ELECTROCHEMICAL TAPE-AND-PAPER-BASED PH SENSORS SUITABLE FOR ORAL PH MEASUREMENTS

by

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Dedicated to my parents, siblings, nieces, and nephew.

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ABSTRACT

Low oral pH (<5.5) has been shown to play an important role in dental erosion. The measurement of oral pH can be useful in preventative care, in aiding the dental caregiver in determining the likeliness of future dental cavities. The measurement of oral pH has become a popular area of research in an effort to develop a more quantitative method for the diagnosis of dental caries. We are developing an electrochemical tape-and-paper-based pH sensor for future applications in oral pH measurements. These tape-and-paper-based devices are low-cost, easy to fabricate, sterilizable, disposable and portable. The presence of intrinsic material defects of the painted graphite electrode generates oxo-groups which are electroactive. Some of these electroactive species, such as quinone, are pH-dependent and allow for the measurement of pH using cyclic voltammetry. There is shift in the potential of the redox peaks corresponding to the sensing species that can be correlated with the pH of a sample. We optimized the assay with a conditioning oxidative potential pre-treatment step and an ionic adjuster to carry out the pH measurements. We characterized the devices in different buffer solutions, as well as commercial pH standards and establish calibration curves. The reproducibility of the electrochemical response of the devices was also successfully across multiple devices and users. Their shelf-life was demonstrated to be at least three months. The devices successfully measured the pH of beverage and mouthwash, and different formulations of artificial saliva. Their performance in the presence bacteria and in growth media was assessed. Some complex matrices such as growth media required some additional optimization.

Towards this objective we fabricated and tested devices with various formulations of carbon paste for the painted working electrode. These flexible tape-and-paper-based devices are promising sensors for pH measurements in oral samples and potentially even for *in vivo* and *in situ* pH measurements.

CHAPTER 1. INTRODUCTION

This project addresses research in an established analytical sector, pH sensors, with different required characteristics and improvements, for new and upcoming applications: oral pH measurements. Low oral pH has become a frequent topic in the discussion of dental caries, one of the most prevalent yet reversible and preventable diseases in the world today. As new studies are conducted and more research is reported, low oral pH, specifically the establishment of a known critical pH for enamel demineralization has highlighted the need for a pH sensor that can be applied to oral preventative care. After describing some background information regarding oral pH and pH measurements, Chapter 2 highlights the different studies done in this research area and the types of sensors that have been developed over the years.

pH sensors are very common in general use. The glass pH probe has been around since the 20th century and is a staple in laboratories at all different levels. Current research has also showcased voltammetric pH sensors. Our tape-and-paper-based devices are voltammetric pH sensors and are further described in Chapter 3. The development and optimization of an earlier version of the device was done in a prior project and results of this work will be briefly introduced when needed. Some of the benefits of our proposed device address the common problems found in other oral pH sensors reported in the literature. Our tape-and-paper-based devices are low-cost, sterilizable, bendable, flexible, and disposable. In Chapter 3, the proof of concept of pH measurement using the devices is demonstrated and calibration curves are established. The reproducibility of the devices across users is also demonstrated in Chapter 3. The devices were then; used to test pH of different solutions from artificial saliva in Chapter 4 to oral samples (complex matrices) in Chapter 5.

Due to the complex nature of the oral environment and the bacterial biofilm that contributes to dental erosion, the devices were tested in bacterial samples and growth media in Chapter 6. Chapter 7 explores the change in composition of the electrodes using carbon paste and the electrochemical optimization of these carbon-paste devices. This project demonstrates the performance and improvements made on these tape-and-paper-based devices towards oral pH sensing. The testing in various samples, buffers, commercial samples, biological and chemical samples provided the data necessary to further optimize the devices for future oral pH measurement.

CHAPTER 2. BACKGROUND

2.1 Oral pH

2.1.1 Defining dental caries in relation to oral pH

To fully understand the relevance of the research that is presented here, it is first important to outline how this research will be useful when it can be applied to oral healthcare. Dental erosion is a very common process that occurs in the mouth and can lead to the development of dental caries[1]. Dental caries are defined as a dietobacterial disease and remain one of the most prevalent diseases in the world[2]. This disease consists of cycles of demineralization and remineralization of the tooth enamel[2, 3]. The critical pH for the erosion of dental enamel is around 5.5, below which, demineralization of the enamel occurs[2]. This low pH is caused by acidic byproducts of the cariogenic bacteria such as *Streptococcus Mutans (S.mutans)*[2]. The enamel of the tooth is labeled in Figure 1.

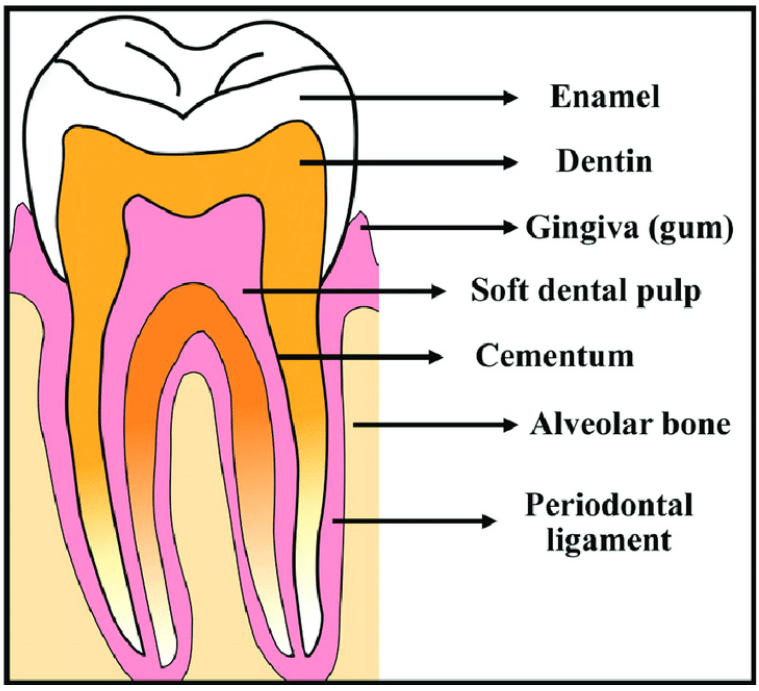

Figure 1. Human tooth structure (adapted from ref. 4) [4]

Since remineralization occurs in cycles, this process is ongoing, which allows for the reversibility of the disease in its early stages[2]. Early stages of dental caries disease begin with

subsurface demineralization, beginning below the enamel surface and therefore is not easily detected[5]. The remineralization process itself does not have to be complete to be effective in reversing the development of caries lesions[6]. Also, it should be noted that the resulting enamel produced from remineralization has been shown to be more resistant to further demineralization. Thus, there is an increased interest in preventative care options and the development of early detection methods have become coveted. Before an effective preventative treatment plan can be recommended, a diagnosis must first be made. The level of progression of demineralization also is important in this diagnosis, particularly if the disease is in its early stages. Factors contributing to demineralization and remineralization are displayed in Figure 2. Currently, early stages of the disease are difficult to detect due to the main methods employed as will be described in more detail later in this chapter[7, 8]. A more quantitative approach can be beneficial in the development of early detection methods. One popular area of research in this area is the measurement of oral pH [7, 9, 10].

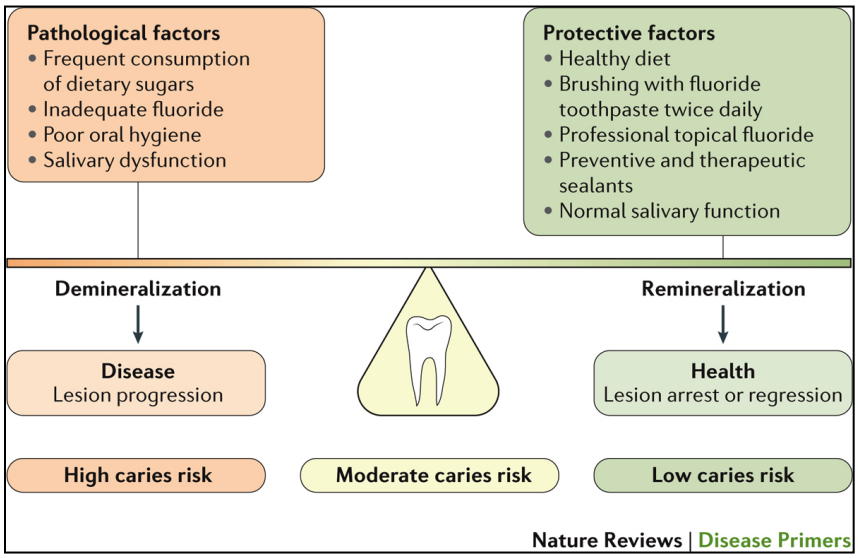

Figure 2. Factors determining equilibria for demineralization and remineralization (adapted from ref. 5)[5]

The International Caries Detection and Assessment System (ICDAS) provides a standard for which providers of dental care can refer to in diagnosing dental caries and planning the right treatment plan[11]. These protocols for caries assessment have been revisited four times over the last 18 years since the ICDAS was first established. These amendments follow the trends as new research is conducted, detection methods are improved, and as preventative care becomes more

popular. According to the most recent guidelines set by ICDAS, the main recommended method for caries detection is visual examination combined with radiography[11]. As those detection methods are based on visual observation, they are prone to human error[10]. Radiography is often used to detect the loss of mineral density and allows for more accurate diagnosis, however, it does not allow for quantitative analysis of dental caries[10]. None of the commonly utilized detection methods include measurement of the oral pH, nor are there any set guidelines for pH measurements when evaluating the stage of caries. As research in this field has become more popular, sensors for oral pH measurements have been developed in various ways. They will be further examined in section 2.3.1, "pH measurement in the oral environment".

2.2 Chemistry of the mouth

There is a complex mixture of fluids in the mouth which makes up saliva. The oral environment is also home to bacteria. In the mouth, a dynamic equilibrium of demineralization and remineralization exists within the oral environment[3]. In a healthy oral environment, there is a balance of this equilibrium therefore there is no net loss of enamel and dental caries does not progress[5]. There are many factors that influence oral pH, specifically the interaction of saliva with the bacterial biofilm on the teeth also known as plaque[3]. This interaction of saliva with the bacterial biofilm impacts where the equilibrium lies and thus also impacts the cycles involved in dental erosion[5]. An acidic environment encourages the demineralization of the dental enamel. Having a gauge as to where the equilibrium lies therefore goes hand in hand with the measurement of oral pH. Whether the pH measurement is of a bacterial sample from the mouth or saliva, either can give insight as to where the equilibria of demineralization and remineralization lies[3, 12].

2.2.1 The role of saliva in the oral environment

Saliva plays an active role in the oral environment and can affect the pH measurement. Understanding the role of saliva in the mouth and in dental caries is necessary for developing the best methods to measure oral pH. Saliva can restore the pH of the mouth and plays an important part in influencing the pH of the dental biofilm. Saliva itself is composed of various ions such as calcium, phosphate, and fluoride, which it supplies to the fluid phase of the biofilm, in close association with the surface of the tooth[13]. . Additionally, the carbonate/bicarbonate buffer system

present in saliva is one of its defensive components[14]. Saliva can also act as a buffer in the mouth, neutralizing the acid formed by biofilm bacteria[5, 15]. Therefore, saliva is said to play a protective role in the mouth in general as these properties make it very influential in the remineralization phase of dental caries. Thus, the salivary flow can influence the formation of cavities positively or negatively[5, 15]. Saliva also contains different proteins such as lysozyme, lactoferrin, lactoperoxidase, immunoglobulins, agglutinin, and mucins, which allow the saliva to carry out multiple physiological functions [13, 14].

Saliva has also been used as a diagnostic biomarker for the detection of the periodontal disease, gingivitis[7]. From a dental perspective, a salivary pH of 7.0 is often a good indication of a health oral environment[16]. The pH of unstimulated saliva lies within the range of 5-7[7, 17]. Unstimulated saliva, or resting saliva, refers to saliva produced when no exogenous or pharmacological stimulation is present[18]. Stimulated saliva refers to the stimulation when the saliva secretion is promoted under stimulation by gustatory, mechanical or pharmacological forces[18].

As previously mentioned, the critical pH threshold has been defined to be at a value of 5.5, this value was established by the many studies carried out in investigating the relationship between oral pH and dental caries[1, 3, 6]. The critical pH of the mouth is inversely proportional to the concentrations of calcium and phosphate in the saliva and the fluid of the plaque[12]. The relationship between the elemental composition of the saliva and the fluid of the plaque shows that there are multiple factors in the oral environment that will affect this critical pH. Research studies on salivary pH have reported that the pH of patients with generalized chronic periodontitis, the salivary pH was more acidic than that of the control group[16]. Thus, the salivary pH could be measured to reflect the oral environment, prior to developing a prototype capable to measure the actual plaque biofilm. The benefits to using saliva for running these diagnostic tests include the availability of saliva, ease of collection and storage[7]. Research studies involving pH of the saliva show that when the samples are collected and tested *ex-vivo* the pH does not change compared to the pH measured when tested inside of the mouth[7].

2.2.2 The effect of bacteria in the oral environment

Beyond saliva, understanding the role bacteria plays in the mouth, particularly in the dental caries, is necessary in developing a quantitative detection method involving pH measurement.

Though a complex topic, studies of the microorganisms present in the oral environment have been conducted and *Streptococcus mutans* is particularly well-studied. In these studies *Streptococcus mutans* had been isolated from ongoing carious lesions in the human mouth[2]. The bacterial environment of the mouth, however, is so diverse that this bacteria is only one of over 500 species found in dental plaque[2]. There are many members of the resident flora in the mouth which do not necessarily contribute to the development of the dental caries[15]. As mentioned earlier, two species of *mutans streptococci* have been found to be most significant in dental caries, however it must be again noted that the oral ecology is very complex. *Streptococcus mutans* and *Streptococcus sobrinus* are just the two most studied species and are considered the principal agents in enamel caries. There is evidence, however, suggesting that, due to the many factors that contribute to the formation of carious lesions, there may be other bacteria that could take the role of *S. mutans* in caries development.

