UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

THE DEVELOPMENT OF REDOX POLYMERS BASED ON LINEAR POLY(ETHYLENIMINE) FOR APPLICATION IN AMPEROMETRIC BIOSENSORS

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

DOCTOR OF PHILOSOPHY

By

STEPHEN MERCHANT
Norman, Oklahoma
2007

UMI Number: 3296163

ProQuest Information and Learning Company
300 North Zeeb Road
P.O. Box 1346
Ann Arbor, MI 48106-1346

THE DEVELOPMENT OF REDOX POLYMERS BASED ON LINEAR POLY(ETHYLENIMINE) FOR APPLICATION IN AMPEROMETRIC BIOSENSORS

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF CHEMICAL, BIOLOGICAL,
AND MATERIALS ENGINEERING

BY

_____
Dr. David Schmidtke

_____
Dr. Daniel Glatzhofer

_____
Dr. Brian Grady

_____
Dr. Vassilios Sikavitsas

_____
Dr. Alberto Striolo

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. David Schmidtke, for giving me the opportunity to work on this project. I would also like to thank Dr. Daniel Glatzhofer for helping us come up with something new to explore, and for all his help along the way. I would also like to thank Dr. Brian Grady, Dr. Vassilios Sikavitsas, and Dr. Alberto Striolo for taking the time to be on my committee. I would also like to thank Terri Colliver for always being so pleasant and helpful.

I would especially like to thank my wife Sara for being so patient. I could not have done this without her. I also have to thank all of my friends and family for reminding me that there is a world outside of the laboratory.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Redox polymers based on linear poly(ethylenimine) were synthesized for use in amperometric biosensors. The first polymer synthesized for this work was methylferrocenyl linear poly(ethylenimine) [MeFc-LPEI]. The solution electrochemistry of this material exhibited redox potential shifts in the opposite direction of what is normally observed for ferrocene modified amines. It was demonstrated here that counter ion effects, in conjunction with the proximity of the ferrocene group to the polymer backbone, may be responsible for the counterintuitive potential shifts. Crosslinked films of this polymer also displayed abnormal behavior. Under certain conditions the films would undergo oxidative degradation which resulted in electrochemical and sensors instability. This phenomena is probably also related to the proximity of the ferrocene group to the polymer backbone, in addition to the morphological changes that occur in crosslinked films. Nonetheless, this material was shown to produce the highest responding glucose sensors (~1200 $\mu A/cm^2$) reported confirming our hypothesis that such materials could be developed into effective sensor components.

The second polymer synthesized was hexylferrocenyl linear poly(ethylenimine) [HxFc-LPEI]. By extending the ferrocene group further away from the polymer backbone HxFc-LPEI eliminates the proximity issue, particularly the morphological confinement in crosslinked films. This dramatically improves both the electrochemical response and the sensor response with respect to stability. The cyclic voltammograms indicate no apparent multiple microenvironments as only single wave redox behavior is observed. Sensor responses with HxFc-LPEI suggest that extending the ferrocene redox couple from the backbone results in less efficient electrical communication with the

enzyme. While, still producing competitive responses to glucose (~600 μA/cm^2), the benefit of the longer tether on the ferrocene couple is observed in a ten-fold increase in the long term stability of the sensor. The half life of HxFc-LPEI based glucose sensors is 30 hours while the half life of MeFc-LPEI based sensors is only 3 hours.

CHAPTER 1:

Introduction and Background Information

INTRODUCTION

Research and development in the area of biosensors has grown almost exponentially over the last 30 years in terms of financial investment, scientific publications, and number of researchers both in academia and industry. The reasons for this increase in research activity are many, but due in part to the broad nature of the subject of biosensors. This multi-disciplinary field of study includes chemists, biologists, physicist, and engineers, among others, often times working in conjunction with each other. In addition, the industries and applications in which biosensors are used are equally varied and include clinical applications, the food and beverage industry, military agencies, and environmental monitoring, to name a few[1]. The dynamic nature of these applications and the inherently complicated task of detecting biologically relevant chemicals continue to drive the demand for improvements in all aspects of functionality of these devices including selectivity, sensitivity, responsiveness, and signal output. These improvements continue to be realized with the development of innovative new materials and processes.

Despite being such a broad topic, biosensor research has been, and should continue to be dominated by the study and development of glucose sensors according to market research reports. In 2005 Freedonia Group[2], an industry market research firm based in Cleveland, OH, estimated that by 2009 the U.S. glucose sensor market will reach about $2.5 billion, which, by their estimates is about 90% of the entire $2.8 billion biosensor market and 60% of the entire $4.2 billion chemical sensor market.

While glucose sensors are used in a variety of applications, the health industry overwhelmingly drives the progress in research for glucose monitoring in diabetics. In 2005 the U.S. prevalence of diabetes in adults was estimated at 20.8 million, while the age-adjusted prevalence of diabetes increased by 54% among adults from 1994 to 2002[3]. While the proliferation of diabetes is a complicated issue, it has been suggested that close monitoring, and hence, close control of blood glucose levels can delay the onset of diabetes, and prevent comorbidities[3,4]. More specifically, there is significant interest in the development of implantable continuous monitoring sensors for the prevention of hypoglycemia in patients with Type 1 diabetes[5-8]. The combination of the demand for improvements in the function of sensors and advances in glucose monitoring provides the motivation for this research.

PURPOSE OF THE WORK

The purpose of this work was to develop new redox polymers based on linear poly(ethylenimine) [LPEI] for application in amperometric biosensors. The selection of LPEI as the polymer backbone of choice for development of new redox polymers may seem peculiar as there are a very large number of polymers from which to choose. So,

the question is: why use LPEI? In general, when designing a new redox polymer, particularly for use in biosensors, there are three key characteristics the polymer backbone should possess: (i) flexibility or a high degree of segmental mobility[9,10], (ii) a high functional density[11], and (iii) the ability to hydrate or dissolve in water[12]. These are, in fact, all qualities of LPEI making it a viable candidate for use as a redox polymer. In addition, there have been a number of reports suggesting that PEI interacts closely with enzymes via electrostatics as it has been used in the construction of layer-by-layer sensors[13]. There have been other reports still which indicate that the interaction PEI has with anionic enzymes acts to stabilize the enzymes whereby their activity or half lives are increased[14-17].

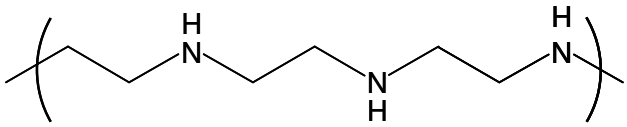


Figure 1.01: Structure of LPEI

Thus, my hypotheses are the following: (i) the highly flexible backbone will allow for an high rate of collision between redox centers and hence a high rate of charge transport through the polymer, and (ii) the ability of LPEI to closely associate itself with enzymes can be exploited by attaching a redox active species to the polymer backbone to form a new redox polymer, which will serve as an effective mediator in amperometric biosensors. In other words, a close interaction between LPEI and an enzyme should translate to efficient electrical communication between a ferrocene modified LPEI (Fc-LPEI) and glucose oxidase; and hence, a highly functional sensor.

A diagram of the signal transduction scheme in amperometric biosensors provides a means by which the proposed benefits of LPEI based redox polymers may be conceptualized.

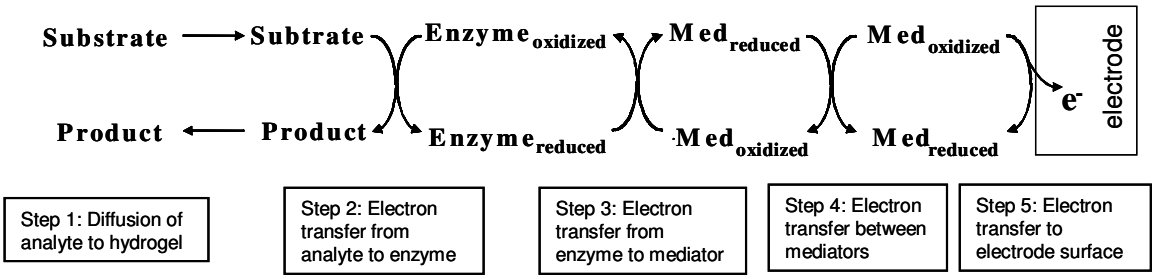


Figure 1.02: Amperometric biosensor signal transduction schematic.

In general, mass transfer (step 1), the enzymatic reaction (step 2), and the transfer of charge to the electrode surface (step 5) are considered to be relatively fast as to not limit the response of the sensor. The hypotheses of this work are directed toward addressing improvements in steps 3 and 4. The remainder of this chapter will provide concise background information necessary for understanding the work presented in the proceeding chapters.

BACKGROUND

Enzyme Electrode Concepts

Enzymes (E) are biological catalysts which facilitate the transformation of a substrate (S) to a product (P) usually via the following set of general reactions:

$$S + E \rightarrow E{\cdot}S$$

$$E{\cdot}S \rightarrow E + P$$

Enzyme electrodes take advantage of the selectivity and reactivity of enzymes towards particular substrates. In general, enzyme electrodes are constructed by immobilizing an enzyme in close proximity to an electrode in such a manner that the substrate and reaction products are able to diffuse through the enzyme layer while the enzyme remains in a fixed location close to the electrode surface. The enzymatic reaction that occurs as the substrate is transported to the enzyme results in changes in the concentrations of the substrate and reaction products. These changes are subsequently measured electrochemically using potentiometric or amperometric techniques. Before discussing specific developments in enzyme electrodes, the topics of enzyme immobilization and electrochemical sensors will be briefly addressed in the proceeding sections. These topics are important in distinguishing different types of sensor construction and modes of detection.

Enzyme Immobilization Techniques

There are a variety of techniques available to immobilize enzymes on the surface of electrodes. The simplest of these is the physical adsorption of the enzyme directly onto the electrode surface. While this procedure eliminates the need for any chemical reactions which are potentially detrimental to the activity of the enzyme, there is a potential for deactivation or denaturation of the enzyme upon adsorption to a surface. Additional disadvantages include sensor instability due to desorption, and irreproducibility[18]. One approach to mitigate these problems is the entrapment of the enzyme against the electrode surface using a semi-permeable membrane which allows for the diffusion of the smaller substrate and product molecules to, and away from the

enzyme, respectively, while inhibiting diffusion of the larger enzyme molecules away from the electrode. This method is not without disadvantages, however. Leeching of the enzyme may still occur depending on the durability and integrity of the membrane[19]. In addition, the membrane may introduce mass transport limitations that could have unfavorable effects on the sensor.

The technique of choice for the immobilization of enzymes in this work is the formation of enzymatic redox hydrogels by crosslinking the enzyme to a redox polymer. This process, sometimes referred to as "wiring" of enzymes, involves the use of bifunctional reagents, such as diepoxides, dihalides, and diacrylates to covalently attach the enzyme to the polymer[9,12,20-24]. An additional interaction occurring in this system by which the enzyme is immobilized at the surface is the electrostatic complexation between the anionic enzyme and the cationic polymer.

After mixing the appropriate amount of enzyme, polymer, and crosslinker, the hydrogel is solvent cast onto the electrode surface. In many cases the crosslinking of these components to each other occurs in the condensed phase, hence a certain amount of time is required for reaction after the solvent has evaporated to obtain a suitable hydrogel. The result is an insoluble film that is physically adsorbed to the surface of the electrode. This film swells in aqueous solution and allows for the diffusion of substrate, product, and electrolyte into and out of the hydrogel matrix[10]. While this technique is simple, relatively reproducible, and minimizes the leeching of sensor components, there are also disadvantages. For example, it is difficult to control, differentiate, and characterize the *inter*molecular and *intra*molecular crosslinking. There is also the ever-present risk of deactivating the enzyme by introducing a chemical reaction with the crosslinker.

There are yet other popular techniques for immobilizing enzymes at electrode surfaces including direct covalent attachment of the enzyme to the electrode using surface pretreatments[18], and layer-by-layer deposition[25] which involves the electrostatic complexation of the enzyme to an alternately charged macromolecule. While these methods have been shown to be viable, they are not the focus of this work and are only mentioned to provide sufficient breadth of background information to the reader.

Potentiometric and Amperometric Sensors

Potentiometric sensors measure potential differences across an ion selective membrane and correlate these measurements to the concentration of the ion or analyte. The most common example of a potentiometric sensor is a glass pH electrode. This type of electrode consists of a glass tube containing a reference electrode and a fill solution, and a silicate glass membrane. When the glass membrane is placed in an aqueous solution the silicate groups exchange their alkaline metal ions (usually Na^+) with H^+ resulting in a potential difference across the membrane which is a linear function of the pH of the solution[26]. This type of electrode can be adapted for use as a biosensor by immobilizing an enzyme whose reaction generates H^+ or OH^- near the glass membrane. The change in pH of the solution can then be correlated to the concentration of the analyte. There are several examples of potentiometric enzyme electrodes in the literature, as well as several review articles which cover this topic[27,28]; however, since the sensor aspect of this work is focused on amperometric sensors, potentiometric sensors will not be discussed any further.

Amperometric sensors measure the flow of current at an electrode surface at a fixed potential between the working and reference electrode. The functionality of these sensors is based on the oxidation or reduction of relevant species at the electrode surface producing either anodic or cathodic currents which correlate linearly to concentration. The most common example of an amperometric sensor is the Clark oxygen electrode[26]. It consists of a platinum electrode poised at E = -600 mV (vs. Ag/AgCl) and covered with an oxygen-permeable membrane. As oxygen diffuses through the membrane it is electrochemically reduced to H_2O_2 at the electrode surface via the following reaction:

$$O_2 + 2\ e^- + 2\ H_2O \rightarrow H_2O_2 + 2\ OH^-$$

The changes in cathodic current that are produced indicate the changes in the concentration of dissolved oxygen in the sample. Alternatively, in systems where H_2O_2 is produced the electrode may be poised at E = +600 mV (vs. Ag/AgCl) to oxidize the peroxide:

$$H_2O_2 \rightarrow O_2 + 2\ e^- + 2\ H^+$$

This electrocatalyitc oxidation produces an anodic current corresponding to the peroxide concentration. These amperometric sensors have also been adapted for use as biosensors by combining their function with the function of enzymes. The next section will provide a brief description of the progression of these sensors, particularly glucose sensors.

The Progression of Amperometric Glucose Electrodes

Since glucose sensors are the focus of the overwhelming majority of biosensor research in industry and academia, as well as this dissertation, the following discussion of the progression of amperometric biosensors will only refer to glucose sensors in

particular. In general the redox enzyme glucose oxidase is immobilized at an electrode surface and the flavin adenine dinucleotide (FAD) active sites in the enzyme catalyze the oxidation of glucose. This process is exploited in different ways to produce a current which ultimately corresponds to the concentration of glucose in the sample.

First Generation: O_2 Mediator

The first generation of amperometric enzyme electrodes involves exploiting the role that dissolved oxygen plays in the catalytic process. After reporting the development of their amperometric oxygen electrode in 1958, Clark et al proposed the concept of combining an enzyme with this device to form an amperometric biosensor; and in 1967 Updike and Hicks reported the first enzyme electrode based on this concept[29]. GOx was immobilized against the Clark oxygen electrode within a poly(acrylamide) gel. The enzyme catalyzed the oxidation of glucose to gluconolactone, subsequently passing the electrons to dissolved oxygen as shown in the equations above. The reaction between glucose oxidase (GOx) and glucose is as follows:

$$GOx(FAD) + Glucose \rightarrow GOx(FADH_2) + Gluconolactone$$

$$GOx(FADH_2) + O_2 \rightarrow GOx(FAD) + H_2O_2$$

With the platinum electrode poised at -600 mV vs. Ag/AgCl the cathodic current of the sensor was actually a measure of the depletion of oxygen due to the enzymatic reaction. Alternatively, as mentioned above, with the platinum held at +600 mV vs. Ag/AgCl the anodic current output of the sensor was actually a measure of the concentration of H_2O_2 produced. The electrodes described here represent the first generation glucose electrodes, where O_2 depletion or H_2O_2 production is measured.

While these types of sensors have been widely used, they also have their disadvantages. In the measurement of O_2 depletion examples of common problems include low oxygen content in the samples, slow diffusion rates, and slow electron transfer kinetics[30]. Measurement of H_2O_2 at an operating potential of +600 mV introduces the potential for interferences from the oxidation of other biologically relevant species such as ascorbic acid and uric acid[5,31]. The research that was preformed to alleviate these issues lead to the development of the second generation of glucose sensors.

Second Generation: Redox Mediator

The second generation of amperometric enzyme electrodes replaces dissolved oxygen as the electron acceptor in the catalytic process with a redox mediator (M), which acts to shuttle electrons to the electrode surface. The reaction scheme in this type of sensor design is as follows:

$$GOx(FAD) + Glucose \rightarrow GOx(FADH_2) + Gluconolactone$$

$$GOx(FADH_2) + 2\ M_{ox} \rightarrow GOx(FAD) + 2\ M_{red} + 2H^+$$

Self exchange of e^- between redox centers: $M_{red} + M_{ox} \rightarrow M_{ox} + M_{red}$

at the electrode surface: $M_{red} \rightarrow M_{ox} + e^-$

Hence, the reoxidation of the mediator at the electrode surface is measured amperometrically. In addition to the greater control over the concentration of the redox mediator, this type of system also allows for the decrease in sensor operating potential. For example, the first artificial redox mediator used was quinone which is reduced to hydroquinone by GOx, and then oxidized back to quinone at a potential of +400 mV vs.

Ag/AgCl[30]. This reduces the possibility of oxidizing other species. Further progress was made with diffusional mediators when organometallic molecules, such as ferrocene and its derivatives, were employed as the electron acceptors (M_{ox})[32-35]. These molecules allow for greater control of certain aspects of the system including operating potential and ionic charge on the mediator.

One of the more significant advancements in amperometric glucose sensors came with the use of redox polymers as fixed mediators, rather than diffusional. Redox polymers consist of organometallic redox couples attached to a polymer backbone. Crosslinking these polymers with glucose oxidase, or "wiring", eliminates mass transport issues concerning the diffusion of the mediator to the enzyme to accept electrons from the cofactor, then to the electrode surface to provide current[36]. Figure 1.03 illustrates the concept of "wiring" an enzyme to an electrode surface. In terms of signal transduction, this method of sensor construction follows the same general scheme depicted in Figure 1.02.

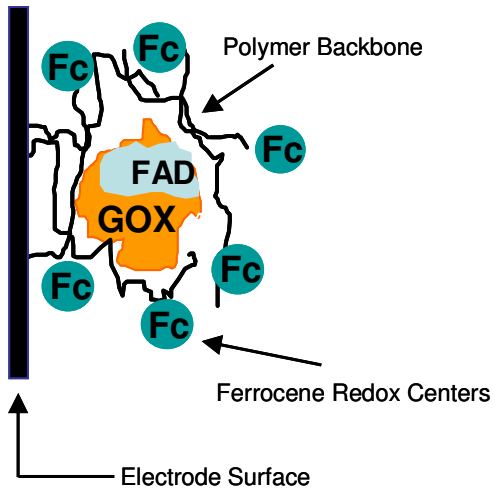


Figure 1.03: Schematic illustrating the concept of molecular "wiring" of glucose oxidase (GOx) to an electrode surface with a redox polymer containing ferrocene (Fc) redox centers.

Wiring also provides a method of reproducibly immobilizing the enzyme and mediator together without the need for additional materials or layers, such as membranes. In addition to the conveniences, wired enzyme sensors have been found to be among the most sensitive and effective amperometric glucose sensors available[9,22,23,36].

Third Generation: Direct Electron Transfer from Enzyme to Electrode

The third generation of amperometric enzyme electrodes circumvents the use of redox mediators all together and uses direct electrical communication between the redox active sites in the enzyme and the electrode surface[37], as shown in Figure 1.04.

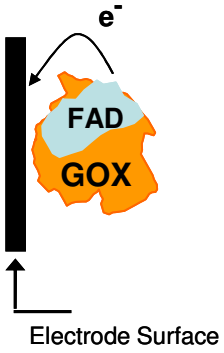


Figure 1.04: Schematic illustrating the concept of direct electron transfer from the enzyme to electrode surface.

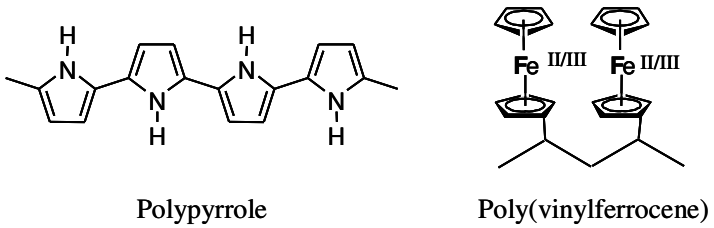
Despite research indicating the promise of the third generation sensors[38-40], there has been relatively little research performed with these types of sensors in comparison to the others discussed here. While all enzyme based amperometric sensors have their issues to overcome, direct electron transfer from the enzyme to the electrode surface is particularly difficult as it, in general, requires some chemical modification of the enzyme, or the electrode surface, or both. For example, removal of the redox active FAD sites from the surrounding protein of the enzyme, then reconstituting the enzyme with the FAD sites

located on the periphery has been shown to be a promising technique to allow direct electron transfer[41]. However, the risk of irreversibly damaging the enzyme activity is abnormally high in these situations.

Redox Polymers

In a similar fashion to biosensors, the subject of polymers tends to be very broad. Even subclasses of polymers such as electroactive polymers can encompass a variety of materials with considerable differences. Theses differences can have significant effects when developing new materials for specific applications. Since the purpose of this work was to develop new redox polymers for use in biosensors, it is important to understand the distinctions between the various types of electroactive polymers.

There are three main types of electroactive polymers: loaded ionomers, electronically conductive polymers (ECP's), and redox polymers. Loaded ionomers are far less commonly used materials, while there is an abundant amount of research involving ECP's and redox polymers. The different structures of these materials give rise to the different charge transport mechanisms exhibited by each, which largely determine the usefulness of each type of polymer for a particular application. Figure 1.05 shows the structure of polypyrrole, a common ECP, and poly(vinylferrocene), a common redox polymer.



Polypyrrole Poly(vinylferrocene)

Figure 1.05: Structures of polypyrrole and poly(vinylferrocene)

Redox polymers are formed by covalently attaching a redox active moiety, such as ferrocene, to an electrochemically inactive polymer backbone. The localization of these redox species results in an electrical conduction, or electron transport mechanism commonly called "electron hopping". In this process, electrons are localized at the sites of the reduced form of the redox species along the polymer, followed by the transfer of the electrons to neighboring oxidized forms of the redox species[42-44]. It is by this mechanism that electrons, and hence charge, can be transported along the backbone of a redox polymer chain, and from one polymer chain to an adjacent polymer chain. Redox polymers are only conductive over a limited range of potentials with the maximum occurring at the redox potential of the polymer, or when the number of reduced sites equals the number of oxidized sites.

Loaded ionomers are comprised of a redox active species, which are electrostatically incorporated in an ion-exchange polymer matrix. This type of electroactive polymer is typically formed by placing an ion-exchange polymer into a solution containing redox active ions. Thus, the redox active moiety becomes the counter ion in the polyionic material. In many instances an electrode coated with the loaded ionomer is desired, in which case the electrode is first coated with the ion-exchange polymer, such as Nafion, then inserted into a solution containing the redox species. Electron transport in loaded ionomers occurs either by physical diffusion of the redox species, or electron hopping, or some combination of the two mechanisms. The range of conductivity for loaded ionomers can vary over a wide range depending upon the specific material being used[45].

Electrically conducting polymers (ECP's) are usually electrochemically synthesized onto an electrode surface from a solution of a redox active monomer;

however, they can also be chemically synthesized and deposited onto either a conductive or non-conductive surface. These materials have conjugated polymer backbones that results in charge delocalization, in contrast to redox polymers whose charge is localized at the redox centers[46]. This delocalized charge results in efficient electron transport along the polymer backbone through polarons and bipolarons; however, interchain electron transfer is limited. ECP's usually remain conductive over a much wider range of potentials than loaded ionomers or redox polymers. In addition, the conductivity can be synthetically altered using dopants, which results in conductivities for these materials ranging from semi-conducting to near metallic.

Due to the electron hopping mechanism, redox polymers have been shown to be the most effective mediators in amperometric biosensors[9,36,37]. Maximum current densities achieved using ECP's such as polypyrrole[47-51] as the electron mediator range from 0.1 to 10 $\mu A/cm^2$ while sensors using redox polymer mediators typically achieve current densities ranging from 500 to 1000 $\mu A/cm^2$. The ability of redox polymers to transfer electrons from a redox site on one polymer chain to a redox site on an adjacent polymer chain also lends itself to the transfer of electrons from enzyme molecules to the redox sites on the polymers. This seemingly subtle mechanism provides a means by which the redox active FAD sites buried within the insulating glycoprotein of the enzyme may transfer the electrons they generate to the mediators, and subsequently to the electrode for measurement.

OVERVIEW

The purpose of this work was to develop new redox polymers based on linear poly(ethylenimine) modified with ferrocene redox moieties for application in amperometric biosensors. The remainder of this dissertation documents the studies performed in the development of the poly(ethylenimine) redox polymers. In addition, the study of these materials as they function in amperometric biosensors is also presented. Each chapter begins with an introduction which provides the motivation and purpose of the corresponding study along with background information with respect to relevant literature.

Chapter 2 focuses on the synthesis and solution electrochemical characterizations of the ferrocene modified polymer. Solution electrochemistry refers to the electrochemical behavior observed at the surface of an electrode that has been placed in a solution containing the redox active species as a solute. As presented in this chapter, my observations are somewhat counterintuitive and required extensive investigation in order to make sense of it. The results presented in Chapter 2 provided me with significant insight into the behavior of the polymer despite what may seem like a deviation of the subject matter from actual biosensor work.

Chapter 3 covers the electrochemical studies of crosslinked films of the redox polymer. Since the intended use of the ferrocene modified LPEI involves crosslinking it with an enzyme, an understanding of the electrochemical behavior of the crosslinked film should provide valuable foresight into the performance of the polymer within the sensor hydrogel matrix. As with the solution electrochemistry, the crosslinked film

electrochemistry resulted in some unusual and previously unreported responses which are discussed in this chapter.

Chapter 4 discusses the use of the redox polymer in amperometric biosensors. This was the ultimate goal of the project and this chapter discusses the ability of the new LPEI based redox polymer to support sufficiently fast electron transport through the films, and to effectively communicate with redox enzymes.

Chapter 5 describes the synthesis of an improved, and somewhat modified version of the redox polymer, along with its function in a sensor. This material answered some of our questions concerning polymer structure and stability.

Chapter 6 includes a summary of the major conclusions of this work. In addition, suggestions for future work are presented.

***REFERENCES*:**

(1) Nakamura, H.; Karube, I. *Analytical and Bioanalytical Chemistry* **2003**, *377*, 446.

(2) Freedonia "Industry Study with Forecasts to 2009 and 2014: Chemical Sensors," Freedonia Group, 2005.

(3) Chetty, V. K.; Zellner, B. B. *Statistics in Medicine* **2007**, *26*, 3213.

(4) Pickup, J.; McCartney, L.; Rolinski, O.; Birch, D. *British Medical Journal* **1999**, *319*, 1289.

(5) Schmidtke, D. W.; Heller, A. *Analytical Chemistry* **1998**, *70*, 2149.

(6) Pickup, J. C.; Shaw, G. W.; Claremont, D. J. *Biosensors* **1989**, *4*, 109.

(7) Pickup, J. C.; Shaw, G. W.; Claremont, D. J. *Diabetologia* **1989**, *32*, 213.

(8) Pickup, J. C.; Shaw, G. W.; Claremont, D. J. *Biosensors* **1988**, *3*, 335.

(9) Mao, F.; Mano, N.; Heller, A. *Journal of the American Chemical Society* **2003**, *125*, 4951.

(10) Aoki, A.; Rajagopalan, R.; Heller, A. *Journal of Physical Chemistry* **1995**, *99*, 5102.

(11) Rajagopalan, R.; Aoki, A.; Heller, A. *Journal of Physical Chemistry* **1996**, *100*, 3719.

(12) Aoki, A.; Heller, A. *Journal of Physical Chemistry* **1993**, *97*, 11014.

(13) Liu, A. H.; Kashiwagi, Y.; Anzai, J. *Electroanalysis* **2003**, *15*, 1139.

(14) Heller, J.; Heller, A. *Journal of the American Chemical Society* **1998**, *120*, 4586.

(15) Chen, Q.; Kenausis, G. L.; Heller, A. *Journal of the American Chemical Society* **1998**, *120*, 4582.

(16) Jezkova, J.; Iwuoha, E. I.; Smyth, M. R.; Vytras, K. *Electroanalysis* **1997**, *9*, 978.

