOT-based detectors in microchip electrophoresis are a competitive material with pyrolized photoresist, palladium, and carbon ink electrodes.

3.5 CONCLUSIONS

The method presented is an alternative to reactive ion etching for patterning thin-layer polymer structures. The technique allows for the fabrication of simple or complex patterns, and can facilitate many electrodes on one substrate. It is less expensive than most other methods of dry etching. Microwave etching requires no exotic gases or regulators, no reactive ion etcher, and no turbomolecular pumps. If desired, the chamber can be backfilled with SF₆, CₓFᵧ, or other common etch gases after pumping down. Using a Bytac mask instead of lithographic masks, the patterning and etching process can be performed in any laboratory. The microwave oven generates an air plasma similar to others published in literature. The feature size of microwave-etched structures is defined by the resolution of the photoresist and the photomask. Common plasma etchants such as SiCl₄, CF₄, SF₆, and BCl₃ should be investigated for etching polymers as well as inorganic semiconductor materials (these etchants are toxic and should only be used with ventilation under strict safety precautions). The geometric area of electrodes fabricated via microwave etching is congruent with the electroactive area indicating that this method etches down to the substrate, and defines features predictably. The optical transparency of the device could be used for simultaneous electrochemical and fluorescent detection. A microchip electrophoresis device with a polymer detection electrode was fabricated and characterized. Additionally, the first microchip electrophoresis separation of dopamine and serotonin with amperometric electrochemical detection has been shown. Polymer electrodes offer unique advantages including optical transparency, low cost, and easy processability in addition to performing similarly to traditional biosensor electrode materials.
3.6 ACKNOWLEDGEMENTS

The authors thank Dr. Brooke Beam, Paul Lee, and Omid Mahdavi, and Dr. Chris Baker for fruitful discussion, technical expertise, and lithography materials. Additionally, we thank the University of Arizona for funding this work.
3.7 REFERENCES


CHAPTER 4

BIOCOMPATIBLE PEDOT:NAFION COMPOSITE ELECTRODE COATINGS FOR
SELECTIVE DETECTION OF NEUROTRANSMITTERS IN VIVO
4.1 ABSTRACT

A Nafion and poly(3,4-ethylenedioxythiophene) (PEDOT) containing composite polymer has been electropolymerized on carbon-fiber microelectrodes with the goal of creating a mechanically stable, robust, and controllable electrode coating that increases the selectivity and sensitivity of in vivo electrochemical measurements. The coating is deposited on carbon-fiber microelectrodes by applying a triangle waveform from + 1.5 V to – 0.8 V and back in a dilute solution of ethylenedioxythiophene (EDOT) and Nafion in acetonitrile. Scanning electron microscopy demonstrated that the coating is uniform and ~100 nm thick. Energy-dispersive x-ray spectroscopy demonstrated that both sulfur and fluorine are present in the coating, indicating the incorporation of PEDOT (poly(3,4-ethylenedioxythiophene) and Nafion. Two types of PEDOT:Nafion coated electrodes were then analyzed electrochemically. PEDOT:Nafion-coated electrodes made using 200 µM EDOT exhibit a 10-90 response time of 0.46 ± 0.09 seconds vs. 0.45 ± 0.11 seconds for an uncoated fiber in response to a 1.0 µM bolus of dopamine. The electrodes coated using a higher EDOT concentration (400 µM) are slower with a 10-90 response time of 0.84 ± 0.19 seconds, but display increased sensitivity to dopamine, at 46 ± 13 nA/µM, compared to 26 ± 6 nA/µM for the electrodes coated in 200 µM EDOT and 13 ± 2 nA/µM for an uncoated fiber. All of these differences are statistically significant. PEDOT:Nafion-coated electrodes were lowered into the nucleus accumbens of a rat, and both spontaneous and electrically evoked dopamine release were measured. In addition to improvements in sensitivity and selectivity, the coating dramatically reduces acute in vivo biofouling.
4.2 INTRODUCTION

Monitoring real-time dynamics of biogenic amines in vivo is essential for understanding the role of chemical communication in cognitive function. These molecules are released at axon terminals in response to salient stimuli and diffuse through the extracellular space where they can either act on distal receptors (volume neurotransmission) or are cleared by reuptake or metabolic mechanisms. Dopamine is of particular interest because of its well-established role in reward-based behavior, memory, addiction, and movement. Most often, carbon-fiber microelectrodes (CFMEs) are utilized for these electrochemical measurements because of their biocompatibility, small size (5 – 10 µm in diameter), and favorable electrochemical properties. Furthermore, neurotransmission occurs on the sub-second timescale, thus for a measurement to probe transmitter dynamics, the temporal resolution of the measurement must be on the order of milliseconds. Because of this, CFMEs have been coupled to fast-scan cyclic voltammetry, which provides adequate temporal resolution and the shape of the resultant voltammogram can be used for analyte identification and quantification. However, during in vivo measurements, the presence of interferents complicates measurements warranting additional modification of the electrode surface to enhance selectivity.

For in vivo measurements of chemical communication the presence of metabolites and antioxidants in the extracellular space can detrimentally affect accurate measurements of chemical communication. Specifically, a large body of work has been directed towards maximizing the selectivity of dopamine over ascorbic acid (AA) and 3,4-dihydroxyphenylacetic acid (DOPAC). These two molecules share a similar oxidation potential with dopamine, and can be present in concentrations 100-fold in excess of dopamine. To address this, researchers have historically relied
predominantly on Nafion, which is a copolymer of polytetrafluoroethylene with perfluorovinyl ether sulfonic acid side chains. The sulfonic acid moiety is stabilized by the electron-withdrawing character of the attached chain, and as such the pK_a of the moiety is estimated at -6, leaving the functional group deprotonated within the physiological pH range. Presumably, a negative charge immobilized at the surface of the electrode will restrict the diffusion of anions to the electrode. Because ascorbic acid and DOPAC are both negatively charged at physiological pH, and dopamine is positively charged, a decrease of interferent signal and an increase of analyte signal is anticipated with successful coating of Nafion on the electrode. Nafion also forms cation-conducting sulfonate networks, which allow the transport of positively charged species to the electrode. Nafion is commonly dip-coated or electro-deposited onto electrodes prior to in vivo measurement in an attempt to minimize current measured from interferents. It has also been successfully used to increase selectivity of serotonin and adenosine measurements, and to reduce the shift in reference electrode potential during chronic implantation. Because Nafion is a fluoropolymer like PTFE (polytetrafluoroethylene), it does not strongly adhere to carbon-fiber surfaces and forms non-uniform layers, both of which limit the usefulness of Nafion coatings. Additionally, a reproducible, robust, and facile means for deposition on to cylindrical carbon-fiber microelectrodes has not yet been achieved.

Here, we deposit Nafion onto carbon-fiber microelectrodes by synthesizing a polyethylenedioxythiophene (PEDOT) and Nafion-containing polymer in a novel scheme on the surface of the electrode. This ensures a thin, even coating of the material, and enhances selectivity towards cations. Additionally, both PEDOT and Nafion have a well-established history in being coated onto biosensors to improve sensor function or biocompatibility. The deposition of a PEDOT:Nafion composite coating is described
and characterized. The chemical space of the coating is explored and optimized, and coated electrodes are shown to yield accurate measurements of dopamine in vivo. Lastly, the coating provides a surface that mitigates biofouling and retains enhanced selectivity and sensitivity for dopamine over interferents following six hours of in vivo implantation.

4.3 MATERIALS AND METHODS

4.3.1 ELECTRODE FABRICATION

Each CFME was fabricated by isolating and aspirating a single T-40 carbon fiber (Cytec Thornel, Woodland Park, NJ) into a standard glass capillary (1.2 mm o.d. x 0.68 mm i.d., 4” long, A-M Systems, Sequim, WA, USA). Each filled capillary was then heated and pulled using a type PE-2, RR50915300 pipet puller (Narishige, Tokyo, Japan). The carbon-fiber electrode was cut to ~ 75 µm in length from the glass seal using a surgical blade. Electrical contact was made by inserting wire-wrap wire coated in alcohol-based graphite conductive adhesive (1.2 kOhm/in², Alfa Aesar, Ward Hill, MA) through the open end of the capillary tube. An epoxy seal using Loctite 1C Hysol epoxy-patch adhesive (Henkel Corporation, Madison Heights, WI, USA) was made around the periphery of the electrical wire protruding from the glass capillary and cured overnight at room temperature. To validate the generality of the electrode coating scheme, some electrodes were fabricated using IM-7 and AS-4 type carbon fibers (Hexcel Corporation, Stamford, CT, USA) to ensure that this chemistry was not specific to T-40 carbon fibers. Electrochemical data is reported for T-40 carbon fibers. T-40 carbon fibers (Cytec Thornel, Woodland Park, NJ), IM-7 carbon fibers, and AS-4 carbon fibers (Hexcel Corporation, Stamford, CT, USA) were coated successfully.

4.3.2 BIOLOGICAL EXPERIMENTS PROTOCOL
Adult, male Sprague-Dawley rats (350 – 450 g; Harlan Laboratories, Harlan, Kentucky, USA) were used. All procedures were performed in accordance with the policies of the National Institutes of Health guidelines for laboratory animals under protocols approved by the University of Arizona Institutional Animal Care and Use Committee. Rats were housed three per cage on a 12-h light–dark cycle with food and water provided for *ad libitum* consumption. Stereotaxic surgeries were performed under isoflurane anesthesia (5 or 2.5 % delivered in air at 1 L/min for the induction and maintenance of anesthesia during surgery, respectively). Several burr holes were drilled in the skull to allow access to the nucleus accumbens shell for microelectrode placement (stereotaxic coordinates from bregma: AP +1.7 mm; ML +1.0 mm; DV -7.2 mm from the skull surface), to the medial forebrain bundle (MFB) for the stimulating electrode placement (from bregma: AP -2.5 mm; ML +1.7 mm; DV -7.5 mm from the skull), and to the contralateral hemisphere (from bregma: AP +2.0 mm; ML +2.0 mm; DV – 3.0 mm from the skull) for the reference electrode placement. Coordinates were based on the rat brain atlas. Body temperature was maintained at 37 °C with a feed-back-controlled heating pad (Harvard Apparatus, USA). A bipolar stimulating electrode (Plastics One, Wallingford, CT) provided constant-current, stimulation pulses to the MFB. To avoid electrical crosstalk, the stimulation was programmed to occur during the rest period between individual cyclic voltammogram recordings. The stimulus was optically isolated from the signal generation apparatus (NeuroLog System, Hertfordshire, England). A 40 pulse, 60 Hz biphasic (± 150 µA, 2 ms per phase) stimulation was used for all experiments. *In vivo* fast-scan cyclic voltammetry was performed with a Dagan ChemClamp and software developed by Knowmad Technologies, LLC.
4.3.3 CHEMICALS

Electrodes were pre-tested prior to coating deposition in an artificial cerebrospinal fluid solution (aCSF) (15 mM Tris HCl, 126 mM NaCl, 2.5 mM KCl, 20 mM Na₂CO₃, 1.2 mM NaH₂PO₄, 2.0 mM Na₂SO₄, 2.4 mM CaCl₂, 1.2 mM MgCl₂, pH = 7.40). Prior to CaCl₂ and MgCl₂ addition, the pH of the aCSF was adjusted to 7.40 using 0.1 N NaOH or a 0.1 N HCl solution. Electrodes were submerged in buffer and a triangle waveform from - 0.4 V to + 1.3 V was applied at 400 V/s for 1 minute; electrodes without a stable background were discarded. PEDOT:Nafion deposition solutions consisted of 100-200 µL of a stock solution of 0.04 M EDOT (Sigma Aldrich, St. Louis, MO, USA) in acetonitrile (prepared by the addition of 43 µL EDOT to 10 mL acetonitrile) and 200 µL of LQ-1105 Nafion (Ion Power Inc., DE, USA) in 20 mL acetonitrile (HPLC grade, EMD Chemicals Inc., Darmstadt, Germany). The final deposition solutions prepared from the stock solution contained either 200 µM EDOT (low-density PEDOT:Nafion coating) or 400 µM EDOT (high-density PEDOT:Nafion coating). Prior to electrodeposition, deposition solutions were mixed for 1 minute and used within 12 hours. Dopamine, ascorbic acid, DOPAC, bovine serum albumin, and all other chemicals, unless otherwise specified, were purchased from Sigma Aldrich. Neurotransmitter measurements were performed in aCSF buffer solution.

4.3.4 ELECTROCHEMISTRY

The voltage for electrodeposition was controlled using a Gamry Instruments Reference 600 potentiostat (Warminster, PA, USA) in a three-electrode configuration. A tightly coiled silver wire was used as the counter electrode, and a straight silver wire was used as the reference electrode. All electrochemical data were recorded using electrodes fabricated with T-40 carbon fibers. Both the reference and counter electrodes were polished using sandpaper and rinsed using 18.2 MΩ doubly-deionized (MilliQ) water.
Deposition was performed by applying a triangle waveform from +1.5 V to -0.8 V at 100 mV/s for 15 cycles, and using an open-circuit potential prior to waveform application. Electrochemical characterization of coated and uncoated electrodes was performed via fast-scan cyclic voltammetry using the WCCV 3.0 software package, (Knowmad Technologies, LLC, Tucson, Arizona). A computer-controlled flow cell with a 6-port valve switch (VICI Valco, Houston, TX, USA) and Dagan ChemClamp potentiostat (Minneapolis, MN, USA) was used for background-subtracted electrochemical measurements of neurotransmitters and interferents.

4.4 RESULTS AND DISCUSSION

4.4.1 ELECTROPOLYMERIZATION OF EDOT WITH NAFION AS THE COUNTERION

The polymerization of monothiophenes such as ethylenedioxythiophene (EDOT) is possible through a variety of oxidant-initiated or electrochemical processes.\textsuperscript{31–34} Commonly, iron (iii) tosylate (the monomeric homologue of polystyrenesulfonate) or iron (iii) chloride are used as oxidants for \textit{in situ} polymerization.\textsuperscript{35} Solutions of EDOT and oxidant in solvent are deposited, dried, and rinsed to form a conductive polymer film. Alternatively, EDOT can be oxidatively electro-polymerized to PEDOT in the presence of a counter ion. Nafion contains sulfonate groups much like iron tosylate or polystyrenesulfonate making it a suitable counter ion. Additionally, because oxidized PEDOT is positively charged, Nafion can be incorporated into the coating as a counter ion. Given this similarity, thick PEDOT:Nafion composite films have been previously synthesized on platinum wires via a galvanostatic deposition in a 5% aqueous dispersion of Nafion with small a volume of EDOT added, though to date, no applications of this composite material have been described.\textsuperscript{36}
Described here is the electro-synthesis of a surface-immobilized PEDOT:Nafion composite polymer. We have optimized solution concentrations to examine two different coating regimes, called low-density (LD) PEDOT:Nafion and high-density (HD) PEDOT:Nafion. Additionally, we employ an information-rich coating deposition method, cyclic voltammetry, which has been shown to increase the nucleation density of electropolymerized conducting polymers when compared to an amperometric deposition. This may lead to an increased Nafion density (and thus an increased repulsion of anionic species) at the electrode.

