DISSERTATION

THE DEVELOPMENT OF PORTABLE ELECTROCHEMICAL SENSORS FOR ENVIRONMENTAL AND CLINICAL ANALYSIS

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ABSTRACT

THE DEVELOPMENT OF PORTABLE ELECTROCHEMICAL SENSORS FOR ENVIRONMENTAL AND CLINICAL ANALYSIS

The ability to perform chemical and biochemical analysis at the point-of-need (PON) has become increasingly sought. PON sensing is critical in both environmental and clinical monitoring applications to reduce cost and time of analysis and achieve early detection of potentially harmful pollution and health indicators. Electroanalysis is very well suited to PON sensing applications with miniaturized instrumentation available, fast analysis times, high sensitivity, low detection limits and the ability to be interfaced with both conventional and paper-based microfluidics (μ PADs). The primary focus of this thesis is to improve electrochemical sensors for PON applications by: 1) reducing the number of liquid handling steps required by the end user, 2) further development of better performing disposable electrode materials and 3) the proper integration of electrodes with disposable microfluidic paper-based devices.

The first half of this thesis, Chapter 2 through Chapter 4, focuses on the development of a new functionality in µPADs coupled with high quality boron doped diamond paste electrodes (BDDPESs). The electrochemical PAD (ePAD) is referred to as the Janus-ePAD after the two-faced Greek god. The Janus-ePAD developed in Chapter 2 takes advantage of the ability to store reagents within porous paper matrices. In the Janus-ePAD, reagents were stored in two separate channels connected by a sample inlet to adjust the sample pH and perform multiplexed electrochemical detection at two analytes' optimal pH conditions. Therefore, the device is able to carry out several liquid handling and operator steps in situ, further simplifying electrochemical

PON sensing. In Chapter 3, fundamental electrochemical characteristics of the BDDPEs are then studied in order to improve their electroanalytical utility, providing a guide to the use of this new composite electrode material.

Then, in Chapter 4, a second generation Janus-ePAD is developed to overcome several problems typically encountered in ePADs, namely, slow flow rates and analysis times and lowered electrochemical detection sensitivities due to the paper-electrode interface. Both of these problems are addressed by developing a multi-layer Janus-ePAD that consists of a wax-patterned paper layer taped to a transparency film layer, generating microfluidic channel in the gap between the two layers. Passive fluid transport is still achieved within the channel gap via capillary action but at much faster flow rates decreasing analysis time by over 20 times compared to a one-layer Janus-ePAD. The paper-electrode interface is removed by placing screen-printed carbon electrodes (SPCEs) on the transparency film layer, providing increased reproducibility and bulk solution sensitivity.

The second main focus of this thesis is the development of better performing electrode materials that retain the simplicity and disposability required for on-site electroanalysis. In Chapter 5, this goal is accomplished by the development of a novel SPCE composition using glassy carbon (GC) microparticles as the active electrode component and a conductive commercial ink as the binder component of this composite electrode material. The GC-SPE is then applied to the detection of the toxic heavy metals Cd and Pb using anodic stripping voltammetry (ASV). The use of GC microparticles as opposed to the widely used graphite powders in the bulk SPCE formulation allows for the GC-SPE to sensitively and quantitatively detect Cd and Pb at environmentally relevant levels without the need for any post-fabrication modification which is typically required for graphite based SPCEs.

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Following the development of the GC-SPE in Chapter 5, in Chapter 6, a systematic study was carried out to understand the relationship between SPCE composition, or carbon particle type, and electrochemical performance with the goal of improving the electrochemical performance of these single-use, mass producible, inexpensive and disposable electrode materials in their native, or unmodified state. Significantly, it was found that SPCE composition can be optimized and tuned to provide electrochemical sensing performance on par with other types of carbon composites historically believed to outperform SPCEs.

The work contained within this thesis achieves the goal of developing better performing PON electrochemical sensing motifs while retaining maximum simplicity of fabrication and operation of ePADs and SPCEs. Through automation of liquid handling steps using a paper-based device, further simplification of sensitive multiplexed electrochemical detection was achieved. The fundamental understanding of the electrochemical performance of SPCEs allowed for further applications without extensive post-fabrication modifications which have historically hindered their translation from academic to real-world settings. The work presented herein can be used to guide further development of electrochemical PON sensors for a variety of environmental and clinical applications.

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CHAPTER 1: INTRODUCTION

There has long been a demand for miniaturized chemical and biochemical sensors for environmental and clinical monitoring at the point-of-need (PON). PON sensing is a billion dollar industry in the USA alone, with the market value of PON clinical diagnostics expected to increase at a compound annual growth rate of 10.4% to USD 46.7 billion by 2024.¹ Compared to instrumental lab-based approaches, analysis of clinical and environmental samples at the PON with fieldable sensing platforms is highly beneficial for a variety of reasons including high spatial and temporal resolution, low-cost, fast time to answer, and early detection.² Much of the initial interest responsible for the boom in PON sensor development was the need for affordable analysis in resource limited settings located in under developed countries, however, they have significant value in developed countries as well.^{2, 3}

Probably the most successful example of a commercialized PON sensor is the portable glucose meter, which is used by millions of individuals suffering from diabetes to monitor their disease on a daily basis.^{4, 5} Other successful clinical PON diagnostics have been developed to detect a variety of health indicators including infectious diseases, tumor and cancer biomarkers, and pregnancy and fertility biomarkers along with many others. For example, in just the last 20 years, several infectious disease outbreaks have threatened human health, many of them emerging in remote areas including the current COVID-19 pandemic, the response to which has suffered due to a lack of affordable, PON diagnostics that can be deployed to rapidly detect and respond to outbreaks.^{2, 6-9} Tools for environmental monitoring at the PON are also sought for a variety of applications where on-site analysis is crucial to rapidly mitigating risk of exposure and safeguarding ecosystem, animal and human health from exposure to toxic pollutants.^{3, 10-12}

Relevant environmental applications in which PON sensors are desirable include water and air quality monitoring. Contaminants that require monitoring in bodies of water include, but are not limited to, heavy metals, waterborne pathogens, industrial pollutants, pharmaceuticals and pesiticides.¹³⁻¹⁵ On the other hand, PON air quality monitoring, especially in workplace settings, typically seeks to quantify potentially harmful gases including nitrogen dioxide (NO₂), volatile organic compounds (VOCs) and ammonia (NH₃) as well as particulate matter (PM).^{15, 16}

Fluorescence, colorimetric, electrochemical and chemiluminescence are the most commonly employed detection motifs in PON sensors due to the availability of miniaturized and portable instrumentation.^{2, 9, 17, 18} Of these, colorimetry is the most common due to its simplistic readout, sometimes requiring only the naked-eye to detect the generated color change.¹⁹ Usually, such devices provide only a yes/no answer to whether an analyte is present, while some can provide qualitative ranges of analyte concentrations. Electroanalysis easily outperforms colorimetry in terms of sensitivity, detection limits, analysis time, and quantitative capabilities. While electrochemical detection does require instrumentation, miniaturized and portable instrumentation that is operable with a smart phone is available. As opposed to any other technique listed above, electrochemistry does not involve the detection of light, therefore, it is insensitive to variable lighting conditions at field sites, sample contaminants such as suspended colloids or colored matrices, and human error in color interpretation.

Electroanalysis is an excellent detection motif for the development of low-cost PON sensors. In particular, carbon electrode materials are specifically suited for PON sensing due to carbon's relative abundance and low-cost.¹⁵ Carbon electrodes can also be integrated with both traditional and paper-based microfluidic devices (μ PADs), providing further assay automation and liquid handling capabilities for simple on-site use.²⁰⁻²⁴ Several forms of carbon are available for

electrode fabrication with easily modifiable surfaces and, as a result, carbon materials are highly adaptable to the sensitive and quantitative detection of a variety of environmentally and clinically relevant analytes.¹⁵

As mentioned previously, a wide variety of carbon materials are available for the development of electrochemical sensors. Carbon electrodes serve as a good alternative to their metallic electrode counterparts because they display several desirable properties for electroanalysis including an extended solvent window, biocompatibility, rich surface chemistry for potential modifications, relative inertness, and low-cost, while also providing the high conductivity and favorable catalytic activity typically associated with metallic electrodes.^{15, 25, 26} Common graphitic, C-sp² hybridized, carbon electrodes include glassy carbon (GC), highly ordered pyrolytic graphite (HOPG), edge plane pyrolytic graphite (EPPG), basal plane pyrolytic graphite (BPPG) and carbon nanotubes (CNTs). Graphitic carbons consist of layers of graphene sheets, where edge planes are the edges of the graphene sheets and basal plane is the face of the graphene sheets.^{26,27} Since the forces holding the individual graphene sheets together are weak, a variety of microstructures due to the arrangements of the layers, distances between layers and crystallite sizes can occur. Graphitic carbon electrodes are highly complex and the exact mechanism governing the structure-activity relationship is still debated in the literature. For example, while edge planes and edge defect sites have long been thought to be more electrocatalytic than the basal plane, recent evidence has shown that clean basal plane graphite is also highly active, however it is more susceptible to adsorption and fouling.^{26, 28} In terms of selecting a graphitic carbon electrode for electroanalysis, several experimental parameters specific to carbon electrode types are important to consider including solvent window, electrocatalytic activity, capacitive currents, background processes, and adsorption/fouling characteristics.^{26, 28, 29}

These properties vary widely between graphitic carbons and depend upon surface chemistry, surface microstructure (e.g. BPPG vs. EPPG vs. GC), electronic structure, electrode cleanliness, and impurities.^{26, 28}

Another allotrope of carbon that has gained popularity is diamond. As a result of complete C-sp³ hybridization, electrically conducting (doped) diamond possesses several superior properties compared to graphitic carbons for electroanalysis.^{30, 31} Boron doped diamond (BDD) electrodes have the widest solvent window of any electrode material in aqueous electrolyte, low capacitive currents, low background faradaic currents and chemical inertness resulting in high fouling and corrosion resistance.^{30, 32-34} As with other carbon electrodes, these properties are dependent on the synthesis, processing, surface microstructure (e.g. grain boundaries), and surface chemistry.^{30, 35-} ³⁷ While electroanalysis with carbon electrodes is highly adaptable to on-site monitoring, with many examples of PON sensors employing the aforementioned carbon electrode materials, the traditional electrode formats are not ideal for user-friendly and inexpensive PON analysis. The difficulty of adapting conventional carbon electrodes to on-site applications lies in their lack of disposability and challenging and cost prohibitive techniques required for their integration with microfluidic devices to remove the need for traditional, cumbersome, electrochemical cell set-ups. To overcome these problems, carbon composite electrodes (CCEs) are a popular alternative to traditional solid carbon electrodes.

CCEs are a mixture of carbon, typically graphite powder, and an inert binder. It is important to note that graphite powders vary widely in preparation, but are typically ball milled versions of the materials discussed previously, resulting in a highly heterogenous surface consisting of both basal and edge planes. Several binders are used to fabricate a diverse library of CCE types including polymers, organic liquids such as paraffin oil and mixtures of several components to

make printable inks.^{25, 38, 39} Depending on the composition (carbon particle and binder) CCEs display an array of physical and electrochemical properties. The main advantage of CCEs is their ease of fabrication with low-cost materials. The most common CCEs employed in PON sensors are screen-printed carbon electrodes (SPCEs). SPCEs are typically fabricated with commercial inks that consist of a mixture of several components to produce a thixotropic fluid that can be printed in the desired geometry using a screen or stencil. As a result, SPCEs are mass producible cost just fractions of a dollar apiece, making them easily disposable. For these reasons, SPCEs have been very successful in PON sensing. For example, many portable glucose meters employ SPCEs for electrochemical sensing.³⁹ Since SPCE inks can be printed onto a variety of substrates including plastic and paper, they are easily integrated with traditional and paper-based microfluidic devices.40,41 Other CCEs fabricated with polymers (thermoplastic electrodes, TPEs) and low molecular weight hydrocarbons (carbon paste electrodes, CPEs) have also been integrated with microfluidic devices, however, not to the extent of SPCEs due to lack of mass producibility and disposability compared to SPCEs.^{33,42,43} Drawbacks to working with CCEs are mainly associated with the presence of the inert binder within the bulk material and at the electrode surface, which can negatively impact conductivity and electrochemical performance.²⁶ Overall, the development and integration of CCEs with microfluidics, especially microfluidic paper-based analytical devices $(\mu PADs)$ has shown great promise for the development of PON sensors that are inexpensive, easy to operate and disposable while providing good sensitivity and detection limits.

While paper has been used as a substrate for analytical testing for over a century, it was first demonstrated as an alternative to traditional microfluidics via the patterning of "microfluidic" channels to direct fluid flow by Whitesides and coworkers in 2007.^{10, 12, 44} Several methods have been adopted for patterning hydrophobic barriers, with wax-printing and subsequent melting of

the wax to define 3-dimensioal barriers in the paper being the most popular.⁹ Microfluidic paperbased analytical devices (μ PADs) are extremely well suited to the development of PON sensors for several reasons. Paper used to fabricate μ PADs is highly abundant and characterized by lowcost, biocompatibility, flexibility, disposability and easy to fold and manipulate into different device geometries.^{21, 45, 46} Paper is composed of a hydrophilic cellulosic fiber network which passively transports fluids, precluding the need for cumbersome external pumps required for the operation of traditional microfluidics.² Due to its porosity, it is also possible to store or immobilize reagents within the paper channels for further assay automation simplifying user operation in PON settings.⁴⁷ For these reasons, the field of μ PAD development has grown significantly in recent years to further advance the performance and analytical capabilities of μ PADs and electrochemical PADs (ePADs).⁴⁸

The work described in this thesis aims to contribute to the field of PON electroanalysis for environmental and clinical applications by presenting new functionalities in ePADs, fundamental and practical studies on several novel compositions for CCEs including SPCEs and boron doped diamond paste electrodes (BDDPEs), as well as better integration practices of CCEs with paperbased devices to maximize the analytical functionality of these inexpensive resources. The ePADs and composite electrodes developed herein can be used to further develop high performance PON sensors while maintaining a high degree of simplicity which is essential for achieving commercialization and wide-spread use.

In Chapter 2 of this thesis, an ePAD is developed which is capable of performing simultaneous electrochemical experiments in distinct solution conditions on a single sample for sensitive multi-analyte detection. The system is referred to as the Janus-ePAD after the two-faced Greek god. This device was developed since many clinically and environmentally relevant samples

contain several species of interest and methods to simultaneously quantify each analyte simultaneously are valuable to further decrease cost, reagent consumption, analysis time and complexity in field settings. However, this is challenging to accomplish at the PON by current techniques, where tuning assay conditions towards one analyte often results in poor selectivity and sensitivity for other species in the mixture, with optimal solution conditions including pH, ionic strength and solvent varying widely between analytes.⁴⁹⁻⁵¹ To overcome this, one option is to carry out multiple experiments with different ePADs and solution preparation procedures. However, in PON sensing, the goal is to minimize any and all liquid handling and preparation steps carried out by users. Multiplexed analysis of all desired analytes with a single sample injection step by the user is a significant goal in PON sensing.⁵²⁻⁵⁶ In aqueous systems, the pH conditions are critical to controlling electrochemical reactions and the resulting analytical sensitivities and detection limits for each analyte present. Solution pH mediates metal speciation, redox potentials due to the concomitant transfer of protons or hydroxide ions, and acid-base equilibria of species whose electroactivity is a function of association or dissociation.^{51, 57, 58} Therefore, the Janus-ePAD was developed to adjust solution pH conditions in situ to perform electroanalysis in two different pH conditions on a single sample simultaneously. In the Janus-ePAD, sample wicks down two channels from a single inlet towards two discreet zones impregnated with reagents to adjust sample pH before flow termination in two electrochemical detection zones. The detection zones feature independent working electrodes and shared reference and counter electrodes, facilitating simultaneous detection of multiple species at each species' optimal solution pH. The device utility and applicability are demonstrated through the simultaneous detection of two biologically relevant species, serotonin and norepinephrine, and a common enzymatic assay product (p-aminophenol) at two different solution pH conditions generated in situ. Janus-ePADs show great promise as an

inexpensive and broadly applicable platform which can reduce the complexity and/or number of steps required in multiplexed analysis at the PON.

The Janus-ePAD developed in Chapter 2 employs boron doped diamond paste electrodes (BDDPEs) for electrochemical detection. Carbon paste electrodes (CPEs) composed of mineral oil and graphite powder were first developed by Ralph Adams in 1958.⁵⁹ Since then, many variations of CPE compositions have been developed and fundamentally characterized.^{38, 60} However, boron doped diamond has not gained popularity as an electrode material until about the last 20 years due to improved technologies for growth of high quality BDD.³⁰ Moreover, since BDD has not gained popularity until recently, its use as the conductive component of composite electrode formulations has been extremely limited, with only a handful of publications since 2011.^{33,61,62} While BDDPEs integrated with the Janus-ePAD performed well in their pre-modified format where reduced graphene oxide was deposited on the surface prior to incorporation with ePADs, some problems were encountered when attempting to use unmodified BDDPEs for quantitative electroanalysis. However, BDD displays several superior properties compared to graphitic carbons and its incorporation with portable sensing platforms is of interest. In order to understand the relationship between BDDPE composition (mineral oil binder and BDD powder) and electrochemical performance, I carried out further fundamental electrochemical characterizations. The characterizations presented in Chapter 3 of this thesis provide a guide to electroanalysis with BDDPEs and as well as their proper integration with ePADs.

While the use of paper to create microfluidic devices has several advantages, mainly, the ability to passively transport fluid in well-defined channels by capillary action, a significant drawback to μ PADs is extremely slow flow rates within the small (μ m) sized capillaries that make up the channels. Also, the ability to pattern reagents and direct fluid flow through multiple channels

that can be modified with specific reagents for multiplexed analysis and assay automation for PON sensors is extremely beneficial. However, slow flow rates in paper lead to very slow analysis times (e.g. 10s of min per device) regardless of detection motif.⁶³ This problem is further exacerbated in ePADs, including the Janus-ePAD developed in Chapter 2, since paper channels also house the electrodes, most commonly SPCEs. The binder components of SPCEs are hydrophobic, and penetrate the paper pores upon printing, acting as a hydrophobic barrier to fluid flow through the channels. The electroactive surface area defined by flow underneath SPCEs at the paper-electrode interface is also variable, and initial ePADs have typically suffered from poor sensitivity and reproducibility.⁴¹ One method employed to overcome the problems associated with SPCEs used in ePADs is the use of microwire electrodes including Au and Pt.⁶⁴⁻⁶⁶ While microwires can be cleaned and/or modified prior to integration with a paper-based device, they are very fragile, difficult to work with and would be very difficult to scale up in the current reported formats for mass production.¹² Another workaround for directly printing SPCEs onto the paper channels includes off-chip development of reusable electrodes. For example, thermoplastic electrodes (TPEs) are templated into a plastic substrate which can then be taped to a µPAD. The TPEs used in previous works showed good electrochemical performance in these formats for flow injection analysis.⁴² However, one could argue that off-chip fabrication of reusable electrodes is not ideal for PON sensing, where one-shot, disposable electrodes are desired to avoid the need to regenerate electrode surfaces, further complicating end user operation.³⁹ Also, in the case of analysis of biological fluids, PON devices must be disposable since reuse of any component can result in cross contamination. Clearly, even with the advancements of electrode incorporation with ePADs made, SPCEs remain the most practical electrode material for electroanalysis at the PON, however, their integration with µPADs needs further optimization.

New research has emerged on the development of fast-flow PADs generated by sandwiching at least one layer of paper with either a second layer of paper, or another substrate such as glass slides, transparency films, and other plastics.^{63, 66-69} The capillary forces generated by the paper layer/layers induce pump-free flow through the gap generated by the two device layers. Flow rates in these 3-dimensional devices are significantly faster than those obtained in a single layer of paper. While the increase in flow rate is dependent on the gap height, which can be controlled by the thickness of the adhesive layer that adheres the top and bottom layer, up to 169 times increase in flow rates have been obtained.⁶³ Another advantage to using multi-layer devices with a channel gap is the opportunity to strategically place SPCEs on the non-paper layers for better reproducibility than SPCEs printed on paper, where bulk solution electrochemistry can be carried out at the SPCE surface exposed to solution contained in the channel gap. Also, printing SPCEs on non-paper substrates affords more opportunities for electrode modification "off-chip" prior to attaching the SPCE layer to the paper layer, similar to the electrode alternatives discussed above, without sacrificing mass producibility.¹² Since the Janus-ePAD developed in Chapter 2 suffered greatly from the aforementioned problems with one-layer ePADs, a second generation Janus-ePAD is developed in Chapter 4. The second generation Janus-ePAD was a hybrid device fabricated with a paper layer and a transparency film layer. Importantly, analysis time was decreased from 20 min to less than one min in the hybrid Janus-ePADs and bulk solution electrochemistry was obtained while maintaining the ability to adjust pH conditions in situ. While Chapters 2 through 4 focus on the development of a first and second generation Janus-ePAD along with fundamental insights into BDDPEs and their integration with Janus-ePADs, the last two chapters of this thesis, and the rest of this introduction, focus on fundamental characterizations to

understand the behavior of SPCEs and guide the applications of novel SPCE compositions, with broad implications in the field of PON electroanalysis with this class of electrodes.

In Chapter 5 of this thesis, a novel GC microparticle stencil printed electrode (GC-SPE) is developed for the detection of cadmium (Cd) and lead (Pb). Heavy metal pollution to toxic levels in environmental matrices is most often due to industrial activities such as mining, smelting, and certain recycling practices.⁷⁰ In Flint, Michigan, city drinking water became contaminated with hazardous waste levels of Pb due to policies which ignored anti-corrosion measures, leading to the corrosion of lead piping, resulting in unsafe drinking water.⁷¹ Heavy metal pollution monitoring is important because heavy metals are non-biodegradable and accumulate in the environment. In humans, chronic exposure to low levels and acute exposure to high levels of several heavy metals has been linked to a variety of severe health effects involving nearly all organ systems and can even result in death.^{72, 73} Thus, identification and quantification is vital for informing remediation efforts, exposure prevention and understanding the dynamic transport of heavy metals in the environment.⁷⁴ Anodic stripping voltammetry (ASV) performed with Bi-film GC electrodes has been extremely successful for multiplexed heavy metal detection at sub-ppb detection limits, however, adapting the method to a portable format with disposable SPCE materials has proven to be difficult. Graphite powders are exclusively employed in SPCE fabrication, yet graphite is inherently heterogeneous, with both edge and basal planes exposed, resulting in heterogeneous electrochemical activity across an SPCE surface. This heterogeneity is problematic in ASV since metal deposition occurs in several chemical environments leading to double stripping peaks for heavy metals, which are then difficult to identify and quantify.^{26, 75} While GC is also a form of graphitic carbon, the microstructure is isotropic, and Bi-film GCEs provide single, well resolved stripping peaks for Cd and Pb detection.^{29, 76} In order to retain the simplicity of SPCEs, without complex post-fabrication modification typically required for heavy metal quantification with ASV at these electrodes, a novel SPCE formulation was developed using GC microparticles as the conductive carbon component. The GC-SPEs developed in Chapter 5 outperformed conventional SPCEs for ASV of Cd and Pb with an in situ plated Bi-film. To demonstrate the analytical utility, Cd and Pb concentrations were quantified in real soil samples. Moreover, due the simplicity and low cost of GC-SPEs, their commercialization is possible. Currently, Access Sensor Technologies located in Fort Collins, CO is producing GC-SPE prototypes for commercial distribution. The development of the GC-SPE in Chapter 5 lead me to believe that the electrochemical properties and analytical utility of SPCEs could be further tuned and enhanced by controlling SPCE composition through fabrication with different carbon particle types and is the focus of Chapter 6 of this thesis.

As mentioned previously, a variety of CCEs exist which are differentiated by the physical properties the binder material imparts on the final electrode.^{25, 38, 42, 59, 60, 77} SPCEs are typically fabricated with commercial inks, whose proprietary formulations have been optimized for screen or stencil printing.³⁹ Therefore, the ink compositions, including carbon particle type, used by researchers are determined by the manufacturer of the inks and are not further modified by researchers before printing. Because other CCEs such as TPEs and CPEs do not rely on thixotropic inks for printing, all components of the composite have been easily controlled by researchers and several studies have been carried out to optimize carbon particle type as well as the optimal carbon particle to binder mass ratio to provide high performance carbon composites that rival the conductivity and electrochemical activity of conventional solid electrodes such as GC, Pt and Au.^{25, 78-81} On the other hand commercially available SPCEs and inks are typically activated postfabrication to improve their electrochemical characteristics via the removal of inhibitive binder

from carbon particle surfaces.⁸²⁻⁸⁷ Even after activation, most SPCEs still suffer from poor electrochemical characteristics due to the high resistance of the bulk material. Owing to the previous results in Chapter 5 showing that SPCEs can be fabricated by the addition of GC microparticles to a commercial ink containing some type of carbon nanomaterial, producing dramatically different electrochemical properties which are better suited for ASV than graphite SPCEs, a study on the effects of SPCEs made with different carbon particle types was carried out. The systematic study revealed that carbon particle size, purity, microstructure and mass loading play a significant role in the quality of the electrochemical response and mechanical stability of SPCEs. Several types of graphite particles and GC microparticles as well as the nanomaterial carbon black were compared for SPCE fabrication. After activation of the SPCEs with plasma treatment, certain SPCE formulations provided electrochemical activity on par with TPEs and CPEs, which have long been thought to inherently outperform SPCEs.^{25, 81}

In summary, the work contained in this thesis is aimed toward the development of better performing PON sensors for electroanalysis with carbon based electrodes for biological and environmental applications. First, a novel Janus-ePAD is developed for simultaneous electrochemical detection in several pH conditions which were generated on-line. Following this, the fundamental electrochemical characteristics of a new composite electrode, BDDPEs, is carried out to understand the composition/performance relationship for further applications in electroanalysis. A hybrid Janus-ePAD is developed to overcome the problems associated with single-layer ePADs, significantly improving analysis times and reproducibility in ePADs fabricated in simple manner with low-cost and disposable materials. A novel SPCE formulation employing GC microparticles (GC-SPE) was used to quantify Cd and Pb in environmental samples at relevant detection limits without complex modification, simply by tuning the carbon particle type uses in the SPCE composition. Finally, novel formulations of SPCEs are made and the relationship between composition and performance is characterized through fundamental electrochemistry, further expanding the capabilities and improving the performance of these commercializable, inexpensive and single-use electrode materials in PON electroanalysis.

REFERENCES

1.Singh, S.; Tripathi, P.; Nara, S., Emerging Point-of-Care Diagnostic Methods for Disease Detection. In *Biomedical Engineering and its Applications in Healthcare*, Springer: 2019; pp 377-397.

2.Gong, M. M.; Sinton, D., Turning the page: advancing paper-based microfluidics for broad diagnostic application. *Chemical reviews* **2017**, *117* (12), 8447-8480.

3. Martinez, A. W.; Phillips, S. T.; Whitesides, G. M.; Carrilho, E., Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices. *Analytical Chemistry* **2010**, *82* (1), 3-10.

4.Xiang, Y.; Lu, Y., Using personal glucose meters and functional DNA sensors to quantify a variety of analytical targets. *Nature chemistry* **2011**, *3* (9), 697.

5. Montagnana, M.; Caputo, M.; Giavarina, D.; Lippi, G., Overview on self-monitoring of blood glucose. *Clinica Chimica Acta* **2009**, *402* (1-2), 7-13.

6.Gates, B., The next epidemic—lessons from Ebola. *New England Journal of Medicine* **2015**, *372* (15), 1381-1384.

7.Yager, P.; Domingo, G. J.; Gerdes, J., Point-of-care diagnostics for global health. *Annual review of biomedical engineering* **2008**, *10*.

8. Piguillem, F.; Shi, L., Optimal COVID-19 quarantine and testing policies. 2020.

9. Akyazi, T.; Basabe-Desmonts, L.; Benito-Lopez, F., Review on microfluidic paper-based analytical devices towards commercialisation. *Analytica chimica acta* **2018**, *1001*, 1-17.

10.Cate, D. M.; Adkins, J. A.; Mettakoonpitak, J.; Henry, C. S., Recent developments in paperbased microfluidic devices. *Analytical chemistry* **2015**, *87* (1), 19-41.

11.Carrell, C.; Kava, A.; Nguyen, M.; Menger, R.; Munshi, Z.; Call, Z.; Nussbaum, M.; Henry, C., Beyond the lateral flow assay: A review of paper-based microfluidics. *Microelectronic Engineering* **2019**, *206*, 45-54.

12.Noviana, E.; McCord, C. P.; Clark, K. M.; Jang, I.; Henry, C. S., Electrochemical paperbased devices: sensing approaches and progress toward practical applications. *Lab on a Chip* **2019**, *20* (1), 9-34.

13.Mandal, N.; Mitra, S.; Bandyopadhyay, D., Sensors for Point-of-Care Monitoring of Drinking Water Quality. *IEEE Sensors Journal* **2019**, *19* (18), 7936-7941.

14.Kim, U.; Ghanbari, S.; Ravikumar, A.; Seubert, J.; Figueira, S., Rapid, affordable, and point-of-care water monitoring via a microfluidic DNA sensor and a mobile interface for global health. *IEEE journal of translational engineering in health and medicine* **2013**, *1*, 3700207-3700207.

15.Hersey, M.; Berger, S. N.; Holmes, J.; West, A.; Hashemi, P., Recent Developments in Carbon Sensors for At-Source Electroanalysis. *Analytical Chemistry* **2019**, *91* (1), 27-43. 16.Cate, D. M.; Noblitt, S. D.; Volckens, J.; Henry, C. S., Multiplexed paper analytical device for quantification of metals using distance-based detection. *Lab on a Chip* **2015**, *15* (13), 2808-2818.

17.Delaney, J. L.; Hogan, C. F.; Tian, J.; Shen, W., Electrogenerated chemiluminescence detection in paper-based microfluidic sensors. *Analytical chemistry* **2011**, *83* (4), 1300-1306. 18.Shen, B.; Xie, Y.; Irawan, R., A novel portable fluorescence detection system for microfluidic card. *Journal of Instrumentation* **2008**, *3* (12), T12001.

19.Meredith, N. A.; Quinn, C.; Cate, D. M.; Reilly, T. H.; Volckens, J.; Henry, C. S., based analytical devices for environmental analysis. *Analyst* **2016**, *141* (6), 1874-1887. 20.Nie, Z.; Deiss, F.; Liu, X.; Akbulut, O.; Whitesides, G. M., Integration of paper-based microfluidic devices with commercial electrochemical readers. *Lab on a Chip* **2010**, *10* (22), 3163-3169.

21.Rackus, D. G.; Shamsi, M. H.; Wheeler, A. R., Electrochemistry, biosensors and microfluidics: a convergence of fields. *Chemical Society Reviews* **2015**, *44* (15), 5320-5340. 22.Nie, Z. H.; Nijhuis, C. A.; Gong, J. L.; Chen, X.; Kumachev, A.; Martinez, A. W.; Narovlyansky, M.; Whitesides, G. M., Electrochemical sensing in paper-based microfluidic devices. *Lab on a Chip* **2010**, *10* (4), 477-483.

23.Adkins, J.; Boehle, K.; Henry, C., Electrochemical paper-based microfluidic devices. *Electrophoresis* **2015**, *36* (16), 1811-1824.

24.Zang, D.; Ge, L.; Yan, M.; Song, X.; Yu, J., Electrochemical immunoassay on a 3D microfluidic paper-based device. *Chemical Communications* **2012**, *48* (39), 4683-4685. 25.Klunder, K. J.; Nilsson, Z.; Sambur, J. B.; Henry, C. S., Patternable Solvent-Processed Thermoplastic Graphite Electrodes. *Journal of the American Chemical Society* **2017**, *139* (36), 12623-12631.

26.McCreery, R. L., Advanced carbon electrode materials for molecular electrochemistry. *Chemical Reviews* **2008**, *108* (7), 2646-2687.

27.McDermott, C. A.; Kneten, K. R.; McCreery, R. L., Electron transfer kinetics of aquated Fe+ 3/+ 2, Eu+ 3/+ 2, and V+ 3/+ 2 at carbon electrodes inner sphere catalysis by surface oxides. *Journal of the Electrochemical Society* **1993**, *140* (9), 2593-2599.

28.Patel, A. N.; Tan, S.-y.; Miller, T. S.; Macpherson, J. V.; Unwin, P. R., Comparison and reappraisal of carbon electrodes for the voltammetric detection of dopamine. *Analytical chemistry* **2013**, *85* (24), 11755-11764.

29. Zoski, C. G., Handbook of electrochemistry. Elsevier: 2006.

30. Macpherson, J. V., A practical guide to using boron doped diamond in electrochemical research. *Physical Chemistry Chemical Physics* **2015**, *17* (5), 2935-2949.

31.Luong, J. H.; Male, K. B.; Glennon, J. D., Boron-doped diamond electrode: synthesis, characterization, functionalization and analytical applications. *Analyst* **2009**, *134* (10), 1965-1979.

32.Hutton, L. A.; Iacobini, J. G.; Bitziou, E.; Channon, R. B.; Newton, M. E.; Macpherson, J. V., Examination of the Factors Affecting the Electrochemical Performance of Oxygen-Terminated Polycrystalline Boron-Doped Diamond Electrodes. *Analytical Chemistry* **2013**, *85* (15), 7230-7240.

33.Nantaphol, S.; Channon, R. B.; Kondo, T.; Siangproh, W.; Chailapakul, O.; Henry, C. S., Boron Doped Diamond Paste Electrodes for Microfluidic Paper-Based Analytical Devices. *Analytical Chemistry* **2017**, *89* (7), 4100-4107.

34.Bennett, J. A.; Wang, J.; Show, Y.; Swain, G. M., Effect of sp2-bonded nondiamond carbon impurity on the response of boron-doped polycrystalline diamond thin-film electrodes. *Journal of The Electrochemical Society* **2004**, *151* (9), E306-E313.

35.Granger, M. C.; Witek, M.; Xu, J.; Wang, J.; Hupert, M.; Hanks, A.; Koppang, M. D.; Butler, J. E.; Lucazeau, G.; Mermoux, M., Standard electrochemical behavior of high-quality, boron-doped polycrystalline diamond thin-film electrodes. *Analytical Chemistry* **2000**, *72* (16), 3793-3804.

36.Patten, H. V.; Lai, S. C.; Macpherson, J. V.; Unwin, P. R., Active sites for outer-sphere, inner-sphere, and complex multistage electrochemical reactions at polycrystalline boron-doped diamond electrodes (pBDD) revealed with scanning electrochemical cell microscopy (SECCM). *Analytical chemistry* **2012**, *84* (12), 5427-5432.

37.Duran, B.; Brocenschi, R. F.; France, M.; Galligan, J. J.; Swain, G. M., Electrochemical activation of diamond microelectrodes: implications for the in vitro measurement of serotonin in the bowel. *Analyst* **2014**, *139* (12), 3160-3166.

38. Svancara, I.; Vytras, K.; Barek, J.; Zima, J., Carbon paste electrodes in modern electroanalysis. *Critical Reviews in Analytical Chemistry* **2001**, *31* (4), 311-345.

39. Metters, J. P.; Kadara, R. O.; Banks, C. E., New directions in screen printed electroanalytical sensors: an overview of recent developments. *Analyst* **2011**, *136* (6), 1067-1076.

40.Randviir, E. P.; Brownson, D. A.; Metters, J. P.; Kadara, R. O.; Banks, C. E., The fabrication, characterisation and electrochemical investigation of screen-printed graphene electrodes. *Physical Chemistry Chemical Physics* **2014**, *16* (10), 4598-4611.

41.Adkins, J. A.; Boehle, K.; Friend, C.; Chamberlain, B.; Bisha, B.; Henry, C. S., Colorimetric and electrochemical bacteria detection using printed paper-and transparency-based analytic devices. *Analytical chemistry* **2017**, *89* (6), 3613-3621.

42.Noviana, E.; Klunder, K. J.; Channon, R. B.; Henry, C. S., Thermoplastic Electrode Arrays in Electrochemical Paper-Based Analytical Devices. *Analytical Chemistry* **2019**, *91* (3), 2431-2438.

43.Pradela-Filho, L. A.; Noviana, E.; Araujo, D.; Takeuchi, R.; Santos, A.; Henry, C. S., Rapid Analysis in Continuous Flow Electrochemical Paper-Based Analytical Devices. *ACS* sensors **2020**.

44.Martinez, A. W.; Phillips, S. T.; Butte, M. J.; Whitesides, G. M., Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angewandte Chemie International Edition* **2007**, *46* (8), 1318-1320.

45. Yetisen, A. K.; Akram, M. S.; Lowe, C. R., based microfluidic point-of-care diagnostic devices. *Lab on a Chip* **2013**, *13* (12), 2210-2251.

46.Xia, Y.; Si, J.; Li, Z., Fabrication techniques for microfluidic paper-based analytical devices and their applications for biological testing: A review. *Biosensors and Bioelectronics* **2016**, *77*, 774-789.

47.Cunningham, J. C.; Scida, K.; Kogan, M. R.; Wang, B.; Ellington, A. D.; Crooks, R. M., Paper diagnostic device for quantitative electrochemical detection of ricin at picomolar levels. *Lab on a Chip* **2015**, *15* (18), 3707-3715.

48.Cunningham, J. C.; DeGregory, P. R.; Crooks, R. M., New functionalities for paper-based sensors lead to simplified user operation, lower limits of detection, and new applications. *Annual Review of Analytical Chemistry* **2016**, *9*, 183-202.

49.Bard, A. J.; Faulkner, L. R.; Leddy, J.; Zoski, C. G., *Electrochemical methods: fundamentals and applications*. wiley New York: 1980; Vol. 2.

50.Chicharro, M.; Zapardiel, A.; Bermejo, E.; Perez, J. A.; Hernandez, L., EPHEDRINE DETERMINATION IN HUMAN URINE USING A CARBON-PASTE ELECTRODE

MODIFIED WITH C-18 BONDED SILICA-GEL. *Analytical Letters* **1994**, *27* (10), 1809-1831. 51.Read, T. L.; Joseph, M. B.; Macpherson, J. V., Manipulation and measurement of pH sensitive metal–ligand binding using electrochemical proton generation and metal detection. *Chemical Communications* **2016**, *52* (9), 1863-1866.

52.Nilghaz, A.; Guan, L.; Tan, W.; Shen, W., Advances of Paper-Based Microfluidics for Diagnostics ☐ The Original Motivation and Current Status. *ACS sensors* **2016**, *1* (12), 1382-1393.

53.Martinez, A. W.; Phillips, S. T.; Butte, M. J.; Whitesides, G. M., Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angew. Chem.-Int. Edit.* **2007**, *46* (8), 1318-1320.

54.Dungchai, W.; Chailapakul, O.; Henry, C. S., Electrochemical detection for paper-based microfluidics. *Analytical chemistry* **2009**, *81* (14), 5821-5826.

55.Martinez, A. W.; Phillips, S. T.; Carrilho, E.; Thomas III, S. W.; Sindi, H.; Whitesides, G. M., Simple telemedicine for developing regions: camera phones and paper-based microfluidic devices for real-time, off-site diagnosis. *Analytical chemistry* **2008**, *80* (10), 3699-3707.

56.Li, X.; Ballerini, D. R.; Shen, W., A perspective on paper-based microfluidics: Current status and future trends. *Biomicrofluidics* **2012**, *6* (1), 011301.