2.3 Theory of pH and its measurement

pH as a theory and corresponding measurements were applied in chemistry as early as the 20th century, while the concept has existed even earlier[19]. The pH of a system is an intensive property, meaning it is not based on the size of the system. Instead, the pH of a system influences the position of equilibria of chemical and biological reactions, hence its importance in these fields[20]. Due to its importance and usefulness in many areas, pH measurements are very common. Initially pH was defined by Sørensen in terms of the concentration of hydrogen ions and later defined in terms of the relative activity of these hydrogen ions present in solution[21]. The relative activity of these hydrogen ions requires a convention for its evaluation[21]. Recommendations for the measurement of pH have been outlined by the International Union for Pure and Applied Chemistry (IUPAC), based on the Harned cell[21, 22].The simplest formula used to define pH is shown below in Equation 1.

$$pH = -\log[H^+]$$

Equation 1

Since measuring pH is very common in the laboratory and beyond, multiple established methods exist for pH measurement. The most conventional quantitative method to measure pH

relies on potentiometry, which mainly utilizes glass electrodes[23, 24]. The benefits of using the glass probes include easy handling as well as a high selectivity towards pH sensing[23]. Potentiometric pH sensors work by measuring the difference in electromotive force between the sensing electrode and a reference electrode[25, 26]. A pH meter consists of this indicative electrode, a reference electrode and some apparatus that can measure the difference in electromotive force[19]. In general, potentiometric pH sensors are popular in many different applications because their size can be reduced, the structure is relatively simple, and the cost of fabrication is relatively low. The relationship between the potential generated and the concentration of H^+ ions in the solution is given by the Nernst equation[8] (Equation 2).

$$E = E_0 - 2.302\left(\frac{RT}{nF}\right)logH^+$$

Equation 2

In Equation 2 , E is the indicative potential, E_0 represents the standard potential, R represents the universal gas constant, T represents the absolute temperature and F is the Faraday constant[27]. When developing an electrochemical pH sensor, the goal is for it to be highly selective to H^+ ions as well as display as close to an ideal Nernstian sensitivity as possible[7].

Potentiometry is not the center of current research for pH measurement devices being developed, instead, sensors are based on amperometry and voltammetry[24]. For amperometric and voltammetric sensors the application of a voltage is the driving force to induce redox reactions[25]. Both techniques offer high sensitivity and accuracy, but are known to have poor selectivity[25]. Other electrochemical method-based pH sensing systems include ion-selective membranes, two terminal microsensors, metal oxide and conductometric pH sensors[23]. Optical methods are also popular[28]. Optical pH sensors make use of indicator dyes, however, the major disadvantage with these sensors is their limited long-term stability[28].

Another common method of testing pH that is less precise, but fast and low cost, is the use of pH paper strips. These strips are made by using pH-responsive color changing molecules and are convenient, highly portable and relatively low cost[29]. These are attractive characteristics for oral application; however, this method is only semi-quantitative as the pH measurement is not decimally accurate[29]. Since these strips are color changing and need to be compared to a standard

to determine the pH, this does not solve the issue of human error which currently exists within the current methods in determination of presence of dental caries. Additionally, these paper strips are not responsive to slight changes in pH, which is very common when measuring different areas of the mouth or even the tooth[29, 30]. They also are not sterile, cannot be sterilized, and can contain hazardous chemicals therefore not applicable to be used in the mouth.

2.4 pH measurements in the oral environment

While measurement of pH is a very common and established practice in other applications, it has not yet been successfully and systemically applied to the field of dental care. Some studies reported measurements of oral pH or pH of oral samples as the connection between pH and demineralization[9, 10, 27]. The main challenge in measuring the pH of dental biofilm on teeth is due to the structural complexity of the biofilm and its heterogeneity[31]. The differences that exist in the composition of the biofilm as well as the overall complexity that exists in the oral environment have proved to be a challenge in developing an assay for the measurement of oral pH. There is also difficulty in measuring the pH *in situ*, at the tooth due to the rough and uneven surface of teeth, especially considering most of the sensors discussed utilize bulk, solid and flat sensing surfaces[7].

The dental biofilm has been found to be essential in the etiology of caries. The dental biofilm of plaque is directly related to the development of carious lesions[15]. Measurement of the pH of dental biofilm can contribute to the determination of the likelihood of dental erosion and the possibility of the progression of dental caries. Dental plaque itself is a complex biofilm in which bacteria can exist[15]. The involvement of this bacteria was previously outlined in Section 2.2.2 which put into context its role in the chemistry of the mouth. The oral environment is an excellent environment for the growth and survival of bacteria[15]. A way to directly measure the pH of this dental biofilm would be more reflective of the stage of caries rather than measuring the saliva. This means the device would need to be able to electrochemically measure pH of bacteria samples in general, as the dental biofilm is very complex. Despite these challenges, there have been several studies that have taken different electrochemical approaches to measure oral pH[7, 10, 30, 32].

Some documented attempts made use of the antimony electrode. However, this presented some issues. Firstly, the plated antimony electrode itself required constant re-standardization with a known buffer which made the process of measuring pH in different areas of the mouth

consecutively difficult[32]. There is considerable variation in the composition of the enamel among different teeth, or even in terms of different areas of a single tooth[15]. Another issue encountered with this method was that there needed to be a heavy deposit of plaque on the tooth surface in order to be certain that there was contact with the sensor for accurate pH measurement[32]. The level of deposited plaque is not consistent, adding to the limitations plaguing these types of methods.

The study by Forscher *et al.* reports the use of different potentiometric methods to study the pH of the biofilm of the plaque. In the study, they use the aforementioned plated antimony electrode *in situ* and a glass electrode *in vitro* to measure pH[32]. One benefit of the antimony electrode was its size which could allow for it to be used in many areas of the mouth due to its small diameter, but the size off the electrode was not reported in this study[9].While the measurement was successful, there were issues with both electrodes making them not a reliable enough method to be integrated into common oral care practice such as the sensitivity of the glass probe[30, 32]. Also, the toxicity of the antimony is an issue which eliminates it for possible use in the mouth[30, 32]. The fragility of the electrode proved to be an issue as well[30, 32]. The glass probe used for comparison in this study was not sensitive enough to be applicable for oral pH measurement[30].

Other recent research conducted led to the development of ion-sensitive field-effect transistors (ISFET) specifically for the application of pH measurement of plaque[7, 30]. The ISFET is mostly used in the field of oral pH research and has been used to develop multiple sensors[7]. In the study by Kuribayashi *et al*., a micro-pH ISFET sensor was used to directly study carious lesions by measuring intra-oral pH[30]. The superficial plaque and debris was scraped off of the tooth and then the sensor was used to directly measure pH[30]. The ISFET sensor was reported to measure pH within a clinically acceptable time. The values for a clinically acceptable time frame were not given however this result was encouraging. An issue, however, resided with the size of the micro-sensor itself, which had a diameter of 0.7 mm and a reference electrode with a 1 mm diameter. Another issue mentioned in this study was again the use of a solid, flat, sensing surface which was not compatible with the rough undulated surface of the tooth[30]. While the results of this study helped demonstrate how useful pH measurement of carious lesions could be in caries diagnosis, the researchers also concluded that this device required further development to be applicable[30].

According to a review on electrochemical pH sensing platforms, one of the more preferred methods for conducting oral pH measurement is the use of the Ir/IrOx electrode, which also used an ISFET configuration[7]. The Ir/IrOx sensors have become popular in research due to their ability

to overcome issues such as size of the sensing surface and ability to be used along a rough surface like the tooth[7]. The diameter of the Ir/IrOx electrode used in studies related to oral pH was 300 μm[7, 27]. The Ir/IrOx sensors were particularly interesting according to this review, due to their near Nernstian response and high repeatability[7]. Repeatability is important in all chemical measurements, but especially when an assay is used in a diagnostic capacity. Additionally, the oral pH varies, studies have shown differences in pH measurements depending on the stage of dental caries or which part of the tooth was tested by sensors[7, 27]. Therefore, a repeatable method of measurement is important to be able to compare the stages of demineralization precisely between different teeth. Our sensors will aim to have this high level of repeatability in measurement. Furthermore, our proposed device has the advantage of being disposable, sterilizable, low-cost and bendable.

In other studies, different metal oxide electrodes have been used for oral pH measurements. For example, tantalum oxide, (Ta_2O_5) was used but was not as popular in oral pH research as Ir/IrOx[28]. This sensor also uses an ISFET configuration. In a study by Murakami *et al*, the micro-pH sensor used was made of a silicon semiconductor, while the surface was made of a layer of tantalum oxide (Ta_2O_5)[9]. This study was somewhat successful as it determined that there was a difference in pH depending on the type of caries and the stage of demineralization. The arrested caries showed higher pH values than the active caries. The arrested caries had pH values ranging from 6.1 to 6.4 while the active caries showed lower values down to 5.3. While this study shows well how the stage of demineralization is directly correlated to pH, it should be noted that this micro-pH sensor was not consistent enough in reporting the pH values. The pH values reported varied by 0.1 to 0.5 depending on the method used to introduce the sensing area to the tooth. More development was required to fully be able to quantitatively assess pH[9].

Additionally, carbon microfiber has also been used in studies for oral pH sensing research[7]. The electrochemical technique used here was amperometry[33]. Unlike the previous two sensors described which focused on oral pH as in the tooth itself, this research reported measurements of saliva, both artificial and human samples[33]. In our work we were able to measure artificial saliva as well which will be discussed in a later chapter. It is important to note that our devices do use carbon materials too, however our measurements use voltammetry. Carbon materials have been commonly used in electrochemically for many reasons due to the benefits that it presents as an electrode.

While the methods outlined above work well in many scenarios, they are not fit for applications in the measurement of oral pH (*in vivo* or *in situ*) due to common issues previously mentioned like size and shape of electrodes, durability of device, and repeatability of measurements. Oral pH sensing, particularly that of saliva or the dental biofilm has additional criteria such as the biological components of the oral environment, for which the conventional pH measurement methods may not fit. There remains a lack of a reliable method of measuring oral pH, specifically of the dental biofilm and to a lesser extent the saliva.

In this research, we will present a tape-and-paper device that can be applied to the measurement of oral samples and therefore later, oral pH. The next chapters will describe the development, optimization, characterization, and testing in different relevant chemical biological and commercial samples of our tape-and-paper-based pH sensors.

CHAPTER 3. MEASURING PH WITH TAPE-AND-PAPER-BASED DEVICES

3.1 Concept of pH measurement.

As described in the previous chapter, the most generally used instrument for pH measurement, the glass pH probe, relies on potentiometry. The developed tape-and-paper-based devices, however, use the electrochemical technique of voltammetry to measure pH of solutions. Specifically, here we performed cyclic voltammetry. Cyclic voltammetry is a very common electrochemical technique where a potential range is scanned linearly, forward and backward, and the resulting current is measured[19].

To measure the oral pH using this technique, the appropriate electrodes must be selected. In this case we used carbon for both the working and counter electrodes and Ag/AgCl as a pseudo reference electrode. The reference electrode is termed as a pseudo reference Ag/AgCl electrode because it is a painted AgCl mixed electrode, not the standard Ag/AgCl electrode. The reference electrode provides a constant potential relative to the potential of the working electrode[19]. Carbon has multiple properties rendering it attractive for electrochemical use such as its wide potential window, low cost and the fact that it is relatively chemically inert[34].

Carbon is utilized for pH sensors due to these attractive electrochemical properties but also because of intrinsic defects on the carbon surface can induce the presence of redox active molecules[35, 36]. The sensing mechanism of our tape-and-paper devices is based on the presence of intrinsic defects on the carbon paint used to fabricate the working and counter electrodes on the devices[35]. These intrinsic defects react with the air to form various redox molecules such as quinones[36]. The study by Compton *et al*. reported the use of these redox molecules formed to measure pH and determined that the redox molecules formed were probably quinones. The study showed that these quinones have redox reactions that are pH-dependent as they undergo a $2H^+/2e^-$ electron transfer[33, 35]. This electron transfer allows for the devices to measure the pH electrochemically, specifically here using cyclic voltammetry. The resulting reduction and oxidation peaks in the voltammogram shift with a change in the pH of the solution. The peaks shift to lower potentials, i.e., more reductive potentials, with an increase in the pH of the solution being tested. The potential window scanned remains the same, and as the pH of the solution varies, the potential of the redox peaks in the cyclic voltammogram (CV) varies as well.

3.2 Initial stages of the project

This project was initiated by a previous graduate student who developed paper-based devices for the measurement of pH. She demonstrated the concept of the earlier prototypes which were paper-based, for the electrochemical measurement of pH by cyclic voltammetry. The optimizations included the determination that an ionic adjuster is required for the assay. An ionic adjuster of either 0.1 M KCl or NaCl was required in the solutions being tested to reach a threshold ionic strength and conductivity. The ionic adjuster was added for the calibration of the devices in commercial buffer solutions across the entire pH range and in all samples tested.

The optimizations also included the use of a pre-conditioning step whereby the devices are exposed to a higher oxidative potential during the first scan. This higher oxidative potential is used to encourage the formation of more oxo-groups on the surface of carbon electrodes which is the basis of the sensing mechanism of the devices. The redox peaks are more visible when the pre-conditioning step is used. Thus, in this work, we also exposed the devices to this oxidative potential by conducting an initial cyclic voltammetry scan from 1 V to – 0.5 V vs. Ag/AgCl. We used the redox peaks of the second scan, from 0.7 V to -0.5 V vs. Ag/AgCl, to determine the pH of the solution being tested.

3.3 Experimental Section for proof of concept of pH measurement with devices

3.3.1 Materials and Reagents

Whatman® qualitative filter paper Grade 2 was purchased from Fisher Scientific. Carbon conductive paint (E3456) and silver/silver chloride (E2908) (Ag/AgCl) conductive paint was obtained from Ercon (Wareham, MA). Potassium Chloride (KCl) was purchased from Fisher BioReagents. Standard buffer solutions were purchased from Sigma Aldrich. Buffers for calibration of the pH meter were purchased from Mettler Toledo.

3.3.2 Preparation of Solutions

A solution of 1 M KCl was prepared and used to adjust commercial buffer solutions to include a final concentration of 0.1 M KCl as the ionic adjuster. The pH of the solutions was

measured with a Mettler Toledo Sven Compact modular pH meter after running cyclic voltammograms.

3.3.3 Electrochemical Measurement

The cyclic voltammetry measurements were performed using an Autolab PGstat128 potentiostat (Nova 2.0). Cyclic voltammograms were run from 1 V to -0.5 V vs. pseudo-Ag/AgCl for the first cycle and from 0.7 V to -0.5 V vs. pseudo-Ag/AgCl for the second cycle with a scan rate of 0.02 V/s. The reference electrode is termed as a pseudo reference Ag/AgCl electrode because it is a painted AgCl mixed electrode, not the standard Ag/AgCl electrode. Analysis of cyclic voltammograms was performed on the ELChemviewer software, in which the peak potential was determined.

