AMPEROMETRIC GLUCOSE BIOSENSOR BY MEANS OF ELECTROSTATIC LAYER-BY-LAYER ADSORPTION ONTO ELECTROSPUN POLYANILINE FIBERS

A Thesis

by

YOUNG JAE SHIN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2009

Major Subject: Electrical Engineering

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Approved by:

Chair of Committee,	Jun Kameoka
Committee Members,	Byung Jun Yoon
	Xing Cheng
	Ibrahim Karaman
Head of Department,	Costas Georghiades

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ABSTRACT

Amperometric Glucose Biosensor by Means of Electrostatic Layer-by-layer Adsorption onto Electrospun Polyaniline Fibers.

(May 2009)

Young Jae Shin, B.S., Korea Advanced Institue of Science and Technology

Chair of Advisory Committee: Dr. Jun Kameoka

An amperometric glucose biosensor was fabricated using electospun polyaniline fibers. Polyaniline was reacted with camphorsulfonic acid to produce a salt, which was then dissolved in chloroform containing polystyrene. Using this solution, fibers were formed and collected by electrospinning. Glucose oxidase was immobilized onto these fibers using an electrostatic layer-by-layer adsorption technique. In this method, poly(diallyldimethylammonium chloride) was used as the counter ion source. The level of adsorption was examined and evidence of layer-by-layer adsorption was obtained using a quartz crystal microbalance technique. A biosensor was fabricated from these fibers as a working electrode, and used to measure the glucose concentration accurately.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Kameoka, and my committee members, Dr. Yoon, Dr. Su, and Dr. Karaman, for their guidance and support throughout the course of this research. I would like to express my appreciation to Dr. Soriaga for using a potentiostat and Dr. Teizer for conducting QCM experiments.

Thanks also go to my friends and colleagues and the department faculty and staff for making my time at Texas A&M University a great experience. I also want to extend my gratitude to the National Science Foundation under Grandt No. NSF-CMMI-0809283, which provided resources, and to all students who were willing to participate in the study.

Finally, thanks to my mother and father for their love and to my brother for his encouragement.

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1. INTRODUCTION

An amperometric biosensor is an analytical device that converts the concentration of an analyte into an electrical signal by integrating biological sensing. Biosensors are portable, simple-to-use and high specificity analytical tools. Therefore, biosensors are expected to have promising applications in a variety of fields, such as pharmacy, health care, pollution monitoring, food and agricultural product processing etc. During the previous 20 years several attempts have been made to create sensitive, selective, reliable, and low cost glucose sensors due to the clinical significance of measuring the blood glucose levels.[1,2]

The reaction in a glucose biosensor used to determine the glucose concentration is as follows.

$$\text{glucose} + O_2 \rightarrow \text{gluconic acid} + H_2O_2 \qquad (1)$$

$$H_2O_2 \rightarrow O_2 + 2\,H^+ + 2\,e^- \qquad (2)$$

The reaction in equation (1) proceeds on glucose oxidase (GOD). The amount of hydrogen peroxide produced in equation (1) is normally measured using an amperometric method by oxidation at the working electrode according to equation (2).

Polyaniline is one of the most studied conducting polymers on account of its excellent stability in different solutions and good electronic properties. Polyaniline has a

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This thesis follows the style of Journal of Applied Polymer Science.

series of structural formulas consisting of oxidized imine and reduced amine. The average oxidation state can be varied from 1 to zero to provide three principal forms of polyaniline: fully oxidized, 'pernigraniline'; half oxidized, 'emeraldine'; and fully reduced, 'leucoemeraldine'. Previously, research on polyaniline was difficult because polyaniline is insoluble in organic solvents. Fifteen years ago, it was reported that polyaniline could be dissolved in organic solvents, such as chloroform, if it was doped with an acid with a large molecular weight, such as camphorsulfonic acid, .[3-6] Since this report, there have been many studies using polyaniline.

Electrospinning is a simple and versatile method for producing fibers from a variety of materials with fiber diameters ranging from tens of nanometers to several micrometers. During the electrospinning process, fibers are produced under a high voltage electrostatic field between the metallic nozzle of a syringe and a metallic collector. The charged polymer solution is jetted from the metal needle to the grounded collector. In the working distance, the polymer jet elongates, solidifies, and deposits on the collector. The fibers are deposited in the form of a non-woven fabric onto the target collector through a random deposition process of a projected jet of the polymer solution. The electrospun fibrous membrane has a high specific area and porous structure, which makes them excellent candidates for filtration, drug delivery systems, tissue engineering, wound dressing, sensors, and enzyme immobilization. Fibrous membranes have many attractive features when used as supports for enzyme immobilization These include a large surface area for the attachment of enzymes, a fibrous morphology to improve the mass-transfer rate of the substrate, and a membrane-like structure for easy recovery from

the reaction media and continuous operations in a bioreactor. The high surface area to volume ratio makes electrospun conducting polymer fibers particularly interesting for sensing applications.[7-10]