(17) Huan, Z. W.; Persson, B.; Gorton, L.; Sahni, S.; Skotheim, T.; Bartlett, P. *Electroanalysis* **1996**, *8*, 575.

(18) Lojou, E.; Bianco, P. *Journal of Electroceramics* **2006**, *16*, 79.

(19) Alegret, S. *Analyst* **1996**, *121*, 1751.

(20) Ohara, T. J.; Rajagopalan, R.; Heller, A. *Analytical Chemistry* **1993**, *65*, 3512.

(21) Ling, Y.; Hammerle, M.; Olsthoorn, A. J. J.; Schuhmann, W.; Schmidt, H. L.; Duine, J. A.; Heller, A. *Analytical Chemistry* **1993**, *65*, 238.

(22) Hale, P. D.; Boguslavsky, L. I.; Inagaki, T.; Karan, H. I.; Lee, H. S.; Skotheim, T. A.; Okamoto, Y. *Analytical Chemistry* **1991**, *63*, 677.

(23) Gregg, B. A.; Heller, A. *Journal of Physical Chemistry* **1991**, *95*, 5976.

(24) Gregg, B. A.; Heller, A. *Journal of Physical Chemistry* **1991**, *95*, 5970.

(25) Wang, Y. D.; Joshi, P. P.; Hobbs, K. L.; Johnson, M. B.; Schmidtke, D. W. *Langmuir* **2006**, *22*, 9776.

(26) Cunningham, A. J. *Introduction to Bioanalytical Sensors*, 1998.

(27) Vadgama, P.; Crump, P. W. *Analyst* **1992**, *117*, 1657.

(28) Rechnitz, G. A. *Journal of Clinical Laboratory Analysis* **1987**, *1*, 308.

(29) Updike, S. J.; Hicks, G. P. *Nature* **1967**, *214*, 986.

(30) Diamond *Principles of Chemical and Biological Sensors*, 1998.

(31) Lowry, J. P.; Mcateer, K.; Elatrash, S. S.; Duff, A.; Oneill, R. D. *Analytical Chemistry* **1994**, *66*, 1754.

(32) Cass, A. E. G.; Davis, G.; Francis, G. D.; Hill, H. A. O.; Aston, W. J.; Higgins, I. J.; Plotkin, E. V.; Scott, L. D. L.; Turner, A. P. F. *Analytical Chemistry* **1984**, *56*, 667.

(33) Marr, G.; Rockett, B. W. *Journal of Organometallic Chemistry* **1982**, *227*, 373.

(34) Schlapfe.P; Mindt, W.; Racine, P. *Clinica Chimica Acta* **1974**, *57*, 283.

(35) Battaglini, F.; Calvo, E. J. *Journal of the Chemical Society-Faraday Transactions* **1994**, *90*, 987.

(36) Heller, A. *Abstracts of Papers of the American Chemical Society* **1990**, *200*, 48.

(37) Habermuller, L.; Mosbach, M.; Schuhmann, W. *Fresenius Journal of Analytical Chemistry* **2000**, *366*, 560.

(38) Azamian, B. R.; Davis, J. J.; Coleman, K. S.; Bagshaw, C. B.; Green, M. L. H. *Journal of the American Chemical Society* **2002**, *124*, 12664.

(39) Besteman, K.; Lee, J. O.; Wiertz, F. G. M.; Heering, H. A.; Dekker, C. *Nano Letters* **2003**, *3*, 727.

(40) Guiseppi-Elie, A.; Lei, C. H.; Baughman, R. H. *Nanotechnology* **2002**, *13*, 559.

(41) Willner, I.; Heleg-Shabtai, V.; Blonder, R.; Katz, E.; Tao, G. L.; Buckmann, A. F.; Heller, A. *Journal of the American Chemical Society* **1996**, *118*, 10321.

(42) Forster, R. J.; Vos, J. G.; Lyons, M. E. G. *Journal of the Chemical Society-Faraday Transactions* **1991**, *87*, 3769.

(43) Forster, R. J.; Vos, J. G.; Lyons, M. E. G. *Journal of the Chemical Society-Faraday Transactions* **1991**, *87*, 3761.

(44) Forster, R. J.; Vos, J. G. *Journal of Electroanalytical Chemistry* **1991**, *314*, 135.

(45) Antolini, E. *Journal of Applied Electrochemistry* **2004**, *34*, 563.

(46) McAndrew, T. P. *Trends in Polymer Science* **1997**, *5*, 7.

(47) Habermuller, K.; Ramanavicius, A.; Laurinavicius, V.; Schuhmann, W. *Electroanalysis* **2000**, *12*, 1383.

(48) Palmisano, F.; Zambonin, P. G.; Centonze, D. *Fresenius Journal of Analytical Chemistry* **2000**, *366*, 586.

(49) Ram, M. K.; Adami, M.; Paddeu, S.; Nicolini, C. *Nanotechnology* **2000**, *11*, 112.

(50) Uang, Y. M.; Chou, T. C. *Electroanalysis* **2002**, *14*, 1564.

(51) Uang, Y. M.; Chou, T. C. *Biosensors & Bioelectronics* **2003**, *19*, 141.

CHAPTER 2:

Unusual Solution Electrochemistry of Ferrocene Modified Linear Poly(ethylenimine)

INTRODUCTION

Ferrocenyl groups have been attached to lipids, surfactants, dendrimers, and polymers to serve as redox centers for a wide variety of applications. Attachment of ferrocenyl groups to cationic lipids have been utilized for reversible condensation of DNA[1] and cell transfection[2,3] while attachments of trimethyl[4]- or trialkyl-ammonium[5] cations allow for anion sensing. Similarly, ferrocenyl attachment to surfactants allows for electrochemical control of micelle formation[6-8], while ferrocene modified dendrimers and polymers are being used in electrochemical current rectifiers[9] and biosensors[10-12].

Ferrocene modified polyamines have recently gained more attention for their abilities to exhibit high rates of electron transport and exchange electrons with redox enzymes. Calvo et al have reported the use of ferrocene modified polyallyamine[13,14] in amperometric glucose sensors using glucose oxidase while Lan et al have reported similar applications with ferrocene modified branched poly(ethylenimine)[15] [BPEI].

Coincidentally, there have been several reports indicating that BPEI may act to stabilize enzymes through electrostatic complexation between the cationic polymer and the anionic proteins[16,17]. In addition, intramolecular hydrogen bonding may facilitate a close interaction in a BPEI – enzyme matrix[18]. Thus, combining the ability of BPEI to interact with enzymes with the attachment of ferrocenyl redox groups to the polymer could prove quite valuable in the development of amperometric enzyme based biosensors.

However, the reports of ferrocene modified BPEI only examine the electrochemical behavior of the polymer films immobilized onto an electrode surface[15,19]. Such analysis does not allow for the examination of the redox behavior of the polymer alone as a function of pH. Since it is well known that polyamines are sensitive to changes in pH[20-29] with respect to ionization and polymer configuration it is vital to understand the implications of these phenomena on the redox behavior of the ferrocenyl modified polymer in order to optimize systems where these materials may be applicable, such as biosensors.

In this chapter I report the first synthesis of a ferrocenyl modified linear poly(ethlylenimine) [LPEI] and its electrochemical behavior in aqueous solution as a function of pH. I show here that the protonation and deprotonation of the LPEI backbone result in unusual electrochemical responses. The linearity and homogeneity of LPEI make it an attractive candidate for investigating the effect of ionization on the redox behavior of the ferrocene groups. However, I also report the synthesis and electrochemical characterization of the branched version of the redox polymer (Fc-BPEI), and a model monomeric compound, for comparison and interpretation of my observations.

EXPERIMENTAL METHODS

Chemicals and Solutions

Ferrocenecarboxaldehyde, poly(2-ethyl-2-oxazoline), and all salts and acids were purchased from Aldrich. (Dimethylaminomethyl)ferrocene was synthesized by methods previously reported in the literature[30]. All other chemicals and solvents were reagent grade and used as received. Water was obtained from a Nanopure® water purification system and had a specific resistance of 18 MΩ cm.

Instrumentation

Cyclic voltammetry experiments were performed with a bipotentiostat (Model 832) and 3mm glassy carbon electrodes purchased from CH Instruments (Austin, TX). Experiments were conducted in a three-electrode cell configuration with a silver silver-chloride reference electrode (Ag/AgCl), and a platinum wire counter electrode. Prior to use, all electrodes were polished successively on three grades of alumina (1.0, 0.3, 0.05 μm) and washed thoroughly with Nanopure water after each polishing step. Constant temperature (25±1°C) was maintained during the experiments by using a water-jacketed electrochemical cell connected to a circulating water bath. An Accumet® AR25 pH meter (Fisher Scientific) was used to determine the pH of all aqueous solutions.

Synthesis and Characterization of Fc-LPEI and Fc-BPEI

Linear poly(ethylenimine) (LPEI) (avg. MW ca. 86,000) was obtained by acidic hydrolysis of poly(2-ethyl-2-oxazoline) (avg. MW 200,000), followed by neutralization with sodium hydroxide[31]. In a round-bottom flask, 0.252 g of LPEI was dissolved in 10

mL methanol and a solution of 0.187g (0.87mmol) ferrocenecarboxaldehyde dissolved in 3 mL methanol was added to it dropwise under constant agitation. The resulting dark red solution was stirred for 2 h and cooled in an ice bath. Sodium borohydride (0.033g, 0.87mmol) was added, upon which the solution lightened in color. After 1 h, the methanol was removed under vacuum and the residue was extracted overnight with diethyl ether to remove any non-reacted aldehyde and ferrocenylmethanol. The ether was then decanted and the residue was washed with diethyl ether before being dried under vacuum. The residue was extracted with benzene and insoluble salts were removed by filtration. Finally the solvent was removed from the filtrate under vacuum to give ca. 0.170 g (40%) Fc-LPEI. The Fc-BPEI was prepared by the same method as the Fc-LPEI[15]. ^{1}H-NMR (300 Mhz, d_6-benzene): δ3.9-4.3 (br, Fc ring H), 3.7-3.3 (br, FcCH_2-, NH), 2.5-2.9 (br, -CH_2N-). The degree of substitution of the amine hydrogens by ferrocenylmethylene moieties was estimated from the integration ratio of the ferrocenyl proton to polymer methylene backbone signals in the ^{1}H-NMR spectra of each resulting polymer and found to be ca. 20% for the Fc-LPEI and Fc-BPEI. The synthesis reactions, along with the proposed structure of both polymers are shown in Figure 2.01 below.

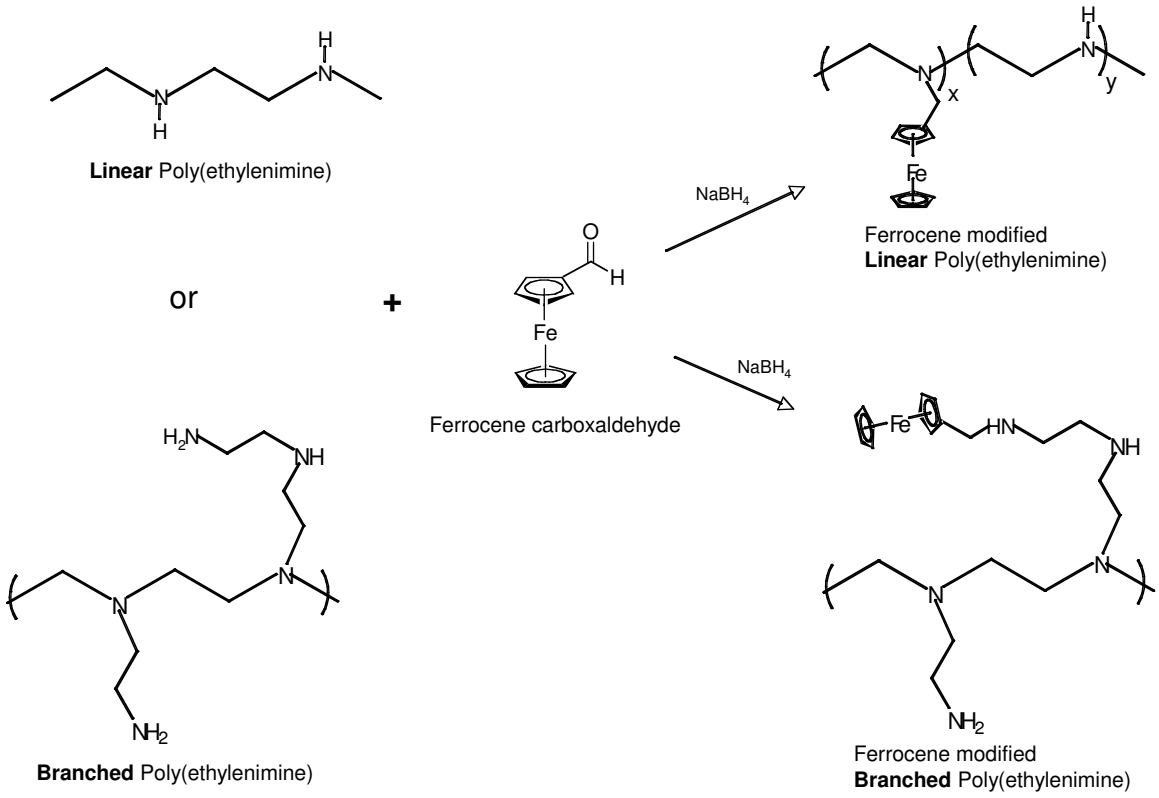


Figure 2.01: The synthesis reactions and proposed structures of Fc-LPEI and Fc-BPEI.

RESULTS AND DISSCUSSION

Electrochemistry of (Dimethylaminomethyl)ferrocene

Before examining the electrochemical behavior of the ferrocene modified linear poly(ethylenimine), cyclic voltammetry measurements were performed using the model compound (dimethylaminomethyl)ferrocene [DMAMF] since the differences between the ionization of monomeric and polymeric amines are well known. Unlike polyamines, the titration of monomeric amines produces an S-shaped curve indicating that the local ionic environment of the ferrocene groups in DMAMF should not vary greatly from each other

at very low or very high pH. Figure 2.02 shows the cyclic voltammetric responses of DMAMF at low and high pH.

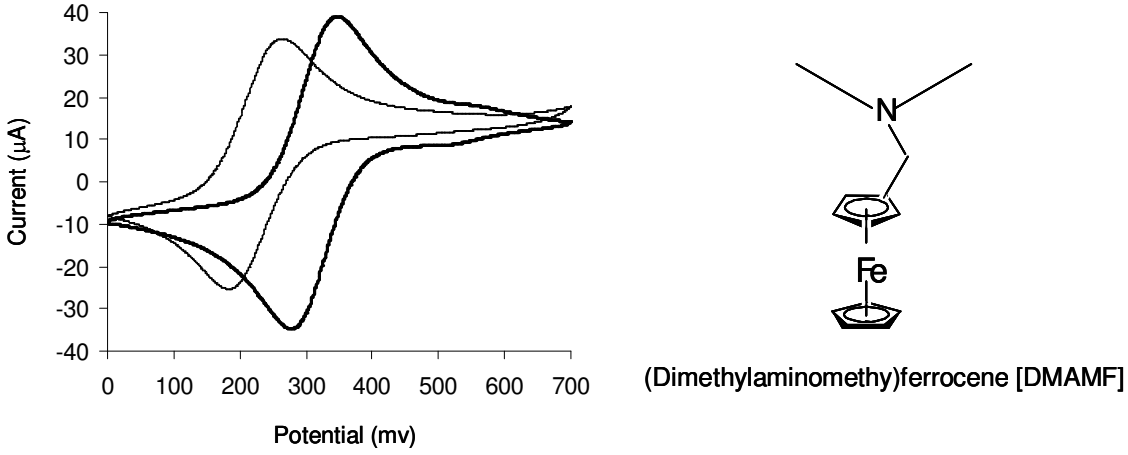


Figure 2.02: Cyclic voltammograms of (Dimethylaminomethyl)ferrocene at pH 1 (Heavy) and pH 11 (Light). $\nu = 50$ mV/s

At pH 1 a single anodic wave at ~350 mV is observed. When the pH is increased to 11 and the tertiary amine is predominantly neutralized a shift to a lower oxidation potential occurs with an anodic wave at ~270 mV. These potential shifts were expected and can be reasonably explained by the following[32,33]: (i) at low pH the nitrogen atoms are protonated hence requiring more energy to oxidize ferrocene (Fc) to the positively charged ferricinium (Fc^{+1}) in close proximity to the protonated monoamine; (ii) at high pH the monoamine is neutral thus requiring less energy to form the positively charged Fc^{+1}.

Unusual potential shifts with protonation/deprotonation of Fc-LPEI

Figure 2.03 shows the cyclic voltammetric behavior of the 15% substituted Fc-LPEI polymer in aqueous solution as a function of pH.

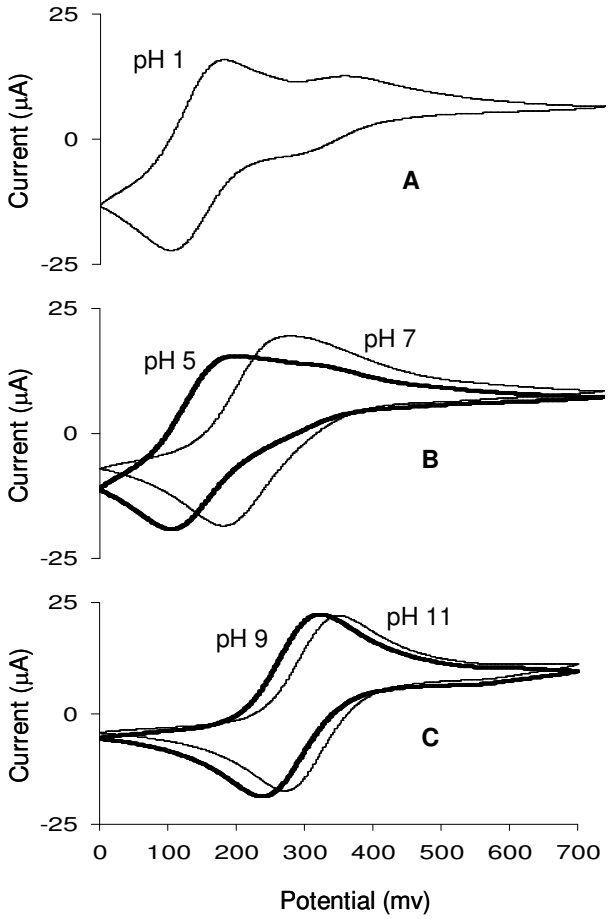


Figure 2.03: Cyclic voltammograms of Fc-LPEI with 0.1M Cl^- as the supporting electrolyte at (A) pH 1, (B) pH 5 and 7, and (C) pH 9 and 11. ν = 50 mV/s

At pH 1 the polymer exhibited a well defined redox couple at ~150 mV and a less-defined couple at 340 mV. As the pH was increased to 5, the two oxidation peaks appeared to overlap, which resulted in a broad oxidation peak that spanned from 150 to 340 mV. When the pH was increased to 7 the oxidation peak narrowed and a single oxidation peak was observed at 265 mV. Increasing the pH further to pH 9 and 11 resulted in a narrowing of the oxidation peak and the oxidation peak potential shifting to 330 mV and 360 mv, respectively. This two-wave behavior and increase in ferrocene oxidation potential with pH was unexpected. Although, previous studies with ferrocene

derivatives have also reported the presence of multiple redox wave behavior with pH changes, they typically have observed the opposite effect, i.e. a decrease in oxidation potential as the pH increases[5,32-34]. Thus in order to investigate the nature of this unusual electrochemical behavior additional solution electrochemistry was performed. When control CV experiments were performed with non-substituted LPEI, redox activity was not observed in the same potential range, which eliminated the possibility that the observed behavior involved oxidation and reduction of the nitrogen atoms on the polymer backbone. To test the possibility that this unusual behavior was due to part of the polymer being adsorbed on the surface of electrode, additional solution chemistry experiments were performed in which the scan rate was varied. Peak oxidation currents correlated linearly with $(\text{scan rate})^{1/2}$, rather than scan rate, over a large range of pH values, indicating that the reaction at the electrode surface is largely a diffusion controlled process and not significantly impacted by adsorption phenomena (data not shown). Based on these results I hypothesized that the multiple redox waves at low pH and the gradual shift of oxidation to higher potentials with deprotonation of the polymer backbone are phenomena related to the proximity of the ferrocene moieties to the highly functional LPEI backbone.

To test this hypothesis I performed the same solution electrochemical experiments with ferrocene modified branched poly(ethylenimine) [Fc-BPEI] since the ferrocene moieties should be primarily attached to the primary amines on the periphery of the branched pol(ethylenimine) structures. A comparison of the electrochemical behavior of the Fc-LPEI in aqueous solution to that of the Fc-BPEI yields a marked difference

between the two polymers. Figure 2.04 shows that at pH 1 the Fc-BPEI exhibits a single oxidation peak at ~400 mV, in contrast to the two oxidation peaks exhibited by Fc-LPEI.

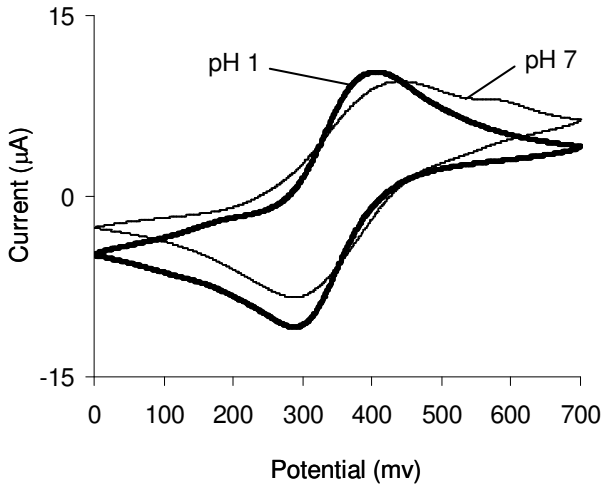


Figure 2.04: Cyclic voltammograms of Fc-BPEI with 0.1M Cl^- as the supporting electrolyte at pH 1 (heavy) and 7 (light). ν = 50 mV/s

In addition the shape of the redox wave and the oxidation peak potential of the Fc-BPEI did not change significantly as the pH was increased to 5 (data not shown). At pH 7 the oxidation peak shifted to ~ 440 mV, and a relatively small shoulder peak appeared around 590 mV. Electrochemical data was only collected up to pH 7 for the Fc-BPEI in solution since this polymer began to precipitate at pH 9, a slightly lower pH than Fc-LPEI precipitated. Nonetheless, this data provides valuable information for the comparison of the electrochemical behavior of the linear and branched ferrocene substituted PEI.

I believe that the structural differences between the two polymers give rise to the discrepancy in their electrochemical behavior in solution. In the branched PEI most of the ferrocene molecules are likely bound to the primary amines, located on the outer edges of the polymer structure. Thus protonation of the nitrogen atoms on the polymer

backbone should not have as great an effect on the local ionic environment of each ferrocene. In contrast, the ferrocene molecules on the linear PEI are directly attached to the secondary amines on the polymer backbone, and thus, their local ionic environment is more affected by changes in pH. The effects of these structural differences are observed at pH 1 where the branched polymer exhibits one oxidation peak while the linear polymer exhibits two. Additionally, the oxidation potential of the ferrocene molecules on the branched polymer remains relatively constant as deprotonation occurs while there is a shift to more positive potentials in the linear polymer.

Low-potential redox behavior at low pH

While the majority of nitrogen atoms in the LPEI backbone are protonated at pH 1, it has been shown that the degree of protonation of LPEI does not reach 100%, even at very low pH[22,23,25,28]. Based on this, I hypothesize that the oxidation peak at 170 mV is due to the oxidation of ferrocene molecules attached to, or in close proximity to positively charged nitrogen atoms, while the oxidation peak at 340 mV corresponds to ferrocenes attached to, or in close proximity to neutral nitrogen atoms.

This explanation is based on the following experiments and observations. First, I performed similar solution electrochemistry experiments with a 5% substituted Fc-LPEI. Figure 2.05 compares the CV response of the 5% vs. 15% substituted polymers. At pH 1 the ratio of the oxidation current at 340 mv to the oxidation current at 170 mV is larger for the 5% substituted polymer (ratio =1.1) then the 15% substituted polymer (ratio =0.8). Hence for the same degree of protonation on the polymer backbone, the polymer with fewer ferrocene sites would be less likely to have ferrocene molecules in proximity to the positively charged nitrogen atoms and their associated counter ions. This produces a

relatively lower oxidation current at 170 mV and a relatively higher oxidation current at 340 mV.

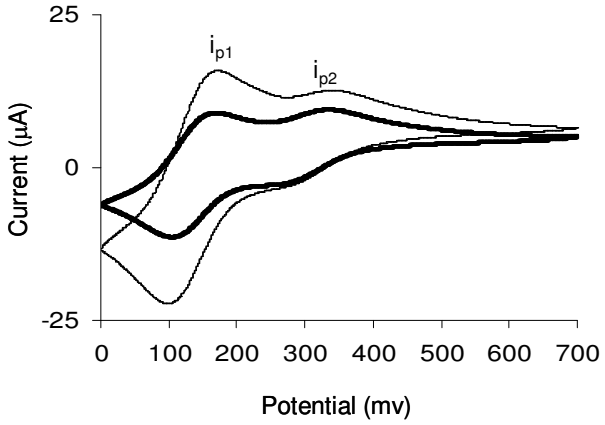


Figure 2.05: Cyclic voltammograms of 5% substituted (heavy) and 15% substituted (light) Fc-LPEI with 0.1M Cl^- as the supporting electrolyte at pH 1. ν = 50 mV/s

Second, solution electrochemistry was also performed with 0.1 M tetrabutylammonium hexafluorophosphate in methylene chloride (CH_2Cl_2). By using an aprotic organic solvent the polymer could be dissolved in a neutral state, and allowed me to systematically investigate the effect of protonation of the polymer backbone on the electrochemistry. The degree of protonation was varied by successively adding Trifluoroacetic acid (CF_3COOH). As the nitrogen-to-hydrogen ratio was systematically varied the peak oxidation potential gradually shifted from 930 mv with no acid to 400 mV when N/H=1 (see Figure 2.06). These results corroborated the trend observed in the electrochemistry of Fc-LPEI in aqueous systems where protonation of the polymer backbone led to a decrease in oxidation potential.

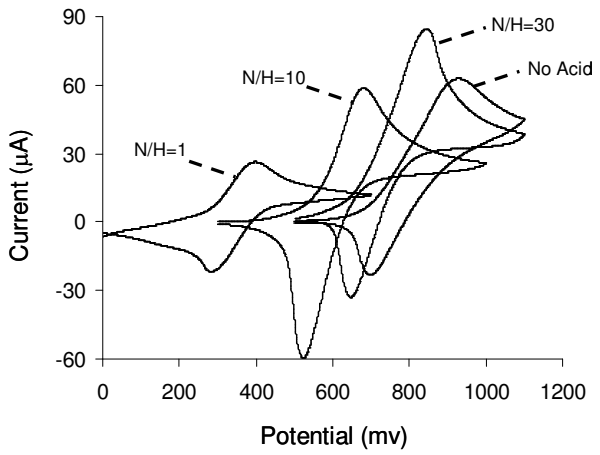


Figure 2.06: Cyclic voltammograms of Fc-LPEI in methylene chloride with 0.1 M tetrabutylammonium hexafluorophosphate as the electrolyte and successive additions of trifluoroacetic acid: no acid, Nitrogen to Hydrogen ratio of N/H=30, N/H=10, and N/H=1. ν = 50 mV/s

Counter ions promote oxidation at lower potentials

I acknowledge that the lower oxidation potential of the ferrocene molecules in regions of high degrees of protonation seems counterintuitive since the resulting ferricinium ion is also positive. However, a possible explanation for the phenomena observed in these studies is that at low pH the combination of the high density of protonated nitrogen atoms and the Cl^- counter ions associated with them may result in an environment with a higher local dielectric constant relative to the environment of ferrocene molecules in regions of neutral nitrogen atoms, effectively creating two populations of ferrocenes. The population of ferrocene molecules in the regions of higher dielectric constant should oxidize at a lower potential, while those in the regions of lower dielectric constant should oxidize at a higher potential. As the pH of the solution is increased, the nitrogen atoms on the polymer backbone are gradually deprotonated and thus the two populations of ferrocene at low pH transition to a single environment at high pH.

To test this explanation, the solution electrochemistry of Fc-LPEI was studied with hexafluorophosphoric acid (HPF_6), since PF_6^- is known to be a weakly coordinating anion[35]. At pH 1 the polymer showed slight secondary anodic behavior at ~ 75 mV and a broad primary anodic peak at 260 mV, as shown in Figure 2.07.