57.Read, T. L.; Bitziou, E.; Joseph, M. B.; Macpherson, J. V., In situ control of local pH using a boron doped diamond ring disk electrode: optimizing heavy metal (mercury) detection. *Analytical chemistry* **2013**, *86* (1), 367-371.

58.Blaho, J. K.; Goldsby, K. A., Redox regulation based on the pH-dependent hydrolysis of 2pyridinecarboxaldehyde coordinated to ruthenium (II). *Journal of the American Chemical Society* **1990**, *112* (16), 6132-6133.

59.Adams, R. N., Carbon paste electrodes. *Analytical chemistry* **1958**, *30* (9), 1576-1576. 60.Švancara, I.; Schachl, K., Testing of unmodified carbon paste electrodes. *Chem. Listy* **1999**, *93*, 490-499.

61.Kondo, T.; Sakamoto, H.; Kato, T.; Horitani, M.; Shitanda, I.; Itagaki, M.; Yuasa, M., Screen-printed diamond electrode: A disposable sensitive electrochemical electrode. *Electrochemistry Communications* **2011**, *13* (12), 1546-1549.

62.Kondo, T.; Udagawa, I.; Aikawa, T.; Sakamoto, H.; Shitanda, I.; Hoshi, Y.; Itagaki, M.; Yuasa, M., Enhanced sensitivity for electrochemical detection using screen-printed diamond electrodes via the random microelectrode array effect. *Analytical chemistry* **2016**, *88* (3), 1753-1759.

63.Channon, R. B.; Nguyen, M. P.; Henry, C. S.; Dandy, D. S., Multilayered Microfluidic Paper-Based Devices: Characterization, Modeling, and Perspectives. *Analytical Chemistry* **2019**, *91* (14), 8966-8972.

64.Fosdick, S. E.; Anderson, M. J.; Renault, C.; DeGregory, P. R.; Loussaert, J. A.; Crooks, R. M., Wire, mesh, and fiber electrodes for paper-based electroanalytical devices. *Analytical chemistry* **2014**, *86* (7), 3659-3666.

65. Adkins, J. A.; Henry, C. S., Electrochemical detection in paper-based analytical devices using microwire electrodes. *Analytica Chimica Acta* **2015**, *891*, 247-254.

66.Adkins, J. A.; Noviana, E.; Henry, C. S., Development of a Quasi-Steady Flow Electrochemical Paper-Based Analytical Device. *Analytical chemistry* **2016**, *88* (21), 10639-10647.

67.Channon, R. B.; Nguyen, M. P.; Scorzelli, A. G.; Henry, E. M.; Volckens, J.; Dandy, D. S.; Henry, C. S., Rapid flow in multilayer microfluidic paper-based analytical devices. *Lab on a Chip* **2018**, *18* (5), 793-802.

68.Soum, V.; Park, S.; Brilian, A. I.; Kwon, O.-S.; Shin, K., Programmable Paper-Based Microfluidic Devices for Biomarker Detections. *Micromachines* **2019**, *10* (8), 516.

69.Jang, I.; Berg, K. E.; Henry, C. S., Viscosity Measurements Utilizing a Fast-Flow Microfluidic Paper-Based Device. *Sensors and Actuators B: Chemical* **2020**, 128240. 70.Control, C. f. D.; Prevention, Agency for Toxic Substances and Disease Registry. Case

studies in environmental medicine: lead toxicity, 2007. 2009.

71.Denchak, M., Flint water crisis: Everything you need to know. *National Resource Defense Council* **2018**.

72.O'Neil, G. D.; Newton, M. E.; Macpherson, J. V., Direct Identification and Analysis of Heavy Metals in Solution (Hg, Cu, Pb, Zn, Ni) by Use of in Situ Electrochemical X-ray Fluorescence. *Analytical Chemistry* **2015**, *87* (9), 4933-4940.

73.EPA Drinking Water Contaminants - Standards and regulations. https://www.epa.gov/dwstandardsregulations#8.

74.Mahamoud Ahmed, A.; Lyautey, E.; Bonnineau, C.; Dabrin, A.; Pesce, S., Environmental concentrations of copper, alone or in mixture with arsenic, can impact river sediment microbial community structure and functions. *Frontiers in microbiology* **2018**, *9*, 1852.

75.Kneten, K. R.; McCreery, R. L., EFFECTS OF REDOX SYSTEM STRUCTURE ON ELECTRON-TRANSFER KINETICS AT ORDERED GRAPHITE AND GLASSY-CARBON ELECTRODES. *Analytical Chemistry* **1992**, *64* (21), 2518-2524.

76.Wang, J.; Lu, J. M.; Hocevar, S. B.; Farias, P. A. M.; Ogorevc, B., Bismuth-coated carbon electrodes for anodic stripping voltammetry. *Analytical Chemistry* **2000**, *72* (14), 3218-3222. 77.Urbaniczky, C.; Lundström, K., Voltammetric studies on carbon paste electrodes: The influence of paste composition on electrode capacity and kinetics. *Journal of electroanalytical*

chemistry and interfacial electrochemistry **1984**, 176 (1-2), 169-182.

78.Klunder, K. J.; Clark, K. M.; McCord, C.; Berg, K. E.; Minteer, S. D.; Henry, C. S., Polycaprolactone-enabled sealing and carbon composite electrode integration into

electrochemical microfluidics. *Lab on a Chip* **2019**, *19* (15), 2589-2597.

79.Mikysek, T.; Svancara, I.; Kalcher, K.; Bartos, M.; Vytras, K.; Ludvik, J., New Approaches to the Characterization of Carbon Paste Electrodes Using the Ohmic Resistance Effect and Qualitative Carbon Paste Indexes. *Analytical Chemistry* **2009**, *81* (15), 6327-6333.

80.Švancara, I.; Hvízdalová, M.; Vytřas, K.; Kalcher, K.; Novotný, R., A microscopic study on carbon paste electrodes. *Electroanalysis* **1996**, *8* (1), 61-65.

81.Wang, J.; Kirgöz, Ü. A.; Mo, J.-W.; Lu, J.; Kawde, A. N.; Muck, A., Glassy carbon paste electrodes. *Electrochemistry communications* **2001**, *3* (4), 203-208.

82.Wang, J.; Tian, B.; Nascimento, V. B.; Angnes, L., Performance of screen-printed carbon electrodes fabricated from different carbon inks. *Electrochimica Acta* **1998**, *43* (23), 3459-3465.

83.González-Sánchez, M. I.; Gómez-Monedero, B.; Agrisuelas, J.; Iniesta, J.; Valero, E., Highly activated screen-printed carbon electrodes by electrochemical treatment with hydrogen peroxide. *Electrochemistry Communications* **2018**, *91*, 36-40.

84.Wang, S.; Chang, K.; Yuan, C., Enhancement of electrochemical properties of screen-printed carbon electrodes by oxygen plasma treatment. *Electrochimica Acta* **2009**, *54* (21), 4937-4943.

85.Wei, H.; Sun, J.-J.; Xie, Y.; Lin, C.-G.; Wang, Y.-M.; Yin, W.-H.; Chen, G.-N., Enhanced electrochemical performance at screen-printed carbon electrodes by a new pretreating procedure. *Analytica chimica acta* **2007**, *588* (2), 297-303.

86.Wang, J.; Pedrero, M.; Sakslund, H.; Hammerich, O.; Pingarron, J., Electrochemical activation of screen-printed carbon strips. *Analyst* **1996**, *121* (3), 345-350.

87.Cui, G.; Yoo, J. H.; Lee, J. S.; Yoo, J.; Uhm, J. H.; Cha, G. S.; Nam, H., Effect of pretreatment on the surface and electrochemical properties of screen-printed carbon paste electrodes. *Analyst* **2001**, *126* (8), 1399-1403.

CHAPTER 2: JANUS ELECTROCHEMISTRY – SIMULTANEOUS ELECTROCHEMICAL DETECTION AT MULTIPLE WORKING CONDITIONS IN A PAPER-BASED ANALYTCIAL DEVICE

2.1 Chapter Overview

In this chapter, an electrochemical paper-based analytical device (ePAD) capable of performing simultaneous electrochemical experiments in distinct solution conditions on a single sample for sensitive multi-analyte detection was developed. The system is referred to as the JanusePAD after the two-faced Greek god since the two solution conditions and two electrochemical experiments can be carried out on a single device. The simultaneous detection of multiple analytes in a single sample is a critical tool for the analysis of real world samples. However, this is challenging to accomplish in the field by current electroanalytical techniques, where tuning assay conditions towards a target analyte often results in poor selectivity and sensitivity for other species in the mixture. In the Janus-ePAD, sample wicks down two channels from a single inlet towards two discreet reagent zones that adjust solution pH, before flow termination in two electrochemical detection zones. These zones feature independent working electrodes and shared reference and counter electrodes, facilitating simultaneous detection of multiple species at each species' optimal solution conditions. The device utility and applicability are demonstrated through the simultaneous detection of two biologically relevant species (norepinephrine and serotonin) and a common enzymatic assay product (p-aminophenol) at two different solution pH conditions. Janus-ePADs show great promise as an inexpensive and broadly applicable platform which can reduce the complexity and/or number of steps required in multiplexed analysis, while also operating under the optimized conditions of each species present in a mixture. This work was completed by myself and a visiting member of the Henry group from Thailand, Siriwan (Pad) Nantaphol. My contributions include the p-aminophenol experiments as well as primary authorship of the manuscript. Fundamental electrochemical studies of the BDDPEs are presented in Chapter 3 and a second iteration of the Janus-ePAD that I developed is presented Chapter 4 of this thesis. The work presented herein is published in *Analytica Chimica Acta*.¹

2.2 Introduction

Methods for detecting multiple analytes simultaneously at the point-of-need are of significant interest in many fields including clinical diagnostics,^{2,3} environmental monitoring,^{4,5} and food safety.^{6,7} Multiplexed analysis typically minimizes the required sample volumes, the ease and time of analysis, and/or cost of sensing. However, it is challenging to quantify multiple species - where each species requires unique conditions, from a single sample in one device using simple analyses. Frequently, target analytes are present at lower concentrations than background species⁸ and matrix effects can inhibit detection.⁹ Moreover, each species may require different detection settings such as detection technique, assay reagents, buffer type, and/or pH conditions, as well as intricate modifications to the sensing surface to impart selectivity. This leads to difficulties in setting experimental conditions for sensing of each analyte, often resulting in diminished sensitivity and selectivity for one or more species in the mixture, or the requirement for altogether separate detection methods or steps.⁸ Integrated platforms or arrays of multiple sensors have been applied to multiplexed detection.¹⁰⁻¹³ These sensors are usually designed with experimental conditions specific to a single target analyte, whereby separate preparation procedures are required. However, extra solution preparation procedures are undesirable in point-of-need (PON) diagnostics as an individual procedure for detection of each analyte increases the analysis time, cost, material requirements and training required for an end-user.

In 2007, Whitesides and coworkers demonstrated the first microfluidic paper-based analytical device (uPAD).¹⁴ The device was used to perform multiplexed bioassays; since this work, µPADs have been developed extensively for use in PON settings. µPADs are an attractive platform for multiplexed PON testing as a result of their low cost, portability, low sample consumption, ease of use, and disposability.¹⁵ Flow is generated via capillary action, precluding the need for mechanical or electrical pumps associated with traditional microfluidic devices.¹⁶ The porous paper matrix allows for storage of dried reagents,¹⁷ facilitating multi-step assays.¹⁸ Through patterning with hydrophobic barriers, multiple fluidic channels can be generated on a single paper device for multiplexing.^{19, 20} Colorimetric µPADs featuring multiple channels have been demonstrated extensively for multiplexed detection, where the intensity or hue of a color change corresponds to the target analyte concentration.²¹⁻²³ Despite their inherent simplicity, colorimetric µPADs are limited by the requirement for a colorimetric substrate and poor detection limits and/or sensitivity.²⁴⁻²⁶ Additionally, splitting of a sample into multiple channels for multiplexed sensing results in a decrease in the total number of moles of analyte available to react and produce a detectable color change, making detection limits a critical variable in this class of µPADs.²⁷

Electrochemical PADs (ePADs) provide a more quantitative detection method with lower detection limits, increased sensitivity, rapid measurement times (<1 min), and amenability to miniaturization.²⁸ Electrochemical detection is an appealing approach for multiplexed detection, as through control of the applied potential, multiple species in a mixture can be detected in one measurement. However, this is insufficient when the target species exhibit similar redox potentials, resulting in unresolved signals. In this case, the signals can sometimes be resolved through pretreatments such as derivatization or chromatographic separations, both of which are unsuited for PON sensing. Simultaneous electrochemical sensing of multiple species without pretreatment

can also be performed using chemically modified electrodes (CMEs).²⁹⁻³² CMEs and arrays of CMEs have been incorporated into ePADs for multiplexed detection of cancer biomarkers,^{33, 34} heavy metals in environmental³⁵ and human serum samples,³⁶ and an array of other environmental and biological analytes. Still, the electrochemical experiments typically take place under a single set of conditions, including pH, ionic strength, and solvent. This becomes problematic for multiple-analyte detection as these parameters influence reaction rates and sensitivities and the optimal conditions are frequently analyte specific.^{37,39} Solution pH is a critical variable, as pH controls metal speciation, redox potentials due to the concomitant transfer of protons or hydroxide ions, and acid-base equilibria of species whose electroactivity is a function of association or dissociation.³⁷⁻³⁹ Often times, sensitivity is sacrificed for one or more species in a mixture by performing analysis at a single pH.⁴⁰ Methods to electrochemically control pH conditions in situ via the electrolysis of water for the detection of a single pH sensitive species have been reported.^{38,41} However, one would ideally conduct electrochemical analysis on a single aliquot, at the optimal detection conditions for each individual analyte present simultaneously.

In this work, we demonstrate a Janus-ePAD for performing multiplexed detection with the capability for in situ pH control for optimized electrochemistry. Janus-ePADs are demonstrated for two applications: the simultaneous detection of norepinephrine (NE) and serotonin (5-hydroxytryptamine, 5-HT) and the detection of p-aminophenol (pAP). NE and 5-HT are of significant interest since their electrochemical behaviors are pH dependent⁴² and low levels of NE and 5-HT are linked to several disorders, including depression, migraine, and anxiety disorders.⁴³ pAP is often detected as the electrochemically active product in a variety of enzymatic assays and is an important clinical and environmental contaminant.²⁴ Since enzyme activity is pH dependent and enzyme specific, performing multiple enzymatic assays at each enzymes' optimal pH
conditions from one sample is challenging. As a proof of concept, the target analytes (NE, 5-HT and pAP) are detected at two different pH values, pH 6.0 and pH 8.0, in a single device featuring in situ pH generation. Janus-ePADs provide a new approach for the fabrication of high-performance multiplexed sensing devices and has broad reaching implications for simultaneous electrochemical detection of multiple species at the PON.

2.3 Experimental

Chemicals, Reagents and Materials

All chemicals were analytical grade and used as received, and all solutions were prepared using purified water (18.2 M Ω cm) from a Milli-Q Millipore water purification system. 5-HT was acquired from Alfa Aesar (Ward Hill, MA). NE, potassium phosphate monobasic, sodium phosphate dibasic, phosphoric acid (H₃PO₄), sodium hydroxide (NaOH) were acquired from Sigma-Aldrich (St. Louis, MO). Potassium ferrocyanide (Fe(CN)₆⁴⁻) was acquired from Mallinckrodt (St. Louis, MO). Graphene oxide (GO) was acquired from XF Nano, Inc. (Nanjing, China). Light mineral oil was acquired from Fischer Scientific (New Jersey). pH-indicator strips with a pH range of 4.0 - 7.0 and 6.5 - 10.0 were acquired from Merck (Darmstadt, Germany). Whatman 4 chromatography paper was acquired from Fisher Scientific (Pittsburgh, PA). A XEROX Phaser 8860 printer was used to print wax patterns on PADs following established protocols. An Isotemp hot plate from Fischer Scientific, set at 150°C, was used to melt the wax on the paper. Ag/AgCl ink from Gwent Group (Torfaen, U.K.) was used to construct the conducting pads and reference electrode (RE). Carbon ink from Ercon Incorporated (Wareham, MA) was used for the construction of the counter electrode (CE). Boron doped diamond (BDD) powder was prepared through a previously reported procedure.⁴⁴ The fabrication of the working electrode (WE) of BDD paste electrode (BDDPE) followed a published protocol.⁴⁵ Stencil-printed Ag/AgCl on a transparency sheet substrate was prepared as a conducting pad. To minimize BDD paste consumption, an electrode body containing three smaller openings (0.1×2 mm rectangles) was fabricated using a laser engraving system (Epilog, Golden, CO). The BDDPE was prepared by mixing BDD powder and mineral oil (70:30, w/w) and filled into the electrode body.

Device Fabrication and Operation

The design, details of the fabrication procedures and operation of the Janus-ePAD is shown in Figure 2.1. Adobe Illustrator CS6 software was used to design device features containing sample reservoir, two reagent zones and two detection zones. After printing the design using a wax printer (Xerox Colorqube 8870), devices were heated on a hot plate at 175 °C for 50 s to melt the wax through the paper, creating a hydrophobic barrier. The backs of the paper devices, except the detection zones, were taped with Scotch packing tape to control fluid flow and prevent leaking during the measurements. The electrochemical detection zone consists of three layers: (i) CE and RE fabricated on 8 mm diameter circular hydrophilic areas at the back side of wax-printed paper by stencil-printing, (ii) Whatman #4 paper pieces inserted between the stencil-printed electrodes paper layer and WE layer to improve the efficiency for the solution flow in the channel, and (iii) the BDDPEs. Two different pH values of pH 6.0 and 8.0 can be generated by adding three $1.4 \mu L$ aliquots of 0.5 M H₃PO₄ to the 1st reagent zone and three $1.4 \mu L$ aliquots of 0.5 M NaOH to the 2nd reagent zone. All reagents and samples were applied on the front (wax-printed) side of device. Between each reagent addition, the device was dried at room temperature.



Figure 2.1 Schematic for (a) fabrication of the BDDPE WE (b) fabrication of the CE and RE on wax-patterned paper (c) attachment of BDDPE WEs to paper device to fabricate Janus-ePAD (d) Janus-ePAD design and (e) operation for multiplexed detection with in situ pH adjustment.

For the measurement step, a polydimethylsiloxane (PDMS) lid was placed on the top of device for applying equal pressure across the paper surface, thus controlling the flow rates. A 60 μ L aliquot of sample solution was gently introduced into the device at the sample reservoir through the hole in the PDMS lid, capillary action carried solution along the channels (the wax barrier served to confine and direct sample flow). As the solution reacted with the H₃PO₄ and NaOH deposited at the reagent zones, pH values of solution were adjusted to pH 6.0 and 8.0, respectively. After the adjusted pH solutions reached the detection zones, the PDMS lid was removed and electrochemical detection was performed.

Electrochemical Detection

All electrochemical experiments were performed using a model CHI832 bipotentiostat (CH Instruments, Austin, TX) with four-electrode configuration including a reference electrode (RE), a counter electrode (CE), and two working electrodes (WEs). All measurements were carried out at room temperature ($22\pm1^{\circ}$ C). For NE and 5-HT detection, an electrochemically reduced

graphene oxide-modified BDDPE (ERGO-BDDPE) was used as the WE for NE and 5-HT detection in an attempt to enable simultaneous detection of both compounds as described in our previous work.⁴⁵ The ERGO-modified electrode preparation followed a previously published protocol.⁴⁵ Briefly, to prepare a 1.0 mg/mL GO solution, 1.0 mg of GO sheet was dissolved in 1.0 mL of 0.1 M phosphate buffer solution (PBS) pH 7.0 with sonication for 1 h to generate a homogeneous solution. Then 50 μ L of GO solution was drop casted onto the electrode surface. Cyclic voltammetry was employed to reduce GO by sweeping the potential from 0.0 to -1.5 V at a scan rate of 50 mV s⁻¹ for 10 cycles. Finally, the modified electrode was rinsed with deionized water and stored at the room temperature to dry. Standard solutions of NE and 5-HT were prepared in 0.1 M phosphate buffer (PB) pH 7.0, and a 60 µL aliquot was used for the experiments. Differential pulse voltammetry (DPV) was employed for NE and 5-HT detection with an amplitude of 60 mV, potential increment of 4 Hz, and a pulse width of 0.05 s. Electrochemical detection of p-aminophenol (pAP, EMD Milipore, Billerica, MA) was carried out using differential pulse voltammetry (DPV). For DPV, a pulse amplitude of 50 mV, potential increment of 4 Hz, and a pulse width of 0.1 s were used.

2.4 Results and Discussion

Electrochemical Characterization of the Janus-ePAD

To validate the Janus-ePAD, Fe(CN)₆⁴⁻ cyclic voltammetry was investigated with the two detection zones / working electrodes. As shown in Figure 2.2, the BDDPEs exhibit well-defined and symmetrical anodic and cathodic peaks, with similar peak currents and peak potentials between the two working electrodes $(4.23 \pm 0.03 (i_{pa1}) \text{ vs } 4.55 \pm 0.18 (i_{pa2}) \mu\text{A}, -4.22 \pm 0.11 (i_{pc1}) \text{ vs } -4.61 \pm 0.09 (i_{pc2}) \mu\text{A}, 0.21 \pm 0.01 (E_{pa1}) \text{ vs } 0.23 \pm 0.01 (E_{pa2}) \text{ V}, \text{ and } -0.10 \pm 0.02 (E_{pc1}) \text{ vs } -0.12 \pm 0.02 (E_{pc2}) \text{ V vs Ag/AgCl}$. The average peak potential separation (ΔE_p) was found to be

 311 ± 12 , and 351 ± 13 mV for BDDPE1 and BDDPE2, respectively (N = 3). Due to its inner sphere electrocatalytic nature, Fe(CN)₆⁴⁻ exhibits electrochemical irreversibility at the BDDPEs. The electron transfer kinetics of this species are impeded at oxygen terminated BDD and large ΔE_p values have been frequently observed at oxygen terminated BDD.^{46,47}



Figure 2.2 Cyclic voltammograms of 4 mM Fe(CN)₆^{4–} in 0.1 M KCl at BDDPE1 (black line) and BDDPE2 (blue line) on Janus-ePAD. Scan rate: 50 mV s⁻¹, WE: BDDPE.

In Situ pH Adjustment in a Janus-ePAD

To carry out on-line adjustment of phosphate buffer pH from pH 7.0 to pH 6.0 or 8.0, 0.5 $M H_3PO_4$ and 0.5 M NaOH were dried on the two reagent zones and pH-indicator strips were used to measure the solution pH in the detection zone. The parameters influencing the adjustment of pH values were optimized including volume of sample/standard solution and volume of H₃PO₄ and NaOH solution.

The sample volume needed to wet the channels, reagent zones, and detection zones, was initially investigated. Three 1.0 μ L aliquots each of 0.50 M H₃PO₄ and 0.50 M NaOH solutions were added into 1st reagent zone and 2nd reagent zone, respectively. Next, 45 to 65 μ L of 0.10 M PB pH 7.0 solution was added into the sample zone. The pH-indicator strips were placed on the

bottom of each detection zones to observe the pH change and the solution homogeneity after pH adjustment. The results are shown in Figure 2.3 a. Homogeneous color change on pH-indicator strips, indicating full wetting, was observed at 65 μ L, and consequently, 65 μ L was chosen as the optimum condition. The amount of H₃PO₄ and NaOH is another important factor in the adjustment of pH value. Therefore, 0.50 μ L to 1.8 μ L of 0.50 M H₃PO₄ and 0.50 M NaOH was added to the 1st reagent zone and the 2nd reagent zone, respectively, and 65 μ L of 0.10 M PB pH 7.0 was applied to the sample reservoir of the device to determine the optimal volume for adjusting pH on each side of detection zones to pH 6.0 and pH 8.0. The pH-indicator strip was used to observe the change in solution pH reaching the detection zones. As shown in Figure 2.3b, the optimal volume of 0.50 M H₃PO₄ and 0.50 M NaOH that can adjust the solution pH from pH 7.0 to pH 6.0 or pH 8.0 was 1.4 μ L.



Figure 2.3 Effect of parameters for in situ pH adjustment of 0.10 M PB from pH 7.0 to pH 6.0 and pH 8.0. (a) Effect of sample/reagent volume and (b) volume of H_3PO_4 and NaOH solutions on the color change of pH-indicator strip.

Neurotransmitter Detection

NE and 5-HT are important catecholamine neurotransmitters in biological samples. The simultaneous detection of these compounds is of great importance since low levels of NE and 5-HT have been correlated to several disorders, including depression, migraines, and anxiety.⁴³ NE and 5-HT have similar oxidation potentials and cannot be discriminated using bare BDDPEs.⁴⁵ In contrast, electrochemically reduced graphene oxide modified BDDPEs (ERGO-BDDPE) can differentiate peak potentials, enabling simultaneous analyte detection.⁴⁵ Figure 2.4a shows the DPVs of NE and 5-HT at ERGO-BDDPE at different pH conditions. The redox behavior of NE and 5-HT are pH dependent, with shifting overpotentials and peak currents as a function of pH. Clearly NE and 5-HT show preference for differing pH conditions, with maximum oxidation currents for NE and 5-HT at pH 6.0 and pH 8.0 respectively as shown in Figure 2.4b.



Figure 2.4 DPVs of 25 μ M NE and 10 μ M 5-HT in 0.1 M PB in the pH range of 5.0 – 9.0 at ERGO-BDDPE, note DPVs are offset for clarity. (b) The relationship of oxidation current of NE and 5-HT against pH (N = 3). DPV measurements were performed at an amplitude of 60 mV, potential increment of 4 Hz, and a pulse width of 0.05 s.

Electrochemical Behavior of NE and 5-HT on Janus-ePAD

Differential pulse voltammetry (DPV) was used to evaluate the performance of the JanusePAD for simultaneous detection of NE and 5-HT under their respective optimal pH conditions. Initially, a solution consisting of 25 µM NE and 10 µM 5-HT was prepared in 0.10 M PB pH 7.0. The solution pH can be simultaneously adjusted to pH 6.0, and pH 8.0 by impregnating the reagent zones with H₃PO₄ and NaOH respectively. DPVs of NE and 5-HT at the ERGO-BDDPE1 and ERGO-BDDPE2 are shown in Figure 2.5. At the ERGO-BDDPE1 (pH 6.0), NE and 5-HT exhibit oxidative peaks at 0.02 ± 0.01 and 0.15 ± 0.04 V vs. Ag/AgCl respectively, with peak oxidation currents of NE and 5-HT was 0.40 ± 0.02 and 0.12 ± 0.04 µA respectively. For the ERGO-BDDE2 (pH 8.0), the oxidation peak of NE and 5-HT occurs at potential of -0.053 ± 0.042 and 0.10 ± 0.02 V vs. Ag/AgCl, respectively, and the oxidation current of NE and 5-HT was 0.31 ± 0.05 and 0.25 $\pm 0.04 \,\mu$ A, respectively. From these results, it can be observed that at ERGO-BDDPE2 (pH 8.0), the oxidation peak potential of NE and 5-HT occurs at more negative values compared to ERGO-BDDPE1 (pH 6.0). The highest peak current of NE was observed at ERGO-BDDPE1 (pH 6.0) while the highest peak current of 5-HT was observed at ERGO-BDDPE2 (pH 8.0). These results were in accordance with the results shown Figure 2.4, indicating that the Janus-ePAD can be used to simultaneously detect NE and 5-HT at each species' optimized pH conditions.



Figure 2.5 DPVs of 25.0 μ M NE and 10.0 μ M 5-HT in 0.1 M PB on Janus-ePAD at WE1 and WE2 where pH of 0.1 M PBS was simultaneously in situ adjusted from pH 7.0 to pH 6.0 (WE1) and 8.0 (WE2), respectively. DPV measurements were performed at an amplitude of 60 mV, potential increment of 4 Hz, and a pulse width of 0.05 s.

Janus-ePAD Detection Zone Design

The design of the Janus-ePAD also influences the performance. Three different designs were fabricated as shown in Figure 2.6a. For the 1st design, the CE and RE were fabricated on the wax-patterned paper and the WE sections were attached on the wax-patterned device side opposite to the screen-printed CE and RE. For the 2nd design, the WEs were attached to the device on the screen-printed CE and RE side. For the 3rd design, the CE and RE section were fabricated on a separate piece of wax-printed paper which was attached on the top of wax-patterned paper and the WE sections were attached on the bottom of the device. Figure 2.6b and 2.6c show the comparison of peak currents of NE and 5-HT at pH 6.0 and 8.0 obtained from different designs of Janus-ePAD. The oxidation peak current of NE and 5-HT for the 2nd design is the highest. This is due to the smallest distance between electrodes and paper and all electrodes being completely covered by sample solution in the 2nd platform. In case of the 1st and 3rd platforms, the low sensitivity may be a result of poor sample solution coverage of the electrodes. The surfaces of the BDD electrodes are hydrophobic as a result of the mineral oil binder, while the commercial inks used render the

CE and RE hydrophobic as well. The hydrophobicity of these surfaces likely leads to poor wetting of the electrode surface at the end of the paper channels. The porous cellulose matrix of the paper channel may also not be 100% saturated as fluid saturation decreases as the fluid front distance increases from the source.⁴⁸



Figure 2.6 Schematic representation for the fabrication of janus-ePADs with different designs (a). The effect of janus-ePAD design on oxidation current of 25 μ M NE (b) and 10 μ M 5-HT (c).

Analytical Performance

Using the optimized conditions, the analytical performance of the device for NE and 5-HT detection was evaluated. As shown in Figure 2.7, the peak currents of NE or 5-HT increased linearly with increasing concentration. For NE detection, at the ERGO-BDDPE1 (pH 6.0), a linear calibration plot was found over a range of $5.0 - 75 \ \mu\text{M}$ with a sensitivity of 0.019 $\ \mu\text{A} \ \mu\text{M}^{-1}$ and correlation coefficient (R²) of 0.9849. At the ERGO-BDDPE2 (pH 8.0), a linear calibration plot was found over a range of $10 - 75 \ \mu\text{M}$ with a sensitivity of 0.012 $\ \mu\text{A} \ \mu\text{M}^{-1}$ and correlation coefficient (R²) of 0.9890.



Figure 2.7 Calibration curves for increasing concentration of (a) NE in the range of $5.0 - 75 \,\mu\text{M}$ (pH 6.0) and $10 - 75 \,\mu\text{M}$ (pH 8.0) and (b) 5-HT in the range of $1.0 - 10 \,\mu\text{M}$ (pH 6.0) and $0.5 - 10.0 \,\mu\text{M}$ (pH 8.0) (*n*=3).

For 5-HT, at the ERGO-BDDPE1 (pH 6.0), a linear calibration plot was obtained over a range of 1.0 - 10 μ M with a sensitivity of 0.017 μ A μ M⁻¹ and R² of 0.9928. For the ERGO-BDDPE2, a linear calibration plot was obtained over a range of 0.5–10 μ M with a sensitivity of 0.027 μ A μ M⁻¹ and R² of 0.9909. The LODs (3SD_{blank}/slope) were 0.71 and 0.54 μ M for NE and 5-HT, respectively, for the ERGO-BDDPE1 (pH 6.0). The LODs of NE and 5-HT were 1.2, and 0.38 μ M, respectively, for the ERGO-BDDPE2 (pH 8.0). The analytical performance of these two electrodes is summarized in Table 2.1.

Electrode	NE			5-HT		
	Linear range	Sensitivity	LOD	Linear range	Sensitivity	LOD
	(µM)	$(\mu A \ \mu M^{-1})$	(µM)	(µM)	$(\mu A \ \mu M^{-1})$	(µM)
ERGO-BDDPE1	5.0 - 75	0.019	0.71	1.0 - 10	0.017	0.54
(pH 6)						
ERGO-BDDPE2	10 - 75	0.012	1.2	0.5 - 10	0.027	0.38
(pH 8)						

Table 2.1 Summary of analytical performance for NE and 5-HT detection on Janus-ePAD.

pAP detection

Enzymatic assays are important detection motifs as a result of an enzymes inherent selectivity toward target analytes.⁴⁹ Important applications of enzymatic assays include clinical diagnostics, in enzyme linked immunosorbent assays (ELISAs), and in bacteria detection, where enzymes produced by bacteria can be monitored in order to detect and identify bacteria.^{24, 49, 50} For example, β -Galactosidase (β -Gal) activity is often monitored to detect E. coli contamination via the production of the electrochemically active molecule p-aminophenol (pAP) from paminophenylgalactopyranoside (pAPG) substrate hydrolysis.²⁴ Multiplexed enzymatic assays have been demonstrated on ePADs, for example, Dungchai et al. simultaneously determined uric acid, lactate, and glucose, however, each system was analyzed at the same pH.⁵¹ It would be ideal to perform the assays at each enzymes' optimal solution pH thus improving both sensitivity and detection limits. This proof of concept is demonstrated as we believe the Janus-ePAD would ultimately be used to perform multiplexed enzymatic assays at each enzymes' optimal pH conditions, and as such, pAP serves as a model analyte for these applications. Here, we demonstrate the simultaneous detection of pAP at two pH conditions in the Janus-ePAD as both the enzymatic activity and the electrochemical detection of pAP are pH dependent processes. The two electron - two proton oxidation of pAP to p-quinoneimine (PQ) is given in Figure 2.8.



Figure 2.8 Reversible 2 electron 2 proton oxidation of p-aminophenol.

A 59.1 mV shift in peak potential per pH unit is predicted since the oxidation of pAP involves a 1:1 ratio of protons to electrons. This relationship is governed by the Nernst equation:⁵²

$$E = E^{\circ} - \frac{0.0591}{n} \log[H^+]^2$$
 Equation 2.1

Here, the measured potential (*E*) is directly proportional to the standard potential of the oxidation of pAP (E°), the number of electrons transferred (*n*), the activity of H^+ , and the proportionality constant equal to 59.1 mV at 298 K.

The optimized conditions for on line pH generation as discussed above were used to detect pAP at BDDPE1 (pH 8.0) and BDDPE2 (pH 6.0). As shown in Figure 2.9, upon in situ pH generation, the peak potential and height for pAP oxidation vary with pH (Figure 2.9b) as compared to the response obtained at BDDPE1 and BDDPE2 when both cells operate at pH 7.0 (Figure 2.9a). As the pH increases, the overpotential required to oxidize pAP decreases. As demonstrated in the calibration curves for pAP obtained under dual pH conditions in the Janus-ePAD (Figure 2.8c), the sensitivity increases by about a factor of 2, increasing the pH from pH 6.0 (0.0022 μ A μ M⁻¹) to pH 8.0 (0.0040 μ A μ M⁻¹). Of note here is the larger standard deviations obtained at unmodified BDDPEs, which range from 15% to 47% of the average peak current (N = 3 devices). Upon further studies, it was found that the presence of the mineral oil pasting liquid at the surface of the BDDPE contributes to two phenomena that impact the reproducibility of the electrode response in the ePAD: i) time-dependent extraction and subsequent pre-concentration of

organic analytes and ii) slow electron transfer kinetics due the inhibitive layers of pasting liquid at the electrode surface. Both of these phenomena are commonly observed with carbon paste electrodes, however, when the electrodes are utilized in bulk solutions, dissolution of these inhibitory pasting liquid molecules occurs immediately upon immersion in solution, and the electrode surface is sufficiently active, while still being prone to analyte extraction.^{53, 54} In the ePAD proposed here, this dissolution and subsequent activation is a slow, time-dependent process as a result of small volumes confined to the cellulose matrix at the electrode surface. Another factor that contributed to the poor reproducibility was surface roughness of the BDDPEs resulting in poor contact between the BDDPE and ePAD. For this reason, further studies were carried out to activate BDDPEs prior to device fabrication and reduce BDDPE surface so as to eliminate this time-dependent and variable activation process. The results of this work are discussed in Chapter 3 of this thesis. This, we believe, is the reason the pre-modified ERGO-BDDPEs provide more reproducible results, as the electrode surface is modified and thus activated prior to attachment and use in the Janus-ePAD. Despite the poor reproducibility obtained as a result of the unmodified BDDPEs, this work, to the best of our knowledge, is the first demonstration of applying multiple solution chemistries to a single sample simultaneously in a paper-based device.



Figure 2.9 (a) DPVs obtained simultaneously in the Janus-ePAD for the oxidation of 60.5 μ M pAP (pH 7.0), (b) DPVs obtained for pAP under pH conditions generated in situ, (c) pAP calibration curves at pH 8.0 and pH 6.0.

2.5 Conclusions

In this chapter, a Janus electrochemical paper-based analytical device (Janus-ePAD) was developed. This device exploits the ability to pattern paper with multiple fluidic channels and store dried reagents in specific zones to perform solution pH adjustment and electrochemical detection in multiple sets of solution conditions on single sample simultaneously. This Janus-ePAD has a wide range of implications in point-of-need paper-based diagnostics where sensitive and selective multiplexed detection is necessary, as is often the case in biomedical diagnostics, environmental monitoring, and food quality analysis.⁵⁵⁻⁵⁷ While multiplexed colorimetric and electrochemical PADs have been demonstrated extensively in the literature for a wide range of analytes, the reported ePADs have not yet addressed the sensitivity of redox reactions to solution chemistry, and have largely relied upon highly analyte specific chemically modified electrode (CME) systems.^{8, 58} To offer a solution to this problem, the solution pH was adjusted on line to carry out electrochemical detection of serotonin and norepinephrine at each species' optimal solution pH. p-Aminophenol was also detected in two pH conditions simultaneously as a proof-of-concept towards the goal of developing multiplexed enzymatic assays in the Janus-ePADs. In future applications, it is important to note that not only can solution conditions be tuned in situ, but each detection zone may also contain either a different working electrode material or working electrodes chemically modified for optimal detection of one analyte in a mixture as well. In future work, the Janus-ePAD can be adopted for sensitive and selective multiplexed detection where analytes require specific experimental conditions such as buffer type, ionic strength, and/or solvent. This ePAD can reduce the complexity and/or time of analysis which an end user must carry out while maintaining the highest degree of selectivity and specificity for each species in a mixture.

REFERENCES

1.Nantaphol, S.; Kava, A. A.; Channon, R. B.; Kondo, T.; Siangproh, W.; Chailapakul, O.; Henry, C. S., Janus electrochemistry: Simultaneous electrochemical detection at multiple working conditions in a paper-based analytical device. *Analytica Chimica Acta* **2019**, *1056*, 88-95.

2.Pregibon, D. C.; Toner, M.; Doyle, P. S., Multifunctional Encoded Particles for High-Throughput Biomolecule Analysis. *Science* **2007**, *315* (5817), 1393.

3.Kingsmore, S. F., Multiplexed protein measurement: technologies and applications of protein and antibody arrays. *Nature Reviews Drug Discovery* **2006**, *5*, 310.

4.Mentele, M. M.; Cunningham, J.; Koehler, K.; Volckens, J.; Henry, C. S., Microfluidic Paper-Based Analytical Device for Particulate Metals. *Anal. Chem.* **2012**, *84* (10), 4474-4480.

5.Kulagina, N. V.; Lassman, M. E.; Ligler, F. S.; Taitt, C. R., Antimicrobial Peptides for Detection of Bacteria in Biosensor Assays. *Anal. Chem.* **2005**, *77* (19), 6504-6508.