3.3.4 Fabrication of the devices

A 10-cm strip of masking tape was cut. Then a hole was punched at each end of the strip of tape using a 5/8-inch hole puncher. Pre-cut disks of P2 filter paper were prepared. One disk was placed over the tape on the sticky side. The strip of tape was then folded in half so that the hole covered the disk. A stencil was prepared with a CO_2 laser cutter. The prepared stencil was placed over the folded tape. Conductive paints were then applied to create the electrodes. The stencil was removed, and the device was left to dry until complete evaporation of the solvent. For convenience, they were typically left to dry for 24 hours under the fume hood. These steps are displayed in Figure 3.

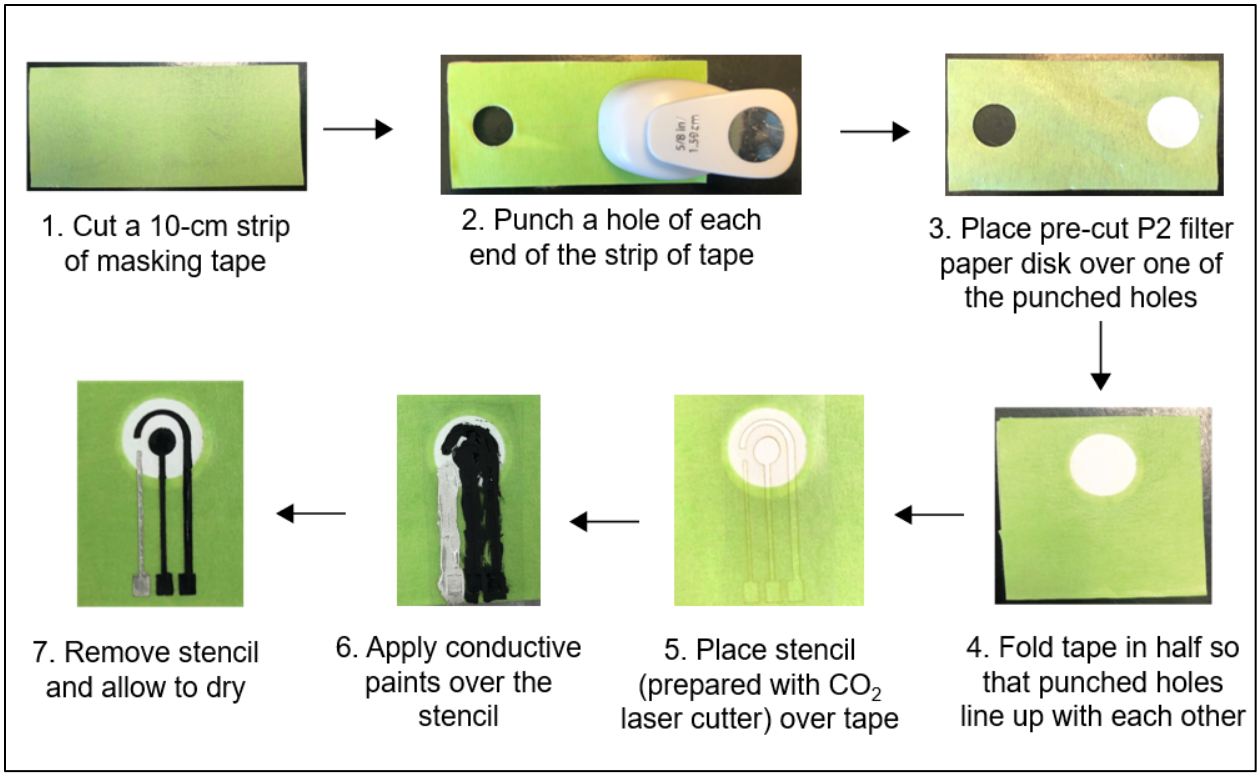

Figure 3. Fabrication steps for tape-and-paper-based-devices.

Prior to conducting electrochemical measurements, all devices were primed. To prime devices, they were dipped in approximately 8 mL of milli-Q H_2O for a minute and then left to completely dry. In between electrochemical measurements, devices were washed according to a specific washing procedure established in our group. To wash a device, it was first rinsed with milli-Q H_2O on each side three times. The device is then dipped in approximately 8 mL of milli-Q H_2O for 30 seconds in a small beaker. This step is repeated for a second time for 30 seconds in a different beaker of milli-Q H_2O.

3.4 Proof of concept using commercial buffer solutions.

The devices were first tested in standard buffer solutions at different pH values. Since the reactions of the quinones formed on the carbon surface are reductions (Figure 4), we use the reduction peak potentials for calibration over a wide range of pH. Additionally, as the redox peaks shift to more negative potentials with an increasing pH, the pH-dependent oxidation peak shifts towards the reference electrode oxidation peaks. The shift in the potential of the reduction and oxidation peaks as the pH changes is clearly visible in Figure 5. This shift in potential is the basis

for the measurement of pH using cyclic voltammetry. The analysis of the potential peaks can be used to determine the pH of the solution along with an established calibration curve equation. The cyclic voltammograms of four commercial buffer solutions can be seen in Figure 5.

There is a large sharp peak around 0.04 V vs. Ag/AgCl, which is believed to be due to the Ag/AgCl reference electrode. As higher pH solutions are tested, the oxidation peaks, for pH in the range 9-12, can overlap with this reference electrode peak.

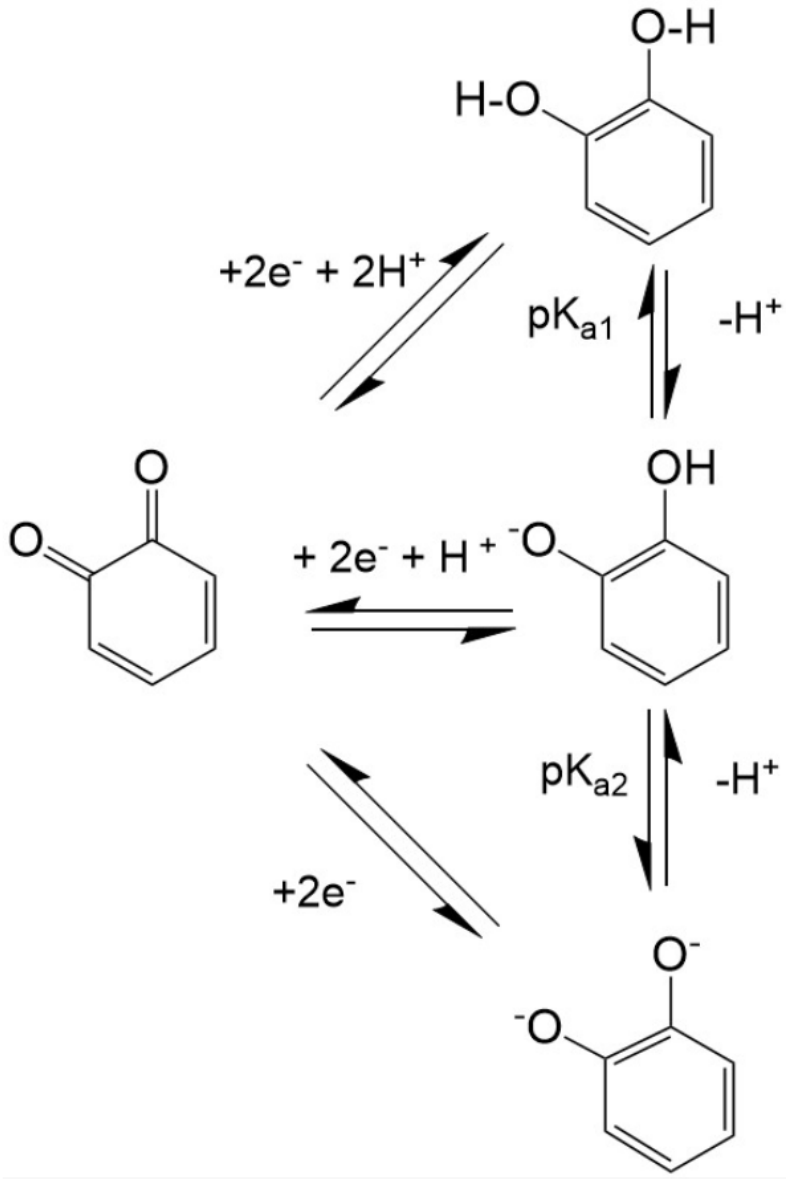

Figure 4. Reduction reaction pathways of ortho-quinone[35]

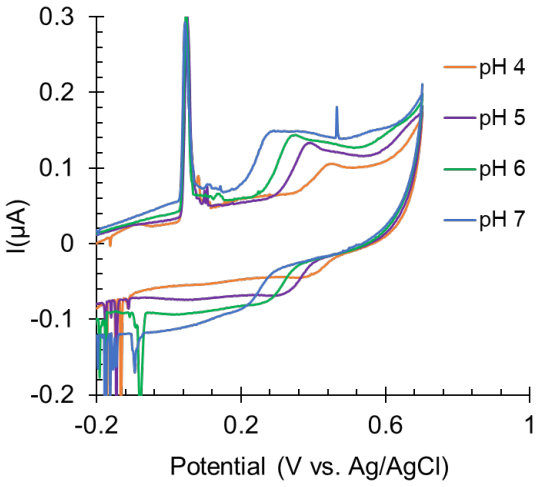

Figure 5. Overlay of cyclic voltammograms in standard buffer solutions pH 4 to 7 with 0.1 M KCl using the same tape-and-paper-based device.

The potential of the pH-dependent peak was determined with the ElChemviewer software. These standards allowed for building of a calibration curve and for the linear correlation to be assessed. The calibration curves of multiple devices over different pH ranges are shown in Figure 6. The devices showed strong linear correlation across multiple devices with a near Nernstian response. A Nernstian response can be described as a potential response that corresponds with the expected response according to the Nernst equation (Equation 2), having a slope around 59 mV/pH for the fitted line of the measured potential vs. the pH.

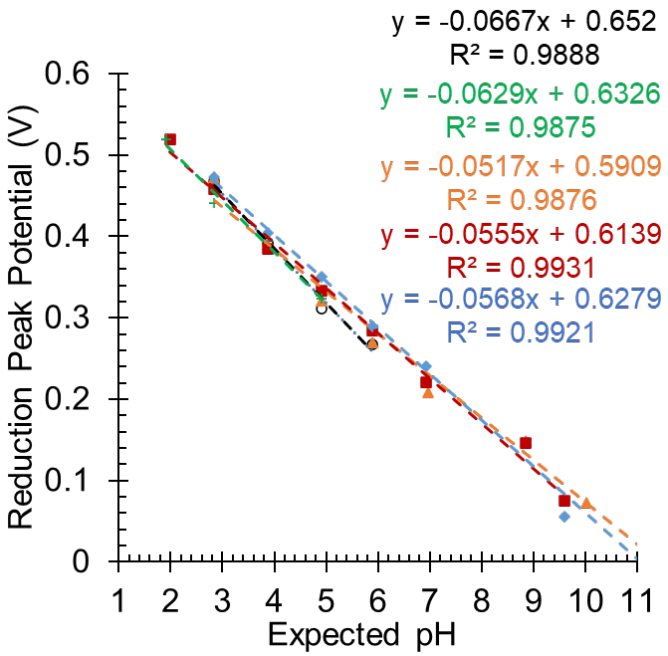

Figure 6. Overlay of calibration curves of reduction peak potentials (vs. Ag/AgCl) of five different tape-and-paper-based devices.

Values of the coefficient of determination R^2 for the five devices values of the curves were 0.9875 and above which corresponds to a good to strong linear correlation. The range of pH tested exceeded the expected range of oral pH, which is on average 6.2-7.6[16]. This pH range is the average range expected in a healthy oral environment. The calibration curves in Figure 6 were based on only the reduction peak potentials; however, both the reduction and oxidation peak potentials from the cyclic voltammograms of the commercial buffer solution can be used to establish calibration curves. A calibration curve over the pH range 3-9 using the oxidation peaks is shown in Figure 7.

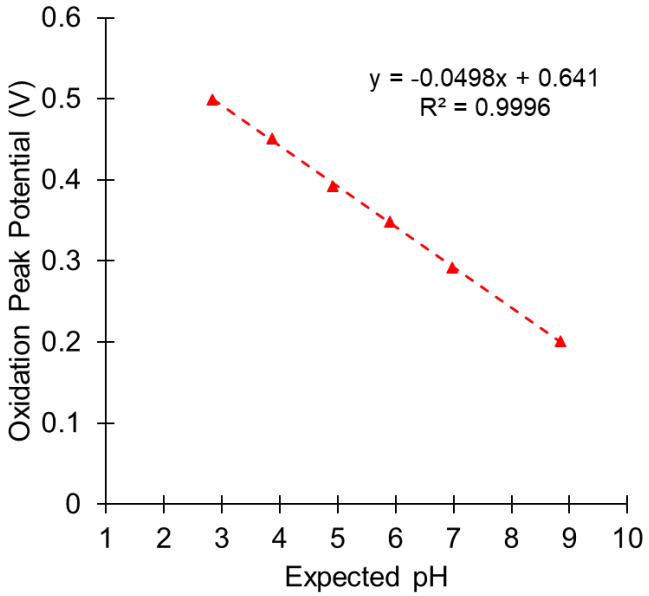

Figure 7. Calibration curve of oxidation peak potentials (vs. Ag/AgCl) of one device in standard buffer solution pH 3 – 9

Though the linear response is excellent with an R^2 value of 0.9996 for the calibration curve made using the oxidation peak potentials, the equation of the fitted line is not comparable to those of the calibration curves performed with the reduction peaks shown in Figure 6. The fitted response is further from the expected Nernstian response with a slope of -0.0498 V/pH and thus also lower sensitivity. As the different data sets shown in Figure 6 overlapped, they were combined and averaged to generate a single calibration curve covering the pH range of 2 to 12 (Figure 8).

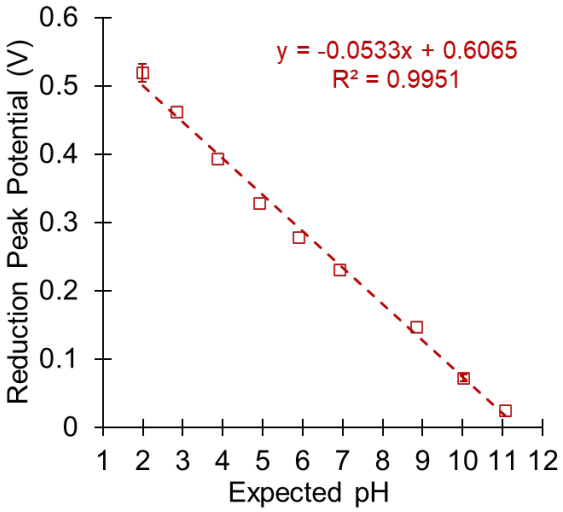

Figure 8. Calibration curve of averaged reduction peak potentials (vs. Ag/AgCl) of multiple devices in standard buffer solutions pH 2 – 12.