We propose that the final structure of the PEDOT has a positive charge which is coordinated by a Nafion sulfonate (Scheme 4-1). A positive charge every three monomer units has been proposed for PEDOT:Tosylate and given the similarity of the counter ion, it is expected that the Nafion sulfonate coordinates similarly. Furthermore, measurements made at other electrochemically polymerized PEDOTs with sulfonate dopants indicate that an excess of sulfonates is present in the coatings. Extending this conclusion, the PEDOT:Nafion coating is likely not charge neutral (i.e. one sulfonate to one PEDOT positive charge) but instead contains an excess of sulfonate groups relative to the positive charges on PEDOT. Given that a PEDOT-associated sulfonate on a Nafion chain is only one of many, this may increase the negative character of the coating, generating selectivity towards cations.

An ordinary deposition trace is shown, with cycles 1, 5, 10, and 15 highlighted in Figure 4-1. Three prominent characteristics of this deposition are apparent. First, an oxidation current near the anodic limit (+1.5 V) is attributed to the oxidation of EDOT that polymerized to form PEDOT. This oxidation current on the deposition voltammogram is the film-forming current, and is an indicator of coating success. Increasing the EDOT
Scheme 4-1: Proposed structure of PEDOT:Nafion. EDOT undergoes electropolymerization in the presence of Nafion, forming a PEDOT:Nafion composite polymer that coats the surface of a carbon-fiber microelectrode.
Figure 4-1: Electropolymerization of EDOT with Nafion counterions on carbon-fiber microelectrodes. 

a) Voltammetric trace of a LD PEDOT:Nafion coating on a carbon-fiber microelectrode. Deposition cycles 1, 5, 10, and 15 are shown. 

b) Energy-dispersive X-ray spectroscopy of a LD PEDOT:Nafion coating on a T-40 carbon-fiber microelectrode indicating the presence of fluorine and sulfur. 

c) Electron micrograph of uncoated carbon-fiber microelectrode with characteristic ~100 nm striations. 

concentration results in a larger oxidative deposition current (Figure 4-2). Second, a reduction wave starting near -0.6 V is apparent; we attribute this wave to the reduction of protons. The cycling of a carbon-fiber microelectrode using a deposition waveform in a solution of acetonitrile and sulfuric acid results in a similar wave shape and reduction potential (Figure 4-2). Third, a small irreversible peak-shaped wave is apparent at 50 mV, and appears in most deposition traces. We attribute this peak to an irreversible oxidation of the PEDOT coating, as observed elsewhere in PEDOT electro-synthesis literature. Voltammetric deposition of EDOT without the presence of a counter ion in solution does not form a coating or generate an oxidative current (Figure 4-2).

Scanning electron microscopy was used to compare the surface morphology of uncoated (Figure 4-1C) and PEDOT:Nafion-coated carbon-fiber microelectrodes (Figure 4-1D). The unmodified carbon fiber exhibits a striated surface, with individual striations measuring between 50 and 200 nm wide. After deposition of a low-density PEDOT:Nafion coating on the electrode, a ~100 nm coating on the electrode obfuscates the striations, and imparts a smoother surface morphology. Energy-dispersive X-ray spectroscopy was utilized to measure the presence of sulfur and fluorine in the coatings. As both PEDOT and Nafion contain sulfur, while only Nafion contains fluorine, the fluorine Kα line was used to confirm the presence of Nafion in the PEDOT:Nafion coatings (Figure 4-1B). Indeed, the fluorine peak is present for polymer-coated electrodes, and is absent for uncoated electrodes (Figure 4-3). Interestingly, given a constant Nafion concentration and an increasing EDOT concentration, the fluorine Kα peak grows with higher EDOT concentrations, indicating that the PEDOT incorporates more counterion Nafion into the coating if there is more PEDOT polymerized during the voltammetric deposition process (data not shown).
Figure 4-2: Electrodeposition control experiments for PEDOT:Nafion at carbon-fiber microelectrodes. Color indicates the deposition cycle number. a) CFME in 20 mL ACN. b) CFME in 20 mL ACN + 100 µL EDOT stock. c) CFME in 20 mL ACN + 200 µL LQ-1105 Nafion. d) CFME in low density PEDOT:Nafion deposition solution. e) CFME in high density PEDOT:Nafion deposition solution. f) CFME in 20 mL ACN + 30 µL conc. H$_2$SO$_4$. 
Figure 4-3: Control EDX spectrum (uncoated electrode). Note the lack of a fluorine peak.
4.4.2 FAST-SCAN CYCLIC VOLTAMMETRY AT PEDOT:NAFION MODIFIED ELECTRODES

To explore the chemical effect of EDOT concentration on coating performance, two concentrations of EDOT (and thus two coating types) were chosen for exploration in the scope of this work. The first coating type (Figure 4-4, panel A) is prepared with the intent of maximizing selectivity while minimizing changes in temporal response or background current. Flow-injection analysis background-subtracted fast-scan cyclic voltammetry was used to characterize the effect of LD PEDOT:Nafion coatings on the temporal response and background current of the electrode (Table 1 and Figure 4-4, panel A). A statistically significant increase in sensitivity from 13 ± 2 nA/µM for uncoated carbon fibers to 26 ± 6 nA/µM for coated carbon fibers was observed (Student’s t-test, n = 6 electrodes, P < 0.01). The 10-90 rise time of a 1.0 µM bolus of dopamine detected at the LD PEDOT:Nafion coated electrode was 0.45 ± 0.11 seconds, compared to 0.46 ± 0.09 seconds from an uncoated electrode. This difference was not statistically significant (Student’s t-test, n = 6 electrodes, P = 0.95). The background shape and current are essentially unchanged. The second coating type (Figure 4-4, panel B), HD PEDOT:Nafion, is prepared with the intent of maximizing selectivity and sensitivity while maintaining a background current under the maximum current threshold of typical FSCV current amplifiers. The HD PEDOT:Nafion coating resulted in a statistically significant 4-fold increase in sensitivity with respect to the uncoated carbon-fiber electrode for dopamine (46 ± 13 nA/µM, Student’s t-test, n = 6 electrodes, P < 0.05). The background current increases nearly 3-fold, though the background shape is similar. The wave shape of a background-subtracted dopamine voltammogram is markedly different as the oxidative current is ~4 times larger while the reduction current increases only by a factor of 1.5 compared to a control electrode. Electron microscopy of HD PEDOT:Nafion-coated electrodes does not indicate an increased geometric area compared to LD...
Figure 4-4: Electrochemical performance of PEDOT:Nafion coated carbon-fiber microelectrodes. Blue line – low-density coated electrode, green line - high-density coated electrode, black line - uncoated electrode.\textbf{a)} Low-density PEDOT:Nafion coated electrodes, \textbf{b)} High-density PEDOT:Nafion coated electrodes. \textit{Left} - background currents recorded at a 75 µm long T-40 carbon-fiber microelectrodes. \textit{Center} - background subtracted voltammograms of 1.0 µM dopamine in aCSF. \textit{Right} - current vs. time traces of a background-subtracted 1.0 µM bolus of dopamine.
PEDOT:Nafion-coated electrodes. We are currently investigating the origin of this sensitivity difference but suspect that differences in proton-transfer equilibrium between the adsorbed dopamine and adsorbed dopamine-orthoquinone could give rise to this effect. The 10-90 rise time of a 1.0 µM bolus of dopamine detected at the higher EDOT coated electrode is 0.84 ± 0.19 seconds, compared to 0.46 ± 0.09 seconds from an uncoated electrode. Clearly, a sacrifice in temporal resolution is made for a substantial increase in sensitivity. This temporal resolution may not be needed for all types of measurements, for example in equilibrium surface coverage measurements of dopamine via fast-scan controlled-adsorption voltammetry, but may be advantageous for low signal recordings, such as in vivo measurements of spontaneous phasic dopamine release, or perhaps for lower concentration transmitters.

4.4.3 VOLTAMMETRY OF DOPAMINE AND INTERFACES AT CARBON-FIBER MICROELECTRODES

Following deposition of Nafion onto the electrode surface, it is expected that mass transfer for a cation (such as dopamine) should be faster than that of an anion (such as DOPAC or ascorbic acid). To validate that the PEDOT:Nafion coating decreased the rate of mass transfer of DOPAC and ascorbic acid, slow scan cyclic voltammetry (20 mV/s) was performed using LD PEDOT:Nafion-coated electrodes on solutions of 1.0 mM dopamine, DOPAC, and AA in 20 mM pH 7.4 phosphate buffered saline (Figure 4-5). The steady-state current for a 1.0 mM dopamine voltammogram is largely unchanged for the coated electrodes, meaning that the coating has a negligible effect on the rate of mass transfer for dopamine. However, a considerable decrease is apparent in both Figure 4-5B and 4-5C which correspond to DOPAC and ascorbic acid (anions at physiological pH). Fast-scan cyclic voltammograms (400V/s) show an increase in dopamine sensitivity and a decrease in sensitivity to interferents (DOPAC and AA).
Figure 4-5: Cyclic voltammetry of dopamine, DOPAC, and ascorbic acid at PEDOT:Nafion-coated electrodes. Solid line – LD PEDOT:Nafion-coated electrode, dashed line – uncoated electrode. Cyclic voltammograms collected with a scan rate of 10 mV/s for a) dopamine (1.0 mM), b) DOPAC (1.0 mM), and c) AA (1.0 mM), exhibit a decrease in the rate of mass transfer for anionic compounds, while dopamine remains comparably unchanged. Representative background-subtracted fast-scan cyclic voltammograms of d) dopamine (1.0 µM), e) DOPAC (20 µM), and f) ascorbic acid (200 µM) (L to R), show that the sensitivity for DOPAC and AA has decreased. Electrode sensitivity has increased for dopamine for PEDOT:Nafion-coated vs. uncoated electrodes.
These signals are dependent on adsorption, and the PEDOT:Nafion coating reduces anion adsorption.

4.4.4 SELECTIVITY AND LIMIT OF DETECTION FOR PEDOT:NAFION-COATED ELECTRODES

Selectivity of PEDOT:Nafion-coated electrodes for dopamine over DOPAC and ascorbic acid was quantified by background-subtracted fast-scan cyclic voltammetry (Figure 4-6). Equimolar current ratios of dopamine and DOPAC or dopamine and ascorbic acid were used to calculate the selectivity of the sensor. The current used in this ratio was measured at peak potential of the oxidation wave for dopamine. Uncoated electrodes had a selectivity of 54 ± 6 for dopamine/AA and 21 ± 4 for dopamine/DOPAC. Electrodes prepared using a traditional dip-coating method are not statistically distinguishable from uncoated electrodes (97 ± 20 for dopamine/AA and 23 ± 7 for dopamine/DOPAC, Student’s t-test, n = 3 electrodes, P > 0.1 for both). Conversely, LD PEDOT:Nafion-coated electrodes exhibit a statistically significant increase in selectivity for DA/AA (530 ± 60, Student’s t-test, n = 15 electrodes, P < 0.001), and at HD PEDOT:Nafion-coated electrodes, the selectivity increased to 1540 ± 150, which was also statistically significant compared to uncoated carbon-fiber electrodes (Student’s t-test, n = 3 electrodes, P < 0.001). The selectivity for DA/DOPAC was increased from 21 ± 4 (uncoated) to 45 ± 6 (LD PEDOT:Nafion coating) and 52 ± 4 (HD PEDOT:Nafion coating). This difference was statistically significant when comparing uncoated to any of the PEDOT:Nafion-coated electrodes (Student’s t-test, n = 3 - 15 electrodes, P < 0.05). It is not yet understood why selectivity for DA over DOPAC plateaus.

Electrode noise was characterized on 15 LD PEDOT:Nafion and 3 HD PEDOT:Nafion-coated electrodes by measuring the RMS (root mean square) current noise of the
Figure 4-6: Chemical selectivity of PEDOT:Nafion-coated, Nafion dip-coated, and uncoated carbon-fiber microelectrodes for dopamine over ascorbic acid and DOPAC. Selectivity is defined as an equimolar current ratio at the peak oxidation potential of dopamine. Error bars are standard error of the mean (SEM) \((n = 3 - 15)\). Statistical significance is marked with asterisks \((\ast P < 0.05, \ast \ast P < 0.01, \ast \ast \ast P < 0.001)\), and selectivity of treated electrodes is compared to uncoated carbon-fiber microelectrodes.
electrode between 0.575 and 0.625 V vs. Ag/AgCl while scanning at 400 V/s in pH 7.4 aCSF. Resultant data was filtered as previously described.\textsuperscript{42} The RMS noise for uncoated carbon fibers was 80 ± 30 pA. LD PEDOT:Nafion-coated electrodes had a statistically significant decrease in noise to 30 ± 10 pA (Student’s t-test, n = 15 electrodes, P < 0.01), while HD PEDOT:Nafion-coated electrodes showed a statistically significant increase to 100 ± 40 pA (Student’s t-test, n = 3 electrodes, P < 0.01).

Background-subtracted fast-scan voltammetry was also used to measure limit-of-detection for dopamine at uncoated, LD PEDOT:Nafion-coated, and HD PEDOT:Nafion-coated electrodes. The limit-of-detection was calculated by measuring the RMS current noise across the oxidation potential for dopamine over a 50 mV window (\textit{vide supra}, Table 1). For uncoated carbon-fiber microelectrodes, this was 20 ± 7 nM. LD PEDOT:Nafion-coated electrodes had a limit of detection of 4 ± 1 nM for dopamine, while HD PEDOT:Nafion-coated electrodes had a limit of detection of 6 ± 1 nM for dopamine. Both of these limits of detection for PEDOT:Nafion-coated carbon-fiber electrodes were statistically different compared to uncoated carbon-fiber electrodes (Student’s t-test, n = 6 electrodes, P < 0.05).