6.Adkins, J. A.; Boehle, K.; Friend, C.; Chamberlain, B.; Bisha, B.; Henry, C. S., Colorimetric and Electrochemical Bacteria Detection Using Printed Paper- and Transparency-Based Analytic Devices. *Anal. Chem.* **2017**, *89* (6), 3613-3621.

7.Jokerst, J. C.; Adkins, J. A.; Bisha, B.; Mentele, M. M.; Goodridge, L. D.; Henry, C. S., Development of a Paper-Based Analytical Device for Colorimetric Detection of Select Foodborne Pathogens. *Analytical Chemistry* **2012**, *84* (6), 2900-2907.

8. Tanner, E. E.; Compton, R. G., How can electrode surface modification benefit electroanalysis? *Electroanalysis* **2018**.

9.Rodthongkum, N.; Ruecha, N.; Rangkupan, R.; Vachet, R. W.; Chailapakul, O., Grapheneloaded nanofiber-modified electrodes for the ultrasensitive determination of dopamine. *Anal. Chim. Acta* **2013**, *804*, 84-91.

10. Washburn, A. L.; Luchansky, M. S.; Bowman, A. L.; Bailey, R. C., Quantitative, Label-Free Detection of Five Protein Biomarkers Using Multiplexed Arrays of Silicon Photonic Microring Resonators. *Anal. Chem.* **2010**, *82* (1), 69-72.

11.Chen, Z.-P.; Zhong, L.-J.; Nordon, A.; Littlejohn, D.; Holden, M.; Fazenda, M.; Harvey, L.; McNeil, B.; Faulkner, J.; Morris, J., Calibration of Multiplexed Fiber-Optic Spectroscopy. *Anal. Chem.* **2011**, *83* (7), 2655-2659.

12.Rebe Raz, S.; Haasnoot, W., Multiplex bioanalytical methods for food and environmental monitoring. *TrAC, Trends Anal. Chem.* **2011**, *30* (9), 1526-1537.

13.Kloth, K.; Niessner, R.; Seidel, M., Development of an open stand-alone platform for regenerable automated microarrays. *Biosens. Bioelectron.* **2009**, *24* (7), 2106-2112.

14. Martinez, A. W.; Phillips, S. T.; Butte, M. J.; Whitesides, G. M., Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angewandte Chemie International Edition* **2007**, *46* (8), 1318-1320.

15.Martinez, A. W.; Phillips, S. T.; Whitesides, G. M.; Carrilho, E., Diagnostics for the developing world: microfluidic paper-based analytical devices. ACS Publications: 2009. 16.Gong, M. M.; Sinton, D., Turning the page: advancing paper-based microfluidics for broad diagnostic application. *Chemical reviews* **2017**, *117* (12), 8447-8480.

17.Rahbar, M.; Nesterenko, P. N.; Paull, B.; Macka, M., Geometrical Alignment of Multiple Fabrication Steps for Rapid Prototyping of Microfluidic Paper-Based Analytical Devices. *Anal. Chem.* **2017**, *89* (22), 11918-11923.

18.Fu, E.; Downs, C., Progress in the development and integration of fluid flow control tools in paper microfluidics. *Lab on a Chip* **2017**, *17* (4), 614-628.

19.Cate, D. M.; Adkins, J. A.; Mettakoonpitak, J.; Henry, C. S., Recent Developments in Paper-Based Microfluidic Devices. *Analytical Chemistry* **2015**, *87* (1), 19-41.

20. Yang, Y.; Noviana, E.; Nguyen, M. P.; Geiss, B. J.; Dandy, D. S.; Henry, C. S., Paper-Based Microfluidic Devices: Emerging Themes and Applications. *Analytical Chemistry* **2017**, *89* (1), 71-91.

21.Sriram, G.; Bhat, M. P.; Patil, P.; Uthappa, U. T.; Jung, H.-Y.; Altalhi, T.; Kumeria, T.; Aminabhavi, T. M.; Pai, R. K.; Madhuprasad; Kurkuri, M. D., Paper-based microfluidic analytical devices for colorimetric detection of toxic ions: A review. *TrAC, Trends Anal. Chem.* **2017**, *93*, 212-227.

22.Creran, B.; Li, X.; Duncan, B.; Kim, C. S.; Moyano, D. F.; Rotello, V. M., Detection of Bacteria Using Inkjet-Printed Enzymatic Test Strips. *ACS Appl. Mater. Interfaces* **2014**, *6* (22), 19525-19530.

23.Wei, X.; Tian, T.; Jia, S.; Zhu, Z.; Ma, Y.; Sun, J.; Lin, Z.; Yang, C. J., Microfluidic Distance Readout Sweet Hydrogel Integrated Paper-Based Analytical Device (µDiSH-PAD) for Visual Quantitative Point-of-Care Testing. *Anal. Chem.* **2016**, *88* (4), 2345-2352.

24.Adkins, J. A.; Noviana, E.; Henry, C. S., Development of a Quasi-Steady Flow Electrochemical Paper-Based Analytical Device. *Analytical chemistry* **2016**, *88* (21), 10639-10647.

25.Hossain, S. M. Z.; Brennan, J. D., β-Galactosidase-Based Colorimetric Paper Sensor for Determination of Heavy Metals. *Anal. Chem.* **2011**, *83* (22), 8772-8778.

26.Rattanarat, P.; Dungchai, W.; Cate, D.; Volckens, J.; Chailapakul, O.; Henry, C. S., Multilayer paper-based device for colorimetric and electrochemical quantification of metals. *Analytical chemistry* **2014**, *86* (7), 3555-3562.

27.Nguyen, M. P.; Meredith, N. A.; Kelly, S. P.; Henry, C. S., Design considerations for reducing sample loss in microfluidic paper-based analytical devices. *Analytica chimica acta* **2018**, *1017*, 20-25.

28.Nie, Z.; Deiss, F.; Liu, X.; Akbulut, O.; Whitesides, G. M., Integration of paper-based microfluidic devices with commercial electrochemical readers. *Lab on a Chip* **2010**, *10* (22), 3163-3169.

29.Fernandes, S. C.; Walz, J. A.; Wilson, D. J.; Brooks, J. C.; Mace, C. R., Beyond wicking: Expanding the role of patterned paper as the foundation for an analytical platform. ACS Publications: 2017.

30.Gao, W.; Nyein, H. Y. Y.; Shahpar, Z.; Fahad, H. M.; Chen, K.; Emaminejad, S.; Gao, Y.; Tai, L.-C.; Ota, H.; Wu, E.; Bullock, J.; Zeng, Y.; Lien, D.-H.; Javey, A., Wearable Microsensor Array for Multiplexed Heavy Metal Monitoring of Body Fluids. *ACS Sens.* **2016**, *1* (7), 866-874.

31.Radha Shanmugam, N.; Muthukumar, S.; Chaudhry, S.; Anguiano, J.; Prasad, S., Ultrasensitive nanostructure sensor arrays on flexible substrates for multiplexed and simultaneous electrochemical detection of a panel of cardiac biomarkers. *Biosens. Bioelectron.* **2017**, *89*, 764-772.

32.Jampasa, S.; Siangproh, W.; Laocharoensuk, R.; Yanatatsaneejit, P.; Vilaivan, T.; Chailapakul, O., A new DNA sensor design for the simultaneous detection of HPV type 16 and 18 DNA. *Sens. Actuators, B* **2018**, *265*, 514-521.

33.Li, L.; Li, W.; Yang, H.; Ma, C.; Yu, J.; Yan, M.; Song, X., Sensitive origami dual-analyte electrochemical immunodevice based on polyaniline/Au-paper electrode and multi-labeled 3D graphene sheets. *Electrochimica Acta* **2014**, *120*, 102-109.

34.Zang, D.; Ge, L.; Yan, M.; Song, X.; Yu, J., Electrochemical immunoassay on a 3D microfluidic paper-based device. *Chemical Communications* **2012**, *48* (39), 4683-4685.

35.Mettakoonpitak, J.; Mehaffy, J.; Volckens, J.; Henry, C. S., AgNP/Bi/Nafion-modified Disposable Electrodes for Sensitive Zn (II), Cd (II), and Pb (II) Detection in Aerosol Samples. *Electroanalysis* **2017**, *29* (3), 880-889.

36.Ruecha, N.; Rodthongkum, N.; Cate, D. M.; Volckens, J.; Chailapakul, O.; Henry, C. S., Sensitive electrochemical sensor using a graphene–polyaniline nanocomposite for simultaneous detection of Zn (II), Cd (II), and Pb (II). *Analytica chimica acta* **2015**, *874*, 40-48.

37.Read, T. L.; Bitziou, E.; Joseph, M. B.; Macpherson, J. V., In situ control of local pH using a boron doped diamond ring disk electrode: optimizing heavy metal (mercury) detection. *Analytical chemistry* **2013**, *86* (1), 367-371.

38.Read, T. L.; Joseph, M. B.; Macpherson, J. V., Manipulation and measurement of pH sensitive metal–ligand binding using electrochemical proton generation and metal detection. *Chemical Communications* **2016**, *52* (9), 1863-1866.

39.Blaho, J. K.; Goldsby, K. A., Redox regulation based on the pH-dependent hydrolysis of 2pyridinecarboxaldehyde coordinated to ruthenium (II). *Journal of the American Chemical Society* **1990**, *112* (16), 6132-6133.

40.Chen, X.; Zhang, G.; Shi, L.; Pan, S.; Liu, W.; Pan, H., Au/ZnO hybrid nanocatalysts impregnated in N-doped graphene for simultaneous determination of ascorbic acid, acetaminophen and dopamine. *Materials Science and Engineering: C* **2016**, *65*, 80-89. 41.Rassaei, L.; Marken, F., Pulse-voltammetric glucose detection at gold junction electrodes.

Analytical chemistry 2010, 82 (17), 7063-7067.

42.Wang, Y.; Wang, S.; Tao, L.; Min, Q.; Xiang, J.; Wang, Q.; Xie, J.; Yue, Y.; Wu, S.; Li, X.; Ding, H., A disposable electrochemical sensor for simultaneous determination of norepinephrine and serotonin in rat cerebrospinal fluid based on MWNTs-ZnO/chitosan composites modified screen-printed electrode. *Biosens. Bioelectron.* **2015**, *65*, 31-38.

43.Graeff, F. G.; Guimaraes, F. S.; De Andrade, T. G.; Deakin, J. F., Role of 5-HT in stress, anxiety, and depression. *Pharmacology, biochemistry, and behavior* **1996**, *54* (1), 129-41.

44.Kondo, T.; Sakamoto, H.; Kato, T.; Horitani, M.; Shitanda, I.; Itagaki, M.; Yuasa, M., Screen-printed diamond electrode: A disposable sensitive electrochemical electrode. *Electrochemistry Communications* **2011**, *13* (12), 1546-1549.

45.Nantaphol, S.; Channon, R. B.; Kondo, T.; Siangproh, W.; Chailapakul, O.; Henry, C. S., Boron Doped Diamond Paste Electrodes for Microfluidic Paper-Based Analytical Devices. *Analytical Chemistry* **2017**, *89* (7), 4100-4107.

46.Hutton, L. A.; Iacobini, J. G.; Bitziou, E.; Channon, R. B.; Newton, M. E.; Macpherson, J. V., Examination of the factors affecting the electrochemical performance of oxygen-terminated polycrystalline boron-doped diamond electrodes. *Analytical chemistry* **2013**, *85* (15), 7230-7240. 47.Granger, M. C.; Swain, G. M., The influence of surface interactions on the reversibility of Ferri/Ferrocyanide at boron-doped diamond thin-film electrodes. *Journal of the Electrochemical Society* **1999**, *146* (12), 4551-4558.

48.Houghtaling, J.; Liang, T.; Thiessen, G.; Fu, E., Dissolvable bridges for manipulating fluid volumes in paper networks. *Analytical chemistry* **2013**, *85* (23), 11201-11204.

49.Adkins, J. A.; Boehle, K.; Friend, C.; Chamberlain, B.; Bisha, B.; Henry, C. S., Colorimetric and electrochemical bacteria detection using printed paper-and transparency-based analytic devices. *Analytical chemistry* **2017**, *89* (6), 3613-3621.

50.Hemalatha, T.; UmaMaheswari, T.; Krithiga, G.; Sankaranarayanan, P.; Puvanakrishnan, R., Enzymes in clinical medicine: an overview. *Indian J. Exp. Biol.* **2013**, *51*, 777-788.

51.Dungchai, W.; Chailapakul, O.; Henry, C. S., Electrochemical Detection for Paper-Based Microfluidics. *Analytical Chemistry* **2009**, *81* (14), 5821-5826.

52.Bard, A. J.; Faulkner, L. R.; Leddy, J.; Zoski, C. G., *Electrochemical methods: fundamentals and applications*. wiley New York: 1980; Vol. 2.

53.Rice, M. E.; Galus, Z.; Adams, R. N., Graphite paste electrodes: Effects of paste composition and surface states on electron-transfer rates. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* **1983**, *143* (1-2), 89-102.

54.Ravichandran, K.; Baldwin, R. P., Enhanced voltammetric response by electrochemical pretreatment of carbon paste electrodes. *Analytical Chemistry* **1984**, *56* (9), 1744-1747.

55.Wu, M. Y.-C.; Hsu, M.-Y.; Chen, S.-J.; Hwang, D.-K.; Yen, T.-H.; Cheng, C.-M., Point-of-care detection devices for food safety monitoring: Proactive disease prevention. *Trends in biotechnology* **2017**, *35* (4), 288-300.

56.Hu, J.; Wang, S.; Wang, L.; Li, F.; Pingguan-Murphy, B.; Lu, T. J.; Xu, F., Advances in paper-based point-of-care diagnostics. *Biosens. Bioelectron.* **2014**, *54*, 585-597.

57.Meredith, N. A.; Quinn, C.; Cate, D. M.; Reilly, T. H.; Volckens, J.; Henry, C. S., based analytical devices for environmental analysis. *Analyst* **2016**, *141* (6), 1874-1887.

58.Adkins, J.; Boehle, K.; Henry, C., Electrochemical paper-based microfluidic devices. *Electrophoresis* **2015**, *36* (16), 1811-1824.

CHAPTER 3: BORON DOPED DIAMOND PASTE ELECTRODES AND THEIR INTEGRATION WITH ELECTROCHEMICAL PAPER-BASED ANALYTICAL DEVICES

3.1 Chapter Overview

This chapter presents follow up work to the research presented in Chapter 2 to further understand the fundamental electrochemistry and use of boron doped diamond paste electrodes (BDDPEs) in electrochemical paper-based analytical devices. All of the content was taken from my orals document and briefly summarized in the publication in *Analytica Chimica Acta* titled "Janus Electrochemistry – Simultaneous Electrochemical Detection at Multiple Working Conditions in a Paper-based Analytical Device." This work highlights the effects of BDDPE composition, a composite of BDD powder and mineral oil, as well as surface roughness on the resulting electroanalytical properties. It was found that BDDPEs require the application of a large pressure to pack the BDD paste into the electrode body and reduce surface roughness to produce adequate contact between the BDDPE and a paper-based device. BDDPEs also require an activation procedure to remove pasting liquid from the BDDPE surface and improve electrochemical response and stability.

3.2 Introduction

There are various methods for electrode fabrication and incorporation with paper-based analytical devices. Metallic electrodes are incorporated by printing metallic ink, nanoparticle growth, ink-jet printing, or the incorporation of microwires - usually gold or platinum.¹ However, carbon materials exhibit an extended solvent window and lower capacitive currents compared to metallic electrode materials.¹⁻³ Carbon electrodes are typically incorporated by stencil printing carbon composite inks. The composite most commonly consists of graphite powder mixed with

several other components, including polymers and solvents, to produce a viscous thixotropic fluid which can then be printed through a screen or stencil onto the paper substrate and are referred to as screen or stencil-printed carbon electrodes (SPCEs).^{3,4} Carbon paste electrodes (CPEs) are an alternative carbon composite type ubiquitous throughout electroanalysis.^{5, 6} CPEs are a mixture of carbon particles, most commonly graphite powder, and a pasting liquid. The pasting liquid used in CPEs, usually paraffin (mineral) oil, remains in the liquid state throughout the lifetime of the electrode. For this reason, CPEs are commonly employed in traditional electrode formats and not interfaced with electrochemical paper-based analytical devices (ePADs). However, the presence of liquid versus dry binder influences the electrochemical response of carbon composite electrodes, with CPEs exhibiting enhanced conductivity and electron transfer kinetics relative to SPCEs.⁷⁻⁹ Since CPEs remain in the liquid state throughout the lifetime of the electrode, they cannot be printed directly onto paper like SPCEs and an electrode body which holds the paste must be fabricated ex situ and attached to a paper-based device, and there is currently only one example of incorporating paste electrodes in the literature.¹⁰ Ex situ electrode preparation is advantageous since electrode pretreatments can be performed outside of an ePAD, eliminating the risk of damaging or contaminating the paper substrate.^{1,3} CPEs also fit the criteria for use in ePADs since they are easy to fabricate, inexpensive, and can be modified by simply mixing modifiers into the paste.⁹ Since the binder is a low molecular weight hydrocarbon which remains a liquid, a variety of carbon particle types, such as boron doped diamond (BDD), that are difficult to homogenize with printing inks used in SPCEs can be employed.^{10,11}

BDD has become a popular alternative to graphitic carbon electrode materials. As a result of complete C-sp³ hybridization, diamond demonstrates several desirable electrochemical characteristics.^{12, 13} BDD has the widest solvent window of any electrode material in aqueous electrolyte, low capacitive currents, low background faradaic currents and reduced susceptibility to fouling.^{14, 15} However, these properties are dependent on the synthesis and processing of synthetic BDD.¹⁶⁻¹⁹ For example, thick film BDD (100s of μms) exhibits lower background currents and a wider solvent window due to minimal presence of defects in the form of non-diamond carbon. However, thick film BDD is difficult and expensive to process, requiring specialized equipment and techniques for sealing and polishing.¹² Thin film BDD exhibits a reduced solvent window, due to increased presence of non-diamond carbon impurities. It is slightly easier to grow but must remain attached to the growth substrate, reducing the obtainable electrode geometries.¹⁰ As was the case with the development of graphitic carbon composite electrodes (e.g. CPEs and SPCEs), a BDD composite electrode material that can be easily, cheaply, and quickly processed into a range of different electrode geometries and integrated into portable electrochemical devices is desirable.^{10, 20}

More recently, BDD powder has been used to fabricate disposable screen-printed composite electrodes with a polyester resin as the binder.²⁰ Initial studies on this material show that it outperforms SPCEs providing increased signal-to-noise ratios, an extended solvent window, and increased fouling resistance.²¹ In Chapter 2 of this thesis, boron doped diamond paste electrodes (BDDPEs) were fabricated with BDD powder and paraffin oil and attached to a paper-based device with an adhesive. The BDDPEs demonstrated desirable electrode properties associated with classical BDD electrodes, along with the aforementioned advantages associated with paste electrodes. Specifically, i) the BDDPE surface can be refreshed by the removal of some amount of the paste and subsequent addition of fresh paste, ii) BDDPEs are amenable to bulk modification, and iii) the BDDPE can be modified prior to attachment into a microfluidic device.^{10, 22} However, as mentioned in Chapter 2, the BDDPEs suffered from poor reproducibility

when the unmodified iteration was employed in the Janus-ePAD due to the fabrication of BDDPEs by hand and the presence of the pasting liquid. Therefore, this chapter focuses on further understanding the fundamental electrochemical characteristics of BDDPEs in order to optimize their fabrication and integration with paper-based analytical devices.

3.3 Experimental

Chemicals and Materials

All chemicals were analytical grade and used as received, and all solutions were prepared using purified water (18.2 M Ω cm) from a Milli-Q Millipore water purification system. Potassium ferrocyanide (Fe(CN)₆⁴⁻) was acquired from Mallinckrodt (Missouri, USA). Ferrocenylmethyl trimethylammonium hexafluorophosphate (FcTMA⁺) was synthesized in-house via a previously reported method.²³ Potassium chloride (KCl) was obtained from Fisher Scientific (New Jersey, USA). Graphite powder ($\leq 20 \mu$ m) was purchased from Sigma-Aldrich (Missouri, USA). Conductive carbon ink (E3178) was purchased from Ercon Inc. (Massachusetts, USA). All electrochemical experiments were performed with a model 660B bipotentiostat (CHI instruments, Austin, TX) at room temperature (22 ± 1 °C). For experiments carried out with individual BDDPEs, a platinum wire counter electrode and a Ag/AgCl or saturated calomel RE were used as specified.

Boron Doped Diamond Paste Electrode Fabrication

The fabrication process of BDDPEs is shown in Figure 3.1. Carbon ink (1.5 grams graphite powder to 3.5 grams ink) was stencil printed onto transparency film (3M, PP2200), as an electrical connection for the BDDPEs. Next, a sheet of transparency film was sandwiched between two sheets of double-sided adhesive (3M, St. Paul, MN). This three-layer system was then laser cut to produce a 14 x 14 mm square containing a geometry of three band electrode bodies (0.1 mm x 2.0

mm) to minimize BDD paste consumption.²³ One side of the double-sided adhesive was used to adhere the electrode body to the carbon ink electrical connection. To formulate the BDD paste, BDD powder (average particle diameter = 366 nm) obtained from Prof. T. Kondo (Univ. of Tokyo) was added to light paraffin oil (70:30 w/w, Fisher Scientific, NJ), homogenized, then packed into the three band electrode body by hand, or covered and stored for later use. Optical profilometry (Zygo Corporation, Berwyn, PA) was used to image the BDDPEs.



Figure 3.1 Schematic showing the fabrication process for boron doped diamond paste electrodes (BDDPEs).

ePAD Fabrication

The ePAD was designed using the graphic design program CorelDRAW (Corel, Ottawa, Ontario). The design was wax printed onto Whatman No. 1 filter paper (Fischer Scientific, Fairlawn, NJ) using a wax printer (Xerox Phaser 8860). The device consists of a sample inlet (d = 7.23 mm) leading to a channel (l = 5.5 mm) that splits into two channels (l = 5.8 mm), leading to a reagent zone (d = 4.80 mm) followed by a channel (l = 5.3 mm) leading to a detection zone (d = 7.60 mm). The microfluidic channels (w = 1.25 mm) were defined by the hydrophobic barrier created by melting the wax through the paper on a hot plate at 140 °C for 120 s. A CO₂ laser cutter (Epilog, Golden, CO) created a stencil from transparency film (3M, St. Paul, MN) of the reference electrode and counter electrode (RE and CE). The RE and CE were printed onto the hydrophilic detection zones with carbon ink.³⁴ Graphite powder (< 20 µm diameter, Sigma, St. Louis, MO) was added

to a commercial carbon ink, homogenized, stencil printed, and then dried at 60 °C for 60 min (Figure 3.2A). A second layer of Whatman No. 1 filter paper, laser cut to cover the entire electrochemical detection zone (d = 7.60 mm), was attached to the top adhesive layer of the BDDPE working electrode (WE, fabrication described in following section). The BDDPEs were then attached to each of the two detection zones. A layer of packing tape (3M, St. Paul, MN) was added to the back and front of the device to prevent leaking and evaporation (Figure 3.2B). Figure 3.2C shows a front view of the final ePAD.



Figure 3.2 Schematic showing (A) wax printed paper with SPC RE and CE, (B) all device layers, (C) front view of final ePAD.

3.4 Results and Discussion

To assess the reproducibility of the electrochemical response in the Janus-ePAD with BDDPEs, cyclic voltammetry of FcTMA⁺ was carried out as shown in Figure 3.3. Here, the variable response observed for FcTMA⁺ at each working electrode demonstrates the poor reproducibility consistently observed in the Janus-ePADs and discussed in Chapter 2. Since BDDPEs are fabricated by hand into a holder with small dimensions, one factor contributing to the poor reproducibility was believed to be poor contact between the BDDPE surface and paper device due to electrode roughness.



Figure 3.3 Cyclic voltammograms recorded in Janus-ePAD at BDDPE 1 and BDDPE 2 for 1.0 mM FcTMA⁺ in 0.1 M KCl at a scan rate of 100 mV s⁻¹.

To assess the surface roughness of BDDPEs, FcTMA⁺ voltammetry was studied since expected peak current for this species can be easily calculated, and is directly proportional to electroactive surface area. At the timescales employed, the diffusion layer thickness at the peak current in CV was estimated to be ~36 μ m.²⁴ This diffusion layer thickness is on a similar scale to the surface roughness of BDDPEs (25 μ m, Figure 10A, B), therefore the electrode surface appears rough, and the electrochemically active surface area is greater than the geometric surface area.^{12,63} CVs recorded for FcTMA⁺ in bulk solution with BDDPES are shown in Figure 3.4. Here, the average experimental peak current was 14.45 ± 1.04 μ A (N = 3), varying by 7.26%, indicating slight variability in electroactive surface areas. The peak currents are higher than the theoretical peak current of 12.70 μ A, indicating an increased electroactive surface area due to surface roughness.



Figure 3.4 Cyclic voltammograms recorded for FcTMA⁺ in bulk 0.1 M KCl solution at a scan rate of 100 mV s⁻¹.

To improve electrode-paper contact a simple experiment was performed in which previously fabricated devices were pressed at 700 pounds with a hydraulic press for 120 s at room temperature. Fe(CN)₆⁴⁻ was employed to characterize the electrochemical response of pressed devices as shown in Figure 3.5A. Here, the average peak current for the oxidation of Fe(CN)₆⁴⁻ was 2.78 \pm 0.17 µA with a coefficient of variation of 6.05%. The reproducibility was significantly improved compared to that obtained for pAP and FcTMA⁺ detection in the ePAD (Figure 2.9 and 3.3). However, the measured currents are significantly lower than the expected peak current of 5.72 µA estimated with Equation 3.1.²⁴

$$(\delta i)_{max} = \frac{nFAD^{\frac{1}{2}}C}{\pi^{\frac{1}{2}}(\tau-\tau')^{\frac{1}{2}}} x \left(\frac{1-\sigma}{1+\sigma}\right)$$
 Equation 3.1

Here, peak height $(\delta i)_{max}$, is proportional to Faraday's constant (*F*; 96,485 C mol⁻¹), the number of electrons transferred (*n*), the surface area of the electrode (*A*; cm²), diffusion coefficient of Fe(CN)₆⁴⁻ (7.26 x 10⁻⁶ cm² s⁻¹) and analyte concentration (*C*; mol cm⁻³) and inversely proportional

to the difference between the first sampled current and the second sampled current of the potential pulse, τ' and τ respectively. σ was calculated using equation 3.2.²⁴

$$\sigma = \exp\left(\frac{nF}{RT}\frac{\Delta E}{2}\right)$$
 Equation 3.2

Here, R is the universal gas constant, T is the temperature, and ΔE is the pulse amplitude.



Figure 3.5 Differential pulse voltammograms recorded of 1.0 mM $Fe(CN)_6^{4-}$ in 0.1 M KCl in pressed Janus ePAD (n = 2). (B) DPVs recorded in Janus ePAD for 1.0 mM $Fe(CN)_6^{4-}$ in 0.1 M KCl at times 25 min, 31 min and 75 min.

Figure 3.5B shows DPVs recorded for $Fe(CN)_6^4$ at various time points after solution wicked to the end of channel. The peak current magnitude and reproducibility of $Fe(CN)_6^4$ -demonstrated a time dependence. Here, sensitivity reached a maximum at 31 min, but was most reproducible between the two channels of the Janus ePAD at longer times with average peak currents of 3.14, 4.66, and 2.58 μ A and RSDs of 14.42, 4.04, and 1.24 % for 25, 31, and 75 min respectively. The increase in sensitivity from 25 to 31 min indicates an electrode activation process. The peak height obtained at 31 min, which is close to the theoretical value, indicates the BDDPE surface is in good contact with the paper. However, the peak current is still slightly lower than theory predicts at 31 min. This is due to the presence of the paper, which occupies a significant

area of the electrode surface.²⁵ At 75 min, evaporation had a more significant effect reducing interfacial electrode-solution contact.

Optical profilometry was used to image the BDDPEs and quantify the surface roughness. Figure 3.6 shows optical profilometry images of a BDDPE before pressing (Figure 3.6A and B), after pressing (Figure 3.6C and 3.6D) and after use in electrochemical experiments (Figure 3.6E and 3.6F). The root-mean-square (RMS) surface roughness of the BDDPE decreased by an order of magnitude from $28.37 \pm 5.86 \,\mu\text{m}$ to $3.35 \pm 0.56 \,\mu\text{m}$ when pressed. This smoothed surface likely lead to a significant increase in electrode-paper contact (Figure 3.6G). After use in electrochemical experiments, the RMS surface roughness increased slightly, by $0.40 \pm 0.20 \,\mu\text{m}$, to $3.75 \pm 0.52 \,\mu\text{m}$. This is likely a result of dissolution of pasting liquid after immersion of electrodes in aqueous solutions and electrochemical cycling.^{6, 26} This dissolution is believed to be the cause of the electrode activation observed in Figure 3.8B. Surface roughness and edge effects should not lead to increased signals at pressed BDDPEs, since the surface roughness is on a smaller scale than a typical diffusion layer thickness (e.g. tens of μ m), and experimental currents should agree with those calculated for the geometric surface area of the BDDPEs.



Figure 3.6 Optical profilometry images recorded for BDDPEs (A) before being pressed (C) after being pressed (700 pounds, 120 s) and (E) after use in electrochemical experiments. (B), (D), and (F) are the 3 dimensional maps of (A), (C), and (E) respectively. (G) is a schematic showing improved electrode contact with paper as a result of pressing.

To confirm that BDDPE activation was occurring within the device, repetitive DPVs were recorded for FcTMA⁺ every two min after the paper channels were visually saturated. FcTMA⁺ peak currents at each time point are shown in Figure 3.7. Here, a similar behavior to that of $Fe(CN)_6^{4-}$ was observed, where both the sensitivity and reproducibility for the detection of FcTMA⁺ were time dependent. The response became reproducible at a time of about 15 min, where RSDs remained at or below 10.0%. The initial increase in sensitivity over the first ten min of measurements is likely the result of electrode activation as well as increasing saturation of the paper with solution.^{27, 28} Compared to the Fe(CN)₆⁴⁻ data, the dissolution of the hydrophobic binder may occur faster here as a result of constant electrochemical cycling (e.g. max current reached in 10 vs. 31 min). The decrease in electrochemical signal over time (after about 20 min), is likely a result of electrode deactivation effects, as well as drying of the paper.



Figure 3.7 Average peak current recorded for the oxidation of 1.0 mM FcTMA⁺ in 0.1 M KCl with DPV as a function of time after solution saturated the device (N = 3).

Studies were carried out in bulk solution to determine the time dependent BDDPE response (Figure 3.8). As discussed previously, the initial increase in peak current, seen clearly in Figure 3.8A, is a result of the dissolution of inhibitive layers of the liquid binder, which likely occurs faster in the larger solution volumes used here. In Figure 3.8B, the electrode was soaked prior to cycling for five min, and significant changes in peak currents were not observed. Electrode (A) was soaked in solution for periods of ten min without cycling and significant increases in peak current that surpassed the expected peak current of 12.70 μ A were observed (Figure 3.8A).



Figure 3.8 (A) and (B) both show the peak currents of 1.0 mM FcTMA^+ in 0.1 M KCl as function of time recorded with CV. In (A) CVs were initially recorded without waiting between runs, then recorded every ten min once the expected response was achieved (1.27×10^{-5} A), after waiting 10 min between measurements, CVs were recorded every two min. In (B) CVs were recorded every two min for 36 min, after initially soaking the electrode for five min. The inset shows a zoomed version of (B).

A similar phenomenon has been observed when using CPEs where the hydrophobic paraffin oil used as a binder extracts organic analytes, such as ferrocene derivatives, into the bulk of the carbon paste matrix.²⁹ Therefore, the increase in peak currents measured at increased times is likely a result of the extraction of FcTMA⁺ into the BDDPE bulk. When the electrode is continuously cycled every two min (Figure 3.8B and after 35 min Figure 3.8A) the response remains stable and very slightly decreases over time, likely a result of a slow extraction process.

Electrode pretreatment was investigated in an attempt to overcome the undesired analyte extraction and initial dissolution of the binding liquid. Surface layers of the binding liquid have been removed by chemically or electrochemically oxidizing CPE surfaces.²⁹ Electrochemical oxidation is simpler, faster, and can be applied in situ.²⁶ Adams et al. showed that electrochemical oxidation of CPEs at high anodic potentials gives rise to various hydrophilic surface oxides, which intensely repel lipophilic binder molecules, decreasing surface coverage, leading to a surface that behaves more like "dry graphite" with enhanced electron transfer kinetics.^{27, 30} Similar to graphite, anodic polarization of BDD electrodes increases surface oxide coverage.^{12, 17, 18} In this study, it was hypothesized that anodic polarization of the BDDPE would likewise increase the density of hydrophilic surface oxides through the generation of reactive oxygen species at the electrode surface (e.g. O₂ and CO₂). While anodic polarization of free-standing BDD electrodes generally involves the application of extremely high positive potentials from +2 to +3 V for several to tens of min, paste electrodes are limited to polarization at potentials between 1.25 and 1.85 V, as higher potentials can destroy structural integrity of a paste electrode due to pasting liquid leakage.²⁹

To oxidize BDDPEs, 1.1, 1.4, 1.6 and 1.8 V pretreatments were applied to BDDPEs for 60 s. A decrease in the time dependent sensitivity for FcTMA⁺ detection was observed as shown in Figure 3.9. However, the electrode response did not stabilize, and a steady increase in sensitivity

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at longer times occurred. The average peak current obtained at the longest time point was $10.03 \pm 0.58 \ \mu\text{A}$ (5.74% RSD), significantly lower than the predicted peak current of 12.70 μA . This is likely due to the initial oxidation of the binder, which can leave product residues (e.g. short chain hydrocarbons) adsorbed to the electrode surface that are removed upon electrochemical cycling.²⁶

Sometimes, after anodic polarization, CPE and BDD electrodes require "equilibration" by a cathodic polarization.^{19, 29} While anodic polarization oxidizes the electrode surface and removes impurities, the cathodic polarization activates the surface toward electron transfer by further removing adsorbed organic impurities and/or decreasing the density of carbon-oxygen functional groups, presumably by introducing surface hydrogen.^{12, 29} Anodic polarization at 1.4 V for 60 s followed by a cathodic "equilibration" at -1.0 V for 15 s was applied to BDDPEs. The results for the peak currents obtained for the detection of FcTMA⁺ after this electrochemical pretreatment are shown in Figure 3.9. Here, peak currents obtained were 12.41 ± 0.47 , 12.22 ± 0.35 , and $12.22 \pm 0.45 \mu$ A at soaking times of 0, 3, and 8 min respectively (theoretical peak current is 12.70μ A). The average peak current obtained for all times is $12.28 \pm 0.11 \mu$ A with an RSD of 0.98%. These results indicate that this pretreatment removes a significant portion of the lipophilic binder present at the electrode surface resulting in a time independent current response that agrees with theory.



Figure 3.9 Average peak current plotted as a function of time for oxidation of 1.0 mM FcTMA⁺ in 0.1 M KCl measured by CV. Electrodes treated either by anodic polarization (1.4 V, 60 s) then cathodic "equilibration" (-1.0 V, 15 s) or anodic polarization (1.4 V, 60 s) only. Error bars indicate ± 1 standard deviation (N = 3 electrodes).

To confirm BDDPE surface oxidation, both the solvent window and Fe(CN)₆⁴⁻ voltammetry were recorded at anodically polarized BDDPEs. Anodic polarization of BDD is known to remove non-diamond carbon impurities, which are catalytic sites for water electrolysis at cathodic and anodic potential extremes, therefore, removal of NDC increases solvent window width.¹² Fe(CN)₆³⁻ oxidation is inhibited at oxygen terminated BDD, resulting in sluggish electron transfer kinetics.¹⁷ The solvent windows and CVs of Fe(CN)₆⁴⁻ recorded before and after anodic polarization of BDDPEs are shown in Figure 3.10A and Figure 3.10B respectively. The solvent window of the BDDPE improves significantly, indicating a decrease in NDC catalytic sites.^{12, 16,} ³¹ In the cathodic window, the oxygen reduction reaction (ORR) currents between -0.9 and -1.5 V are decreased compared to the non-polarized BDDPE indicating an increase in surface oxide coverage, which act as a blocking layer inhibiting ORR.⁵⁷ Fe(CN)₆⁴⁻ exhibits irreversible voltammetry at both untreated and anodically polarized BDDPEs. However, the irreversibility is more extreme at the anodically polarized electrode, where ΔE_p increased from 450 mV to 550 mV, indicating even slower electron transfer kinetics as a result of an increase in the density of surface oxides. These data indicate increased surface oxide coverage of the anodically polarized BDDPE.



Figure 3.10 (A) Solvent windows recorded in 0.1 M KCl (pH 6.5) for an untreated (—) and anodically polarized (—) BDDPE at a scan rate of 0.1 V s⁻¹. (B) CVs recorded for 1.0 mM $Fe(CN)_{6^{4-}}$ in 0.1 M KCl with an untreated (—) and anodically polarized (—) BDDPE.

3.5 Conclusions

In this chapter, further characterization of the boron doped diamond paste electrodes (BDDPEs) employed in Chapter 2 is presented. Early iterations of the Janus-ePAD suffered from variability in device and electrode fabrication, leading to poor reproducibility for the detection of pAP at multiple pH conditions. The BDDPE fabrication was optimized to decrease surface roughness and improve contact upon attachment of the BDDPE to paper-based device. The effects of the presence of the lipophilic pasting liquid on the electrochemical response of BDDPEs were studied, leading to new insights into the proper use of this novel electrode material. An electrochemical pretreatment which eliminates the unwanted effects of extraction of less polar species into the electrode bulk, and initial dissolution of binding liquid was developed. These fundamental insights will allow for future developments of high quality ePAD applications employing BDDPEs.

REFERENCES

1.Adkins, J. A.; Noviana, E.; Henry, C. S., Development of a Quasi-Steady Flow Electrochemical Paper-Based Analytical Device. *Analytical chemistry* **2016**, *88* (21), 10639-10647.

2.Adkins, J.; Boehle, K.; Henry, C., Electrochemical paper-based microfluidic devices. *Electrophoresis* **2015**, *36* (16), 1811-1824.

3. Adkins, J. A.; Henry, C. S., Electrochemical detection in paper-based analytical devices using microwire electrodes. *Analytica Chimica Acta* **2015**, *891*, 247-254.

4.Dungchai, W.; Chailapakul, O.; Henry, C. S., Electrochemical detection for paper-based microfluidics. *Analytical chemistry* **2009**, *81* (14), 5821-5826.

5.Olson, C.; Adams, R. N., CARBON PASTE ELECTRODES APPLICATION TO ANODIC VOLTAMMETRY. *Analytica Chimica Acta* **1960**, *22* (6), 582-589.

6.Svancara, I.; Vytras, K.; Barek, J.; Zima, J., Carbon paste electrodes in modern electroanalysis. *Critical Reviews in Analytical Chemistry* **2001**, *31* (4), 311-345.

7.Kalcher, K.; Kauffmann, J. M.; Wang, J.; Svancara, I.; Vytras, K.; Neuhold, C.; Yang, Z., SENSORS BASED ON CARBON-PASTE IN ELECTROCHEMICAL ANALYSIS - A REVIEW WITH PARTICULAR EMPHASIS ON THE PERIOD 1990-1993. *Electroanalysis* **1995**, *7* (1), 5-22.