Various enzyme immobilization methods have been used in the field of biosensors, e.g. the crosslinking method with glutaraldehyde, reactions to produce covalent bonding, entrapment by an electrostatic interaction and a sol-gel reaction. All of these methods have their own merits and demerits. Hence, many studies have been carried out to increase their individual merits.[11-21]

Layer-by-layer films assembled by the alternate adsorption of a charged protein and oppositely charged species from solutions through electrostatic interactions have been reported.[22] Under the appropriate conditions, the protein can be assembled with many oppositely charged materials, such as polyions,[23-25] inorganic nanoparticles,[26-29] sol-gel-derived inorganic materials,[30-33] etc.

In this study, an amperometric glucose biosensor was fabricated using electrospun polyaniline fibers. Glucose oxidase was immobilized on the polyaniline fibers using an electrostatic layer-by-layer adsorption method.

2. EXPERIMENTAL

2.1 Materials and instrument

Polystyrene (M_w 350,000, M_n 170,000), camphorsulfonic acid (CSA), titanium(IV) butoxide, poly(diallyldimethylammonium chloride) (PDDA), glucose oxidase (GOD), glucose, chloroform, polyaniline (emeraldine base, M_w 300,000), sulfuric acid, hydrogen peroxide, 2-mercaptoethanol, ethanol, toluene were purchased from Sigma-Aldrich Co.

A 0.400 M stock glucose solution was prepared daily and stored overnight to reach mutarotational equilibrium prior to use.

A syringe pump (Harvard Apparatus) and a high-voltage power supplier (Bertan 230) were used in the electrospinning apparatus. Quartz resonators (USI System, Fukuoka, Japan), power supplier (Eslaw S20TT), and frequency counter (hp 5315A) were used in the quartz crystal microbalance (QCM) technique. Chronoamperometric detection was carried out using a potentiostat (BAS Votammograph CV-27) and digital converter (e-corder 201 eDAQ). Scanning electron microscopy (SEM) was performed using a Jeol 6400 scanning electron microscope.

2.2 Electrospinning of polyaniline with polystyrene

The solution for electrospinning was formed as follows. 0.66 g of polyaniline and 0.84 g of CSA were dissolved in 10 mL of chloroform. Subsequently, 2.0 g of polystyrene was added to this solution. The solution was stirred magnetically for 18 hours to produce the solution for electrospinning. The apparatus for electrospinning included a glass syringe, an 18 gauge stainless-steel needle, a syringe pump, a high-voltage power supplier, and an aluminum foil as the collector. The polymer solution was drawn from the needle tip with an electrostatic force generated from the high voltage applied between the tip and grounded collector. The polymer solution formed a Taylor cone and jetted through the tip of the needle to the collector. The flow rate of the polymer solution was 0.5 mL/hr, the applied voltage was 15 kV, and the distance between the needle tip and collector was 20 cm. The nanofiber was deposited on the collector in the form of a non-woven mat. These fibers were used after drying in a vacuum oven at 50 ℃ for 5 hr.

In the case of using polystyrene only, 3 g of polystyrene was dissolved in 10 mL of chloroform. This solution was used for electrospinning under the above conditions.

2.3 Immobilization of glucose oxidase

0.10 g of the dried fibers was placed into 20 mL of a 0.10 M titanium(IV) butoxide in ethanol solution for 3 min. The fibers were then rinsed with in pure ethanol

for 30 sec, followed by another rinse with fresh ethanol. The fibers were then placed into distilled water for 1 min. These fibers were then added to 20 mL of 2.0 wt% PDDA aqueous solution for 30 min, rinsed with distilled water for 1 min, followed by a further rinse with fresh distilled water. The fibers were then placed into 20 mL of 2.0 mg/mL GOD in a pH 7 phosphate buffer solution for 60 min, rinsed with distilled water for 30 sec, followed by another rinse with fresh distilled water.

When several layers of GOD were required, both PDDA and GOD adsorption processes were repeated alternately.

2.4 QCM experiment

QCM measurements were used to monitor film growth after each adsorption step. In this measurement, the resonator was immersed in a polyelectrolyte solution for a given period, dried with nitrogen, and the change in frequency was measured. The quartz resonators were covered with gold electrodes on both faces and their resonance frequency was 9 MHz (AT-cut). The surface roughness factor of these electrodes was previously estimated to be 1.1 (± 5%) by scanning electron microscopy.[33] The reproducibility was ± 2 Hz over 2 hrs.