The averaged data displays a strong linear correlation with a R^2 of 0.9951. This great correlation on the averaged data from multiple devices was important to note as our devices are meant to be disposable. While they had the capability to be re-used, the data presented shows evidence that the pH measured using multiple devices is consistent across those devices.

The reproducibility of the devices across multiple users was tested by comparing the averaged data obtained by three different users. Figure 9 shows the calibration curves obtained with devices made by two previous researchers on the project. Table 1 shows the analysis of the results from the three users.

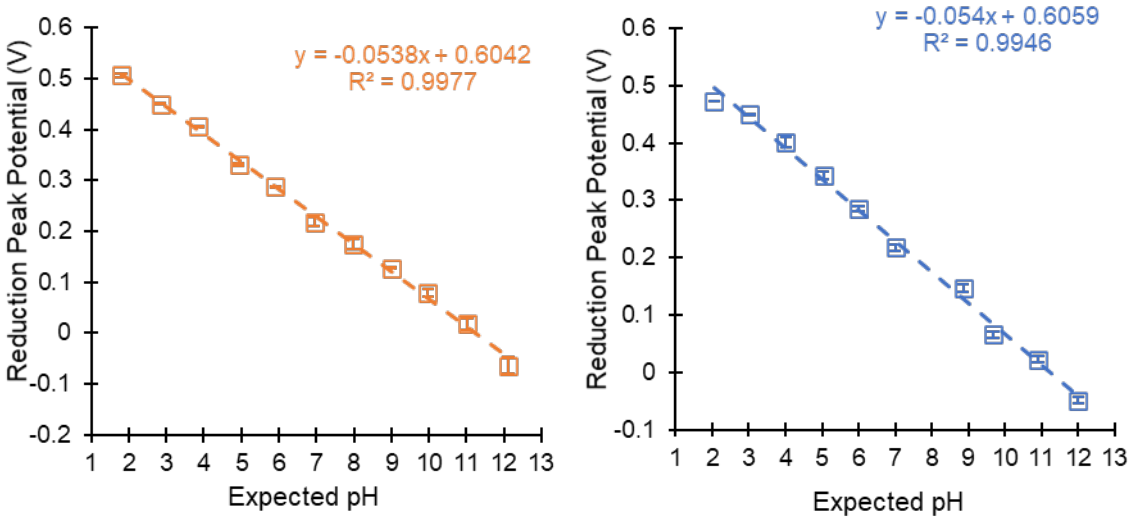

Figure 9. Comparison of calibration curves of devices made and tested by user 2 (a) and user 3 (b)

Table 1. Comparison of equations and linear correlations of calibration curves from different users.

User	Equation	R^2	Standard Deviation of Slope
User 1	y = -0.0533x + 0.6065	0.9951	0.0004
User 2	y = -0.0538x + 0.6042	0.9977	
User 3	y = -0.054x + 0.6059	0.9946	

Despite measurements being conducted on different handmade devices, fabricated by different people over two years, there was strong linearity across devices and users. Once the proof of concept and reproducibility were established, we explored shelf-life. To assess shelf-life, an experiment was conducted after a 3-month period: "old" devices were day 0 and "new" devices were made 3 months later (day 90). All devices were tested over the same pH range of standard buffer solutions made with the ionic adjuster added. Figure 10 shows how all the calibration curves from old and new devices overlapped and thus confirmed that the devices were not affected by a 3-month storage. Results show that there is no significant difference between old devices and new devices as they all had comparable equations. All devices displayed very good linear correlation

with R^2 values ranging from 0.9833 to 0.9987. Data from the calibration curves in Figure 10 is displayed in Table 2.

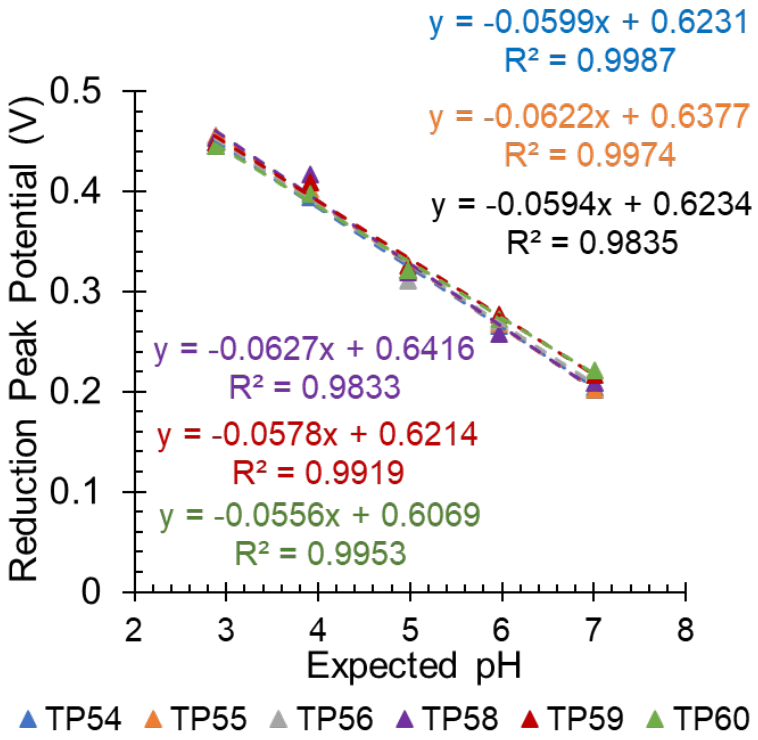

Figure 10. Shelf-life study overlay of calibration curves of reduction peak potentials. Commercial buffer solutions prepared with 0.1 M KCl as ionic adjuster. Data collected from six different devices, three devices were fabricated on day 0 and the other three devices were fabricated 3 months later.

Table 2. Comparison of calibration equations of old devices and new devices from shelf test, as well as comparisons of R^2 values.

Day 0	**Equations**	**R^2**	Standard Deviation of slope
TP54	y = -0.0591x + 0.6176	0.9998	0.003
TP55	y = - 0.0612x + 0.6304	0.9992	
TP56	y = - 0.0569x + 0.6055	0.9949	
Day 90	**Equations**	**R^2**	
TP58	y = - 0.0599x + 0.6218	0.9962	
TP59	y = - 0.0558x + 0.6080	0.9992	
TP60	y = - 0.0546x + 0.5995	0.9974	

The data from the shelf-life experiment were combined into one calibration curve. The reduction peak potentials for Day 0 and Day 90 devices were averaged and plotted in Figure 11.

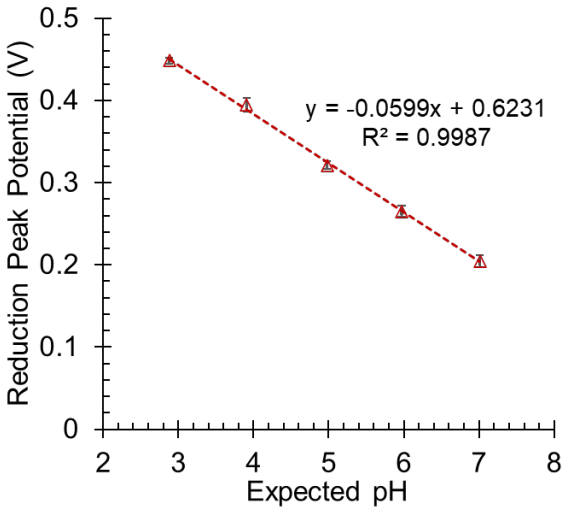

Figure 11. Shelf-life study calibration curve of averaged reduction peak potentials (vs. Ag/AgCl) in commercial buffer solutions, pH 3 to 7 commercial buffer solutions prepared with 0.1 M KCl (ionic adjuster). Data collected using six different devices, detailed in Table 2.

The resulting equation had a Nernstian response with a slope of 59.9 mV/pH. Additionally, the standard deviation on each potential was small, demonstrating the repeatability of the assay and the great shelf-life of the devices. Altogether, this chapter demonstrated the proof of concept of pH measurement of the tape-and-paper-based devices in commercial buffer solutions. The devices repeatability was also reported, and shelf-life was determined to be at least 3 months.

CHAPTER 4. PH DETERMINATION OF ARTIFICIAL SALIVA

4.1 Relevance of the saliva measurements.

When performing oral pH measurements, the environment of the tooth must be considered. This environment is mainly saliva which is a complex mixture and can encourage remineralization[5, 13]. Indeed, the tooth is surrounded by saliva which can carry away the harmful substances and simultaneously introduces restorative chemicals to the tooth's environment[13]. The role of saliva in the mouth is thus very integral to maintaining oral health. The close interaction between saliva and the tooth also means that the measurement of saliva can reflect the oral pH or locally the pH of the tooth. As seen in Chapter 2, multiple research studies in this area have targeted the measurement of salivary pH as an indicator for oral pH and dental erosion[18, 33, 37].

In the developmental stage, artificial saliva is typically used. Although less complex, it reflects the chemical composition of saliva, in particular, regarding the ionic composition which would be the source of main interferents with electrochemical measurements. Artificial saliva does not include the biological components found in real saliva. In the mouth there are a lot of proteins and different bacteria that exist naturally. These biological components make real saliva and the oral environment very complex. The salivary proteins vary in the mouth and play a protective role in the cycles of demineralization and remineralization[13]. According to the Food & Drug Administration (FDA), oral care givers can prescribe certain formulations of artificial saliva to help with xerostomia also known as "dry mouth".

In this work, we used two different artificial saliva formulations, indicated in tables 3 and 4, and adjusted their pH to represent a range of situations. As stated previously, the critical pH for dental erosion is around 5 – 5.5, therefore we adjusted the artificial saliva accordingly so that the solutions measured were higher and lower than the critical pH.

4.2 Experimental section pertaining to artificial saliva tests

4.2.1 Materials and Reagents

Potassium chloride (KCl) was purchased from Fisher Bioreagents. Potassium thiocyanate (KSCN), sodium chloride (NaCl), and potassium phosphate monobasic (KH_2PO_4) were purchased

from Fisher Chemical. Sodium phosphate monobasic (Na_2HPO_4) and sodium hydroxide (NaOH) were purchased from Fisher Scientific. Calcium chloride ($CaCl_2$), ammonium chloride (NH_4Cl), and citric acid ($C_6H_8O_7$) were purchased from Acros Organics. Hydrochloric acid (HCl) was purchased from Sigma Aldrich. Sodium carbonate ($NaHCO_3$) was purchased from Alfa Aesar.

4.2.2 Artificial Saliva Preparation

Artificial saliva stock solutions were prepared according to the formulations indicated in Tables 3 and 4. A stock solution of each artificial saliva formulation was prepared and then the pH was adjusted as needed. Artificial saliva solutions were adjusted with 1 M HCl and 1 M NaOH as needed. KCl was added as an ionic adjuster to the solutions after the pH was adjusted so that there was 0.1 M KCl added to the final solution. The pH was measured with Mettler Toledo pH meter after performing cyclic voltammetry.

Table 3. Formulation of artificial saliva #1. Stock solution of artificial saliva was prepared using these chemicals and concentrations (AFNOR standard: S90-701)[33].

Chemicals	Concentration	
	g/L	mmol/L
Na_2HPO_4	0.260	1.00
KH_2PO_4	0.200	1.50
$NaHCO_3$	1.50	18.0
KSCN	0.330	3.00
NaCl	6.70	115
KCl	1.20	16.0

Table 4. Formulation of artificial saliva #2. Stock solution of artificial saliva was prepared using these chemicals and concentrations[38].

Chemicals	Concentration	
	g/L	mmol/L
$C_6H_8O_7$	0.192	1.00
$CaCl_2$	0.111	1.00
KSCN	0.107	1.10
NH_4Cl	0.214	4.00
NaCl	0.292	5.00
KCl	1.118	15.0

4.2.3 Electrochemical Measurements

All electrochemical measurements were performed on Autolab PGstat204 potentiostat. The first scan of the cyclic voltammogram measurement was used as conditioning step, scanning the potential from 1 V to -0.5 V vs. Ag/AgCl. The potential range applied for the second scan was from 0.7 V to -0.5 V vs. Ag/AgCl. CVs were analyzed with the EL Chemviewer software using the second scan.

4.3 Artificial saliva recipe comparison

The artificial saliva #1 was mainly used in preliminary stages of the project. The pH of this artificial saliva, however, was not easily adjusted. The stock solution of artificial saliva #1 has a neutral pH, of around 7 and adjustment of the pH the solutions were attempted using strong base and acid to measure a range of 3 to 8. Given that artificial saliva acts as a buffer, however, great volumes of acid/base were required to adjust the pH of this formulation. This adjustment of pH therefore decreased the different ionic concentrations too much. Furthermore, redox peaks were not observed as consistently as would have been desired. Redox peaks were not observed in most CVs analyzed. Therefore, another formulation of commonly used artificial saliva was tested.

There are few notable differences between the artificial saliva formulations which could be explained when examining the variation in their chemical components. The artificial saliva #2 formulated in its stock had an acidic pH value, of 3.3, thus, much lower than the previous formulation used, and, well below the critical pH for the oral environment[12]. Thus, this low pH, more suited to our test, can be explained when looking at the different chemicals used in the formulation in Table 4. There are more acids used in the chemical composition of artificial saliva #2. This artificial saliva formulation was much less resistant to pH adjustment. Since the stock solution pH was very acidic, only NaOH, as a strong base, was used for pH adjustment to achieve higher values of pH.

The electrochemical measurements of artificial saliva were conducted in the same way that the commercial buffer solutions were measured. As previously stated, the pH-dependent redox peaks observed in these CVs were less defined. Initially, there was no ionic adjuster added to the artificial saliva since the formulations both already contain KCl and NaCl. However, after CVs were repetitively run with the lack of detection of pH-dependent peaks, the solutions were adjusted with the ionic adjuster in the same manner that the commercial buffer solutions were. This confirmed that concentration of NaCl/KCl < 20 mM does not have a sufficient ionic adjuster effect. Representative CVs for artificial saliva #2 are shown in the Figure 12.