8. Svancara, I.; Zima, J., Possibilities and Limitations of Carbon Paste Electrodes in Organic Electrochemistry. *Current Organic Chemistry* **2011**, *15* (17), 3043-3058.

9.Mikysek, T.; Svancara, I.; Kalcher, K.; Bartos, M.; Vytras, K.; Ludvik, J., New Approaches to the Characterization of Carbon Paste Electrodes Using the Ohmic Resistance Effect and Qualitative Carbon Paste Indexes. *Analytical Chemistry* **2009**, *81* (15), 6327-6333.

10.Nantaphol, S.; Channon, R. B.; Kondo, T.; Siangproh, W.; Chailapakul, O.; Henry, C. S., Boron Doped Diamond Paste Electrodes for Microfluidic Paper-Based Analytical Devices. *Analytical Chemistry* **2017**, *89* (7), 4100-4107.

11.Stefan, R.-I.; Bairu, S. G., Diamond paste based electrodes for the determination of Pb (II) at trace concentration levels. *Talanta* **2004**, *63* (3), 605-608.

12.Hutton, L. A.; Iacobini, J. G.; Bitziou, E.; Channon, R. B.; Newton, M. E.; Macpherson, J. V., Examination of the factors affecting the electrochemical performance of oxygen-terminated polycrystalline boron-doped diamond electrodes. *Analytical chemistry* **2013**, *85* (15), 7230-7240.

13.McCreery, R. L., Advanced carbon electrode materials for molecular electrochemistry. *Chemical Reviews* **2008**, *108* (7), 2646-2687.

14.Read, T. L.; Joseph, M. B.; Macpherson, J. V., Manipulation and measurement of pH sensitive metal–ligand binding using electrochemical proton generation and metal detection. *Chemical Communications* **2016**, *52* (9), 1863-1866.

15.Read, T. L.; Bitziou, E.; Joseph, M. B.; Macpherson, J. V., In situ control of local pH using a boron doped diamond ring disk electrode: optimizing heavy metal (mercury) detection. *Analytical chemistry* **2013**, *86* (1), 367-371.

16.Macpherson, J. V., A practical guide to using boron doped diamond in electrochemical research. *Physical Chemistry Chemical Physics* **2015**, *17* (5), 2935-2949.
17.Granger, M. C.; Swain, G. M., The influence of surface interactions on the reversibility of Ferri/Ferrocyanide at boron-doped diamond thin-film electrodes. *Journal of the Electrochemical Society* **1999**, *146* (12), 4551-4558.

18.Patten, H. V.; Lai, S. C.; Macpherson, J. V.; Unwin, P. R., Active sites for outer-sphere, inner-sphere, and complex multistage electrochemical reactions at polycrystalline boron-doped diamond electrodes (pBDD) revealed with scanning electrochemical cell microscopy (SECCM). *Analytical chemistry* **2012**, *84* (12), 5427-5432.

19.Duran, B.; Brocenschi, R. F.; France, M.; Galligan, J. J.; Swain, G. M., Electrochemical activation of diamond microelectrodes: implications for the in vitro measurement of serotonin in the bowel. *Analyst* **2014**, *139* (12), 3160-3166.

20.Kondo, T.; Sakamoto, H.; Kato, T.; Horitani, M.; Shitanda, I.; Itagaki, M.; Yuasa, M., Screen-printed diamond electrode: A disposable sensitive electrochemical electrode. *Electrochemistry Communications* **2011**, *13* (12), 1546-1549.

21.Kondo, T.; Udagawa, I.; Aikawa, T.; Sakamoto, H.; Shitanda, I.; Hoshi, Y.; Itagaki, M.; Yuasa, M., Enhanced sensitivity for electrochemical detection using screen-printed diamond electrodes via the random microelectrode array effect. *Analytical chemistry* **2016**, *88* (3), 1753-1759.

22.Nantaphol, S.; Kava, A. A.; Channon, R. B.; Kondo, T.; Siangproh, W.; Chailapakul, O.; Henry, C. S., Janus electrochemistry: Simultaneous electrochemical detection at multiple working conditions in a paper-based analytical device. *Analytica Chimica Acta* **2019**, *1056*, 88-95.

23.Lemay, S. G.; van den Broek, D. M.; Storm, A. J.; Krapf, D.; Smeets, R. M. M.; Heering, H. A.; Dekker, C., Lithographically fabricated nanopore-based electrodes for electrochemistry. *Analytical Chemistry* **2005**, *77* (6), 1911-1915.

24.Bard, A. J.; Faulkner, L. R.; Leddy, J.; Zoski, C. G., *Electrochemical methods: fundamentals and applications*. wiley New York: 1980; Vol. 2.

25.Nie, Z.; Nijhuis, C. A.; Gong, J.; Chen, X.; Kumachev, A.; Martinez, A. W.; Narovlyansky, M.; Whitesides, G. M., Electrochemical sensing in paper-based microfluidic devices. *Lab on a Chip* **2010**, *10* (4), 477-483.

26.Chi, Q.; Göpel, W.; Ruzgas, T.; Gorton, L.; Heiduschka, P., Effects of pretreatments and modifiers on electrochemical properties of carbon paste electrodes. *Electroanalysis* **1997**, *9* (5), 357-365.

27.Rice, M. E.; Galus, Z.; Adams, R. N., Graphite paste electrodes: Effects of paste composition and surface states on electron-transfer rates. *Journal of Electroanalytical Chemistry and Interfacial Electrochemistry* **1983**, *143* (1-2), 89-102.

28.Houghtaling, J.; Liang, T.; Thiessen, G.; Fu, E., Dissolvable bridges for manipulating fluid volumes in paper networks. *Analytical chemistry* **2013**, *85* (23), 11201-11204.

29. Svancara, I.; Kalcher, K.; Walcarius, A.; Vytras, K., *Electroanalysis with carbon paste electrodes*. Crc Press: 2012.

30.Ravichandran, K.; Baldwin, R. P., Enhanced voltammetric response by electrochemical pretreatment of carbon paste electrodes. *Analytical Chemistry* **1984**, *56* (9), 1744-1747. 31.Williams, O. A., Nanocrystalline diamond. *Diamond and Related Materials* **2011**, *20* (5-6), 621-640.

CHAPTER 4: DEVELOPMENT OF A FAST FLOW HYBRID JANUS-EPAD FOR SIMULTANEOUS ELECTROCHEMICAL DETECTION AT MULTIPLE PH CONDITIONS

4.1 Chapter Overview

Traditional microfluidic paper-based analytical devices (μ PADs) rely on a single layer of paper to wick sample via capillary action. In these devices, flow rates are slow resulting in long analysis times, regardless of detection motif. In electrochemical PADs, this problem is further exacerbated by the presence of hydrophobic stencil-printed carbon electrodes (SPCEs) in the channel further inhibiting fluid flow. SPCEs in ePADs also suffer from poor reproducibility and sensitivity due the paper at the SPCE surface, which is a sporous heterogeneous surface that blocks a significant portion of the SPCE electroactive surface area. In order to overcome the aforementioned limitations of traditional ePADs, the following chapter describes the development of a multi-layer hybrid Janus-ePAD. The hybrid Janus-ePAD consists of a paper layer, several layers of double-sided adhesive and a top layer of transparency film. The channel gap induced significantly faster flow rates, decreasing analysis time by over 20x compared to a one-layer device. The placement of SPCE working electrodes on the transparency film layer resulted in bulk solution electrochemistry of FcTMA⁺. In situ pH adjustment is also characterized colorimetrically by impregnating paper channels with a universal pH indicator and quantified using a pH sensitive redox probe. Poor mixing of an injected buffer and a strong base was observed resulting in a pH gradient in the electrochemical detection zones. Impregnation of the paper channels with a buffer for in situ pH adjustment of unbuffered solution is demonstrated as an alternative, providing heterogeneous pH conditions at the electrochemical detection zone over a wide range of pH values.

4.2 Introduction

The fabrication of electrochemical paper-based analytical devices (ePADs) with stencilprinted carbon electrodes (SPCEs) for the development of low-cost and portable environmental and clinical diagnostics has become increasingly popular. ePAD designs have become progressively more complex, enabling a wide variety of functions to be carried out on the device to both enhance ePAD performance and ease of operation in field settings.¹⁻³ For example, in Chapter 2 of this thesis, a Janus-ePAD was developed for simultaneous electrochemical detection in multiple solution conditions generated in situ.⁴ The development of the Janus-ePAD hinged on the ability to pattern a paper substrate to direct fluid flow in microfluidic paper channels through wax-printing as well as impregnate the paper substrate with reagents for in situ sample pH adjustment. While promising, ePADs including the Janus-ePAD developed in Chapter 2, still face several challenges, limiting their wide spread use and commercialization.

The majority of first generation ePAD designs have been 2-dimensional, consisting of a single layer of paper that contains several components including stored reagents, SPCEs, and wax printed barriers. However, such devices pose several challenges for the development of high performance ePADs.⁵⁻⁷ First, the electroactive sensing area of SPCEs printed on paper channels is controlled by the interfacial area of the SPCE and paper. Paper consists of a heterogeneous porous cellulose fiber network; therefore, the electroactive surface area determined by the interface between the SPCE and paper is highly heterogeneous resulting in poor inter-electrode reproducibility.⁸ Also, even in the best case scenario, the paper fibers occupy a significant portion of the electroactive surface area further reducing sensitivity compared to bulk solution electrochemical detection where the entire electrode surface is in direct contact with solution.⁹ Second, SPCEs are hydrophobic due to the polymeric binder components, and SPCEs act as a

barrier to solution imbibition at the SPCE-paper interface resulting in slower flow rates and longer analysis times. Third, reagents stored in the paper to carry out any number of functions such as pH adjustment are difficult to mix upon rehydration with sample. Since flow is typically laminar in paper channels, the mixing mechanism is by diffusion only, which is slow.¹⁰ Several methods have been proposed to improve mixing in paper-based devices including reagent patterning, dissolvable bridges to slow flow and allow for mixing by diffusion, surface acoustic waves, as well as the use of air permeable vents to allow air bubbles into a mixing chamber for convective mixing. However, these methods increase device fabrication and operation complexity, reducing the feasibility of ePADs, including the Janus-ePAD, of reaching commercialization and widespread use.¹¹⁻¹⁴

Finally, while paper-based devices take advantage of capillary forces generated by the hydrophilic cellulose matrix to imbibe solution within the device channels negating the need for external pumps and in many cases extra pipetting steps, flow rates within a paper channel decrease as the distance the fluid front travels increases due to increased viscous drag forces.¹⁵ This relationship is governed by the Lucas-Washburn equation:¹⁶

$$l(t) = \sqrt{\left(\frac{\gamma r cos\theta}{2\mu}\right)t}$$
 Equation 4.1

where the distance the fluid front travels as a function of time, l(t), is proportional to the square root of time (t), cosine of the solution contact angle with paper (θ), effective pore radius of the paper (r), and solution surface tension (γ), as well as inversely proportional to the square root of solution viscosity (μ).¹⁶ This decay in flow rate significantly increases analysis times in ePADs. Due to the long paper channels, changing channel geometry and SPCEs, the Janus-ePAD developed in Chapter 2 required nearly 25 min for solution to saturate the entire length of the channel. Moreover, an additional 20 min was required to allow solution to fully saturate the detection zone at the end of the channels since paper saturation decreases as the distance the fluid front has traveled from the source increases.¹³

In order to overcome the aforementioned problems associated with ePADs, 3-dimensional paper-based devices, in which the paper channel is replaced with a gap, have been developed. In their seminal work, Adkins et al. fabricated a 3-dimensional ePAD by sandwiching Pt microwire electrodes between two paper layers. The device consisted of an inlet followed by a straight channel leading to a 270° fan-shaped paper outlet to generate quasi-steady flow for flow injection analysis. The gap located between the paper layers acts a large pore, increasing the effective pore radius of the device.¹⁷ The increase in pore radius due to the channel gap generated a 237% increase in flow rate relative to a single paper layer device.⁶ In this work, Au microwire electrodes were located in the center of the channel as opposed to SPCEs directly printed onto the paper used in most ePADs. Accordingly, the aforementioned problems associated with SPCEs in microfluidic paper-based channels did not arise. However, microwire electrodes are extremely fragile and difficult to work with rendering them less suitable than SPCEs for mass production of commercial devices. Further studies to characterize "fast-flow" in multi-layer paper-based devices carried out by Channon et al. showed that flow rates are proportional to the gap height. Compared to the gap heights employed by Adkins et al. of about 25 µm, larger gap heights (up to 300 µm) can generate even faster flow rates in PADs of up to about 170x faster relative to flow rates generated in single layer PADs. The gap height and flow rate can be easily tuned and defined by the addition of tape layers between the top and bottom paper layer.¹⁸

Herein, a second generation hybrid Janus-ePAD was developed to overcome the problems associated with the 2-dimensional iteration of the Janus-ePAD initially developed in Chapter 2.

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The device developed here consists of a bottom layer of wax-patterned paper attached to polyethylene terephthalate (PET) layer with varying layers of double-sided adhesive to control the channel height. SPCEs are used in this device since the BDDPEs used previously are not a printable material and cannot be integrated within the microfluidic channel for bulk solution electrochemistry. The PET layer used as the top layer results in several advantages relative to a second paper layer including: 1) PET acts as a seal, negating the necessity for a top layer of tape; 2) SPCEs printed on PET provide a more reproducible electroactive surface area by removing the SPCE-paper interface; 3) SPCEs printed on PET can be pretreated prior to device fabrication without damage or contamination of the PET substrate. pH adjustment is carried out in situ in the paper-plastic hybrid Janus-ePAD by the addition of base to adjust the pH of an injected buffer as in Chapter 2, and the mixing dynamics were characterized. pH adjustment was characterized both colorimetrically and electrochemically revealing that spotting strong acid or base on the paper channel results in a large pH gradient due to slow mixing. In order to minimize poor mixing effects on the resultant pH, the channels were impregnated with buffers of the desired pH, resulting in homogeneous pH adjustment of downstream sample when unbuffered sample is injected. The optimal gap height for fast analysis times is also discussed. Finally the hybrid Janus-ePAD is applied to the electrochemical detection of the model redox molecule FcTMA⁺, providing bulk solution sensitivity in an ePAD.

4.3 Experimental

Chemicals, Materials and Equipment

All chemicals were analytical grade and used as received. All solutions were prepared using purified water (18.2 M Ω ·cm) from a Milli-Q Millipore water purification system. Ferrocenylmethyl trimethylammonium hexafluorophosphate (FcTMA⁺) was synthesized in-house using a previously reported method.¹⁹ Potassium chloride (KCl), glacial acetic acid (TraceMetalTM Grade) and boric acid were purchased from Fisher Scientific (New Jersey, USA). Sodium hydroxide (NaOH) and potassium phosphate monobasic were purchased from Sigma-Aldrich (Massachusetts, USA). Thymol blue obtained from Sigma Aldrich (Missouri, USA), bromothymol blue obtained from JT Baker (New Jersey, USA) methyl red obtained from Sigma-Aldrich, phenolphthalein obtained from Finn Scientific (Illinois, USA) and 95% ethanol obtained from Sigma-Aldrich were used to make Yamada's universal indicator.²⁰ Methyl 4-Hydroxybenzoate (methyl paraben) was obtained from Tokyo Chemical Company (Tokyo, Japan). Graphite powder $(\leq 20 \ \mu m)$ purchased from Sigma-Aldrich and conductive carbon ink (E3178) purchased from Ercon Inc. (Massachusetts, USA) were used to fabricated SPCEs via a previously reported protocol.²¹ Ag/AgCl ink was purchased from Gwent Group (Torfaen, U.K.) for reference electrode (RE) construction. The design of the paper-based layer was printed onto Whatman #1 filter paper obtained from Fisher Scientific with a Xerox Phaser 8860 wax printer (Connecticut, USA). PET transparency film (PP 2200) and double-sided adhesive (467 MP) were obtained from 3M (Minnesota, USA). All device components consisting of these materials were laser cut using a 30 W Epilog Engraver Zing Laser Cutter and Engraver (Colorado, USA). All electrochemical experiments were performed with a model 660B bipotentiostat (CHI instruments, Austin, TX) at room temperature (22 ± 1 °C).

Hybrid Janus-ePAD Fabrication

The ePAD was designed using CorelDraw software (Ontario, CA). The paper-based layer employed the same design as the Janus-ePAD developed in Chapter 2, however, the wax barriers of the device here consisted of only the outline of the channel geometry as opposed to filling in the entire device with ink as shown in Figure 4.1B. This strategy for generating wax barriers was

used because the double-sided adhesive used to adhere the two layers did not stick to wax coated paper. The line thickness was 4-pt to ensure enough wax was present to form the barrier. After printing, the wax was melted through the paper on a 160 °C hot plate for 90 s to generate a 3dimensional barrier. Briefly, the paper channels defined by the wax-barrier consist of a sample inlet leading to straight channel, which splits into two channels, leading to two separate reagent zones, followed by straight channels which lead to two detection zones containing three electrode electrochemical cells. The RE was then stencil-printed onto the paper layer as shown in Figure 4.1B. The RE was designed to be printed on the paper layer in order to avoid printing at the inlet to the electrochemical zone from the channel so as not to act as a further barrier to sample imbibition from the straight channel into the increasing area detection zone. The T-Shape of the RE consisted of a horizontal strip 6 mm x 1 mm. The electrical connection was made with the vertical strip which was 2 mm x 10 mm. The ink was then dried in a 65 °C oven for 30 min. After this time a metal spatula was used to paint Ag/AgCl ink onto the portion of the RE inside the paper channels to serve as the pseudo-RE. Carbon ink was stencil-printed onto transparency (PET) film to generate working electrode 1 (WE1), working electrode 2 (WE2), and the counter electrode as shown in Figure 4.1A. The electrodes were then cured in a 65 °C oven for 30 min. The WEs had a geometric surface area contained within the channel of 0.17 cm². The transparency and paper layers were attached to one another with double-sided adhesive laser cut to contain the same channel geometry bound by the wax-printed paper layer excluding the sample well inlet (Figure 4.1B). Three gap heights defined by the thickness of this layer were tested. One layer of doublesided adhesive, two layers of double-sided adhesive, and a layer of transparency film sandwiched between two layers of double-sided adhesive generated gap heights of 60 µm, 120 µm, and 230 um respectively. Finally, Scotch[®] brand heavy duty packing tape (3M) was used to seal the bottom paper layer of the device. A top view of the hybrid Janus-ePAD is shown in Figure 4.1C. The solution volume required to saturate the device was 120μ L.



Figure 4.1 Schematic of hybrid Janus-ePAD fabrication showing (A) top transparency film with SPCE WEs and CE, (B) all layers of the device, and (C) the top-view of a hybrid Janus-ePAD.

Colorimetric Analysis of pH Adjustment

NaOH reagent or buffer reagent mixing dynamics with an injected buffered sample or unbuffered sample in the channel gap was visualized using Yamada's universal indicator. Yamada's universal indicator was prepared by dissolving 10.0 Thymol blue, 60.0 mg bromothymol blue, 25.0 mg methyl red and 100.0 mg phenolphthalein in 200.0 mL 95% ethanol. This was then neutralized to pH 7.0 with 0.1 M NaOH and diluted up to 400 mL with water. The colorimetric response of Yamada's indicator dried on paper was then calibrated with a PAD containing wax-printed spots. Yamada's indicator was dried on each spot. 0.1 M acetate buffers with a pH of 3.50, 4.00, and 5.00 were prepared by titrating 0.1 M acetic acid with 1.0 M NaOH. Phosphate buffers with a pH of 6.00, 6.35 and 7.10 were prepared by titrating 0.1 M potassium phosphate monobasic with 1.0 M NaOH. pH 9.38 0.1 M borate buffer was prepared by titrating 0.1 M boric acid with 1.0 M NaOH. For colorimetric imaging in the hybrid Janus-ePAD, the entire channel area downstream of the reagent zones was then saturated with Yamada's Indicator by pipetting 5.0 μ L aliquots of solution for a total of 20 μ L. The device was then allowed to dry in ambient conditions prior to full ePAD fabrication. The exact concentration of NaOH solution employed to adjust buffer pH in situ was determined via a phenolphthalein titration to be 1.087 M. NaOH was pipetted onto one of the reagent zones with the specified volume and the second reagent zone was left unmodified to serve as a control. For pH adjustment and determination of unbuffered sample, Yamada's indicator was first pipetted onto the specified zones of the device and allowed to dry. This was followed by pipetting the specified buffers onto the reagent zones in three 5.0 μ L aliquots which were allowed to dry at room temperature. After device fabrication, unbuffered sample was added to the injection zone. An iPhone 10 was used to capture images of the devices.

Electrochemical Analysis of pH Adjustment and FcTMA⁺ Calibration

For quantitative determination of the pH generated in the device detection zone the pH sensitive redox probe, methyl paraben, and pH insensitive redox probe, FcTMA⁺, were employed. 1.0 mM methyl paraben and 1.0 mM FcTMA⁺ solutions were prepared in 25 v/v% methanol and 75 v/v% water with 0.1 M KCl as the supporting electrolyte. The peak potential difference between methyl paraben and FcTMA⁺ was measured using square wave voltammetry (SWV). In SWV, the frequency was 15 Hz, the amplitude was 25 mV, and the increment was 4 mV. To determine the pH after diffusive mixing in the device when NaOH was employed to adjust buffer pH, SWV voltammetry was carried out for separate devices at varying time points as discussed below. A calibration curve for change in peak potential of methyl paraben vs. FcTMA⁺ was generated in the device by injecting samples with a known pH. Cyclic voltammetry (CV) at a scan rate of 100 mV s⁻¹ was employed to generate a 0.025 to 1.0 mM FcTMA⁺ calibration curve.

4.4 Results and Discussion

Fast Flow in Hybrid Janus-ePADs

One of the main drawbacks associated with microfluidic paper-based analytical devices (μ PADs), regardless of detection motif, is slow flow rates and long analysis times. Recent reports propose the use of multi-layer paper or paper/plastic devices to overcome slow flow rates in single layer μ PADs, referred to as fast flow-PADs (ff-PADs).^{6, 18, 22} The fast flow phenomenon was first observed and modeled in multilayer PADs by Martinez et al. The fast flow rates were found to result mainly from the gap between the device layers with small gap heights (12 μ m). The flow rates obtained for these devices were treated using equation 4.1 using a modified calculation for effective pore radius generated by the gap. The pore radius is defined using equation 4.2:¹⁷

$$r = \frac{2r'(hw + R(gw))}{2hw + gw}$$
 Equation 4.2

where r' is mean capillary radius of the paper, *h* is the paper thickness, *w* is the channel width, *g* is the channel height, and R = g / 2 is the half channel height with all units in meters. Equation 4.2 was derived treating the channel gap as consecutive large pores of radius R. While the modified Lucas-Washburn model agreed with channel gaps up to 25 µm, recent work by Channon et al. showed that Equations 4.1 and 4.2 severely overpredict flow rates in devices containing large channel gaps.²³ Following their initial study, Channon et al. developed a model to describe flow in channel gaps greater than 75 µm. In this model, the net pressure driving force in the Lucas-Washburn formulation was the capillary pressure generated in the gap counteracted by viscous dissipation.¹⁸ Figure 4.2 shows the time required for solution to wick to the end of hybrid Janus-ePADs with varying gap heights or a single layer Janus-ePAD. The time required for solution to

wick the full length of the device was 46.7 ± 2.9 s, 160.0 ± 8.6 s, 336.7 ± 7.6 s, and 660 ± 120 s for 230 µm, 120 µm, and 60 µm gap heights and single layer device, respectively. While 660 s, or 11 min, is required for solution to saturate a one layer Janus-ePAD, the initial iteration of the device contained an SPCE at the detection zone inlet, which acted as a hydrophobic barrier to fluid flow, further increasing device saturation time to over 20 min. The SPCEs only slightly increased wet-out times to 53.3 ± 6 s for a 230 µm gap height (Figure 4.2). The ability to print SPCEs on both layers of the two layer device allowed for the removal of the hydrophobic RE from the detection zone inlet. Based on these times and the length of the paper channels, experimental flow rates (calculated with the time required to wick the entire channel distance of 26 mm) were 0.056 cm s⁻¹, 0.016 cm s⁻¹, 0.008 cm s⁻¹ for decreasing gap heights. While these flow rates represent a significant increase in flow rate obtained in the one layer Janus-ePAD of 0.004 cm s⁻¹, they are slower, yet reasonably so, than those predicted and experimentally obtained by Channon et al. for these gap heights.¹⁸ A stipulation necessary for the Lucas-Washburn theory to hold requires the solution reservoir to be non-limiting feeding a straight channel of constant cross-sectional area.²⁴ However, in the Janus-ePAD, straight channels feed solution into increasing cross-sectional area (e.g. the reagent zone and detection zone). Therefore, the greater decrease in distance traveled with time compared to straight channels was expected.²⁵ While experimental flow rates in the hybrid Janus-ePAD do not agree with any current model due to changes in cross-sectional area, flow rates are remarkably faster resulting in decreased wet-out and analysis times from over 20 min for a single layer device to less than one min in the hybrid Janus-ePAD. This decrease in wet-out time bodes well for further practical applications of the Janus-ePAD.



Figure 4.2 Relationship between full channel saturation time in the Janus-ePAD and gap height. **In situ pH Adjustment**

Figure 4.3 shows the colorimetric response of Yamada's indicator on Whatman #1 chromatography paper. A discernable color change was observed in the pH range of 3.50 to 9.38. The image also indicates that Yamada's indicator components stick well to paper, with a relatively homogeneous color intensity across the spotted zones. This is critical for visualizing local pH conditions throughout the microfluidic channels in the Janus-ePAD during device wet-out.



Figure 4.3 Colorimetric response of Yamada's universal pH indicator on Whatman #1 chromatography paper. The following colors were obtained: pH 3.50 is red/pink, pH 4.01 is red/orange, pH 5.02 is orange, pH 6.01 is yellow/orange, pH 6.35 is yellow, pH 7.10 is yellow/green, pH 7.82 is green and pH 9.38 is blue.

To visualize the pH generated in a pH 4.00 sample, the reagent zone of one channel was

impregnated with 5.0 µL of 1.0 M NaOH solution and the paper channel downstream of both the

modified and unmodified reagent zones was impregnated with Yamada's universal indicator.

Figure 4.4 shows the subsequent color change after injection of a pH 4.00 buffered sample.



Figure 4.4 pH changes in a hybrid Janus-PAD generated by the addition of $5.0 \,\mu\text{L}$ of $1.0 \,\text{M}$ NaOH to the first reagent zone. The second channel and reagent zone serve as a pH control. Inset shows the a zoomed image of the reagent modified channel.

As demonstrated in Figure 4.4, the pH change generated in a pH 4.00 acetate buffer upon rehydration of NaOH in the reagent zone results in a steep gradient in pH throughout the channel. In the unmodified channel, the color is consistent with a pH 4.00 solution throughout. In the modified channel, the color change ranges from the red/orange of a pH 4.00 solution up to blue and even purple indicating a highly alkaline pH. These images are consistent with immediate rehydration of the dried NaOH during channel wet-out, forming a high NaOH concentration plug at the fluid front. After full wet-out, videos of the pH conditions in the device were recorded over the course of one hour. In these videos, the pH gradient becomes less steep, indicating diffusional mixing. However, the pH this was not enough time for pH to homogenize and the pH gradient remained after a full hour (images not shown). The pH gradient generated in the device is consistent with slow diffusional mixing of rehydrated reagents, which has been a major disadvantage to performing in situ solution chemistry in microfluidic paper-based devices.^{3,10} This pH gradient is very likely another contributing factor to the poor reproducibility of the first generation Janus-ePAD presented in Chapter 2, where concentrated NaOH or H₃PO4 were

employed to generate higher or lower pH conditions at the detection zones, respectively. The same experiment was carried out with one layer Janus-ePADs and the steep pH gradient was observed, indicating that poor mixing is not due to the channel gap in the hybrid Janus-ePAD. While, the colorimetric method employed above provides valuable information about in situ pH adjustment in the Janus-ePAD, it only provides qualitative information about the actual pH in the detection zone.

To quantify the solution pH generated in situ, electrochemical calibration of pH was employed. The electrochemical oxidation of methyl paraben is pH sensitive and undergoes the following proposed electron transfer reaction.²⁶



Figure 4.5 Proposed oxidation reaction for methyl paraben in aqueous solution.

According to the Nernst equation, a one electron – one proton transfer oxidation results in a peak potential shift of 59.1 \pm 10 mV per pH unit (Equation 2.1).²⁷ Therefore, a pH calibration curve was generated using SWV of FcTMA⁺ and methyl paraben at various pH conditions in the device. The peak potential of methyl paraben was measured vs. the peak potential of FcTMA⁺ as opposed to the Ag/AgCl RE since a change in solution pH could result in potential shifts of Ag/AgCl pseudo-RE.²⁸ Figure 4.6A shows representative voltammograms for FcTMA⁺ and methyl paraben at a pH of 4.00 and 5.04. While FcTMA⁺ is oxidized at the same potential regardless of pH, a clear shift to more facile potentials for methyl paraben oxidation is observed as pH increases as predicted by equation 2.1. Figure 4.6B shows the calibration curve generated for oxidation peak potential of methyl paraben over the pH range 3.50 to 8.00. Higher pH conditions were not included since methyl paraben oxidation is not pH sensitive above a pH of about 8 due to deprotonation ($pK_a = 8.87$).²⁹ Methyl paraben peak potential oxidation shifted cathodically by 69.48 mV per pH unit vs. FcTMA⁺, in agreement with the Nernst equation.



Figure 4.6 (A) SWVs recorded in Janus-ePADs for the oxidation of FcTMA⁺ and methyl paraben at pH 4.00 (green trace) and pH 5.04 (black trace). (B) Calibration curve generated for methyl paraben oxidation peak potential as a function of solution pH vs. FcTMA⁺ peak potential in Janus-ePADs (N = 3).

Next, the same experiment that was performed colorimetrically above was carried out in the hybrid Janus-ePAD and characterized using electrochemical detection. Here, 5.0μ L of 1.0 M NaOH was dried on reagent zone one while reagent zone two served as the blank control. 0.1 M pH 4.0 acetate buffer containing 0.5 mM FcTMA⁺, 0.5 mM methyl paraben and 0.1 M KCl was injected at the sample inlet. SWV was carried out at both WEs simultaneously at time intervals of 3, 4.5, 6, and 10 min after full device wet-occurred. Separate devices were used for each time point to avoid repetitive electrochemical measurements in a single device due to methyl paraben's electrode

fouling characteristics. In Figure 4.7, the equilibration of pH conditions in the detection zone over time due to diffusive mixing of NaOH with the pH 4.00 buffer is evident. Here, the methyl paraben peak shifts significantly to less positive potentials at increasing equilibration indicating pH is increasing with time. This is consistent with the colorimetric data, where the narrow NaOH plug generated during wet-out slowly broadens as diffusion occurs.

Increasing Equilibration Time



Figure 4.7 SWVs recorded at WE1 (gold trace) and WE2 (black trace) of 0.5 mM FcTMA⁺ and methyl paraben in 0.1 M pH 4.00 acetate buffer and 0.1 M KCl for increasing equilibration times after full wet-out of the Janus-ePAD. NaOH was added to the reagent zone upstream of WE1 and WE2 served as the known pH control.

This experiment was carried out in triplicate and the peak potential difference of methyl paraben between WE1 and WE2 versus FcTMA⁺ was employed to calculate the solution pH in the modified electrochemical detection zone using the calibration curve generated in Figure 4.6. The methyl paraben peak potential differences between the pH 4.00 channel and the adjusted pH channel were 54 ± 14 mV and 88 ± 17 mV corresponding to a pH increase in the NaOH channel from pH 4.00 to pHs of 4.81 ± 0.22 , 5.34 ± 0.21 , for the 3 and 4.5 min equilibration times respectively. These results confirm the visual data obtained within the device, where mixing occurred over time by diffusion, resulting in a visual color change.

The SWVs shown in Figure 4.7 recorded in the NaOH modified channel for the 6 and 10 min time points show evidence of other redox processes which were not observed when generating the calibration in curve in Figure 4.5B for pHs between 3.5 and 8.0. Therefore, it is possible that

the pH in the detection zones at these times is much greater than 8.0. Water oxidation also occurs in these systems to a much higher extent indicated by the larger currents and lower onset potentials at about +1.0V in the gold traces in Figure 4.6. Water oxidation generates protons, therefore this reaction is more favorable at high pHs (low H⁺ concentration).³⁰ This is also confirmed in the colorimetric data, where locations of pH greater than 8.0 are indicated by the purple and blue colors. Nonetheless, these data further confirm non-uniform pH adjustment of an injected buffer with concentrated acid or base. As a means to obtain uniform pH conditions in the detection zones of the hybrid Janus-ePAD, buffers of the desired pH were dried in the reagent zones, and unbuffered water was injected at the sample inlet. According to the Henderson-Hasselbalch equation, buffer pH is controlled by the ratio of conjugate weak acid and weak base. Therefore, upon rehydration of dried buffer during wet-out in a paper device, concentration gradients will not affect the solution pH since the ratios of conjugate acid and base should remain uniform.³¹ Figure 4.8 shows the colorimetric and electrochemical responses for adjusting the pH of an unbuffered sample with buffer impregnated channels. Figure 4.8A and 4.8B show the colorimetric response in the electrochemical detection zone where pH 9.38 borate buffer, pH 4.00 acetate buffer, and pH 7.00 and 6.00 phosphate buffers were dried on the reagent zones as denoted in the image. Here, the color produced in the detection zones is homogeneous and in agreement with the known pH responses shown in Figure 4.3. In order to confirm pH electrochemically methyl paraben and FcTMA⁺ voltammetry was employed. Here, pH 7.00 buffer was stored in channel one and pH 4.00 buffer was stored in channel two. Unbuffered 0.1 M KCl containing 0.5 mM FcTMA⁺ and methyl paraben was injected at the sample inlet and SWVs were recorded once solution saturated each detection zone as shown in Figure 4.8C. The peak potential difference for methyl paraben between the two channels was 204 mV vs. FcTMA⁺. Based on the calibration curve in Figure 4.6B, a 204 mV peak potential difference corresponds to a pH difference of 2.94, which is in agreement with the known pH difference of 3.00 pH units. The colorimetric response is also shown in Figure 4.8C and correlates with the expected color response. Clearly, buffer impregnated channels are better suited for in situ pH adjustment in μ PADs. In the device shown in Figure 4.8A, a pH difference of 5.0 was generated between the two channels. Since buffers are only useful between plus or minus one pH unit of the weak acid pKa, a larger pH difference than two would be very difficult to achieve with the injection of a buffer and subsequent pH adjustment via mixing with a strong acid or base, assuming a mechanism for reagent mixing was also incorporated.³¹



Figure 4.8 (A) and (B) are images of Janus-ePADs showing the colorimetric response of Yamada's indicator dried in the detection zones. In (A) pH 9.3 borate buffer and pH 4.00 acetate buffer were used. In (B) pH 7.00 and 6.00 phosphate buffer were used. (C) Shows the SWVs recorded for 0.5 mM FcTMA⁺ and methyl paraben in 0.1 M KCl and pH 7.00 or pH 4.00 buffer stored in the reagents zones and the corresponding colorimetric response for these pH conditions.

FcTMA⁺ Calibration

Lastly, to demonstrate the analytical utility of the hybrid Janus-ePAD, a calibration curve for FcTMA⁺ was generated. Figure 4.9 shows the peak currents measured with CV for the oxidation of 0.25mM to 1.0 mM FcTMA⁺ in 0.1 M KCl. CVs were recorded one min after sample injection, compared to 45 min required in the one layer Janus-ePADs. The peak currents were also in agreement with those predicted by the Randles-Sevcik equation for a diffusion limited, one-electron process indicating bulk solution electrochemistry is achieved at the SPCE surface within the channel gap.²⁷ Good correlation (correlation coefficient = 0.993) between peak current and concentration was obtained over the tested concentration range.



Figure 4.9 0.025 - 1.0 mM FcTMA⁺ calibration curve generated using CV in the hybrid JanusePAD (N = 3 devices, 6 WEs) in 0.1 M KCl at a scan rate of 100 mV s⁻¹.

4.5 Conclusions

In this chapter, a second generation hybrid Janus-ePAD was developed. The device is fabricated using traditional ePAD fabrication methods such as wax-printing and stencil-printing with the addition of multiple layers of double-sided adhesive to adhere the paper device component to a transparency film component for sample flow within the channel gap. Flow rates within the 3-dimensional channels were slower than those predicted and observed in straight channel devices

due to changing channel geometries. However, device wet-out times are about 20 times faster in the hybrid Janus-ePAD (230 µm gap height) than in the one layer Janus-ePAD when all SPCE components are present. Since SPCEs can be printed on both device layers, more options for SPCE placement are available allowing for the removal of the SPCE printed across the inlet of the electrochemical detection zone which further impeded fluid imbibition in the one layer device. In situ pH adjustment was visualized colorimetrically by drying a universal pH indicator onto paper channels and quantified with a pH sensitive electrochemical probe, revealing heterogeneous pH adjustment of an injected buffer with strong base due to lack of efficient mixing. As an alternative, impregnating the paper with buffer for in situ pH adjustment was shown to be effective for a wide range of pH conditions. Finally, bulk solution electrochemistry of FcTMA⁺ was achieved at SPCEs located in the device channels. This work represents a simple fabrication method for significant improvement in the analytical utility of Janus-ePADs. The device can be adopted for a wide variety of analyses where different pH conditions are required for the optimal electrochemical detection of each analyte present in a mixture. Examples include heavy metal detection, multiplexed enzymatic assays, and clinical diagnoses of biologically relevant analytes.

REFERENCES

1.Noviana, E.; Klunder, K. J.; Channon, R. B.; Henry, C. S., Thermoplastic Electrode Arrays in Electrochemical Paper-Based Analytical Devices. *Analytical Chemistry* **2019**, *91* (3), 2431-2438. 2.Noviana, E.; McCord, C. P.; Clark, K. M.; Jang, I.; Henry, C. S., Electrochemical paper-based devices: sensing approaches and progress toward practical applications. *Lab on a Chip* **2019**, *20* (1), 9-34.

3.Cunningham, J. C.; DeGregory, P. R.; Crooks, R. M., New functionalities for paper-based sensors lead to simplified user operation, lower limits of detection, and new applications. *Annual Review of Analytical Chemistry* **2016**, *9*, 183-202.

4. Nantaphol, S.; Kava, A. A.; Channon, R. B.; Kondo, T.; Siangproh, W.; Chailapakul, O.; Henry, C. S., Janus electrochemistry: Simultaneous electrochemical detection at multiple working conditions in a paper-based analytical device. *Analytica Chimica Acta* **2019**, *1056*, 88-95.

5.Adkins, J.; Boehle, K.; Henry, C., Electrochemical paper-based microfluidic devices. *Electrophoresis* **2015**, *36* (16), 1811-1824.

6.Adkins, J. A.; Noviana, E.; Henry, C. S., Development of a Quasi-Steady Flow Electrochemical Paper-Based Analytical Device. *Analytical chemistry* **2016**, *88* (21), 10639-10647.

7.Adkins, J. A.; Boehle, K.; Friend, C.; Chamberlain, B.; Bisha, B.; Henry, C. S., Colorimetric and electrochemical bacteria detection using printed paper-and transparency-based analytic devices. *Analytical chemistry* **2017**, *89* (6), 3613-3621.