The increase in mass [M (g)] for adsorption was estimated from the QCM frequency shift [$\triangle$F (Hz)] of the dry films using the Sauerbery equation.[34] The following equation was derived considering the resonator characteristics:

$$\triangle F = -1.832 \times 108\ M/A$$

where A = 0.16 ± 0.01 cm^2, which is the apparent area of the microbalance electrodes. A 1 Hz change in $\triangle F$ corresponds to 0.87 ng. The film thickness (d) on both sides of the electrode was estimated using the following equation:[34]

$$d\ (nm) \approx -0.16\ \triangle F\ (Hz)$$

The density for polyion films and proteins was assumed to be 1.2 ± 0.1 g/cm3 and 1.3 ± 0.1 g/cm^3, respectively.

2.5 Measurement of adsorbed amount using QCM technique

The surface of the resonator covered with gold was cleaned with a piranha solution (sulfuric acid: hydrogen peroxide = 3:1, v/v). The surface was then washed with distilled water and dried with nitrogen gas. The resonator was placed into 3 mL of a 10 mM 2-mercapotoethanol in ethanol solution for 12 hr. The resonator was immersed in distilled water for 30 min. The resonator surface was rinsed 3 times with distilled water, and dried with nitrogen gas. The change in frequency was then measured.

The resonator coated with 2-mercapotoethanol was placed into 3 mL of a 100 mM titanium(IV) butoxide in ethanol solution for 3 min. The resonator was then rinsed twice with pure ethanol for 30 sec each. The resonator was then immersed in distilled water for 1 min and dried with nitrogen gas. The change in frequency was measured.

After measuring the change in frequency, the resonator was placed into an aqueous 2 wt% PDDA solution for 30 min. The resonator was then rinsed twice with

distilled water for 1 min each and dried with nitrogen gas. The change in frequency was then measured.

The resonator coated with PDDA was placed into 3 mL of a pH 7 phosphate buffer solution containing 2.0 mg/mL GOD for 60 min to immobilize the GOD on the fibers. The resonator was rinsed twice with distilled water for 30 sec each, and dried with nitrogen gas. The change in frequency was then measured.

Many GOD layers were obtained by repeating the PDDA coating and GOD immobilizing processes alternately. In every step, the resonator was dried with nitrogen gas, and the change in frequency was measured.

2.6 Characterization of glucose biosensor

A glucose biosensor was fabricated in 40 mL of a pH 7 phosphate buffer solution using the fiber mat onto which GOD had been immobilized as a working electrode. All the electrochemical experiments were carried out using a standard one-compartment three-electrode cell. The reference electrode was Ag/AgCl (3M KCl) and the counter electrode was a platinum wire (20 cm). All electrode potentials were referred to the reference electrode. A glucose stock solution was prepared and allowed to stand for 24 hr prior to use in order to allow the α and β anomers to equilibrate.

3. RESULTS AND DISCUSSION

3.1 Electrospinning of polyaniline with polystyrene

A biosensor was fabricated using the electrospun fibers. The polymer solution for electrospinning was made by dissolving polystyrene and polyaniline in chloroform. Polystyrene forms a fiber by electrospinning and polyaniline is a conducting polymer. However, polyaniline alone cannot form a fiber with sufficient mechanical properties by electrospinning. Therefore, polystyrene was used as a support to form a fiber with conducting polyaniline. Polyaniline is a conducting polymer with several molecular formulas according its oxidation state. Previously, there was considerable difficulty in applying polyaniline because polyaniline is insoluble in organic solvents. Fifteen years ago, it was reported that polyaniline could be made soluble in organic solvents, such as chloroform, by forming a salt with an acid with a long or large alkyl chain structure, such as CSA. Since then, there have been a considerable number of studies on polyaniline.

In this study, a solution of polyaniline and CAS in chloroform was prepared and polystyrene was added. The resulting solution was used as a polymer solution to make a fiber by electrospinning.

Polyaniline was reacted with CSA to produce a salt. The molar ratio of polyaniline : CSA was 2 : 1, in which polyaniline was calculated using the aniline

monomeric unit. At this ratio, the salt of polyaniline and CSA was quite soluble in chloroform.

The polyaniline : polystyrene weight ratio used in this experiment was 1 : 3, because this was the minimum ratio of polyaniline at which this polymer blend can show sufficient conductivity. The reason for choosing the minimum ratio was to produce a fiber with sufficient conductivity but with the minimum amount of polyaniline.

Electrospinning was carried out using electrospinning equipment with the above polymer solution. Figure 1 shows SEM images of the fibers formed.

Figure 1 (a) shows the fibers of polystyrene alone, and (b) shows the fiber of a blend of polyaniline and polystyrene. The fibers had a comparatively homogeneous thickness.

Figure 2 shows the thickness distribution of the fibers estimated from the SEM images.