\textbf{4.4.5 EVALUATION OF PEDOT:NAFION-COATED ELECTRODES FOR \textit{IN VIVO} DOPAMINE MEASUREMENTS}

To validate the sensor’s response to a typical voltammetric stimulated release experiment, a stainless steel stimulating electrode was implanted in the medial forebrain bundle and a LD PEDOT:Nafion-coated carbon-fiber microelectrode was implanted in the nucleus accumbens of an anesthetized Sprague-Dawley rat. Prior to implantation, the coated electrode was cycled from -0.4 V to +1.3 V at 400 V/s in pH 7.4 aCSF buffer for 10 minutes. Stimulated release of dopamine was measured with a concentration of
approximately 300 nM (Figure 4-7A). A color plot recorded during stimulation shows typical stimulated release and reuptake (Figure 4-7A). Additionally, a current vs. time trace of the current at the oxidation wave for dopamine, and an extracted voltammogram are shown (Figure 4-7A, inset). To further validate the use of this sensor in vivo, physiological dopamine release was monitored in the nucleus accumbens of an anesthetized rat (Figure 4-7B). This is, to our knowledge, the first recording of dopamine transient release events in anesthetized animals without administration of reuptake inhibitors. Compared to recordings from anesthetized rats published elsewhere, we see more transients over a 20 second timeframe than were reported in a five-minute bin of transient recordings (1-5). While there are differences in anesthesia (urethane vs. isofluorane) and recording location (caudate putamen vs. nucleus accumbens) between the two recordings, we believe this arises at least in part due to the increased sensitivity of the PEDOT:Nafion-coated electrode. Several dopamine transients ranging from 5 - 200 nM are present. A representative cyclic voltammogram is taken at the white vertical dashed line and displayed in the inset. To validate that these recordings were enabled by the coating, a proper control experiment with matched implantation and measurement sites, anesthesia, and surgical preparations could be performed.

4.4.6 INVESTIGATION OF PEDOT:NAFION COATING ON IN VIVO AND IN VITRO FOULING

The PEDOT:Nafion-coated sensors were characterized for their ability to resist synthetic and in vivo biofouling. In the context of these experiments, we define biofouling as the sensor’s decrease in sensitivity to dopamine as measured by flow cell background-subtracted FSCV after being placed in a challenging chemical environment. To assess resistance to biofouling, we compare pre-calibration DA sensitivity with post-calibration DA sensitivity. While other important work has shown that the sensitivity of tyramine-
Figure 4-7: *In vivo* measurements of dopamine release using a PEDOT:Nafion coated carbon-fiber microelectrode. Current vs. time traces are extracted from the color plot at the peak oxidation potential for dopamine (~600 mV). Representative cyclic voltammograms are taken from the vertical white dashed line on the color plots. a) Dopamine release was electrically evoked by stimulation (black bar, 40 pulses at 60 Hz, ± 150 µA, 2 ms per phase) of the medial forebrain bundle and monitored in the nucleus accumbens of a Sprague-Dawley rat. b) Spontaneous transients recorded in the nucleus accumbens of an isoflurane-anesthetized Sprague-Dawley rat.
fouled carbon fiber microelectrodes is renewed with application of the + 1.3 V waveform for 15 minutes,\textsuperscript{44} due to practical limitations electrode pre- and post-calibrations are infrequently performed by modern practitioners of \textit{in vivo} voltammetry.

Fouling of the electrode in biological tissue was simulated by implanting the electrode into 40 g/L solution of bovine serum albumin (BSA) in pH 7.4 aCSF and applying the previously described detection waveform. This solution has been used elsewhere to mimic the fouling capacity of the brain environment.\textsuperscript{45,46} Uncoated and three LD PEDOT:Nafion-coated CFMEs were submerged in this solution for 2 hours. Three uncoated CFMEs were also submerged in pH 7.4 aCSF and cycled using the same waveform for 2 hours as a control for sensor degradation via application of the waveform.

For the \textit{in vitro} fouling procedure in BSA, uncoated CFMEs lost 40 ± 5 % of their sensitivity to a 1 µM bolus of dopamine (Figure 4-8, panel A). LD PEDOT:Nafion-coated CFMEs lost only 5 ± 6 % of their sensitivity, and electrodes cycled in pH 7.4 aCSF lose only 1 ± 3 % of their sensitivity. This sensitivity loss of approximately 40% for uncoated fibers is also reported elsewhere.\textsuperscript{46} The difference between coated and uncoated fibers is statistically significant (Student's t-test, \( P < 0.01, n = 3 \)), while the difference between LD PEDOT:Nafion-coated CFMEs and control electrodes in pH 7.4 aCSF is not statistically significant (Student's t-test, \( P > 0.3, n = 3 \)).

For the \textit{in vivo} fouling procedure, uncoated electrodes were implanted in the prefrontal cortex (PFC) and nucleus accumbens (NAcc) of a male Sprague-Dawley rat for 30 minutes and compared to LD PEDOT:Nafion coated electrodes that were implanted for 6 hours to demonstrate the capacity of this coating to mitigate biofouling. Uncoated
Figure 4-8: PEDOT:Nafion electrodes resist a) synthetic fouling (40 g/L BSA in pH 7.4 aCSF) and b) in vivo biofouling. The dopamine detection waveform (-0.4 V to +1.3 V) was continuously applied for the duration of synthetic and in vivo biofouling experiments. Asterisks indicate statistical significance when compared to uncoated CFMEs. Error bars are SEM (n = 3 - 4) c) Electron micrograph of two representative uncoated CFMEs removed after 30 minute implantations in the nucleus accumbens or prefrontal cortex show large accumulations of biomaterial on the surface of the electrode. d) Electron micrograph of a LD PEDOT:Nafion coated carbon fiber microelectrode implanted in the nucleus accumbens for six hours shows decreased accumulation of biomaterial when compared to uncoated CFMEs. Statistical significance is marked with asterisks (* P < 0.05, ** P < 0.01), and selectivity of treated electrodes is compared to uncoated carbon-fiber microelectrodes.
carbon fibers lost 60 ± 19 % and 33 ± 9 % of their sensitivity to dopamine over the course of 30 minutes of implantation in the PFC and NAcc, respectively (Figure 4-8, panel B). LD PEDOT:Nafion-coated CFMEs lost only 9 ± 5 % of the pre-calibration sensitivity, despite being implanted for 5.5 hours longer than the uncoated fibers. Differences in sensitivity between uncoated implanted electrodes (in the PFC and NAcc) and uncoated electrodes cycled in aCSF for 6 hours were statistically significant (Student’s T-test, P < 0.05, n = 3 - 4 for both implant sites), while LD PEDOT:Nafion-coated electrodes did not lose a statistically significant amount of sensitivity (Student’s T-test, P > 0.1, n = 3).

The origin of large uncertainty in uncoated CFME sensitivity loss could be due to differences in surgical preparation. Recently, two-photon mapping measurements made during implantation of microelectrodes showed that whether the microelectrode ruptures a blood vessel as it is lowered into the cortex can dramatically impact the performance of the microelectrode.47 Because few laboratories are equipped to guide the microelectrode into the brain with advanced blood vessel imaging techniques, there is value in mitigating fouling originating from ruptured intracranial blood vessels or other sources not described in literature.

Scanning electron microscopy with energy-dispersive X-ray spectroscopy was also performed with the intent of examining the surface morphology and fluorine content of coated and uncoated electrodes post-implantation. A representative micrograph of uncoated CFMEs implanted in either the PFC or NAcc show large accumulations of biomaterial, perhaps dried blood, on the electrodes after just 30 minutes of implantation (Figure 4-8, panel C). In contrast, a representative electron micrograph of a LD PEDOT:Nafion coated CFME implanted for 6 hours shows little accumulation of
biomaterial on the electrode. The coated electrode also retained fluorine and sulfur (and therefore PEDOT:Nafion) on the electrode surface following implantation (Figure 4-9). The sensors retained selectivity for dopamine over ascorbic acid after 6 hours of implantation (pre-implantation DA/AA selectivity: 530 ± 40, post-implantation: 520 ± 180, no statistical difference, Student’s t-test, P > 0.1, n = 3 electrodes). The post-implantation selectivity for dopamine over DOPAC for the coated electrode was also retained (pre-implantation DA/DOPAC selectivity: 45 ± 6, post-implantation: 35 ± 3, no statistical difference, Student’s t-test, P > 0.1, n = 3 electrodes). Thus, the performance of the electrode coating did not change during an acute in vivo measurement.

4.5 CONCLUSIONS

A Nafion and PEDOT containing composite polymer has been electropolymerized in a novel scheme on carbon-fiber microelectrodes. The robust and reproducible coating is applied voltammetrically in a solution of EDOT and Nafion. Coated electrodes show increased electrochemical sensitivity (2 - 5x) and selectivity (2 - 30x), a comparable temporal response, lower noise, and mechanical stability. Coated electrodes do not lose selectivity and sensitivity after being implanted in the brain for 6 hours, which is an improvement over uncoated electrodes. From these data, we posit that LD PEDOT:Nafion-coated electrodes have a desirable combination of characteristics that lead us to recommend it over an uncoated electrode for in vivo dopamine measurements. The LD PEDOT:Nafion coating is also preferable to the HD PEDOT:Nafion coating because it retains electrochemical information by preserving the voltammogram shape. However, in circumstances where high sensitivity is required, the HD PEDOT:Nafion-coated electrodes may prove useful. Here, we exhibit the advantages of the coating with respect to dopamine measurements, but it may also be advantageous for in vivo measurements of other neurotransmitters. In the future, we

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Figure 4-9: EDX spectrum of a PEDOT:Nafion coated electrode post-implantation. The presence of fluorine and sulfur indicate that the PEDOT:Nafion coating is present.
would like to investigate chronic-implant performance and to establish a better mechanistic understanding of the sensor performance.

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4.7 REFERENCES


CHAPTER 5

SYNTHESIS AND CHARACTERIZATION OF DISPERSED OLIGO-EDOT:NAFION NANOPARTICLES FOR QUANTITATIVE WATER SENSING
5.1 ABSTRACT

Oligo-EDOT:Nafion composite nanoparticles are easily prepared via a one pot synthesis in ambient conditions from commercially available reagents. These particles form uniform dispersions in acetonitrile with a diameter of approximately 100 nm, and we characterize them by visible absorption spectroscopy, thermogravitational analysis, transmission electron microscopy, and dynamic light scattering. When suspended in acetonitrile, these particles form a stable suspension of low molecular weight PEDOT and Nafion, as the particles have a zeta potential of -49 ± 11 mV. Exposure to water or other polar protic solvents induces chemical and physical changes in the nanoparticles, resulting in a bathochromic shift in the absorption spectrum and a constriction of the nanoparticle geometry from a diameter of 111 ± 8 nm to 71 ± 6 nm. Here, we adapt this supramolecular behavior to inexpensively (<2$ USD) and rapidly (a few seconds) detect the water content of acetonitrile with UV-VIS absorption spectroscopy. The limit-of-detection of water in acetonitrile is 125 ppm, and the linear dynamic range extends up to 2500 ppm.
5.2 INTRODUCTION

Nanoparticles are an important class of functional materials with potential to impact a variety of problems of interest. The field of nanotechnology is rapidly growing, with applications of polymer and inorganic nanoparticles seen in imaging,\textsuperscript{1} drug delivery systems and medicine,\textsuperscript{2-4} biosensors,\textsuperscript{5-9} and electronic materials.\textsuperscript{10-13} While the nanoparticle synthesis field is maturing, there is still an absence of functional polymer nanoparticle backbones. Polythiophenes such as poly(3,4-ethylenedioxythiophene) (PEDOT) or poly(3,4-propylenedioxythiophene) (ProDOT) are of particular interest because of their conductivity and ubiquity in electronic devices,\textsuperscript{14-16} electrochromic devices,\textsuperscript{17} supercapacitors,\textsuperscript{18,19} medical coatings,\textsuperscript{20} and biosensors.\textsuperscript{21,22} The ethylene (EDOT) or propylene (ProDOT) bridge provides a site to incorporate modifiers and linkers making polythiophenes an excellent scaffold upon which to incorporate biotherapeutics or electronic interface modifiers.\textsuperscript{23}

When polymerized, PEDOT is a blue solid; intractable in all solvents regardless of small molecule doping. As a result, PEDOT is most commonly used as a polymeric blend with a polystyrenesulfonate (PSS) counterion.\textsuperscript{24} The deprotonated sulfonate groups on PSS interact with positively charged polarons on PEDOT chains through a coulombic attraction, establishing a strong intermolecular interaction in which PSS chains entangle PEDOT.\textsuperscript{25,26} With this interaction, PEDOT and PSS form a stable and soluble dispersion, which is easily processed by spin coating, spray coating, doctor blading, or dip coating.\textsuperscript{24}

Here, we draw inspiration from polystyrenesulfonate and form a stable nanoparticle dispersion of PEDOT by polymerizing EDOT in the presence of an aqueous dispersion of perfluorinated sulfonated polymer (Nafion). The solubility of the PEDOT is greatly
enhanced, and the fluorinated backbone of the Nafion gives rise to unique solution-dispersed behavior. After the addition of polar protic solvents such as water or methanol, these nanoparticle dispersions undergo a chemical change, and irreversibly change conformation to minimize free energy in the new solvation environment. We exploit this behavior to use these nanoparticle dispersions as quantitative detectors of local solvation environment in acetonitrile.

A promising application of solvation environment-sensitive nanoparticles is to use them to detect impurities in solvents. Modern synthetic methodology often requires anhydrous conditions, and many important commercial compounds decompose in the presence of water. The Karl Fischer (KF) titration is the gold standard of water analysis for organic solvents, utilizing a quantitative reaction with iodine and sulfur dioxide in either the coulometric or volumetric variations. These titrations are accurate from the parts per million up to the parts per hundred range, but require an expensive ($5000 USD) instrument that must be operated by an expert and is only useful for the task of KF-titrations. Other quantitative methods exist, such as by $^{19}$F nuclear magnetic resonance spectroscopy or gas chromatography, but these methods require internal standards and also expensive instruments. Molecular sieves, distillation, and liquid-liquid extractions are commonly employed to dehydrate solvents, but each method has drawbacks – molecular sieves rely on the slow diffusion of water into 3 – 4 Å pores, distillation requires enormous amounts of energy on an industrial scale and can pose serious safety concerns, and liquid-liquid extraction generates large amounts of waste. Unnecessary or wasteful purifications may be avoided if a fast, easy, and inexpensive assay for the water content of solvents were available. Here, we present the synthesis and characterization of a PEDOT:Nafion nanoparticle solution which provides an inexpensive ($2 USD per calibration and measurement), simple, rapid, and
quantitative assay for water in acetonitrile. In addition to this practical application, these nanoparticles may have additional utility in serving as a scaffold for drug delivery, electronic device interface modifiers, or sensors for other types of molecules.