8. Punjiya, M.; Moon, C. H.; Matharu, Z.; Nejad, H. R.; Sonkusale, S., A three-dimensional electrochemical paper-based analytical device for low-cost diagnostics. *Analyst* **2018**, *143* (5), 1059-1064.

9.Nie, Z.; Nijhuis, C. A.; Gong, J.; Chen, X.; Kumachev, A.; Martinez, A. W.; Narovlyansky, M.; Whitesides, G. M., Electrochemical sensing in paper-based microfluidic devices. *Lab on a Chip* **2010**, *10* (4), 477-483.

10.Carrell, C.; Kava, A.; Nguyen, M.; Menger, R.; Munshi, Z.; Call, Z.; Nussbaum, M.; Henry, C., Beyond the lateral flow assay: A review of paper-based microfluidics. *Microelectronic Engineering* **2019**, *206*, 45-54.

11.Rezk, A. R.; Qi, A.; Friend, J. R.; Li, W. H.; Yeo, L. Y., Uniform mixing in paper-based microfluidic systems using surface acoustic waves. *Lab on a Chip* **2012**, *12* (4), 773-779.

12.Fridley, G. E.; Le, H. Q.; Fu, E.; Yager, P., Controlled release of dry reagents in porous media for tunable temporal and spatial distribution upon rehydration. *Lab on a Chip* **2012**, *12* (21), 4321-4327.

13.Houghtaling, J.; Liang, T.; Thiessen, G.; Fu, E., Dissolvable bridges for manipulating fluid volumes in paper networks. *Analytical chemistry* **2013**, *85* (23), 11201-11204.

14.Lafleur, L.; Stevens, D.; McKenzie, K.; Ramachandran, S.; Spicar-Mihalic, P.; Singhal, M.; Arjyal, A.; Osborn, J.; Kauffman, P.; Yager, P., Progress toward multiplexed sample-to-result detection in low resource settings using microfluidic immunoassay cards. *Lab on a Chip* **2012**, *12* (6), 1119-1127.

15.Cate, D. M.; Adkins, J. A.; Mettakoonpitak, J.; Henry, C. S., Recent developments in paperbased microfluidic devices. *Analytical chemistry* **2015**, *87* (1), 19-41. 16.Washburn, E. W., The dynamics of capillary flow. *Physical review* **1921**, *17* (3), 273. 17.Camplisson, C. K.; Schilling, K. M.; Pedrotti, W. L.; Stone, H. A.; Martinez, A. W., Two-ply channels for faster wicking in paper-based microfluidic devices. *Lab on a Chip* **2015**, *15* (23), 4461-4466.

18.Channon, R. B.; Nguyen, M. P.; Henry, C. S.; Dandy, D. S., Multilayered Microfluidic Paper-Based Devices: Characterization, Modeling, and Perspectives. *Analytical Chemistry* **2019**, *91* (14), 8966-8972.

19.Lemay, S. G.; van den Broek, D. M.; Storm, A. J.; Krapf, D.; Smeets, R. M. M.; Heering, H. A.; Dekker, C., Lithographically fabricated nanopore-based electrodes for electrochemistry. *Analytical Chemistry* **2005**, *77* (6), 1911-1915.

20.Jahanshahi-Anbuhi, S.; Kannan, B.; Pennings, K.; Ali, M. M.; Leung, V.; Giang, K.; Wang, J.; White, D.; Li, Y.; Pelton, R. H., Automating multi-step paper-based assays using integrated layering of reagents. *Lab on a Chip* **2017**, *17* (5), 943-950.

21.Berg, K. E.; Adkins, J. A.; Boyle, S. E.; Henry, C. S., Manganese Detection Using Stencilprinted Carbon Ink Electrodes on Transparency Film. *Electroanalysis* **2016**, *28* (4), 679-684. 22.Jang, I.; Berg, K. E.; Henry, C. S., Viscosity Measurements Utilizing a Fast-Flow

Microfluidic Paper-Based Device. Sensors and Actuators B: Chemical 2020, 128240.

23.Channon, R. B.; Nguyen, M. P.; Scorzelli, A. G.; Henry, E. M.; Volckens, J.; Dandy, D. S.; Henry, C. S., Rapid flow in multilayer microfluidic paper-based analytical devices. *Lab on a Chip* **2018**, *18* (5), 793-802.

24.Gong, M. M.; Sinton, D., Turning the page: advancing paper-based microfluidics for broad diagnostic application. *Chemical reviews* **2017**, *117* (12), 8447-8480.

25.Fu, E.; Ramsey, S. A.; Kauffman, P.; Lutz, B.; Yager, P., Transport in two-dimensional paper networks. *Microfluidics and nanofluidics* **2011**, *10* (1), 29-35.

26.Naik, K. M.; Nandibewoor, S. T., Electroanalytical method for the determination of methylparaben. *Sensors and Actuators A: Physical* **2014**, *212*, 127-132.

27.Bard, A. J.; Faulkner, L. R.; Leddy, J.; Zoski, C. G., *Electrochemical methods: fundamentals and applications*. wiley New York: 1980; Vol. 2.

28.Kava, A. A.; Beardsley, C.; Hofstetter, J.; Henry, C. S., Disposable Glassy Carbon Stencil Printed Electrodes for Trace Detection of Cadmium and Lead. *Analytica Chimica Acta* **2019**. 29.Angelov, T.; Vlasenko, A.; Tashkov, W., HPLC determination of pKa of parabens and investigation on their lipophilicity parameters. *Journal of liquid chromatography & related technologies* **2007**, *31* (2), 188-197.

30.Read, T. L.; Bitziou, E.; Joseph, M. B.; Macpherson, J. V., In situ control of local pH using a boron doped diamond ring disk electrode: optimizing heavy metal (mercury) detection. *Analytical chemistry* **2013**, *86* (1), 367-371.

31. Harris, D. C., Quantitative chemical analysis. Macmillan: 2010.

CHAPTER 5: DISPOSABLE GLASSY CARBON STENCIL-PRINTED ELECTRODES FOR TRACE DETECTION OF CADMIUM AND LEAD

5.1 Chapter Overview

Cadmium (Cd) and lead (Pb) pollution are significant environmental and human health concerns, and methods to detect Cd and Pb at the point-of-need (PON) are valuable. Stencil-printed carbon electrodes (SPCEs) are an attractive electrode material for PON applications due to their low cost, ease of fabrication, disposability and portability. At present, SPCEs are nearly exclusively formulated from graphitic carbon powder and conductive carbon ink. However, graphitic carbon SPCEs are not ideal for heavy metal sensing due to the heterogeneity of graphitic SPCE surfaces. Moreover, SPCEs typically require extensive modification to provide suitable detection limits and sensitivity at the PON, significantly increasing cost and complexity of analysis. While there are many examples of chemically modified SPCEs, the bulk SPCE composition has not been studied for heavy metal detection. Here, a glassy carbon microparticle stencil-printed electrode (GC-SPE) was developed. The GC-SPEs were first characterized with SEM and cyclic voltammetry and then optimized for Cd and Pb detection with an in situ plated Bi-film. The GC-SPEs require no chemical modification or pretreatment significantly decreasing the cost and complexity of fabrication. The detection limits for Cd and Pb were estimated to be 0.46 μ g L⁻¹ and 0.55 μ g L⁻¹, respectively, which are below EPA limits for drinking water (5 μ g L⁻¹ Cd and 10 µg L⁻¹ Pb).¹ The reported GC-SPEs are advantageous with their low cost, ease of fabrication and use, and attractive performance. The GC-SPEs can be used for low-level metal detection at the PON as shown herein. This work was carried out under the guidance of several people at Access Sensor Technologies (AST) in Fort Collins, CO. Those that contributed support include Dr. Josephine Hofstetter, Dr. Tom Reilly, Dr. John Wydallis, Dr. Dan Miller-Leonberg and Kai Fuller. Chloe Beardsley, an undergraduate at CSU and an intern at AST developed the soil extraction method and prepared the soil extractions for Cd and Pb quantification. For clarity, the main text of the publication is included along with the some of the supporting figures throughout. The remaining supporting figures are provided in Appendix I of this thesis. The work presented in this chapter is published in *Analytica Chimica Acta*.²

5.2 Introduction

Heavy metal pollution, originating from both natural and industrial sources, is a significant human and environmental health concern. Heavy metals are persistent in the environment, non-biodegradable, and bioaccumulate.³ While several heavy metals are essential for biochemical and physiological pathways, other heavy metals such as cadmium (Cd) and lead (Pb) are highly toxic and serve no biochemical function.⁴ Cd and Pb accumulate in human tissues, adversely affecting numerous organs and organ systems causing respiratory, cardiovascular, renal, neurological, developmental, reproductive, and hematological symptoms.^{3, 5} As a result, federal agencies have set strict limits and regulations on the acceptable levels of Cd and Pb in media including water, soil and air in both environmental and occupational settings.^{6, 7} Since distribution of Cd and Pb in the environment is widespread, these metals should be continuously monitored.³

Atomic absorption spectroscopy (AAS), inductively coupled plasma mass spectrometry (ICP-MS) and variations thereof are the certified, standard methods for highly sensitive Cd and Pb determination in a variety of complex sample matrices.⁸⁻¹⁰ However, these techniques are not suitable for large scale environmental studies or on-site monitoring. Samples must be distributed to a centralized laboratory and analyzed by trained personnel. Therefore, the time to answer is slow and costs are high using conventional methods.¹¹ Electrochemical techniques including

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potentiometry and anodic/cathodic stripping voltammetry are beneficial when rapid, on-site determination of Cd and Pb is necessary. With portable, miniaturized instrumentation becoming more accessible, electrochemical methods are well suited for PON monitoring of Cd and Pb, providing fast analysis times (e.g. several min) with minimal training.^{12, 13} Commercially available ion selective electrodes (ISEs) provide detection of limits of 10 μ g L⁻¹ and 210 μ g L⁻¹ for Cd and Pb, respectively.^{14, 15} These detection limits are not relevant in many applications. For example, 5 μ g L⁻¹ Cd and 10 μ g L⁻¹ Pb are the maximum permissible levels in drinking water.¹⁶ In addition, multiplexing is difficult because different ISEs are required for the detection of various ions.

Anodic stripping voltammetry (ASV) is widely used in trace and ultra-trace metal determination. Historically, mercury (Hg) (mercury film or hanging mercury drop electrode) was the standard electrode material for stripping voltammetry.^{17, 18} Hg provides a wide negative potential window, which is useful for the large negative potentials required to deposit metals. Hg forms a stable amalgam with other metals and multiplexed detection of up to six metals is possible. In optimized systems, ASV can provide as low as 10⁻¹¹ M detection limits.^{11, 17} However, Hg is a toxic metal, and its use has been banned and/or restricted in certain countries.¹⁹ Significant efforts have been made to construct alternative, non-toxic, electrode materials. In 2000, Joseph Wang and coworkers reported the first bismuth-film electrode. In this work, bismuth (Bi) was deposited in situ onto a glassy carbon electrode (GCE), providing similar stripping performance to Hg-film electrodes.¹⁷ Bi-film GCEs are attractive for trace metal analysis since Bi forms stable alloys with other metals, is non-toxic, and Bi-film GCEs are not sensitive to dissolved oxygen, eliminating the need to purge solutions prior to analysis.^{17, 20} However, macro GCEs are not suitable for PON detection because they are bulky, non-disposable, expensive, and require large sample volume.

Stencil-printed carbon electrodes (SPCEs), a common composite carbon electrode material, have been widely adopted for PON testing of metals. SPCEs are inexpensive, simple to fabricate, mass producible, require small sample volumes (e.g. $< 100 \mu$ L), and disposable.^{13,21} Moreover, carbon composite electrodes are frequently integrated with both traditional and paper-based microfluidics, with the goal of increasing portability and field use.^{22, 23} Several reports performed ASV with Bifilm SPCEs. However, Bi-film SPCEs have displayed a number of undesirable characteristics including double stripping peaks for a single metal, poor resolution of neighboring metal peaks, high background currents and lower sensitivities than macro GCEs.²⁴⁻²⁷ Graphite powders are exclusively employed in SPCE fabrication, yet graphite is inherently heterogeneous, with both edge and basal planes exposed, resulting in heterogeneous electrochemical activity at a single electrode surface.^{28, 29} The heterogeneity is suggested to contribute to the aforementioned problems with stripping voltammetry.¹⁸ While glassy carbon is also a form of graphitic carbon, the microstructure differs from graphite and Bi-film GCEs provide single, well resolved stripping peaks for Cd and Pb detection.¹⁷ Significant work has been done to overcome the problems with Bi-film SPCEs by modifying the electrode surface with nanomaterials and/or polymers such as silver nanoparticle/Bi/Nafion,²⁴ Nafion/ionic liquid/Graphene,³⁰ multiwalled carbon nanotubes,³¹ and many others.³² Cd and Pb quantification has been successful with these methods, nevertheless, chemical modification is complex, time consuming, expensive, and often reduces electrode stability.

To date, there are no published works studying the use of other carbon materials and/or graphite types for the SPCE bulk electrode composition for Cd and Pb detection. There are a large number of reports on modifying the surface of SPCEs with alternative forms of carbon.³³⁻³⁶ However, modifying the bulk material is of interest since there are many forms of carbon available

for fabricating carbon composite electrodes, including SPCEs. Here, we developed a glassy carbon microparticle stencil-printed electrode (GC-SPE). The proposed GC-SPE is similar to macro GCEs for the detection of Cd and Pb in terms of stripping peak characteristics, allowing an inexpensive, simple, disposable and portable detection platform. The GC-SPEs were fabricated in the same fashion as traditional SPCEs; GC microparticles were mixed with a commercial ink, then printed through a stencil onto a polyethylene terephthalate (PET) substrate. Electrochemical characteristics including solvent window, double layer capacitance, and cyclic voltammetry of several redox mediators, both surface sensitive and insensitive, are discussed. Next, the optimal detection conditions for square wave anodic stripping voltammetry (SWASV) for trace detection of Cd and Pb with the GC-SPEs are presented. Here, the GC-SPE is capable of rapid, sensitive, and simultaneous detection of Cd and Pb. Detection limits were estimated based on 3S/N as 0.46 μg L⁻¹ (Cd) and 0.54 μg L⁻¹ (Pb) after 6 min deposition.³⁷ The system was then used to determine Cd and Pb in soil extractants. This work provides steps toward simplified fabrication of stencilprinted electrodes for ASV of heavy metals, whereby the carbon type can be tailored to improve the performance of this class of electrodes for PON applications.

5.3 Experimental

Reagents and Materials

Potassium chloride (KCl) and glacial acetic acid (TraceMetalTM Grade) were purchased from Fisher Scientific (New Jersey, USA). Potassium hexacyanoferrate(III) (K₃Fe(CN)₆), potassium hexacyanoferrate(II) trihydrate (K₄Fe(CN)₆), sodium hydroxide (NaOH, 99.99% trace metal basis), atomic absorption spectroscopy standards of Bi, Cd, and Pb (1000 mg L⁻¹ in 5 wt% nitric acid), and certified reference standard soil (Trace metals sand 1) were purchased from Sigma-Aldrich. p-Aminophenol (pAP), glassy carbon microparticles (spherical, $0.4 - 12 \mu m$) and glassy carbon microparticles (spherical, $10 - 20 \ \mu m$) were purchased from Alfa-Aesar (Massachusetts, USA). Commercial carbon ink (E3178) was purchased from Ercon Inc. (Massachusetts, USA). Silver (Ag) paint was purchased from SPI supplies (Pennsylvania, USA). Silver/Silver Chloride ink was purchased from Gwent Group (Torfean, UK). Ferrocenylmethyl trimethylammonium hexafluorophosphate (FcTMA⁺) was synthesized in house using a previously reported method.³⁸ All solutions were prepared fresh daily using 18.2 M Ω ·cm water purified using a Milli-Q system. Transparency film (polyethylene terephthalate/PET) and double-sided adhesive (467 MP) were purchased from 3M (Minnesota, USA). The electrode stencil and solution reservoir were designed using CorelDRAW (Corel; Ontario, Canada) and cut using a 30 W Epilog Engraver Zing CO₂ laser cutter and engraver (Colorado, USA). Carbon electrodes (ItalSens IS-C) were purchased from Basi Inc. (Indiana, USA). The stir plate (EquathermTM, 267-914) used in stirred ASV experiments was purchased from Great Lakes Graphite (Ontario, Canada). Soil samples (#14, #21, and Gold King Mind (GKM)) were obtained from commercial and private sources.

GC-SPE and SPCE Fabrication

The GC-SPEs were fabricated according to a previously reported three electrode cell design which was laser cut into a PET sheet to form the stencil.^{24, 33} The fabrication scheme is shown in Figure S5.1. To investigate the effect of particle size on electrochemical performance of GC-SPEs, two different GC microparticle sizes, $0.4 - 12 \mu m$ and $10 - 20 \mu m$, were used for fabrication of GC-SPEs. A hand-mixed composite of GC microparticles and commercial carbon ink (Ercon) (0.8 g ink to 1.0 g GC) was then stencil-printed onto a second PET sheet. This ratio was chosen due to the best consistency of the ink for stencil printing. The electrodes were then cured in a 65 °C oven for 30 min. The working electrode (WE) was 3 mm in diameter. Both WE

and the counter electrode (CE) were GC-SPEs. Ag/AgCl ink (Gwent) was painted onto the exposed GC-SPE surface to serve as the pseudo-reference electrode (RE). Ag/AgCl ink produced the most stable potential over time compared to a GC-SPE RE and Ag paint RE (SPI). The same procedure was carried out to fabricate Ercon ink only SPCEs and graphite SPCEs (3.5 g ink to 2.0 g graphite). To fabricate the solution reservoir, a PET sheet was sandwiched between two layers of double-sided adhesive (3M) and laser cut. The solution reservoir was attached to the SPEs via the double-sided adhesive and aligned with the electrode connection pads to define a reproducible interelectrode surface area of 0.112 cm^2 . The solution volume required to fill the solution reservoir was 50 µL.

Electrode Characterization

All electrochemical experiments were carried out using a CH Instruments 660B potentiostat (Texas, USA) at room temperature ($22 \pm 1 \, ^{\circ}$ C). For electrode characterization, Ag/AgCl ink served as the RE, a GC-SPE served as the CE, and all solutions contained 0.1 M KCl as the supporting electrolyte. A solution volume of 50 µL was used to fill the solution reservoir. Cyclic voltammetry experiments were carried out with 1.0 mM FcTMA⁺, 1.0 mM Fe(CN)₆³⁻ /Fe(CN)₆⁴⁻ and 0.5 mM pAP at a scan rate of 100 mV s⁻¹. Solvent windows were recorded in 0.1 M KCl (pH 6.5) at a scan rate of 200 mV s⁻¹ between +2.0 and -2.0 V. Capacitance measurements were carried out in 0.1 M KCl at scan rate of 100 mV s⁻¹ between +0.1 and -0.1 V for a total of 10 cycles. The electrodes were imaged using a JSM-6500F field emission scanning electron microscope (JEOL, Tokyo, Japan) with a 2 kV acceleration voltage.

Cd and Pb Detection

The RE used was either a saturated calomel electrode (SCE) or Ag/AgCl ink. The Ag/AgCl was compared to the SCE to demonstrate the feasibility of these sensors for in field use. pH optimization studies for ASV were carried out in 0.1 M acetate buffers with pH values varying from pH 3.6 to 5.5. All metal (Cd, Pb, and Bi) solutions were prepared by diluting the AAS standards in acetate buffer. A deposition potential of -1.2 V and -1.4 V vs. SCE and Ag/AgCl were used for deposition in ASV, respectively. In SWV the optimized parameters of the waveform were a frequency of 14 Hz, an amplitude of 80 mV, and an increment of 20 mV. The Bi-film was plated in situ by spiking the Cd and Pb solutions with the appropriate concentrations of Bi. Cd and Pb were extracted from the real soil samples using a previously described extraction method.³⁹ Standard addition was used to quantify Cd and Pb in the extraction solutions. 3 μ M Fe(CN)₆³⁻ was added to the standard addition solutions to remove copper (Cu) interference as previously reported.²⁴ To generate a calibration curve in stirred solutions, the stir plate was set to 5.

5.4 Results and Discussion

Reference Electrode Stability

ASV consists of a two-step process. The first step requires the electrodeposition of labile or free metal ion to its zero-valence state on the electrode surface for preconcentration. The second step is the subsequent oxidation, or stripping, of metal from the electrode surface. The stripping peak current is proportional to the concentration of analyte, which is directly proportional to the amount of analyte deposited on the electrode surface during the deposition step. The deposition step is typically carried out at a potential more negative than the most negative formal potential, E^{0° , of the metal ions of interest to drive the reduction and deposition of all metals being analyzed.^{40,41} The applied potential at the WE controls the extent to which metal deposition occurs;

therefore, the applied potential, defined by the RE, during a deposition step must be stable over time and consistent between devices. Conventional REs are designed so that all components that determine their potential are stable regardless of the sample, with an inner filling solution that defines the developed potential separated from the sample electrolyte. However, stencil-printed REs are directly exposed to the sample electrolyte. When a RE lacks an inner filling solution, and is in direct contact with the sample solution, such as the stencil-printed REs, it is termed a quasi-RE. Quasi-REs do not have a well-defined potential and the potential can drift over time even in a defined electrolyte solution.^{9, 42, 43} Since a stable potential at the WE is critical for reproducible ASV measurements, the stability of several stencil-printed REs were tested. The stability of a GC-SPE RE, Ag paint RE, Ag/AgCl paint RE, and a DropSens Ag RE were determined by recording their open circuit potentials as a function of time in pH 3.6 acetate buffer versus an SCE as shown in Figure 5.1. Here, the Ag paint (homemade RE and PalmSens RE) and GC-SPE exhibited significant potential drift, possibly due to the slight solubility of these inks in aqueous solution. The large changes in potential of 30 to 40 mV over the course of the REs' first 400 sec exposed to the electrolyte would be detrimental to reproducible electrodeposition of metal leading to poor reproducibility in the stripping peak currents. The Ag/AgCl RE produced a stable potential over time in pH 3.6 buffer, possibly due to slight dissolution of AgCl producing a very low but consistent chloride ion concentration. This is further indicated by the slight potential instability in the first 100 s (Figure 5.1D). Chloride ions could not be added directly to establish the potential of Ag/AgCl due to interference with ASV of Cd and Pb. Ag/AgCl ink was selected as the RE for further ASV experiments. It is important to note that while the inter-electrode potential defined by the Ag/AgCl ink is consistent in pH 3.6 acetate buffer, the potential was pH dependent, shifting

by about 20 mV between pH 3.6 and pH 4.0 (Figure 5.1D). Since Ag/AgCl provides a stable potential in defined electrolyte solution, it is termed a pseudo-RE.



Figure 5.1 Open circuit potential recorded in 0.1 M acetate buffer, pH 3.6, for (a) an Ag paint RE, (b) an Ag PalmSens RE, (c) a GC-SPE RE and (d) Ag/AgCl ink RE (black) and an Ag/AgCl RE in 0.1 M pH 4.0 buffer (gold).

Electrochemical Characterization and Imaging of GC-SPEs

To investigate which GC microparticle size provides better electrochemical performance, cyclic voltammetry (CV) was used to determine peak-to-peak separation (ΔE_p) and peak currents of several well understood redox mediators. First, CVs of Fe(CN)₆³⁻/Fe(CN)₆⁴⁻ were obtained (Figure 5.2A). Fe(CN)₆³⁻/Fe(CN)₆⁴⁻ is an electrochemically reversible redox species, however, it has been shown to undergo a surface sensitive electrochemical reaction and irreversible voltammetry is often observed at carbon electrode materials.^{22, 28, 29} Here, the measured ΔE_p values were 441 ± 12 mV and 512 ± 7 mV for the 0.4 – 12 µm and 10 – 20 µm GC-SPEs respectively.

The large peak separations for both are likely a result of deactivation of the electrode surface due to the ink coating as well as ohmic resistance effects.^{13,44,45} A scan rate study was carried out with each electrode type as shown in Figure S5.2. The peak separation of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ increases with increasing scan rate at both electrode types, however, peak separation s at the $10 - 20 \,\mu m$ GC-SPE exhibited greater dependence on the scan rate than the smaller particle size. CV of pAP, an electrochemically reversible but surface sensitive redox species is shown in Figure 5.2B. The measured ΔE_p values were 246 ± 14 and 421 ± 18 mV for the 0.4 – 12 µm and 10 – 20 µm GC-SPEs respectively, again indicating lower electrochemical activity of the $10 - 20 \,\mu m$ GC-SPE. Next, CV of FcTMA⁺ was investigated as shown in Figure 5.2C. FcTMA⁺ is surface insensitive and undergoes an outer-sphere electron transfer reaction.⁴⁶ Therefore, the voltammetry is insensitive to the electronic and chemical structure of the electrode surface and Nernstian behavior is observed at sufficiently conductive electrode materials.^{46, 47} Here, ΔE_p values were $107 \pm 8 \text{ mV}$ and 105 ± 15 mV for the 0.4 – 12 µm and 10 – 20 µm GC-SPEs respectively. There is no difference in FcTMA⁺ voltammetry between the two electrodes, indicative of the surface insensitive electron transfer reaction of FcTMA⁺.⁴⁶ The peak separations indicate ohmic resistance effects due to the lower conductivity associated with composite electrode materials.48 Since FcTMA+ is an electrochemically reversible redox probe, an ideal peak-to-peak separation of 59 (±10) mV is expected under diffusion limited conditions.^{46, 49} The 0.4 – 12 μ m GC-SPE outperformed the $10 - 20 \ \mu m$ GC-SPE indicated by the smaller ΔE_p values and higher peak currents obtained for both surface sensitive species. Therefore, the $0.4 - 12 \mu m$ GC-SPE was selected for further studies. We hypothesize that the smaller particle size outperforms the larger particle size due to easier mixing of the particles and the ink, producing better particle to particle contact and reduced ohmic resistance effects.50



Figure 5.2 Cyclic voltammograms recorded at a $0.4 - 12 \ \mu m$ (gold traces) and $10 - 20 \ \mu m$ (blue traces) GC-SPE in (a) 1 mM Fe(CN)₆³⁻/Fe(CN)₆⁴⁻ (b) 0.5 mM pAP and (c) 1 mM FcTMA⁺ in 0.1 M KCl. (d) Solvent windows recorded with the two GC-SPE types and a homemade SPCE (MG1599) in 0.1 M KCl, pH 6.5.

The HER and oxygen reduction reaction (ORR) are typical reactions occurring in the cathodic potential region employed in ASV, and can have deleterious effects on both the deposition and stripping of Cd and Pb.^{9, 17} Therefore, both electrochemical reactions must be considered when selecting an electrode material for ASV. The solvent window for each type of GC-SPE was measured and compared to a homemade SPCE (MG1599) (Figure 5.1D). Here, both GC-SPE solvent windows are almost featureless with only a small background peak at ~1 V, which is attributed to sp² carbon oxidation.⁴⁶ The SPCE current density for hydrogen evolution (HER) at potentials negative of -1.5 V is significantly greater than both GC-SPEs. HER is a catalytic reaction and requires surface binding sites to occur to an appreciable extent. Greater HER currents at the graphite SPCE indicates the presence of a higher density of catalytic sites, likely on graphite edges.^{28, 46} Additionally, a reduction wave for ORR is not observed at the GC-SPE. Figure 5.3. shows the potential windows of a macro GCE, a GC-SPE, and the graphite SPCEs used in previous
works for ASV of Cd and Pb.^{51, 52} At the macro-GCE, ORR was observed at an onset potential of \sim -1.5 V. These results indicate the GC-SPE has a wider operational cathodic potential window than graphite SPCEs and macro GCEs, rendering the GC-SPE suitable for ASV.



Figure 5.3 Solvent windows recorded in 0.1 M KCl (pH 5.5) for a 0.4 to 12 μ m GC-SPE (grey line), macro glassy carbon electrode (green line) and homemade SPCE (red line).

The background current is an important parameter to consider in electrochemical sensing applications as it limits the achievable detection limits.⁴⁶ The capacitance of an electrode contributes significantly to the background current; therefore, the capacitance of the GC-SPEs was measured experimentally by recording CVs in 0.1 M KCl between -0.1 and 0.1 V as shown in Figure S5.3. Equation 5.1 was used to calculate the apparent capacitance of the GC-SPEs ($0.4 - 12 \mu m$ and $10 - 20 \mu m$), traditional SPCEs, and a macro GCE.

$$C_{dl} = \frac{i_{average}}{vA}$$
 Equation 5.1

 $i_{average}$ is the average absolute value of the measured anodic and cathodic currents in A, v is the scan rate in V/s and A_{geometric} is the geometric surface area in cm².⁴⁷ C_{dl} values for the GC-SPEs were 6.05 ± 0.59 µF cm⁻² and 6.60 ± 1.21 µF cm⁻² for the 0.4 – 12 µm and 10 – 20 µm GC-SPEs

respectively. The low capacitance obtained is due to the low fraction of electrochemically reactive (GC) surface area in contact with solution, while the remainder of the surface is occupied by the ink.²⁸ This low capacitance is a highly desirable property for sensing applications as it directly effects the signal to noise characteristics. The experimentally measured capacitance of a macro GCE was 66.1 μ F cm⁻². This large capacitance is likely a result of polishing the electrode, which can increase the number of redox active moieties on the electrode surface. Commercially available SPCEs have capacitance around 37 μ F cm⁻² which is consistent with the data presented in Figure 5.5.⁴⁴ The MG1599 had a measured capacitance of 7.95 ± 0.59 μ F cm⁻².

Scanning electron microscopy (SEM) was used to image the surface morphology of the 0.4 $-12 \mu m$ and the SPCE as shown in Figure 5.4. The GC particles appear as smooth spheres (Figure 5.4A), while the graphitic flakes appear rougher and both basal and edge planes are visible. The surface of the outermost GC particles do not all appear to be uniformly coated in the ink. However, the coating on the graphite particles on the SPCE surface appear to be thinner and more homogeneous, and a much higher magnification is required to image uncoated particles (Figure 5.4B inset). We hypothesize that the difference in particle morphology clearly evident in the SEM images contributes to the stripping behavior shown in Figure 5.4.



Figure 5.4 SEM images of (a) $0.4 - 12 \mu m$ GC-SPE and (b) the MG1599 SPCE.

To compare the behavior of the GC-SPEs for ASV to conventional stencil-printed electrode materials, SWASV was carried out with four different electrode types: 1) GC-SPE, 2) SPCE (MG-1599), 3) Ercon ink only SPCE, and 4) PalmSens SPCE as shown in Figure 5.5. Here, the GC-SPE produces symmetric and well-resolved peaks for stripping voltammetry of Cd and Pb at -0.87 V and -0.65 V (vs Ag/AgCl) respectively. In contrast, the stripping peaks for Cd and Pb obtained using the homemade graphite SPCE are split into unresolved double peaks indicated by the small shoulder on the Cd peak at about -0.88 V and the broad double peaks obtained for Pb at about -0.65 V and -0.59 V. These results are indicative of Cd and Pb being deposited and subsequently stripped from different chemical environments, possibly the basal and edge planes of the graphite.^{24, 53, 54} These double peaks would likely become more evident at lower concentrations, as larger peaks obscure this effect.⁵⁴ Stripping peaks are observed for the Ercon ink SPCE, however, the peaks are not well resolved and quantification via SWASV of Cd and Pb at this SPCE material without further modification would be difficult. Cd and Pb stripping peaks are also evident with the PalmSens electrode, however, in contrast to all other SPCE types, the background current is an order of magnitude higher, significantly affecting the achievable signal-to-noise ratio. The increase is likely a result of the higher capacitance of commercial SPCEs compared to the GC-SPEs as discussed previously.⁴⁴ A high background and low signal to noise ratio is undesirable in electrochemical sensing as the detection limit is calculated using this parameter.³⁷ Here, the GC-SPE provides the highest currents for SWASV for the simultaneous detection of Cd and Pb compared to the other SPCE types as evident in the voltammograms shown in Figure 5.5.



Figure 5.5 SWASV for a 100 μ g L⁻¹ solution of Cd and Pb in 0.1 M acetate buffer (pH 3.6) with an in situ plated Bi-film GCSPE (blue line), SPCE (green line), Ercon ink only SPCE (gold line) and PalmSens SPCE (black line). Deposition time for all ASVs was 3 min.

Cd and Pb Detection

Prior to generating calibration curves for Cd and Pb with the GC-SPE, we first optimized the SWV parameters and solution conditions. The square wave frequency, amplitude, increment and deposition time were selected for optimal reproducibility and maximum peak currents while still retaining well-resolved peaks. The optimal square wave parameters of 80 mV for the amplitude, 20 mV for the increment, and 14 mV for the frequency were selected (Figure 5.6). While the peak current increased at higher frequencies ($i_p \propto f^{1/2}$), higher frequencies also increased the peak width, decreasing Cd and Pb peak resolution.⁴⁰ We selected a deposition time of 6 min. While sensitivity increases at increasing deposition times and we did not observe saturation up to 15 min deposition times, we chose 6 min to minimize analysis time and maintain sensitivity. Deposition time optimization data is shown in Appendix I (Figure S5.4).



Figure 5.6 Optimization of a) amplitude, b) increment and c) frequency for square wave stripping voltammetry of 100 μ g L⁻¹Cd and Pb in 0.1 M acetate buffer, pH 3.6. Deposition time 180 seconds.

The concentration of Bi precursor present in the metal plating solution has been shown to be a critical parameter for optimal SWASV sensitivity. We hypothesize this to be a result of the thickness of the Bi film formed during the deposition step.⁵⁵ As demonstrated previously, the Bi to Cd and Pb ratio has significant effects on the sensitivity of the stripping peak signals. For ASV with carbon paste electrodes (CPEs), the optimal Bi to Cd and Pb ratio has been shown to range between 1 and 10, with a decrease in sensitivity at ratios greater than 10.55 To study these effects at GC-SPEs, the Bi ion concentration in the Cd and Pb plating solution was varied from 1 to 5 mg L^{-1} . The resulting peak currents for 100 µg L^{-1} Cd and Pb are shown in Figure 5.7A. Here, the peak current reaches a maximum at 2.5 mg L⁻¹ Bi, or a Bi to Cd and Pb ratio of 12.5. At higher Bi concentrations, both peak current and reproducibility of Cd and Pb decrease. This is likely a result of increasing Bi-film thickness at high concentrations, leading to Cd and Pb occlusion during codeposition.⁵⁵ While the Bi to Cd and Pb ratio of 12.5 was optimal for 100 µg L⁻¹ Cd and Pb, this concentration is at the higher end of the concentrations we are interested in detecting, therefore, a ratio of 10 Bi to Cd and Pb (2 mg L⁻¹ Bi to 100 µg L⁻¹ Cd and Pb) was selected for calibration of Cd and Pb in the 7.5 to 200 μ g L⁻¹ range. Figure 5.7B shows the peak currents for 10 μ g L⁻¹ Cd and Pb co-deposited with varying concentrations of Bi. Again, the greatest peak currents were obtained at a Bi to metal ratio of 12.5 (250 μ g L⁻¹ Bi). Therefore, we selected 250 μ g L⁻¹ Bi for the calibration of 2.5 to 50 μ g L⁻¹ Cd and Pb.



Figure 5.7 SWASV peak currents obtained for (a) 100 μ g L⁻¹ Cd and Pb and (b) 10 μ g L⁻¹ Cd and Pb as a function of Bi precursor concentration carried out in 0.1 M acetate buffer, pH 3.6. Deposition times were 3 min and 12 min for 100 μ g L⁻¹ and 10 μ g L⁻¹ Cd and Pb respectively.

Solution chemistry, especially pH, is critical to the sensitive detection of Cd and Pb with Bi-film electrodes.⁵⁶⁻⁵⁹ Therefore, SWASV was carried out in 0.1 M acetate buffers ranging in pH from 3.6 to 5.5. The peak currents for 100 μ g L⁻¹ Cd and Pb as a function of pH are shown in Figure 5.8A. Interestingly, the highest peak currents occurred at a pH of 3.6. This diverges from the literature where acetate buffer, pH 4.5, is widely accepted as the optimal supporting electrolyte for both in situ and ex situ Bi-film electrodes.¹⁷ For further investigation, the Bi-film stripping characteristics were studied as a function of solution pH shown in Figure 5.8B and 5.8C. Here, the

peak current for Bi is significantly higher at a pH of 3.6 than all other pH conditions, decreasing exponentially as a function of increasing pH. The pH dependence of Cd and Pb ASV is likely controlled by the properties of the Bi-film formed on the GC-SPE surface. Based on the voltammetry shown in Figure 5.8B, Bi-film deposition at a pH of 3.6 yields the thickest Bi-film, which is likely more uniform, and provides a reproducible surface for Cd and Pb co-deposition. Higher pH conditions have been reported in the literature for ASV of Cd and Pb but not when employing Bi-film electrodes. For example, a copper electrode provided the greatest sensitivity in pH 5.5 acetate buffer.⁹ As mentioned previously, the HER limits the cathodic potential window for an electrode material. HER is an inner-sphere electron transfer reaction and sensitive to the electrocatalytic properties of the electrode surface, producing H₂ gas via the reduction of H⁺. At lower pH (increasing H⁺ concentration), HER is increasingly thermodynamically favorable and occurs at less negative over potentials. In ASV, significant HER is detrimental due to the formation of gas bubbles on the electrode surface which interferes with metal deposition. Moreover, Bi hydrolysis occurs in solution pHs as low as 1 to 2.60 Metal speciation, including Bi speciation, controls lability and consequently, deposition efficiency.^{32, 57, 59} To mitigate HER while maintaining the highest percentage of labile Bi in solution, pH 4.5 is typically employed in SWASV with in situ Bi-film carbon electrodes. However, HER does not occur to an appreciable extent at the GC-SPEs compared to graphite (Figure 5.2D) and macro GC electrodes (Figure 5.3). Potential windows were recorded in acetate buffer pH 3.6 to 5.5. The HER currents did not increase with acidity at the GC-SPEs. The GC-SPEs are functional in more acidic solutions, and the deposition step can be carried out in increasingly acidic conditions, increasing the stability of Bi in the plating solutions.⁹



Figure 5.8 SWASV peak currents obtained in 0.1 M acetate buffers ranging from pH 3.6 to 5.5 for (a) 100 μ g L⁻¹ Cd and Pb. SWASV results obtained for 2 mg L⁻¹ Bi plated in 0.1 M acetate buffers ranging from pH 3.6 to 5.5, (b) voltammograms and (c) peak currents.