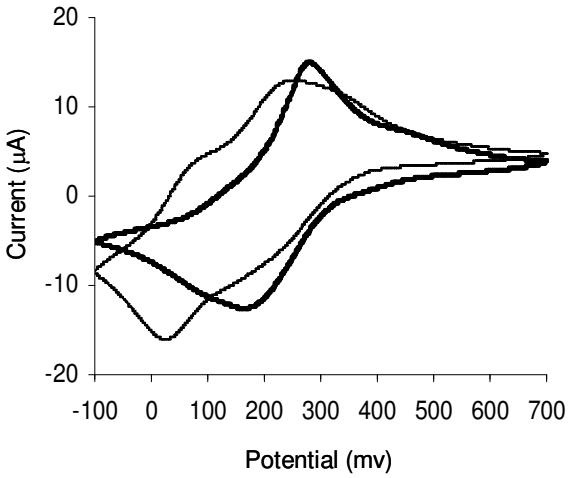


Figure 2.07: Cyclic voltammograms of Fc-LPEI in hexafluorophosphoric acid, HPF_6: (light) pH 1, and (heavy) pH 5. ν = 50 mV/s

As the pH was increased to 5 the secondary anodic behavior near 75 mV disappeared while the primary anodic peak narrowed and shifted to a more positive value of 285 mV. Although the relative magnitudes of the anodic peak currents and potential shifts were different in HPF_6 than in HCl, the general trend was very similar. The lower potential oxidation wave was more pronounced with the smaller, more densely charged Cl^- counter ion, than with PF_6^- which is a much larger and less densely charged counter ion[35]. This should allow for the chloride to be more closely associated with the polymer and its positive charges, thus providing a greater means of charge compensation following the oxidation of ferrocene.

CONCLUSIONS

In this study I report the unique solution electrochemistry of ferrocene modified linear poly(ethylenimine) [Fc-LPEI]. The unusual characteristics of this material include (i) multiple redox waves at low pH, and (ii) progression to a single redox wave at a higher potential with an increase in pH. The shift to higher redox potentials with deprotonation, or increasing pH, is contrary to the majority of electrochemical studies of ferrocene modified amines making this the first work, to the best of my knowledge, to systematically address the nature of this atypical behavior. Solution electrochemical measurements of Fc-BPEI do not exhibit the unusual characteristics of Fc-LPEI indicating that the proximity of the ferrocene groups to the highly functional backbone of LPEI results in the noted phenomena. I also address the specific nature of the multiple redox waves and the atypical shift with deprotonation. Voltammetry measurements with less-substituted LPEI and organic phase measurements suggest that the lower potential redox wave is a result of ferrocene moieties located near positively charged nitrogen atoms on the polymer backbone; while the higher potential redox waves correspond to ferrocenes located near neutral nitrogen atoms. Finally, experiments incorporating the less coordinating PF_6^- counter ion and the use of the model compound (dimethylaminomethyl)ferrocene [DMAMF] suggest that the association of the counter ions with the positively charged nitrogen atoms on the LPEI backbone in conjunction to their proximity to the ferrocene groups are responsible for the counterintuitive low-potential redox wave at low pH.

Acknowledgements: I would like to thank Dr. Glatzhofer for his wisdom involving this work, and Matt Meridith for synthesizing the model compound for me.

REFERENCES

(1) Hays, M. E.; Jewell, C. M.; Lynn, D. M.; Abbott, N. L. *Langmuir* **2007**, *23*, 5609.

(2) Abbott, N. L.; Jewell, C. M.; Hays, M. E.; Kondo, Y.; Lynn, D. M. *Journal of the American Chemical Society* **2005**, *127*, 11576.

(3) Jewell, C. M.; Hays, M. E.; Kondo, Y.; Abbott, N. L.; Lynn, D. M. *Journal of Controlled Release* **2006**, *112*, 129.

(4) Reynes, O.; Moutet, J. C.; Pecaut, J.; Royal, G.; Saint-Aman, E. *New Journal of Chemistry* **2002**, *26*, 9.

(5) Reynes, O.; Moutet, J. C.; Royal, G.; Saint-Aman, E. *Electrochimica Acta* **2004**, *49*, 3727.

(6) Saji, T.; Hoshino, K.; Aoyagui, S. *Journal of the Chemical Society-Chemical Communications* **1985**, 865.

(7) Saji, T.; Hoshino, K.; Aoyagui, S. *Journal of the American Chemical Society* **1985**, *107*, 6865.

(8) Griffiths, P. C.; Fallis, I. A.; Chuenpratoom, T.; Watanesk, R. *Advances in Colloid and Interface Science* **2006**, *122*, 107.

(9) Oh, S. K.; Baker, L. A.; Crooks, R. M. *Langmuir* **2002**, *18*, 6981.

(10) Losada, J.; Zamora, M.; Armada, P. G.; Cuadrado, I.; Alonso, B.; Casado, C. M. *Analytical and Bioanalytical Chemistry* **2006**, *385*, 1209.

(11) Armada, M. P. G.; Losada, J.; Zamora, M.; Alonso, B.; Cuadrado, I.; Casado, C. M. *Bioelectrochemistry* **2006**, *69*, 65.

(12) Kwon, S. J.; Kim, E.; Yang, H.; Kwak, J. *Analyst* **2006**, *131*, 402.

(13) Battaglini, F.; Calvo, E. J.; Danilowicz, C.; Wolosiuk, A. *Analytical Chemistry* **1999**, *71*, 1062.

(14) Calvo, E. J.; Etchenique, R.; Danilowicz, C.; Diaz, L. *Analytical Chemistry* **1996**, *68*, 4186.

(15) Chuang, C. L.; Wang, Y. J.; Lan, H. L. *Analytica Chimica Acta* **1997**, *353*, 37.

(16) Chen, Q.; Kenausis, G. L.; Heller, A. *Journal of the American Chemical Society* **1998**, *120*, 4582.

(17) Jezkova, J.; Iwuoha, E. I.; Smyth, M. R.; Vytras, K. *Electroanalysis* **1997**, *9*, 978.

(18) Andersson, M. M.; Hatti-Kaul, R.; Brown, W. *Journal of Physical Chemistry B* **2000**, *104*, 3660.

(19) Liu, A. H.; Kashiwagi, Y.; Anzai, J. *Electroanalysis* **2003**, *15*, 1139.

(20) Kokufuta, E. *Langmuir* **2005**, *21*, 10004.

(21) Kokufuta, E.; Wang, B. L.; Yoshida, R.; Khokhlov, A. R.; Hirata, M. *Macromolecules* **1998**, *31*, 6878.

(22) Kokufuta, E.; Suzuki, H.; Yoshida, R.; Yamada, K.; Hirata, M.; Kaneko, F. *Langmuir* **1998**, *14*, 788.

(23) Smits, R. G.; Koper, G. J. M.; Mandel, M. *Journal of Physical Chemistry* **1993**, *97*, 5745.

(24) Weyts, K. F.; Goethals, E. J. *Die Makromolekulare Chemie, Rapid Communications* **1989**, *10*, 299.

(25) Chang, D. R.; Harden, S.; Loverro, N. *Journal of Macromolecular Science, Chemistry* **1986**, *A23*, 801.

(26) Lewis, E. A.; Barkley, J.; Stpierre, T. *Macromolecules* **1981**, *14*, 546.

(27) Chatani, Y.; Tadokoro, H.; Saegusa, T.; Ikeda, H. *Macromolecules* **1981**, *14*, 315.

(28) Bloys van Treslong, C. J.; Staverman, A. J. *Recueil, Journal of the Royal Netherlands Chemical Society* **1974**, *93*, 171.

(29) Shepherd, E. J.; Kitchener, J. A. *Journal of the Chemical Society* **1956**, 2448.

(30) Lednicer, D. H., Charles, R. *Organic Syntheses* **1960**, *40*, 31.

(31) York, S.; Frech, R.; Snow, A.; Glatzhofer, D. *Electrochimica Acta* **2001**, *46*, 1533.

(32) Alvarez, J.; Ren, T.; Kaifer, A. E. *Organometallics* **2001**, *20*, 3543.

(33) Alvarez, J.; Kaifer, A. E. *Organometallics* **1999**, *18*, 5733.

(34) Suzaki, Y.; Horie, M.; Sakano, T.; Osakada, K. *Journal of Organometallic Chemistry* **2006**, *691*, 3403.

(35) Khorasani-Motlagh, M.; Safari, N.; Noroozifar, M.; Saffari, J.; Biabani, M.; Reboucas, J. S.; Patrick, B. O. *Inorganic Chemistry* **2005**, *44*, 7762.

CHAPTER 3:

Effects of Electrolyte and pH on the Behavior of Crosslinked Films of Ferrocene-Modified Poly(ethylenimine)

Reproduced with permission from: Merchant, S.A., Glatzhofer, D.T., and Schmidtke, D.W., *Langmuir*, 2007, 23(22), 11295-11302, American Chemical Society.

INTRODUCTION

The development of new and novel redox polymers has been the focus of considerable research over the last 20 years due to their potential use in electrocatalysis[1-3], photoelectrochemistry[4], biosensing applications[5-8], drug delivery[9,10], batteries[11], and biofuel cells[12,13]. Various polymer backbones and redox couples have been utilized in the development of these novel redox polymers. Among the more popular polymer backbones utilized are poly(vinylpyridine)[1,5,14], poly(vinylimidazole)[15-17], and poly(allylamine)[18,19], while ferrocene, osmium, and ruthenium are the most popular redox mediators. Previous studies have suggested that the following characteristics are among the most desired for redox polymers: (i) polymer flexibility or high segmental mobility[14,20,21], (ii) a high degree of functional density on the polymer to facilitate modification and crosslinking[22], and (iii) a polymer that hydrates well[23].

Poly(ethylenimine) (PEI) is an attractive candidate to serve as a redox polymer backbone for all of the reasons listed above. PEI can be obtained in either a branched (BPEI) or a linear (LPEI) form. It has been shown that BPEI consists of approximately

equal numbers of primary and tertiary amines, and these account for about half of the amino groups in the polymer with the other half of the total number of amino groups being secondary amines[24,25]. On the other hand, the amino groups in LPEI are almost exclusively secondary amines, with the exception of the primary amines at each end of the polymer chains[26,27]. Both the linear and branched forms have glass transition temperatures (LPEI T_g = -35°C, BPEI T_g= -50°C) that are below that of currently utilized redox polymer backbones such as poly(vinylpyridine) (T_g = +142°C)[28] or poly(vinylimidazole) (T_g = +163°C)[29]. This suggests that at room temperature or higher they should exhibit a high degree of segmental mobility and thus have enhanced electron transport. A second attractive feature of PEI polymers is that they have a high density of amines in their structure that allows one to easily modify the polymer. As shown in Figure 3.01, different redox moieties can be easily attached to the polymer (e.g. Ferrocene, Ruthenium, or Osmium complexes) through the primary (BPEI) and secondary (LPEI) amine groups.

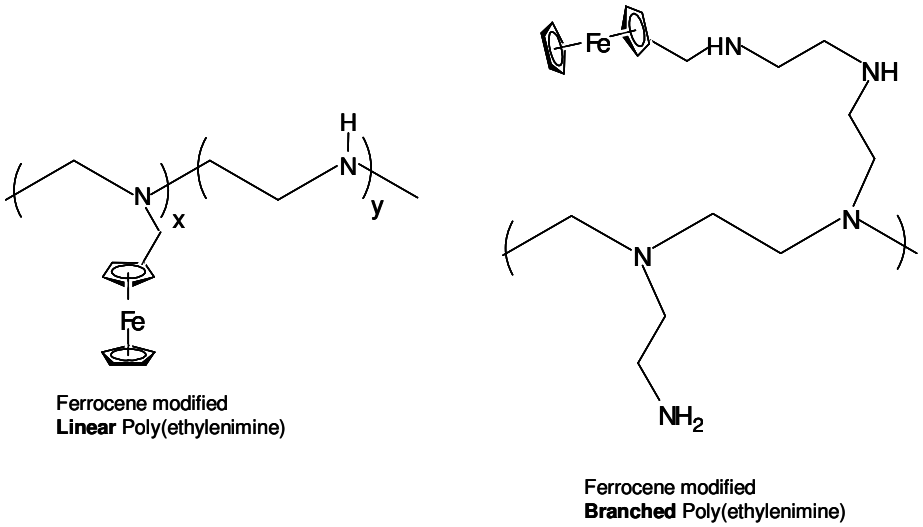


Figure 3.01: Structure of Linear Poly(ethylenimine) and Branched Poly(ethylenimine) modified with ferrocene (ferrocene carboxaldehyde)

This should also allow one to vary the degree of redox center substitution and control crosslink density. An additional attractive feature of PEI is that it appears to enhance several enzyme properties. PEI by itself has been shown to enhance the long-term storage stability of enzymes in solution and during freeze drying[30], as well as increasing the sensitivity and stability of enzymes in a number of biosensor designs[31-35].

Recently there have been a few reports on the development of new redox polymers based on poly(ethylenimine) backbones[36-38]. Huan et al. coupled Toluidine Blue O moieties to the primary amines of BPEI through a terephthaloyl coupling in the development of sensors for the electrocatalytic oxidation of NADH[37]. Alternatively, Lui coupled ferrocene carboxaldehyde to BPEI and incorporated these redox polymers into polyelectrolyte multilayer films via a layer-by-layer deposition technique[36]. Chuang et al. also coupled ferrocene carboxaldehyde to BPEI but incorporated these redox polymers into carbon paste electrodes for glucose sensing[38]. Although the study by Chuang reported the effect of pH on the electrocatalytic response of their sensors to glucose, they did not systematically investigate the effect of pH or crosslinking on the electrochemical behavior of the redox polymer alone. Understanding the influence of pH on PEI-based redox polymers is essential since it is well known that both uncrosslinked[27,39,40] and crosslinked[41,42] forms of PEI undergo a volume collapse at high pH due to hydrogen bonding between PEI chain segments and surrounding water molecules which results in the formation of crystalline hydrates. In contrast, protonated forms of PEI at low pH are in an extended state due to the electrostatically repulsive positive charges on the polymer backbone, and have been shown to be quite soluble in water.

In this chapter I report the synthesis of ferrocene redox polymers based on LPEI and BPEI. I demonstrate that these redox polymer films can exhibit multi-wave redox behavior that is dependent upon pH, electrolyte, and degree of crosslinking. Electron diffusion through these films was in some cases approximately 40-fold higher then those reported for ferrocene based polyallylamine redox polymers. To my knowledge this is the first report of electron diffusion measurements for ferrocene modified poly(ethylenimine) redox polymers. Finally I demonstrate that these redox polymers are capable of electrically communicating with the redox centers of enzymes exhibiting saturation current densities between 240-480 $\mu A/cm^2$.

EXPERIMENTAL SECTION

Chemicals and Solutions

Both redox polymers, Fc-LPEI and Fc-BPEI were synthesized as previously described in Chapter 2. Ethylene glycol diglycidyl ether (EGDGE) was purchased from Polysciences Inc., Warrington, PA. All other chemicals and solvents were reagent grade and used as received. Water was obtained from a Nanopure® water purification system and had a specific resistance of 18 MΩ cm. Phosphate buffer saline solution (PBS, pH 7.4) was prepared by dissolving 8.0 g of NaCl, 1.0 g of Na_2HPO_4, 0.2 g of KCl, and 0.2 g KH_2PO_4 in 1000 ml of water.

Instrumentation

Cyclic voltammetry and constant potential experiments were performed with a bipotentiostat (Model 832) and 3mm glassy carbon electrodes purchased from CH

Instruments (Austin, TX), while electrochemical impedance measurements were performed with a Solartron SI 1260 impedance/gain-phase analyzer in conjunction with a SI 1287 potentiostat. Unless otherwise noted, experiments were conducted in a three-electrode cell configuration with a saturated calomel reference electrode (SCE), and a platinum wire counter electrode. Prior to use, all electrodes were polished successively on three grades of alumina (5, 1, 0.3 μm) and washed thoroughly with Nanopure water after each polishing step. Constant temperature (25±1°C) was maintained during the experiments by using a water-jacketed electrochemical cell connected to a circulating water bath. An Accumet® AR25 pH meter (Fisher Scientific) was used to determine the pH of the larger volume electrochemical solutions, whereas pH test strips were used to indicate the pH of the lower volume polymer solutions used to prepare the redox hydrogels.

Redox Hydrogel Film Preparation/Electrochemistry

The Fc-LPEI and Fc-BPEI were each dissolved in aqueous solution at pH 3, and the solution was subsequently neutralized with a NaOH solution until the final concentration of the polymer solution was 10 mg/ml. The polymer solutions were mixed with ethylene glycol diglycidyl ether (EGDGE) such that the ratio of nitrogen atoms in the polymer to the epoxide groups on the crosslinker (2 per molecule) was 5:1 corresponding to a theoretical degree of crosslinking of 20%, or 29 wt% EGDGE in the films. The electrode surfaces were coated with 3 μl aliquots of each polymer/EGDGE mixture and allowed to dry for 12-14 hours in ambient conditions before electrochemically testing in aqueous solutions of varying pH. The pH of the

electrochemical medium was adjusted using the strong acid corresponding to the background electrolyte used (NaCl/HCl, $NaClO_4/HClO_4$) and NaOH, and a new electrode film was tested at each pH. Cyclic voltammetry experiments were performed on the polymer films at pH values of 1, 3, 5, 7, 9, and 11, using a saturated calomel electrode (SCE) as the reference and a scan rate of 50 mV/s. Electrochemical impedance spectroscopy measurements were used to determine $cD_e^{1/2}$, where D_e is the electron diffusion coefficient and C is the concentration of accessible redox sites in the film.

Gel Preparation and Swelling

Highly concentrated polymer solutions (165 mg/ml) were prepared to create gels suitable for handling during swelling experiments. In this case 100-150 mg of polymer was placed in a vial and 0.1 M HCl was added to half the volume required to achieve 165 mg/ml. The vial was placed in a hot water bath and heated until the polymer dissolved. The vial was removed from the heat and HCl was added in small increments. When the solution cooled and the polymer phase separated, the solution was reheated until dissolution and more HCl was added. This process was repeated until the polymer remained dissolved at room temperature. The remaining volume required to achieve 165 mg/ml was added using 0.2 M HCl to lower the pH of the final polymer solution between 5 and 6.

The concentrated polymer solutions were mixed with EGDGE such that the ratio of nitrogen atoms on the polymer to the epoxide groups on the crosslinker was 5:2 corresponding to a 40% theoretical degree of crosslinking. The resulting polymer/crosslinker mixture was placed in a 1ml syringe whose tuberculin slip tip had been cut off with a razor. Special care was taken to ensure that bubbles were not created

when transferring the polymer/crosslinker mixture to the modified syringe. After curing for 48 hours the gels were carefully pushed out of the syringe using the plunger and were allowed to dry in ambient conditions for another 24 hours. The diameter of the gel cylinder was measure at approximately 3 mm using digital calipers and discs measuring approximately 1 mm in length were cut from the cylinder using a razor.

Glucose Sensor Preparation and Testing

Glucose sensors were prepared by crosslinking glucose oxidase to either Fc-BPEI or Fc-LPEI to form enzymatic redox hydrogels. 45 μl of polymer solution (10 mg/ml), 35 μl of glucose oxidase solution (10 mg/ml), and 0.29 μl of EGDGE were mixed together and 3 μl aliquots were placed onto the glassy carbon electrode surface. The mixture was allowed to dry for 12-14 hours. Cyclic voltammetry was performed on the sensors to determine their electrochemical behavior. The response of each sensor to glucose was determined using amperometric analysis, where the working potential of the sensors was held constant at 400 mV in 0.1 M $NaClO_4$ background electrolyte. Aliquots of a 2 M glucose solution were added to the stirring electrochemical medium and the resulting current was monitored as a function of time to determine the steady-state current at each glucose concentration.

RESULTS AND DISCUSSION

Electrochemistry of Crosslinked Films of Fc-LPEI and Fc-BPEI

With Phosphate Buffered Saline as the Electrolyte

Crosslinked films of both Fc-BPEI and Fc-LPEI were investigated electrochemically in order to determine their usefulness in applications such as

biosensors and biofuel cells. The films were initially tested in phosphate buffered saline (PBS) at pH 7.4 since this is a commonly used background electrolyte for these applications. Figure 3.02 shows typical cyclic voltammograms for crosslinked films of Fc-BPEI (Fig. 3.02A) and Fc-LPEI (Fig. 3.02B).

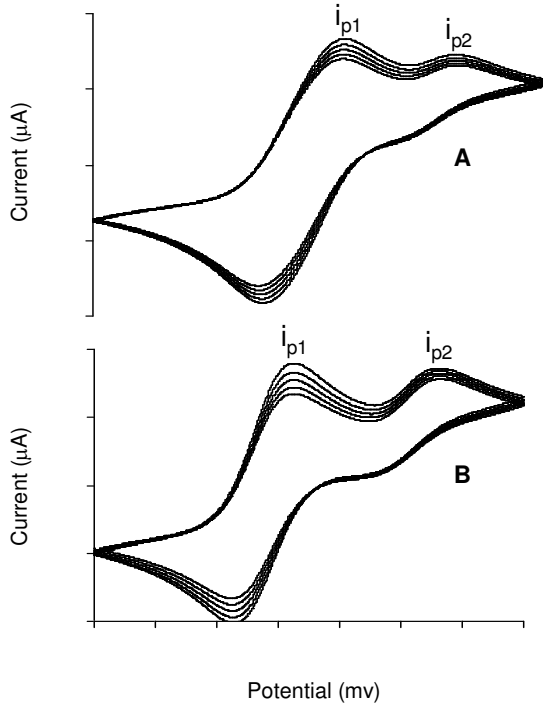


Figure 3.02: Cyclic voltammograms of crosslinked films of (A) Fc-BPEI and (B) Fc-LPEI in phosphate buffered saline (PBS) background electrolyte at pH 7.4 at 50 mV/s, scans 10-15.

Both types of films exhibited an initial oxidation peak at ~300-400 mV and a secondary oxidation peak at ~ 600 mV. In addition, the electrochemical response exhibited instability such that the current response decreased with each subsequent scan. The instability effects were somewhat more dramatic in the linear polymer. For example, the percent decrease in the primary oxidation peak current (i_{p1}) from scan 10 to 15 for the Fc-BPEI film was 15% while the percent decrease for Fc-LPEI film was 25%. In addition, the ratio of the secondary oxidation peak current to the primary peak current is greater in

the linear polymer (i_{p2}/i_{p1}=1.16 ± 0.026) than the branched polymer (i_{p2}/i_{p1}=0.94 ± 0.015). This behavior was unexpected since this phenomena has not previously been reported in the literature for similar materials and conditions[38].

Studies have shown that poly(ethylenimine) and its corresponding hydrogels are highly sensitive to pH with respect to polymer configuration and gel morphology[26,27,41,44]. In addition, ferricinium (Fc^+) has been shown to be susceptible to nucleophilic attack resulting in the decomposition of ferrocene[45,46]. Thus, in order to address the nature of the phenomena observed in the electrochemistry of the crosslinked films, both redox hydrogels were tested over the pH range from 1 to 11, and in a variety of electrolytes including ClO_4^- which is non-nucleophilic and has been shown not to attack Fc^+[45].

With Perchlorate as the Electrolyte

Figure 3.03 shows the cyclic voltammograms for crosslinked Fc-BPEI in 0.15 M ClO_4^- as a function of pH. In contrast to the results in phosphate buffered solutions, the electrochemical response for this polymer was the same in the pH range from 1 to 9 and is represented in Figure 3.03A at pH 7. Only at pH 11 did the crosslinked polymer show signs of a second oxidation peak at 550 mV and begin to exhibit a significant instability.

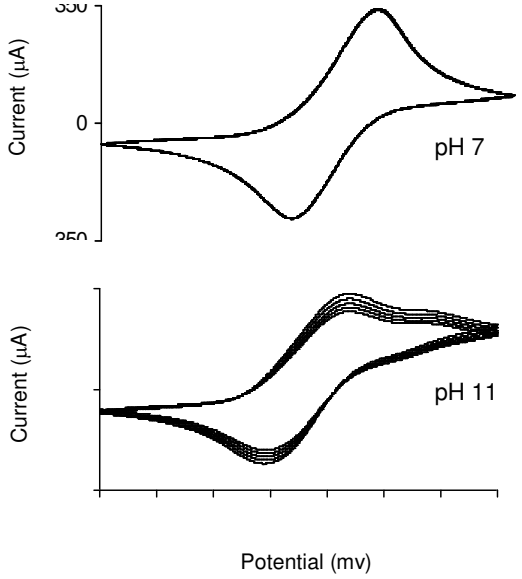


Figure 3.03: Cyclic voltammograms of crosslinked Fc-BPEI films with 0.15 M perchlorate as electrolyte, 50 mV/s, scans 10-15.

Figure 3.04 (below) shows the cyclic voltammograms for crosslinked Fc-LPEI in 0.15 M ClO_4^- as a function of pH. Unlike the behavior of the branched redox polymer, the linear redox polymer showed some variation in its response over the pH range studied. At pH 1 the polymer exhibited some slight redox behavior at 200 mV and a broad oxidation peak at 400 mV. The response of the polymer between pH 3 and 9 remained essentially the same. Although signs of the second oxidation peak at 550 mV began to appear at pH 7 (Figure 3.04), it was not until pH 11 that the second oxidation peak developed dramatically and the electrochemical response became unstable. It should also be noted that the relative magnitude of the second peak (i_{p2}/i_{p1}) at pH 11 was greater in the linear redox polymer than the branched. To determine whether other electrolytes would result in the same electrochemical behavior, crosslinked films of both the Fc-BPEI and Fc-LPEI were additionally tested in Cl^-, NO_3^-, and SO_4^-. Both polymer films exhibited similar trends as a function of pH as they did in ClO_4^- (data not shown).

However, the response in Cl^- was less stable then in the other electrolytes, with the peak current slightly decreased with each subsequent oxidation-reduction cycle. This may be attributed to the known instability of the ferricinium ion (Fc^+) due to its decomposition in the presence of nucleophiles such as the chloride ion; whereas, the perchlorate, nitrate, and sulfate ions are non-nucleophilic[45,46]. In addition, the peak oxidation and reduction currents were consistently higher for both polymers when tested in perchlorate.

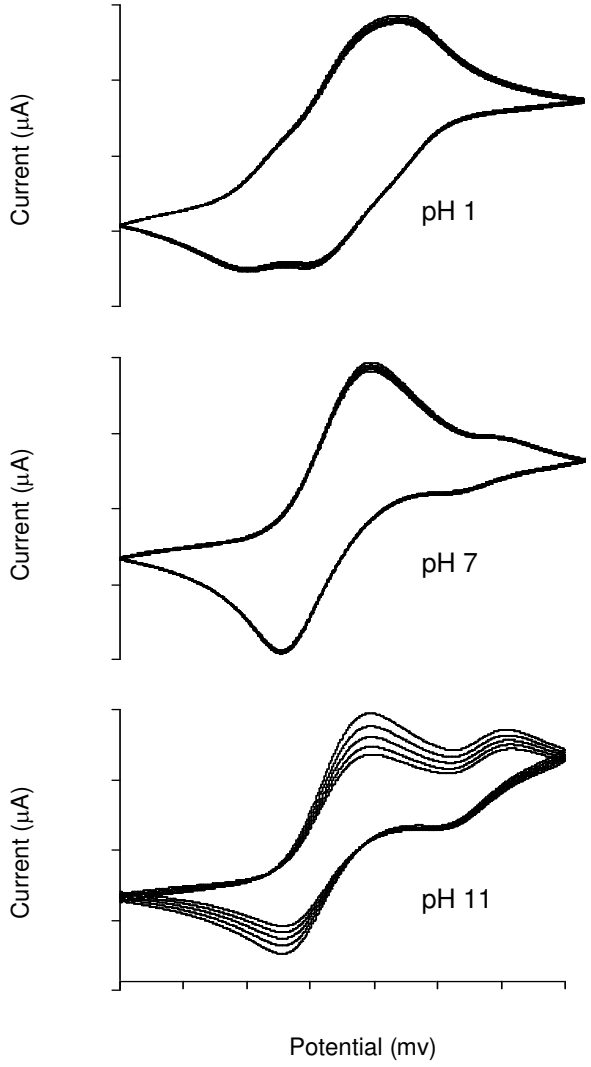


Figure 3.04: Cyclic voltammograms of crosslinked Fc-LPEI films with 0.15 M perchlorate as electrolyte, 50 mV/s, scans 10-15.