5.3 MATERIALS AND METHODS

Nanoparticles were prepared by solvent evaporation oligomerization in borosilicate glass 20 mL scintillation vials. Unless otherwise specified, 150 µL of 3,4-ethylenedioxythiophene (EDOT) (Sigma Aldrich, St. Louis, MO, USA) and 150 µL of Nafion (LQ-1105, 5% w/w in solution of lower aliphatic alcohols and water) (Ion Power, New Castle, DE, USA) was added to 10 mL of HPLC grade acetonitrile (EMD Millipore, Darmstadt, Germany) and the vial was left uncapped in a laboratory fume hood for 18 hours. A purple solid formed at the bottom of the vials which turned red upon the addition of 20 mL acetonitrile. This solution was split evenly into separate glass vials, and spiked with 10-100 µL of a nanopure water/acetonitrile stock solution to bring the concentration of added water to between 125 ppm (parts per million) and 16 ppt (parts per thousand). Vials were capped and mixed for 5 seconds, and then UV/Vis absorbance measurements were made in a capped quartz 1 cm path length cuvette. ICP-MS was performed using a Perkin Elmer ELAN DRC II (Perkin Elmer, Waltham, MA, USA). All reagents were trace metal grade, and quantitation was done relative to an indium internal standard. An Agilent 8453 diode array UV/Visible spectrophotometer was used for all UV/Vis measurements. A Tecnai Spirit transmission electron microscope operated at 100 kV was used for all electron microscopy. Samples were spotted 5-10 times onto a 300 mesh carbon-coated copper TEM grid (Electron Microscopy Sciences, Hatfield, PA, USA) and dried in air. Dynamic light scattering (DLS) was performed on a Zetasizer ZS (Malvern Instruments, Worcestershire, United Kingdom). 3 mL of aliquots of the particles were analyzed in borosilicate glass cuvettes.
at 25°C using the automatic mode (n = 3 batches of particles). The z-average radius
(z.average) and polydispersity index (PDI) were calculated from the correlation function
using the Malvern Zetasizer Software version 7.03 (Malvern Instruments,
Worcestershire, United Kingdom). The zeta potential of the particles was measured in
ethanol because our zeta potential cell was not compatible with acetonitrile.

5.4 RESULTS AND DISCUSSION

5.4.1 SYNTHESIS AND THERMOGRAVITATIONAL ANALYSIS OF
NANOPARTICLES

The solvent evaporation oligomerization of PEDOT:Nafion nanoparticles is depicted in
Figure 5-1. A dilute solution of EDOT and Nafion in acetonitrile is added to a scintillation
vial, which is left exposed to atmosphere and evaporated in a fume hood for 18-24 hours
(a). After evaporation, a solid mass of particles is formed on the container (b), which is
readily solubilized by the addition of acetonitrile (c). Varying the EDOT and Nafion
concentrations yields differently colored solids, ranging from brown to red to purple to
blue, with higher concentrations of EDOT and Nafion resulting in dark brown colored
material. After exposure to ambient humidity for hours to days (depending on airflow
and humidity), nanoparticle suspensions that start brown, red, or purple will shift in color
to blue. After preparation, the nanoparticles will remain suspended in acetonitrile for
several months with a maximum solubility of approximately 5-10 mg/mL.

In order to probe the reagents for adventitious metals which might catalyze the reaction
by oxidation, the acetonitrile, Nafion, and EDOT were quantitatively analyzed for the
elements Be, Al, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd, Sn, Sb, Ba, and
Pb by ICP-MS by evaporating 1 mL of each sample and re-suspending them in 10 mL of
0.1 M nitric acid, followed by filtration through a 0.45 um nylon syringe filter. No metals
Figure 5-1: Synthesis of oligo-EDOT:Nafion nanoparticles by solvent evaporation. X, Y, and Z represent polymer subunits. On average, there are 18 CF₂ units between each sulfonate for the Nafion used. 

a) A mixture of EDOT and an aqueous dispersion of Nafion are dispersed in acetonitrile. This solution is mixed and left uncapped in a fume hood for 18 hours. 

b) Red and purple particles are formed on the bottom and sides of the vial. 

c) Upon re-suspension in acetonitrile, a uniform dispersion of oligo-EDOT:Nafion nanoparticles appear red in color.
were found at concentrations above 10 ppb in any of the reagents. Three freeze-pump-thaw cycles were performed on a solution of EDOT and Nafion in an oxygen-free glovebox, where the synthesis was repeated successfully, indicating that oxygen is not required for oligomerization, and confirming that the formation of PEDOT is likely not proceeding through the standard oxidative route. The Nafion in some manner acts as an oligomermerizing agent for EDOT, though sulfonate groups are not known to be oxidizing. When the synthesis is repeated with a lithiated (instead of protonated) Nafion analog (LiTHion, Ion Power, New Castle, DE, USA) in place of Nafion, no particles or colored solids form, suggesting that a low pH is needed for nanoparticle synthesis.

Thermogravitational analysis of nanoparticles (Figure 5-2) shows a large decrease in mass (~65 %) of the particles between 100 °C and 170 °C corresponding to the evaporation of excess EDOT and low order EDOT oligomer (on the order of 5 monomer units). The remaining mass of the nanoparticles is mostly lost between 380 °C and 410 °C, which is due to the thermal degradation of Nafion.

5.4.2 UV/VISIBLE ABSORBANCE SPECTROSCOPY MEASUREMENTS

The synthetic space of the particles was explored by modifying the concentration of the starting reagents. The aim of these experiments was to optimize the synthesis conditions. UV/Vis absorption spectra of a panel of synthesis conditions are shown in Figure 5-3. Nanoparticles were synthesized by varying the EDOT concentration from 665 ppm to 66500 ppm with Nafion held at a constant 310 ppm, or by varying the Nafion concentration from 5 ppm to 3125 ppm with EDOT held constant at 16625 ppm. The particles were synthesized as described in Figure 5-1, and suspended in 20 mL acetonitrile prior to measurement. Absorption below 300 nm corresponds to EDOT, while protonated EDOT oligomers are present between approximately 325 nm and 450
**Figure 5-2**: Thermogram of 2.1 mg nanoparticles and 2.2 mg solid Nafion. Range = 25 – 800 °C, rate = 10 °C/minute, stabilized at 25 °C for 10 minutes prior to run. EDOT monomer, dimer, trimer, and oligomer are evaporated at 120 – 190 °C, while the solid Nafion backbone degrades at approximately 350 – 550 °C.
Figure 5-3: UV/Visible absorption spectra of nanoparticles prepared in different synthesis conditions. a) At a fixed EDOT concentration, with variable Nafion concentrations. b) At a fixed Nafion concentration, with variable EDOT concentrations.
nm. The dimer absorbs at maxima of 325 nm and 334 nm, the trimer at 353 nm, 382 nm, and 408 nm, and the tetramer at 400 nm, 426 nm, and 447 nm. In synthesis conditions with higher EDOT or Nafion concentrations (above 310 ppm Nafion and 6650 ppm EDOT), nanoparticles approximately 100 nm in size become measurable by dynamic light scattering (DLS), and absorbance maxima at both 491 nm and 589 nm are visible.

To examine the effect of various solvents on the particle’s absorption maximum wavelength, two separate batches of nanoparticles were prepared with 125 µL Nafion, 125 µL EDOT, and 10 mL acetonitrile. These particles were re-solvated in 20 mL of acetonitrile and combined into a 50 mL beaker. From this beaker, nine 3 mL aliquots of nanoparticles in acetonitrile were removed and added to quartz cuvettes. To each of these cuvettes, between 2 and 10 drops of solvent were added. The solvents varied in polarity hydrogen bonding capacity (as measured by the solvatochromic pyridinium N-phenolate betaine dye),\(^\text{32}\) and proticity, and including diethyl ether, dichloromethane, 1-butanol, ethyl acetate, acetone, acetonitrile (control), methanol, ethanol, and water. The results are summarized in Table 1. A large volume of solvent (10 drops) was added to vials that did not appear to change color upon the addition, and a small amount (2 drops) was added to cuvettes where a large shift in absorption occurred. In general, aprotic solvents do not shift the absorption maximum of the particles when compared to the control. Protic solvents shift the absorption maximum by between 13 nm (butanol) and 46 nm (water). Additionally, the absorption maximum shift of water is greater than less polar protic solvents.
Table 5-1: Shifts in absorbance caused by the addition of solvents to the nanoparticle dispersion (n = 3 sets of particles split up into 4 mL aliquots). $E_T(30)$ is an indicator of polarity and hydrogen bonding capacity adapted from C. Reichardt.32

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$E_T(30)$ (kcal·mol$^{-1}$)</th>
<th>Max. Abs. λ (nm)</th>
<th>Protic</th>
<th>Drops added</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonitrile (control)</td>
<td>46</td>
<td>531 ± 4</td>
<td>no</td>
<td>10</td>
</tr>
<tr>
<td>diethyl ether</td>
<td>34.5</td>
<td>535 ± 3</td>
<td>no</td>
<td>10</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>38</td>
<td>535 ± 4</td>
<td>no</td>
<td>10</td>
</tr>
<tr>
<td>dichloromethane</td>
<td>41</td>
<td>533 ± 3</td>
<td>no</td>
<td>10</td>
</tr>
<tr>
<td>acetone</td>
<td>42</td>
<td>531 ± 2</td>
<td>no</td>
<td>10</td>
</tr>
<tr>
<td>1-butanol</td>
<td>50</td>
<td>544 ± 5</td>
<td>yes</td>
<td>2</td>
</tr>
<tr>
<td>ethanol</td>
<td>52</td>
<td>570 ± 2</td>
<td>yes</td>
<td>2</td>
</tr>
<tr>
<td>methanol</td>
<td>55</td>
<td>557 ± 6</td>
<td>yes</td>
<td>2</td>
</tr>
<tr>
<td>water</td>
<td>63</td>
<td>577 ± 4</td>
<td>yes</td>
<td>2</td>
</tr>
</tbody>
</table>

5.4.3 TRANSMISSION ELECTRON MICROSCOPY MEASUREMENTS

Transmission electron microscopy (TEM) was used to visualize the nanostructure of the particles and to examine the effects of hydration on particle structure. A batch of nanoparticles were prepared with 125 µL Nafion, 125 µL EDOT, and 10 mL acetonitrile. These particles were re-suspended in 20 mL acetonitrile, and appeared red in color. The particles were split into two 10 mL aliquots, and to one solution, 2500 ppm water was added. After the addition of water, the color of this solution changed from red to blue. Both aliquots were then filtered with 0.2 µm nylon syringe filters, and spotted onto carbon TEM grids within 10 minutes of re-suspension.

Representative transmission electron micrographs are shown in Figure 5-4, with (A) depicting a nanoparticle prepared at [EDOT] = 1.12 mM in an unhydrated solution of
Figure 5-4: Two representative nanoparticles prepared from the same pot of solution.  

**a)** Half of the particles were drop-cast onto a TEM grid within 10 minutes of re-suspension. These particles exhibit an open conformation.  

**b)** The other half of the particles were exposed to 2500 ppm H$_2$O prior to being drop-cast. The particle exhibits a surface-energy minimizing conformation, wrapping itself into a spherical shape.  

**c)** This size change was confirmed with dynamic light scattering measurements, which show a statistically significant decrease (36 %, p < 0.01) in particle size upon hydration.
acetonitrile, (B) depicting a nanoparticle prepared at [EDOT] = 1.12 mM in a hydrated (2500 ppm H₂O) solution of acetonitrile. Both A and B show a similar microstructure, with nodules 10-30 nm in diameter connected in a twisted chain configuration. The dry nanoparticles exhibit an open configuration while the wet nanoparticles typically exhibit a closed configuration, perhaps to minimize surface energy. No particles were identified by TEM when Nafion or EDOT alone was dispersed in acetonitrile or ethanol and drop cast onto carbon grids.

A solution of nanoparticles (prepared with 9 µM EDOT, 200 µL Nafion in 10 mL ACN) left capped in a hood for 60 days produced some nanoparticles with more interesting morphologies (Figure 5-5) including a branched configuration visually similar to a teddy-bear cholla cactus. Phase or crystal boundaries on the order of 5 nm are readily apparent in the micrograph, indicating that there are areas of differing electron density in the branches.

5.4.4 DYNAMIC LIGHT SCATTERING AND ZETA POTENTIAL MEASUREMENTS

In the interest of understanding the reproducibility of the synthesis and painting a clearer picture of solution-phase particle dynamics, the size of the nanoparticles was evaluated by dynamic light scattering (DLS). A batch of nanoparticles were prepared with 125 µL Nafion, 125 µL EDOT, and 10 mL acetonitrile. These particles were brought up in 20 mL acetonitrile, and appeared red in color. The particles were filtered with a 0.2 µm nylon syringe filter, split into equal volumes, and stored in separate containers. To one container, 3 drops of water were added, and the hydrated particles changed color from red to blue in a few seconds. To the other container, 3 drops of acetonitrile were added. The hydrodynamic diameter (size) of both the hydrated and unhydrated solutions was measured by dynamic light scattering (DLS). Unhydrated nanoparticles had an average diameter of 111 ± 8 nm (n = 3 batches of particles) with a polydispersity index (PDI) of
Figure 5-5: Transmission electron micrographs of oligo-EDOT:Nafion nanoparticles prepared with [EDOT] = 9 µM, 200 µL Nafion in 10 mL ACN, left capped in a hood for 60 days, and filtered with a 0.45 µm syringe filter prior to spotting on a TEM grid. The solution was light blue in color.
0.31 ± 0.09, and hydrated nanoparticles had an average diameter of 71 ± 6 nm with a PDI of 0.26 ± 0.05. The difference between average diameter is statistically significant (Student’s t test, p < 0.01), while the difference in PDI is not. The average hydrodynamic diameter of the nanoparticles decreased by 36% upon the addition of water to the acetonitrile. A reasonable explanation for this behavior is a solvent-induced minimization of surface energy that manifests in a conformational change. The zeta potential of particles synthesized with 125 µL Nafion, 125 µL EDOT, and 10 mL acetonitrile and re-suspended in 10 mL ethanol was measured to be -49 ± 11 mV (n = 3 batches of particles). This zeta potential is consistent with the observation that these particles do not settle in solution, as a highly charged surface will resist aggregation. No particles were observed by DLS and no zeta potential measurements could be made when EDOT or Nafion alone was dispersed in acetonitrile or ethanol, respectively.