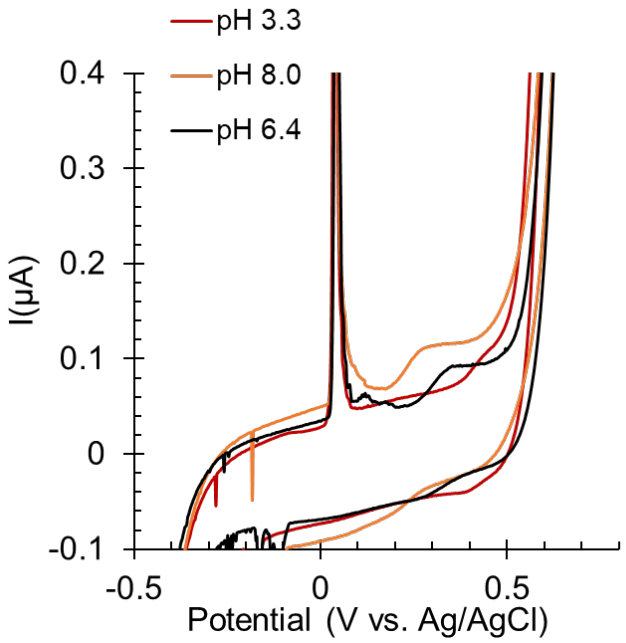

Figure 12. CVs of artificial saliva #2. Artificial saliva pH adjusted with 1 M HCl and then with 0.1 M KCl (ionic adjuster). CVs ran on three different tape-and-paper-based devices. pH measured with pH meter after CVs were ran.

Figure 12 shows well-defined peaks obtained when testing artificial saliva #2. The CVs for artificial saliva have a slightly different shape compared to the CV of commercial buffer solutions previously discussed in Chapter 3. The solvent wall appeared at a lower potential of 0.6 V vs. Ag/AgCl here compared to 0.7 V vs. Ag/AgCl of the commercial buffer solutions. Additionally, the peak for the solution at pH 3.3 is barely visible compared to the peaks for artificial saliva at pH 8.0 and pH 6.4. Furthermore, the reduction peak on the CV for the solution at pH 3.3 is more defined relative to the oxidation peak. Unlike the measurements of commercial buffer solutions, the devices could not be repeatedly used when testing artificial saliva. Therefore, a new device was used for each measurement. As the devices are designed to be disposable, and they have shown good repeatability across devices, this degradation of the electrodes was not an issue.

The reduction peak potentials of the CVs collected with artificial saliva were analyzed as described in the experimental section. The potentials of the peaks were used to calculate the pH from the devices. This was done by plugging the potential values in Equation 3 from a previously established calibration curve with commercial buffer solutions, not presented in the previous chapter. To determine the pH, the measured potential of the reduction peaks was used in Equation

3. Figure 12 shows the comparison between the pH as determined by the tape-and-paper-based devices and the pH measured with the pH meter.

$$y = - 0.0536x + 0.5986$$

Equation 3

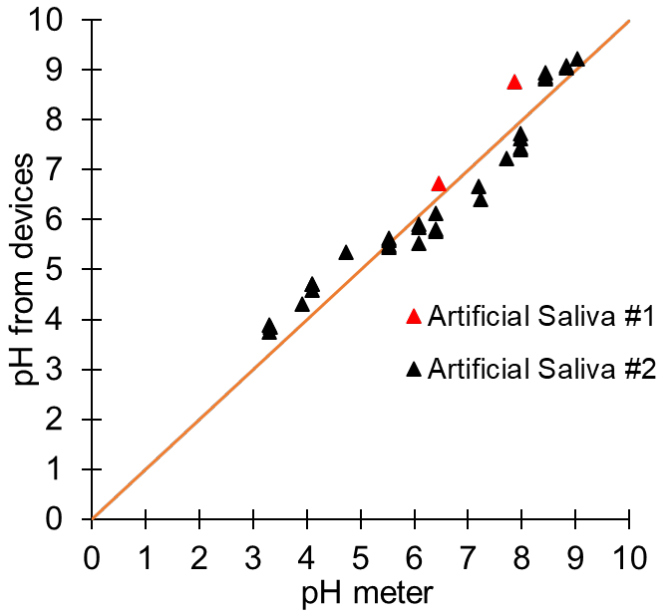

Figure 13. pH measurements of artificial saliva. CV of artificial saliva run on tape-and-paper-based devices. Orange line represents the 1:1 line for pH measurements from pH meter values vs. pH values from devices. pH of artificial saliva adjusted with 1 M NaOH and then with 0.1 M KCl (ionic adjuster). Data collected using eighteen different devices. PH meter measurements performed after CV.

The pH measurements of the artificial saliva with the devices corresponded with the measurements with the pH meter. Artificial saliva #1 was successfully measured by the devices at pH values close to its original neutral pH. There is more data for artificial saliva #2 as this formulation produced observable redox peaks more consistently than the artificial saliva #1. The pH range measured reflects the range that is typically expected in the mouth, and a few data points at the extreme pH values. The pH of artificial saliva #2 was successfully measured with the devices at a wider range for pH 3 to 9.

This chapter demonstrated the tape-and-paper-based devices capability to measure pH in artificial saliva samples of two different artificial saliva formulations and yielded values of pH comparable to the values obtained using a pH meter.

CHAPTER 5. TESTING COMMERCIAL SAMPLES

In this chapter, we report the electrochemical pH measurement of commercial samples with the tape-and-paper-based devices. The samples chosen are consumed via the mouth and interact with the oral environment. The oral environment is impacted by the foods and drinks we consume daily[5]. These drinks can affect the pH of the mouth, especially right after consumption[5]. Too many acidic drinks or foods can have an adverse effect on the enamel[5]. The cycles of demineralization or re-mineralization can be aided by these foods and drinks we consume, depending on their pH and how often they are consumed.

5.1 pH measurement of oral sample experimental section

5.1.1 Materials and Reagents

Crest© mouthwash and Simple Organic© Blueberry Ginger kombucha were purchased from a commercial grocery store. Potassium chloride (KCl) was purchased from Fisher Bioreagents. Sodium hydroxide (NaOH) was purchased from Fisher Scientific.

5.1.2 Preparation of samples for pH measurement

Samples were prepared with 0.1 M KCl as an ionic adjuster before running electrochemical measurements. The pH of the samples was measured with a Mettler Toledo pH meter after cyclic voltammograms were run.

5.1.3 Electrochemical Measurements

Electrochemical measurements were carried out on the same instrument indicated in previous chapter. A volume of 400 μL of sample was pipetted on to the front of the devices. CVs were performed with same method outlined in the previous chapters. A conditioning step was performed using the first scan, and the second scan was used for pH measurement. Analysis of the CV was done using EL Chemviewer software.

5.2 Kombucha

Kombucha is a fermented tea with live probiotics that has become more popular in recent years. The drink itself is very acidic, therefore after its consumption, the oral environment would have to readjust the equilibria to restore the pH of the mouth to a more neutral level. Kombucha has a pH of 3.3 when measured with the pH meter. The CVs of the kombucha were initially run without any treatment besides the addition of the ionic adjuster. The resulting CV had multiple redox peaks, but no peaks were visible at the potential expected to correspond to the pH of the kombucha observed. The kombucha was then diluted with milli-Q water to try to decrease the concentration of their electroactive species and thus the current of their respective redox peaks. This would render the pH-dependent peak more visible. As the kombucha has a pH of 3.3, the diluted kombucha was expected to have a higher value of pH and thus the potential of the redox pH-dependent peak should be more oxidative. Despite dilutions, however, the pH-dependent peak was still not observable, and the samples remained very acidic with pH below 4.

Considering kombucha is one of the most complex samples tested so far, we used it as an example of a complex matrix sample, to explore whether pH-dependent peaks could be observed for this type of sample. Using 1 M NaOH, the pH of two separate samples was adjusted. Therefore, the redox peaks were expected to be observed at a lower potential relative to the potential expected for the original kombucha sample. These attempts were successful, and the pH-dependent peak was observed for kombucha adjusted both to pH 8.1 and 6.1. The CV for the kombucha adjusted to pH 8.1 can be seen in Figure 14.

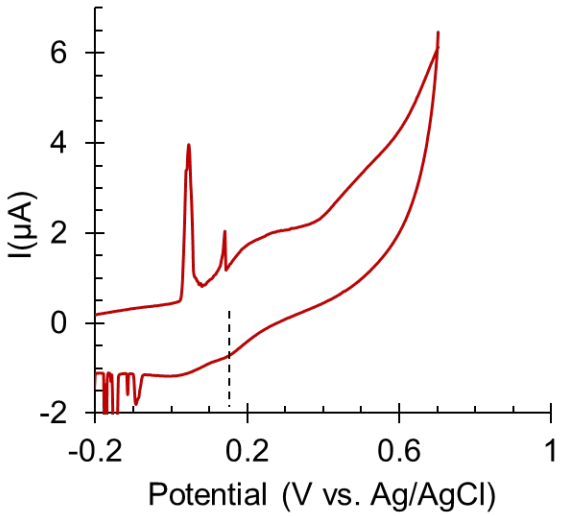

Figure 14. CV of Simple Organic© Kombucha with 0.1 M KCl (ionic adjuster), adjusted to pH 8.1 with 1 M NaOH. Scan rate 0.02 V/s from 0.7 V to -0.5 V.

The black vertical dashed line indicates the location of the pH-dependent reduction peak. The ElChemviewer software was used to precisely determine the potential of this peak. The pH measured with the devices was calculated from the potentials using Equation 3 from Chapter 4. These values matched the pH measured with the pH meter within 3%. The CV still displayed a few other peaks from some electrochemically active species in kombucha but demonstrated the ability of our pH sensor to test a sample with a complex matrix.

5.3 Mouthwash

Mouthwash is used primarily as an oral care product. It should not be swallowed but swished around in the mouth, and thus it also impacts the pH of the mouth. It is formulated to have a protective role in the little time it is in the mouth. The Crest© mouthwash was chosen here for its alcohol-free characteristic. Mouthwash is often used in day-to-day oral care. The mouthwash had a pH of 5.0 when measured with the pH meter. This pH value was surprising as the known critical pH for enamel demineralization, is pH 5. According to the website for another mouthwash brand, Listerine ©, mouthwash does not have an effect on salivary pH[39]. Instead, the low pH of mouthwash is intended to help kill bad breath germs.

Similar to the kombucha in the previous section, the CV of the mouthwash was initially run without any pH adjustments to the sample. The CV is shown in Figure 14.

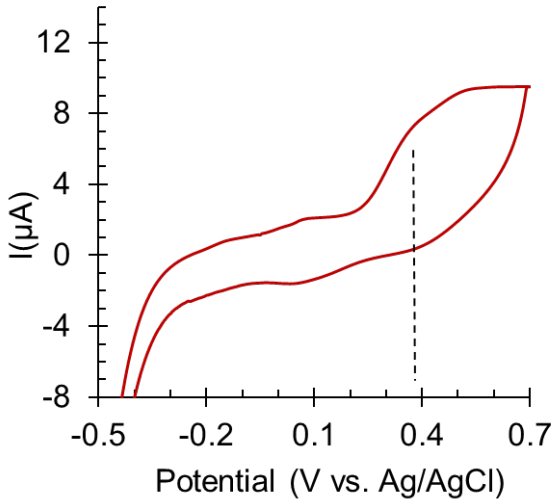

Figure 15. CV of Crest© mouthwash at pH 4.99 with 0.1 M KCl (ionic adjuster). Scan rate 0.02 V/s from 0.7 V to - 0.5V.

The dashed vertical line indicates the location of the pH-dependent reduction peak. The ElChemviewer software was used to precisely determine the potential of this peak. The pH measured with the devices was calculated from the potentials using Equation 3 from Chapter 4. These values matched the pH measured with the pH meter with an error of 10%.

The devices have been demonstrated to work in real samples, relevant to oral pH measurements, demonstrating the capability of the tape-and-paper-based devices for pH sensing in samples with complex matrix.

CHAPTER 6. MEASURING PH OF BACTERIAL SAMPLES

6.1 Relevance of Bacteria to the project

Due to the complex nature of the mouth, both chemically and biologically, the measurement of pH in bacteria samples is an important developmental stage of our devices so that they can later be applied to real oral pH measurements. For convenience at this early stage, non-pathogenic bacteria were used. This provided a suitable test for the devices with a more biological sample compared to the commercial buffer solutions and artificial saliva which more so reflected the chemistry of the oral environment.

6.2 Experimental section specific to bacterial media sample preparation

6.2.1 Materials and Reagents

Agar was purchased from Acros Organics. Bacto Tryptone was purchased from Becton Dickinson. Sodium Chloride was obtained from Fisher Chemical. Phosphate Buffer Saline 10X was obtained from Fisher BioReagents. A stock of E4104S *Escherichia coli (E. coli)* K12 ER2738 was obtained from New England Biolabs (NEB).

6.2.2 Bacteria Culture Preparation

Lysogeny Broth (LB) media was prepared with 1% (w/v) Tryptone, 0.5% (w/v) Yeast extract and 0.17 M NaCl. Agar was prepared using Tryptone, Yeast extract, NaCl and Agar powder. The solution was sterilized in the autoclave at 121°C for 50 minutes. The Agar was then left to cool down and then poured into petri dishes. A master colony plate was prepared using by swabbing 1 µL of the stock suspension of *E. coli* K12 ER2738 on a tetracycline agar plate. One colony from this master plate was placed in 25 mL of LB media and placed in the incubator at 37 °C at 200 rpm for 24 hours. After the 24-hour incubation, the overnight solution was tittered on agar plates. The agar plates were placed in the incubator at 37 °C overnight and the colonies were counted the next day to determine the concentration of bacteria used in the experiment. For measurements of PBS with bacteria, the overnight suspension was diluted by 1000 and suspended in PBS.

6.2.3 Preparation of Bacterial Media Sample for pH Measurement

LB media was added to a 20 mL scintillation vial and adjusted with KCl for a final solution that contained 0.1 M KCl. The pH of the LB media was measured with a Mettler Toledo Sven Compact modular pH meter after the CVs were ran.

Phosphate Buffer Saline (PBS) 10X was diluted to PBS 1X with milli-Q H_2O to have a final concentration as shown in Table 5. PBS was tested with the 0.1 M KCl as the ionic adjuster and the pH was measured with the pH meter as descried previously.

Table 5. Final concentrations of components in PBS 1X

Phosphate Buffer Saline (PBS) 1X	
NaCl	137 mM
KCl	2.7 mM
Na_2HPO_4	10 mM
KH_2PO_4	1.8 mM

6.2.4 Electrochemical Measurement of Bacteria

Prior to some of the electrochemical measurements, gas treatments were performed on the LB media with 0.1 M KCl. For the oxygen treatment, O_2 gas was bubbled in 20 mL of LB media with 0.1 M KCl for 10 minutes before running the CV. For the nitrogen gas treatment, N_2 gas was bubbled in 20 mL of LB Media with 0.1 M KCl for 10 minutes before the running the CV.