The changes in the electrochemical behavior of Fc-BPEI and Fc-LPEI hydrogels with pH in solutions without phosphate may be attributed to the well documented changes in polyelectrolyte configuration with pH, as discussed above, and for corresponding hydrogels. Kokufuta et al showed that LPEI hydrogels crosslinked with EGDGE are in a swollen state until pH 10.7 at which point the gels collapse as a result of the deprotonation of the nitrogen atoms on the polymer backbone and subsequent formation of hydrogen bonds between these nitrogen atoms and water molecules[41]. This corresponds well with the electrochemical behavior of the ferrocene modified polyelectrolyte hydrogels (in the absence of phosphate) in this work where the response remains the same until pH 11 at which point a second region of redox behavior appears. Since the Fc-PEI gels should be collapsed at this pH, it is proposed that the portion of the ferrocene molecules in this second region are more confined within the collapsed gel structure resulting in limited local segmental mobility and an increased redox potential, whereas those ferrocene molecules that are not confined produce the same redox behavior over the entire pH range studied. Furthermore, the collapse of the hydrogel also coincides with the expulsion of solvent and ions, which would also result in a decreased local dielectric constant and increased redox potential for the confined ferrocene molecules. Finally, it is evident that the second region of redox behavior at pH 11 and the instability are directly related to each other since the current response of the films is stable under all other conditions. Therefore, it is proposed that the oxidation and reduction of the confined ferrocene molecules between 500 and 600 mV within the collapsed gel causes some type of damage to the films such that the peak current decreases with each subsequent potential scan. It may, however, be argued that the

hydroxide ion is in great enough concentration at pH 11 to cause the decomposition of Fc^+ via nucleophilic attack[45,46].

With Phosphate as the Electrolyte

While the electrochemical behavior of both polymers as a function of pH is similar in the presence of the electrolytes discussed above, using phosphate as the electrolyte produces a different trend with pH. Figure 3.05 shows the response of crosslinked Fc-BPEI in 0.15 M phosphate where it remains the same from pH 1 to 5.

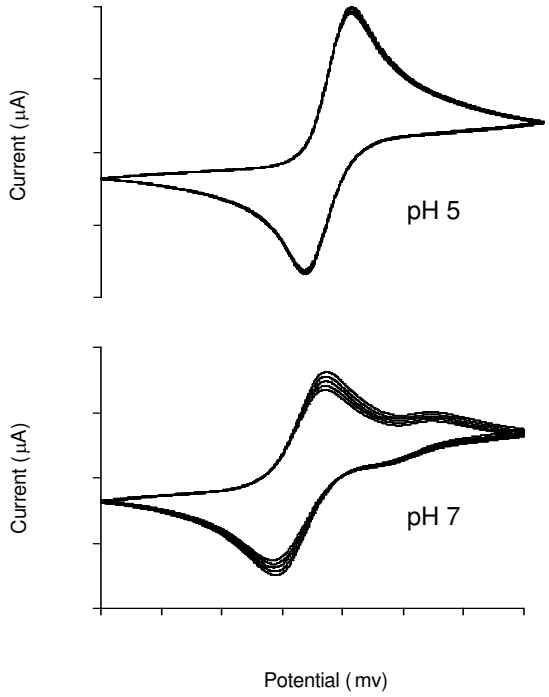


Figure 3.05: Cyclic voltammograms of crosslinked Fc-BPEI films with 0.15 M phosphate as electrolyte, 50mV/s, scans 10-15.

At pH 7 the second oxidation peak appears and becomes more defined as the pH is increased, and the instability with each subsequent scan begins, unlike the behavior in other electrolytes where these phenomena were not observed until pH 11. The behavior of the linear polymer follows the same trend, however, the emergence of the second

oxidation peak and the instability are more dramatic. This corresponds well to the behavior observed in PBS, suggesting that phosphate is the cause of the unusual responses.

The difference in the trend in the electrochemical behavior with pH of the Fc-BPEI and Fc-LPEI in non-phosphate electrolytes versus that in phosphate can be attributed to the fact that phosphate is the only one of the electrolytes examined that changes species at approximately the same pH at which the second region of redox behavior appears. Species predominance charts suggest that pH 7 is the point at which monobasic phosphate ($H_2PO_4^{-1}$) begins to form dibasic phosphate (HPO_4^{-2}). Additionally, dibasic phosphate has been shown to both bind well to PEI and to cause the deswelling of hydrogels[47-49]. This corresponds well with the hypothesis that the collapse of the redox hydrogel results in confinement of a portion of ferrocene molecules, and the oxidation and reduction of these particular moieties occurs at a higher potential and causes instability in the film. Also, the fact that the polymers exhibit these two phenomena at pH 7 in phosphate suggests that it is not the nucleophilic attack of Fc^+ by OH^- that produces this instability.

Effect of Crosslinker

Since the secondary redox peaks at 550 mV are readily observed in a PBS solution, this background electrolyte was used to determine the effect of increasing the crosslink density on the redox behavior. Figure 3.06 shows the results for Fc-LPEI films made with 29, 44, and 62 wt % EGDGE, and demonstrates that increasing the amount of

crosslinking in the gels increases the relative magnitude of the second oxidation peak (i_{p2}/i_{p1}=0.96, 1.25, and 2.24 respectively).

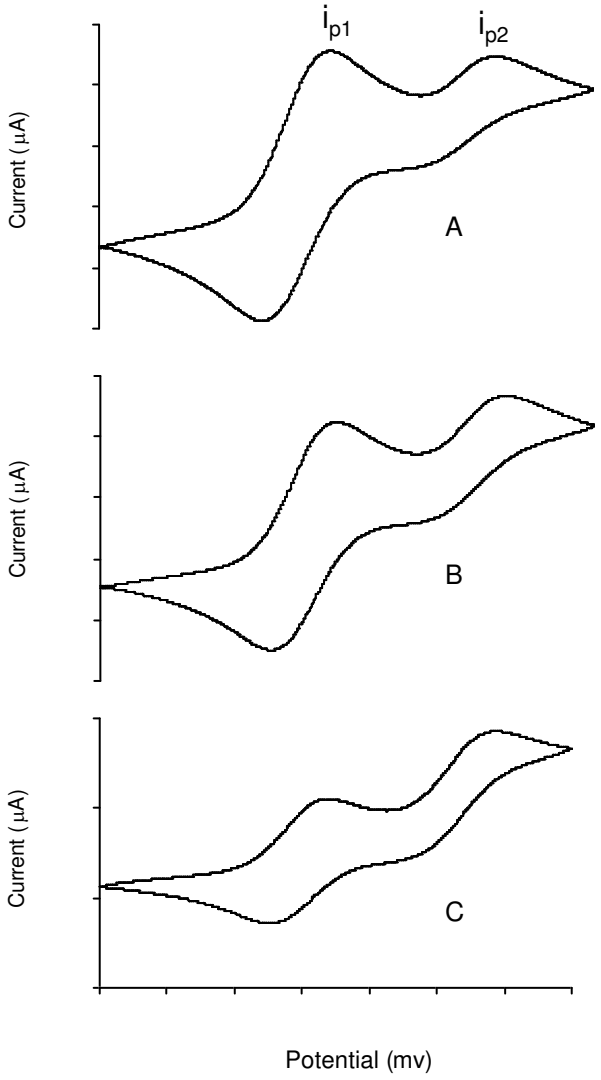


Figure 3.06: Cyclic voltammograms of crosslinked Fc-LPEI films in PBS at pH 7.4 with increasing amounts of crosslinker in the film, 50 mV/s, scans 10-15 (a) 29 wt% (b) 44 wt% (c) 62 wt%.

Similar results were obtained when the crosslink density was increased in Fc-BPEI films (data not shown), however, as noted previously; the emergence of the second oxidation peak was more dramatic in the linear polymer. Several studies have shown that increased crosslinking ratios decrease the segmental mobility of redox moieties[23,50] which

appears to support the hypothesis that the emergence of the secondary redox peaks at 550 mV is related to confinement of the redox moieties.

Electrochemical Impedance Spectroscopy: Dependence of $cD_e^{1/2}$ on pH and electrolyte

In order to assess the effects of pH and counterion type on electron transport through the crosslinked redox polymer films electrochemical impedance spectroscopy (EIS) at a DC potential of E=0.35 V and an AC perturbation of 10 mV was performed. In the low-frequency range the impedance response was analyzed using the Randles circuit by plotting the imaginary impedance, Im(Z), versus the inverse square root of frequency, $\omega^{-1/2}$ (Figure 7), with a slope equal to the Warburg coefficient, σ_w:

$$Im(Z)= \sigma_w \omega^{-1/2} \quad (1)$$

The value $cD_e^{1/2}$ was determined directly from σ_w:

$$\sigma_w=RT/n^2F^2cD_e^{1/2} \quad (2)$$

The linearity of the data confirms semi-infinite diffusion behavior of electron transfer through the redox hydrogels[18,51].

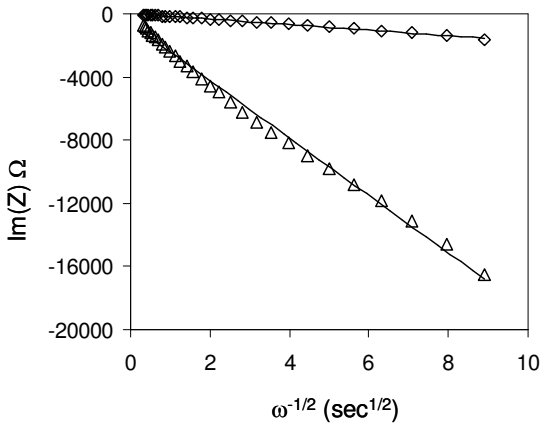


Figure 3.07: Randles plot Im(Z) vs. w-1/2 for Fc-LPEI in (◊) perchlorate, and (Δ) phosphate at pH 5.

Figure 3.08 shows estimates of $cD_e^{1/2}$ for Fc-BPEI and Fc-LPEI as a function of pH in perchlorate, chloride, and phosphate solutions. When either perchlorate or chloride was used as the supporting electrolyte, the $cD_e^{1/2}$ for both polymers remained relatively constant over the entire pH range.

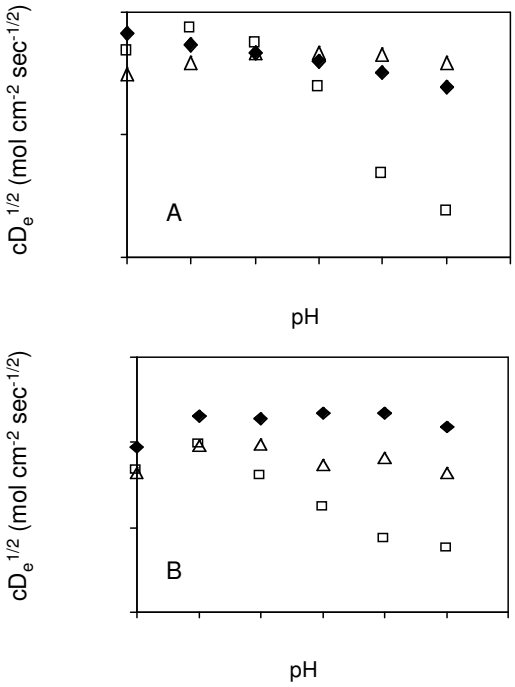


Figure 3.08: pH dependence of the apparent electron diffusion coefficient $cDe^{1/2}$ of (a) Fc-BPEI and (b) Fc-LPEI in 0.15 M (◆) perchlorate, and (□) phosphate. n=3 under each condition, values in charts are averages showing the trend with pH.

The $cD_e^{1/2}$ values for the Fc-BPEI films in either perchlorate (~4.1 x 10^{-8} mol/cm^2•s$^{1/2}$) or chloride (~4.4 x 10^{-8} mol/cm^2•s$^{1/2}$) were nearly identical and approximately 40-fold higher then those reported for ferrocene based polyallylamine redox polymers ($cD_e^{1/2}$ = 11 x 10^{-10} mol/cm^2•s$^{1/2}$)[18]. In contrast, when crosslinked films of Fc-LPEI were tested there were subtle differences in the $cD_e^{1/2}$ values for perchlorate (~1.8 x 10^{-8} mol/cm^2•s$^{1/2}$) and chloride (~6.5 x 10^{-9} mol/cm^2•s$^{1/2}$). These results would appear to

confirm my initial assumption that the rate of electron transport would be enhanced through the use of a flexible backbone.

In contrast to the results in perchlorate and chloride, which were relatively constant over the entire pH range, there was a significant decrease (about one order of magnitude) in $cD_e^{1/2}$ for both the Fc-BPEI and Fc-LPEI polymers with increasing pH when phosphate was the supporting electrolyte. Similar decreases in $cD_e^{1/2}$ with pH in the presence of phosphate have been observed by others[15,23,52]. This decrease is thought to be due to the fact that as the polymer backbone becomes deprotonated, the repulsive forces acting within the polymer network are diminished causing the polymer network to collapse. As the polymer network collapses there is a reduction in the chain segment mobility, which leads to a decrease in the transport of electrons through the film[15,23]. I propose that the differences between the pH independence of $cD_e^{1/2}$ in perchlorate and chloride solutions and the pH dependence in phosphate solutions are related to the binding nature of HPO_4^{-2} to PEI which results in an increased confinement of the ferrocene groups and, hence, a less efficient electron transfer process.

Redox Hydrogel Deswelling

To determine the effect of electrolyte type on the swelling/deswelling behavior, cylindrical gels (diameter ~3mm) of crosslinked Fc-LPEI were prepared and exposed to different electrolytes and changes in their size were imaged with optical microscopy. Similar to previous deswelling studies with unmodified LPEI[41], Fc-LPEI gels were completely swollen at low pH in HCl (Figure 3.09 A) and collapsed to ~55% of their swollen diameter upon addition of NaOH to pH 11 (Figure 3.09 B). These results suggest

that modification with ferrocene does not significantly effect the conformational changes PEI hydrogels undergo with protonation or deprotonation of the polymer backbone.

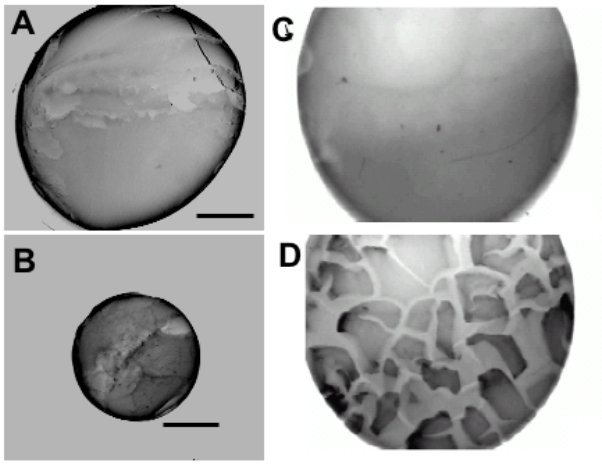


Figure 3.09: Effects of electrolyte and pH on macroscopic gel structure. (A) Optical image of a crosslinked gel of Fc-LPEI in HCl at pH 1 (diameter = 8.1 mm); (B) Image of the gel in Figure A after the pH has been changed to pH 11 (diameter = 4.5 mm); (C) Optical of image of a crosslinked gel of Fc-LPEI in water; (D) Image of the gel in Figure C one minute after 0.1 M perchlorate has been added (pH 5.0).

The behavior of the Fc-LPEI hydrogels in the presence of other electrolytes is not as well understood at this point. Both $H_2PO_4^-$ and HPO_4^{-2} induce a uniform deswelling of the gels, similar to that caused by OH^-, such that the gel maintains its macroscopic structure but reduces in size. Fc-LPEI gels exposed to 0.1 M monobasic phosphate (pH=5) collapse to about 80% of the swollen diameter, while gels exposed to 0.1 M dibasic phosphate (pH=9) collapse to about 70% of the swollen diameter. Although there appears to be little difference in the size of the gels with mono- and dibasic phosphate, the electrochemistry of these gels is quite different, as discussed above. Below pH 7, where monobasic phosphate is predominant, the films are electrochemically stable and exhibit one oxidation peak; above pH 7, where dibasic phosphate is predominant, the films are less stable and exhibit two oxidation peaks. These results suggest that it is not the deswelling of the gels alone that is responsible for the unusual electrochemical

phenomena, but some combination of collapse and "binding" within the gel structure, such as the hydrogen bonds formed between water molecules and the deprotonated nitrogen atoms on the backbone (at high pH), or the binding between nitrogen atoms by dibasic HPO_4^{-2}, that results in the unusual electrochemical behavior.

The behavior of the Fc-LPEI gels in perchlorate, while unusual, further illustrates the importance of binding within the redox hydrogels. While exposure of swollen gels to OH^-, $H_2PO_4^-$, and HPO_4^{-2} results in uniform deswelling, exposure of the gels to ClO_4^- results in almost immediate cracking of the gel followed by deswelling to about 60% of the swollen diameter. Figure 3.09 C shows the Fc-LPEI gel completely swollen in water before the addition of any electrolyte; while Figure 3.09 D shows the cracking of the gel one minute after the addition of 0.1 M ClO_4^- (pH=5). Drastic changes in polyelectrolyte structure in the presence of perchlorate have been recently reported and are thought to be due to the transition in the gel from hydrophilic to hydrophobic, and the subsequent expulsion of water[53]. As with the gels in monobasic phosphate, the gels exposed to perchlorate exhibit a significant decrease in size, but the cyclic voltammetric response is stable and exhibits only one region of redox behavior. Based on these results we propose that while monobasic $H_2PO_4^-$ and ClO_4^- both produce deswelling of the gels, neither forms hydrogen bonds, or binds nitrogen atoms on the polymer backbone; thus confinement of the ferrocene moieties in the presence of these electrolytes is not an issue.

It should be noted that while the same trends were observed in Fc-BPEI gels the changes were much less dramatic, as the Fc-BPEI gels appeared to be more rigid and swelled/deswelled to a lesser extent. In addition, the cracking phenomena observed in the Fc-LPEI gels in the presence of perchlorate appears to be a non-issue with respect to

the electrochemistry of the films since the Fc-BPEI gels did not exhibit the same effect when exposed to $NaClO_4$; yet the electrochemistry of the two materials is very similar. The exact nature of the gel cracking, in addition to the structural changes and chemical nature of the gels in the presence of the different electrolytes are currently being studied in order to determine the interactions occurring at the molecular level.

Glucose Sensor Response

In order to estimate the potential usefulness of Fc-LPEI and Fc-BPEI in applications such as biosensors and biofuel cells, I immobilized the enzyme glucose oxidase in these films and studied the ability of these redox polymers to electrically communicate with the redox centers of enzymes. Since the electrochemical behavior of these redox polymers was shown to be dependent upon pH and the electrolyte type, I tested the response of these sensors to glucose in both phosphate and perchlorate solutions and compared it to sensors fabricated with a more commonly used redox polymer poly[(vinylpyridine)Os(bipyridyl)$_2$Cl$^{2+/3+}$] (PVP-Os)[5,54-56]. Figure 3.10 shows the steady-state current response of these films to glucose when poised at 0.4 V vs SCE. I first tested the sensors in solutions of perchlorate at pH 7.0, since our previous experiments demonstrated that the crosslinked Fc-LPEI films exhibited a stable electrochemical response. As shown in Figure 3.10 and Table 3.01, both the Fc-LPEI and PVP-Os sensors displayed Michaelis-Menten behavior over all of the conditions tested. In perchlorate, the Fc-LPEI sensors were highly responsive to glucose both at low concentrations and at saturating conditions (~350 μA cm^{-2}) and exhibited the highest Km (24.4 mM) of all the conditions tested. In contrast the PVP-Os sensors showed very

little response to glucose in perchlorate. Other researchers have also noted that sensors formed with PVP-Os do not perform well with ClO_4^- as the background electrolyte[23]. In order to compare these sensors under conditions in which the PVP-Os sensors do respond well, we also tested these sensors in phosphate solutions at pH 7.0. Once again the

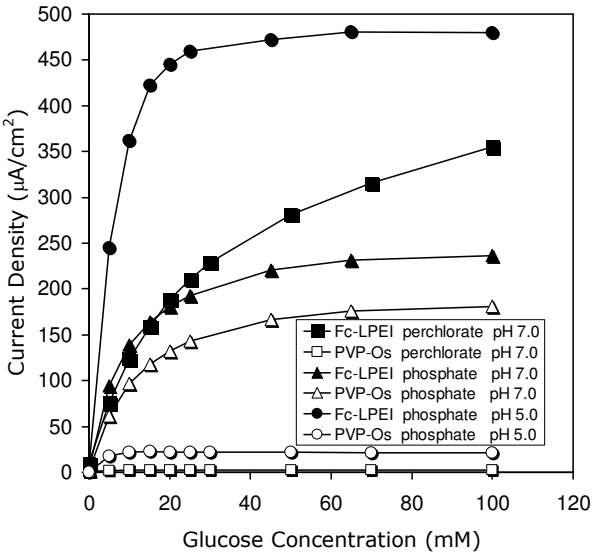


Figure 3.10: Enzymatic current response to glucose for sensors made from Fc-LPEI and PVP-Os in either 0.1 M $NaClO_4$ (pH 7.0), 0.1 M phosphate (pH 5.0), or 0.1 M phosphate (pH 7.0). T = 25°C, E = 0.4 V vs SCE.

Fc-LPEI sensors responded with a higher response to glucose then the PVP-Os sensors, both at low and saturating conditions (Table 3.01) despite the fact that the phosphate was shown to degrade the electrochemical response at this pH. However the PVP-Os sensors did exhibit a higher K_m. Finally, I tested both sensors in phosphate at pH 5, because this was a condition under which the electrochemical response of the Fc-LPEI sensors was stable in phosphate. Under these conditions, the response of the Fc-LPEI sensors was the highest, exhibiting the highest sensitivity (48.0 $\mu A/cm^2 \bullet mM$) and the highest current density ((480 $\mu A/cm^2$) observed, while the PVP sensors showed a minimal response (~20 $\mu A\ cm^{-2}$). These results suggest that despite the unusual electrochemical behavior in

phosphate solutions at pH 7.0 or higher, crosslinked films of Fc-LPEI are highly efficient at electrically communicating with FAD center of Glucose Oxidase, and may be excellent alternatives for applications and conditions (e.g. low pH) in which more widely used redox polymers such as PVP-Os will not work.

Table 3.01: Effects of pH, Electrolyte, and Redox Polymer Type on Biosensor Response

Redox Polymer (n = # of electrodes)	Electrolyte	pH	J_{Max} ($\mu A/cm^2$)	K_m (mM)	Sensitivity ($\mu A/cm^2 \bullet mM$)
PVP-Os (n=6)	Phosphate	7	181 ± 19	11.9 ± 0.4	12 ± 1
Fc-LPEI (n=6)	Phosphate	7	237 ± 57	8.8 ± 1.5	18 ± 3
PVP-Os (n=4)	Phosphate	5	21 ± 10	0.5 ± 0.06	3.6 ± 1.6
Fc-LPEI (n=6)	Phosphate	5	480 ± 20	6.8 ± 0.4	48 ± 1
PVP-Os (n=4)	Perchlorate	7	2.6 ± 1.0	0.5 ± 0.2	0.13 ± 0.08
Fc-LPEI (n=6)	Perchlorate	7	355 ± 45	24.4 ± 10.0	10.3 ± 1.3

J_{Max} is the maximum current obtained experimentally at saturating glucose concentrations. K_m was determined graphically from a Lineweaver-Burke plot. Sensitivity was determined from the experimental current response at 5 mM glucose concentration. Values are expressed as Mean ± standard error of the mean

CONCLUSIONS

A key factor affecting electron transport through redox polymer films is the segmental mobility of the polymer backbone. Increased segmental mobility results in a greater number of collisions between redox centers and hence a higher rate of electron transport through these materials. Traditionally, researchers have investigated the effects of segmental mobility on electron transport by varying temperature[57,58], the degree of crosslinking[23,59,60], the length of the redox center spacer arm[21], or the polymer backbone[61,62]. However, very few if any have examined the effect of increasing flexibility or segmental mobility by using a polymer which has a high functional density along its backbone. As mentioned in the Introduction, the most commonly used

polymers are PVP, PVI, and PAA; all of which are polyamines whose nitrogen atoms are connected to one another by a hydrocarbon backbone. PEI on the other hand has a polyamine backbone and this feature lends itself to the many interesting characteristics of this polymer; one of which is its high degree of flexibility as noted by the extremely low Tg. The observation that electron diffusion through these films is in some cases approximately 40-fold higher then those reported for ferrocene based polyallylamine redox polymers supports the initial hypothesis that the increased flexibility of PEI would lead to higher electron transport. To my knowledge this is the first report of electron diffusion measurements for ferrocene modified Poly(ethylenimine) redox polymers.

Systematic investigation of the effects of pH, electrolyte species, and crosslinking on both Fc-LPEI and Fc-BPEI revealed multiple redox wave behavior at high pH and/or in the presence of Dibasic Phosphate, HPO_4^{-2}. Although other groups have constructed films of crosslinked Fc-BPEI, to my knowledge there have been no reports on this multiple redox wave behavior or on the synthesis of Fc-LPEI redox polymers. Knowledge of this sensitivity to phosphate and the related instability is significant because it could potentially pose problems in using ferrocene based PEI redox polymers for biological applications since dibasic phosphate exists under physiological conditions. However, this sensitivity to phosphate could also be used as an advantage in developing anionic sensors to phosphate particularly in organic solvents. This data is also important because it suggests that may be a trade-off between the flexibility gained via a high functional density backbone and the susceptibility of the backbone conformation to electrolyte/pH effects.

Finally I demonstrated that LPEI-Fc redox polymers could be used as molecular wires to connect the redox centers of an enzyme (GOX) to an electrode surface. The current densities at saturating glucose concentrations (~ 480 $\mu A/cm^2$) were significantly higher then those reported for other Ferrocene redox polymers with polyallylamine (100 $\mu A/cm^2$)[18], polyacrylamide (13.5 $\mu A/cm^2$)[63], or polysiloxane (275 $\mu A/cm^2$)[64] backbones. In addition, the LPEI-Fc redox polymer was as good or better in communicating with the buried FAD redox centers of GOX then the commonly used PVP-Os redox polymer under a variety of conditions. In particular the LPEI-Fc redox polymer was much more effective at low pH. This is particularly encouraging because at pH 5, the LPEI-Fc redox polymer is extremely stable and suggests that it may be useful in the wiring of other redox enzymes with peak enzymatic activities at pH 5 or less. These PEI based redox polymers may be useful for in vivo biosensing and biofuel cell applications. PEI is routinely used in gene delivery[65-68] and also in the construction of biocompatible coatings[69,70], and drug delivery[71-73].

Acknowledgements: This work was supported in part by a NSF Career Award to DWS (BES-0547619). I would also like to thank Youdan Wang for help in the glucose calibration studies, and Dr. Adam Heller for valuable discussions concerning this work.

REFERENCES

(1) Andrieux, C. P.; Haas, O.; Saveant, J. M. *Journal of the American Chemical Society* **1986**, *108*, 8175.

(2) Barton, S. C.; Kim, H. H.; Binyamin, G.; Zhang, Y. C.; Heller, A. *Journal of Physical Chemistry B* **2001**, *105*, 11917.

(3) Barton, S. C.; Kim, H. H.; Binyamin, G.; Zhang, Y. C.; Heller, A. *Journal of the American Chemical Society* **2001**, *123*, 5802.

(4) Willner, I.; Eichen, Y.; Frank, a. J.; Fox, M. a. *Journal of Physical Chemistry* **1993**, *97*, 7264.

(5) Gregg, B. A.; Heller, A. *Anal Chem* **1990**, *62*, 258.

(6) Sirkar, K.; Pishko, M. V. *Analytical Chemistry* **1998**, *70*, 2888.

(7) Calvo, E. J.; Wolosiuk, A. *Chemphyschem* **2005**, *6*, 43.

(8) Ishikawa, M.; Schmidtke, D. W.; Raskin, P.; Quinn, C. A. P. *Journal of Diabetes and Its Complications* **1998**, *12*, 295.

(9) Pernaut, J. M.; Reynolds, J. R. *Journal of Physical Chemistry B* **2000**, *104*, 4080.

(10) Low, L. M.; Seetharaman, S.; He, K. Q.; Madou, M. J. *Sensors and Actuators B-Chemical* **2000**, *67*, 149.

(11) Novak, P.; Muller, K.; Santhanam, K. S. V.; Haas, O. *Chemical Reviews* **1997**, *97*, 207.

(12) Heller, a. *Physical Chemistry Chemical Physics* **2004**, *6*, 209.

(13) Chen, T.; Barton, S. C.; Binyamin, G.; Gao, Z. Q.; Zhang, Y. C.; Kim, H. H.; Heller, A. *Journal of the American Chemical Society* **2001**, *123*, 8630.

(14) Guschin, D. a.; Sultanov, Y. M.; Sharif-Zade, N. F.; Aliyev, E. H.; Efendiev, A. A.; Schuhmann, W. *Electrochimica Acta* **2006**, *51*, 5137.