5.4.5 CONDUCTIVITY MEASUREMENTS

Particles from all tested synthesis conditions were not conductive when drop-cast onto glass slides and rinsed with water. This could be a product of the low-order EDOT oligomers having a high charge hopping barrier, or perhaps due to the synthesis conditions favoring oligomers with an interrupted pi-conjugation system.

5.4.6 OLIGO-EDOT:NAFION NANOPARTICLES FOR THE QUANTITATIVE DETECTION OF WATER IN ACetonitrile

With the addition of water or other solvents to red solutions of nanoparticles suspended in acetonitrile, a bathochromic shift in the absorption maximum wavelength of the solution is observed over the course of 1-2 seconds when gently shaken or stirred. This bathochromic shift is not reversible when nanoparticle dispersions used for water quantitation are dried with molecular sieves. These nanoparticles undergo structural (as
observed by TEM) and chemical (as observed by spectroscopy) changes upon the addition of polar protic solvents. Here, we exploit these changes to employ the particles as a sensor for water in acetonitrile. Solutions with a red color upon suspension in acetonitrile were chosen for water detection as they displayed the largest bathochromic shift in absorption maximum upon the addition of water, and therefore had the highest sensitivity. Figure 5-6a shows the absorption spectra of a single pot synthesis of particles (prepared with 125 µL Nafion, 125 µL EDOT, and 10 mL acetonitrile), separated into six vials (3 mL per vial). A volume of 10-100 µL of a water/acetonitrile mixture was spiked into the vials, and they were quickly mixed for 5 seconds then measured on the spectrophotometer. Figure 5-6b shows the UV/visible absorption spectra maximum in the visible range plotted against the water concentration, where error bars are standard error of the mean, with replicate measurements coming from a new solution of nanoparticles with new water spikes. The limit of detection is probably dictated by the 1 nm resolution of our spectrophotometer at a concentration of 125 ppm H₂O. The linear dynamic range extends up to 2500 ppm H₂O, though quantitation is possible via non-linear fits up to 10000 ppm H₂O.

The spectrum contains three peaks, one at ~380 nm, one at ~410 nm, and one at ~510 nm. The first two peaks correspond to the protonated EDOT trimer, and the latter peak corresponds to oligo-EDOT (>5 monomer units). Upon the addition of water, the peak at 380 nm grows in intensity, and the peak at 510 nm shifts bathochromically while decreasing in magnitude. This suggests a decomposition of higher order oligomer into trimer with the addition of water, or perhaps the absorbing species crashing out of solution. Because no precipitate was observed, we believe that the de-polymerization hypothesis is more plausible. The shoulder to the right of the peak at 510 nm (centered on 565 nm) does not decrease in intensity.
Figure 5-6: Oligo-EDOT:Nafion nanoparticles as a quantitative water sensor. a) UV/Visible absorption spectra of acetonitrile-dispersed nanoparticles with different concentrations of water in solution. b) Linear region of the calibration plot of water concentration vs. wavelength maxima of visible absorbance. (n = 3, error bars are standard error of the mean) Inset: Entire range of tested water concentrations shows a roll-off in linearity after 2500 ppm H₂O. c) Solutions of nanoparticles dispersed into 4 mL aliquots of acetonitrile. L to R – increasing concentrations of water added, 0 ppm, 1000 ppm, 2000 ppm, 3000 ppm, 4000 ppm, 5000 ppm, 6000 ppm, 7000 ppm.
When a 20 mL vial containing 4 mL of nanoparticles dispersed in acetonitrile is left capped after the quantitative addition of water, the absorbance of the dispersed particles changes slowly with time. Figure 5-7 illustrates this time-dependent absorption of water by monitoring the wavelength of maximum absorbance for solutions of water-spiked acetonitrile over the course of 12 hours. After solutions were spectroscopically measured, they were stored under dry argon. The acetonitrile blank's absorbance wavelength maxima increases by ~3 nanometers per hour. Additionally, in solutions left to equilibrate for an extended period of time, the absorbance maxima increases more rapidly at water concentrations under 5000 ppm, and rises to a slightly higher plateau. This effect has practical consequences for utilizing these nanoparticles as quantitative sensors. Measurements must be performed within the first 5-10 minutes of nanoparticle-solvent interaction to achieve maximum sensitivity as defined by $\lambda_{\text{max, sample}} - \lambda_{\text{max, blank}}$, otherwise changes in $\lambda_{\text{max}}$ may be incorrectly interpreted as arising from changes in water concentration.

5.5 CONCLUSIONS

Herein, we have characterized a novel synthesis of EDOT oligomer composite nanoparticles. These nanoparticles are easily synthesized from two inexpensive, commercially available materials in a one-step room temperature procedure requiring no special equipment. The oligomerization proceeds even under strict anaerobic conditions, indicating oxygen is not required for nanoparticle genesis. Given the absence of other possible oxidizing species, this would seem to indicate a non-oxidative mechanism is at play. This oligomerization mechanism is of great general synthetic interest, as to our knowledge; there are no outstanding examples of non-oxidative oligomerization of non-halogenated polythiophenes. By manipulating synthesis
Figure 5-7: Time-dependent wavelength of maximum absorbance for nanoparticle dispersions with varying concentrations of added water. Nanoparticle dispersions were stored under dry argon between measurements to minimize the effect of atmospheric water.
conditions, increasing the length of oligomers could potentially provide a new route to polythiophenes. A non-oxidative polymerization of thiophenes could potentially provide a more homogeneous conductive material than what is prepared by oxidative polymerization with a Lewis acid such as FeCl$_3$ due to the complete exclusion of any trace metal impurities. The mechanism of the oligomerization may be of high general synthetic interest and is under investigation.

The nanoparticles have a tunable starting color, and because of both chemical and physical changes, their absorbance and morphology change predictably upon encountering protic polar solvents while suspended in acetonitrile. We have exploited this interesting supramolecular behavior to employ these nanoparticles as a quantitative water sensor in acetonitrile using a UV/Visible spectrophotometer. The linear dynamic range of water detection extends from 125 ppm up to 2500 ppm, which is a concentration range of interest for trace water analysis.

5.6 ACKNOWLEDGEMENTS
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5.7 REFERENCES


CHAPTER 6

INTEGRATED INJECTION-MOLDED MICROCHIPS FOR STIMULATION AND MEASUREMENT OF EXOCYTOSIS FROM SINGLE PC12 CELLS USING A POLYMER MICROELECTRODE
6.1 ABSTRACT

Polymer electrodes have recently emerged as an alternative to traditional electrode materials like carbon or gold for the measurement of neurotransmitters. These electrodes are easy to fabricate, offer excellent electrochemical properties for the measurement of biogenic amines, and are easily incorporated into microfluidic geometries. Here, we have fabricated the first injection molded polymer microchip for the capture, stimulation, and measurement of exocytosis from single PC12 cells. This 2-inch device was fabricated by the thermal bonding of two TOPAS cyclic olefin copolymer substrates. The top TOPAS substrate includes luer lock fittings, a serpentine channel for separating clumps of cells, and a straight channel for cell selection. The device also incorporates an auxiliary 3 µm-wide channel where suction is applied to capture and immobilize a PC12 cell over a PEDOT electrode for stimulation and measurement. The bottom TOPAS substrate contains 500 nm cavities etched via reactive ion etching. In these cavities, a thin film of gold and chromium serves as contact leads to a 3 µm x 8 µm PEDOT band electrode where measurements of exocytosis from single cells or neurons can be made. We characterize the device by measuring ferrocene and dopamine using slow-scan cyclic voltammetry, and demonstrate the use of the device for easily monitoring exocytosis from single PC12 cells.
6.2 INTRODUCTION

The fundamental of neurotransmission is the synapse, where two neurons meet. Here, a propagating action potential traveling down the axon of the presynaptic neuron is translated to a chemical signal by the release of neurotransmitter into the synapse. This occurs when the electrical impulse depolarizes the cell, opening voltage-gated calcium channels localized near the synapse in the cell membrane. Over the course of a few hundred microseconds, the presynaptic cell's intracellular calcium concentration locally increases from 100 nM to 100 µM.\textsuperscript{1,2} With this rapid change in concentration, calcium binds to Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II (CaM kinase II), a protein complex that phosphorylates synapsin I.\textsuperscript{3} The phosphorylation of synapsin I changes the conformation of the protein, decreasing the binding affinity of synaptic vesicles to the cytoskeleton and releasing bound vesicles.\textsuperscript{4} Released vesicles are trafficked to the active zone of the presynaptic neuron, where they are docked, tethered, and primed for fusion via a SNARE complex, consisting of synaptobrevin, syntaxin, and SNAP-25.\textsuperscript{5,6}

The subsequent vesicle fusion process relies on the rapid complex multi-step interaction of a large number of proteins and complexes to fuse the vesicle with the membrane. Once the vesicles release their contents, these molecules diffuse across the synapse to the postsynaptic neuron, where they bind to receptors and propagate the electrical signal further.\textsuperscript{7} Given the complexity of this system, dysfunction in only one of the proteins in this system can lead to serious neurological disease states. These diseases include a diverse set of symptoms, ranging from mood disorders (\textit{via} a mutant SNAP-25 promoter) to movement disorders (\textit{via} α-synuclein aggregates),\textsuperscript{8,9} are mostly incurable,\textsuperscript{10} affect millions of people around the world,\textsuperscript{11} and burden society with enormous emotional and economic costs.\textsuperscript{12,13}
Of these, Lambert-Eaton Myasthenic Syndrome (LEMS) stands out as a clear example. The symptoms of LEMS include weakness in the limbs, double vision, and difficulty swallowing.\cite{14} It has been demonstrated that LEMS can be caused by autoimmune responses or inherited, and that the symptoms in genetic cases are a direct result of a mutation in the synaptotagmin 2 (SYT2) gene, which codes for a protein that acts as a regulator of spontaneous exocytosis.\cite{15} Drosophila with a mutant SYT2 gene display a greatly increased frequency of spontaneous exocytosis events, manifesting in the loss of motor control.\cite{16,17} LEMS is incurable, and the symptoms are poorly managed by existing pharmaceutical drugs.\cite{18} Another model system, the rat adrenal pheochromocytoma (PC12) cell variant of this mutant is also available, and these cells contain much of the same exocytosis machinery as primary neurons do.\cite{19-23} Mutant PC12 disease model cells are also available with a variety of other neuropathic diseases including Huntington’s disease,\cite{24-26} Alzheimer’s disease,\cite{27-29} and Parkinson’s disease.\cite{30-32} Given the availability of high-throughput synthesis of small molecule and peptide pharmaceutical drugs,\cite{33-35} a rapid diagnostic assay for the analysis of vesicle exocytosis from mutant PC12 cells with neurodegenerative diseases before and after treatment would be an excellent way to identify successful drug candidates for LEMS or other diseases. While high-throughput assays of PC12 neurite outgrowth and proliferation have been developed,\cite{36,37} there is no existing methodology to rapidly screen the effects of pharmaceutical agents on exocytosis from PC12 cells.

The most commonly employed method of measuring exocytosis from single PC12 cells involves the manual fabrication of carbon-fiber disc electrodes, a multi-step low-throughput process outlined in Figure 6-1. To fabricate these electrodes, a 5 µm-diameter carbon fiber is aspirated into a glass capillary, and it is pulled into two parts by a capillary puller. The electrodes are then cut under a microscope, dipped into epoxy,
Figure 6-1: The fabrication of a carbon fiber disc electrode for use in amperometric measurements of exocytosis. **a)** A single carbon fiber is aspirated into a glass capillary tube, which is pulled by applying a high current through a nichrome wire. **b)** The pulled capillary is cut crudely with scissors, and two parts are formed. **c)** The electrode is cut to a short (< 10 µm) length cylinder. **d)** The electrode is dipped into epoxy to form a seal at the glass-carbon interface. **e)** Electrodes are polished on a rotary pad until a flat, angled geometry is obtained.
cured, and then polished using a rotary beveler for 20+ minutes per electrode. The multi-step manual nature of this process introduces many opportunities for failure, and because they are made by hand, no two electrodes are exactly the same. They must be pre-tested prior to use, and then an expert must align the electrode and stimulating pipette using micromanipulators on a microscope stage. The measurement scientist must then gently come into contact with the cell, applying a delicate pressure to ensure proximity of the electrode to the cell membrane. Thus, to make measurements from treated and untreated cells, the measurement scientist must manually select cells and repeat this process dozens of times to gain statistical significance. This disc electrode is an excellent low-throughput tool, but does not adequately address the challenges posed by high-throughput screening.

Here, we introduce an attractive high-throughput alternative to the widely used carbon-fiber amperometry technique. Using novel microfabrication technology, we have fabricated a disposable injection-molded microchip capable of individuating, capturing, stimulating, and measuring exocytotic release from single PC12 cells. This chip is robust, inexpensively produced in large numbers, and easily interfaced with automated equipment. By incorporating a capture channel with an integrated electrode, the microchip takes the approach of bringing the cell to the electrode with suction instead of relying on manual alignment of the electrode on the cell by the measurement scientist. This approach means that measurements made with this device are more comparable to each other, as error induced by positioning are not present in the data. We describe the fabrication of the device, electrochemical and physical characterization, and show that the device can be used to make measurements from single PC12 cells.

6.3 MATERIALS AND METHODS
6.3.1 CHEMICALS

Ferrocene carboxylic acid, calcium chloride, dopamine, glucose, and magnesium chloride were purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium chloride and sodium bicarbonate were purchased from EMD (Gibbstown, NJ, USA). Perchloric acid, sodium phosphate monobasic and sodium sulfate were purchased from Mallinckrodt (Hazelwood, MO, USA). Stock solutions of neurotransmitters were prepared in 0.1 M HClO₄, and then diluted to the appropriate concentration in an isotonic HEPES buffer (150 mM NaCl, 5 mM KCl, 1.2 mM MgCl₂, 2 mM CaCl₂, 5 mM glucose, 10 mM HEPES, pH 7.4). Stimulation of cells was performed by exchanging the buffer for an isotonic HEPES buffer at 105 mM KCl and 50 mM NaCl. All water used was purified to a resistivity of 18.2 MΩ·cm using MilliQ Gradient A10 water purification system (EMD Millipore, Darmstadt, Germany).