Next, calibration curve were generated for Cd and Pb with the optimized SWASV parameters and solution conditions described above. While the GC-SPEs are disposable, it was also found that they are reusable up to at least 10 times when the electrode was cleaned between by applying a potential of +0.4 V for 100 seconds in pH 3.6 acetate buffer as shown in Figure S5.5. Therefore, we generated two sets of calibration curves for 7.5 to 200 µg L⁻¹ Cd and Pb. The first calibration curve was generated with a brand new electrode for every run (N = 3 per calibration point). In these experiments, an SCE RE was employed and the calibration curve is shown in Figure 5.9A and 5.9C. Next, a calibration curve was generated with a total of six electrodes. The electrodes were reused after applying a cleaning step between each ASV experiment in acetate buffer, pH 3.6, by holding the potential at +0.4 V for 100 seconds. One electrode was employed to test each standard concentration once. These calibration curves are shown in Figure 5.9B and 5.9D. The Ag/AgCl ink RE was used to demonstrate the applicability and reusability of the sensor for use in the field. The sensitivities of the calibration curves generated with an SCE RE and an Ag/AgCl RE were similar but statistically different for both Cd and Pb. The observed difference in sensitivities is attributed to reusing the electrodes for the Ag/AgCl calibration curve. Over the course of many ASV experiments, the GC-SPE undergoes significant cathodic polarization to deposit the metals, then is anodically polarized at +0.4 V in acetate buffer to clean the surface for

the next experiment. Electrochemical polarization at high anodic potentials generates reactive oxygen species such as O₂ and CO₂, that can further oxidize the carbon electrode surface. Also, oxidation of organic surface impurities (e.g. the ink) and their subsequent removal from the electrode surface can occur, resulting in further changes to the composite electrode surface.⁶¹ While some change in the surface chemistry is evident, the correlation for all calibration curves is greater than 0.99, and both methods are suitable for Cd and Pb calibration. As the results in Figure 5.9 show, the GC-SPEs provide sharp, well-resolved, and reproducible peaks for the simultaneous detection of Cd and Pb over a wide linear range of 7.5 to 200 µg L⁻¹ Cd and Pb. The sensitivities were 0.122 μ A L μ g⁻¹ (R² = 0.992) and 0.101 μ A L μ g⁻¹ (R² = 0.992) for Cd and Pb, respectively. The sensitivities obtained for the developed electrodes are higher than other SPCEs reported in the literature. For example, the sensitivities achieved for Cd and Pb with a graphitic Bi-film SPCE modified with multi-walled carbon nanotubes were 0.0066 μ A L μ g⁻¹ and 0.0068 μ A L μ g⁻¹, respectively.³³ The deposition time in this study was slightly shorter at 4 min, however, this small difference would not account for the almost 20 times increase in sensitivity at the GC-SPEs. Detection limits were calculated using $3\sigma/m$, where σ is the standard deviation of the background current for the lowest measured concentration and m is the slope of the calibration curve. Detection limits were obtained as 0.46 µg L⁻¹ and 0.55 µg L⁻¹ for Cd and Pb respectively. To the best of our knowledge, these detection limits have only been reported for chemically modified Bi-film SPCEs and/or bare Bi-film SPCEs operated under hydrodynamic conditions



Figure 5.9 Square wave voltammograms recorded for varying concentrations of Cd and Pb in 0.1 M acetate buffer, pH 3.6, vs. a) SCE and b) Ag/AgCl. Deposition potential was -1.4 V vs. SCE and -1.6 V vs. Ag/AgCl. Resulting calibration curves for c) Cd and Pb vs. SCE and d) Cd and Pb vs. Ag/AgCl with cleaned and reused electrodes.

In hydrodynamic conditions, the deposition step is carried out under forced convection.^{22, 23, 62} Therefore, mass transport is not limited by diffusion, deposition is more efficient, and higher sensitivities are achieved. To further increase sensitivity and lower detection limits, while also decreasing deposition time, the GC-SPEs could be adapted into portable systems that operate under flow such as traditional and paper-based microfluidics.⁶³⁻⁶⁶ To illustrate the effect of forced convection on stripping analysis at the GC-SPEs, calibration curves were generated for Cd and Pb in stirred solution. Figure 5.10 compares the calibration curves generated for Cd (Figure 5.10A) and Pb (Figure 5.10B). The sensitivity increased by a factor of ~ 6 for both Cd and Pb utilizing a 3 min deposition. The increase in sensitivity resulted in lower detection limits of 0.098 μ g L⁻¹ Cd and 0.084 μ g L⁻¹ Pb. A third calibration curve (Figure S5.6) for 2.5 to 50 μ g

 L^{-1} Cd and Pb employing 250 µg L^{-1} Bi and a 20 min deposition was generated to demonstrate the flexibility of the sensor in terms of sensitivity and detection limits by tuning the deposition parameters in quiescent solution. Under these conditions, detection limits were lowered by a factor of two to 0.18 µg L^{-1} and 0.29 µg L^{-1} for Cd and Pb, respectively.



Figure 5.10 Calibration curves for Cd (a) and Pb (b) in stirred solution (blue) and quiescent solution (red). Cd exhibited a linear relationship between peak current and concentration from 5 μ g L⁻¹ to 80 μ g L⁻¹ and 5 μ g L⁻¹ to 200 μ g L⁻¹ in stirred and quiescent solution, respectively. Pb exhibited a linear relationship from 20 μ g L⁻¹ to 100 μ g L⁻¹ and 7.5 to 200 μ g L⁻¹ in stirred and quiescent solution, respectively.

Finally, the GC-SPEs were employed for quantitative determination of Cd and Pb in several soil samples via the standard addition method. The amounts of Cd and Pb were validated using ICP-MS and the results obtained using ASV and ICP-MS are shown in Table 5.1. Prior to ASV detection the samples were exposed to a mild extraction solution that is designed for PON or in-field use with quick extraction times and low hazard. The amounts found using the mild extraction solution with ASV detection were lower than those found using ICP-MS as expected. Traditionally, samples are fully digested using strong acidic conditions at high temperatures in order to free all Cd and Pb and measure total amounts. The extraction method used here likely extracts other organic matter that in turn can complex Cd and Pb in the sample.⁵⁹ However, ASV only measures labile Cd and Pb.⁵⁹ Despite the differences observed, the concentrations of Cd and

Pb determined by ASV are comparable to those obtained with ICP and are appropriately sensitive for PON or in-field detection.

 Sample	Cd (µg L ⁻¹) ASV	Cd (µg L ⁻¹) ICP-MS	Pb (µg L ⁻¹) ASV	Pb (µg L ⁻¹) ICP-MS
Standard Soil	2630 ± 110	4080	$1410\pm\ 180$	2310
Soil Sample #14	46.3 ± 9.3	63.2	2440 ± 200	7150
Soil Sample #21	Below LOD	0	$213~\pm~58$	450
Gold King Mine	Below LOD	0	79.7 ± 31.3	121

Table 5.1 Concentrations of Cd and Pb determined by ASV and ICP-MS sample extractants.

5.5 Conclusions

In this chapter, the fabrication and characterization of a glassy carbon stencil-printed electrode (GC-SPE) is demonstrated for the first time. The GC-SPEs exhibit highly desirable properties for sensing the heavy metals Cd and Pb. These properties include low background and capacitive currents, inhibited hydrogen evolution reaction (HER) in acidic pH conditions, and a more homogeneous electrochemically active surface for metal deposition leading to single, well resolved peaks for the anodic stripping voltammetry of Cd and Pb. Fabrication of the GC-SPEs is simple and no pre-treatment or chemical modification is required to detect trace levels of Cd and Pb which bodes well for a PON sensor as it significantly reduces the cost of the sensor. The use of fabricate the GC-SPE lead to a cost of about \$0.25 per sensor. Further work can include the integration of the GC-SPEs into both traditional and paper-based microfluidics, permitting the deposition step to be carried out under hydrodynamic conditions, further increasing the sensitivity of Cd and Pb detection at the PON.

REFERENCES

1.EPA Drinking Water Contaminants - Standards and regulations. https://www.epa.gov/dwstandardsregulations#8.

2.Kava, A. A.; Beardsley, C.; Hofstetter, J.; Henry, C. S., Disposable Glassy Carbon Stencil Printed Electrodes for Trace Detection of Cadmium and Lead. *Analytica Chimica Acta* **2019**. 3.Tong, S.; von Schirnding, Y. E.; Prapamontol, T., Environmental lead exposure: a public health problem of global dimensions. *Bulletin of the World Health Organization* **2000**, 78 (9), 1068-1077.

4.Patra, R. C.; Ruatray, A. K.; Swarup, D., Oxidative Stress in Lead and Cadmium Toxicity and Its Amelioration. *Veterinary Medicine International* **2011**.

5.Buruiana, D. L.; Lefter, D.; Tiron, G. L.; Balta, S.; Bordei, M.; In *TOXICITY OF HEAVY METALS ON THE ENVIRONMENT AND HUMAN HEALTH*, 15th International

Multidisciplinary Scientific Geoconference (SGEM), Albena, BULGARIA, Jun 18-24; Technology Ltd: Albena, BULGARIA, 2015; pp 565-571.

6.Guidelines for Drinking-Water Quality. 4th ed.; Organization, W. H., Ed. WHO Press: Geneva, Switzerland, 2011.

7.Dobson, S., Cadmium: Environmental Aspects. World Health Organization: Geneva Switzerland, 1992.

8.Tuzen, M., Determination of heavy metals in fish samples of the middle Black Sea (Turkey) by graphite furnace atomic absorption spectrometry. *Food Chem.* **2003**, *80* (1), 119-123.

9.Kang, W. J.; Pei, X.; Rusinek, C. A.; Bange, A.; Haynes, E. N.; Heineman, W. R.; Papautsky, I., Determination of Lead with a Copper-Based Electrochemical Sensor. *Analytical Chemistry* **2017**, *89* (6), 3345-3352.

10.Goulle, J. P.; Mahieu, L.; Castermant, J.; Neveu, N.; Bonneau, L.; Laine, G.; Bouige, D.; Lacroix, C., Metal and metalloid multi-elementary ICP-MS validation in whole blood, plasma, urine and hair - Reference values. *Forensic Science International* **2005**, *153* (1), 39-44.

11.Arduini, F.; Calvo, J. Q.; Amine, A.; Palleschi, G.; Moscone, D., Bismuth-modified electrodes for lead detection. *Trac-Trends Anal. Chem.* **2010**, *29* (11), 1295-1304.

12.Martinez, A. W.; Phillips, S. T.; Whitesides, G. M.; Carrilho, E., Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices. *Analytical Chemistry* **2010**, 82 (1), 3-10.

13.Adkins, J.; Boehle, K.; Henry, C., Electrochemical paper-based microfluidic devices. *Electrophoresis* **2015**, *36* (16), 1811-1824.

14.<u>https://hannainst.com/hi4112-lead-sulfate-combination-ion-selective-electrode.html</u>. 15.Hannah, Hannah Instruments.

16.Association, W. Q., Water Quality Association.

17.Wang, J.; Lu, J. M.; Hocevar, S. B.; Farias, P. A. M.; Ogorevc, B., Bismuth-coated carbon electrodes for anodic stripping voltammetry. *Analytical Chemistry* **2000**, *72* (14), 3218-3222. 18.Hutton, L. A.; Newton, M. E.; Unwin, P. R.; Macpherson, J. V., Factors Controlling Stripping Voltammetry of Lead at Polycrystalline Boron Doped Diamond Electrodes: New Insights from High-Resolution Microscopy. *Analytical Chemistry* **2011**, *83* (3), 735-745. 19.Balistreri, E. J.; Worley, C. M., Mercury: The good, the bad, and the export ban. *Resour.*

Policy **2009**, *34* (4), 195-204.

20.Economou, A., Bismuth-film electrodes: recent developments and potentialities for electroanalysis. *Trac-Trends Anal. Chem.* **2005**, *24* (4), 334-340.

21.Dungchai, W.; Chailapakul, O.; Henry, C. S., Electrochemical Detection for Paper-Based Microfluidics. *Analytical Chemistry* **2009**, *81* (14), 5821-5826.

22.Nantaphol, S.; Channon, R. B.; Kondo, T.; Siangproh, W.; Chailapakul, O.; Henry, C. S., Boron Doped Diamond Paste Electrodes for Microfluidic Paper-Based Analytical Devices. *Analytical Chemistry* **2017**, *89* (7), 4100-4107.

23.Nie, Z. H.; Nijhuis, C. A.; Gong, J. L.; Chen, X.; Kumachev, A.; Martinez, A. W.; Narovlyansky, M.; Whitesides, G. M., Electrochemical sensing in paper-based microfluidic devices. *Lab on a Chip* **2010**, *10* (4), 477-483.

24.Mettakoonpitak, J.; Mehaffy, J.; Volckens, J.; Henry, C. S., AgNP/Bi/Nafion-modified Disposable Electrodes for Sensitive Zn(II), Cd(II), and Pb(II) Detection in Aerosol Samples. *Electroanalysis* **2017**, *29* (3), 880-889.

25.Shen, L. L.; Zhang, G. R.; Li, W.; Biesalski, M.; Etzold, B. J. M., Modifier-Free Microfluidic Electrochemical Sensor for Heavy-Metal Detection. *Acs Omega* **2017**, *2* (8), 4593-4603.

26.Martin-Yerga, D.; Alvarez-Martos, I.; Blanco-Lopez, M. C.; Henry, C. S.; Fernandez-Abedul, M. T., Point-of-need simultaneous electrochemical detection of lead and cadmium using low-cost stencil-printed transparency electrodes. *Analytica Chimica Acta* **2017**, *981*, 24-33. 27.Wang, J.; Lu, J. M.; Anik, U.; Hocevar, S. B.; Ogorevc, B., Insights into the anodic stripping voltammetric behavior of bismuth film electrodes. *Analytica Chimica Acta* **2001**, *434* (1), 29-34.

28.McCreery, R. L., Advanced carbon electrode materials for molecular electrochemistry. *Chemical Reviews* **2008**, *108* (7), 2646-2687.

29.Kneten, K. R.; McCreery, R. L., EFFECTS OF REDOX SYSTEM STRUCTURE ON ELECTRON-TRANSFER KINETICS AT ORDERED GRAPHITE AND GLASSY-CARBON ELECTRODES. *Analytical Chemistry* **1992**, *64* (21), 2518-2524.

30.Chaiyo, S.; Mehmeti, E.; Zagar, K.; Siangproh, W.; Chailapakul, O.; Kalcher, K., Electrochemical sensors for the simultaneous determination of zinc, cadmium and lead using a Nafion/ionic liquid/graphene composite modified screen-printed carbon electrode. *Analytica Chimica Acta* **2016**, *918*, 26-34.

31.Fu, L.; Li, X. L.; Yu, J. S.; Ye, J. S., Facile and Simultaneous Stripping Determination of Zinc, Cadmium and Lead on Disposable Multiwalled Carbon Nanotubes Modified Screen-Printed Electrode. *Electroanalysis* **2013**, *25* (2), 567-572.

32.Hersey, M.; Berger, S. N.; Holmes, J.; West, A.; Hashemi, P., Recent Developments in Carbon Sensors for At-Source Electroanalysis. *Analytical Chemistry* **2019**, *91* (1), 27-43. 33.Rattanarat, P.; Dungchai, W.; Cate, D.; Volckens, J.; Chailapakul, O.; Henry, C. S., Multilayer Paper-Based Device for Colorimetric and Electrochemical Quantification of Metals. *Analytical Chemistry* **2014**, *86* (7), 3555-3562.

34.Niu, X. H.; Zhao, H. L.; Lan, M. B., Disposable screen-printed antimony film electrode modified with carbon nanotubes/ionic liquid for electrochemical stripping measurement. *Electrochim. Acta* **2011**, *56* (27), 9921-9925.

35.Ali, T. A.; Mohamed, G. G., Multi-walled carbon nanotube and nanosilica chemically modified carbon paste electrodes for the determination of mercury(II) in polluted water samples. *Analytical Methods* **2015**, *7* (15), 6280-6289.

36.Deshmukh, M. A.; Shirsat, M. D.; Ramanaviciene, A.; Ramanavicius, A., Composites Based on Conducting Polymers and Carbon Nanomaterials for Heavy Metal Ion Sensing (Review). *Critical Reviews in Analytical Chemistry* **2018**, *48* (4), 293-304.

37.Long, G. L.; Winefordner, J. D., LIMIT OF DETECTION. Analytical Chemistry **1983**, 55 (7), A712-+.

38.Lemay, S. G.; van den Broek, D. M.; Storm, A. J.; Krapf, D.; Smeets, R. M. M.; Heering, H. A.; Dekker, C., Lithographically fabricated nanopore-based electrodes for electrochemistry. *Analytical Chemistry* **2005**, *77* (6), 1911-1915.

39.Beardsley, C. A.; Reilly, T. H. I.; Hofstetter, J. C., Fieldable Soil Preparation Methods for In-Situ Site Characterization of Heavy Metal Pollution. *Submitted Manuscript* **2019**.

40.Bard, A. J.; Faulkner, L. R.; Leddy, J.; Zoski, C. G., *Electrochemical methods: fundamentals and applications*. wiley New York: 1980; Vol. 2.

41.Borrill, A. J.; Reily, N. E.; Macpherson, J. V., Addressing the practicalities of anodic stripping voltammetry for heavy metal detection: a tutorial review. *Analyst* **2019**, *144* (23), 6834-6849.

42.Shinwari, M. W.; Zhitomirsky, D.; Deen, I. A.; Selvaganapathy, P. R.; Deen, M. J.; Landheer, D., Microfabricated reference electrodes and their biosensing applications. *Sensors* **2010**, *10* (3), 1679-1715.

43.Zoski, C. G., Handbook of electrochemistry. Elsevier: 2006.

44.Klunder, K. J.; Nilsson, Z.; Sambur, J. B.; Henry, C. S., Patternable Solvent-Processed Thermoplastic Graphite Electrodes. *Journal of the American Chemical Society* **2017**, *139* (36), 12623-12631.

45.Adkins, J. A.; Henry, C. S., Electrochemical detection in paper-based analytical devices using microwire electrodes. *Analytica Chimica Acta* **2015**, *891*, 247-254.

46.Macpherson, J. V., A practical guide to using boron doped diamond in electrochemical research. *Physical Chemistry Chemical Physics* **2015**, *17* (5), 2935-2949.

47.Hutton, L. A.; Iacobini, J. G.; Bitziou, E.; Channon, R. B.; Newton, M. E.; Macpherson, J. V., Examination of the Factors Affecting the Electrochemical Performance of Oxygen-

Terminated Polycrystalline Boron-Doped Diamond Electrodes. *Analytical Chemistry* **2013**, 85 (15), 7230-7240.

48.NavarroLaboulais, J.; Trijueque, J.; GarciaJareno, J. J.; Vicente, F., Ohmic drop effect on the voltammetric behaviour of graphite plus polyethylene composite electrodes. *Journal of Electroanalytical Chemistry* **1997**, *422* (1-2), 91-97.

49.Simonov, A. N.; Morris, G. P.; Mashkina, E. A.; Bethwaite, B.; Gillow, K.; Baker, R. E.; Gavaghan, D. J.; Bond, A. M., Inappropriate Use of the Quasi-Reversible Electrode Kinetic Model in Simulation-Experiment Comparisons of Voltammetric Processes That Approach the Reversible Limit. *Analytical Chemistry* **2014**, *86* (16), 8408-8417.

50.Nemcova, L.; Barek, J.; Zima, J., A voltammetric comparison of the properties of carbon paste electrodes containing glassy carbon microparticles of various sizes. *Journal of Electroanalytical Chemistry* **2012**, *675*, 18-24.

51.Mettakoonpitak, J.; Miller-Lionberg, D.; Reilly, T.; Volckens, J.; Henry, C. S., Low-cost reusable sensor for cobalt and nickel detection in aerosols using adsorptive cathodic square-wave stripping voltammetry. *Journal of Electroanalytical Chemistry* **2017**, *805*, 75-82.

52.Mettakoonpitak, J.; Mehaffy, J.; Volckens, J.; Henry, C. S., AgNP/Bi/Nafion-modified Disposable Electrodes for Sensitive Zn (II), Cd (II), and Pb (II) Detection in Aerosol Samples. *Electroanalysis* **2017**, *29* (3), 880-889.

53.Foster, C. W.; de Souza, A. P.; Metters, J. P.; Bertotti, M.; Banks, C. E., Metallic modified (bismuth, antimony, tin and combinations thereof) film carbon electrodes. *Analyst* **2015**, *140* (22), 7598-7612.

54.Lu, M.; Toghill, K. E.; Compton, R. G., Simultaneous Detection of Trace Cadmium(II) and Lead(II) Using an Unmodified Edge Plane Pyrolytic Graphite Electrode. *Electroanalysis* **2011**, *23* (5), 1089-1094.

55.Baldrianova, L.; Svancara, I.; Vlcek, M.; Economou, A.; Sotiropoulos, S., Effect of Bi(III) concentration on the stripping voltammetric response of in situ bismuth-coated carbon paste and gold electrodes. *Electrochimica Acta* **2006**, *52* (2), 481-490.

56.Schonberger, E. A.; Pickering, W. F., INFLUENCE OF PH AND COMPLEX-FORMATION ON THE ASV PEAKS OF PB, CU AND CD. *Talanta* **1980**, *27* (1), 11-18.

57.Read, T. L.; Joseph, M. B.; Macpherson, J. V., Manipulation and measurement of pH sensitive metal-ligand binding using electrochemical proton generation and metal detection. *Chemical Communications* **2016**, *52* (9), 1863-1866.

58.Baldrianova, L.; Agrafiotou, P.; Svancara, I.; Jannakoudakis, A. D.; Sotiropoulos, S., The effect of acetate concentration, solution pH and conductivity on the anodic stripping voltammetry of lead and cadmium ions at in situ bismuth-plated carbon microelectrodes. *Journal of Electroanalytical Chemistry* **2011**, *660* (1), 31-36.

59.Yang, Y. Y.; Pathirathna, P.; Siriwardhane, T.; McElmurry, S. P.; Hashemi, P., Real-Time Subsecond Voltammetric Analysis of Pb in Aqueous Environmental Samples. *Analytical Chemistry* **2013**, *85* (15), 7535-7541.

60.Ayala, R.; Martinez, J. M.; Pappalardo, R. R.; Refson, K.; Marcos, E. S., Effect of Basicity on the Hydrolysis of the Bi(III) Aqua Ion in Solution: An Ab Initio Molecular Dynamics Study. *Journal of Physical Chemistry A* **2018**, *122* (7), 1905-1915.

61.Wang, J.; Pedrero, M.; Sakslund, H.; Hammerich, O.; Pingarron, J., Electrochemical activation of screen-printed carbon strips. *Analyst* **1996**, *121* (3), 345-350.

62.Wang, J.; Lu, J. M.; Hocevar, S. B.; Ogorevc, B., Bismuth-coated screen-printed electrodes for stripping voltammetric measurements of trace lead. *Electroanalysis* **2001**, *13* (1), 13-16.

63.Renault, C.; Anderson, M. J.; Crooks, R. M., Electrochemistry in Hollow-Channel Paper Analytical Devices. *Journal of the American Chemical Society* **2014**, *136* (12), 4616-4623.

64.Channon, R. B.; Nguyen, M. P.; Henry, C. S.; Dandy, D. S., Multilayered Microfluidic Paper-Based Devices: Characterization, Modeling, and Perspectives. *Analytical Chemistry* **2019**, *91* (14), 8966-8972.

65.Noviana, E.; Klunder, K. J.; Channon, R. B.; Henry, C. S., Thermoplastic Electrode Arrays in Electrochemical Paper-Based Analytical Devices. *Analytical Chemistry* **2019**, *91* (3), 2431-2438.

66.Carrell, C.; Kava, A.; Nguyen, M.; Menger, R.; Munshi, Z.; Call, Z.; Nussbaum, M.; Henry, C., Beyond the lateral flow assay: A review of paper-based microfluidics. *Microelectronic Engineering* **2019**, *206*, 45-54.

CHAPTER 6: EXPLORING CARBON PARTICLE TYPE AND PLASMA TREATMENT TO IMPROVE ELECTROCHEMICAL PERFORMANCE OF STENCIL-PRINTED CARBON ELECTRODES

6.1 Chapter Overview

Stencil-printing conductive carbon inks has revolutionized the development of inexpensive, disposable and portable electrochemical sensors. However, stencil-printed carbon electrodes (SPCEs) typically suffer from poor electrochemical properties. While many surface pretreatments and modifications have been tested to improve the electrochemical activity of SPCEs, the bulk composition of the inks used for printing has been largely ignored. Recent studies of other carbon composite electrode materials show significant evidence that the conductive carbon particle component is strongly related to electrochemical performance. However, such a study has not been carried out with SPCEs. In this work, we perform a systematic characterization of SPCEs made with different carbon particle types including graphite particles, glassy carbon microparticles and carbon black. The relationship between carbon particle characteristics including particle size, particle purity, and particle morphology as well as particle mass loading on the fabrication and electrochemical properties of SPCEs is studied. SPCEs were plasma treated for surface activation and the electrochemical properties of both untreated and plasma treated SPCEs are also compared. SPCEs displayed distinct analytical utilities characterized through solvent window and double layer capacitance. Cyclic voltammetry (CV) of several standard redox probes, FcTMA⁺, ferri/ferrocyanide, and pAP was used to establish the effects of carbon particle type and plasma treatment on electron transfer kinetics of SPCEs. CV of the biologically relevant molecules uric acid, NADH and dopamine was employed to further illustrate the differences in sensing and

fouling characteristics of SPCEs fabricated with different carbon particle types. SEM imaging revealed significant differences in the SPCE surface microstructures. This systematic study demonstrates that the electrochemical properties of SPCEs can be tuned and significantly improved through careful selection of carbon particle type and plasma cleaning with a goal toward the development of better performing electrochemical point-of-need sensors. I carried out this work on my own. Dr. Pat McCurdy at CSU helped with obtaining SEM images. The work presented in this chapter has been submitted as a manuscript to *Talanta*. Supporting information is provided in Appendix II of this thesis.

6.2 Introduction

Carbon electrodes are widely employed in sensing applications due to their favorable electrochemical activity, high conductivity, extended solvent window, biocompatibility, rich surface chemistry, relative inertness and low cost.¹⁻³ More recently, a significant focus has been placed on the development of easy to fabricate and inexpensive carbon electrodes for point-of-need (PON) sensing. Carbon electrodes such as glassy carbon (GC), pyrolytic graphite, or boron doped diamond (BDD) are difficult to fabricate, thus, their use is limited in PON sensing. In order to improve ease of fabrication and lower costs, carbon composite electrodes (CCEs), made from conductive carbon particles held together by an inert binder have been the electrode of choice.².^{4,5} A variety of binder materials have been used for composite electrode fabrication including high molecular weight polymers such as poly(methyl methacrylate) and poly(caprolactone), referred to as thermoplastic electrodes (CPEs)⁶⁻⁸ and mixtures of materials to generate printable inks, referred to as screen/stencil printed carbon electrode (SPCEs).^{9, 10}

A variety of CCEs exist which are differentiated by the physical properties the binder material imparts on the final electrode.^{2, 4, 8, 11-13} For example, after homogenizing a dissolved polymer and graphite powder, TPEs form a material with a "gum-like" consistency that can then be pressed into a template until excess solvent has evaporated, forming a solid electrode. The solid TPE is mechanically robust and able to withstand abrasive polishing and sanding.² Alternatively, the low molecular weight hydrocarbons used in CPEs remain a liquid throughout the lifetime of the electrode, rendering CPEs mechanically fragile. After homogenization, CPEs are packed into electrode bodies by hand, reducing interelectrode reproducibility. The surface can be easily refreshed by removing outer layers of the paste, eliminating more complicated pretreatments that may be required for surface renewal after electrochemical cycling. While both CPEs and TPEs are inexpensive, can be fabricated simply and have high electrochemical activity, their use in PON electrochemical sensing has remained limited by lack of disposability, mass producibility and facile integration with portable sensing platforms such as paper-based analytical devices.

Screen or stencil-printed electrodes (SPCEs) have transformed the development of electrochemical PON sensors. SPCEs are characterized by low cost, small size, low sample volume requirement, disposability - precluding the need for treatments to return an electrode to its original state - good sensitivity, and mass producibility.^{14, 15} SPCEs are a composite of various substances including graphite, carbon black, solvents, and a polymeric binder such as polyvinyl chloride, however, the exact composition of commercial inks is proprietary.^{15, 16} Prefabricated, commercial SPCEs are used in a significant portion of sensor development due to the availability of commercial flow cells and ease of integration with portable instrumentation allowing for more seamless commercialization and field use. SPCEs are also printed in-house, most commonly using commercially available conductive carbon inks since their thixotropic properties are required for

screen or stencil printing.¹⁷⁻¹⁹ A squeegee is used to print inks onto the desired substrate either through a mesh screen or stencil. After printing, the electrodes are typically cured in an oven, generating solid electrodes. In this way, 100s of electrodes can be produced quickly using low cost materials. In-house printing of SPCEs is critical in portable sensor development because the inks can be bulk modified with electrocatalysts or biorecognition elements prior to printing, precluding the need for post-fabrication modifications which add complexity and cost to sensor development. Moreover, the inks can be printed in many planar geometries onto microfluidic paper-based analytical devices, which can carry out tasks such as mixing, timing, and sequential fluid delivery, further improving assay automation in PON sensing.²⁰⁻²³ While SPCEs have been a critical component in the development of sophisticated PON sensors, SPCEs suffer from poor electrochemical activity, higher detection limits, and lower sensitivity than other CCE types.^{2, 13, 24, 25}

SPCE electrochemistry can be improved through pretreatment or surface modification with nanomaterials. Pretreatments include plasma treatment,^{26, 27} soaking in organic solvents,²⁸ exposure to UV/ozone,²⁹ and electrochemical cycling in various solvents.³⁰⁻³² Nanomaterial modification of SPCEs via drop-casting is widely used to control the electrochemical active portion of the electrode surface and resulting electrochemistry. Common nanomaterials include metallic nanoparticles,³³⁻³⁵ carbon nanotubes,^{36, 37} carbon black,^{38, 39} and graphene/graphene oxide.^{40, 41} While these methods have been successful, pretreatment and/or post-fabrication modification to improve the basic electrochemical functionality of a disposable, single-use, electrode is not ideal. On the other hand, it is well known that particle characteristics including size, purity, and aspect ratio as well as the carbon to binder ratio are related to CCE performance, with some electrode compositions providing as good or better electrochemistry than commercial

electrodes such as GC, Au, or Pt macro-electrodes.^{2, 24, 42} The electrochemical activity of both prefabricated SPCEs and SPCEs made in-house with commercially available inks from a variety of vendors have been characterized, however, little is known about the exact composition of commercial SPCEs and its relationship to electrochemical and physical quality.^{14-16, 40, 43} Recently, we reported the fabrication of glassy carbon microparticle SPCEs (GC-SPEs) and applied them to detecting Cd and Pb. In this work, GC-SPEs demonstrated dramatic improvement for ASV of Cd and Pb compared to graphite SPCEs due to the relationship between carbon particle and electrochemical response.⁴⁴

Here, we report a systematic study on the relationship between SPCE bulk composition, specifically carbon particle type and carbon particle mass loading, and the physical and electrochemical properties of SPCEs. Different carbon particles were added to a starting commercial carbon ink that contains an unknown carbon nanomaterial, but has required the addition of graphite for use in electrochemical sensing in previous works. Such a study is relevant to further understand and improve the quality of SPCEs without increasing time or fabrication cost. We explore graphite particles, glassy carbon microparticles and carbon black which vary in size, microstructure, purity and vendor for SPCE fabrication. Plasma treatment is carried out and optimized for each SPCE type. Plasma treatment was chosen since it is effective in activating the electrochemical characteristics of both untreated and plasma treated SPCEs were determined with several inner and outer sphere redox species, double-layer capacitance, and aqueous solvent window. Herein, a benchmark for researchers wishing to work with SPCEs which have significantly higher electrochemical activity than previous SPCE iterations, while maintaining low

background currents and wide solvent windows, without complex and time-consuming modification procedures is provided.

6.3 Experimental

Solutions

All solutions were prepared fresh daily using 18.2 M Ω · cm water purified using a Milli-Q system (MilliporeSigma, USA). To test the electrochemical characteristics of all SPCEs, solutions were prepared in 0.1 M potassium chloride (KCl, Fisher Scientific, New Jersey, USA) with 1 mM potassium hexacyanoferrate(III) ($K_3Fe(CN)_6$) and potassium hexacyanoferrate(II) $(K_4(CN)_6 \cdot 3H_2O)$ trihydrate (Sigma-Aldrich) as well 1mM ferrocenylmethyl as trimethylammonium hexafluorophosphate (FcTMA⁺, synthesized in-house).⁴⁵ 0.5 mM paminophenol (pAP, Sigma-Aldrich) 1.0 mM ascorbic acid (AA, Sigma-Aldrich), 1 mM betanicotinamide adenine dinucleotide disodium salt (NADH, Sigma-Aldrich), 1mM uric acid (UA, Sigma-Aldrich), and 1.0 mM dopamine hydrochloride (DA, Sigma-Aldrich) were all prepared in 0.1 M, pH 7.4 phosphate buffer. Phosphate buffer was prepared by titrating a 0.2 M potassium phosphate monobasic (KH₂PO₄, Sigma-Aldrich) solution with 1.0 M sodium hydroxide (NaOH, Sigma-Aldrich) to a pH of 7.4. All chemicals were used as received and were of analytical grade.

SPCE Materials and Fabrication

Five carbon types, listed in Table 6.1, were used to fabricate SPCEs. 0.4 to 12 μ m glassy carbon (GC) microparticles (spherical, Type 1) were purchased from Alfa-Aesar (Massachusetts, USA). 2 to 12 μ m GC microparticles were purchased from Sigma-Aldrich, acetylene carbon black powder was purchased from Strem Chemicals (Massachusetts, USA), graphite powder (< 20 μ m, sigma graphite) was purchased from Sigma-Aldrich, and 3569 graphite powder was purchased from Asbury Carbons (New Jersey, USA). To prepare the composites, the carbon types were hand-

mixed with commercial carbon ink (E3178) purchased from Ercon Inc. (Massachusetts, USA) in the ratios indicated in Table 6.1. Ratios were selected to produce the best consistency for stencil printing of the resulting inks. To prepare the stencil, a poly(ethylene) terephthalate (PET) sheet, purchased from 3M (Minnesota, USA) was laser cut using a 30 W Epilog Engraver Zing CO₂ laser cutter and engraver (Colorado, USA). The PET stencil was taped to another PET sheet and a squeegee was used to print the carbon inks. After printing, the SPCEs were cured in a 65 °C oven for 30 min. A previously reported three electrode design was used for the stentil.^{44, 46} The solution reservoir was fabricated by sandwiching a PET sheet between two layers of double sided adhesive (3M) and laser cutting. The reservoir was attached to the SPCEs with the double sided adhesive and aligned with the connection pads to define the electrode area reproducibly at 0.112 cm² as reported previously.⁴⁴ SPCE plasma treatment was carried out with an LF-5 system purchased from Mercator Control Systems INC. (CA, USA) at a power of 125 W and a pressure of 500 (± 50) mTorr for various times specified in the relevant figures.

Table 6.1 Carbon particle types and their characteristics used to fabricate SPCEs.	,

Carbon Particle Type	Particle Size	Purity	Manufacturer	Ink to Carbon Ratio (g)
Alfa GC	0.4 to 12 µm	Ash content 0.0042%	Alfa-Aesar	0.8 to 1.0
Sigma GC	2 to 12 µm	99.95%	Sigma	0.9 to 1.0
Carbon Black	42 nm	99.9%	Strem Chemicals	3.7 to 0.5
Sigma Graphite	$<$ 20 μm	Unavailable	Sigma	3.5 to 1.5
3569 Graphite	150 μm	99.8%	Asbury Carbon	3.5 to 2.5 & 3.5 to 1.5

SPCE Electrochemical Characterization and Imaging

All electrochemical experiments were performed using a CH Instruments 660B potentiostat (Texas, USA) at room temperature (22 ± 1 °C). The reference electrode (RE) was either a saturated calomel electrode (SCE) or Ag/AgCl ink (Gwent Group, Torfean, UK) painted

directly onto the SPC RE and allowed to dry at room temperature. The RE used is denoted in each figure. Cyclic voltammograms (CVs) were recorded in 1.0 mM solutions of FcTMA⁺, Fe(CN)₆⁴⁻/Fe(CN)₆³⁻, AA, DA, UA, and NADH and in 0.5 mM solutions of pAP at a scan rate of 100 mV s⁻¹. Solvent windows were recorded in 0.1 M KCl (pH 6.5) at a scan rate of 200 mV s⁻¹. Solvent windows were also recorded in 0.1 M KCl (pH 6.5) at a scan rate of 100 mV s⁻¹ for quantification of the potential range. Capacitance was quantified by recording CVs in 0.1 M KCl (pH 6.5) between +0.1 and -0.1 V for a total of 5 cycles. Scanning electron microscopy images of the electrodes were acquired with a JSM-6500F field emission scanning electron microscope (JEOL, Tokyo, Japan) with a 2 kV acceleration voltage.

6.4 Results and Discussion

SPCE Fabrication and Imaging

To explore the effects of carbon particle type on the electrochemical response of SPCEs several carbon types were selected for SPCE fabrication. Carbon particles were selected in a range of particle sizes, microstructures and purities as indicated in Table 6.1. Two different graphite particles, Sigma graphite and 3569 were selected since graphite is by far the most common carbon particle type used for CCE fabrication. The Sigma graphite SPCE used here is the same composition of SPCE employed in several previous works so as to compare the performance to the new 3569 graphite SPCEs developed here.^{46,49} Carbon black is often used to modify SPCE surfaces; therefore, it was selected to determine its suitability in bulk SPCE formulations. Two GC microparticles, Alfa GC (0.4 to $12 \,\mu$ m) and Sigma GC (2 to $12 \,\mu$ m) were selected due to GC's distinct physical and electrochemical properties and recent successful use in SPCEs.⁴⁴ 10 to 20 μ m Afla GC microparticles were characterized in our previous work, and were not studied further here because of their poor electrochemical performance in SPCEs.⁴⁴

Prior to further characterization, the carbon to binder ratio was optimized, since conductive particle loadings have significant implications on composite electrode performance.^{2,4} The optimal carbon to binder ratio is physically limited by the amount of carbon particle that can be homogenized with the ink as well as the final consistency of the composite for stencil printing. If too thin, the ink will easily leak between the stencil boundary and the substrate. On the other hand, if the ink is too thick, it is difficult to achieve a defect-free surface. Particle mass loading is also important for improving the conductivity of composite electrodes since the contact resistance between particles as well as the number of contact points delimits composite electrode conductivity.^{2, 6, 15} Since commercial inks are typically employed for SPCE fabrication, carbon particle mass loading and the effects on electrochemical properties of SPCEs has not been studied. However, ohmic resistance characterized by slow apparent electron transfer rates and substandard electrochemical properties is a significant drawback to working with conventional SPCEs.^{15,43,50} For these reasons, particle mass loading was studied in order to maximize carbon particle loading for best conductivity. Among the graphitic carbon and carbon black particles, particle size significantly contributed to mass loading capabilities. As indicated in Table 6.1, carbon black particles are 42 nm while the 3569 graphite particles are 150 μ m. The maximum carbon black to binder ratio was limited to 0.5 g to 3.7 g. However, the maximum mass loading of the 3569 graphite was 2.5 g graphite in 3.5 g binder. The Sigma graphite particles, with an intermediate size of ≤ 20 μ m, exhibited maximum mass loading at a graphite to binder ratio of 1.5 g to 3.5 g. As particle size increases, higher mass loadings can be achieved. The dependence of mass loading on particle size has been seen with other CCEs as well.² In contrast to the graphite and carbon black particles, GC microparticles required less binder, exhibiting optimal particle to binder ratios of 1.0 g to 0.8 g for Alfa GC and 1.0 g to 0.9 g for Sigma GC microparticles.