(15) Ohara, T. J.; Rajagopalan, R.; Heller, A. *Anal Chem* **1993**, *65*, 3512.

(16) Ju, H. X.; Leech, D. *Analytica Chimica Acta* **1997**, *345*, 51.

(17) Mikeladze, E.; Schulte, A.; Mosbach, M.; Blochl, A.; Csoregi, E.; Solomonia, R.; Schumann, W. *Electroanalysis* **2002**, *14*, 393.

(18) Calvo, E. J.; Etchenique, R.; Danilowicz, C.; Diaz, L. *Analytical Chemistry* **1996**, *68*, 4186.

(19) Koide, S.; Yokoyama, K. *Journal of Electroanalytical Chemistry* **1999**, *468*, 193.

(20) Aoki, a.; Rajagopalan, R.; Heller, a. *Journal of Physical Chemistry* **1995**, *99*, 5102.

(21) Mao, F.; Mano, N.; Heller, A. *J Am Chem Soc* **2003**, *125*, 4951.

(22) Rajagopalan, R.; Aoki, A.; Heller, A. *Journal of Physical Chemistry* **1996**, *100*, 3719.

(23) Aoki, a.; Heller, a. *Journal of Physical Chemistry* **1993**, *97*, 11014.

(24) Kobayashi, S.; Hiroishi, K.; Tokunoh, M.; Saegusa, T. *Macromolecules* **1987**, *20*, 1496.

(25) Bloys van Treslong, C. J.; Staverman, A. J. *Recueil, Journal of the Royal Netherlands Chemical Society* **1974**, *93*, 171.

(26) Weyts, K. F.; Goethals, E. J. *Makromolekulare Chemie-Rapid Communications* **1989**, *10*, 299.

(27) Smits, R. G.; Koper, G. J. M.; Mandel, M. *Journal of Physical Chemistry* **1993**, *97*, 5745.

(28) Brandup, J.; Immergut, E. *Polymer Handbook*, 3rd ed.; John Wiley and Sons, 1989.

(29) Li, X.; Goh, S. H.; Lai, Y. H.; Wee, A. T. S. *Polymer* **2001**, *42*, 5463.

(30) Andersson, M. A.; Hatti-Kaul, R. *Journal of Biotechnology* **1999**, *72*, 21.

(31) Mcmahon, C. P.; Rocchitta, G.; Serra, P. A.; Kirwan, S. M.; Lowry, J. P.; O'Neill, R. D. *Analyst* **2006**, *131*, 68.

(32) Mcmahon, C. P.; Rocchitta, G.; Kirwan, S. M.; Killoran, S. J.; Serra, P. A.; Lowry, J. P.; O'Neill, R. D. *Biosensors & Bioelectronics* **2007**, *22*, 1466.

(33) Gorton, L.; Jonssonpettersson, G.; Csoregi, E.; Johansson, K.; Dominguez, E.; Markovarga, G. *Analyst* **1992**, *117*, 1235.

(34) Jezkova, J.; Iwuoha, E. I.; Smyth, M. R.; Vytras, K. *Electroanalysis* **1997**, *9*, 978.

(35) Wang, J.; Liu, J.; Chen, L.; Lu, F. *Analytical Chemistry* **1994**, *66*, 3600.

(36) Liu, A. H.; Kashiwagi, Y.; Anzai, J. *Electroanalysis* **2003**, *15*, 1139.

(37) Huan, Z. W.; Persson, B.; Gorton, L.; Sahni, S.; Skotheim, T.; Bartlett, P. *Electroanalysis* **1996**, *8*, 575.

(38) Chuang, C. L.; Wang, Y. J.; Lan, H. L. *Analytica Chimica Acta* **1997**, *353*, 37.

(39) Chatani, Y.; Tadokoro, H.; Saegusa, T.; Ikeda, H. *Macromolecules* **1981**, *14*, 315.

(40) Chang, D. R.; Harden, S.; Loverro, N. *Journal of Macromolecular Science, Chemistry* **1986**, *A23*, 801.

(41) Kokufuta, E.; Suzuki, H.; Yoshida, R.; Yamada, K.; Hirata, M.; Kaneko, F. *Langmuir* **1998**, *14*, 788.

(42) Kokufuta, E.; Suzuki, H.; Yoshida, R.; Kaneko, F.; Yamada, K.; Hirata, M. *Colloids and Surfaces a-Physicochemical and Engineering Aspects* **1999**, *147*, 179.

(43) York, S.; Frech, R.; Snow, A.; Glatzhofer, D. *Electrochimica Acta* **2001**, *46*, 1533.

(44) Kokufuta, E.; Wang, B. L.; Yoshida, R.; Khokhlov, A. R.; Hirata, M. *Macromolecules* **1998**, *31*, 6878.

(45) Prins, R.; Korswage.Ar; Kortbeek, a. G. *Journal of Organometallic Chemistry* **1972**, *39*, 335.

(46) Holecek, J.; Handlir, K.; Klikorka, J.; Dinhbang, N. *Collection of Czechoslovak Chemical Communications* **1979**, *44*, 1379.

(47) Birnbaum, E. R.; Rau, K. C.; Sauer, N. N. *Separation Science and Technology* **2003**, *38*, 389.

(48) Okazaki, Y.; Ishizuki, K.; Kawauchi, S.; Satoh, M.; Komiyama, J. *Macromolecules* **1996**, *29*, 8391.

(49) Nakamae, K.; Miyata, T.; Hoffman, a. S. *Makromolekulare Chemie-Macromolecular Chemistry and Physics* **1992**, *193*, 983.

(50) Rajagopalan, R.; Heller, A. *Electrical `Wiring` of Glucose Oxidase in Electron Conducting Hydrogels*; Blackwell Science Publishers: Cambridge, MA, 1997.

(51) Battaglini, F.; Calvo, E. J.; Danilowicz, C.; Wolosiuk, A. *Analytical Chemistry* **1999**, *71*, 1062.

(52) Gregg, B. a.; Heller, a. *Journal of Physical Chemistry* **1991**, *95*, 5970.
(53) Azzaroni, O.; Moya, S.; Farhan, T.; Brown, A. A.; Huck, W. T. S. *Macromolecules* **2005**, *38*, 10192.
(54) Pishko, M. V.; Michael, A. C.; Heller, A. *Anal Chem* **1991**, *63*, 2268.
(55) Joshi, P. P.; Merchant, S. A.; Wang, Y.; Schmidtke, D. W. *Anal Chem* **2005**, *77*, 3183.
(56) Wang, Y.; Joshi, P. P.; Hobbs, K. L.; Johnson, M. B.; Schmidtke, D. W. *Langmuir* **2006**, *22*, 9776.
(57) Lyons, M. E. G.; Fay, H. G.; Vos, J. G.; Kelly, a. J. *Journal of Electroanalytical Chemistry* **1988**, *250*, 207.
(58) Forster, R. J.; Kelly, a. J.; Vos, J. G.; Lyons, M. E. G. *Journal of Electroanalytical Chemistry* **1989**, *270*, 365.
(59) Oh, S. M.; Faulkner, L. R. *Journal of the American Chemical Society* **1989**, *111*, 5613.
(60) Forster, R. J.; Walsh, D. A.; Mano, N.; Mao, F.; Heller, A. *Langmuir* **2004**, *20*, 862.
(61) Doherty, a. P.; Stanley, M. a.; Arana, G.; Koning, C. E.; Brinkhuis, R. H. G.; Vos, J. G. *Electroanalysis* **1995**, *7*, 333.
(62) Leech, D.; Forster, R. J.; Smyth, M. R.; Vos, J. G. *Journal of Materials Chemistry* **1991**, *1*, 629.
(63) Bu, H. Z.; Mikkelsen, S. R.; English, a. M. *Analytical Chemistry* **1995**, *67*, 4071.
(64) Hale, P. D.; Boguslavsky, L. I.; Inagaki, T.; Karan, H. I.; Lee, H. S.; Skotheim, T. a.; Okamoto, Y. *Analytical Chemistry* **1991**, *63*, 677.
(65) Boussif, O.; Lezoualch, F.; Zanta, M. a.; Mergny, M. D.; Scherman, D.; Demeneix, B.; Behr, J. P. *Proceedings of the National Academy of Sciences of the United States of America* **1995**, *92*, 7297.
(66) Kichler, a.; Leborgne, C.; Coeytaux, E.; Danos, O. *Journal of Gene Medicine* **2001**, *3*, 135.
(67) Thomas, M.; Klibanov, A. M. *Proceedings of the National Academy of Sciences of the United States of America* **2002**, *99*, 14640.
(68) Godbey, W. T.; Wu, K. K.; Mikos, A. G. *Journal of Controlled Release* **1999**, *60*, 149.
(69) He, W.; Bellamkonda, R. V. *Biomaterials* **2005**, *26*, 2983.
(70) Ai, H.; Meng, H. D.; Ichinose, I.; Jones, S. A.; Mills, D. K.; Lvov, Y. M.; Qiao, X. X. *Journal of Neuroscience Methods* **2003**, *128*, 1.
(71) Tiyaboonchai, W.; Woiszwillo, J.; Sims, R. C.; Middaugh, C. R. *International Journal of Pharmaceutics* **2003**, *255*, 139.
(72) Tiyaboonchai, W.; Woiszwillo, J.; Middaugh, C. R. *Journal of Pharmaceutical Sciences* **2001**, *90*, 902.
(73) Backer, M. V.; Aloise, R.; Przekop, K.; Stoletov, K.; Backer, J. M. *Bioconjugate Chemistry* **2002**, *13*, 462.

CHAPTER 4:

High Current Density Amperometric Biosensor Based on a Ferrocene Modified Linear Poly(ethylenimine) Redox Polymer Wire

INTRODUCTION

The "molecular wiring" of the redox centers of enzymes to electrode surfaces via redox polymers has attracted considerable attention due to the high current densities (0.1 – 1.0 mA/cm^2) obtained and their nonleachable nature. These high current densities are particularly attractive because they allow for miniaturization of biosensors designed for the in vivo monitoring of glucose in diabetes[1-3], glutamate and ascorbate in brain tissue[4,5], and peroxides in neurodegenerative disorders[6]. High current densities have also been exploited in the detection of hybridization reactions in RNA and DNA assays[7-9], and antigen-antibody binding in immunoassays[10-12]. Finally these increased current densities have been exploited in developing miniaturized biofuel cells[13-16].

The flow of electrons between the analyte and the electrode surface in redox polymer-enzyme based systems is a multi-step process (Figure 4.01). Depending upon

the sensor design, several of these transduction steps may limit the overall reaction rate and consequently the maximal current density.

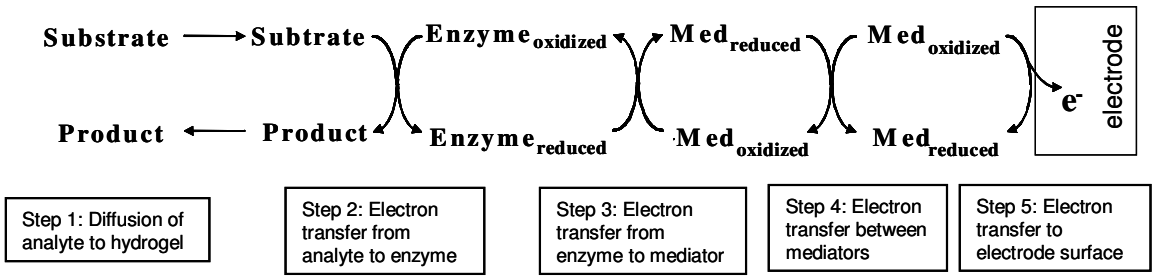


Figure 4.01: Amperometric biosensor signal transduction schematic.

Previous studies[17-19] have suggested that normally either step (3) or (4) is rate determining. The rate at which electrons are transferred between the enzyme and the mediator is dependent on the distance between them and whether an electrostatic complex forms between the redox polymer and enzyme[20,21]. The rate at which electrons are transported through the film (i.e. self-exchange between identical redox centers) is dependent on both charge propagation along the polymer's backbone (through σ and other chemical bonds) and collisions between polymer segments. Increasing the charge density on the polymer backbone results in (a) making the polymer films more hydrophilic and allowing for enhanced permeation of substrates and products; (b) increases the strength of the electrostatic complex formed with the enzyme; and (c) increases the rate of electron transport through the films; all of which result in increased current density[17,19]. In addition, the rate of electron transfer between the enzyme and the mediator is significantly enhanced when (a) the polymer backbone is flexible[21]; (b) the

spacer arm coupling the redox center to the polymer backbone are flexible[22]; and (c) the length of the spacer arm is increased[23,24].

In chapter 3, I reported the synthesis and characterization of a novel redox polymer based on attaching methylferrocene (MeFc) redox centers to a LPEI backbone[25]. These initial studies with crosslinked films of the redox polymer alone demonstrated that electron diffusion through these MeFc-LPEI based films was approximately 40-fold higher then those reported for ferrocene based polyallylamine redox polymers. I also demonstrated that MeFc-LPEI redox polymers could be used as molecular wires to connect the redox centers of an enzyme (GOX) to an electrode surface. The current densities at saturating glucose concentrations (~ 480 $\mu A/cm^2$) were significantly higher then those reported (13.5 - 275 $\mu A/cm^2$) for other ferrocene based redox polymers[26-28], and compare favorably with osmium based redox polymers[29,30]. Initially these high current densities were attributed to the high rate of electron transfer in these films. However electron diffusion coefficients for crosslinked redox polymer films that contained enzyme were not measured.

In this chapter, I investigate the relationship between electron diffusion through crosslinked MeFc-LPEI films containing enzyme and the sensor response. I demonstrate that a slight modification of our synthesis procedure of MeFc-LPEI results in a dramatic increase in the current density for glucose oxidase based sensors (i_{max} = 1.2 mA/cm^2). To the best of my knowledge, this value is the highest current density achieved for "wired" glucose oxidase based sensors on 3mm diameter electrodes. Heller's groups have achieved current densities on the same order of magnitude using either pyrroloquinoline quinone (PQQ) modified glucose dehydrogenase, or ultra micro electrodes where radial

diffusion at the electrode surface is possible[22,31]. In addition, Binyamin et al were able to achieve similar current densities by incorporating graphite particles into the hydrogel matrix, and Schmidtke et al showed that current densities at saturation of ~ 1mA could be achieved by incorporating single wall carbon nanotubes into the hydrogel[32,33]. In all of the studies mentioned above an osmium modified redox polymer was used to "wire" the enzyme. I also demonstrate that these high current densities are not entirely dependent on increases in electron diffusion coefficients, but that other interactions, such as the interaction between PEI and the enzyme, maybe more important. Finally, I demonstrate the versatility of MeFc-LPEI as a "wire" for enzyme electrodes by crosslinking the polymer with horseradish peroxidase. These hydrogen peroxide sensors also exhibit among the highest current densities for HRP electrodes.

EXPERIMENTAL

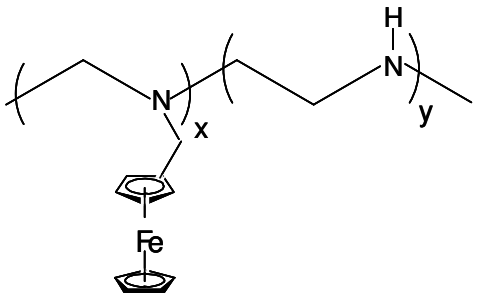
Materials and Instruments

The materials and instrumentation used in for the work in this chapter is the same as that indicated in the previous chapter/s. While the synthesis of the redox polymer used here is also the essentially the same, a slight modification to the process by which the polymer is isolated from the by-products allows for a cleaner product. A description of the synthesis is repeated below with the additional steps of purification.

Polymer Synthesis

Linear poly(ethylenimine) (LPEI) (avg. MW ca. 86,000) was obtained by acidic hydrolysis of poly(2-ethyl-2-oxazoline) (avg. MW 200,000), followed by neutralization

with sodium hydroxide[34]. In a round-bottom flask, 0.252 g of LPEI was dissolved in 10 mL methanol and a solution of 0.187g (0.87mmol) ferrocenecarboxaldehyde dissolved in 3 mL methanol was added to it dropwise under constant agitation. The resulting dark red solution was stirred for 2 h and cooled in an ice bath. Sodium borohydride (0.033g, 0.87mmol) was added, upon which the solution lightened in color. After 1 h, the methanol was removed under vacuum and the residue was extracted overnight with diethyl ether to remove any non-reacted aldehyde and ferrocenylmethanol. The ether was then decanted and the residue was washed with diethyl ether before being dried under vacuum. The residue was dissolved in methanol and the solution was vacuum filtered. This process was repeated three times and replaces the benzene extraction step that we previously reported[25]. This method results in a clearer product and a polymer which exhibits higher current densities. Finally the solvent was removed from the filtrate under vacuum to give ca. 0.170 g (40%) MeFc-LPEI. ^{1}H-NMR (300 Mhz, d_6-benzene): δ3.9-4.3 (br, Fc ring H), 3.7-3.3 (br, FcCH_2-, NH), 2.5-2.9 (br, -CH_2N-). The degree of substitution of the amine hydrogens by ferrocenylmethylene moieties was estimated from the integration ratio of the ferrocenyl proton to polymer methylene backbone signals in the ^{1}H-NMR spectra of each resulting polymer and found to be ca. 15% for the MeFc-LPEI. Differential scanning calorimetry showed the MeFc-LPEI to have a glass transition temperature of ca. –14 °C. The proposed structure for MeFc-LPEI is shown in Figure 4.02.



Ferrocene modified
Linear Poly(ethylenimine)

Figure 4.02: Proposed structure of ferrocene modified linear poly(ethylenimine) [MeFc-LPEI].

Glucose Sensor Preparation and Testing

The MeFc-LPEI was dissolved in aqueous/HCl solution at pH 3, and the solution was subsequently neutralized with a NaOH solution until the final concentration of the polymer solution was 10 mg/ml. Glucose sensors were prepared by crosslinking glucose oxidase to MeFc-LPEI to form enzymatic redox hydrogels: 14 μl of polymer solution (10 mg/ml), 6 μl of glucose oxidase solution (10 mg/ml), and 0.75 μl of EGDGE solution (10% $^{v}/_{v}$) were mixed together and 3 μl aliquots were placed onto the glassy carbon electrode surface. The mixture was allowed to dry for 18-24 hours. Cyclic voltammetry was performed on the sensors to determine their electrochemical behavior. The response of each sensor to glucose was determined using amperometric analysis, where the working potential of the sensors was held constant at 400 mV in either 0.1 M NaH_2PO_4, or a mixture of 0.05 M NaH_2PO_4 + 0.05 M NaCl background electrolyte. Aliquots of a 2 M glucose solution were added to the stirring electrochemical medium and the resulting current was monitored as a function of time to determine the steady-state current at each glucose concentration.

RESULTS AND DISSCUSSION

0.1 M Phosphate Solution

I performed cyclic voltammetry on electrodes coated with crosslinked MeFc-LPEI/GOx films in a 0.1 M phosphate solution at pH 5 and pH 7. As shown in Figure 4.03 the results are varied. At pH 5 (Figure 4.03A-Heavy) the film exhibits a single anodic peak at ~355 mV, while the film tested at pH 7 (Figure 4.03B-Heavy) exhibits multiple anodic waves at ~340 mV and ~550 mV, respectively.

I have previously reported this multi-wave behavior for crosslinked films of MeFc-LPEI alone (no GOx) tested in solutions containing phosphate with a pH ≥ 7 where monobasic phosphate ($H_2PO_4^{-1}$) begins to form dibasic phosphate (HPO_4^{-2}). This species of inorganic phosphate has been shown to cause deswelling or collapse of polymer gels, in this case, resulting in morphological confinement of ferrocene molecules attached to the LPEI backbone[35,36]. This produces two apparent populations of ferrocenes, and hence, two anodic peaks as the more confined ferrocene molecules require more energy to oxidize.

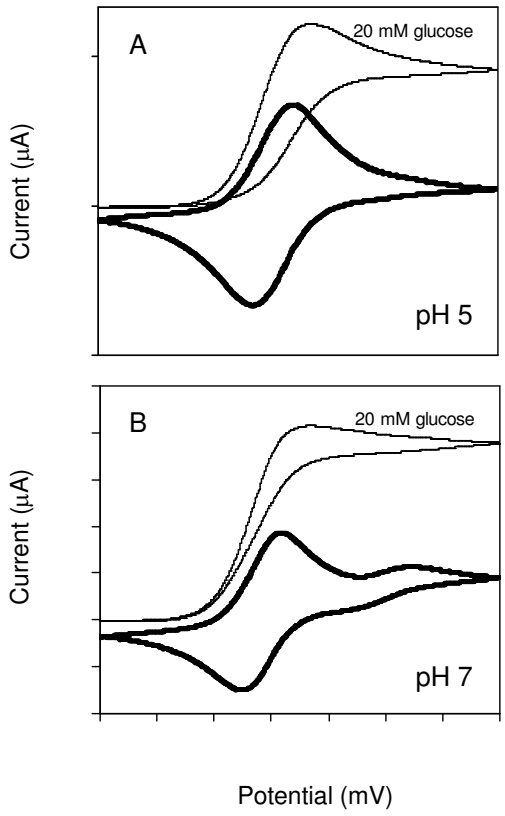


Figure 4.03: Cyclic voltammograms of MeFc-LPEI/Glucose Oxidase enzymatic redox hydrogels with no glucose **(heavy)** and with 20 mM glucose added (light) in 0.1 M phosphate at (A) pH 5 and (B) pH 7. ν=5 mV/sec

The difference in the electrochemistry of these films extends to the catalytic electro-oxidation of glucose when 20 mM glucose is added to each solution. This process is described by the following set of reactions:

$$GOx(FAD) + Glucose \rightarrow GOx(FADH_2) + gluconolactone \quad (1)$$

$$GOx(FADH_2) + 2\ Fc^{+1} \rightarrow GOx(FAD) + 2\ Fc + 2\ H^+ \quad (2)$$

Self exchange of e^- **:** $Fc + Fc^+ \rightarrow Fc^+ + Fc$ (3)

At the electrode surface **:** $Fc \rightarrow Fc^+ + e^-$ (4)

In 0.1 M phosphate at pH 5 (Figure 4.03A-Light) the redox active ferrocene sites on the polymer exhibit significant communication with the enzymatic reaction as indicated by:

(i) minimal electrode driven reduction as electrons are supplied to the ferricinium (Fc^{+3}) by the reduced form of the enzyme [$GOx(FADH_2)$], and (ii) the elevated and relatively constant anodic current as a result of the constant regeneration of ferrocene (Fc^{+2}) by $GOx(FADH_2)$. In other words, as the electrode oxidizes ferrocene to ferricinium, the ferricinium are immediately reduced back to ferrocene by the enzyme since they are in good communication with each other, as opposed to being reduced by the electrode itself. Despite the apparent confinement of ferrocene molecules in 0.1 M phosphate at pH 7 (Figure 4.03B-Light) there is increased anodic behavior and even less electrode driven reduction than at pH 5.

Amperometric glucose response curves for these sensors in 0.1 M phosphate at pH 5 and 7 are shown in Figure 4.04. The maximum current density at enzyme saturation, i_{max} (taken at 100 mM glucose) at pH 5 is 440 ± 50 μA/cm^2 and the sensitivity taken at 5 mM glucose (SEN) is 50 ± 5 μA/cm^2·mM.

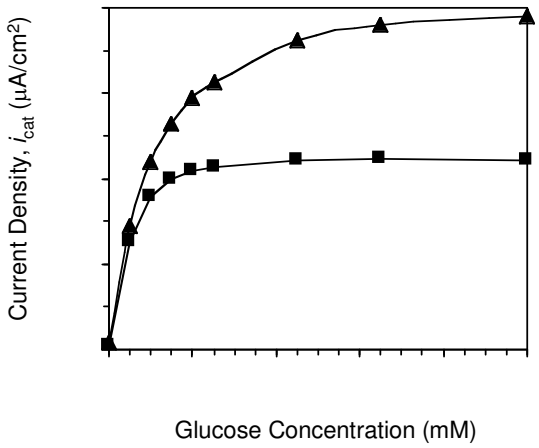


Figure 4.04: Glucose response curves for glassy carbon electrodes coated with MeFc-LPEI/Glucose Oxidase enzymatic redox hydrogels in 0.1 M phosphate at (■) pH 5 and (▲) pH 7. E=0.4 V

As suggested by the cyclic voltammograms in Figure 4.03 the sensor response at pH 7 is enhanced with an i_{max} of 780 ± 80 μA/cm^2 and a SEN of 55 ± 3 μA/cm^2·mM.

Lineweaver-Burk analysis of the experimental data at pH 5 and 7 produces Michaelis-Menten constants, K_m, of 5.5 ± 0.5 mM and 10.0 ± 1.8 mM respectively, and J_{max} values of 540 ± μA/cm^2 and 870 ± 120 μA/cm^2 respectively.

0.05 M Phosphate + 0.05 M Chloride Buffer Solution

When phosphate and chloride were combined in equimolar concentrations such that an ionic strength of 0.1 M was maintained, we observed a synergistic effect. The voltammograms in Figure 4.05 suggest increased electrical communication between the redox polymer and the enzyme at pH 7 as noted above; however, there appears to be more electrode driven reduction than with phosphate alone at either pH value. Despite this, as shown in Figure 4.06, the amperometric glucose responses for the sensors in the phosphate/chloride mixture exhibit higher maximum current densities than the sensors in phosphate at either pH. The i_{max} at pH 5 and 7 is 660 ± 35 μA/cm^2 and 1200 ± 70 μA/cm^2 respectively, while the SEN is 58 ± 3 μA/cm^2·mM and 74 ± 3 μA/cm^2·mM respectively. The K_m's for the sensors under these conditions are 7.0 ± 0.4 mM and 12.0 ± 0.6 mM respectively. The response of the MeFc-LPEI/GOx based glucose sensors reported here is exceptional, particularly in the phosphate/chloride buffer solution. To the best of our knowledge, the i_{max} of 1200 μA/cm^2 at pH 7 is the highest current density achieved for "wired" glucose oxidase based sensors on 3mm diameter electrodes.

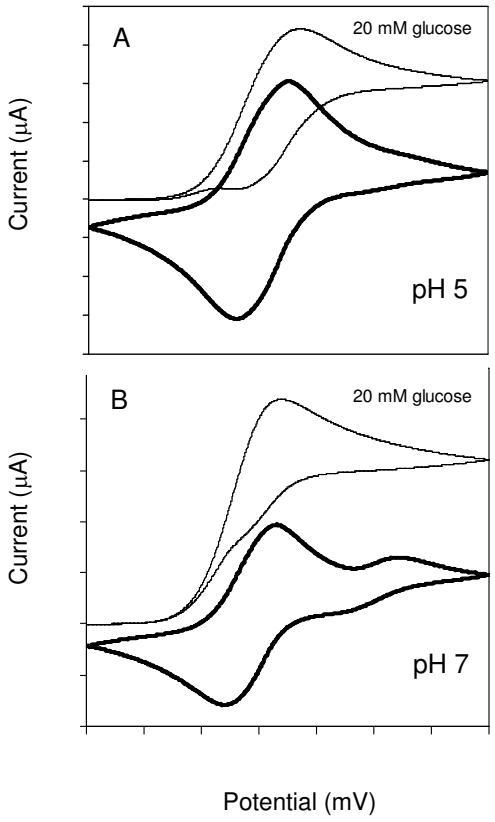


Figure 4.05: Cyclic voltammograms of MeFc-LPEI/Glucose Oxidase enzymatic redox hydrogels with no glucose (**heavy**) and with 20 mM glucose added (light) in 0.05 M phosphate + 0.05 M chloride at (A) pH 5, (B) pH 7; ν=5 mV/sec

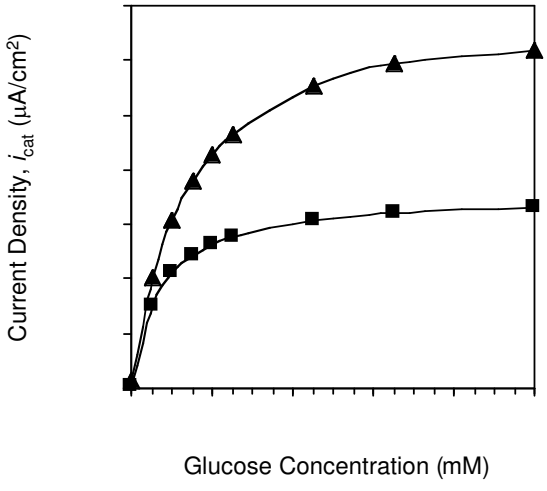


Figure 4.06: Glucose response curves for glassy carbon electrodes coated with MeFc-LPEI/Glucose Oxidase enzymatic redox hydrogels in 0.05 M phosphate + 0.05 M chloride at (■) pH 5 and (▲) pH 7. E=0.4 V

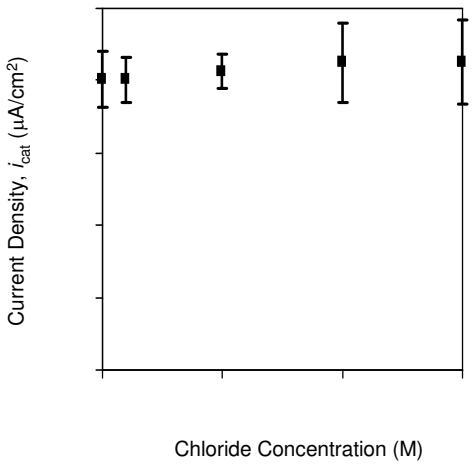


Figure 4.07: Maximum glucose sensor at enzyme saturation in phosphate/chloride buffered solutions at pH 7. The phosphate concentration was held constant at 50 mM while a range of chloride concentrations were used ranging from 0 to 150 mM.