The cyclic voltammetry measurements were performed using an Autolab PGstat128 potentiostat. Cyclic voltammograms were run from 1 V to -0.5 V for the first cycle and from 0.7 V to -0.5 V for the second cycle with a scan rate of 0.02 V/s. Analysis of cyclic voltammograms was performed on the ELChemviewer software.

6.3 Measurements of bacterial media samples

CVs for LB media were performed as for all previous samples. Despite using the same method there was no pH-dependent peak observed in any of the multiple CVs attempted using multiple devices. Besides the absence of the desired peaks, overall, the CVs of LB media were similar to the CVs seen for other samples (Figure 16). The main difference is that the solvent wall

starts at 0.5 V vs. Ag/AgCl which is lower than with the standard buffer solutions where the solvent wall started at 0.7 V vs. Ag/AgCl.

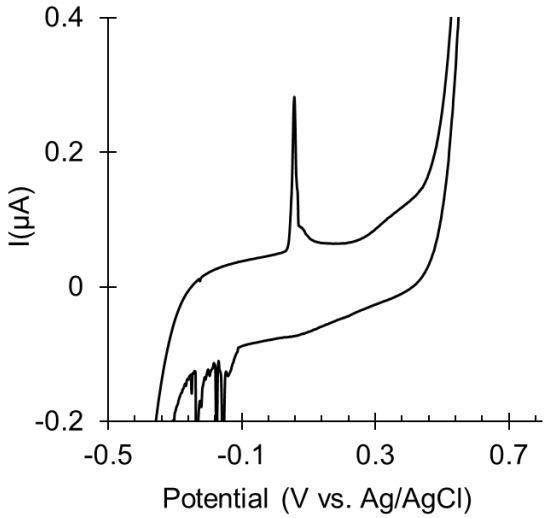

Figure 16. CV of LB Media with 0.1 M KCl (ionic adjuster) at pH 6.63. Scan rate 0.02 V/s from 0.7 V to - 0.5V.

To attempt to determine why the pH-dependent peak was not observed, we examined the composition LB media. The main difference noted between the various buffers and the LB media was the presence of yeast and tryptone. The ionic adjuster used in all samples was at the same concentration, however the composition of most of the other samples tested included more salts in the samples themselves. Therefore, we increased the concentration of the ionic adjuster, KCl, from 0.1 M to 1 M in LB media. However, this increased concentration of KCl did not yield a pH-dependent peak. We then modified the range the of scanned potentials and increased it up to 1 V vs. Ag/AgCl. While the range of 0.7 V to -0.5 V vs. Ag/AgCl worked for previous samples, the solvent redox peak shifted to the lower potential of 0.5 V vs. Ag/AgCl for the LB media. The hypothesis was that the solvent wall shift prevented the observation of the expected peak. The overall shape of the CVs, however, did not improve despite these slight changes to the method, and remained as seen Figure 16.

The shift of the solvent wall to a lower potential than with other samples could be linked to higher level of oxygenation of the LB media. To try to decrease the presence of oxygen in the sample we bubbled nitrogen for 10 minutes in the LB media prior to performing the electrochemical measurements. This pre-treatment would remove the oxygen, and thus hypothetically, decrease the background current and therefore allow the observation of the pH-dependent peak. Figure 17 compares the CV of the LB media without any treatment to the CV of the LB media after the nitrogen treatment.

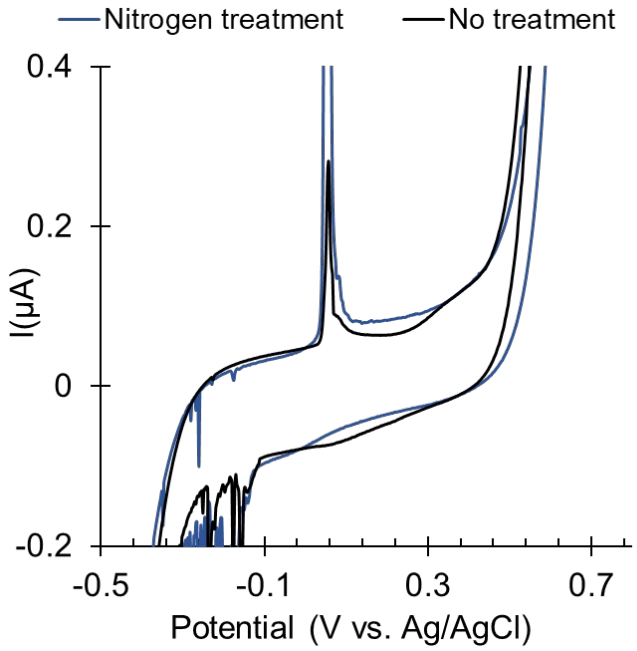

Figure 17. CV of LB media after bubbling of nitrogen for 10 minutes (blue line) compared to CV of LB media without (black line)

There was no significant difference in the current response with or without the nitrogen treatment as in both cases the pH-dependent peak of the LB media was not observed. Thus, instead of decreasing the capacitive current, i.e., the baseline current resulting from the overall electronic resistance of the electrochemical cell, we attempted to increase the Faradaic current, i.e., the current resulting from the targeted redox reaction. The main way to increase a Faradaic current is by increasing the concentration of the targeted electroactive species, in this case, the pH-dependent species formed by the presence of the oxo-groups.

By bubbling oxygen, the hypothesis was that more oxo-groups could be formed on the carbon electrodes would be increased and thus yield more pH-dependent redox species and a pH-

dependent peak with a greater current. The oxygen was bubbled in the LB media sample prepared with the 0.1 M KCl.

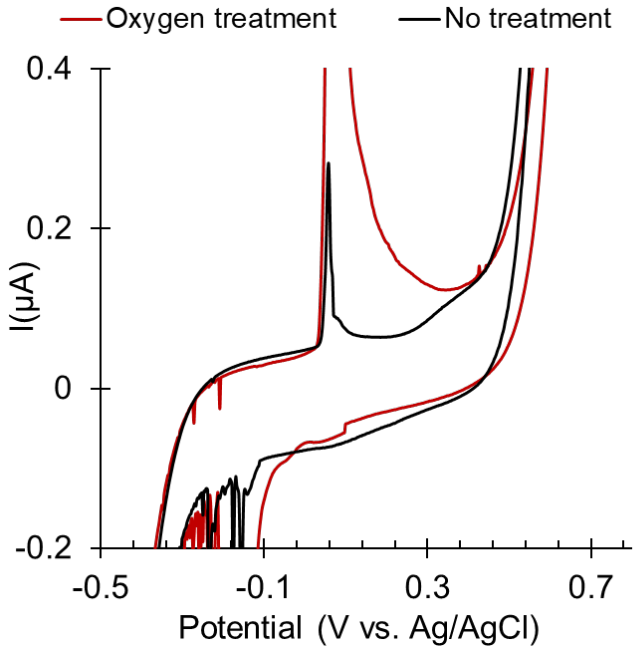

Figure 18. CV of LB media after bubbling of oxygen for 10 minutes (red line) compared to CV of LB media without.

The envisioned result of the oxygen treatment was that the intensity of the Faradaic current would be sufficiently great due to the added oxo-groups that the potential increase in the capacitive current due to the added oxygen in the system could be ignored. As seen in Figure 18, the oxygen treatment was unsuccessful to induce an observable pH-dependent peak. As a control, the same gaseous treatments were performed in the commercial buffer solutions to identify if these results were due to the LB media or would be common to any sample.

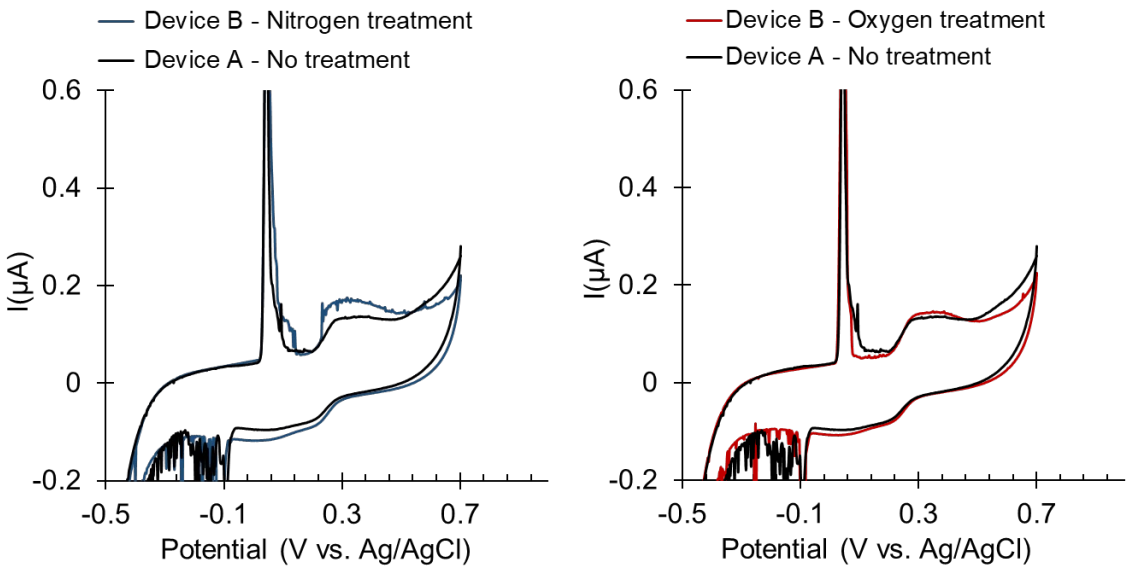

Figure 19. CVs of commercial buffer solution pH 7.00 after bubbling nitrogen for 10 minutes (blue line), after bubbling oxygen for 10 minutes (red line) compared to CV without treatment (black line). CVs performed using two different devices.

Figure 19 shows that for the commercial buffer solutions, the pH-dependent peak is observed in all conditions. Neither treatment affected the intensities of the currents, Faradaic or capacitive. Although not helpful for the pH measurement in LB media, these results demonstrated that for measurable samples, the results from the tape-and-paper-based devices were not affected by the different level of oxygenation of the samples.

The failure of all these attempts to measure the pH of the LB media lead to the hypothesis that LB media might degrade the sensing elements on the electrodes or the electrodes themselves. To test this hypothesis 3 CVs were ran successively on the same device: (i) in commercial buffer, pH 6.0, (ii) in LB media, pH 6.6, and again (iii) in commercial buffer pH 6.0 (Figure 20).
The chosen standard buffer solution was at a pH of 6.0, as it was close to the pH 6.6 of the LB media as measured with the pH meter.

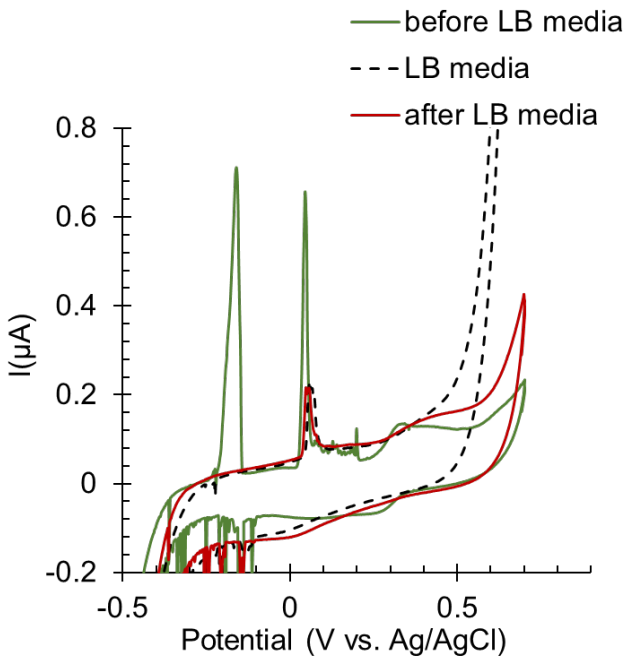

Figure 20. CVs of commercial buffer solution with 0.1 M KCl pH 6.0 before (green line) and after (red line) CV of LB media (dashed black line). All CVs were ran on the same device.

Figure 20 shows that the device cannot be used for pH measurement after a CV performed in LB media. The pH-dependent peak visible in the standard buffer CV at 0.27 V vs. Ag/AgCl before the LB media is no longer visible after the LB media run. This test was repeated on five different devices to confirm the results. After each run in the LB media, the device could no longer measure the pH of the standard buffer solution. Thus, it was concluded that the LB media does indeed damage the tape-and-paper-based devices. The attempt to measure the pH of LB media was done for this developmental stage of the project, as bacteria is typically cultured in LB media in the laboratory, however, this problem does not affect the overall goal of this project as LB media is not present in the mouth.

6.4 pH measurements of Phosphate Buffer Saline

Phosphate buffer saline (PBS) is a biological buffer that is often used in microbiology as it provides a stable environment for bacteria. As previously stated, LB media damages the electrodes on the devices, therefore, PBS was tested to assess whether this was a suitable option for future bacterial sample tests. The solution was tested with and without the ionic adjuster. There is already

about 137 mM NaCl in the PBS solution that was tested, which may provide enough ions for the electrochemical pH measurement, however, the ionic adjuster was added for consistency with our previous measurements.

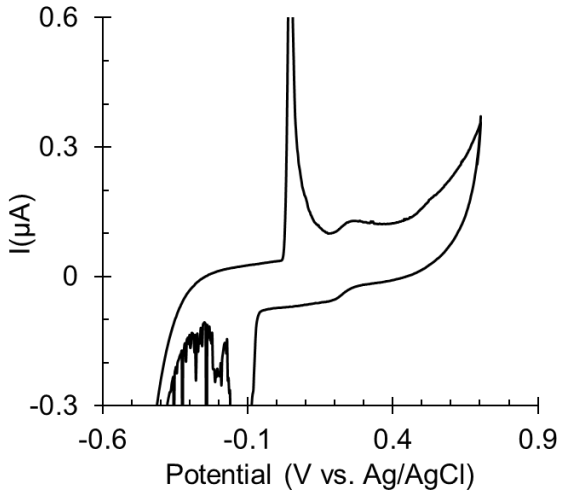

Figure 21. CV of PBS at pH 7.50 with 0.1 M KCl (ionic adjuster). Scan rate 0.02 V/s from 0.7 V to -0.5 V.