The mean thickness of the polystyrene and blend of the polyaniline and polystyrene fibers was 1,016 nm and 1,220 nm, respectively. The fibers of the blend have a larger mean thickness and thickness distribution than the polystyrene fibers.

The electrochemical properties of the blended polymer fibers were examined by coating the resonator of the QCM with the blended polymer solution and carrying out cyclicvoltametry in 0.5 M H2SO4. The results are shown in Figure 3.

Peaks were observed at 0.22 V and 0.76 V in the oxidation process, and at 0.68 V and 0.06 V in the reduction process. Two peaks in both processes mean that the polyaniline exists in three states in this range, as shown in Scheme 1.

There was a positive shift in the oxidation peaks in the oxidation process and a negative shift in the reduction peaks in the reduction process (figure 3), for which the internal resistance of the electrode was responsible.

3.2 Immobilization of glucose oxidase

The adhesion ability of the fiber mat that had been formed from the above experiments, was increased using titanium butoxide. Titanium butoxide was hydrolyzed in water in the last step, and a Ti-O-Ti bond was formed to increase the molecular weight. There were many hydroxyl groups remaining, particularly at the surface of the fiber. Subsequently, one layer of PDDA was stacked on this surface. This layer was sustained by an interaction between the ammonium cation in PDDA and the hydroxyl group at the under layer of TiO_2. GOD, which contains many anionic functional groups at the surface, was placed on the PDDA layer containing many ammonium cations. It was very difficult to remove the layer because there are considerable electrostatic interactions with the large number of counter ions. A layer structure with more than 10 layers could be prepared using this counter ionic interaction.

Because it is very difficult to measure the amount of material adsorbed on the fibers, an attempt was made to measure the adsorbed materials under similar conditions using the QCM technique. First, a layer structure was made on the resonator of QCM. 2-mercaptoethanol was then placed on the gold surface, which changed the surface to a hydroxyl functional group. PDDA was then placed onto this layer, and GOD was loaded

onto the PDDA layer. The layer thickness could be increased by repeating the alternate PDDA layer and GOD layer deposition processes. The decrease in frequency was checked each time.

Although the resonator of the QCM has a different figure with the fibers, the surface of the resonator of the QCM had the same functional group with the fibers. Therefore, the amount adsorbed on the fiber and the formation of a layer-by-layer structure could be estimated from the results obtained from the resonator of the QCM.

When one layer of TiO_2-titanium butoxide was loaded on the resonator, there was a 12-22 Hz decrease in frequency. This means that 11-20 ng of TiO_2 layer had formed on the resonator. The decrease in frequency was measured on 14 alternate layers of PDDA and GOD. The results are shown in figure 4.

In the case of the PDDA layer, there was a 48-71 Hz decrease in frequency. This means that 44-64 ng or 7.7-10 Å (thickness) of PDDA had adsorbed onto the surface in a single adsorption step. In case of the GOD layer, there was a 403-481 Hz decrease in frequency. This means that 363-433 ng or 58-69 Å (thickness) of GOD had adsorbed onto the surface in a single adsorption step.

Using the blend of polyaniline and polystyrene, a fiber was formed by electrospinning. One layer of TiO_2 was coated onto this fiber, and three alternate cycles of PDDA and GOD adsorption were carried out. The resulting fibers were observed by SEM. The results are shown in figure 5.

Most fibers in figure 5 had a similar thickness to that before GOD adsorption. Using the GOD-immobilized fiber mat as a working electrode, a glucose biosensor was fabricated in a 50 mL beaker. The reference electrode was Ag/AgCl (3M KCl) and the counter electrode was a platinum wire (20 cm). The system was charged with 0.500 V and a glucose stock solution was added every 20-30 sec to make a consecutive 1 mM increase in concentration every 20-30 sec. Figure 6 shows the increase in amperes as a function of time. Figure 6 a) shows the case of using the fiber mat onto which two cycles of GOD had been deposited. Figure 6 b) shows the case of using the fiber mat on to which three cycles of GOD had been deposited.

The results in figure 6 b) were more accurate and showed less deviation than the results shown in figure 6 a). Accurate data could not be obtained using the fiber mat onto which one cycle of GOD had been deposited. The best results were obtained when three cycles of GOD had been deposited on the fiber mat.

4. CONCLUSIONS

Fibers were formed by electrospinning a blended polymer solution of polyaniline and polystyrene. The affinity of the fibers was increased using titanium butoxide. More than 3 layers of GOD were immobilized on these fibers using an electrostatic layer-by-layer adsorption technique. PDDA was used as the counter ionic polymer. The amounts of the adsorption on the fibers were estimated using QCM techniques. An amperometric glucose biosensor was fabricated using these fibers as a working electrode. A precise response to the sucrose concentration was obtained when three cycles of GOD had been deposited on the fibers mat.