Following fabrication, scanning electron microscopy (SEM) was used to image SPCE surfaces. Representative SEM images are shown in Figure 6.1. Here, the different carbon particles and the resulting SPCEs are characterized by significant morphological differences. Graphite flakes can be seen clearly in the 3569 and Sigma graphite images with a relatively thick coating of the binder present on the graphite surfaces. The 1.5 to 3.5 3569 did not show any visible differences at lower magnifications compared to the 2.5 to 3.5 3569 SPCE and is included in Appendix II, Figure S6.1. However, at higher magnifications, the lower mass loading 3569 SPCE exhibited more charging effects, voids between carbon particles, and appear to be less densely packed (Figure 6.2), consistent with the electrochemical characterizations discussed below. An additional difference between the 3569 (larger) and Sigma graphite (smaller) particles is the orientation of particles observed in the SEM images. The 3569 particles appear horizontally aligned and densely packed together while the Sigma graphite particles appear randomly oriented and less densely packed. This is further supported in CV experiments discussed below, in which the 3569 SPCEs exhibited less ohmic resistance, indicating a higher number of particle contacts than the Sigma graphite SPCEs. The morphology of the carbon black SPCE surface is characterized by significant cracking as indicated in the SEM image. Some cracks in the electrode surfaces were also visible with the naked eye. At higher curing temperatures, some commercial inks used for SPCEs have generated electrode surfaces with cracks due to the rate of evaporation of solvent components, thus, the carbon black SPCEs were cured at room temperature as opposed to 60 °C to determine if a defect free surface was achievable.⁵¹ Decreasing the curing temperature did not reduce cracking and this affect is likely a result of the small particle size.

In contrast to the graphite particles, GC microparticles SPCE surface contained much less binder on the surface. This is expected since GC is non-porous and little to no penetration of particles with binder occurs resulting in weaker particle-binder interactions.^{52, 53} However, graphite is soft and porous, so binder components can physically penetrate and stick to graphite particles, resulting in the thick binder coating observed. A similar phenomenon was observed when GC and graphite paste electrodes were compared.⁵⁴ This observation is also consistent with the higher mass loading necessary for GC particles compared to graphite particles. While the Alfa GC (0.4 to 12 μ m) and the Sigma GC (2 to 12 μ m) particles have a similar manufacturer reported size distribution, SEM imaging (Figure S6.2) revealed that the Sigma GC particles have a narrower size distribution with less frequency of particles greater than 10 μ m. The effects of this difference in particle size distribution on the electrochemical characteristics of the GC-SPEs is discussed in the following sections.



Figure 6.1 SEM images of 2.5 to 3.5 3569, Sigma graphite, carbon black, Alfa GC, and Sigma GC SPCE surfaces. SPCE types are denoted in the figure.

SPCE Plasma Treatment and Stability

SPCEs typically suffer from poor electrochemical characteristics due to the presence of non-electroactive components of the binder as well as other impurities on the electrode surface.^{28, 30} Several pretreatments have been adopted to etch polymeric binder and other impurities

from the SPCE surface to further expose the electrochemically active particles including oxygen²⁶ or argon²⁷ plasma treatment, electrochemical (galvanostatic or cycling),^{30, 55} soaking in NaOH³² or organic solvents²⁸ for tens to hundreds of minutes. Both electrochemical and chemical pretreatment are time consuming, labor intensive and carry a risk of contamination of the electrode surface via adsorption of impurities.^{56, 57} Therefore, we selected plasma treatment as a simple and fast method to etch surface binder and/or impurities and expose the underlying carbon particles at SPCE surfaces.

To optimize plasma treatment parameters, SPCEs were plasma treated at the same pressure (500 mTorr) and plasma power (125 W) for varying time intervals of 1, 1.5 and 2 min. Carbon black SPCEs were plasma treated for time intervals of 0.5, 1 and 1.5 min due to significant increases in surface cracking at longer treatment times. The optimal treatment time was selected based on the voltammetry of FcTMA⁺, Fe(CN)₆⁴⁻/Fe(CN)₆³⁻ (ferri/ferrocyanide) and pAP assessing anodic peak to cathodic peak separation (ΔE_p) and peak current (Figure S6.3 – S6.5). Based on these parameters, the optimal plasma treatment time was two min for the Sigma GC, Alfa GC, 3569 graphite, and Sigma graphite SPCEs and 30 s for carbon black SPCEs. The carbon black SPCE electrochemistry became worse at longer treatment times, likely due to excessive etching of binder verified by visibly larger surface cracks.

CV was carried out with FcTMA⁺, ferri/ferrocyanide and pAP at increasing time intervals after plasma treatment to ensure the electrochemical responses were stable, with the longest time tested being 10 days (Figure S6.6 – S6.7). Since the electrodes were stored in ambient conditions, some deactivation resulting in larger ΔE_p is expected due to adsorption of small hydrocarbons to the surface.^{3, 58} Increased ΔE_p s were obtained for nearly all plasma treated SPCEs with FcTMA⁺, ferri/ferrocyanide and pAP after 10 days of aging. However, the Sigma GC-SPE only exhibited a significant increase in ΔE_p for pAP. While ΔE_p increased to varying degrees for each electrode, the peak currents for all redox mediators remained stable for at least 10 days, with any discrepancies attributable to batch-to-batch reproducibility (Table S6.1 and S6.22). Plasma treated SPCEs can be stored for long periods of time without requiring a reactivation procedure due to airborne hydrocarbon contamination, however, care should be taken in some electrochemical experiments to ensure currents are being measured at the appropriate potential. Attention can also be given to electrode storage conditions to reduce any surface contamination.⁵⁸ Figure 6.2 shows SEM images of the plasma treated SPCEs compared to their untreated counterparts. Briefly, binder is visibly etched from the graphitic and carbon black SPCE surfaces. However, the untreated GC surfaces are not as well coated with the binder (Figure 6.1), and the binder is not visibly removed after plasma treatment. The resulting activation of the GC-SPEs then is likely due to removal of other adsorbed impurities.



Figure 6.2 SEM images of SPCE surfaces before and after plasma treatment.

Capacitance and Potential Window

An advantage to working with composite electrodes is the small contributions double layer capacitance (C_{dl}) makes to background currents. C_{dl} is determined by the fraction of electroactive carbon exposed to solution; typically a small fraction of the geometric surface area of native CCEs.³ To determine optimal carbon particle type, the effects of carbon particle type and plasma treatment on SPCE C_{dl} were assessed. Figure 6.3 shows CVs recorded in 0.1 M KCl between -0.1 V and +0.1 V for untreated (dashed lines) and plasma treated (solid lines) SPCEs.



Figure 6.3 Averaged CVs (N = 3) recorded in 0.1 M KCl at a scan rate of 100 mV s⁻¹ over the potential range -0.1 V to 0.1 V for (a) 2.5 to 3.5 3569 (green), 1.5 to 3.53569 (black), Sigma graphite (orange), carbon black (grey) and (b) Alfa GC (red) and sigma GC (blue) at untreated (dashed) and plasma (solid) (ai and bi) and untreated only (aii and bi).

C_{dl} values were estimated for all SPCE types and plasma treatment times using Equation 6.1.⁵⁹

$$C_{dl} = \frac{i_{average}}{vA_{geometric}}$$
 Equation 6.1

Here, $i_{average}$ is the absolute value of current (A) recorded between 0.1 V and -0.1 V, v is scan rate (V s⁻¹) and A_{geometric} is geometric surface area (cm²). Greater currents in the CVs shown in Figure 6.3 for plasma treated electrodes are expected since etching and removal of binder coating exposes a higher surface area of carbon particles.³ C_{dl} also increases with increasing plasma treatment times, but to varying degrees for each carbon type. C_{dl} values of 5.9 ± 1.1 and $6.8 \pm 3.0 \,\mu\text{F cm}^{-2}$ were determined for the untreated Alfa GC and Sigma GC-SPEs, respectively and 13.2 ± 3.0 , 3.3 ± 2.1 , 4.8 ± 2.2 , and $9.0 \pm 2.2 \,\mu\text{F cm}^{-2}$ for the untreated Sigma graphite, 1.5 to 3.5 3569, 2.5 to 3.5 3569, and carbon black SPCEs, respectively. Untreated SPCE C_{dl} are lower than C_{dl} values for

conventional glassy carbon electrodes ($24 - 36 \ \mu F \ cm^{-2}$), edge plane graphite electrodes ($60 \ \mu F \ cm^{-2}$), bare commercial DropSens SPCEs ($37 \ \mu F \ cm^{-2}$), as well as CPEs ($62 \ \mu F \ cm^{-2}$).^{2, 3, 60} Compared to the 3569 graphite SPCEs, the Sigma graphite and carbon black SPCEs exhibit slightly larger C_{dl}. The larger C_{dl} of the Sigma graphite SPCE could be due to a higher proportion of edge plane character exposed due to the much smaller particle size. The larger carbon black C_{dl} is likely due to surface cracks significantly increasing the actual surface area in contact with solution.⁶¹

An increase in C_{dl} (Figure 6.3ai and 6.3bi) after plasma treatment was observed for all SPCEs, however the extent of the increase varied among the carbon types and plasma treatment times (Figure 6.4). While low C_{dl} is advantageous in electrochemical sensing in order to reduce background currents, a 2 min plasma treatment time was selected since the electrochemical response of these SPCEs was the most reproducible (Figure S6.3 - S6.5) as well as the most stable over time (data not shown). While C_{dl} does increase with increasing plasma treatment times, this is expected due to additional removal of binder exposing a larger fraction of electrochemically active carbon particles. C_{dl} increased by about 20 times after 2 min plasma treatment to 230 ± 7 , 73.2 ± 3.6 , 72.3 ± 13.3 and $195 \pm 13 \ \mu\text{F cm}^{-2}$ for the Sigma graphite, 1.5 to 3.5 3569, 2.5 to 3.5 3569, and carbon black SPCEs respectively. Cdl of the GC-SPEs also increased by about 20 times to 102 ± 13 , 149 ± 40 and $75.6 \pm 10.9 \,\mu\text{F cm}^{-2}$ for the Alfa GC and Sigma GC SPEs respectively. The plasma treated 3569 SPCEs and Sigma GC-SPEs C_{dl} are closest to, but still greater than what is expected for typical planar carbon electrodes, pointing toward a rough electrode surface. The exact mechanism governing the much larger C_{dl} of the other SPCEs is unclear. However, it is likely a combination of surface roughness, as well as defect site type and density, and/or electroactive

surface groups, both of which could be intrinsic to the carbon type and exposed after plasma cleaning, or introduced during plasma treatment.^{3, 62}



Figure 6.4 Calculated C_{dl} of untreated and plasma treated electrodes as a function of plasma treatment time for 2.5 to 3.5 3569 (green), 1.5 to 3.5 3569 (black), Sigma graphite (orange), carbon black (grey), Alfa GC (red) Sigma GC (blue) SPCEs.

Aqueous potential window is defined by the potential limits at which water electrolysis occurs, where oxygen evolution (OER) occurs at positive potential extremes and hydrogen evolution (HER) occurs at negative potential extremes.⁶³ Since HER and OER can interfere with electroanalysis of certain analytes, it is important to establish a given electrode material's potential window. The aqueous potential windows for both untreated and plasma treated electrodes were recorded to define operable potential windows for each SPCE, as well as determine any other faradaic background reactions. Potential windows recorded at a pH of 6.5 (0.1 M KCl) are shown in Figure 6.5A and 6.5B with the CVs vertically offset for clarity. The potential window was defined as the anodic and cathodic potential limits where a current density greater than 0.5 mA cm⁻² at a scan rate of 100 mV s⁻¹ (CVs not shown) is passed due to water oxidation or reduction as shown in Table 6.2.⁶⁴



Figure 6.5 Potential windows recorded in aerated 0.1 M KCl (pH 6.5) at a scan rate of 200 mV s⁻¹ from 2.0 V to -2.0 V for (a) 3569 2.5 to 3.5 (green), 3569 1.5 to 3.5 (black), Sigma graphite (orange), carbon back (grey) and (b) Alfa GC (red) and Sigma GC (blue) at (i) untreated electrodes and (ii) plasma treated electrodes.

A few trends in the potential window widths are evident. First, after plasma treatment, the potential window of all SPCEs decreases. This is indicative of increased exposure of catalytic sites on the electrode surface after etching of the binder. Another trend, particularly evident with the plasma treated SPCEs, is the potential window dependence on particle purity. Impurities such as carbonaceous substances and metallic impurities are typically present in manufactured carbons.⁶⁵ Common metallic impurities such as Ni, Cu, Fe, and Mn are catalytic toward HER. The Sigma GC particles have the lowest metal content of any particle at 99.95% purity, and exhibit the widest potential window of the plasma treated SPCEs by about 0.4 V. This is evident in the CVs (Figure 6.5aii and 6.5bii) where HER is hindered at negative potential extremes at the Sigma GC-SPE. The Alfa GC is not analyzed for metallic content, therefore a lower purity and higher concentration of metallic impurities could lead to the more facile HER observed at Alfa GC-SPEs. While the

GC-SPEs are more active toward HER than the 3569 graphite with onset potentials of about -1.0 V and -1.6 V respectively, the 3569 graphite appears to be more active toward OER, passing much higher currents for this process, indicating the catalytic sites types vary between SPCE surfaces.

The Sigma graphite SPCE, carbon black SPCE and Sigma GC-SPE show evidence of a reduction peak between -1.3 and -1.8 V and -1.4 and -2.0 V. This peak is attributed to reduction of dissolved oxygen, a common faradaic reaction that occurs at graphitic carbon electrodes.^{63, 64} Since oxygen reduction can interfere with the detection of some analytes at negative potentials, and is difficult to purge in field settings, this should also be considered when selecting an appropriate carbon for SPCE fabrication. Another reduction peak is evident in the CVs of the Sigma GC-SPE, 3569 graphite and Sigma graphite SPCEs at about +0.2 V for the untreated SPCEs which shifts to a potential of about +0.5 V after plasma treatment. This is likely attributable to the reduction of surface quinone groups.^{66, 67} The shift to a more facile potential after plasma treatment could be due to increased electrocatalytic activity and/or an increase in the quinone coverage. In the oxidative potential region, a redox couple is evident at the Sigma graphite SPCEs at a potential of about +1.0 V and 0.0 V for the untreated and plasma treated electrodes respectively. The reaction that produces this faradaic current is unclear and could be due to impurities in the graphite or further oxidation of the graphite.⁶³

Carbon Type	Untreated Potential Window (V)	Plasma Potential Window (V)
Alfa GC	2.32	2.15
Sigma GC	2.91	2.50
3569 2.5 to 3.5	2.47	1.95
3569 1.5 to 3.5	3.36	2.08
Carbon Black	2.63	2.11
Sigma Graphite	2.45	1.93

Table 6.2 Estimated potential windows in 0.1 M KCl (pH 6.5) for untreated and plasma treated SPCEs (N = 3).
Electrochemically Reversible Redox Probes

To further assess the effects of both carbon type and plasma pretreatment on SPCE performance, CV was carried out with 1.0 mM solutions of FcTMA⁺, Fe(CN)₆⁴⁻/Fe(CN)₆³⁻ and pAP as shown in Figure 6.6ai-iii (graphite and carbon black SPCEs) and Figure 6.6bi-iii (GC-SPEs). These redox species were selected because they typically exhibit electrochemical reversibility at sufficiently conductive and clean electrode surfaces.



Figure 6.6 Averaged CVs (N = 3) recorded with (a) 3569 2.5 to 3.5 (green), 3569 1.5 to 3.5 (black), Sigma graphite (orange), carbon black (grey) and (b) Alfa GC (red) and Sigma GC (blue) at untreated (dashed lines) and plasma treated (solid lines) for (i) the oxidation of 1mM FcTMA⁺, (ii) the oxidation of 1 mM ferri/ferrocyanide and (iii) the oxidation of 0.5 mM pAP in 0.1 M KCl at a scan rate of 100 mV s⁻¹.

FcTMA⁺ was tested first to gauge ohmic resistance since it undergoes an outer sphere electron transfer process exhibiting Nernstian behavior (ΔE_p 59 ± 10 mV) under diffusion limited conditions (Figure 6.6ai and 6.6bi insets).⁶³ All untreated electrodes exhibit irreversible ΔE_p for FcTMA⁺, indicating a deactivated surface (Table 6.3).^{2, 59} The 3569 graphite SPCEs exhibit the smallest ΔE_p of 72 ± 2 mV and 76 ± 4 mV for the 2.5 to 3.5 and 1.5 to 3.5 SPCEs respectively. The Sigma graphite SPCE exhibited a less reversible ΔE_p of 94 ± 7 mV. Here, an increase in ohmic

resistance is believed to be a direct result of the less dense packing of Sigma graphite compared to the 3569 graphite observed in Figure 6.1. Carbon black exhibited the largest ΔE_p of 125 ± 8 mV, which is consistent with the lowest mass loading of particles as well as the presence of cracks on the electrode surface. The GC-SPE types exhibited slightly larger ΔE_p of 110 ± 6 mV and 92 ± 4 mV for the Alfa GC and Sigma GC-SPEs respectively. While both particle types have a similar manufacturer reported size distribution, SEM imaging revealed the Sigma GC microparticles are characterized by a narrower size distribution (Figure S6.2). The narrower size distribution and smaller average particle size of the Sigma GC microparticles likely results in additional particle to particle contacts throughout the bulk composite compared to the Alfa GC.

After plasma treatment, all electrodes except for the Alfa GC-SPE exhibited greater reversibility (Table 6.3). While FcTMA⁺ voltammetry is not sensitive to surface chemistry or microstructure, the removal of the binder, which acts as a barrier to electron tunneling, slightly improves electron transfer kinetics.³ Of significance, reversible voltammograms were obtained for both plasma treated 3569 SPCEs with ΔE_p of 53 ± 1 mV and 56 ± 2 mV for the high and low mass loading respectively. This reversibility is typically not observed at SPCEs for outer sphere redox couples, indicating that there is a relationship between both the bulk composition and surface cleanliness and the quality of the electrochemical response at SPCEs.⁶⁸ Expected peak currents for FcTMA⁺ were estimated using Equation S6.1 with the geometric surface area of the SPCEs. All untreated SPCEs, excluding carbon black, exhibited greater peak currents than the expected peak currents for all electrodes increased slightly (Table S6.3), indicating a greater surface roughness.²⁶

Ferri/ferrocyanide is an inner-sphere redox couple commonly applied to electrode characterizations.^{3, 40, 69} The electron transfer kinetics of ferri/ferrocyanide are sensitive to surface

chemistry, microstructure, density of electronic states and electrode cleanliness.^{53, 70} Therefore, it was used to assess variation in SPCE surface conditions before and after plasma pretreatment (Figure 6.6aii and 6.6bii). Here, the surface sensitive nature of ferri/ferrocyanide is demonstrated by large ΔE_p (Table 6.3) and low peak currents for all untreated SPCEs. Upon plasma treatment, the electrocatalytic activity of the SPCEs toward ferri/ferrocyanide increases drastically, indicated by decreased ΔE_p and increased peak current, however, the magnitude of improvement depended on carbon particle type. The 2.5 to 3.5 3569 graphite SPCE exhibited a decrease of ΔE_p from 572 \pm 96 mV to 105 \pm 13 mV after plasma treatment. In contrast, the Sigma graphite SPCE exhibited a decrease of ΔE_p from 297 ± 8 mV to 127 ± 4 mV. The untreated Sigma graphite SPCE exhibits a much smaller ΔE_p than the untreated 3569 graphite SPCE. This is possibly a result of the smaller particle sizes resulting in a higher density of catalytic edge planes and defect sites on the untreated electrode which would affect the capacitance discussed previously as well. However, upon plasma treatment, the 3569 graphite SPCE exhibits a smaller ΔE_p by about 20 mV. These results are consistent with FcTMA⁺ voltammetry, suggesting Sigma graphite SPCEs are more resistive than 3569 graphite SPCEs.

While the electrocatalytic activity of GC-SPE types toward ferri/ferrocyanide improves with plasma treatment, the kinetics were sluggish compared to the graphite SPCEs. ΔE_p of 219 ± 8 m and 183 ± 3 mV were obtained for the plasma treated Alfa and Sigma GC-SPEs respectively. This is likely influenced by the microstructure of GC microparticles compared to graphite particles, where graphite contains a high proportion of edge plane like defects to support fast electron transfer of ferri/ferrocyanide.⁷⁰ The theoretical ΔE_p of ferri/ferrocyanide is 59 ± 10 mV, indicating electron transfer is still slightly impeded for this couple at all plasma treated SPCEs. Surface oxygen functionalities are introduced during plasma treatment and surface oxides are believed to impede electron transfer of ferri/ferrocyanide at carbon electrodes, possibly by blocking of adsorption sites or electrostatic repulsion between negatively charged oxides and negatively charged ferri/ferrocyanide molecules.^{69, 70} Nearly reversible voltammetry ($\Delta E_p \sim 70$ mV) was obtained for ferri/ferrocyanide at argon plasma treated SPCEs²⁷, while irreversible voltammetry ($\Delta E_p \sim 225 \text{ mV}$)²⁶ was obtained for oxygen plasma treated SPCEs and quasireversible voltammetry ($\Delta E_p \sim 86 \text{ mV}$) was obtained at alumina polished GC electrodes,⁷¹ consistent with the data provided here.

Sensor development for the electrochemical detection of p-aminophenol (pAP) is important because pAP serves as the electroactive product in immunoassays and is used in several industries leading to environmental pollution.^{72, 73} Typically, sluggish electron transfer kinetics resulting in low sensitivity are obtained at conventional and pretreated SPCEs.⁷⁴ Therefore, voltammetry of pAP at the SPCEs developed in this work was assessed. CVs for pAP at untreated and plasma treated SPCEs are shown in Figure 6.6aiii and 6.6biii. Again, significant improvement in the electrocatalytic activity of plasma treated SPCEs is observed. pAP undergoes a two electron, two proton oxidation with a theoretical peak separation of 29.5 mV (59/n mV). A wide range of $\Delta E_{\rm p}$ values have been reported for pAP at carbon electrodes with most clean/activated electrodes exhibiting quasireversible voltammetry. ΔE_p of about 60 mV for TPEs,⁵ 140 mV for bare GC macro electrodes,⁷⁵ and 64 mV for electrochemically activated SPCEs⁷⁴ have been reported. Here, as expected, the untreated SPCEs exhibit irreversible voltammetry (Table 6.3). However, remarkably, the plasma treated 2.5 to 3.5 3569 SPCEs exhibit nearly reversible voltammetry with ΔE_p of 39 ± 2 mV. 3569 graphite TPEs, which also require an activation step to remove excess polymer from coated particles, did not exhibit this level of reversibility toward pAP.⁵ These results indicate that optimizing SPCE composition can drastically improve electrochemical performance toward relevant analytes to be on par with electrodes typically considered to be higher quality.^{2, 5} At the GC-SPEs, pAP voltammetry remained consistent with the trend observed for FcTMA⁺ and ferri/ferrocyanide, where the Sigma GC-SPEs provided better reversibility than the Alfa GC-SPEs (Table 6.3).

Table 6.3 Peak-to-peak separation for CV of 1.0 mM FcMTA⁺, ferri/ferrocyanide, and 0.5 mM pAP at untreated and plasma treated SPCEs.

Carbon Particle Type	FcTMA ⁺ ΔE _p (mV)	FcTMA ⁺ ∆E _p (mV) plasma	Ferri/Ferrocyanide ΔE _p (mV)	Ferri/Ferrocyanide ∆E _p (mV) plasma	pAP ∆E _p (mV)	pAP ∆E _p (mV) plasma
Alfa GC	110 (±6)	110 (±3)	532 (±46)	219 (±8)	222 (±9)	116 (±3)
Sigma GC	92 (±4)	80 (±2)	719 (±163)	183 (±3)	350 (±14)	67 (±2)
3569 2.5 to 3.5	72 (±2)	53 (±1)	572 (±96)	105 (±13)	376 (±17)	39 (±2)
3569 1.5 to 3.5	76 (±4)	56 (±2)	753 (±16)	204 (±16)	455 (±8)	48 (±4)
Carbon Black	125 (±8)	102 (±6)	398 (±50)	174 (±3)	415 (±27)	92 (±4)
Sigma graphite	94 (±7)	69 (±2)	297 (±8)	127 (±4)	222 (±20)	62 (±1)

To further understand any ohmic drop effects due to conductivity difference of different SPCE formulations CV of FcTMA⁺ at varying scan rates was carried out. Figure 6.7 shows ΔE_p as a function of scan rate for all SPCEs. Here, ΔE_p increases to varying degrees for each SPCE with increasing scan rate. The low and high mass loading 3569 graphite SPCEs exhibited the smallest increase in ΔE_p , while the Sigma graphite SPCE exhibited a slightly larger ΔE_p increase, consistent with the higher resistance of this SPCE due to lower mass loading of particles and particle orientation. Sigma GC-SPE ΔE_p exhibits a smaller scan rate dependence than the Alfa GC-SPE, further supporting the hypothesis of increased particle-to-particle contacts. The carbon black SPCE ΔE_p exhibited the greatest scan rate dependence, consistent with the low mass loading of carbon black particles and the cracking of the SPCE surface.



Figure 6.7 Peak-to-peak separation for 1.0 mM FcTMA⁺ recorded with CV as a function of scan rates in 0.1 M KCl with 2.5 to 3.5 3569 (green), 1.5 to 3.5 3569 (black), Sigma graphite (orange), carbon black (grey), Alfa GC (red) and Sigma GC (blue) SPCEs (N = 3).

Surface Sensitive and Irreversible Redox Probes

To further elucidate the effects of carbon particle type and plasma treatment on the electroanalytical utility of SPCEs, several biologically relevant probes were studied. Figure 6.8a and 6.8b show the averaged CVs recorded for uric acid (UA, Figure 6.8ai and 6.8bi) and nicotinamide adenine dinucleotide (NADH, Figure 6.8aii and 6.8bii) at both untreated and plasma treated SPCEs. Peak current and peak oxidation potential are listed in Table S6.4 and S6.5. Plasma treatment was found to dramatically improve the response at SPCEs, with increased peak currents and lowered detection potentials in both cases. Since plasma treatment is carried out with ambient air, it is expected to etch binder as well as increase surface oxide coverage on the SPCE surfaces.², ^{26,69} UA is known to be highly sensitive to surface oxides, therefore, the increased catalytic activity of plasma SPCEs toward this probe is attributed to additional surface oxides. The plasma treated Sigma GC-SPE provided the highest catalytic activity of the two GC-SPE types, consistent with previous results. The plasma treated 2.5 to 3.5 3569 SPCE exhibited the highest catalytic activity toward UA compared to the other graphite and carbon black SPCEs. Again, the carbon black SPCEs exhibited the poorest voltammetry at both the untreated and plasma treated versions.



Figure 6.8 Averaged CVs (N = 3) recorded with (a) 3569 2.5 to 3.5 (green), 3569 1.5 to 3.5 (black), Sigma graphite (orange), carbon black (grey) and (b) Alfa GC (red) and Sigma GC (blue) at untreated (dashed lines) and plasma treated (solid lines) for (i) the oxidation of 1mM UA and (ii) the oxidation of 1 mM NADH in 0.1 M phosphate buffer (pH 7.4) at a scan rate of 100 mV s⁻¹.

The electrooxidation of NADH is of interest since has wide application in dehydrogenase based biosensors.⁷⁶⁻⁷⁸ NADH oxidation proceeds at relatively high potentials at unmodified carbon electrodes (greater than +0.5 V), reducing specificity.^{78, 79} Typically, SPCEs are modified with nanomaterials, such as carbon nanotubes, to increase edge plane coverage since untreated SPCEs are relatively inactive.^{80, 81} Here, similar to other carbon electrodes, all untreated SPCEs exhibit a peak potential greater than +0.5 V for NADH oxidation, however, the catalytic activity of the SPCEs significantly improves after plasma treatment. Upon plasma treatment, the 2.5 to 3.5 3569 SPCE exhibits the lowest peak potential of 350 ± 15 mV, a decrease of about 175 mV. The 1.5 to 3.5 3569 SPCE demonstrated lower activity than its higher mass loading counterpart, likely due to a combination of increased resistance and fewer catalytic sites at the electrode surface. The Sigma graphite SPCE again exhibited a slightly higher peak potential and lower peak current than the high mass loading 3569 SPCE, however, this is probably related to the lower mass loading and

particle orientation resulting in additional resistance as discussed previously. The GC-SPEs did not perform as well as their graphite SPCE counterparts for this probe. This is consistent with literature where peak potentials of ~ 400 mV (vs. SCE) and ~ 600 mV (vs. SCE) were obtained at an edge plane pyrolytic graphite (EPPG) and GC electrode respectively.⁸⁰ The peak current for the plasma treated 2.5 to 3.5 3569 SPCEs was closest to the theoretically predicted peak current of 22.6 μ A estimated using Equation S6.2.

Surface Adsorption Dependent Species and SPCE Fouling

The electrochemical detection of dopamine (DA) is of substantial interest. DA is a neurotransmitter with excesses and deficiencies linked to neurological illnesses including Parkinson's, autism and schizophrenia.^{82, 83} The oxidation of DA is a surface adsorption dependent two-electron, two-proton process after which several side reactions can occur generating dopaminergic products that can adsorb to and block the electrode surface.⁸⁴ It is widely accepted that both the kinetics of DA oxidation and the susceptibility to surface passivation is highly dependent on carbon electrode surface microstrucutre.⁸⁴ Therefore, the oxidation and fouling characteristics of DA were studied on untreated and plasma treated SPCEs by recording successive CVs for a total of seven cycles. CV cycle #1 and #7 are shown in Figure 6.9a for (i) 2.5 to 3.5 3569 SPCEs and (ii) Sigma GC-SPEs. Figure 6.9b compares the change in peak current for successive voltammetric cycling for (i) 2.5 to 3.5 3569 SPCEs and (ii) Sigma GC-SPEs. The peak current data is shown as the ratio of the nth cycle to the first cycle (*in/imitia*). Consistent with previous results, plasma treatment significantly enhanced electron transfer kinetics for this surface sensitive probe indicated by the decreased peak separation and increased peak currents for both SPCEs.



Figure 6.9 Averaged (a) CVs (N = 3) for cycle #1 and cycle #7 of 1 mM DA in 0.1 M phosphate buffer (pH 7.4) at (i) 2.5 to 3.5 3569 SPCE and (ii) Sigma GC-SPE at untreated (black) and plasma treated (red) SPCEs. Plot (b) of the ratio of a given cycle # peak current (i_n) to initial peak current ($i_{initial}$) for (i) 2.5 to 3.5 3569 SPCE and (ii) Sigma GC-SPE at untreated (black) and plasma treated (red) SPCEs.

Initially, the 2.5 to 3.5 3569 SPCEs exhibit greater reversibility and sensitivity than Sigma GC-SPEs with ΔE_p 65 ± 6 mV and 90 ± 4 mV respectively. Commercial graphite SPCEs exhibited significantly more irreversible voltammetry of DA with ΔE_p of 232 mV after undergoing oxygen plasma treatment, providing further evidence of the enhanced quality of SPCEs fabricated in this work.²⁶ However, after seven cycles, ΔE_p at the 3569 SPCE decreases by 28% to 91 ± 6 mV while the Sigma GC-SPE decreases by only 14% to 103 ± 8 mV. This trend is also apparent in Figure 6.9bi and 6.9bii, where the peak current decreases less upon voltammetric cycling of DA with the Sigma GC-SPE compared to the 3569 SPCE. The peak current decreases, stabilizing after 3 cycles to about 72% of the initial peak current for the GC-SPE while peak current stabilization occurs after cycle four at the 3569 SPCE to about 66% of the initial peak current. These results are consistent with literature reports of GC electrodes exhibiting less susceptibility to fouling and

lowered sensitivity than both EPPG and BPPG electrodes at these DA concentrations.⁸⁴ Fouling has been found to be more severe at BPPG than both EPPG and GC, suggesting edge planes and defect sites are more resistant to fouling than basal planes. However, the graphite used here is highly heterogeneous consisting of both edge and basal planes and cannot be directly compared to EPPG or BPPG; therefore, the exact mechanism for differences in fouling severity at 3569 SPCEs and GC-SPEs is unclear at this time.⁸⁴ However, these results indicate that fouling severity at SPCEs can be tuned by careful selection of carbon particle. Surface oxide coverage also plays a role in stability toward DA cycling, with increased surface oxide coverage, this is likely partially responsible for the increased stability toward DA cycling of the plasma treated SPCEs.

6.5 Conclusions

The work presented in this chapter demonstrates that there is a significant relationship between bulk SPCE composition and electrochemical and physical properties of the final electrode. The carbon particle types used in this work result in SPCEs with widely different electron transfer properties toward a range of redox probes, with the responses related to binder content, surface oxides, carbon particle microstructure, particle purity, and particle size. Of significance, it was found among graphite types that larger carbon particles can achieve higher mass loadings in the binder, resulting in more particle to particle contacts and less resistive effects typically associated with SPCEs. The electrochemical properties and electron transfer rates of the 3569 graphite SPCEs were comparable to other CEs typically considered to be higher quality than SPCEs (e.g. TPEs). Carbon nanomaterials, such as carbon black, are limited to low carbon particle to binder ratios resulting in more resistive and mechanically unstable SPCEs. Therefore, it is recommended that carbon nanomaterials, such as carbon black, carbon nanotubes and graphene are better suited for use as surface modifications of SPCEs as opposed to serving as the main conductive component of SPCE formulations. In terms of GC microparticle SPEs, SEM imaging revealed that a narrower size distribution of particles results in a less resistive and higher quality GC-SPE. However, due to morphological differences, GC-SPEs are still more resistive than their graphite counterparts and are better suited to applications in which a surface with homogeneous electrochemical activity is beneficial as demonstrated in our previous work for anodic stripping voltammetry of heavy metals. Several redox probes were studied on all SPCE surfaces providing insights into the electrochemical reactivity of SPCEs fabricated with different carbon particles. Results indicate that SPCE electrochemical properties including catalytic activity, ohmic drop, and fouling characteristics can be tuned and improved with the careful selection of carbon particle type. Plasma treatment, which is fast and simple, was found to significantly enhance electrochemical characteristics of SPCEs through removal of surface binder and impurities, exposing the underlying, electrochemically active carbon particles. Increased double layer capacitance and decreased solvent window after plasma treatment can be minimized by employing carbon particles with a known high purity. Here, we present a systematic guide to SPCE composition that can be taken into account when developing new, high performance electrochemical PON sensors with printable carbon inks. Significantly, the improvement in electrochemical responses at SPCEs was achieved without complex surface modifications, which has broad implications for improving the ability to improve electrochemical PON sensors. Furthermore, these inks can be mass produced and printed into several geometries onto a variety of substrates including paper, PET films, glass, and plastics providing adaptability to a wide array of PON sensing applications.

REFERENCES

1.Hersey, M.; Berger, S. N.; Holmes, J.; West, A.; Hashemi, P., Recent Developments in Carbon Sensors for At-Source Electroanalysis. *Analytical Chemistry* **2019**, *91* (1), 27-43. 2.Klunder, K. J.; Nilsson, Z.; Sambur, J. B.; Henry, C. S., Patternable Solvent-Processed Thermoplastic Graphite Electrodes. *Journal of the American Chemical Society* **2017**, *139* (36), 12623-12631.

3.McCreery, R. L., Advanced carbon electrode materials for molecular electrochemistry. *Chemical Reviews* **2008**, *108* (7), 2646-2687.

4.Noviana, E.; Klunder, K. J.; Channon, R. B.; Henry, C. S., Thermoplastic Electrode Arrays in Electrochemical Paper-Based Analytical Devices. *Analytical Chemistry* **2019**, *91* (3), 2431-2438. 5.Klunder, K. J.; Clark, K. M.; McCord, C.; Berg, K. E.; Minteer, S. D.; Henry, C. S.,

Polycaprolactone-enabled sealing and carbon composite electrode integration into

electrochemical microfluidics. *Lab on a Chip* **2019**, *19* (15), 2589-2597.

6.Mikysek, T.; Svancara, I.; Kalcher, K.; Bartos, M.; Vytras, K.; Ludvik, J., New Approaches to the Characterization of Carbon Paste Electrodes Using the Ohmic Resistance Effect and Qualitative Carbon Paste Indexes. *Analytical Chemistry* **2009**, *81* (15), 6327-6333.

7. Svancara, I.; Hvizdalova, M.; Vytras, K.; Kalcher, K.; Novotny, R., A microscopic study on carbon paste electrodes. *Electroanalysis* **1996**, *8* (1), 61-65.

8. Svancara, I.; Vytras, K.; Barek, J.; Zima, J., Carbon paste electrodes in modern electroanalysis. *Critical Reviews in Analytical Chemistry* **2001**, *31* (4), 311-345.

9. Ataide, V.; Mendes, L.; Gama, L.; Araujo, W.; Paixao, T. R. L. C., Electrochemical paperbased analytical devices: Ten years of development. *Analytical Methods* **2020**.

10.Dungchai, W.; Chailapakul, O.; Henry, C. S., Electrochemical Detection for Paper-Based Microfluidics. *Analytical Chemistry* **2009**, *81* (14), 5821-5826.

11.Urbaniczky, C.; Lundström, K., Voltammetric studies on carbon paste electrodes: The influence of paste composition on electrode capacity and kinetics. *Journal of electroanalytical chemistry and interfacial electrochemistry* **1984**, *176* (1-2), 169-182.

12.Švancara, I.; Schachl, K., Testing of unmodified carbon paste electrodes. *Chem. Listy* **1999**, *93*, 490-499.

13.Adams, R. N., Carbon paste electrodes. Analytical chemistry 1958, 30 (9), 1576-1576.

14.Choudhry, N. A.; Kadara, R. O.; Jenkinson, N.; Banks, C. E., Screen printed electrodes provide micro-domain sites for fabricating disposable electro-catalytic ensembles. *Electrochemistry communications* **2010**, *12* (3), 406-409.

15.Kadara, R. O.; Jenkinson, N.; Banks, C. E., Characterisation of commercially available electrochemical sensing platforms. *Sensors and Actuators B: Chemical* **2009**, *138* (2), 556-562. 16.Morrin, A.; Killard, A. J.; Smyth, M. R., Electrochemical characterization of commercial and home-made screen-printed carbon electrodes. *Analytical letters* **2003**, *36* (9), 2021-2039.

17. Adkins, J. A.; Henry, C. S., Electrochemical detection in paper-based analytical devices using microwire electrodes. *Analytica Chimica Acta* **2015**, *891*, 247-254.

18. Mousavi, M. P.; Ainla, A.; Tan, E. K.; El-Rahman, M. K. A.; Yoshida, Y.; Yuan, L.; Sigurslid, H. H.; Arkan, N.; Yip, M. C.; Abrahamsson, C. K., Ion sensing with thread-based potentiometric electrodes. *Lab on a Chip* **2018**, *18* (15), 2279-2290.

19.Wang, J.; Tian, B.; Nascimento, V. B.; Angnes, L., Performance of screen-printed carbon electrodes fabricated from different carbon inks. *Electrochimica Acta* **1998**, *43* (23), 3459-3465. 20.Noviana, E.; McCord, C. P.; Clark, K. M.; Jang, I.; Henry, C. S., Electrochemical paper-based devices: sensing approaches and progress toward practical applications. *Lab on a Chip* **2019**, *20* (1), 9-34.