The PBS solution was successfully tested with the tape-and-paper-based devices. The pH-dependent peak at 0.20 V vs. Ag/AgCl for the pH value of 7.5 was observed in Figure 21, and it corresponded with the pH value obtained with the pH meter. Thus, PBS was noted as a possible solution to use for preparation for bacterial measurements. This idea was tested by suspending *E. coli* K12 ER2738 in PBS and performing the CV on a device (Figure 22).

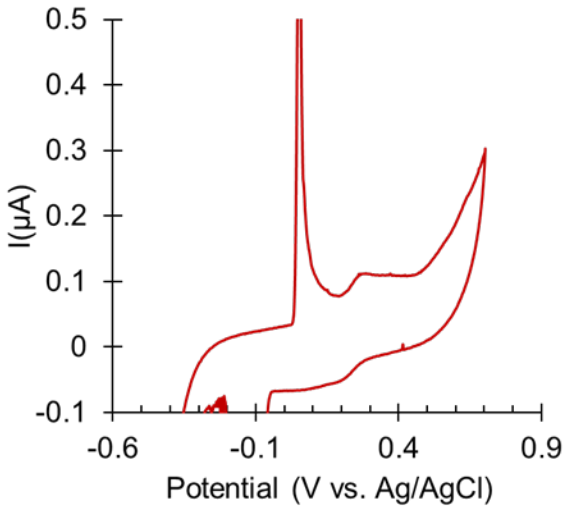

Figure 22. CV of PBS with 0.1 M KCl at pH 7.52 in the presence of *E. coli K12 ER2738*

The CV in Figure 22 shows that the presence of the bacteria does not interfere with the pH measurement using the tape-and-paper-based devices. These results are promising for further development of the devices and measuring pH of bacteria.

CHAPTER 7. TAPE-AND-PAPER-BASED DEVICES MADE WITH CARBON PASTE

7.1 Carbon Paste in Electrochemistry

In this chapter we describe the fabrication, characterization and testing of devices made using carbon paste for the working and counter electrodes. Carbon paste consists of carbon powder mixed with a liquid binder[19, 40, 41]. Typically, carbon paste is used in a mold, such as a Teflon rod, which allows for the paste to be compacted and held together [42]. Carbon-paste electrodes have often been used in electroanalysis for affordability, ease of preparation and high potential for further modification[40]. Different modifiers can be added to the paste in order to optimize the working electrode for the studied analyte [40]. Additionally, carbon-paste electrodes are low cost and yield low capacitance currents which make them an attractive option for electrochemical analysis[19, 41]. For further development, we to explored different carbon formulations as working electrodes for our devices by using carbon paste for the electrodes.

7.2 Experimental Section specific to carbon paste

7.2.1 Materials and Reagents

Carbon conductive paint (E3456) and Silver/Silver chloride (E2908) (Ag/AgCl) conductive paint were obtained from Ercon (Wareham, MA). Nujol for IR Spectroscopy and graphite powder (7-11 micron) were purchased from Alfa Aesar. Hexane was purchased from Fisher Scientific. Potassium Chloride (KCl) was purchased from Fisher BioReagents. Potassium ferrocyanide ($K_4Fe(CN)_6$) was purchased from Acros Organics.

7.2.2 Fabrication of device with carbon paste

Devices were prepared according to the fabrication steps in Chapter 3 while varying the composition of the paint used. The carbon paste was prepared three different ways. The first method involved directly weighing out graphite powder and the commercial carbon conductive paint and mixing them before painting the mixture on the stencil. The second carbon paste formulation was made by mixing graphite powder and mineral oil in a weight ratio of 30/70. This

carbon paste formulation was applied to the stencil using a cotton swab. The third carbon paste formulation was made by adding graphite powder, to a mixture of mineral oil and hexane in a weight ratio of 30/70 powder to solvent. This carbon paste formulation was applied using craft sticks to slather the paste on to the stencil. The various compositions will be detailed in Sections 7.3 and 7.4.

7.2.3 Preparation of solutions

A solution of 0.1 M KCl was prepared and then used to make a stock solution of 5 mM $K_4Fe(CN)_6$ in 0.1 M KCl. This solution was used to electrochemically characterize the carbon-paste devices.

7.2.4 Electrochemical Measurements using devices made with carbon paste

The cyclic voltammograms were performed on the same instrument as in Chapters 3-6 however the potential was sweeped from – 0.85 V to 0.9 V vs. Ag/AgCl for measurements of the solvent $K_4Fe(CN)_6$ at a concentration of 5 mM in 0.1 M KCl.

7.3 Devices made with mixture of carbon paste and commercial paint

Graphite powder was added to the commercial carbon paint in an attempt to increase the formation of the oxo-groups by increasing the amount of graphite of the working electrode. Table 7 shows the different compositions used for the electrode fabrication of the devices used in Figure 23.

There was a slight increase in the current response (Figure 23). This small increase from the added amount of graphite powder may be due to the carbon conductive paint being already close to saturation in terms of how much graphite was in it. The difference between the potentials of the oxidation peak and the reduction peak also decreased from 0.55 V to 0.35 V, closer to the ideal 59 mV derived from the Nernst equation. A mixture with less graphite powder was used to fabricate more devices and then tested in potassium ferrocyanide to compare their current response. Device B (Figure 23) has an oxidation current of values 82.5 μA, between paint device (77.6 μA) and Device A (86.7 μA), however this difference is minimal. This enhancement of the efficiency of

the electrochemical cell could be due to the added lose powder of graphite increasing the conductivity of the electrode.

Table 6. Different compositions of three devices used in Figure 23.

Device	Composition	Ratio (weight)
Paint Device	carbon conductive paint	-
Device A	graphite powder / carbon conductive paint	21/79
Device B	graphite powder / carbon conductive paint	15/85

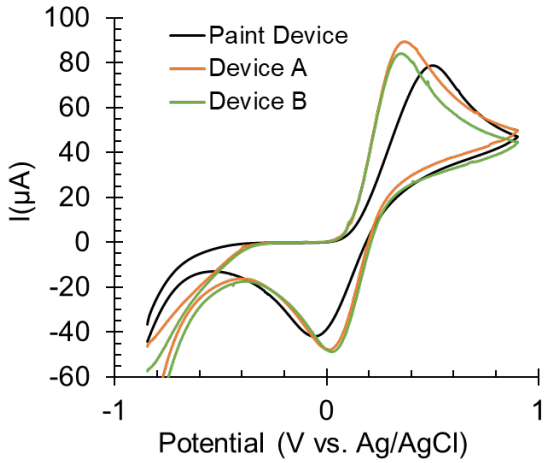

Figure 23. Overlay of CVs in a solution of 5 mM $K_4Fe(CN)_6$ in 0.1 M KCl (ionic adjuster) using different carbon paste tape-and-paper-based devices, Device C & D and an original device with only the commercial paint

Adding graphite powder to the carbon conductive paint for the electrode fabrication did not yield a significant enough change to continue exploring more compositions of carbon paste/commercial paint.

7.4 Optimization of the composition of the liquid binder used in the carbon paste

For the second type of carbon paste prototypes, only graphite powder and a solvent as a liquid binder were used. In literature, carbon paste is often made from graphite powder and mineral oil[40, 42]. The most common weight ratio of graphite powder to mineral oil reported was 70/30. As previously stated, carbon paste is typically placed in a mold, not applied on flexible devices. Thus, when this ratio was tested, and applied with a cotton swab to our devices, the consistency was not compatible with the overall design and specifically the bendability of our devices. The consistency was very crumbly which can be overcome when compacted into a mold but is not compatible to being used in place of paint on a flexible device. In an attempt to achieve a better consistency, the weight ratio was reversed to 30/70 graphite powder: mineral oil (Table 7). This prototype was characterized by CVs in potassium ferrocyanide and compared to the original painted devices (Figure 24).

Table 7. Different compositions of three devices used in Figure 24.

Device	Composition	Ratio (weight)
Paint Device	carbon conductive paint	-
Device C	graphite powder / mineral oil	30/70

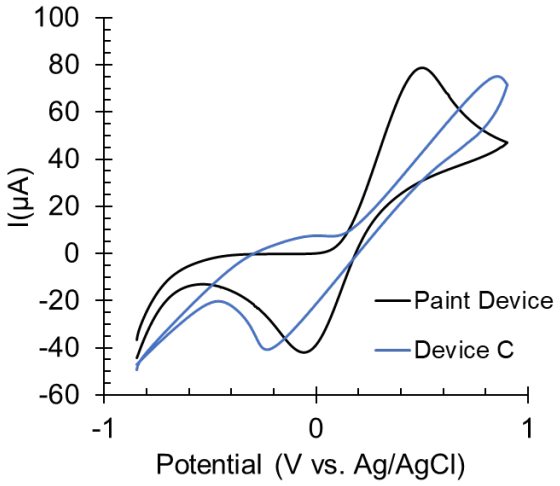

Figure 24. Overlay of CVs in a solution of 5 mM $K_4Fe(CN)_6$ in 0.1 M KCl using standard paint device (black line) compared to Device C made with carbon-paste electrodes (blue line).

While the consistency of the carbon paste used for Device C in Figure 24 was better for our devices than the literature used ratio, the electrochemical characterization was not satisfactory. The oxidation peak was not observed, and there was a shift in the potential of the reduction peak towards more negative potentials. The reduction peak was observed at -0.1 V vs. Ag/AgCl for the device made with carbon paste compared to a peak at 0 V vs. Ag/AgCl for the standard painted device. There was, however, no significant difference in the intensity of the reduction current between the devices (Figure 24). Issues with the consistency of the carbon paste also still existed, as the carbon paste did not fully dry like the standard devices do, i.e., even after 3 days, devices still appeared wet.

Despite some improvements, there was a need for development in terms of electrochemical response of devices made with carbon paste and their final physical characteristics. To improve the carbon paste consistency, different liquid binders were tested to determine the optimal composition. In particular, the two other solvents tested were acetone and hexane, along with a mixture of hexane with mineral oil. Acetone was chosen as it is typically used as a cleaning solvent for the conductive paints. By adding one of these organic solvents, the expectation was to obtain a paste fluid enough to apply easily to the stencils, and able to quickly dry down after application. Both formulations with organic solvents, however, yielded a similar crumbly paste and therefore

did not apply well. The consistency was at the opposite end of the spectrum compared to the 30/70 carbon paste made with just mineral oil as the liquid binder from Device C. This meant that the consistency was so dry that it crumbled off of the tape-and-paper-based devices.

The next paste composition tested was therefore a mixture of hexane with mineral oil. The next step was to figure out the best ratio of hexane to mineral oil. The different liquid binder ratios attempted are listed by weight ratio as well as volume ratio in Table 8.

Table 8. Different compositions of the hexane to mineral oil solvent ratios for carbon paste

Device	Composition	Powder/Solvent Ratio (weight)	Solvent Ratio (hexane/mineral oil) (weight)	Solvent Ratio (hexane/mineral oil) (volume)
Device D	graphite powder / hexane / mineral oil	29/71	53/47	59/41
Device E	graphite powder / hexane/ mineral oil	30/70	41/59	47/53

The target ratio for Device D was approximately an even amount of mineral oil to hexane and resulted to a calculated as 53/47 (by weight). For comparison, the next paste was made with less hexane than mineral oil and was calculated to be 41/59 (by weight). Figure 25 shows the comparison between Devices D and E, both tested by CV using 5 mM $K_4Fe(CN)_6$ in 0.1 M KCl.

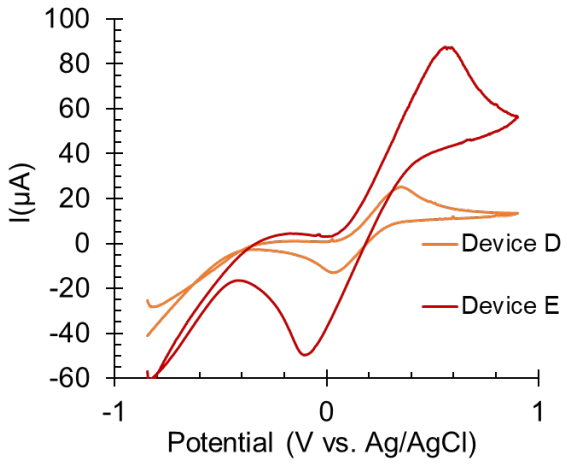

Figure 25. Overlays of CV in a solution of 5 mM $K_4Fe(CN)_6$ in 0.1 M KCl using carbon paste tape-and-paper-based devices. Devices were fabricated using different solvent ratios.

Figure 25 shows that the CVs of both protypes with the newly formulated carbon paste displayed a better electrochemical response than the previous carbon paste prototype. There is a difference in the CVs as Device D in Figure 25 has the better shape, with a smaller difference between the potentials of the oxidation and reduction peaks. This device was made with the carbon paste ratio that had less hexane than mineral oil. The CV of Device E had a higher intensity of current than Device D, though the shape of the CV of Device C is preferred regarding the splits of potentials between oxidation and reduction peaks. Given that the overall expectation of using carbon paste for these modifications was to increase the current response and possibly the oxo-groups formed on the electrode, the carbon paste formulation with less hexane, 41/59 ratio (by weight) of hexane to mineral oil was chosen to move forward. Additionally, these devices statistically worked more often than the devices with the other ratio. The 41/59 ratio (by weight) devices worked 100% of the time (n=3) compared to the other 53/47 ratio (by weight) devices which worked 33% of the time (n=3).

Once a usable formulation of carbon paste for electrodes was determined, we further investigated the quality of the electrode. The tape-and-paper-based devices made with carbon paste were characterized by CV in a solution of 5 mM $K_4Fe(CN)_6$ in 0.1 M KCl over different scan rates to further evaluate the system (Figure 26).