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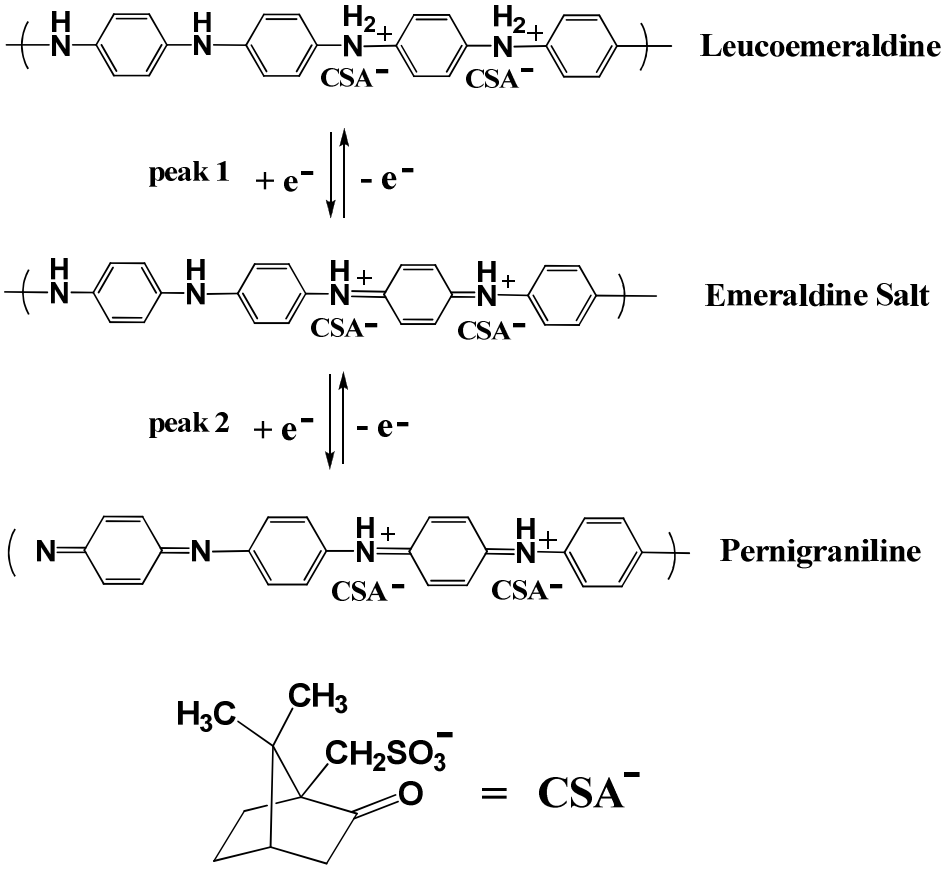
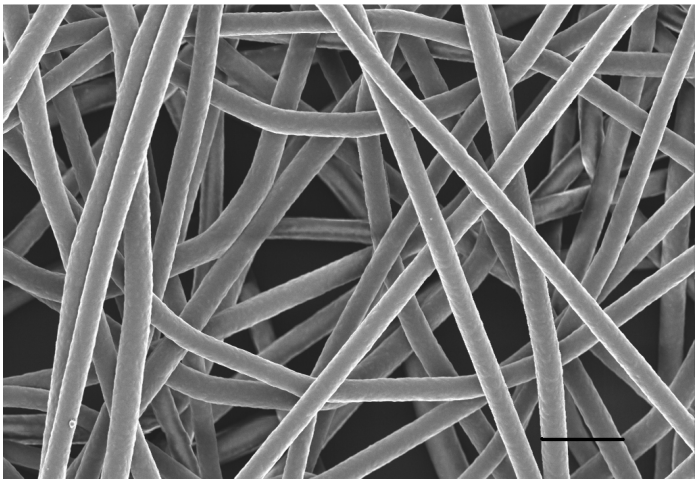
APPENDIX A

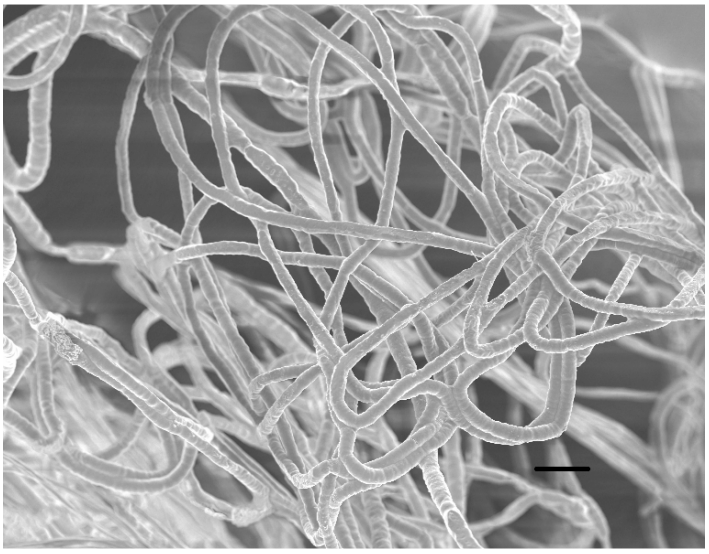


Scheme 1. Molecular formulas of polyaniline according to the oxidation state during cyclovoltametry.