21.Carrell, C.; Kava, A.; Nguyen, M.; Menger, R.; Munshi, Z.; Call, Z.; Nussbaum, M.; Henry, C., Beyond the lateral flow assay: A review of paper-based microfluidics. *Microelectronic Engineering* **2019**, *206*, 45-54.

22.Cunningham, J. C.; DeGregory, P. R.; Crooks, R. M., New functionalities for paper-based sensors lead to simplified user operation, lower limits of detection, and new applications. *Annual Review of Analytical Chemistry* **2016**, *9*, 183-202.

23.Gong, M. M.; Sinton, D., Turning the page: advancing paper-based microfluidics for broad diagnostic application. *Chemical reviews* **2017**, *117* (12), 8447-8480.

24. Švancara, I.; Hvízdalová, M.; Vytřas, K.; Kalcher, K.; Novotný, R., A microscopic study on carbon paste electrodes. *Electroanalysis* **1996**, *8* (1), 61-65.

25.Wang, J.; Kirgöz, Ü. A.; Mo, J.-W.; Lu, J.; Kawde, A. N.; Muck, A., Glassy carbon paste electrodes. *Electrochemistry communications* **2001**, *3* (4), 203-208.

26.Wang, S.; Chang, K.; Yuan, C., Enhancement of electrochemical properties of screen-printed carbon electrodes by oxygen plasma treatment. *Electrochimica Acta* **2009**, *54* (21), 4937-4943. 27.Ghamouss, F.; Luais, E.; Thobie-Gautier, C.; Tessier, P.-Y.; Boujtita, M., Argon plasma treatment to enhance the electrochemical reactivity of screen-printed carbon surfaces. *Electrochimica Acta* **2009**, *54* (11), 3026-3032.

28. Washe, A. P.; Lozano-Sánchez, P.; Bejarano-Nosas, D.; Katakis, I., Facile and versatile approaches to enhancing electrochemical performance of screen printed electrodes. *Electrochimica Acta* **2013**, *91*, 166-172.

29.Wang, J.; Xu, Z.; Zhang, M.; Liu, J.; Zou, H.; Wang, L., Improvement of electrochemical performance of screen-printed carbon electrodes by UV/ozone modification. *Talanta* **2019**, *192*, 40-45.

30.Wang, J.; Pedrero, M.; Sakslund, H.; Hammerich, O.; Pingarron, J., Electrochemical activation of screen-printed carbon strips. *Analyst* **1996**, *121* (3), 345-350.

31. Sundaresan, P.; Chen, T. W.; Chen, S. M.; Tseng, T. W.; Liu, X. H., Electrochemical Activation of Screen Printed Carbon Electrode for the Determination of Antibiotic Drug Metronidazole. *International Journal of Electrochemical Science* **2018**, *13* (2), 1441-1451.

32.Cui, G.; Yoo, J. H.; Lee, J. S.; Yoo, J.; Uhm, J. H.; Cha, G. S.; Nam, H., Effect of pretreatment on the surface and electrochemical properties of screen-printed carbon paste electrodes. *Analyst* **2001**, *126* (8), 1399-1403.

33.Popa, A.; Abenojar, E. C.; Vianna, A.; Buenviaje, C. Y.; Yang, J.; Pascual, C. B.; Samia, A. C. S., Fabrication of metal nanoparticle-modified screen printed carbon electrodes for the evaluation of hydrogen peroxide content in teeth whitening strips. *Journal of Chemical Education* **2015**, *92* (11), 1913-1917.

34.Jirasirichote, A.; Punrat, E.; Suea-Ngam, A.; Chailapakul, O.; Chuanuwatanakul, S., Voltammetric detection of carbofuran determination using screen-printed carbon electrodes modified with gold nanoparticles and graphene oxide. *Talanta* **2017**, *175*, 331-337.

35.Antuña-Jiménez, D.; González-García, M. B.; Hernández-Santos, D.; Fanjul-Bolado, P., Screen-Printed Electrodes Modified with Metal Nanoparticles for Small Molecule Sensing. *Biosensors* **2020**, *10* (2), 9.

36.Muhammad, A.; Hajian, R.; Yusof, N. A.; Shams, N.; Abdullah, J.; Woi, P. M.; Garmestani, H., A screen printed carbon electrode modified with carbon nanotubes and gold nanoparticles as a sensitive electrochemical sensor for determination of thiamphenicol residue in milk. *RSC advances* **2018**, *8* (5), 2714-2722.

37.Mentele, M. M.; Cunningham, J.; Koehler, K.; Volckens, J.; Henry, C. S., Microfluidic paper-based analytical device for particulate metals. *Analytical chemistry* **2012**, *84* (10), 4474-4480.

38.Talarico, D.; Arduini, F.; Constantino, A.; Del Carlo, M.; Compagnone, D.; Moscone, D.; Palleschi, G., Carbon black as successful screen-printed electrode modifier for phenolic compound detection. *Electrochemistry Communications* **2015**, *60*, 78-82.

39.Cinti, S.; Mazzaracchio, V.; Cacciotti, I.; Moscone, D.; Arduini, F., Carbon black-modified electrodes screen-printed onto paper towel, waxed paper and parafilm M®. *Sensors* **2017**, *17* (10), 2267.

40.Randviir, E. P.; Brownson, D. A.; Metters, J. P.; Kadara, R. O.; Banks, C. E., The fabrication, characterisation and electrochemical investigation of screen-printed graphene electrodes. *Physical Chemistry Chemical Physics* **2014**, *16* (10), 4598-4611.

41.Ibáñez-Redín, G.; Wilson, D.; Gonçalves, D.; Oliveira Jr, O., Low-cost screen-printed electrodes based on electrochemically reduced graphene oxide-carbon black nanocomposites for dopamine, epinephrine and paracetamol detection. *Journal of colloid and interface science* **2018**, *515*, 101-108.

42.Kalcher, K.; Kauffmann, J. M.; Wang, J.; Švancara, I.; Vytřas, K.; Neuhold, C.; Yang, Z., Sensors based on carbon paste in electrochemical analysis: a review with particular emphasis on the period 1990–1993. *Electroanalysis* **1995**, *7* (1), 5-22.

43. Metters, J. P.; Kadara, R. O.; Banks, C. E., New directions in screen printed electroanalytical sensors: an overview of recent developments. *Analyst* **2011**, *136* (6), 1067-1076.

44.Kava, A. A.; Beardsley, C.; Hofstetter, J.; Henry, C. S., Disposable Glassy Carbon Stencil Printed Electrodes for Trace Detection of Cadmium and Lead. *Analytica Chimica Acta* **2019**. 45.Lemay, S. G.; van den Broek, D. M.; Storm, A. J.; Krapf, D.; Smeets, R. M. M.; Heering, H. A.; Dekker, C., Lithographically fabricated nanopore-based electrodes for electrochemistry. *Analytical Chemistry* **2005**, *77* (6), 1911-1915.

46.Mettakoonpitak, J.; Mehaffy, J.; Volckens, J.; Henry, C. S., AgNP/Bi/Nafion-modified Disposable Electrodes for Sensitive Zn (II), Cd (II), and Pb (II) Detection in Aerosol Samples. *Electroanalysis* **2017**, *29* (3), 880-889.

47.Adkins, J. A.; Boehle, K.; Friend, C.; Chamberlain, B.; Bisha, B.; Henry, C. S., Colorimetric and electrochemical bacteria detection using printed paper-and transparency-based analytic devices. *Analytical chemistry* **2017**, *89* (6), 3613-3621.

48.Berg, K. E.; Adkins, J. A.; Boyle, S. E.; Henry, C. S., Manganese Detection Using Stencilprinted Carbon Ink Electrodes on Transparency Film. *Electroanalysis* **2016**, *28* (4), 679-684. 49.Mettakoonpitak, J.; Volckens, J.; Henry, C. S., Janus Electrochemical Paper-Based

Analytical Devices for Metals Detection in Aerosol Samples. *Analytical Chemistry* **2020**. 50.NavarroLaboulais, J.; Trijueque, J.; GarciaJareno, J. J.; Vicente, F., Ohmic drop effect on the voltammetric behaviour of graphite plus polyethylene composite electrodes. *Journal of Electroanalytical Chemistry* **1997**, *422* (1-2), 91-97.

51.Fanjul-Bolado, P.; Hernández-Santos, D.; Lamas-Ardisana, P. J.; Martín-Pernía, A.; Costa-García, A., Electrochemical characterization of screen-printed and conventional carbon paste electrodes. *Electrochimica Acta* **2008**, *53* (10), 3635-3642.

52.Stozhko, N. Y.; Malakhova, N. A.; Fyodorov, M. V.; Brainina, K. Z., Modified carboncontaining electrodes in stripping voltammetry of metals. *Journal of Solid State Electrochemistry* **2008**, *12* (10), 1185-1204.

53.Zoski, C. G., Handbook of electrochemistry. Elsevier: 2006.

54.Mikysek, T. s.; Švancara, I.; Kalcher, K.; Bartoš, M.; Vytřas, K.; Ludvík, J. i., New approaches to the characterization of carbon paste electrodes using the ohmic resistance effect and qualitative carbon paste indexes. *Analytical chemistry* **2009**, *81* (15), 6327-6333.

55.Wei, H.; Sun, J.-J.; Xie, Y.; Lin, C.-G.; Wang, Y.-M.; Yin, W.-H.; Chen, G.-N., Enhanced electrochemical performance at screen-printed carbon electrodes by a new pretreating procedure. *Analytica chimica acta* **2007**, *588* (2), 297-303.

56.Zhou, J.; Wipf, D. O., UV/ozone pretreatment of glassy carbon electrodes. *Journal of Electroanalytical Chemistry* **2001**, *499* (1), 121-128.

57.Ranganathan, S.; Kuo, T.-C.; McCreery, R. L., Facile preparation of active glassy carbon electrodes with activated carbon and organic solvents. *Analytical chemistry* **1999**, *71* (16), 3574-3580.

58.Kozbial, A.; Zhou, F.; Li, Z.; Liu, H.; Li, L., Are graphitic surfaces hydrophobic? *Accounts of chemical research* **2016**, *49* (12), 2765-2773.

59.Bard, A. J.; Faulkner, L. R.; Leddy, J.; Zoski, C. G., *Electrochemical methods: fundamentals and applications*. wiley New York: 1980; Vol. 2.

60.Nantaphol, S.; Channon, R. B.; Kondo, T.; Siangproh, W.; Chailapakul, O.; Henry, C. S., Boron Doped Diamond Paste Electrodes for Microfluidic Paper-Based Analytical Devices. *Analytical Chemistry* **2017**, *89* (7), 4100-4107.

61.Arenhart, R.; Barra, G.; Fernandes, C., Simulation of percolation threshold and electrical conductivity in composites filled with conductive particles: Effect of polydisperse particle size distribution. *Polymer Composites* **2016**, *37* (1), 61-69.

62. Yuan, W.; Zhou, Y.; Li, Y.; Li, C.; Peng, H.; Zhang, J.; Liu, Z.; Dai, L.; Shi, G., The edge-and basal-plane-specific electrochemistry of a single-layer graphene sheet. *Scientific reports* **2013**, *3*, 2248.

63.Macpherson, J. V., A practical guide to using boron doped diamond in electrochemical research. *Physical Chemistry Chemical Physics* **2015**, *17* (5), 2935-2949.

64.Hutton, L. A.; Iacobini, J. G.; Bitziou, E.; Channon, R. B.; Newton, M. E.; Macpherson, J. V., Examination of the factors affecting the electrochemical performance of oxygen-terminated polycrystalline boron-doped diamond electrodes. *Analytical chemistry* **2013**, *85* (15), 7230-7240. 65.Smith, J. P.; Foster, C. W.; Metters, J. P.; Sutcliffe, O. B.; Banks, C. E., Metallic Impurities in Graphene Screen-Printed Electrodes Can Influence Their Electrochemical Properties. *Electroanalysis* **2014**, *26* (11), 2429-2433.

66.Ayres, Z. J.; Borrill, A. J.; Newland, J. C.; Newton, M. E.; Macpherson, J. V., Controlled sp(2) Functionalization of Boron Doped Diamond as a Route for the Fabrication of Robust and Nernstian pH Electrodes. *Analytical Chemistry* **2016**, *88* (1), 974-980.

67.Galdino, F. E.; Smith, J. P.; Kwamou, S. I.; Kampouris, D. K.; Iniesta, J.; Smith, G. C.; Bonacin, J. A.; Banks, C. E., Graphite screen-printed electrodes applied for the accurate and reagentless sensing of pH. *Analytical chemistry* **2015**, *87* (23), 11666-11672.

68.Martín-Yerga, D.; Costa-García, A. n.; Unwin, P. R., Correlative Voltammetric Microscopy: Structure–Activity Relationships in the Microscopic Electrochemical Behavior of Screen Printed Carbon Electrodes. *ACS sensors* **2019**, *4* (8), 2173-2180. 69.Keeley, G. P.; McEvoy, N.; Nolan, H.; Holzinger, M.; Cosnier, S.; Duesberg, G. S., Electroanalytical sensing properties of pristine and functionalized multilayer graphene. *Chemistry of Materials* **2014**, *26* (5), 1807-1812.

70.Shang, N. G.; Papakonstantinou, P.; McMullan, M.; Chu, M.; Stamboulis, A.; Potenza, A.; Dhesi, S. S.; Marchetto, H., Catalyst-free efficient growth, orientation and biosensing properties of multilayer graphene nanoflake films with sharp edge planes. *Advanced functional materials* **2008**, *18* (21), 3506-3514.

71.Chen, Q.; Swain, G. M., Structural characterization, electrochemical reactivity, and response stability of hydrogenated glassy carbon electrodes. *Langmuir* **1998**, *14* (24), 7017-7026.

72.Adkins, J. A.; Noviana, E.; Henry, C. S., Development of a Quasi-Steady Flow Electrochemical Paper-Based Analytical Device. *Analytical chemistry* **2016**, *88* (21), 10639-10647.

73.Singh, M.; Sahu, A.; Mahata, S.; Singh, P. K.; Rai, V. K.; Rai, A., Efficient electrochemical determination of p-aminophenol using a novel tricomponent graphene-based nanocomposite. *New Journal of Chemistry* **2019**, *43* (37), 14972-14978.

74.Su, W.-Y.; Wang, S.-M.; Cheng, S.-H., Electrochemically pretreated screen-printed carbon electrodes for the simultaneous determination of aminophenol isomers. *Journal of Electroanalytical Chemistry* **2011**, *651* (2), 166-172.

75.Vilian, A. E.; Veeramani, V.; Chen, S.-M.; Madhu, R.; Huh, Y. S.; Han, Y.-K., Preparation of a reduced graphene oxide/poly-l-glutathione nanocomposite for electrochemical detection of 4-aminophenol in orange juice samples. *Analytical Methods* **2015**, *7* (13), 5627-5634.

76.Rao, T. N.; Yagi, I.; Miwa, T.; Tryk, D.; Fujishima, A., Electrochemical oxidation of NADH at highly boron-doped diamond electrodes. *Analytical chemistry* **1999**, *71* (13), 2506-2511.

77.Pariente, F.; Tobalina, F.; Moreno, G.; Hernandez, L.; Lorenzo, E.; Abruna, H., Mechanistic studies of the electrocatalytic oxidation of NADH and ascorbate at glassy carbon electrodes modified with electrodeposited films derived from 3, 4-dihydroxybenzaldehyde. *Analytical chemistry* **1997**, *69* (19), 4065-4075.

78.Prieto-Simón, B.; Fàbregas, E., Comparative study of electron mediators used in the electrochemical oxidation of NADH. *Biosensors and Bioelectronics* **2004**, *19* (10), 1131-1138. 79.Bartlett, P. N.; Birkin, P.; Wallace, E., Oxidation of β -nicotinamide adenine dinucleotide (NADH) at poly (aniline)-coated electrodes. *Journal of the Chemical Society, Faraday Transactions* **1997**, *93* (10), 1951-1960.

80.Banks, C. E.; Compton, R. G., Exploring the electrocatalytic sites of carbon nanotubes for NADH detection: an edge plane pyrolytic graphite electrode study. *Analyst* **2005**, *130* (9), 1232-1239.

81.Sahin, M.; Ayranci, E., Electrooxidation of NADH on modified screen-printed electrodes: effects of conducting polymer and nanomaterials. *Electrochimica Acta* 2015, *166*, 261-270.
82.Venton, B. J.; Wightman, R. M., Psychoanalytical electrochemistry: dopamine and behavior. ACS Publications: 2003.

83.Li, J.; Lin, X., Simultaneous determination of dopamine and serotonin on gold nanocluster/overoxidized-polypyrrole composite modified glassy carbon electrode. *Sensors and Actuators B: Chemical* **2007**, *124* (2), 486-493.

84.Patel, A. N.; Tan, S.-y.; Miller, T. S.; Macpherson, J. V.; Unwin, P. R., Comparison and reappraisal of carbon electrodes for the voltammetric detection of dopamine. *Analytical chemistry* **2013**, *85* (24), 11755-11764.

85.Hadi, M.; Rouhollahi, A., Simultaneous electrochemical sensing of ascorbic acid, dopamine and uric acid at anodized nanocrystalline graphite-like pyrolytic carbon film electrode. *Analytica chimica acta* **2012**, *721*, 55-60.

CHAPTER 7: CONCLUSIONS AND FUTURE DIRECTIONS

The ability to perform analytical measurements at the point-of-need (PON) has become increasingly sought for both environmental and clinical monitoring applications to reduce cost and achieve early detection. PON sensing platforms must be small in size, easy to transport, require minimal equipment, be user friendly, provide fast time to answer and be disposalbe.^{1, 2} Electroanalysis can be adapted to fit all of these criteria, while also being highly sensitive and quantitative compared to other portable detection motifs, such as colorimetry. However, there are still challenges associated with adapting traditional electrochemical measurements to field deployable sensors which this thesis aimed to resolve.

One challenge that has limited the analytical utility of electrochemical PON sensors is the ability to minimize the number of liquid handling steps carried out by the final user, especially when multiplexed detection is required. The Janus-ePAD developed in Chapters 2 and 4 helped to address this issue by integrating electrodes with microfluidic paper-based analytical devices. Paper microfluidics can store reagents for rehydration upon sample addition and direct fluid flow from a single inlet to several wax-patterned channels by capillary action. In the Janus-ePAD developed in Chapter 2, a single sample's pH was adjusted in situ by storing reagents in the ePAD channels upstream from two separate electrochemical detection zones, allowing for solution preparation to be carried out in situ rather than through several liquid handling steps by an end user. Upon reaching the detection zones, electrochemical analysis was carried out on a single sample in two different pH conditions simultaneously. While this device was only applied to the detection of serotonin, norepinephrine, and pAP, there are a wide variety of applications in which a Janus-ePAD would be advantageous including multiplexed enzymatic assays and the pH dependent

detection of heavy metals. While the Janus-ePAD showed good results for multiplexed analysis in several solution conditions on one sample in the proof-of-concept stage, several challenges associated with the practical utility of the Janus-ePAD emerged. The first challenge was associated with inconsistent signals obtained with boron doped diamond paste electrodes (BDDPEs) integrated with the µPAD. Chapter 3 sought to further understand the electrochemical behavior of BDDPEs and improve their integration with the Janus-ePAD. It was found that pressing the BDDPEs at a high pressure to smooth the surface followed by an electrochemical pretreatment to remove the mineral oil binder from the surface solved these problems. However, due to the binder component of the BDDPEs, they are very difficult to mass produce, and are not an ideal electrode material for PON sensing applications. Other challenges encountered include poor reagent mixing upon reagent rehydration and long analysis times due to slow flow rates in paper.

To overcome these problems, Chapter 4 sought to develop a next generation hybrid JanusePAD. Multi-layered paper-based devices consisting of at least one layer of paper attached to either another layer of paper or a plastic material with layers of tape adhering the two layers have been developed to significantly increase flow rates in paper-based microfluidics through sample flow in the channel gap.^{3, 4} To solve the problem of slow flow rates in the one-layer Janus-ePAD, this concept was adopted for the development of a next generation hybrid Janus-ePAD in Chapter 4. The hybrid Janus ePAD consisted of a wax-patterned paper layer attached to a transparency film layer with several layers of double sided adhesive. This device provided significant improvements in flow rates, decreasing analysis time from around 40 min in the one layer device to less than 1 min. To solve the problem of heterogeneous dissolution and reagent rehydration which led to a pH gradient in the electrochemical detection zones of the one layer device, unbuffered sample was injected and buffers of the desired pH were stored in the paper channel, eliminating any mixing effects on pH adjustment. While this method for pH generation was successful since the pH a buffer is dependent on the ratio of conjugate weak acid and base pair, proper mixing would need to be addressed in other systems. Mixing of rehydrated reagents stored in paper microfluidics remains difficult and further methods to achieve uniform mixing need to be developed.⁵ For example, future work could involve the generations of a standard addition calibration curve with a Janus-ePAD storing standards in each of several channels on a Janus-ePAD, however, uniform mixing would be required to generate the desired standard concentrations at the detection zones for a calibration curve to be generated based on the electrochemical signals. The use of multiple layers in the hybrid Janus-ePAD also allowed for electrodes to be strategically placed on multiple layers so as to not impede fluid flow into the electrochemical detection and allowing for bulk solution electrochemical detection to be carried out in the channel gap, improving electrochemical signals. The hybrid-Janus ePAD could be applied to a wide variety of multiplexed sensing applications in which solution pH control is necessary to improve analytical performance.

Another challenge associated with electrochemical PON sensors is the development of high performing, mass producible, yet inexpensive and disposable electrode materials. Carbon composite electrodes, specifically stencil printed carbon electrodes (SPCEs), have been the electrode of choice for portable sensor applications. However, SPCEs rely on certain physical properties for stencil-printing and researchers typically rely on commercial SPCEs or commercial carbon inks for sensor development. Historically, has been widely accepted that SPCEs suffer from poor electrochemical characteristics compared to other carbon composite and traditional macro electrodes. For this reason, most SPCE sensor development has relied on extensive post-fabrication surface modifications to control the electrochemical activity of the surface. However, this is highly problematic when a sensor is meant to be cheap and disposable, limiting the actual

use of most SPCEs developed in academic settings. To solve this problem and improve the electrochemical characteristics of the innate SPCE, the work presented in Chapters 5 and 6 seek to develop better performing disposable carbon composite electrodes by adapting the components of the bulk material.

In Chapter 5, a novel SPCE composition was developed using glassy carbon microparticles and conductive ink (GC-SPE) for anodic stripping voltammetry (ASV) of Cd and Pb. The GC-SPE was able to overcome previous issues associated with using SPCEs for ASV by simply controlling the bulk composition of the SPCE. The GC-SPE did not require any post-fabrication modifications which have severely limited the commercial adoption of most SPCEs developed in academic settings due to cost prohibitive and time consuming surface modifications. The innate GC-SPE surface provided good sensitivity and environmentally relevant detection limits. The GC-SPEs are mass producible, easy to operate and are currently being produced by Access Sensor Technologies in Fort Collins, CO for commercial distribution. Further work with GC-SPEs should look into integrating GC-SPEs with traditional or paper-based microfluidics to carry out ASV under hydrodynamic conditions to decrease analysis times and improve sensitivity. Simple electrode pretreatments, such as plasma treatment could also be adopted to activate the GC-SPE surfaces and further improve sensitivity and detection limits.

Following the exciting development of the GC-SPE, Chapter 6 of this thesis aimed to further study the relationship between SPCE composition and electrochemical and physical properties using cyclic voltammetry and SEM imaging. Similar to other carbon composites differentiated from SPCEs by the material, SPCEs require surface activation to remove inhibitory binder from the electroactive carbon particle surfaces. This was achieved with plasma treatment which can be carried out on several electrodes simultaneously. A significant relationship was

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found between SPCE composition and electrochemical performance and physical integrity that can be used as a guide in future SPCE development geared to a variety of applications. It was found that through optimizing the carbon particle type and carbon particle to binder ratio, that SPCEs can provide similar electrochemical behavior to other carbon composites traditionally thought to outperform SPCEs. In Chapter 6, GC microparticles obtained from two different manufacturers (Alfa GC and Sigma GC) were used to fabricate SPCEs. Upon electrochemical characterization the Alfa and Sigma GC-SPEs provided significantly different electrochemical characteristics, especially after plasma pretreatment in terms of electrochemical reactivity and double layer capacitance. Preliminary work was carried out to study the effects of the two GC microparticles on ASV of Cd and Pb after plasma pretreatment. Figure 7.1 shows anodic stripping voltammograms obtained for 100 μ g L⁻¹ Cd and Pb at the two types of GC-SPEs after plasma treatment. The Sigma GC-SPE provided higher peak currents and lower background currents than the Alfa GC-SPE after plasma treatment, consistent with the data presented in Chapter 6. Compared to the native GC-SPEs, the peak current magnitudes for Cd and Pb increased by 2 times and 3 times for the Alfa and Sigma GC-SPEs, respectively. Further work would aim to optimize the plasma treated Sigma GC-SPEs for ASV detection of heavy metals. These preliminary data also illustrate the value of studying and optimizing bulk composition to improve electroanalytical utility of SPCEs.



Figure 7.1 Square wave anodic stripping voltammograms for $100 \ \mu g \ L^{-1}$ Cd and Pb at in situ plated Bi-film in 0.1 M pH 3.6 acetate buffer at a plasma treated Sigma (gold) and Alfa (green) GC-SPEs.

Further future work would aim to understand the relationship between carbon particle microstructure and electrochemical activity. Local scanning electrochemical cell microscopy (SECCM) and micro-Raman spectroscopy of the SPCE surfaces are critical to achieve the goal of understanding the structure-activity relationship of SPCE surfaces fabricated with different carbon particle types. For example, it would be interesting to understand why GC microparticle, a form of graphitic carbon, based SPCEs provide a homogeneously active surface for metal deposition during stripping voltammetry leading to single stripping peaks, while graphite powder based SPCEs lead to double stripping peaks.^{6, 7} Overall, this work has broad implications for the development of better performing disposable electrochemical PON sensors while retaining maximum simplicity and cost effectiveness.

REFERENCES

1.Gong, M. M.; Sinton, D., Turning the page: advancing paper-based microfluidics for broad diagnostic application. *Chemical reviews* **2017**, *117* (12), 8447-8480.

2. Martinez, A. W.; Phillips, S. T.; Whitesides, G. M.; Carrilho, E., Diagnostics for the developing world: microfluidic paper-based analytical devices. ACS Publications: 2009. 3. Noviana, E.; McCord, C. P.; Clark, K. M.; Jang, I.; Henry, C. S., Electrochemical paper-based devices: sensing approaches and progress toward practical applications. *Lab on a Chip* **2019**, *20* (1), 9-34.

4.Channon, R. B.; Nguyen, M. P.; Henry, C. S.; Dandy, D. S., Multilayered Microfluidic Paper-Based Devices: Characterization, Modeling, and Perspectives. *Analytical Chemistry* **2019**, *91* (14), 8966-8972.

5.Carrell, C.; Kava, A.; Nguyen, M.; Menger, R.; Munshi, Z.; Call, Z.; Nussbaum, M.; Henry, C., Beyond the lateral flow assay: A review of paper-based microfluidics. *Microelectronic Engineering* **2019**, *206*, 45-54.

6.Martín-Yerga, D.; Costa-García, A. n.; Unwin, P. R., Correlative Voltammetric Microscopy: Structure–Activity Relationships in the Microscopic Electrochemical Behavior of Screen Printed Carbon Electrodes. *ACS sensors* **2019**, *4* (8), 2173-2180.

7.Klunder, K. J.; Nilsson, Z.; Sambur, J. B.; Henry, C. S., Patternable Solvent-Processed Thermoplastic Graphite Electrodes. *Journal of the American Chemical Society* **2017**, *139* (36), 12623-12631.

APPENDIX I

SUPPORTING INFORMATION FOR CHAPTER 5: DISPOSABLE GLASSY CARBON STENCIL-PRINTED ELECTRODES FOR TRACE DETECTION OF CADMIUM AND LEAD



Figure S5.1 Schematic representation of GC-SPE fabrication. (a) Shows the stencil printing step, (b) shows an illustration of a GC-SPE, and (c) shows the final GC-SPE 3 electrode system with the solution reservoir attached.



Figure S5.2 Cyclic voltammograms for 1 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ in 0.1 M KCl at a 0.4 – 12 µm (solid line) and 10 – 20 µm (dashed line) GC-SPE (a). (b) Relationship between peak separation and scan rate and (c) relationship between peak current and square root scan rate.



Figure S5.3 Cyclic voltammograms recorded in 0.1 M KCl (pH 5.5) at a 0.4 to 12 μ m (gold line) and 10 to 20 μ m (blue line) GC-SPE.



Figure S5.4 Relationship between deposition time for and peak current obtained with SWASV for 100 μ g L⁻¹Cd and Pb in 0.1 M acetate buffer, pH 3.6. Deposition potential was -1.4 V vs. SCE.



Figure S5.5 Relationship between peak current and number of runs recorded with one electrode for 100 μ g L⁻¹Cd and Pb employing a 3 min deposition in 0.1 M acetate buffer, pH 3.6. Electrodes were cleaned between runs in acetate buffer by holding the potential at +0.4 V for 100seconds.



Figure S5.6 (a) SWASVs recorded for varying concentrations $(2.5 - 50 \ \mu g \ L^{-1})$ of Cd and Pb at GC-SPEs employing a 20 min deposition at -1.2 V vs. Ag/AgCl. (b) Calibration curves generated for Cd and Pb (N = 3).

APPENDIX II

SUPPORTING INFORMATION FOR CHAPTER 6: EXPLORING CARBON PARTICLE TYPE AND PLASMA TREATMENT TO IMPROVE ELECTROCHEMICAL PERFORMANCE OF STENCIL-PRINTED CARBON ELECTRODES



Figure S6.1 SEM image of 1.5 to 3.5 3569 SPCE.



Figure S6.2 SEM images of Alfa GC and Sigma GC SPEs. Particles $\geq 10 \ \mu m$ are denoted with red arrows.



Figure S6.3 Relationship between plasma treatment time of (a) 1.5 to 3.5 3569 (black), 2.5 to 3.5 3569 (green), and Sigma graphite SPCEs and (b) Alfa GC (red) and Sigma GC (blue) and peakto-peak separation (ΔE_p) of (i) FcTMA⁺, (ii) ferri/ferrocyanide and (iii) pAP.



Figure S6.4 Relationship between plasma treatment time of (a) 1.5 to 3.5 3569 (black), 2.5 to 3.5 3569 (green), and Sigma graphite SPCEs and (b) Alfa GC (red) and Sigma GC (blue) and peak current of (i) FcTMA⁺, (ii) ferri/ferrocyanide and (iii) pAP.



Figure S6.5 Relationship between plasma treatment time of carbon black SPCEs and (a) peakto-peak separation and (b) peak current for 1 mM (i) FcTMA⁺, (ii) ferri/ferrocyanide in 0.1 M KCl and (iii) 0.5 mM pAP in 0.1 M phosphate buffer (pH 7.4).



Figure S6.6 Relationship between elapsed time since plasma treatment for (a) 1.5 to 3.5 3569 (black), 2.5 to 3.5 3569 (green), and Sigma graphite SPCEs and (b) Alfa GC (red) and Sigma GC (blue) and peak-to-peak separation (ΔE_p) of (i) FcTMA⁺, (ii) ferri/ferrocyanide and (iii) pAP.



Figure S6.7 Relationship between elapsed time since plasma treatment for (a) carbon black SPCEs and peak-to-peak separation of (i) FcTMA⁺, (ii) ferri/ferrocyanide and (iii) pAP.

Table S6.1 Peak-to-peak separation for plasma treated SPCEs after 1 day and 10 days for CV of FcTMA⁺, ferri/ferrocyanide, and pAP.

Carbon Particle Type	FcTMA ⁺ ∆E _p (mV) Day 1	FcTMA ⁺ ∆E _p (mV) Day 10	ferri/ferrocyanide ∆E _p (mV) Day 1	Ferri/ferrocyanide ∆E _p (mV) Day 10	pAP ΔE _p (mV) Day 1	pAP ΔE _p (mV) Day 10
Alfa GC	110 (±3)	139 (±6)	219 (±8)	266 (±7)	116 (±3)	137 (±10)
Sigma GC	80 (±2)	75 (±2)	183 (±3)	186 (±11)	67 (±2)	86 (±2)
3569 2.5 to 3.5	53 (±1)	59 (±1)	105 (±13)	156 (±33)	39 (±2)	45 (±2)
3569 1.5 to 3.5	56 (±2)	65 (±1)	204 (±16)	216 (±12)	48 (±4)	59 (±3)
Carbon Black	102 (±6)	134 (±7)	174 (±3)	237 (±4)	92 (±4)	143 (±2)
Sigma Graphite	69 (±2)	77 (±4)	127 (±4)	152 (±9)	62 (±1)	66 (±3)

Carbon Particle Type	FcTMA ⁺ i _p (μA) Day 1	FcTMA ⁺ i _p (μA) Day 10	Ferri/ferrocyanide i _p (μΑ) Day 1	Ferri/ferrocyanide i _p (µA) Day 10	pAP i _p (μA) Day 1	pAP i _p (μA) Day 10
Alfa GC	30.2 (±0.5)	29.6 (±0.3)	35.3 (±2.5)	34.3 (±0.1)	33.5 (±1.1)	34.9 (±0.2)
Sigma GC	27.5 (±0.4)	36.6 (±0.6)	34.1 (±0.9)	39.4 (±1.6)	43.0 (±3.4)	42.4 (±0.8)
3569 2.5 to 3.5	30.2 (±0.9)	34.7 (±2.2)	50.2 (±3.4)	43.1 (±7.8)	44.6 (±1.3)	47.6 (±2.3)
3569 1.5 to 3.5	31.0 (±1.3)	30.3 (±0.6)	39.3 (±2.4)	30.9 (±1.1)	39.3 (±1.6)	40.6 (±4.6)
Carbon Black	31.6 (±3.0)	32.8 (±1.3)	41.8 (±1.7)	37.0 (±1.8)	32.7 (±3.3)	36.6 (±1.0)
Sigma Graphite	31.1 (±1.8)	35.7 (±1.9)	45.2 (±0.6)	41.8 (±1.9)	44.6 (±1.6)	42.1 (±2)

Table S6.2 Peak oxidation current for plasma treated SPCEs after 1 day and 10 days for CV of FcTMA⁺, ferri/ferrocyanide, and pAP.

Equation S6.1

The Randles-Sevcik equation predicts the magnitude of the peak current resulting in a cyclic voltammetry experiment under diffusion limited conditions. For a macroscopic electrode, the current is given by the following equation:¹

$$i_p = (2.69x10^5)n^{2/3}AD^{1/2}v^{1/2}C$$
 Equation S6.1

Where i_p = peak current (A) n = number of electrons transferred A = electrode area (cm²) D = diffusion coefficient (D_{FcTMA+} = 6.71 x 10⁻⁶ cm² s⁻¹)² v = scan rate (V s⁻¹) C = concentration of analyte (mol cm⁻³) **Table S6.3** Experimental peak oxidation currents for FcTMA⁺ recorded with CV at untreated and plasma treated SPCEs fabricated with different particle types 0.1 M KCl at a scan rate of 100 mV s^{-1} .

Carbon Particle Type	FcTMA ⁺ i _p (μA) Untreated	FcTMA ⁺ i _p (μA) Plasma
Alfa GC	24.3 (±0.4)	30.2 (±0.5)
Sigma GC	27.9 (±0.4)	27.5 (±0.4)
3569 2.5 to 3.5	27.8 (±1.6)	30.2 (±0.9)
3569 1.5 to 3.5	26.5 (±1.0)	31.0 (±1.3)
Carbon Black	21.4 (±0.5)	31.6 (±3.0)
Sigma Graphite	25.7 (±3.0)	31.1 (±1.8)

Table S6.4 Peak current recorded for UA and NADH at untreated and plasma treated SPCEs with CV in 0.1 M phosphate buffer (pH 7.4) at a scan rate of 100 mV s⁻¹.

Carbon Particle Type	UA i _p (μΑ)	UA i _p (μA) plasma	NADH i _p (μA)	NADH i _p (μA) plasma
Alfa GC	30.5 (±1.8)	41.1 (±1.1)	15.3 (±0.3)	19.4 (±2.2)
Sigma GC	26.1 (±5.3)	42.3 (±1.5)	13.5 (±0.7)	18.4 (±1.4)
3569 2.5 : 3.5	32.3 (±3.2)	48.6 (±3.9)	14.8 (±0.7)	21.6 (±2.3)
3569 1.5 : 3.5	30.0 (±1.3)	48.3 (±2.4)	13.9 (±0.4)	18.6 (±0.8)
Carbon Black	25.1 (±0.9)	44.5 (±4.0)	13.9 (±0.7)	18.8 (±1.4)
Sigma Graphite	30.8 (±1.1)	42.9 (±4.1)	13.1 (±1.3)	15.3 (±1.7)

Equation S6.2

The expected peak current for NADH was calculated using the following equation for an electrochemically irreversible, diffusion limited reaction for which the first electron is rate-determining:³

$$i_p = (2.99 \ x \ 10^5) ACD^{1/2} v^{1/2} n(\alpha n_a)^{1/2}$$
 Equation S6.2

where i_p = peak current (A) A = electrode area (cm²) C = analyte concentration (mol cm⁻³) D = diffusion coefficient (D_{NADH} = 3.24 x 10⁻⁶ cm² s⁻¹ in 0.1 M phosphate buffer)⁴ v = scan rate (V s⁻¹) n = number of electrons transferred (2) α = transfer coefficient (0.35)³ n_a = number of electrons transferred in the rate-determining step (1)

Carbon Particle Type	UA E _p (mV)	UA E _p (mV) plasma	NADH E _p (mV)	NADH E _p (mV) plasma
Alfa GC	450 (±25)	261 (±9)	533 (±22)	451 (±7)
Sigma GC	448 (±10)	218 (±7)	562 (±12)	400 (±30)
3569 2.5 : 3.5	450 (±10)	197 (±15)	513 (10)	350 (±15)
3569 1.5 : 3.5	454 (±11)	211 (±8)	533 (±15)	415 (±62)
Carbon Black	376 (±32)	261 (±12)	525 (±51)	370 (±13)
Sigma Graphite	405 (±26)	220 (±5)	512 (±18)	374 (±18)

Table S6.5 Peak oxidation potential recorded for UA and NADH at untreated and plasma treated SPCEs with CV in 0.1 M phosphate buffer (pH 7.4) at a scan rate of 100 mV s⁻¹.
REFERENCES

1.Bard, A. J.; Faulkner, L. R.; Leddy, J.; Zoski, C. G., *Electrochemical methods: fundamentals and applications*. wiley New York: 1980; Vol. 2.

2.Noviana, E.; Klunder, K. J.; Channon, R. B.; Henry, C. S., Thermoplastic Electrode Arrays in Electrochemical Paper-Based Analytical Devices. *Analytical Chemistry* **2019**, *91* (3), 2431-2438. 3.Banks, C. E.; Compton, R. G., Exploring the electrocatalytic sites of carbon nanotubes for NADH detection: an edge plane pyrolytic graphite electrode study. *Analyst* **2005**, *130* (9), 1232-1239.

4.Zare, H.; Golabi, S., Caffeic acid modified glassy carbon electrode for electrocatalytic oxidation of reduced nicotinamide adenine dinucleotide (NADH). *Journal of Solid State Electrochemistry* **2000**, *4* (2), 87-94.