6.3.2 ELECTROCHEMICAL MEASUREMENTS

Amperometry was performed by holding the PEDOT:Tosylate electrode potential at +450 mV vs Ag/AgCl using an Axopatch 200B (Axon Instruments, Foster City, CA). The analog signal was recorded at 250 kHz, hardware-filtered with a low pass fourth-order Bessel filter at 2 kHz, and digitized at 10 kHz using a real-time oversampling filter.⁴²

6.3.3 OPTICAL AND ATOMIC FORCE MICROSCOPY

Atomic force microscopy was performed using a Veeco Dimension 3100 (Veeco, Plainview, NY) in tapping mode using a Mikro Masch NSC-15 n-type silicon tip (Mikromasch USA, San Jose, CA). Measured topography was software low-pass filtered once (Nanoscope V531r1 software). Optical micrographs and videos were collected using a Nikon Ti- 2000 microscope (Nikon Corporation, Tokyo, Japan) with a Lumenera INFINITY USB camera (Lumenera, Ottowa, ON, Canada).
6.3.4 CELL CULTURE
PC12 cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in RPMI-1640 medium supplemented with 10% heat inactivated horse serum, 5% fetal bovine serum, and 1% penicillin–streptomycin solution in a 5% CO₂ atmosphere at 37 °C and 100% humidity. PC12 cells were grown in cell culture flasks (BD Biosciences, San Jose, CA) and subcultured every 7-10 days. See Supporting Information for more details. Cells were stimulated with high-K+ solution to evoke exocytosis. Prior to exocytosis measurements, electrodes were pre-tested in a 1 mM ferrocene carboxylic acid solution (pH = 7.4 HEPES buffer), and electrodes with a stable electrochemical response and RMS current noise at or below 1.5 pA were selected for use. All electrochemical measurements were made on a vibration-isolated table inside an earth-grounded Faraday cage. The data was collected and written to file using in-house LabVIEW software.

6.3.5 MICROCHIP DESIGN
All photomasks were designed in the L-EDIT software (Tanner EDA, Monrovia, CA, USA) and purchased from Delta Mask b.v. (Enschede, Netherlands) as 4-inch chromium masks on quartz substrates.

6.3.6 INJECTION MOLDING OF TOPAS SUBSTRATES
Injection molding of both halves of the microchip was performed on an Engel VC330/100 injection-molding machine. Internally lubricated injection molding grade TOPAS 5013 (TOPAS Advanced Polymers GmbH, Frankfurt-Höchst, Germany) pellets were loaded into the hopper of the injection molder, which was heated and injected to the mold with a
duty cycle of about 30 seconds per substrate including heating and cooling. Edge burrs were removed by hand with a scalpel.

6.3.7 MICROCHIP FABRICATION – TOP HALF

Nickel shims were fabricated as relief molds for the top half of the microchips. The fabrication procedure is outlined in Figure 6-2. These shims needed relief at two heights, 1 µm for the cell capture channel, and 100 µm for the carrier channel and suction channels, and were thus produced by a multilevel dry etching and electroplating process. The native oxide layer of undoped silicon wafers was removed by treatment in a 6:1 volume ratio of 40% NH₄F in water to 49% HF in water for 3 minutes. Wafers were then rinsed in water for 5 minutes and dried. A 10 µm layer of AZ4562 photoresist (AZ Electronic Materials, Luxembourg, Luxembourg) was spin-coated on the wafers (2000 RPM, 60 seconds), and wafers were exposed on a Karl Suss MA6 Mask Aligner (SUSS MicroTec, Garching, Germany) for 30 seconds at 7.0 mW/cm² in hard contact mode with a chromium photomask outlining the cell capture channel only. Wafers were then developed in AZ351 photoresist developer (AZ Electronic Materials, Luxembourg, Luxembourg) for 3 minutes, rinsed in water for 5 minutes, and dried. The small channel was etched into the silicon wafer via reactive ion etching (Surface Technology Systems) at a depth of 1 µm (SF₆/O₂ = 32/8 sccm; W_RF = 30 W; t = 4 min; P = 80 mTorr). The wafer was then stripped of residual photoresist in a plasma asher for 20 minutes (O₂ flow rate = 240 sccm, N₂ flow rate = 70 sccm, at a W_RF of 1 W). The second photoresist layer was then deposited on the wafer in the same manner, while using the mask for the main and suction channels. The photoresist was exposed and developed in the same manner. Deep reactive ion etching was utilized to achieve a depth of 100 µm in these two channels (SF₆/O₂/Ar = 180/160/100 sccm; P = 246, W Coil = 2.8 kW, Bias = 170 V, t = 2.6 min). Residual photoresist was stripped off in the same manner. A 10 nm titanium
Figure 6-2: Process flow for the fabrication of the top half of the microchip. Red: AZ4562 photoresist, Dark grey: silicon wafer, Dark blue: titanium and gold seed layer, Light grey: Electroplated nickel, Light blue: TOPAS 5013. Steps a) through g) are for fabrication of the nickel shim for injection molding, and h) through i) are injection molding steps. 

- a) A silicon wafer is coated with photoresist and lithographically patterned with the small channel geometry.
- b) Reactive ion etching is used to etch into the silicon (depth = 1 µm), and residual resist is stripped off.
- c) The wafer is again lithographically patterned with the suction channel and carrier channel geometries.
- d) Deep reactive ion etching is used to etch into the silicon (depth = 100 µm), and residual resist is stripped off.
- e) The wafer is sputter coated with titanium and gold (10 nm and 65 nm) as a seed layer for electroplating.
- f) The wafer is electroplated with nickel.
- g) The silicon wafer is dissolved in a hot potassium hydroxide solution, leaving only the nickel shim. The shim is then coated with a fluorinated anti-stick coating (FTDS).
- h) Molten polymer is shot into the mold in an injection-molding machine.
- i) The final top half geometry of the TOPAS microchip has been defined.
and 65 nm gold seed layer was sputtered on the surface of the etched wafer using a Lesker LAB Line 5 (Kurt J. Lesker Company, Pittsburgh, PA, USA). A 300 µm nickel layer was electroplated on the seeded wafer at a charge of 18.1 Ampere hours. The silicon wafer was then dissolved in a solution of 50% KOH for 10 hours at 80°C, releasing the nickel shim.

### 6.3.8 MICROCHIP FABRICATION – BOTTOM HALF

The bottom half of the microchip includes three photolithographically aligned layers: PEDOT:Tosylate electrodes for exocytosis measurement, gold/chromium contacts for interface with the electrochemical instrumentation, and a groove for these two features to sit in. A graphical representation of these steps is available in Figure 6-3. The groove was used to prevent breaking electrical contact to the electrode upon thermal bonding of the top and bottom halves of the microchip. The gold/chromium contacts were used to prevent IR drop between the connection to the potentiostat and the electrode.

50 mm Ø flat TOPAS wafers were injection molded using the same parameters described above. First, the grooves were photolithographically defined by spin coating a 10-µm layer of AZ4562 photoresist onto the wafers. The photoresist was exposed with a custom mask defining the groove geometry for 4.5 seconds at 7.0 mW/cm². Wafers were then soft baked at 100°C for 90 seconds, and then developed in AZ351 MIF developer for 3 minutes, followed by a 5 minute rinse in a circulating water bath. Grooves were milled 300 nm deep using reactive ion etching, and remaining resist was removed in an acetone bath. Next, the gold/chromium contacts were generated in the groove. In order to establish a negative sidewall profile, a 1.5-µm thick layer of AZ 5214E image reversal photoresist (AZ Electronic Materials, Luxembourg City,
Figure 6-3: Process flow for the fabrication of the bottom half of the microchip. *Light blue:* TOPAS 5013 wafer, *Pink:* AZ-5241E photoresist, *Gold:* chromium and gold electrode leads, *Dark blue:* PEDOT:Tosylate electrode.  

**a)** A photoresist layer is spin coated and patterned on the bare wafer. A reactive ion etching process forms a 300 nm groove for the electrodes to be recessed in the injection-molded 2-inch TOPAS 5013 wafer.  

**b)** An image reversal photoresist lift-off layer with negatively-sloped sidewalls is generated on the wafer.  

**c)** A 20 nm chromium and 200 nm gold contact layer is deposited using an electron beam evaporation technique. The wafer is sonicated in acetone to remove excess materials.  

**d)** A 100 nm layer of PEDOT:Tosylate is spin-coated onto the wafer, and a photolithography step defines a protective structure on the PEDOT:Tosylate. Reactive ion etching is then used to remove unprotected PEDOT:Tosylate.  

**e)** The protective resist structure is removed by rinsing the wafer in acetone.
Luxembourg) was spin-coated (4200 RPM, 60 seconds) onto the surface. The negative side wall ensures that no gold/chromium material would be present on the edge of the groove. Wafers were then soft-baked for 5 minutes at 90 °C. Aligned exposure was performed for 4.5 seconds at 7.0 mW/cm² with hard contact on the front side of the wafer. Wafers were then post-exposure baked for 20 minutes to initiate the image reversal at 120 °C in an oven. Wafers were then removed from the oven and allowed to cool to room temperature for 5 minutes prior to a 1 minute flood exposure at 7.0 mW/cm². Wafers were finally developed in AZ351 photoresist for 3 minutes prior to a 5 minute water rinse, and then 20 nm of chromium (for adhesion) and 200 nm of gold were evaporated onto the surface (EVA600 evaporator, Alcatel, Russia). Residual gold and chromium were lifted off in an acetone bath with light sonication for 30 minutes, leaving the gold/chromium electrode leads sitting in the grooves. Lastly, the active electrode material, PEDOT:Tosylate, was deposited onto the surface. PEDOT:Tosylate was deposited by mixing 2 mL butanol, 150 μL pyridine and 220 μL Clevios™ M V2 in a small vial, and depositing this onto the TOPAS substrates. The substrates were then spun at 1000 rpm for 30 seconds to evenly distribute the PEDOT:Tosylate. The substrates were baked on a hot plate at 70 °C to remove the remaining solvent and then washed in deionized water. The edge bead was removed manually with a Kim-wipe. One final photolithography and etching step was used to define the geometry of the PEDOT layer. AZ5214E photoresist was spin-coated on the PEDOT:Tosylate-coated substrates at 4500 rpm for 30 seconds. The samples were soft baked on a hot plate at 95 °C for 5 minutes before being aligned and exposed on a Karl Suss MA6/BA6 Mask Aligner for 3 seconds at 7.0 mW/cm². Wafers were then developed in AZ351 MIF developer for 3 minutes, followed by a 5 minute rinse in a circulating water bath.

6.3.9 ASSEMBLY OF THE COMPLETE MICROCHIP VIA THERMAL BONDING
Following the fabrication of the top and bottom halves of the microchip, they were aligned using an optical microscope, and a soldering iron was used to create a temporary bond on the outside of the chip. The final thermal bonding process was performed on a custom machine, in a milled aluminum disc to distribute heat evenly. The thermal bonding machine was equilibrated for 30 minutes prior to bonding, and bonding was performed at 120 °C, with 5 kN of pressure per chip, for 10 minutes total.

6.4 RESULTS AND DISCUSSION

6.4.1 MICROCHIP DESIGN AND RATIONALE

These microchips were designed to have several important features facilitating cell capture, stimulation, and measurement of exocytosis from single PC-12 cells. The top half of the chip containing the fluidic systems is outlined in Figure 6-4, and the prominent features include luer taper ports for easy interfacing with automated equipment or syringes, a serpentine segment for individuation of cells, a carrier channel for cell or pharmaceutical agent transport, two suction channels for multiplexed measurement, and a small channel for cell capture. The width and height of all channels except the small channel are 100 μm. The width and height of the small channel are 3 μm and 1 μm, respectively. The size of this channel is minimized to reduce the accidental suction of cells from the carrier channel into the suction channels. The bottom half of the chip containing the electrodes is outlined in Figure 6-5. This half of the chip incorporates several PEDOT:Tosylate electrodes, gold/chromium contacts, and a groove to protect the electrodes. In addition to the two cell measurement electrodes (8 μm wide), each microchip also has three larger auxillary electrodes (20 μm wide).
Figure 6-4: a) Schematic of the microfluidic system on the top half of the two part microchip. Inset: zoomed in portion of part b). b) Schematic of the suction channel for cell capture. The top half of the chip is fabricated by injection molding against a custom electroplated nickel shim. c) Optical micrograph of the top half of the chip. Luer connections allow the fluidic channels of the device to be interfaced with syringes, pumps, or automated equipment.
6.4.2 CHARACTERIZATION BY ATOMIC FORCE MICROSCOPY

Atomic force microscopy was used to probe the success of the fabrication techniques, and to investigate and characterize the interface of the electrode groove, the gold/chromium electrode leads, and the PEDOT:Tosylate electrode. The interface of these three features is shown in Figure 6-5c. The thickness of the chromium and gold layer was measured to be 222 ± 6 nm, within the measurement error of the expected thickness of 220 nm (20 nm chromium under 200 nm gold). The reactive ion-etched TOPAS trench had a depth of 304 ± 3 nm, which was large enough to recess the chromium and gold leads as well as the PEDOT:Tosylate electrode when bonded to the top half of the chip. The thickness of the PEDOT:Tosylate layer was measured to be 65 ± 7 nm in the TOPAS trench, and 119 ± 2 nm on top of the chromium and gold layer. This difference in PEDOT:Tosylate film thickness suggests that the recessed channel is perhaps more difficult to deposit PEDOT:Tosylate in during the spin-coating process, but this did not affect the success of the measurements made at the electrode.