APPENDIX B



(a)



(b)

Figure 1. SEM of fibers. (a) polystyrene, (b) blend of polyaniline and polystyrene. (bar represents 5 μm)

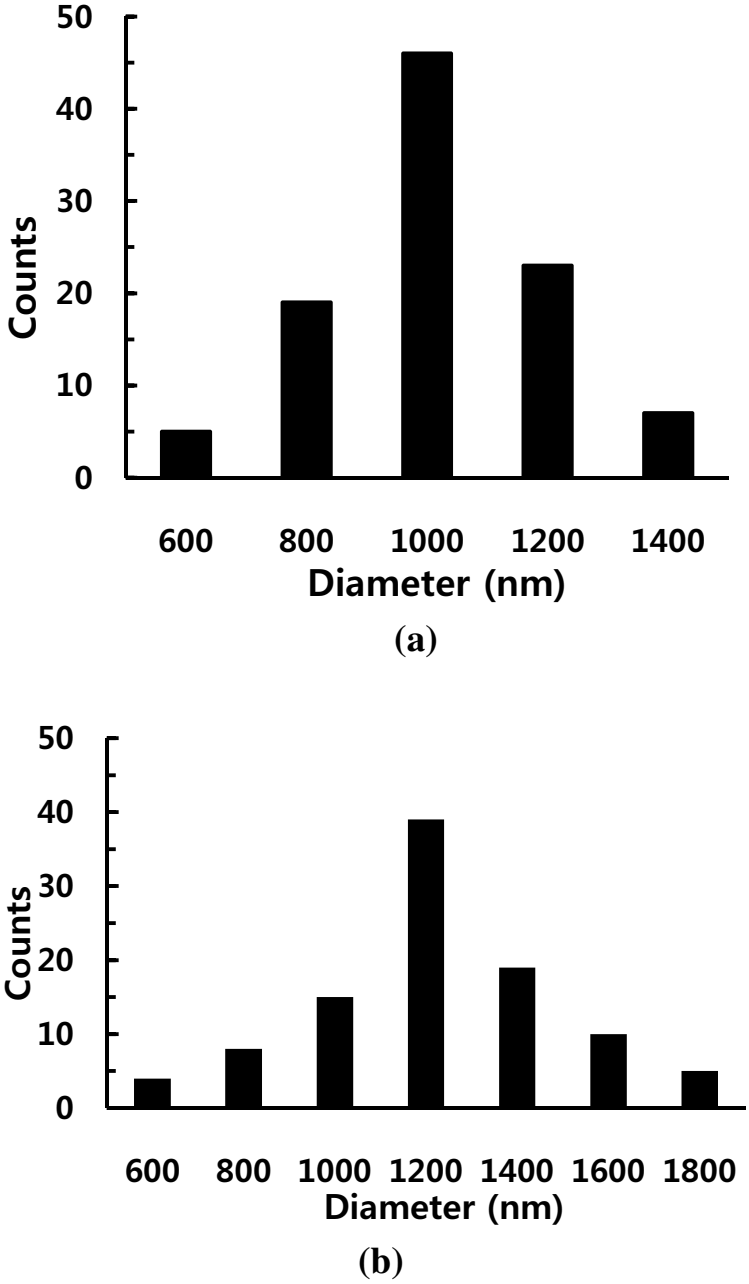


Figure 2. The thickness distribution of the fibers. (a) polystyrene, (b) blend of polyaniline and polystyrene