These MeFc-LPEI based glucose sensors are also able to produce high current densities over a range of chloride concentrations in a phosphate buffered solution, as shown in Figure 4.07. Typical results in the literature for glucose sensors based on ferrocene modified polymers including polyallylamine, polyacrylamide, polysiloxane, and branched poly(ethylenimine)[26-28,37] range from ~ 10 to ~300 $\mu A/cm^2$. Along with the high current density at pH 7, the response of the sensor at pH 5 is also encouraging. To the best of my knowledge these are also the highest current densities achieved at pH 5.

These results appear to suggest that MeFc-LPEI allows for efficient electron transfer from the enzyme to the redox sites on the polymer, and from one ferrocene to another. However, only minimal information can be obtained from these experiments with respect to the changes in the physical nature of the films or charge transport. Thus, I employed two additional electroanalytical techniques to provide further insight into the physical changes these films may experience: (i) electrochemical impedance spectroscopy (EIS) to estimate changes in the apparent electron diffusion coefficient

through the films, and (ii) rotating disk electrode (RDE) voltammetry to estimate changes in the permeability of the films.

Apparent Electron Diffusion Coefficient

Apparent electron diffusion coefficients, $D_e c^2$, for the films under the different solution conditions were estimated using electrochemical impedance spectroscopy (EIS) at a DC potential of E=0.35 V and an AC perturbation of 10 mV. In the low-frequency range the impedance response was analyzed using the Randles circuit by plotting the imaginary impedance, Im(Z), versus the inverse square root of frequency, $\omega^{-1/2}$, with a slope equal to the Warburg coefficient, σ_w:

$$\mathrm{Im}(Z) = \sigma_w \, \omega^{-1/2} \qquad (4)$$

The value $D_e c^2$ was determined directly from σ_w:

$$\sigma_w = RT/n^2F^2cD_e^{1/2} \qquad (5)$$

The linearity of the data confirms semi-infinite diffusion behavior of electron transfer through the redox hydrogels[26,38]. Within the apparent electron diffusion coefficients provided in Table 4.01 are three major distinctions to be discussed.

First, the decrease in $D_e c^2$ observed from pH 5 to pH 7 in both buffer systems is a commonly encountered trend in redox active polyamines in the presence of phosphate[19,39]. This, along with the multi-wave redox behavior, appears to support my suggestion that the dibasic phosphate species results in the morphological confinement of some of the ferrocene molecules thereby limiting the rate of electron transport through the films.

	0.1 M phosphate pH 5	0.1 M phosphate pH 7	0.05 M chloride + 0.05 M phosphate pH 5	0.05 M chloride + 0.05 M phosphate pH 7
D_ec^2 x 10^{18} (mol^2/cm^4s)	3.9 ± 0.4	1.4 ± 0.3	23.6 ± 3.2	1.9 ± 0.4
κD/d x 10^2 (cm/s)	1.3 ± 0.3	1.1 ± 0.4	0.7 ± 0.2	0.7 ± 04
Max. Sensor Response ($\mu A/cm^2$)	440 ± 50	780 ± 80	660 ± 35	1200 ± 70

Table 4.01: Summary of data obtained for measurements of apparent electron diffusion coefficient, D_ec^2, solute permeability through the hydrogel, κD/d, and the sensor response at saturation, i_{max}, in each solution condition.

Second, the D_ec^2 is an order of magnitude greater in the phosphate/chloride mixture at pH 5 which suggests that the incorporation of hydrated chloride ions into the hydrogel increases the segmental mobility of the ferrocenes on the polymer backbone. Kobayashi et al have proposed that the association of hydrated chloride ions with the positively charged nitrogen atoms on the LPEI backbone may result the stretching of the polymer chains[40]. Alternatively, an increase in segmental mobility may be explained by an osmotic effect: the incorporation of chloride ions into the hydrogel may result in an uptake of water. The elevated hydration of the gel should increase segmental mobility, and hence D_ec^2.

Finally, there is not a significant increase in D_ec^2 in the phosphate/chloride solution compared to the phosphate-only solution at pH 7, despite the large increase observed with the addition of chloride ions at pH 5. I propose that the morphological confinement that may be caused by dibasic phosphate limits the rate of electron transport through the hydrogel despite the possible effects of the addition of chloride ions to the system. I also believe that the relatively low D_ec^2 at pH 7, in conjunction with the high response to glucose, is another indication that polymer interaction with the enzyme

makes significant contributions to the behavior and response of the sensors. In other words, the response of the sensors is not only related to the rate of electron transport through the films, but also the rate at which electrons are exchanged between the enzyme and polymer.

Permeability of the Enzymatic Redox Hydrogels

Rotating disk electrodes (RDEs) were used to estimate the permeability of the enzymatic redox hydrogels to a neutral solute in the various solution conditions by measuring the cathodic current produced by the reduction of the model compound *p*-benzoquinone over a range of rotation rates. Figure 4.08 shows a typical rotated disk voltammogram for the reduction of *p*-benzoquinone at a Fc-LPEI/Gox coated glassy carbon RDE. Since the reduction potential of this analyte lies ~300 mv negative of the

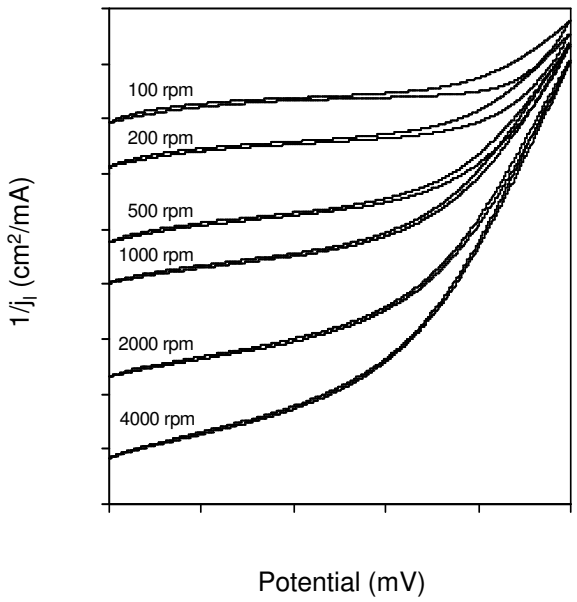


Figure 4.08: Typical reduction of 1.0 mM benzoquinone at a MeFc-LPEI/Glucose Oxidase coated glassy carbon rotating disk electrode at ν= 20 mV/sec over a range of rotation speeds.

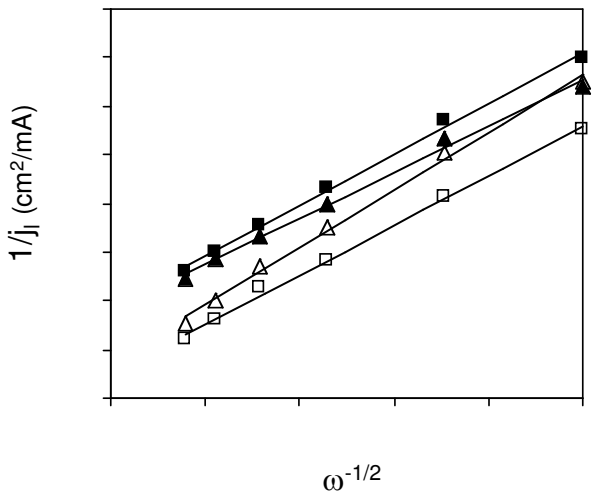


Figure 4.09: Inverse Levich plots of benzoquinone reduction currents at -0.5 V at a MeFc-LPEI/Glucose Oxidase coated glassy carbon rotating disk electrode. (■) 0.05M chloride + 0.05M phosphate pH 5, (▲) 0.05M chloride + 0.05M phosphate pH 7, (□) phosphate pH 5, (△) phosphate pH 7.

ferrocene couple on the redox polymer, electroactivity of the MeFc-LPEI in this potential range was neglected, and all of the cathodic current is considered to be due to the reduction of *p*-benzoquinone at the surface of the electrode. The limiting current density at -500 mv, j_l, is given by the following equation:

$$1/j_l = d/nFC_a^o \kappa D_s + 1/0.62nFC_a^o D^{2/3} \nu^{-1/6} \omega^{1/2} \qquad (6)$$

where the two terms on the right hand side represent the diffusion of the solute through the polymer/enzyme film, and through the Levich depletion layer in solution, respectively[39,41]. As shown in Figure 4.09, inverse Levich plots, j_l^{-1} vs. $\omega^{-1/2}$, exhibit linearity suggesting that diffusion of the *p*-benzoquinone through the film is the rate limiting mass transport process. Thus, permeability, ($\kappa D_s / d$), can be evaluated from the intercept $d/nFC_a^o \kappa D_s$, where d is film thickness, F is Faraday's constant, C_a^o is the bulk analyte concentration, κ is the partition coefficient of the analyte into the film, and

D_s is the diffusion coefficient of analyte through the polymer film. Permeability values for the films in all solution conditions are also given in Table 4.01.

There is no significant change in the permeability of these films as the pH is changed from 5 to 7, which implies that there is no significant degree of hydrogel collapse. This corresponds well with gel swelling studies performed on LPEI hydrogel, and our own gel studies on MeFc-LPEI[42-44]. Hence, I do not expect that the sensor response is controlled by the rate of diffusion of glucose through the hydrogel. These studies, in conjunction with the sensor responses, illustrate the distinction between gel collapse and morphological changes affecting the microenvironment of a portion of the ferrocene molecules. While the presence of dibasic phosphate may result in morphological changes that allow for greater contact with the enzyme and limited segmental mobility, it does not necessarily cause a significant collapse of the film.

Peroxide Sensors based on HRP

In order to determine the versatility of MeFc-LPEI as a molecular "wire" for enzymes I also made peroxide sensors by crosslinking the polymer with horseradish peroxidase (HRP). As shown in Figure 4.10 MeFc-LPEI/HRP sensors show excellent sensitivity to hydrogen peroxide and also exhibit among the highest current densities in comparison to similarly constructed HRP based sensors[45,46]. In addition, the linear range of our sensor is extended to higher concentrations of peroxide. For example, HRP based sensors wired with PVP-Os[46] begin to saturate (plateau in current density output) at ~ 0.3 mM hydrogen peroxide where the maximum current density is ~350 μA/cm^2. HRP

sensors wired with MeFc-LPEI exhibit linearity to a peroxide concentration of 1.5mM where the current density is ~700 μA/cm^2.

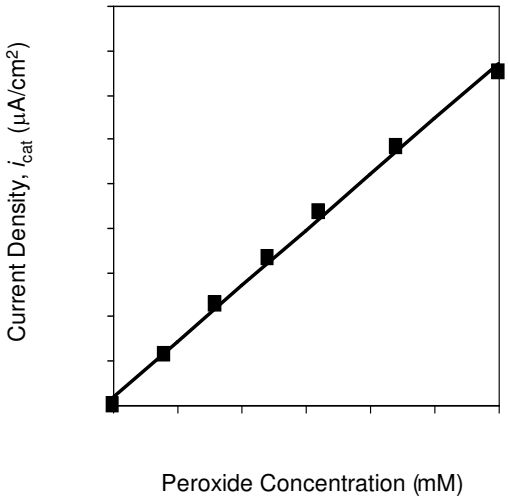


Figure 4.10: Peroxide response curve for glassy carbon electrodes coated with MeFc-LPEI/HRP enzymatic redox hydrogels in 0.05 M phosphate + 0.05 M chloride pH 7. E=0.0 V

Nonetheless, the results with HRP demonstrate that the efficacy of MeFc-LPEI as an electrical mediator in enzymatic redox hydrogels is not exclusive to glucose oxidase indicating that this redox polymer has potential for use in other biosensors and biofuel cells.

CONCLUSIONS

In this chapter I report the development of glucose sensors based on the molecular wiring of GOx with MeFc-LPEI. The exceptionally high current densities exhibited by the sensors suggests that close interactions between the enzyme and LPEI backbone result in efficient electron exchange between the enzyme and the ferrocene redox centers of MeFc-LPEI. These interactions include electrostatic complexation between the

cationic LPEI and the anionic GOx, along with potential hydrogen bonding between the two molecules.

In addition to producing the highest current density glucose oxidase based glucose sensors on 3mm diameter electrodes at pH 7, MeFc-LPEI is also the most effective molecular wire for glucose oxidase at pH 5. I also report apparent electron diffusion coefficients for MeFc-LPEI based sensors that are comparable to other redox polymer systems. These similar rates of self exchange of electrons between ferrocene centers on the polymer further suggests that interactions between the enzyme and LPEI backbone may contribute to the high signal responses of the sensors.

Finally, I demonstrate the versatility of MeFc-LPEI to function as a molecular wire for enzyme electrodes with peroxide sensors based on HRP. These sensors also exhibit the highest current densities of HRP based sensors. The ability of MeFc-LPEI to efficiently communicate with the redox active sites of multiple enzymes has implications for a variety of biosensors, bienzyme based biosensors, and biofuel cells.

Acknowledgements: I would like to thank Dr. Glatzhofer for all of his suggestions and valuable conversations concerning this work. I would also like to thank Brian Howard for his help with peroxide sensor testing.

REFERENCES

(1) Wagner, J. G.; Schmidtke, D. W.; Quinn, C. P.; Fleming, T. F.; Bernacky, B.; Heller, A. *Proceedings of the National Academy of Sciences of the United States of America* **1998**, *95*, 6379.

(2) Schmidtke, D. W.; Heller, A. *Analytical Chemistry* **1998**, *70*, 2149.

(3) Schmidtke, D. W.; Freeland, A. C.; Heller, A.; Bonnecaze, R. T. *Proceedings of the National Academy of Sciences of the United States of America* **1998**, *95*, 294.

(4) Kulagina, N. V.; Shankar, L.; Michael, A. C. *Analytical Chemistry* **1999**, *71*, 5093.

(5) Oldenziel, W. H.; Dijkstra, G.; Cremers, T. I. F. H.; Westerink, B. H. C. *Analytical Chemistry* **2006**, *78*, 3366.

(6) Kulagina, N. V.; Michael, A. C. *Analytical Chemistry* **2003**, *75*, 4875.

(7) de Lumley-Woodyear, T.; Campbell, C. N.; Freeman, E.; Freeman, A.; Georgiou, G.; Heller, A. *Analytical Chemistry* **1999**, *71*, 535.

(8) Campbell, C. N.; Gal, D.; Cristler, N.; Banditrat, C.; Heller, A. *Analytical Chemistry* **2002**, *74*, 158.

(9) Zhang, Y. C.; Pothukuchy, A.; Shin, W.; Kim, Y.; Heller, A. *Analytical Chemistry* **2004**, *76*, 4093.

(10) Lu, B.; Iwuoha, E. I.; Smyth, M. R.; OKennedy, R. *Analytica Chimica Acta* **1997**, *345*, 59.

(11) Lu, B.; Iwuoha, E. I.; Smyth, M. R.; OKennedy, R. *Biosensors & Bioelectronics* **1997**, *12*, 619.

(12) Calvo, E. J.; Danilowicz, C.; Lagier, C. M.; Manrique, J.; Otero, M. *Biosensors & Bioelectronics* **2004**, *19*, 1219.

(13) Tamaki, T.; Ito, T.; Yamaguchi, T. *Journal of Physical Chemistry B* **2007**, *111*, 10312.

(14) Heller, A. *Physical Chemistry Chemical Physics* **2004**, *6*, 209.

(15) Mano, N.; Mao, F.; Shin, W.; Chen, T.; Heller, A. *Chemical Communications* **2003**, 518.

(16) Chen, T.; Barton, S. C.; Binyamin, G.; Gao, Z. Q.; Zhang, Y. C.; Kim, H. H.; Heller, A. *Journal of the American Chemical Society* **2001**, *123*, 8630.

(17) Rajagopalan, R.; Aoki, A.; Heller, A. *Journal of Physical Chemistry* **1996**, *100*, 3719.

(18) Aoki, A.; Rajagopalan, R.; Heller, A. *Journal of Physical Chemistry* **1995**, *99*, 5102.

(19) Aoki, A.; Heller, A. *Journal of Physical Chemistry* **1993**, *97*, 11014.

(20) Katakis, I.; Ye, L.; Heller, A. *Journal of the American Chemical Society* **1994**, *116*, 3617.

(21) Oh, S. M.; Faulkner, L. R. *Journal of Electroanalytical Chemistry* **1989**, *269*, 77.

(22) Mao, F.; Mano, N.; Heller, A. *Journal of the American Chemical Society* **2003**, *125*, 4951.

(23) Mao, F.; Mano, N.; Heller, A. *J Am Chem Soc* **2003**, *125*, 4951.
(24) Schuhmann, W.; Ohara, T. J.; Schmidt, H. L.; Heller, a. *Journal of the American Chemical Society* **1991**, *113*, 1394.
(25) Merchant, S. A. G., D. T.; Schmidtke, D. W. *Langmuir* **2007**, *23*, 11295.
(26) Calvo, E. J.; Etchenique, R.; Danilowicz, C.; Diaz, L. *Analytical Chemistry* **1996**, *68*, 4186.
(27) Bu, H. Z.; Mikkelsen, S. R.; English, A. M. *Analytical Chemistry* **1995**, *67*, 4071.
(28) Hale, P. D.; Boguslavsky, L. I.; Inagaki, T.; Karan, H. I.; Lee, H. S.; Skotheim, T. A.; Okamoto, Y. *Analytical Chemistry* **1991**, *63*, 677.
(29) Kenausis, G.; Taylor, C.; Katakis, I.; Heller, A. *Journal of the Chemical Society-Faraday Transactions* **1996**, *92*, 4131.
(30) Gregg, B. A.; Heller, A. *Journal of Physical Chemistry* **1991**, *95*, 5976.
(31) Ling, Y.; Hammerle, M.; Olsthoorn, A. J. J.; Schuhmann, W.; Schmidt, H. L.; Duine, J. A.; Heller, A. *Analytical Chemistry* **1993**, *65*, 238.
(32) Joshi, P. P.; Merchant, S. A.; Wang, Y. D.; Schmidtke, D. W. *Analytical Chemistry* **2005**, *77*, 3183.
(33) Binyamin, G.; Cole, J.; Heller, A. *Journal of the Electrochemical Society* **2000**, *147*, 2780.
(34) York, S.; Frech, R.; Snow, A.; Glatzhofer, D. *Electrochimica Acta* **2001**, *46*, 1533.
(35) Wang, B. Z.; Anzai, J. *Langmuir* **2007**, *23*, 7378.
(36) Okazaki, Y.; Ishizuki, K.; Kawauchi, S.; Satoh, M.; Komiyama, J. *Macromolecules* **1996**, *29*, 8391.
(37) Chuang, C. L.; Wang, Y. J.; Lan, H. L. *Analytica Chimica Acta* **1997**, *353*, 37.
(38) Battaglini, F.; Calvo, E. J.; Danilowicz, C.; Wolosiuk, A. *Analytical Chemistry* **1999**, *71*, 1062.
(39) Gregg, B. A.; Heller, A. *Journal of Physical Chemistry* **1991**, *95*, 5970.
(40) Kobayashi, S.; Hiroishi, K.; Tokunoh, M.; Saegusa, T. *Macromolecules* **1987**, *20*, 1496.
(41) Ikeda, T.; Schmehl, R.; Denisevich, P.; Willman, K.; Murray, R. W. *Journal of the American Chemical Society* **1982**, *104*, 2683.
(42) Kokufuta, E. *Langmuir* **2005**, *21*, 10004.
(43) Kokufuta, E.; Wang, B. L.; Yoshida, R.; Khokhlov, A. R.; Hirata, M. *Macromolecules* **1998**, *31*, 6878.
(44) Kokufuta, E.; Suzuki, H.; Yoshida, R.; Yamada, K.; Hirata, M.; Kaneko, F. *Langmuir* **1998**, *14*, 788.
(45) Wollenberger, U.; Bogdanovskaya, V.; Bobrin, S.; Scheller, F.; Tarasevich, M. *Analytical Letters* **1990**, *23*, 1795.
(46) Vreeke, M.; Maidan, R.; Heller, A. *Analytical Chemistry* **1992**, *64*, 3084.

CHAPTER 5:

Improvement of Redox Polymer and Biosensor Stability via Extension of Ferrocene Redox Couple Further Away from the Linear Poly(ethylenimine) Backbone

INTRODUCTION

When developing biosensors there are a number of characteristics onto which researchers have focused their efforts in pursuit of optimization. These characteristics include selectivity, sensitivity, and stability, among others. One method of biosensor construction aimed at addressing each of these issues is the "wiring" of enzymes to electrode surfaces with redox polymers to produce amperometric sensors. Heller and other researchers have shown that electrodes coated with theses enzymatic redox hydrogels are capable of producing selective, sensitive, and stable biosensors. While a variety of enzymes have been used to produce glucose[1,2], lactate[3], peroxide[4,5], and many other sensors[6,7], much of the work in this area has focused on the development of redox polymers capable of efficiently mediating the electron exchange between the enzyme and the electrode surface to improve the signal output, or sensitivity of the device[8-11].

Studies have shown that increasing the rate at which the redox polymers self exchange electrons from the reduced form of the redox mediator to the oxidized form

results in sensors which exhibit higher current responses[9,11-13]. This self exchange, or electron diffusion, can be enhanced by increasing the segmental mobility of the redox couples such that the frequency of collision between reduced and oxidized forms may be increased[14]. In addition, research has shown that extension of the redox couple from the polymer backbone[9] allows for increased contact with the redox active cofactors of the enzyme which also provides a means by which enhanced current responses, or sensitivity may be improved.

Recently, I have developed a new redox polymer by attaching methylferrocene to linear poly(ethylenimine) [MeFc-LPEI][15], and has shown that glucose sensors based on "wiring" glucose oxidase with MeFc-LPEI exhibited the highest current densities reported on 3mm diameter electrodes. These elevated responses are potentially due to in part to the electrostatic attraction between the anionic glucose oxidase and the cationic LPEI backbone resulting in a close association between the two molecules[16-18]. In lieu of the close interaction between LPEI and glucose oxidase, locating redox active ferrocene groups in close proximity to the LPEI backbone allows for efficient electron transfer from the enzyme to the redox site on the polymer. In addition, I have been able to achieve sufficiently high rates of electron diffusion through the hydrogels without using flexible tethers to extend ferrocene from the backbone due to the highly flexible LPEI backbone.

Despite the ability of this redox polymer to function within the enzymatic redox hydrogel, I have also shown that crosslinked films of MeFc-LPEI exhibit oxidative degradation, which leads to an unstable electrochemical response. This instability occurs at pH ~11 in non-phosphate containing solutions. Coincidentally, researchers have

shown that at this pH LPEI molecules transition from an extended state due to electrostatically repulsive charges on the backbone to a collapsed polymer conformation containing intermolecular hydrogen bonding[19-22]. Moreover, Kokufuta et al have shown that crosslinked LPEI gels deswell and collapse near this pH as well[23,24]. Based on this I propose that the instability in the electrochemical response of MeFc-LPEI may be due to a morphological confinement of a portion of the ferrocene groups in the collapsed gel matrix.

In addition, I observe a similar instability in solutions containing phosphate at pH ≥ 7 where the dibasic phosphate (HPO_4^{-2}) species is known to exist. This suggests that the dibasic phosphate found in common physiological buffers at pH 7.4 may result in a similar morphological confinement of the ferrocene groups located in close proximity to the LPEI backbone. I believe this instability to be unique to MeFc-LPEI since this behavior has not been noted with other ferrocene redox polymers[25,26]. Based on this, I hypothesize that we can mitigate the instability we observe in our crosslinked films by extending the ferrocene group further away from the LPEI backbone, thereby preventing any potential confinement of the redox group.

In this chapter I report the synthesis of a new LPEI based redox polymer where the ferrocene groups has been extended six carbon atoms away from the backbone (HxFc-LPEI) rather than one carbon atom away (MeFc-LPEI). I will show that crosslinked films of HxFc-LPEI exhibit stable responses both at high pH and in the presence of dibasic phosphate. In addition, I will show that the electrochemical stability of the new polymer translates to highly stable glucose sensor responses.

EXPERIMENTAL

Materials and Instruments

(6-Bromohexyl)ferrocene and all salts and acids were purchased from Aldrich. Ethylene glycol diglycidyl ether (EGDGE) was purchased from Polysciences Inc., Warrington, PA. All other chemicals and solvents were reagent grade and used as received. Phosphate buffered saline (PBS), pH 7.4, was prepared from 8 g/L NaCl, 0.2 g/L KCl, 0.2 g/L KH_2PO_4, and 1.15 g/L Na_2HPO_4. Water was obtained from a Nanopure® water purification system and had a specific resistance of 18 MΩ cm.

Polymer Synthesis

Linear poly(ethylenimine) [LPEI] and MeFc-LPEI were both synthesized according to previously described methods[27]. Hexylferrocenyl-LPEI [HxFc-LPEI] was synthesized by adding 10 ml of acetonitrile (CH_3CN) to 300 mg of LPEI and refluxed for 10 minutes. 2 ml of methanol (CH_3OH) was added and the solution changed from cloudy and non-homogeneous to clear and homogeneous. 380 mg of (6-Bromohexyl)ferrocene was added slowly to the polymer solution with a pipette. The solution was allowed to reflux overnight and the solvent was removed under vacuum. The residue was rinsed with diethyl ether and dried under vacuum to yield ~ 600 mg of HxFc-LPEI.

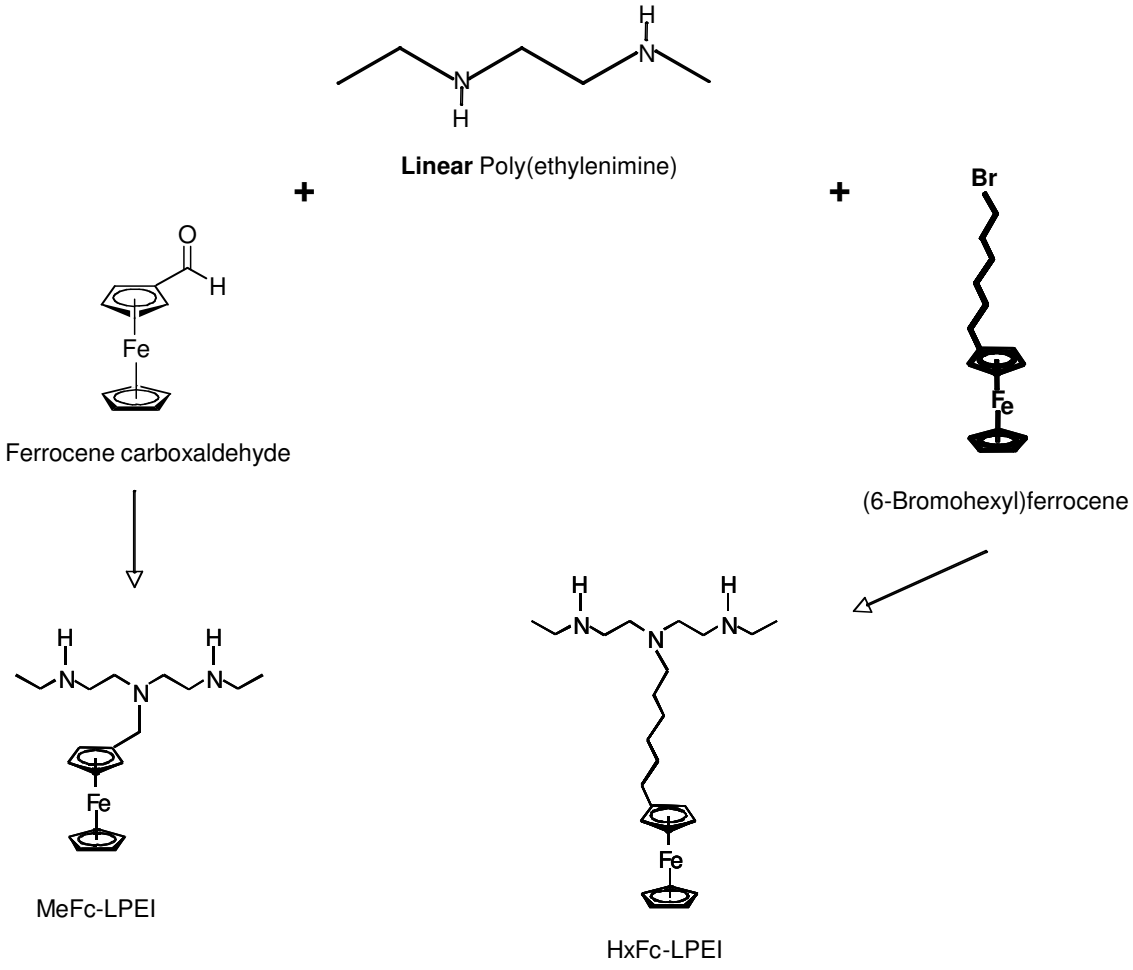


Figure 5.01: Summary of synthetic routes and structures of MeFc-LPEI and HxFc-LPEI redox polymers.