6.4.3 ELECTROCHEMICAL CHARACTERIZATION OF FABRICATED ELECTRODES

Electrodes were characterized with steady state cyclic voltammetry to validate the function and measure the electroactive area of the cell measurement electrode. Ferrocene carboxylic acid and dopamine were used as reporters, one fast outer sphere redox reporter, and the other being the relevant biogenic catecholamine released by PC12 cells. A HEPES buffer (150 mM NaCl, 5 mM KCl, 1.2 mM MgCl₂, 2 mM CaCl₂, 5 mM glucose, 10 mM HEPES, pH 7.4) containing 1 mM ferrocene carboxylic acid was perfused through the small channel from the suction channel, and steady-state cyclic voltammetry (scan rate = 2 mV/s, scanned from 0 V to 0.5 V vs. Ag/AgCl) was used to quantify the electroactive area of the electrode and compare it to the geometric area of the electrode. Figure 6-6a shows a background-subtracted steady state oxidation of 1
Figure 6-5:  a) Schematic of the microchip with top half and bottom half assembled together. Blue: fluidic channels, Gold: Chromium/gold contact pads for the polymer microelectrodes, Black: PEDOT electrodes, Inset: zoomed in portion of microchip, shown in more detail in part b). b) Cell capture channel (small channel) with PEDOT electrode laid across. c) 3-dimensional representation of an atomic force micrograph at the interface of the gold/chromium electrode leads, the PEDOT:Tosylate electrode, and the TOPAS electrode groove which the PEDOT electrode sits in. Atomic force micrograph collected from the region highlighted with a red box in b).
mM ferrocene carboxylic acid at the 3 µm x 8 µm band electrode in the cell capture channel. Approximately 275 pA of current is measured at the plateau of the first cyclic voltammogram, in line with the predicted value of 280 pA for an electrode of this geometry (Equation 6-1). Because this electrode was confined in a 1 µm-tall channel, subsequent voltammograms show a decrease in current as a depletion region in the diffusion-controlled oxidation of ferrocene is created around the electrode (data not shown).

\[
i_{\text{qss}} = \frac{2\pi nF \bar{I}DC}{\ln \left(\frac{64Dt}{w^2}\right)}
\]

Equation 6-1

Slow scan cyclic voltammetry was also performed on a solution of 1 mM dopamine in HEPES buffer as a sensor validation (Figure 6-6b), where approximately 600 pA of current were measured. Given the two-electron oxidation of dopamine, the experimental current is within 10% of the predicted value, indicating that the biosensor is capable of quantitative measurements of dopamine.

6.4.4 ELECTROCHEMICAL CHARACTERIZATION OF ELECTRODE NOISE

In order to successfully resolve amperometric spikes (zeptomoles of dopamine per spike) from the baseline, the root-mean-square (RMS) of the current must be on the order of 1-2 pA. The RMS noise in the current at the cell capture electrode was recorded during the application of a potential sufficient to oxidize dopamine (+450 mV vs. Ag/AgCl, shown in Figure 6-6c). Data was recorded using in-house LabVIEW software that performed real-time oversampling and signal averaging, digitized at 2 kHz and hardware low-pass filtered at \( f_c = 1 \text{ kHz} \). Previous work has shown that 99% of the frequency content of a typical exocytosis peak is contained in the 0 Hz to 1 kHz band.
Figure 6-6: Electrochemical characterization of the electrode.  

a) Background-subtracted steady-state slow scan cyclic voltammogram of 1 mM ferrocene in HEPES buffer at the cell capture electrode (3 µm x 8 µm).  
b) Background-subtracted steady-state slow scan cyclic voltammogram of 1 mM dopamine in HEPES buffer at the cell capture electrode (3 µm x 8 µm).  
c) Amperogram of a potential step at the cell capture electrode from 0.00 V to +0.45 V vs. Ag/AgCl.  
Inset: Amperogram showing the RMS noise at the electrode after double-layer changes.
After signal processing, the RMS current noise was 0.9 pA RMS, sufficiently low to quantitate exocytosis events.

6.4.5 CELL CAPTURE

Cells were loaded into the inlet well of the carrier channel of the microchip (Figure 6-4) and suction was applied to the end of the outlet well of the carrier channel with a syringe or with a custom LabVIEW-controlled 8 channel pressure regulator. Still captures from a video recording show a clump of cells pulled into the channel and separating with the rapid change in linear velocity (Figure 6-7). Cells then traveled through a serpentine segment of the carrier channel where they were individuated further. After a suitable cell was selected for analysis (single cell with a round, healthy appearance), negative pressure was applied to one or both of the suction channels when the cell was within 20 µm of the small capture channel (Figure 6-8). Still captures from a video recording of cell capture on a microscope are shown below. Cells are captured into the small channel within 2-3 seconds of pressure application. When captured into this geometry, a portion of the cell is inside of the small channel as the cell membrane is pulled in. The remaining surface area of the cell is exposed to the main channel, where it can be subjected to pharmacological challenges by flowing them through the carrier channel.

6.4.6 MEASUREMENT OF EXOCYTOSIS FROM A SINGLE PC12 CELL

After the successful capture of a single PC12 cell, the cell was stimulated by the perfusion of an isotonic elevated potassium buffer through the carrier channel. After stimulation, the electrode in the small channel detected several baseline-resolved exocytosis events. These events are shown in Figure 6-9, with the highlighted segment showing one release event in detail. The quantal content was an average of ~190,000 ± 50,000 dopamine molecules per event. While this quantal content of release is close to
Figure 6-7: Optical micrographs from a video showing the suction of cells into the microchip. A clump of cells is pulled into the chip, moving from a) to b) clumped together, and in c) shown while starting to individuate before the serpentine channel. Channel width is 100 µm.
Figure 6-8: Optical micrographs from a video showing the suction of a single PC12 cell from the carried channel into the small channel of the microchip for exocytosis measurement.  

a) A PC12 cell flows through the carrier channel, approaching the small channel.  
b) Negative pressure is applied through the top of the small channel and the PC12 cell begins to migrate towards the small channel.  
c) The PC12 cell is captured into the channel, and a small portion of the cell membrane fills the small channel, creating a seal. The remainder of the cell is exposed to the carrier channel for pharmaceutical challenge or stimulation.
Figure 6-9: Preliminary data exhibiting the first ever measurements of exocytosis from a single PC12 cell in a polymer microchip. *Black trace:* Amperometric recording from captured PC12 cell. *Red trace:* baseline for integration. PC12 cells were incubated in 200 μM L-DOPA for one hour prior to capture and measurement, and stimulated with an isotonic HEPES buffer with elevated potassium to depolarize the cells. Three release events are present in this trace, with an average of \( \sim 190,000 \pm 50,000 \) dopamine molecules per release event.
expected values for L-DOPA incubated PC12 cells, relatively few events are observed. There may be a few reasons for this behavior. First, the total surface area of the cell exposed to the 3 µm x 8 µm electrode is dependent on how far the cell is pulled into the channel. The area of contact with the cell is likely much lower than in a traditional carbon-fiber disc electrode measurement (maybe calculate a cylinder volume here and comment on the difference between it and carbon fibers). Second, mechanical stress from cell membrane deformation into a small cylinder changes the cell membrane curvature substantially. While the SNARE complex facilitates exocytosis in an active manner, membrane curvature has been shown elsewhere to be a critical factor in the mechanics of exocytosis.

6.5 CONCLUSIONS

Here, we have designed and fabricated the first microchip that allows for high-throughput time-resolved amperometric measurements of exocytosis from single PC12 cells. The chip is fabricated on a cyclic olefin copolymer substrate, and several novel microfabrication techniques were integrated, including multi-layer injection-molded plastic substrates, chromium/gold electrode leads to minimize voltage drop between the potentiostat and the electrode, and luer adapter fittings for easy interfacing with automated equipment, syringes, or pumps. These microchips are an attractive alternative to the traditional carbon-fiber amperometry technique because they remove many of the user-induced variability of exocytosis measurements and allow for direct pharmaceutical challenge during measurements. Once loaded into the chip, cells are sucked into a serpentine channel in which they are individuated by forces induced by rapid changes in linear velocity and direction. After moving to the carrier channel, cells are captured into a small capture channel by the application of negative relative pressure through auxiliary suction channels. Once a cell has been captured, buffer with an
elevated potassium concentration is pumped through the carrier channel, which depolarizes the cell membrane and triggers exocytosis. Perpendicular to the cell capture channel lies a 3 µm PEDOT:Tosylate electrode, which oxidizes the released dopamine. We have measured the first single-cell exocytosis events from a PC12 cell using an all-polymer microchip, and the quantitative parameters of exocytosis are in line with expected values for from L-DOPA-incubated PC12 cells. In the future, the capture geometry of the small channel will be modified to facilitate cell capture without deformation of the cell membrane. This modification should yield devices that will more easily capture cells and measure exocytosis. Additionally, these microchips will be used to rapidly screen the efficacy of pharmaceutical agents on the function of the vesicle fusion machinery inherent to the PC12 cell line.

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6.7 REFERENCES


CHAPTER 7

CONCLUSIONS AND FUTURE DIRECTIONS
7.1 FUTURE DIRECTIONS

The easy processability, electronic compatibility, and tunability of conducting polymers will result in their incorporation into a variety of biomolecule detection schemes. For implanted biosensors, even the most sophisticated surface-modified biosensors still induce fibrous encapsulation and inflammatory response, although this is reduced compared to uncoated biosensors. Adverse implant-induced biological responses remain a challenge in a variety of measurement systems. It is likely that coatings and surface modifications will need to be tailored for a particular biological interface in order to achieve maximum device/coating performance and integrity. Therefore, significant efforts must be directed toward advancing the surface chemistry of implantable biosensors. In particular, the development of smart polymeric materials may be the next leap forward in surface coatings for implantable sensors. These smart materials could possess bio-mimetic properties such as an antibody coating or could be designed to actively respond to inflammation or encapsulation by releasing chemicals which minimize these responses. These chemicals include angiogenics to restructure vasculature, anti-inflammatory drugs, signaling molecules such as peptides, or hydrophilic surfaces to mask the presence of foreign implants under a layer of solvated water. The PEDOT:Nafion coating described in Chapter 4 demonstrably reduces the non-specific adsorption of biomolecules to the biosensor, reduces in vivo biofouling, and introduces a biocompatible PEDOT material at the surface of the electrode. These advantages over uncoated sensors are amenable to long-term measurements in the brain. While acute measurements can help neuroscientists establish the connectivity of the brain or understand acute responses to stimulus, probing systems such as learning or memory require measurements made over a period of days or weeks. In the future, PEDOT:Nafion coatings will be applied to electrodes used for chronic recordings of
dopamine release to deepen our understanding neurotransmission in the context of learning and memory.

The versatility of the ethylene bridge on 3,4-ethylenedioxythiophene (EDOT) also permits covalent attachment of enzymes such as glucose oxidase (GOx). This oxidoreductase enzyme functions by converting glucose and oxygen into a lactone and hydrogen peroxide. By immobilizing this enzyme at the surface of a carbon-fiber microelectrode, detection of an otherwise non-electroactive species becomes possible via the oxidation of hydrogen peroxide. This scheme would allow for the first in vivo measurements of glucose on millisecond timescales in small (~5 µL total volume) regions of the brain.

Another promising direction of this work is the development of disposable diagnostic tools for rapid measurements of neurotransmitters from biological models such as the drosophila melanogaster. Compared to existing microchip electrophoresis platforms, the microchip described in Chapter 3 can be fabricated at less than 1/10th of the cost, without special equipment, and in a higher-throughput manner. Given their sensitivity to electroactive neurotransmitters, these microchips could be fabricated to rapidly assess differences in whole-brain dopamine metabolism for d. melanogaster, an organism frequently employed as a model system for neurological diseases including Alzheimer's disease and Parkinson's disease. The microchips described in Chapter 6 could be used to rapidly screen the effect of small molecule drugs on exocytosis from PC-12 cells. These cells are a well-established model system for neurotransmitter release, and a large variety of genetic mutants are available which mirror the dysfunction in synaptic vesicle release machinery observed in humans with debilitating neurological diseases such as Lambert-Eaton Myasthenic syndrome. With modifications to the chip design,
capture and neurotransmitter measurement from primary neuron cultures could also be performed.

Thus far, PEDOT-only electrodes have only been employed in in vitro neurotransmitter detection schemes. In the near future, these materials will be used to fabricate multi-electrode arrays (MEAs) for neurotransmitter detection in vivo. These MEAs can be fabricated in an inexpensive and high-throughput manner, and will be used to evaluate differences in signaling among brain sub-regions such as the nucleus accumbens core and shell simultaneously, reducing the number of animal sacrifices needed for experiments.

7.2 IMPACT

The complicated nature of the brain necessitates better tools for in vivo and in vitro measurements of neurotransmitters. Here, we have increased the accessibility of stand-alone PEDOT electrodes via the development of microwave plasma etching, characterized their electrochemical behavior, and established that they are outstanding electrochemical detectors for microchips. Compared to existing materials, PEDOT is amenable to a larger variety of substrates, easier to process, inexpensive, and has excellent electrochemical behavior for the detection of neurotransmitters. We have demonstrated the utility of PEDOT by fabricating and characterizing the first device for the separation of biogenic amines, and the first device for high-throughput measurements of exocytosis from single PC12 cells. These devices will allow scientists to inexpensively and rapidly study the effects of pharmacological challenges to model systems in disease states.
By electrochemically applying a PEDOT and Nafion composite polymer coating to carbon-fiber microelectrodes, enormously increased sensitivity and selectivity towards dopamine has been obtained, resulting in the first in vivo electrochemical measurements of dopamine transients without administration of a reuptake inhibitor. The coating also increases the stability of the sensor in vivo, whereas uncoated electrodes biofoul in just a short period of time. These materials enable measurement scientists to mitigate or resist the complex biological response to an implanted electrode. Given these benefits, this technology should enable extended chronic in vivo recordings of dopamine, which are important to learn about long-term processes such as learning or memory.

Lastly, we have expanded the chemistry of polythiophenes by developing the synthesis of oligo-EDOT:Nafion nanoparticles. The assembly of oligo-EDOT from monomer occurs via a non-traditional route, perhaps of high general synthetic interest. These particles exploit the tunability of conducting polymers to respond to changes in solvent polarity and hydrogen bonding capacity with a change in absorbance. Moreover, these nanoparticles are easily prepared, inexpensive, and enable quantitative spectroscopic interrogations of water content in organic solvents.

7.3 A NEW SYNTHETIC ROUTE TO PEDOT AND PEDOT:PSS

The preparation of oligo-EDOT:Nafion nanoparticles was a serendipitous effort. When a lithiated Nafion (LITHion) was exchanged for the protonated form (Nafion), no nanoparticles were synthesized. Additionally, all reagents were analyzed for oxidizing species such as iron (III), and no oxidant was found. From there, by exchanging the Nafion for the simplest homologous molecule, trifluoromethanesulfonic (triflic) acid, we realized that PEDOT could be rapidly synthesized in the absence of an oxidizing material. After probing the synthesis conditions and surveying the literature, we may
have stumbled upon the first described non-oxidative synthesis of ethylenedioxythiophene. This synthesis also works for polymerizing other common thiophenes including 3-hexylthiophene (3-HT), a molecule of interest in the solar devices community.