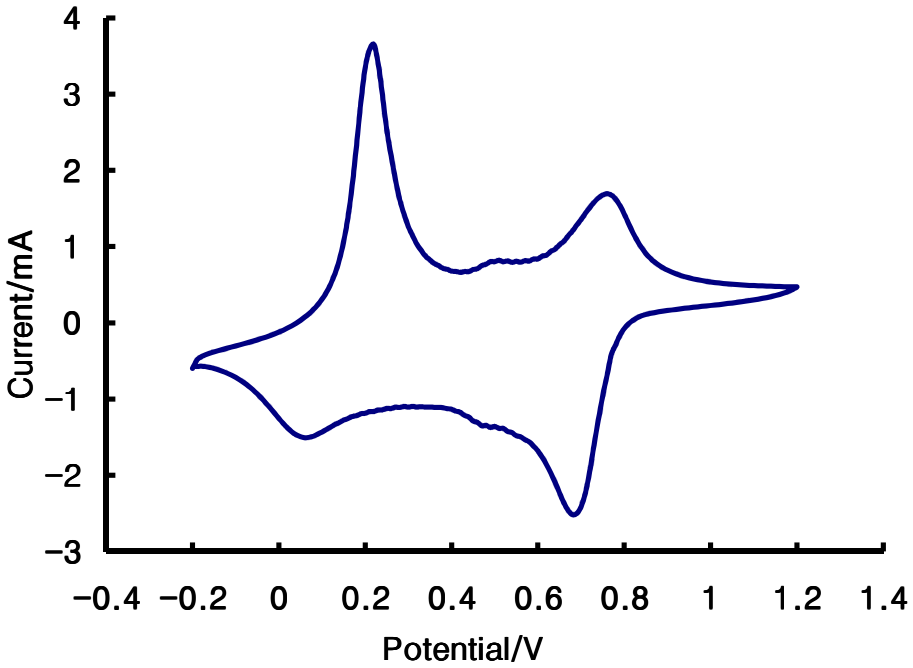


Figure 3. Cyclicvoltametry of the blend of polyaniline and polystyrene.

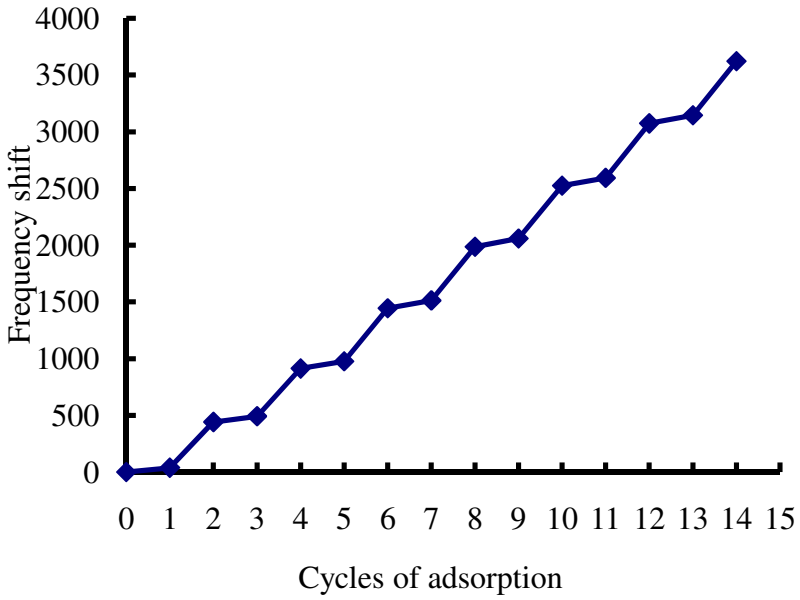


Figure 4. The frequency shift according to the adsorption of PDDA and GOD alternately.