RESULTS AND DISCUSSION

Crosslinked MeFc-LPEI and HxFc-LPEI films in PBS

Before comparing the performance of glucose sensors using MeFc-LPEI and HxFc-LPEI as molecular wires for GOx, the electrochemical behavior of crosslinked films of each polymer without the enzyme was examined. Cyclic voltammograms of crosslinked MeFc-LPEI films in PBS at pH 3, 7, and 11 are shown in Figure 5.02. As reported previously[15], at pH < 7 in solutions containing phosphate the electrochemical

response of MeFc-LPEI hydrogels exhibits a single redox wave and is relatively stable (Figure 5.02A). Figure 5.02B illustrates the multiple redox wave behavior and instability of MeFc-LPEI at pH 7 in PBS. The instability refers to the oxidative degradation of the anodic peak current (i_{p1}) at 335 mV with each subsequent potential cycle. At pH 7 there is a ~ 30% loss of current at i_{p1}. At pH 11 this phenomena is amplified as there is a ~ 70% loss of current from scan 1 to 5.

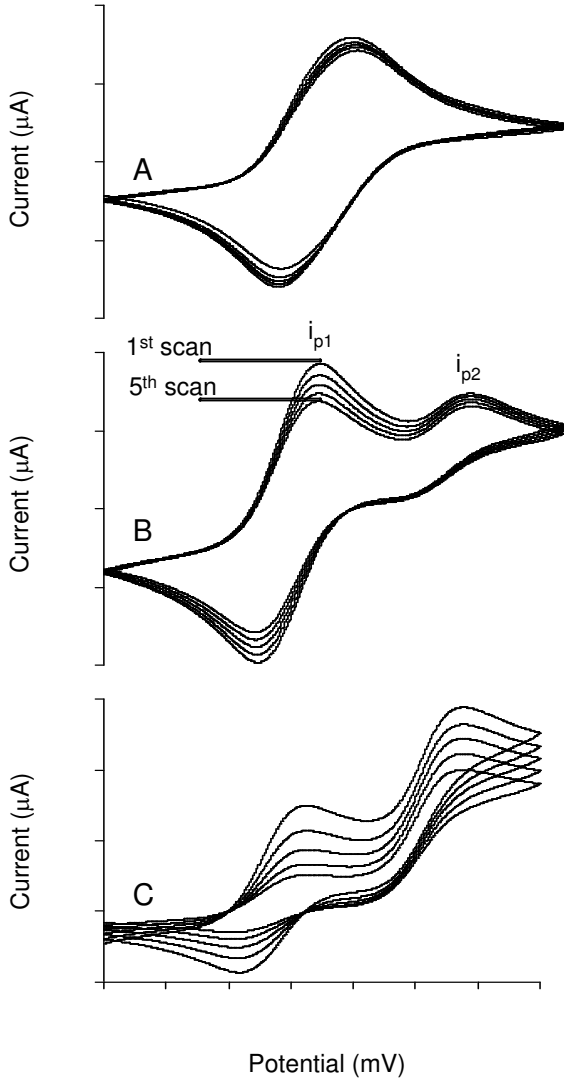


Figure 5.02: Cyclic voltammograms of crosslinked MeFc-LPEI films in PBS at (A) pH 3, (B) pH 7, and (C) pH 11. Potential scans 1-5, scan rate ν = 50 mV/s.

I believe that this electrochemical instability is a result of morphological confinement of a portion of the ferrocene groups attached to the LPEI backbone. Since the ferrocene atoms of MeFc-LPEI are only separated from the polymer backbone by one carbon atom they are closely affected by changes in polymer conformation due either to binding between dibasic phosphate[28] and the nitrogen atoms on the polymer, or to the collapse of the polymer as a result of deprotonation of the secondary amines[19,24]. Hence, extension of the ferrocene groups further away from the LPEI backbone should eliminate these effects.

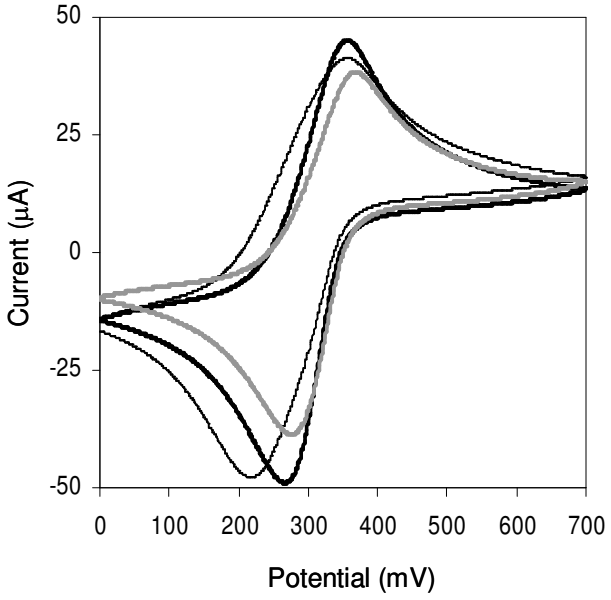


Figure 5.03: Cyclic voltammograms of crosslinked films of HxFc-LPEI in PBS background electrolyte at (——) pH 3, (▬) pH 7, and (▬) pH 11. Scan rate ν = 50 mV/s.

The cyclic voltammograms of HxFc-LPEI are shown in Figure 5.03, and as expected the electrochemical phenomena observed with MeFc-LPEI are absent. These films do not exhibit multi-wave redox behavior, and are stable over the entire pH range. The electrochemical stability of the films at pH 7 in the presence of phosphate is particularly important since biosensors will most likely be employed under physiological

conditions. Additionally, the stability of HxFc-LPEI hydrogels at pH 11 suggest that even under conditions where the polymer is probably deprotonated and in a collapsed configuration the six carbon (hexyl) tether provides sufficient separation of the ferrocene and LPEI backbone as to not result in confinement.

Wired GOx Glucose Sensors

HxFc-LPEI was crosslinked with GOx to determine whether the polymer could communicate electrically, or exchange electrons, with the FAD centers of the enzyme via the following set of reactions:

$$GOx(FAD) + Glucose \rightarrow GOx(FADH_2) + gluconolactone \quad (1)$$

$$GOx(FADH_2) + 2\ Fc^{+1} \rightarrow GOx(FAD) + 2\ Fc + 2\ H^{+} \quad (2)$$

Self exchange of e^- **:** $Fc + Fc^{+} \rightarrow Fc^{+} + Fc$ (3)

At the electrode surface **:** $Fc \rightarrow Fc^{+} + e^{-}$ (4)

I have previously demonstrated the ability of MeFc-LPEI to effectively communicate with GOx. MeFc-LPEI based sensors on 3mm diameter electrodes produced the highest current densities in response to glucose under a variety of conditions. However, since these results were obtained in phosphate buffers containing either 50 or 100mM phosphate, we also report MeFc-LPEI based glucose sensors in this work since the phosphate concentration in PBS is ~10mM. This allows for a side-by-side comparison of the performance of both polymers.

Figure 5.04 shows cyclic voltammograms of both MeFc-LPEI and HxFc-LPEI based sensors with and without glucose. With glucose in solution both sensors appear to maintain significant electrode driven reduction as opposed to reduction of the ferrocene

groups by the GOx($FADH_2$). This is evident by the pronounced reduction peaks in both figures. The results in Figure 5.04B for MeFc-LPEI based sensors are in contrast to those reported in chapter 4 where relatively little electrode driven reduction in the presence of glucose was observed. This may be explained by the differences in buffering solutions since the PBS used here is at a slightly higher pH (7.4 vs. 7) and a lower phosphate concentration as mentioned above.

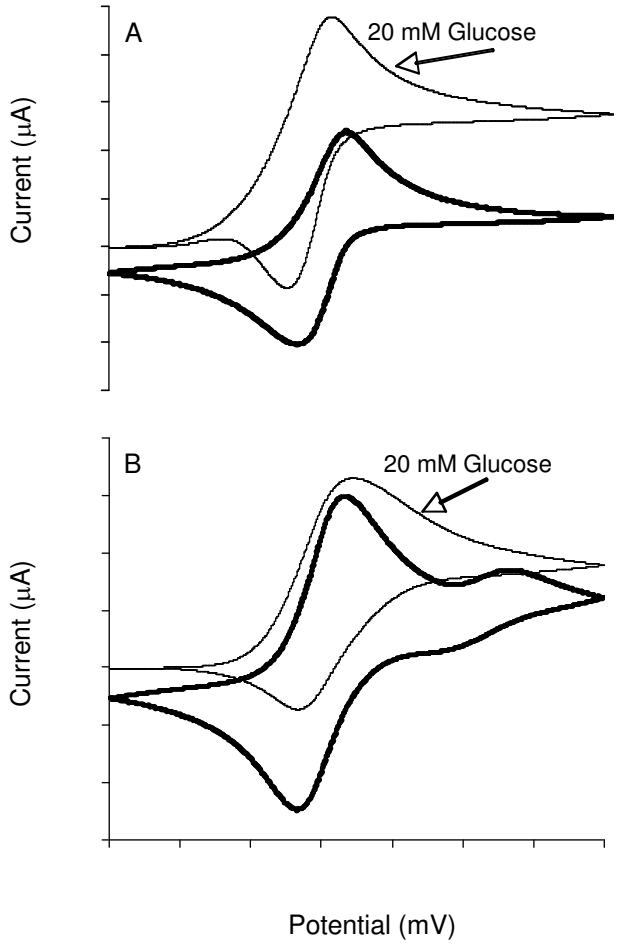


Figure 5.04: Cyclic voltammograms of (A) HxFc-LPEI/GOx, and (B) MeFc-LPEI/GOx based glucose sensors in PBS at pH 7.4 with no glucose in solution (**heavy**) and with 20mM glucose in solution (light).

Despite an elevated degree of communication with the electrode, Figure 5.05 shows that sensors based on both polymers exhibit high current densities in response to glucose. Maximum current density at enzyme saturation, i_{max}, for the MeFc-LPEI based sensor was 900 ± 170 $\mu A \cdot cm^{-2}$, while i_{max} for HxFc-LPEI based sensors was 600 ± 30 $\mu A \cdot cm^{-2}$. Lineweaver-Burke analysis of these glucose response curves yields K_m values of 9 ± 3 mM and 20 ± 5 mM, respectively. Finally, the inset of Figure 5.05 demonstrates the linearity of HxFc-LPEI based sensors in the glucose concentration range from 0-10 mM while the MeFc-LPEI does not display linearity.

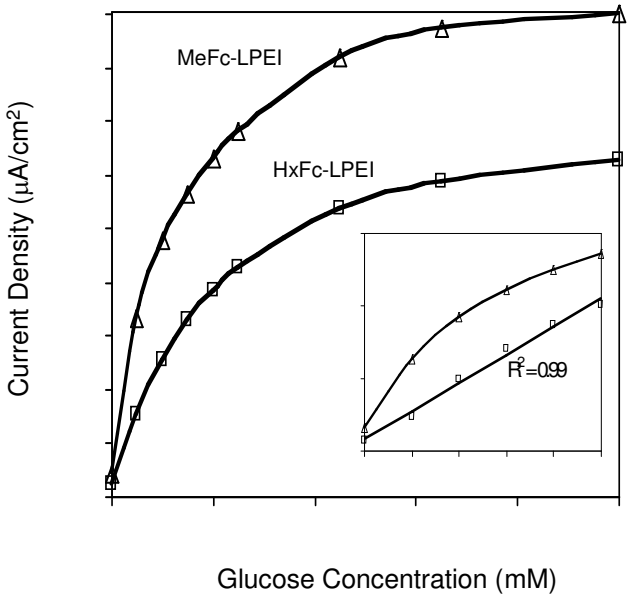


Figure 5.05: Glucose sensor response curves for amperometric sensors based on MeFc-LPEI and HxFc-LPEI. E=0.4 V.

Although the linear range of operation of these types of biosensors can be extended, or imposed in the case of MeFc-LPEI based sensors, by depositing a glucose permeable membrane over the sensing layer, it is encouraging to note that HxFc-LPEI based sensors inherently display linearity.

Long Term Stability of Wired GOx Sensors

Finally, long term stability tests were performed to determine whether the electrochemical stability observed in the cyclic voltammetric response of the HxFc-LPEI hydrogels would translate to stability of glucose sensors based on this polymer. Figure 5.06 shows continuous operation stability tests for sensors made with both polymers.

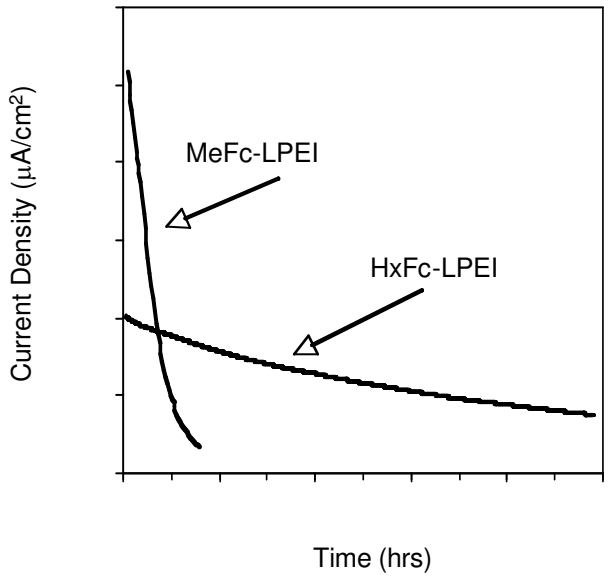


Figure 5.06: Continuous operation long term stability tests for glucose sensors based on MeFc-LPEI and HxFc-LPEI in PBS at pH 7.4, 10 mM glucose, E=0.4 V, T=20 degrees C. n=2 for each condition.

It is evident that there is a dramatic difference in stability. The half life of the MeFc-LPEI based sensor is ~3 hours while the half life of the HxFc-LPEI based sensor is ~30 hours. These results clearly indicate that in addition to the electrochemical stability, extension of the ferrocene group from the LPEI backbone with a longer tether also results in a more stable glucose sensor.

CONCLUSIONS

In this chapter I report the synthesis of hexylferrocenyl linear poly(ethylenimine) [HxFc-LPEI]. I demonstrate that the extension of the ferrocene groups further away from the LPEI backbone eliminates the multi-wave redox behavior and the oxidative degradation observed in crosslinked films of MeFc-LPEI in the presence of dibasic phosphate, and at high pH. This is probably due to the mitigation of morphological confinement of the ferrocene groups as the polymer transitions to a collapsed state. In addition, I also show that HxFc-LPEI is able to exchange electrons with the FAD centers in GOx as glucose sensors formed with this polymer produced current densities at saturation of ~600 $\mu A/cm^2$. Finally, I show that the stability observed in the electrochemical response of the crosslinked HxFc-LPEI films translates to an enhanced stability in the sensor response under continuous long-term operation in comparison to the MeFc-LPEI.

Acknowledgements: I would like to thank Dr. Glatzhofer for his valuable insight and suggestions concerning this work.

REFERENCES

(1) Gregg, B. A.; Heller, A. *Analytical Chemistry* **1990**, *62*, 258.

(2) Ling, Y.; Hammerle, M.; Olsthoorn, A. J. J.; Schuhmann, W.; Schmidt, H. L.; Duine, J. A.; Heller, A. *Analytical Chemistry* **1993**, *65*, 238.

(3) Kenausis, G.; Taylor, C.; Katakis, I.; Heller, A. *Journal of the Chemical Society-Faraday Transactions* **1996**, *92*, 4131.

(4) Kulagina, N. V.; Michael, A. C. *Analytical Chemistry* **2003**, *75*, 4875.

(5) Vreeke, M. S.; Yong, K. T.; Heller, A. *Analytical Chemistry* **1995**, *67*, 4247.

(6) Kulagina, N. V.; Shankar, L.; Michael, A. C. *Analytical Chemistry* **1999**, *71*, 5093.

(7) Oldenziel, W. H.; Dijkstra, G.; Cremers, T. I. F. H.; Westerink, B. H. C. *Analytical Chemistry* **2006**, *78*, 3366.

(8) Calvo, E. J.; Etchenique, R.; Danilowicz, C.; Diaz, L. *Analytical Chemistry* **1996**, *68*, 4186.

(9) Mao, F.; Mano, N.; Heller, A. *Journal of the American Chemical Society* **2003**, *125*, 4951.

(10) Ohara, T. J.; Rajagopalan, R.; Heller, A. *Analytical Chemistry* **1993**, *65*, 3512.

(11) Rajagopalan, R.; Aoki, A.; Heller, A. *Journal of Physical Chemistry* **1996**, *100*, 3719.

(12) Aoki, A.; Heller, A. *Journal of Physical Chemistry* **1993**, *97*, 11014.

(13) Aoki, A.; Rajagopalan, R.; Heller, A. *Journal of Physical Chemistry* **1995**, *99*, 5102.

(14) Oh, S. M.; Faulkner, L. R. *Journal of Electroanalytical Chemistry* **1989**, *269*, 77.

(15) Merchant, S. A. G., D. T.; Schmidtke, D. W. *Langmuir* **2007**, *23*, 11295.

(16) Andersson, M. M.; Hatti-Kaul, R.; Brown, W. *Journal of Physical Chemistry B* **2000**, *104*, 3660.

(17) Chen, Q.; Kenausis, G. L.; Heller, A. *Journal of the American Chemical Society* **1998**, *120*, 4582.

(18) Jezkova, J.; Iwuoha, E. I.; Smyth, M. R.; Vytras, K. *Electroanalysis* **1997**, *9*, 978.

(19) Chatani, Y.; Tadokoro, H.; Saegusa, T.; Ikeda, H. *Macromolecules* **1981**, *14*, 315.

(20) Lewis, E. A.; Barkley, J.; Stpierre, T. *Macromolecules* **1981**, *14*, 546.

(21) Chang, D. R.; Harden, S.; Loverro, N. *Journal of Macromolecular Science, Chemistry* **1986**, *A23*, 801.

(22) Weyts, K. F.; Goethals, E. J. *Die Makromolekulare Chemie, Rapid Communications* **1989**, *10*, 299.

(23) Kokufuta, E.; Suzuki, H.; Yoshida, R.; Kaneko, F.; Yamada, K.; Hirata, M. *Colloids and Surfaces a-Physicochemical and Engineering Aspects* **1999**, *147*, 179.

(24) Kokufuta, E.; Suzuki, H.; Yoshida, R.; Yamada, K.; Hirata, M.; Kaneko, F. *Langmuir* **1998**, *14*, 788.

(25) Chuang, C. L.; Wang, Y. J.; Lan, H. L. *Analytica Chimica Acta* **1997**, *353*, 37.
(26) Liu, A. H.; Kashiwagi, Y.; Anzai, J. *Electroanalysis* **2003**, *15*, 1139.
(27) York, S.; Frech, R.; Snow, A.; Glatzhofer, D. *Electrochimica Acta* **2001**, *46*, 1533.
(28) Nakamae, K.; Miyata, T.; Hoffman, A. S. *Makromolekulare Chemie-Macromolecular Chemistry and Physics* **1992**, *193*, 983.

CHAPTER 6:

Conclusions and Recommendations for Future Work

CONCLUSIONS

The development of redox polymers based on linear poly(ethylenimine) modified with ferrocene redox couples was presented in this dissertation. These materials were shown to exhibit some unusual and unique characteristics, particularly in their electrochemical responses. In addition to novelty, they were also shown to be quite useful in biosensor applications.

The first polymer synthesized for this work was a methylferrocenyl linear poly(ethylenimine). The solution electrochemistry of this material exhibited redox potential shifts in the opposite direction of what is normally observed for ferrocene modified amines. It was demonstrated here that counter ion effects, in conjunction with the proximity of the ferrocene group to the polymer backbone, may be responsible for the counterintuitive potential shifts. Crosslinked films of this polymer also displayed abnormal behavior. Under certain conditions the films would undergo oxidative degradation which resulted in electrochemical and sensors instability. This phenomena is probably also related to the proximity of the ferrocene group to the polymer backbone, in

addition to the morphological changes that occur in crosslinked films. Nonetheless, this material was shown to produce the highest responding glucose sensors reported confirming my hypothesis that such materials could be developed into effect sensor components.

The culmination of this work lies in the synthesis of another new material which addresses many of the issues with the MeFc-LPEI. By extending the ferrocene group further away from the polymer backbone hexylferrocenyl linear poly(ethylenimine) eliminates the proximity issue, particularly the morphological confinement in crosslinked films. This dramatically improves both the electrochemical response and the sensor response with respect to stability.

RECOMMENDATIONS FOR FUTURE WORK

There are three issues of particular importance to be addressed in future studies. First, the nature of the interaction between linear poly(ethylenimine) and glucose oxidase should be addressed. Experiments which could quantify, or qualify on a comparative basis the enzyme-polymer interaction, would lend weight to the proposal that close interactions between the two molecules facilitates efficient electron exchange. Quartz crystal microbalance is one technique that could potentially be used to probe this interaction. For example, the immobilization of LPEI on the crystal surface followed by adsorption of glucose oxidase could provide an idea of the quantity of polymer and enzyme associated with each other by changes in mass. Performing these experiments at varying degrees of protonation of the LPEI backbone would provide insight into the degree to which electrostatics influence the polymer enzyme interaction. Modifications

to the LPEI backbone could also be examined with respect to changes in this interaction. For example, carboxyl or thiol groups could be added to determine if they enhance or impede the association of the enzyme with the polymer. Finally, other polymer backbones could be used such as poly(vinylpyridine), poly(allylamine), and branched poly(ethylenimine) to examine the role that the structure of the polymer backbone plays in the interaction.

Second, the distance between the ferrocene redox couples and the polymer backbone should be optimized. For example, a propylferrocenyl- or a butylferrocenyl-LPEI may allow for both highly sensitive and highly stable devices. In addition, there may be a particular formulation of polymers that would meet this optimal condition. For example, there may be a certain ratio of MeFc-LPEI and HxFc-LPEI that would produce a synergistic, or at least, moderating effect. Along the same lines as this is the concept of modifying LPEI with a different redox couple such as an osmium or ruthenium complex. Preliminary results with an Os-LPEI suggest that stable responses may be achieved with this material.

Third, the application of these polymers with different, and/or multiple enzyme systems should be investigated. This could lead to additional sensors or biofuel cell devices. For example, glucose sensors that use a layer of glucose oxidase to react with glucose, and a wired peroxidase layer to detect the resulting hydrogen peroxide produce have been shown to be effective devices. Since I have shown that MeFc-LPEI to be an excellent wire for horseradish peroxidase, a sensor constructed in this manner using MeFc-LPEI may exhibit relatively enhanced responses. This would also allow for the

sensor to be operated at 0 mV, which could have implications on the stability of the redox polymer and sensor.

BIBLOGRAPHY

Abbott, N. L., C. M. Jewell, et al. (2005). "Ferrocene-containing cationic lipids: Influence of redox state on cell transfection." Journal of the American Chemical Society **127**(33): 11576-11577.

Alegret, S. (1996). "Rigid carbon–polymer biocomposites for electrochemical sensing. A review." Analyst **121**: 1751.

Alvarez, J. and A. E. Kaifer (1999). "Structural and pH control on the electronic communication between two identical ferrocene sites." Organometallics **18**(26): 5733-5734.

Alvarez, J., T. Ren, et al. (2001). "Redox potential selection in a new class of dendrimers containing multiple ferrocene centers." Organometallics **20**(16): 3543-3549.

Andersson, M. M., R. Hatti-Kaul, et al. (2000). "Dynamic and static light scattering and fluorescence studies of the interactions between lactate dehydrogenase and poly(ethyleneimine)." Journal of Physical Chemistry B **104**(15): 3660-3667.

Andrieux, C. P., O. Haas, et al. (1986). "Catalysis of Electrochemical Reactions at Redox Polymer Coated Electrodes - Mediation of the Fe(Iii)/Fe(Ii) Oxidoreduction by a Polyvinylpyridine Polymer Containing Coordinatively Attached Bisbipyridine Chlororuthenium Redox Centers." Journal of the American Chemical Society **108**(26): 8175-8182.

Antolini, E. (2004). "Recent developments in polymer electrolyte fuel cell electrodes." Journal of Applied Electrochemistry **34**(6): 563-576.

Aoki, A. and A. Heller (1993). "Electron-Diffusion Coefficients in Hydrogels Formed of Cross-Linked Redox Polymers." Journal of Physical Chemistry **97**(42): 11014-11019.

Aoki, A., R. Rajagopalan, et al. (1995). "Effect of Quaternization on Electron-Diffusion Coefficients for Redox Hydrogels Based on Poly(4-Vinylpyridine)." Journal of Physical Chemistry **99**(14): 5102-5110.

Armada, M. P. G., J. Losada, et al. (2006). "Electrocatalytical properties of polymethylferrocenyl dendrimers and their applications in biosensing." Bioelectrochemistry **69**(1): 65-73.

Azamian, B. R., J. J. Davis, et al. (2002). "Bioelectrochemical single-walled carbon nanotubes." Journal of the American Chemical Society **124**(43): 12664-12665.

Azzaroni, O., S. Moya, et al. (2005). "Switching the properties of polyelectrolyte brushes via "Hydrophobic collapse"." Macromolecules **38**(24): 10192-10199.

Battaglini, F. and E. J. Calvo (1994). "Enzyme Catalysis at Hydrogel-Modified Electrodes with Soluble Redox Mediator." Journal of the Chemical Society-Faraday Transactions **90**(7): 987-995.

Battaglini, F., E. J. Calvo, et al. (1999). "Effect of ionic strength on the behavior of amperometric enzyme electrodes mediated by redox hydrogels." Analytical Chemistry **71**(5): 1062-1067.

Besteman, K., J. O. Lee, et al. (2003). "Enzyme-coated carbon nanotubes as single-molecule biosensors." Nano Letters **3**(6): 727-730.