Interestingly, in the synthesis conditions currently employed, cationized matrix-assisted laser desorption/ionization mass spectral analysis (MALDI-MS) of the polymer indicates that dihydro-PEDOT is the form being synthesized with a separation of 142 m/z units (Figure 7-1). The reaction appears to proceed through an acid-catalyzed disproportionation (Figure 7-2). This reaction yields a dispersed blue or red liquid for PEDOT and P3-HT, respectively (Figure 7-3). While this configuration of double bonds is not inherently conductive, the addition of re-aromatizing agents easily re-conjugates the system, bringing the m/z to 140, permitting conductivity (Figure 7-4). Furthermore, the triflic acid must be quenched following polymerization for safety concerns. When we quench this reaction with an 18% aqueous dispersion of polystyrenesulfonate (PSS), we easily form a solution-processable conjugated form of PEDOT. MALDI-MS analysis of PSS-quenched PEDOT shows that 1) the fragmentation pathway likely proceeds through a radical cation and matches the fragmentation spectrum of commercial PEDOT:PSS, and 2) in comparison to a commercial PEDOT:PSS solution, the average molecular weight is about 30% higher extending up to a 15-mer plus one proton and a terminal hydroxyl group at a m/z of 2118 (Figure 7-5). Both of these results are promising developments towards a new disproportionation polymerization mechanism for thiophenes. The great advantage of this synthesis scheme is that theoretically, large quantities of more homogenous PEDOT can be catalytically prepared because oxidizing material (or electrochemical oxidation) is not needed. This work is of great general
Figure 7-1: MALDI-MS analysis of the di-hydro form of PEDOT prepared via the novel polymerization scheme. Peaks are separated by 142 m/z units, indicating the presence of a hydrogen on the carbons alpha to the sulfur.

Chemical Formula: C₆H₆O₂S²⁺  
Exact Mass: 142.01
Figure 7-2: The proposed mechanism for the polymerization of PEDOT from EDOT and triflic acid proceeds through a disproportionation reaction.
Figure 7-3: a) (L) P3-HT and (R) PEDOT synthesized via the novel polymerization scheme. UV/Vis spectroscopy of dilute solutions of P3-HT and PEDOT.
Figure 7-4: MALDI-MS analysis of the conjugated form of PEDOT prepared via the novel polymerization scheme with the addition of a re-aromatization agent (trityl cation). Peaks are separated by 140 m/z units, indicating the absence of a hydrogen on the carbons alpha to the sulfur.
Figure 7-5: MALDI-MS analysis of the conjugated form of PEDOT prepared via the novel polymerization scheme with the addition of a re-aromatization agent (trityl cation), and quenched with 18% w/w polystyrenesulfonate. Peaks are separated by 140 m/z units, indicating the absence of a hydrogen on the carbons alpha to the sulfur, and extend out to at least 15-unit polymers.
synthetic interest because to our knowledge, no existing methodology has prepared non-halogenated PEDOT without the presence of oxidants.  

7.4 DECREASED CAPACITANCE AT STAND-ALONE PEDOT MICROELECTRODES FOR FAST-SCAN CYCLIC VOLTAMMETRY

While amperometry at stand-alone PEDOT electrodes has been well-developed in this work, the chemical resolution of this technique is limited. In order to resolve several electroactive species simultaneously, or to deploy these electrodes in the brain, cyclic voltammetry is needed. Because chemical communication occurs on the millisecond timescale, it is ideal to make measurements on that timescale. PEDOT has a high specific capacitance of 1800 µF/cm², which generates a large background current when scanning at high rates (above 1 V/s). Additionally, this high capacitance increases the amount of time the electrode takes to establish a potential drop across the double layer. This charging time limits the speed of repeated measurements, so lowering the capacitance is desired. Using a vapor-phase polymerization scheme (Figure 7-6), we have demonstrated that the capacitance can be lowered by modulating the pyridine to iron (iii) tosylate ratio. (Figure 7-7). The PEDOT:Tosylate we synthesize has nearly a 90% reduction in specific capacitance. This synthetic modification allows for the generation of PEDOT:Tosylate films that could potentially be used for fast-scan cyclic voltammetry.

7.5 AN IMPROVED MICROCHIP FOR CELL CAPTURE AND EXOCYTOSIS MEASUREMENTS

The microchip described in Chapter 6 is an excellent new tool for high-throughput measurements from PC12 cells, a model system for neurotransmission. The number of exocytosis peaks measured is currently limited by 1) the success rate of the suction
Figure 7-6: Vapor-phase synthesis of PEDOT:Tosylate. **a)** a 40% iron (III) tosylate solution and pyridine in 2-butanol is spin-coated onto substrates. **b)** The substrate is exposed to EDOT vapor, and an oxidative polymerization occurs. **c)** The substrate is removed from the vapor and rinsed with ethanol and water.
Figure 7-7: Adjusting the Pyridine to iron (III) tosylate ratio during the vapor phase polymerization decreases the apparent capacitance of the material, permitting faster scan rates at voltammetric electrodes.
process and 2) the positioning of the PC12 cell over the electrode. To increase the
success rate of cell capture and measurement, a geometric modification of the top half
(the fluidics) of the microchip will be made. In this new geometry, the membrane
curvature is not strained during cell capture, gentler pressure can be used to capture
cells, and a larger area of the cell is immobilized over the electrode. An X-Y cross
section of the old (a) and new (b) geometry are shown in Figure 7-8, and a Y-Z cross
section is shown in Figure 7-9. Both of these improvements should yield a device that
makes higher-throughput measurements of exocytosis from PC12 cells.

7.6 CONCLUDING REMARKS

Our understanding of neuroscience is pushed forward by leaps in measurement
technology. Biological systems, particularly the brain, are remarkably challenging
matrices for millisecond-timescale quantitative measurements of neurotransmitters. The
complex biological response to foreign bodies such as implanted sensors warrants the
need for surface modifications on microelectrodes. Furthermore, a great deal of useful
mechanistic information can be gleaned from quantitative measurements from model
systems such as PC12 cells in microfluidic devices. Until this work, these devices
incorporated 2D electrodes which restricted the choice of substrate, were not optically
transparent, or had poor electron transfer kinetics for neurotransmitters.

Here, we have demonstrated that conducting polymers can be employed to enhance the
detection of neurotransmitters in neuroanalytical systems that rely on the sensor's
surface interaction with a biological matrix. These systems include single cells, sub-
regions of the brain, and whole-brain homogenate. Compared to existing alternatives,
these materials are highly biocompatible, easily processed, optically transparent, more
sensitive to analytes of interest, and inexpensive.
Figure 7-8: X-Y cross section of the microchip showing the a) old design and b) revised design of the cell capture chip. *Blue:* TOPAS wafer, *Gold:* Au/Cr contacts, *Grey:* PEDOT electrode.
Figure 7-9: Y-Z cross section of the microchip showing the a) old design and b) revised design of the cell capture chip. Blue: TOPAS wafer. The left hole is the suction channel, the right hole is the carrier channel, and the small channel is between them. In the new design, cells are given 5 µm of Z-space to fit into, as opposed to 1 µm in the old design.
The first ever stand-alone conducting polymer electrodes for the detection of neurotransmitters have been fabricated and characterized. The fabrication and processing of these conducting polymer electrodes is made more accessible to the average laboratory by introducing microwave-generated plasma etching. Furthermore, we introduce the first polymer and glass microchip for the rapid sensitive separation and detection of biogenic amines from whole-brain homogenates. The field of in vivo electrochemical measurements of dopamine is moved forward with a novel and modular conductive polymer-enhanced surface chemistry. These improvements extend the lifetime and sensitivity of the implanted electrode without sacrificing the temporal resolution of the measurement, paving the way for better chronic measurements of dopamine dynamics. The increased sensitivity of the electrode permitted the first ever measurements of transient dopamine release in the nucleus accumbens without the administration of a dopamine reuptake inhibitor. We have also expanded the field of microfluidics and microsensor fabrication by assembling and characterizing the first microchip capable of capture, stimulation, and measurement of exocytosis from single PC12 cells. This microchip allows for high-throughput screening of drug candidates against disease model systems. Lastly, we have exploited the physical properties of conducting polymers to develop a new sensor platform based on easily synthesized polymer nanoparticles, and demonstrate their utility in the rapid, inexpensive optical detection of solvation environment.

7.7 ACKNOWLEDGEMENTS

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Matteucci and Dr. Rafael Taboryski for assistance in engineering the improved microchip for cell capture.
7.8 REFERENCES

SUMMARY: Self-directed, productive, and versatile analytical chemist with a passion for multidisciplinary problem-solving. Managed and organized a productive international collaboration and mentored four younger scientists. Award-winning visual and verbal communication. Primary inventor on patented neural interface technology. Specific technical expertise includes electrochemistry, in vivo measurements of small molecule neurotransmitters, neuroscience, microfluidic systems, biological assay development, nanomaterials, LC-MS/MS, statistical analysis, spectroscopy, optical and electron microscopy, electronics, analytical instrumentation, LabVIEW, and project management.

EDUCATION:

The University of Arizona (Tucson, AZ) 2010 – 2015
Ph.D. Candidate in Chemistry

Towson University (Towson, MD) 2006 – 2010
Bachelor of Science in Chemistry (ACS Certified)

WORK EXPERIENCE:

Graduate Research Assistant – December 2010 – Present
University of Arizona, Laboratory of Dr. Michael L. Heien. Tucson, AZ, USA

- Designed, formulated and characterized a novel polymerization scheme to anchor biocompatible coatings onto carbon fiber microelectrodes for increased dopamine sensitivity during in vivo detection.
- Measured the first dopamine transient release events in anesthetized rats without re-uptake inhibitors.
- Developed a microwave-generated plasma etching system for inexpensive etching of conductive polymers into electrodes. Characterized etch rate and microstructure morphology.
- Developed and characterized the first all-polymer-and-glass microchip electrophoresis separation system for the analysis of neurotransmitters.
- Synthesized and characterized novel fluorinated conductive polymer nanoparticles.
- Discovered a new synthetic route to poly(3,4-ethylenedioxythiophene) (PEDOT) without oxidation.

Technical University of Denmark, Laboratory of Dr. Rafael J. Taboryski. Kgs. Lyngby, Denmark

- Designed and fabricated multi-level microchips for high-throughput neurological drug screening.
- Managed and organized a team of international engineers and scientists.
- Captured single PC-12 cells for the first cell-immobilized measurements of exocytosis.
- Optimized positive and negative lithography processes on 2 inch injection-molded polymer substrates.
- Optimized a photoresist lift-off process for a 200 nanometer electron beam-evaporated gold layer.
- Developed a reactive ion etch process for 500 nanometer anisotropic etch into polymer substrates.

W.M. Keck Center Facility Research Assistant – Fall 2011 – Spring 2012.
University of Arizona, W.M. Keck Center for Surface and Interface Imaging. Tucson, AZ, USA

- Responsible for operation, troubleshooting, and maintenance of three atomic force microscopes (AFM), one scanning electron microscope (SEM) with EDS, and one confocal microscope.
- Trained 50+ students in engineering, chemistry, and physics in advanced operation of instruments.
- Consulted with companies and universities to provide a variety of atomic force and electron imaging services for biological and semiconductor samples with micro or nano-structure submitted to the facility.
- Principal operator of these instruments for biological and semiconductor samples submitted to the facility by industry and students.

Available to start work on May 15, 2015
HONORS AND AWARDS:

Carl Zeiss Electron Microscopy Award (2014)

*Carl Zeiss AG, Arizona Imaging and Microscopy Society. Awarded for technical expertise in electron microscopy and ability to verbally convey complex technical information with ease.*

Distinguished Teaching Award (2014)

*University of Arizona, Department of Chemistry and Biochemistry. Awarded for outstanding performance as a teaching assistant (top 3% of 100+) and unique contributions to the curriculum.*

1st Place, Annual Graduate Research Symposium Poster Award (2014)

*University of Arizona, Department of Chemistry and Biochemistry. Awarded for effective visual and verbal communication of a project to a panel of Ph.D. scientists.*

W.M. Keck Center Facility Research Assistant Position (2011)

*University of Arizona, Department of Chemistry and Biochemistry. Awarded for an excellent teaching record, communication skills, and interest in atomic force microscopy and electron microscopy.*

PUBLICATIONS AND PATENTS:


TEACHING EXPERIENCE:

*Teaching Assistant. Hands-on laboratory instruction of 200+ students in instrumental analytical chemistry. Responsible for pre-testing experiments, maintenance and calibration of measurement tools (HPLC, IC, TGA, DSC, GC-MS, ICP-AES, IR, UV/Vis), and writing standard operating procedures.*

PROFESSIONAL EXPERIENCE:

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<tr>
<td>Member, Second-Year Graduate Student Seminar Committee</td>
<td>Fall 2012 – 2013</td>
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<tr>
<td>Presider, Pittsburgh Conference on Analytical Chemistry (Biosensors)</td>
<td>Spring 2012</td>
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<tr>
<td>Member, Graduate Recruiting and Admissions Committee</td>
<td>Fall 2013 – Present</td>
</tr>
<tr>
<td>Member, Graduate Teaching Assistant Evaluation Committee</td>
<td>Fall 2013 – Present</td>
</tr>
<tr>
<td>Mentor, Adam R. Meier, Sean M. Lambert, Wilfred S. Russell, Gil Wondrak</td>
<td>Spring 2013</td>
</tr>
</tbody>
</table>
VOLUNTEER ACTIVITIES:

**Wings for Women**, a 501(C)(3) community-funded organization dedicated to provide a safe and positive environment to homeless and impoverished women and their children in Tucson, Arizona. 
*Volunteer event planning, management, and photography, 2012 - Present.*

**American Chemical Society Autism Outreach in Chemistry Program**, a camp designed to increase educational and professional opportunities for children ages 11-15 with autism in Tucson, Arizona.  
*Volunteer chemistry staff, 2011.*

PRESENTATIONS AND INVITED LECTURES:


10. **Vreeland RF**, Heien ML. "Exploring conducting polymer electrodes for sensitive electrochemical neurotransmitter detection." Towson University Department of Chemistry, Towson, MD, USA. 2014.


REFERENCES


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CHAPTER 3


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**CHAPTER 5**


### CHAPTER 6


**CHAPTER 7**