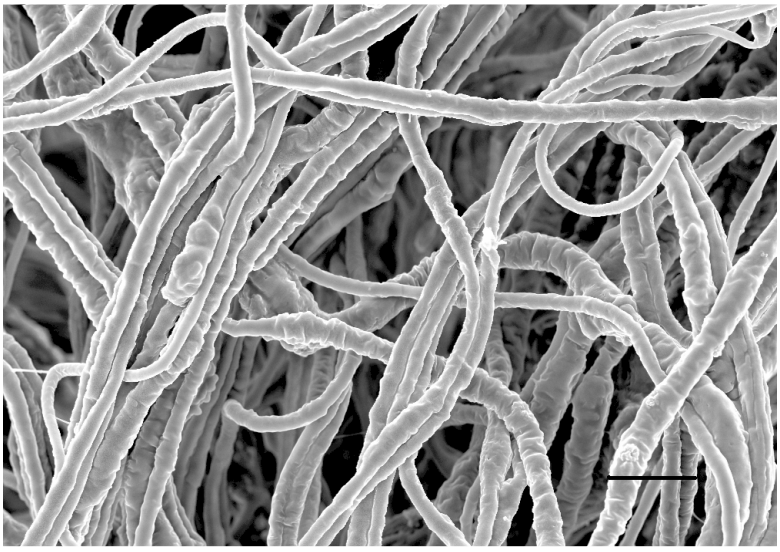
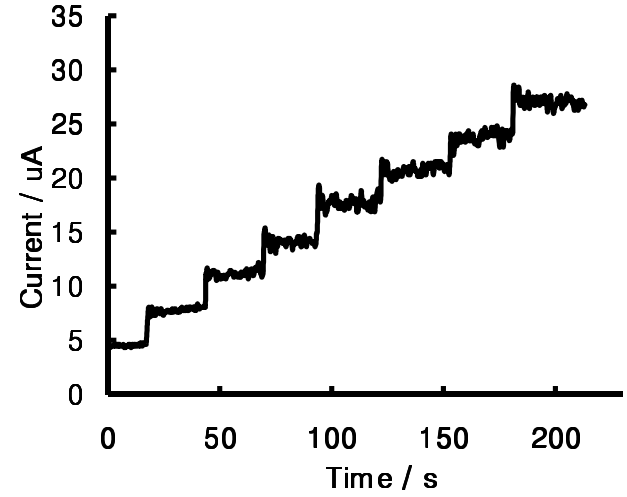
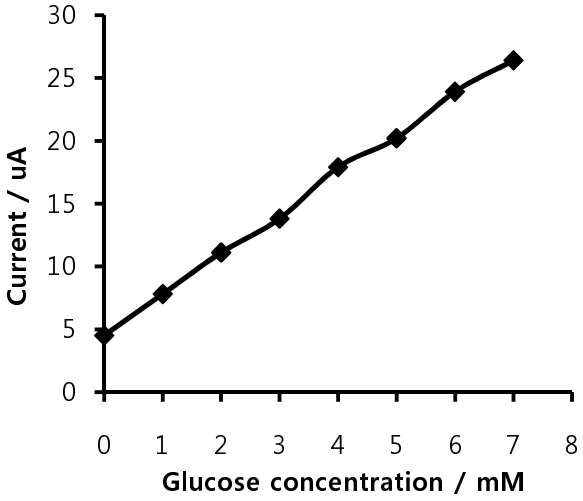


Figure 5. SEM of fibers that GOD was immobilized.
(bar represents 5 μm)

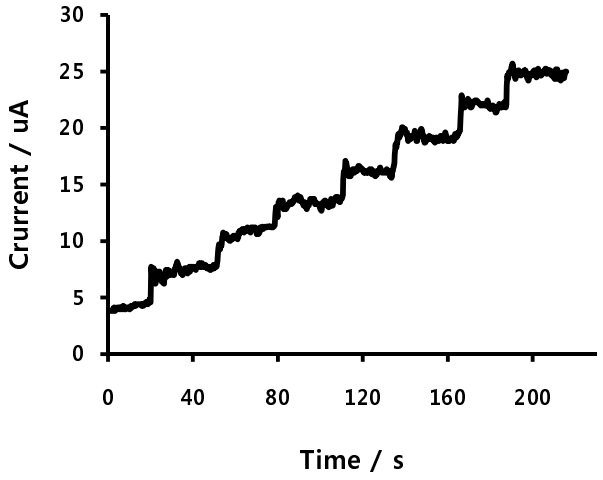


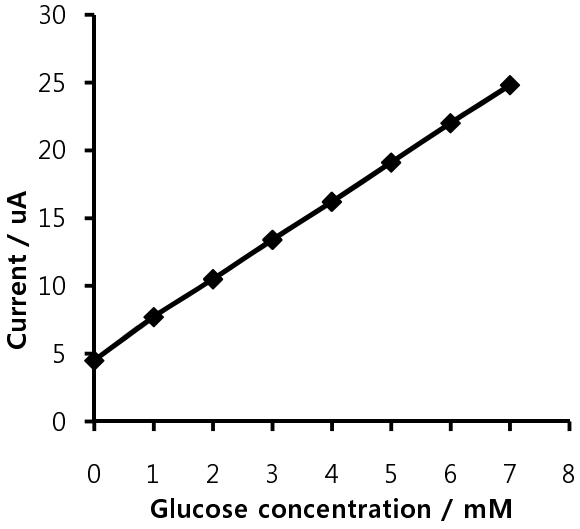


(a)

Figure 6. Amperometric detection on a consecutive 1 mM increase in glucose concentration.

a) 2 cycles of GOD adsorption
b) 3 cycles of GOD adsorption





(b)

Figure 6. Continued.

VITA

Young Jae Shin received his Bachelor of Science degree in electrical and computer engineering from The Korea Advanced Institute of Science and Technology in 2007. He entered the Electrical Engineering Department at Texas A&M University in August 2007 and received his Master of Science degree in May 2009. He wants to strengthen his interdisciplinary approach to chemistry and electrical engineering. His research interests include synthesizing and handling conducting polymeric materials, organic light-emitting materials, polymeric electrolytes, and carbon nanotube composites.

He was diagnosed with Leukemia at the age of 12 and had to have a bone marrow transplant. Leukemia taught him how to overcome adversity rather than allowing it to become a source of discouragement. Through overcoming leukemia, he knows that he can overcome any challenge in life.

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