Binyamin, G., J. Cole, et al. (2000). "Mechanical and electrochemical characteristics of composites of wired glucose oxidase and hydrophilic graphite." Journal of the Electrochemical Society **147**(7): 2780-2783.
Birnbaum, E. R., K. C. Rau, et al. (2003). "Selective anion binding from water using soluble polymers." Separation Science and Technology **38**(2): 389-404.
Bloys van Treslong, C. J. and A. J. Staverman (1974). "Poly(ethylenimine) II Potentiometric titration behaviour in comparison with other weak polyelectrolytes." Recueil, Journal of the Royal Netherlands Chemical Society **93**(6): 171-178.
Brandup, J. I., E. (1989). Polymer Handbook, John Wiley and Sons.
Bu, H. Z., S. R. Mikkelsen, et al. (1995). "Characterization of a Ferrocene Containing Polyacrylamide-Based Redox Gel for Biosensor Use." Analytical Chemistry **67**(22): 4071-4076.
Calvo, E. J., C. Danilowicz, et al. (2004). "Characterization of self-assembled redox polymer and antibody molecules on thiolated gold electrodes." Biosensors & Bioelectronics **19**(10): 1219-1228.
Calvo, E. J., R. Etchenique, et al. (1996). "Electrical communication between electrodes and enzymes mediated by redox hydrogels." Analytical Chemistry **68**(23): 4186-4193.
Calvo, E. J. and A. Wolosiuk (2005). "Wiring enzymes in nanostructures built with electrostatically self-assembled thin films." Chemphyschem **6**(1): 43-47.
Campbell, C. N., D. Gal, et al. (2002). "Enzyme-amplified amperometric sandwich test for RNA and DNA." Analytical Chemistry **74**(1): 158-162.
Cass, A. E. G., G. Davis, et al. (1984). "Ferrocene-Mediated Enzyme Electrode for Amperometric Determination of Glucose." Analytical Chemistry **56**(4): 667-671.
Chang, D. R., S. Harden, et al. (1986). "Protonation of Polyethylenimine." Journal of Macromolecular Science, Chemistry **A23**(6): 801-804.
Chatani, Y., H. Tadokoro, et al. (1981). "Structural Studies of Poly(Ethylenimine) .1. Structures of 2 Hydrates of Poly(Ethylenimine) - Sesquihydrate and Dihydrate." Macromolecules **14**(2): 315-321.
Chen, Q., G. L. Kenausis, et al. (1998). "Stability of oxidases immobilized in silica gels." Journal of the American Chemical Society **120**(19): 4582-4585.
Chen, T., S. C. Barton, et al. (2001). "A miniature biofuel cell." Journal of the American Chemical Society **123**(35): 8630-8631.
Chen, T. L. and H. S. Weng (1986). "A Method for the Determinations of the Activity and Optimal Ph of Glucose-Oxidase in an Unbuffered Solution." Biotechnology and Bioengineering **28**(1): 107-109.
Chetty, V. K. and B. B. Zellner (2007). "Use of survey and clinical data for screening and diagnosis." Statistics in Medicine **26**(17): 3213-3228.
Chuang, C. L., Y. J. Wang, et al. (1997). "Amperometric glucose sensors based on ferrocene-containing B-polyethylenimine and immobilized glucose oxidase." Analytica Chimica Acta **353**(1): 37-44.
Cunningham, A. J. (1998). Introduction to Bioanalytical Sensors.
de Lumley-Woodyear, T., C. N. Campbell, et al. (1999). "Rapid amperometric verification of PCR amplification of DNA." Analytical Chemistry **71**(3): 535-538.

Deng, Q. and S. J. Dong (1994). "Redox Reaction of Peroxidase at Poly(O-Phenylenediamine) Modified Electrode." Electroanalysis **6**(10): 878-881.
Diamond (1998). Principles of Chemical and Biological Sensors.
Dick, C. R. and G. E. Ham (1970). "Characterization of Polyethylenimine." Journal of Macromolecular Science-Chemistry **A 4**(6): 1301-&.
Forster, R. J. and J. G. Vos (1991). "Redox Site Loading, Electrolyte Concentration and Temperature Effects on Charge Transport and Electrode-Kinetics of Electrodes Modified with Osmium Containing Poly(4-Vinylpyridine) Films in Sulfuric-Acid." Journal of Electroanalytical Chemistry **314**(1-2): 135-152.
Forster, R. J., J. G. Vos, et al. (1991). "Controlling Processes in the Rate of Charge Transport through [Os(Bipy)2(Pvp)Ncl]Cl Redox Polymer-Modified Electrodes." Journal of the Chemical Society-Faraday Transactions **87**(23): 3761-3767.
Forster, R. J., J. G. Vos, et al. (1991). "Thermodynamics and Kinetics of Heterogeneous Electron-Transfer at Glassy-Carbon Osmium-Containing Metallopolymer Interfaces." Journal of the Chemical Society-Faraday Transactions **87**(23): 3769-3774.
Freedonia (2005). Industry Study with Forecasts to 2009 and 2014: Chemical Sensors. F. Group. Cleveland, OH, Freedonia Group.
Fuji-Keizai (2004). Biosensor Market, R&D and Commercial Implication, Fuji-Keizai USA.
Gough, D. A., J. Y. Lucisano, et al. (1985). "Two-Dimensional Enzyme Electrode Sensor for Glucose." Analytical Chemistry **57**(12): 2351-2357.
Gregg, B. A. and A. Heller (1989). "Direct Amperometric Biosensors from Enzyme-Containing Cross-Linked Redox Polymers Bound to Electrodes." Abstracts of Papers of the American Chemical Society **198**: 2-Anyl.
Gregg, B. A. and A. Heller (1990). "Cross-Linked Redox Gels Containing Glucose-Oxidase for Amperometric Biosensor Applications." Analytical Chemistry **62**(3): 258-263.
Gregg, B. A. and A. Heller (1991). "Redox Polymer-Films Containing Enzymes .1. A Redox-Conducting Epoxy Cement - Synthesis, Characterization, and Electrocatalytic Oxidation of Hydroquinone." Journal of Physical Chemistry **95**(15): 5970-5975.
Gregg, B. A. and A. Heller (1991). "Redox Polymer-Films Containing Enzymes .2. Glucose-Oxidase Containing Enzyme Electrodes." Journal of Physical Chemistry **95**(15): 5976-5980.
Griffiths, P. C., I. A. Fallis, et al. (2006). "Metallosurfactants: Interfaces and micelles." Advances in Colloid and Interface Science **122**(1-3): 107-117.
Grunlan, J. C., L. Liu, et al. (2006). "Tunable single-walled carbon nanotube microstructure in the liquid and solid states using poly(acrylic acid)." Nano Letters **6**(5): 911-915.
Guiseppi-Elie, A., C. H. Lei, et al. (2002). "Direct electron transfer of glucose oxidase on carbon nanotubes." Nanotechnology **13**(5): 559-564.
Habermuller, K., A. Ramanavicius, et al. (2000). "An oxygen-insensitive reagentless glucose biosensor based on osmium-complex modified polypyrrole." Electroanalysis **12**(17): 1383-1389.

Habermuller, L., M. Mosbach, et al. (2000). "Electron-transfer mechanisms in amperometric biosensors." Fresenius Journal of Analytical Chemistry **366**(6-7): 560-568.
Hale, P. D., L. I. Boguslavsky, et al. (1991). "Amperometric Glucose Biosensors Based on Redox Polymer-Mediated Electron-Transfer." Analytical Chemistry **63**(7): 677-682.
Hays, M. E., C. M. Jewell, et al. (2007). "Reversible condensation of DNA using a redox-active surfactant." Langmuir **23**(10): 5609-5614.
Heller, A. (1990). "Electrical Wiring of Redox Enzymes." Abstracts of Papers of the American Chemical Society **200**: 48-Phys.
Heller, A. (1996). "Amperometric biosensors." Current Opinion in Biotechnology **7**(1): 50-54.
Heller, A. (2004). "Miniature biofuel cells." Physical Chemistry Chemical Physics **6**(2): 209-216.
Heller, J. and A. Heller (1998). "Loss of activity or gain in stability of oxidases upon their immobilization in hydrated silica: Significance of the electrostatic interactions of surface arginine residues at the entrances of the reaction channels." Journal of the American Chemical Society **120**(19): 4586-4590.
Holecek, J., K. Handlir, et al. (1979). "Decomposition of Ferricenium Cation in Alkaline-Medium." Collection of Czechoslovak Chemical Communications **44**(5): 1379-1387.
Hu, H., Y. C. Ni, et al. (2005). "Polyethyleneimine functionalized single-walled carbon nanotubes as a substrate for neuronal growth." Journal of Physical Chemistry B **109**(10): 4285-4289.
Huan, Z. W., B. Persson, et al. (1996). "Redox polymers for electrocatalytic oxidation of NADH - Cationic styrene and ethylenimine polymers." Electroanalysis **8**(6): 575-581.
Ikeda, T., R. Schmehl, et al. (1982). "Permeation of Electroactive Solutes through Ultrathin Polymeric Films on Electrode Surfaces." Journal of the American Chemical Society **104**(10): 2683-2691.
Ishikawa, M., D. W. Schmidtke, et al. (1998). "Initial evaluation of a 290-mu m diameter subcutaneous glucose sensor: Glucose monitoring with a biocompatible, flexible-wire, enzyme-based amperometric microsensor in diabetic and nondiabetic humans." Journal of Diabetes and Its Complications **12**(6): 295-301.
Jewell, C. M., M. E. Hays, et al. (2006). "Ferrocene-containing cationic lipids for the delivery of DNA: Oxidation state determines transfection activity." Journal of Controlled Release **112**(1): 129-138.
Jezkova, J., E. I. Iwuoha, et al. (1997). "Stabilization of an osmium bis-bipyridyl polymer-modified carbon paste amperometric glucose biosensor using polyethyleneimine." Electroanalysis **9**(13): 978-984.
Joshi, P. P., S. A. Merchant, et al. (2005). "Amperometric biosensors based on redox polymer-carbon nanotube-enzyme composites." Analytical Chemistry **77**(10): 3183-3188.
Ju, H. X. and D. Leech (1997). "[Os(bpy)(2)(PVI)(10)Cl]Cl polymer-modified carbon fiber electrodes for the electrocatalytic oxidation of NADH." Analytica Chimica Acta **345**(1-3): 51-58.

Katakis, I., L. Ye, et al. (1994). "Electrostatic Control of the Electron-Transfer Enabling Binding of Recombinant Glucose-Oxidase and Redox Polyelectrolytes." Journal of the American Chemical Society **116**(8): 3617-3618.

Katakis, I., L. Ye, et al. (1994). "Design of Redox Polyelectrolyte Wires for Glucose Electrodes." Abstracts of Papers of the American Chemical Society **208**: 321-PMSE.

Kenausis, G., C. Taylor, et al. (1996). "'Wiring' of glucose oxidase and lactate oxidase within a hydrogel made with poly(vinyl pyridine) complexed with [Os(4,4'-dimehtoxy-2,2'-bipyridine)(2)Cl](+/2+)." Journal of the Chemical Society-Faraday Transactions **92**(20): 4131-4136.

Khorasani-Motlagh, M., N. Safari, et al. (2005). "New class of verdoheme analogues with weakly coordinating anions: The structure of (mu-oxo)bis[(octaethyloxoporphinato)iron(III)] hexafluorophosphate." Inorganic Chemistry **44**(22): 7762-7769.

Kim, H. H., N. Mano, et al. (2003). "A miniature membrane-less biofuel cell operating under physiological conditions at 0.5 V." Journal of the Electrochemical Society **150**(2): A209-A213.

Kobayashi, S., K. Hiroishi, et al. (1987). "Chelating Properties of Linear and Branched Poly(Ethylenimines)." Macromolecules **20**(7): 1496-1500.

Kober, E. M., J. V. Caspar, et al. (1988). "Synthetic Routes to New Polypyridyl Complexes of Osmium(Ii)." Inorganic Chemistry **27**(25): 4587-4598.

Koide, S. and K. Yokoyama (1999). "Electrochemical characterization of an enzyme electrode based on a ferrocene-containing redox polymer." Journal of Electroanalytical Chemistry **468**(2): 193-201.

Kokufuta, E. (2005). "Polyelectrolyte gel transitions: Experimental aspects of charge inhomogeneity in the swelling and segmental attractions in the shrinking." Langmuir **21**(22): 10004-10015.

Kokufuta, E., H. Suzuki, et al. (1999). "Volume collapse of a cationic poly(ethyleneimine) gel induced by the binding of anionic surfactants." Colloids and Surfaces a-Physicochemical and Engineering Aspects **147**(1-2): 179-187.

Kokufuta, E., H. Suzuki, et al. (1998). "Role of hydrogen bonding and hydrophobic interaction in the volume collapse of a poly(ethylenimine) gel." Langmuir **14**(4): 788-795.

Kokufuta, E., B. L. Wang, et al. (1998). "Volume phase transition of polyelectrolyte gels with different charge distributions." Macromolecules **31**(20): 6878-6884.

Kulagina, N. V. and A. C. Michael (2003). "Monitoring hydrogen peroxide in the extracellular space of the brain with amperometric microsensors." Analytical Chemistry **75**(18): 4875-4881.

Kulagina, N. V., L. Shankar, et al. (1999). "Monitoring glutamate and ascorbate in the extracellular space of grain tissue with electrochemical microsensors." Analytical Chemistry **71**(22): 5093-5100.

Kwon, S. J., E. Kim, et al. (2006). "An electrochemical immunosensor using ferrocenyl-tethered dendrimer." Analyst **131**(3): 402-406.

Lakard, S., G. Herlem, et al. (2004). "Adhesion and proliferation of cells on new polymers modified biomaterials." Bioelectrochemistry **62**(1): 19-27.

Lednicer, D. H., Charles, R. (1960). "N,N-Dimethylaminomethylferrocene methiodide." Organic Syntheses **40**: 31-33.
Lei, C. X., H. Wang, et al. (2004). "Immobilization of enzymes on the nano-Au film modified glassy carbon electrode for the determination of hydrogen peroxide and glucose." Electroanalysis **16**(9): 736-740.
Lewis, E. A., J. Barkley, et al. (1981). "Calorimetric Titration of Poly(Vinylamine) and Poly(Iminoethylene)." Macromolecules **14**(3): 546-551.
Li, X., S. H. Goh, et al. (2001). "Miscibility and interactions in blends of carboxyl-containing polysiloxane with poly(1-vinylimidazole)." Polymer **42**(12): 5463-5469.
Ling, Y., M. Hammerle, et al. (1993). "High-Current Density Wired Quinoprotein Glucose-Dehydrogenase Electrode." Analytical Chemistry **65**(3): 238-241.
Liu, A. H., Y. Kashiwagi, et al. (2003). "Polyelectrolyte multilayer films containing ferrocene: Effects of polyelectrolyte type and ferrocene contents in the film on the redox properties." Electroanalysis **15**(13): 1139-1142.
Lojou, E. and P. Bianco (2006). "Application of the electrochemical concepts and techniques to amperometric biosensor devices." Journal of Electroceramics **16**(1): 79-91.
Losada, J., M. Zamora, et al. (2006). "Bienzyme sensors based on novel polymethylferrocenyl dendrimers." Analytical and Bioanalytical Chemistry **385**(7): 1209-1217.
Low, L. M., S. Seetharaman, et al. (2000). "Microactuators toward microwaves for responsive controlled drug delivery." Sensors and Actuators B-Chemical **67**(1-2): 149-160.
Lowry, J. P., K. Mcateer, et al. (1994). "Characterization of Glucose Oxidase-Modified Poly(Phenylenediamine)-Coated Electrodes in-Vitro and in-Vivo - Homogeneous Interference by Ascorbic-Acid in Hydrogen-Peroxide Detection." Analytical Chemistry **66**(10): 1754-1761.
Lu, B., E. I. Iwuoha, et al. (1997). "Development of an "electrically wired" amperometric immunosensor for the determination of biotin based on a non-diffusional redox osmium polymer film containing an antibody to the enzyme label horseradish peroxidase." Analytica Chimica Acta **345**(1-3): 59-66.
Lu, B., E. I. Iwuoha, et al. (1997). "Effects of acetonitrile on horseradish peroxidase (HRP) anti HRP antibody interaction." Biosensors & Bioelectronics **12**(7): 619-625.
Luque, R., M. Orejas, et al. (2004). "pH Control of the production of recombinant glucose oxidase in Aspergillus nidulans." Journal of Applied Microbiology **97**(2): 332-337.
Mano, N. and A. Heller (2003). "A miniature membraneless biofuel cell operating at 0.36 V under physiological conditions." Journal of the Electrochemical Society **150**(8): A1136-A1138.
Mano, N., F. Mao, et al. (2003). "Characteristics of a miniature compartment-less glucose-O-2 biofuel cell and its operation in a living plant." Journal of the American Chemical Society **125**(21): 6588-6594.
Mano, N., F. Mao, et al. (2003). "A miniature biofuel cell operating at 0.78 V." Chemical Communications(4): 518-519.

Mao, F., N. Mano, et al. (2003). "Long tethers binding redox centers to polymer backbones enhance electron transport in enzyme "wiring" hydrogels." Journal of the American Chemical Society **125**(16): 4951-4957.
Marr, G. and B. W. Rockett (1982). "Ferrocene - Annual Survey Covering the Year 1980." Journal of Organometallic Chemistry **227**(Mar): 373-440.
McAndrew, T. P. (1997). "Corrosion prevention with electrically conductive polymers." Trends in Polymer Science **5**(1): 7-12.
Merchant, S. A. G., D. T.; Schmidtke, D. W. (2007). "Effects of Electrolyte and pH on the Behavior of Cross-Linked Films of Ferrocene-Modified Poly(ethylenimine)." Langmuir **23**(22): 11295-11302.
Meyer, T. J., B. P. Sullivan, et al. (1987). "Synthesis and Coordination Chemistry of Poly(4-Vinyl-4'-Methyl-2,2'-Bipyridine) Films on Electrode Surfaces." Inorganic Chemistry **26**(25): 4145-4147.
Miller, L. L. and Q. X. Zhou (1987). "Poly(N-Methylpyrrolylium) Poly(Styrenesulfonate) - a Conductive, Electrically Switchable Cation Exchanger That Cathodically Binds and Anodically Releases Dopamine." Macromolecules **20**(7): 1594-1597.
Munoz, E., D. S. Suh, et al. (2005). "Highly conducting carbon nanotube/polyethyleneimine composite fibers." Advanced Materials **17**(8): 1064-+.
Nakamae, K., T. Miyata, et al. (1992). "Swelling Behavior of Hydrogels Containing Phosphate Groups." Makromolekulare Chemie-Macromolecular Chemistry and Physics **193**(4): 983-990.
Nakamura, H. and I. Karube (2003). "Current research activity in biosensors." Analytical and Bioanalytical Chemistry **377**(3): 446-468.
Nishiumi, T., Y. Chimoto, et al. (2004). "First redox polymer bearing one-step successive two-electron-transfer process based on redox potential inversion." Macromolecules **37**(7): 2661-2664.
Novak, P., K. Muller, et al. (1997). "Electrochemically active polymers for rechargeable batteries." Chemical Reviews **97**(1): 207-281.
Oh, S. K., L. A. Baker, et al. (2002). "Electrochemical rectification using mixed monolayers of redox-active ferrocenyl dendrimers and n-alkanethiols." Langmuir **18**(18): 6981-6987.
Oh, S. M. and L. R. Faulkner (1989). "Electron-Transport Dynamics in Partially Quaternized Poly(4-Vinylpyridine) Thin-Films Containing Ferri Ferrocyanide." Journal of Electroanalytical Chemistry **269**(1): 77-97.
Ohara, T. J., R. Rajagopalan, et al. (1993). "Glucose Electrodes Based on Cross-Linked [Os(Bpy)(2)](+/2+) Complexed Poly(L-Vinylimidazole) Films." Analytical Chemistry **65**(23): 3512-3517.
Okazaki, Y., K. Ishizuki, et al. (1996). "Ion-specific swelling and deswelling behaviors of ampholytic polymer gels." Macromolecules **29**(26): 8391-8397.
Oldenziel, W. H., G. Dijkstra, et al. (2006). "Evaluation of hydrogel-coated glutamate microsensors." Analytical Chemistry **78**(10): 3366-3378.
Palmisano, F., P. G. Zambonin, et al. (2000). "Amperometric biosensors based on electrosynthesised polymeric films." Fresenius Journal of Analytical Chemistry **366**(6-7): 586-601.

Pernaut, J. M. and J. R. Reynolds (2000). "Use of conducting electroactive polymers for drug delivery and sensing of bioactive molecules. A redox chemistry approach." Journal of Physical Chemistry B **104**(17): 4080-4090.

Pickup, J., L. McCartney, et al. (1999). "In vivo glucose sensing for diabetes management: progress towards non-invasive monitoring." British Medical Journal **319**(7220): 1289-U31.

Pickup, J. C., G. W. Shaw, et al. (1988). "Implantable Glucose Sensors - Choosing the Appropriate Sensing Strategy." Biosensors **3**(6): 335-346.

Pickup, J. C., G. W. Shaw, et al. (1989). "Invivo Molecular Sensing in Diabetes-Mellitus - an Implantable Glucose Sensor with Direct Electron-Transfer." Diabetologia **32**(3): 213-217.

Pickup, J. C., G. W. Shaw, et al. (1989). "Potentially-Implantable, Amperometric Glucose Sensors with Mediated Electron-Transfer - Improving the Operating Stability." Biosensors **4**(2): 109-119.

Pishko, M. V., I. Katakis, et al. (1990). "Direct Electrical Communication between Graphite-Electrodes and Surface Adsorbed Glucose-Oxidase Redox Polymer Complexes." Angewandte Chemie-International Edition in English **29**(1): 82-89.

Pizzariello, A., M. Stred'ansky, et al. (2002). "A glucose/hydrogen peroxide biofuel cell that uses oxidase and peroxidase as catalysts by composite bulk-modified bioelectrodes based on a solid binding matrix." Bioelectrochemistry **56**(1-2): 99-105.

Prins, R., Korswage.Ar, et al. (1972). "Decomposition of Ferricenium Cation by Nucleophilic Reagents." Journal of Organometallic Chemistry **39**(2): 335-&.

Rajagopalan, R., A. Aoki, et al. (1996). "Effect of quaternization of the glucose oxidase "wiring" redox polymer on the maximum current densities of glucose electrodes." Journal of Physical Chemistry **100**(9): 3719-3727.

Ram, M. K., M. Adami, et al. (2000). "Nano-assembly of glucose oxidase on the in situ self-assembled films of polypyrrole and its optical, surface and electrochemical characterizations." Nanotechnology **11**(2): 112-119.

Ramachandran, K. B. and D. D. Perlmutter (1976). "Effects of Immobilization on Kinetics of Enzyme-Catalyzed Reactions .1. Glucose Oxidase in a Recirculation Reactor System." Biotechnology and Bioengineering **18**(5): 669-684.

Rechnitz, G. A. (1987). "Biosensors - an Overview." Journal of Clinical Laboratory Analysis **1**(3): 308-312.

Reynes, O., J. C. Moutet, et al. (2002). "(Ferrocenylmethyl)trimethylammonium cation: a very simple probe for the electrochemical sensing of dihydrogen phosphate and ATP anions." New Journal of Chemistry **26**(1): 9-12.

Reynes, O., J. C. Moutet, et al. (2004). "Electrochemical sensing of anions by (ferrocenylmethyl)trialkylammonium cations in homogeneous solution and in polymer films." Electrochimica Acta **49**(22-23): 3727-3735.

Rhoads, H. L., O. Matarredona, et al. (2004). "Dispersion study of Single Walled Carbon Nanotubes in sodium dodecylbenzenesulfonate." Abstracts of Papers of the American Chemical Society **227**: U461-U461.

Ruegg, U. T. and F. Hefti (1984). "Growth of Dissociated Neurons in Culture Dishes Coated with Synthetic Polymeric Amines." Neuroscience Letters **49**(3): 319-324.

Saji, T., K. Hoshino, et al. (1985). "Reversible Formation and Disruption of Micelles by Control of the Redox State of the Head Group." Journal of the American Chemical Society **107**(24): 6865-6868.
Saji, T., K. Hoshino, et al. (1985). "Reversible Formation and Disruption of Micelles by Control of the Redox State of the Surfactant Tail Group." Journal of the Chemical Society-Chemical Communications(13): 865-866.
Saveant, J. M. (1980). "Catalysis of Chemical-Reactions by Electrodes." Accounts of Chemical Research **13**(9): 323-329.
Schlapfe.P, W. Mindt, et al. (1974). "Electrochemical Measurement of Glucose Using Various Electron-Acceptors." Clinica Chimica Acta **57**(3): 283-289.
Schmidtke, D. W., A. C. Freeland, et al. (1998). "Measurement and modeling of the transient difference between blood and subcutaneous glucose concentrations in the rat after injection of insulin." Proceedings of the National Academy of Sciences of the United States of America **95**(1): 294-299.
Schmidtke, D. W. and A. Heller (1998). "Accuracy of the one-point in vivo calibration of "wired" glucose oxidase electrodes implanted in jugular veins of rats in periods of rapid rise and decline of the glucose concentration." Analytical Chemistry **70**(10): 2149-2155.
Shepherd, E. J. and J. A. Kitchener (1956). "The Ionization of Ethylenimine and Polyethylenimine." Journal of the Chemical Society: 2448-2452.
Sirkar, K. and M. V. Pishko (1998). "Amperometric biosensors based on oxidoreductases immobilized in photopolymerized poly(ethylene glycol) redox polymer hydrogels." Analytical Chemistry **70**(14): 2888-2894.
Smits, R. G., G. J. M. Koper, et al. (1993). "The Influence of Nearest-Neighbor and Next-Nearest-Neighbor Interactions on the Potentiometric Titration of Linear Poly(Ethylenimine)." Journal of Physical Chemistry **97**(21): 5745-5751.
Sullivan, B. P., D. J. Salmon, et al. (1978). "Mixed Phosphine 2,2'-Bipyridine Complexes of Ruthenium." Inorganic Chemistry **17**(12): 3334-3341.
Suzaki, Y., M. Horie, et al. (2006). "Structure and properties of protonated N-alkyl-2-aza[3]ferrocenophanes." Journal of Organometallic Chemistry **691**(15): 3403-3407.
Tamaki, T., T. Ito, et al. (2007). "Immobilization of hydroquinone through a spacer to polymer grafted on carbon black for a high-surface-area biofuel cell electrode." Journal of Physical Chemistry B **111**(34): 10312-10319.
Uang, Y. M. and T. C. Chou (2002). "Criteria for designing a polypyrrole glucose biosensor by galvanostatic electropolymerization." Electroanalysis **14**(22): 1564-1570.
Uang, Y. M. and T. C. Chou (2003). "Fabrication of glucose oxidase/polypyrrole biosensor by galvanostatic method in various pH aqueous solutions." Biosensors & Bioelectronics **19**(3): 141-147.
Updike, S. J. and G. P. Hicks (1967). "Enzyme Electrode." Nature **214**(5092): 986-&.
Vadgama, P. and P. W. Crump (1992). "Biosensors - Recent Trends - a Review." Analyst **117**(11): 1657-1670.
Valincius, G., G. Niaura, et al. (2004). "Anion effect on mediated electron transfer through ferrocene-terminated self-assembled monolayers." Langmuir **20**(16): 6631-6638.

Vreeke, M., R. Maidan, et al. (1992). "Hydrogen-Peroxide and Beta-Nicotinamide Adenine-Dinucleotide Sensing Amperometric Electrodes Based on Electrical Connection of Horseradish-Peroxidase Redox Centers to Electrodes through a 3-Dimensional Electron Relaying Polymer Network." Analytical Chemistry **64**(24): 3084-3090.

Vreeke, M. S., K. T. Yong, et al. (1995). "A Thermostable Hydrogen-Peroxide Sensor-Based on Wiring of Soybean Peroxidase." Analytical Chemistry **67**(23): 4247-4249.

Wagner, J. G., D. W. Schmidtke, et al. (1998). "Continuous amperometric monitoring of glucose in a brittle diabetic chimpanzee with a miniature subcutaneous electrode." Proceedings of the National Academy of Sciences of the United States of America **95**(11): 6379-6382.

Wang, B. Z. and J. Anzai (2007). "Redox reactions of ferricyanide ions in layer-by-layer deposited polysaccharide films: A significant effect of the type of polycation in the films." Langmuir **23**(13): 7378-7384.

Wang, J., J. Liu, et al. (1994). "Highly Selective Membrane-Free, Mediator-Free Glucose Biosensor." Analytical Chemistry **66**(21): 3600-3603.

Wang, Y. D., P. P. Joshi, et al. (2006). "Nanostructured biosensors built by layer-by-layer electrostatic assembly of enzyme-coated single-walled carbon nanotubes and redox polymers." Langmuir **22**(23): 9776-9783.

Weyts, K. F. and E. J. Goethals (1989). "Back titration of linear polyethylenimine: Decrease of pH by addition of sodium hydroxide." Die Makromolekulare Chemie, Rapid Communications **10**(6): 299-302.

Willner, I., Y. Eichen, et al. (1993). "Photoinduced Electron-Transfer Processes Using Organized Redox-Functionalized Bipyridinium Polyethylenimine Tio2 Colloids and Particulate Assemblies." Journal of Physical Chemistry **97**(28): 7264-7271.

Willner, I., V. Heleg-Shabtai, et al. (1996). "Electrical wiring of glucose oxidase by reconstitution of FAD-modified monolayers assembled onto Au-electrodes." Journal of the American Chemical Society **118**(42): 10321-10322.

Wollenberger, U., V. Bogdanovskaya, et al. (1990). "Enzyme Electrodes Using Bioelectrocatalytic Reduction of Hydrogen-Peroxide." Analytical Letters **23**(10): 1795-1808.

York, S., R. Frech, et al. (2001). "A comparative vibrational spectroscopic study of lithium triflate and sodium triflate in linear poly(ethylenimine)." Electrochimica Acta **46**(10-11): 1533-1537.

Zhang, Y. C., A. Pothukuchy, et al. (2004). "Detection of similar to 10(3) copies of DNA by an electrochemical enzyme-amplified sandwich assay with ambient O-2 as the substrate." Analytical Chemistry **76**(14): 4093-4097.