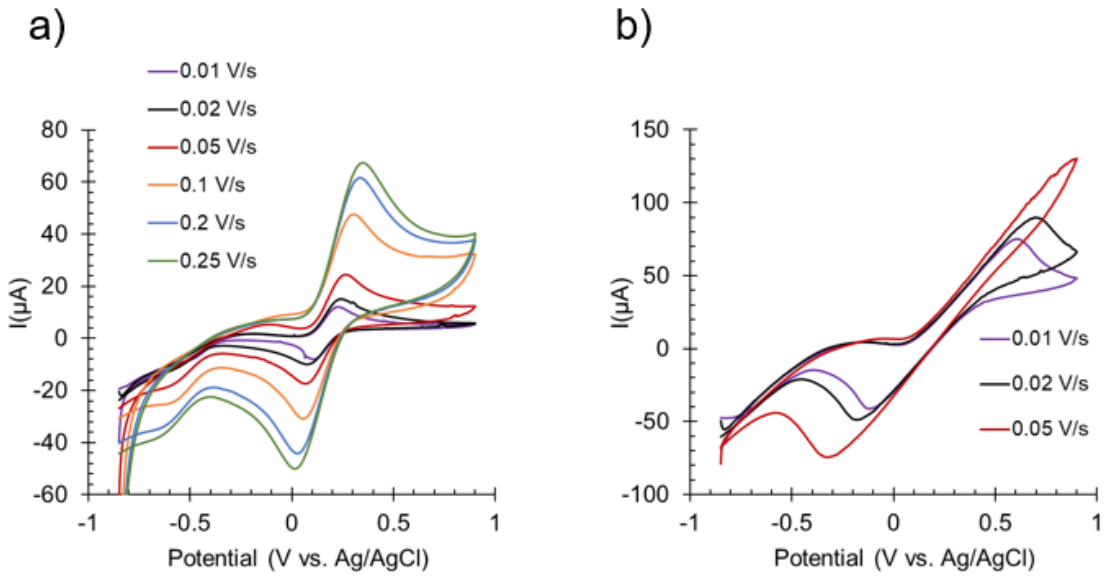

Figure 26. Overlay of CVs of 5 mM $K_4Fe(CN)_6$ in 0.1 M KCl at different scan rates using devices made with carbon-paste electrodes. (a) CVs using Device F. (b) CVs using Device E.

Indeed, for an optimal electrode in a diffusive electrochemical system, the intensity of the peak current is expected to be proportional to the square root of the scan rate, following the Randles-Sevcik equation (Equation 4).

$$i_p = 0.4463\, nFAC \left(\frac{nF\nu D}{RT}\right)^{\frac{1}{2}}$$

Equation 4

Figure 26 (b) shows the CVs of a device prepared in the same batch as the device which resulted in the CVs in Figure 26 (a). The CV at a scan rate of 50 mV/s displays a poorly performing device, whereas at 10 and 20 mV/s, even if not an ideal shape, both oxidation and reduction peaks were observed. These results highlight the need to adjust the electrochemical parameters such as the scan rate for a complete characterization. Although both devices functioned, the device characterized by CVs in Figure 26 (a) performed better than the device characterized by CVs in Figure 26 (b), in particular when considering the split in potentials between the oxidation peak and the reduction peak.

To verify if the best device (Figure 26 (a)) followed the Randles-Sevcik equation, the intensity of the current of the oxidation peak was plotted versus the square root of the scan rate (Figure 27).

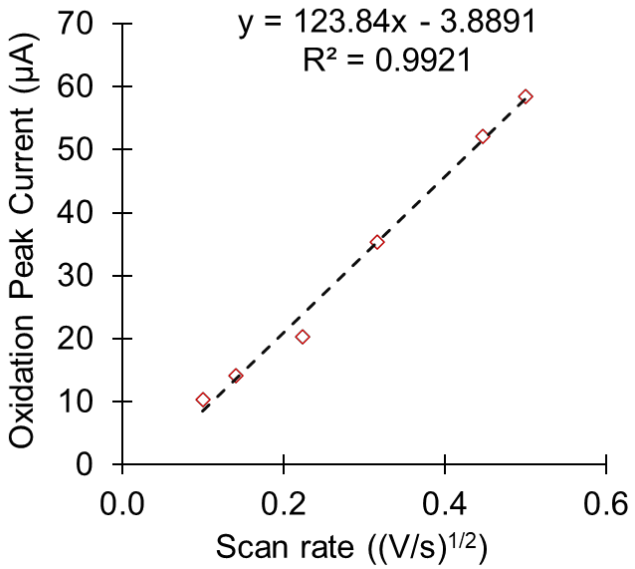

Figure 27. Graph of the oxidation peaks plotted vs. square root of the scan rate for tape-and-paper-based device with carbon-paste electrodes. Data from plot obtained from CVs of 5 mM $K_4Fe(CN)_6$ in 0.1 M KCl in Figure 25 (a).

Figure 27 shows strong linear correlation, indicating that the device acted as an optimal electrochemical cell. These results also confirmed that the analyte was not adsorbed on the surface of the carbon electrode but diffused from the solution to the electrode over time, demonstrating that the devices indeed probed the environment of the solution.

Figure 28 shows the comparison of prototypes made with the formulations of carbon paste with a standard glassy carbon electrode (GCE) as well as with other prototypes of tape-and-paper-based devices.

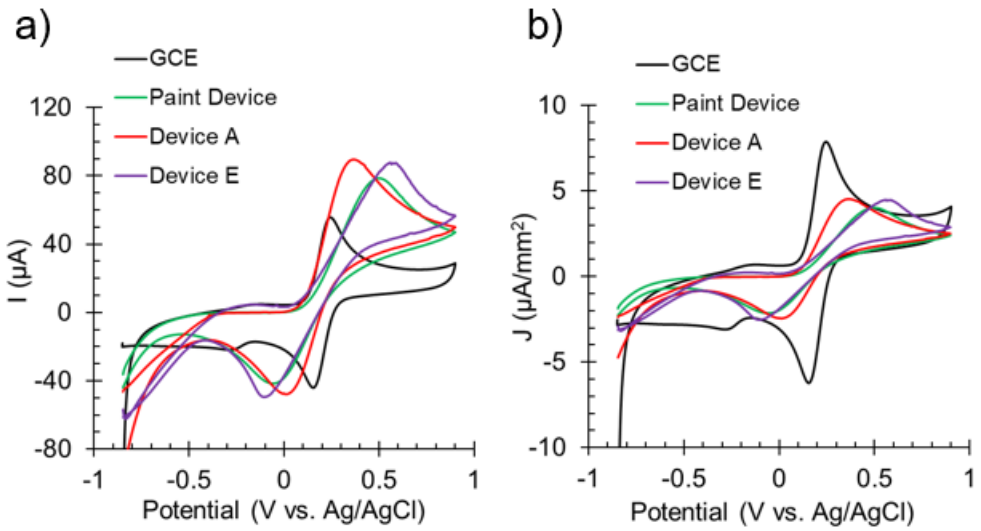

Figure 28. Overlay of CVs in a solution of 5 mM $K_4Fe(CN)_6$ in 0.1 M KCl using glassy carbon electrode (GCE) and tape-and-paper-based devices plotting the current in μA(a) and the . current density in $\mu A/mm^2$ (b).

Figure 28 (a) demonstrates that the various prototypes of the tape-and-paper-based devices all show similar current intensity. The formulations for the carbon paste used for Devices A and C are reported in Tables 6 and 8, respectively. The GCE has a lower intensity of current relative to the devices; however, the GCE has a smaller conductive area. Figure 28 (b) shows the current density to compare the electrode independently of the size of the area of the working electrode. The area could additionally be approximated as the roughness of the paper gave additional microstructure to the electrode surface that was not accounted for in our calculations. These results are expected, given that the GCE is a commercial electrode made with solid graphite, which is treated to be in an efficient electrode. Notably, the performance our carbon-paste electrodes were comparable to that of the electrodes painted using the commercial carbon paint, both in terms of current and current density. These results are promising for further development and optimization of our devices with carbon-paste electrodes.

CHAPTER 8. FUTURE WORK

8.1 Analysis of real saliva samples

As presented in this work, the tape-and-paper-based devices made with commercial carbon paint work well with buffer solutions, commercial standard solutions, and have been able to measure pH of artificial saliva in the range that would be expected for real saliva. Therefore, the next step for the development of our sensor for oral pH would be to optimize the devices for real saliva samples. Measurements of salivary pH have been used in the development of other oral pH sensors as they give an indication of the pH environment around the tooth and thus are useful by themselves while also being a step towards *in situ* measurements. In addition to collecting saliva samples directly, saliva samples linked to specific diagnosed conditions could be obtained through a collaboration with the IU School of Dentistry. These tests and their results would be ideal in terms of further optimizing the prototype devices from both a chemical, biological and dental caregiver standpoints.

The different avenues envisioned to achieve measurements of pH in real saliva are to improve the sensing performance of the devices by (i) using different pre-treatments of the electrodes, (ii) developing further the carbon-paste devices, and (iii) exploring other electroanalytical techniques such as differential pulse voltammetry (DPV), square-wave voltammetry (SWV), or amperometry, and magneto-electrochemistry.

8.1.1 Possible pre-treatment for the carbon electrodes

In one pre-treatment that would be used to attempt to increase the presence of the oxo-groups on our carbon electrode surface, we would directly add an oxidizer to the carbon electrodes[33]. Specifically, we would use potassium permanganate as it is a known oxidizer. The addition of the oxidizer would possibly increase these oxo-groups, and thus increase the Faradaic current.

8.2 Optimization of the carbon-paste electrode

In this work, we described the development of our own formulations of carbon paste to be suitable for our tape-and-paper-based devices. While the best formulation, to date, as outlined in Section 7.4 was used to characterize the carbon-paste electrodes, additional testing for pH

measurements is necessary. Further optimization of the carbon paste could be done and would be useful to improve the performance of the pH sensors. We will optimize the ratio of hexane to mineral oil used in the carbon paste formula by testing more ratios around the current usable formula and establish the tolerance for the optimized ratio of carbon paste. We will also assess the procedure of fabrication of the carbon paste electrodes, specifically the method of application which could be improved. The fabrication steps were previously optimized for the commercial carbon paint, not for carbon paste electrodes. Indeed, so far, the carbon paste seems to be less adhering to the paper than the commercial paint and thus the electrodes made of carbon paste are more fragile. Once satisfied with the new carbon-paste prototypes, they will be tested in other solutions and samples.

8.2.1 Alternative electroanalytical techniques and magneto-electrochemistry

With the satisfactory carbon-paste prototypes, other electroanalytical techniques would be used for tests in other solutions and samples to optimize the assay. These other electroanalytical techniques include differential pulse voltammetry (DPV), square-wave voltammetry (SWV) and amperometry. The use of these electroanalytical techniques may provide more sensitive electrochemical measurements. Another possible avenue would be to explore the application of magneto-electrochemistry to our carbon-paste electrodes[43]. Magneto-electrochemistry research has shown that magnetic fields increase electrochemical flux[43]. We would utilize this property to fabricate magnetically modified carbon-paste electrodes and increase the flux and improve the quality of the carbon-paste electrode.

8.3 Analysis of bacterial samples

The initial stages of the development of the devices for bacteria measurement have been presented in Chapter 6. Further tests need to be conducted with the non-pathogenic *E. coli* K12 ER2738 to assess the potential obstacles of microbiological samples and continue optimizing the devices for pH measurements of bacteria samples. One of these experiments would be to measure the pH of bacterial biofilm grown on the paper-based devices and follow its changes over time. Bacteria can be cultured on paper-based devices[44]. Once the pH measurements are optimized with different types of samples from the non-pathogenic *E. coli* K12 ER2738, we will move on to other

strains of bacteria in our tests. *S. mutans* would be of particular interest to us considering its reported role in inducing cavities, its recorded presence in human saliva, and the impact saliva has on the oral environment[2]. These next steps will bring us closer to the long-term goal of measuring oral pH as the acidic byproduct of the bacterial biofilm on the tooth that promotes dental erosion[45].

8.3.1 Measuring pH with sterile tape-and-paper-based devices

To perform some of the tests mentioned earlier in this chapter, the devices need to be sterile. Additionally, for future oral care, a sterile environment is essential. Thus, we will need to demonstrate further that an efficient sterilization does not negatively impact the devices. The testing of bacterial media samples as well as other buffers commonly used in bacteria culturing experiments performed in the work presented here was not done on devices that were sterile. A preliminary test was, however, performed on sterilized devices and showed that the sterilized devices can indeed be used to measure the pH of PBS in the presence of bacteria, specifically *E. coli* K12 ER2738 (Figure 29).

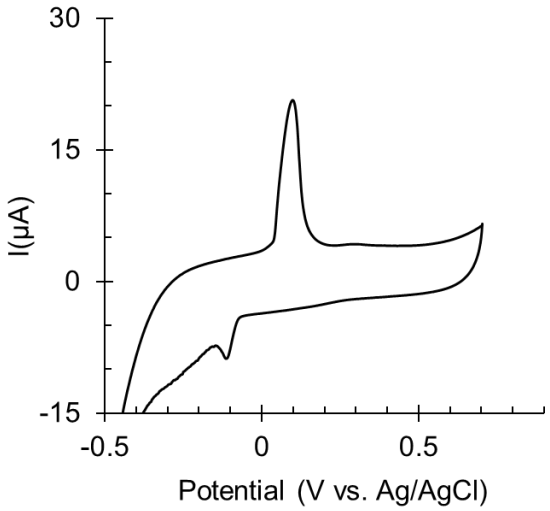

Figure 29. CV of PBS with 0.1 M KCl at pH 7.52 in the presence of *E. coli* K12 ER2738

The tape-and-paper-based device was sterilized in the autoclave in a glass petri dish with 5 mL of milli-Q H_2O at 121°C for 50 minutes and left to dry in the sterile biosafety cabinet. Note that this device was already used prior to the sterilization which could contribute to the less defined

shape of the pH-dependent redox peaks. The pH measured with the devices was calculated to be 7.3 using the Equation 3 and corresponded with the pH meter value of 7.5. This experiment will be repeated on previously unused sterilized tape-and-paper devices. Bacterial growth will also be assessed on the sterilized devices to ensure that no toxicity is developed by the tape, or if applicable by the carbon paste during the autoclaving cycle.

CHAPTER 9. CONCLUSION

This thesis reported the development of electrochemical tape-and-paper-based pH sensors suitable for applications in oral preventive care. The proof of concept of the pH measurement using these tape-and-paper-based devices was demonstrated in buffers and commercial pH solutions, which were used for calibration purposes. The devices showed a strong linear correlation and reproducibility across multiple devices and users. The shelf-life was determined to be at least three months. The devices were used to measure the pH of different samples such commercial mouthwash and then different formulations of artificial saliva at various pH values. The resulting measurements corresponded with the values obtained using a pH meter. The devices were also applied to test more biological samples. The pH of PBS was successfully measured, but it was determined that LB media damaged the sensing ability of the electrodes. The pH of PBS in the presence of bacteria was also measured, and thus demonstrated the potential of the electrochemical tape-and-paper-based devices for more advanced pH measurements in bacterial samples during the subsequent stages of the development of the sensors. A new class of prototypes using carbon-paste electrodes was also designed, fabricated, and characterized. Some of the usable carbon paste already yielded great electrochemical responses when tested with potassium ferrocyanide and showed promise for further optimized formulations. The last chapter presented the envisioned future steps of the project including the new types of samples to be tested such as real saliva, bacterial biofilms or cultured bacteria, and different potential treatments and electrochemical techniques to improve the performance of the electrochemical tape-and-paper-based pH sensors in complex biological samples.

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