DETECTION OF COX-2 ENZYME USING HIGHLY SENSITIVE ELECTROSPUN POLYANILINE NANOFIBER-BASED BIOSENSOR

A Thesis by

Zeinab Veisi

Bachelor of Science, Shiraz University 2010

Submitted to the Department of Mechanical Engineering
and the faculty of the Graduate School of
Wichita State University
in partial fulfillment of
the requirements for the degree of
Master of Science

May 2013

ELECTROSPUN POLYANILINE NANOFIBER BASED BIOSESNOR FOR SENSITIVE COX-2 ENZYME DETECTION

The following faculty members have examined the final copy of this thesis for form and content, and recommend that it be accepted in partial fulfillment of the requirement for the degree of Master of Science with a Major in Mechanical Engineering.

Ramazan Asmatulu, Committee Chair

Anil Mahapatro, Committee Member

Hamid Lankarani, Committee Member

DEDICATION

To my family and friends

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Ramazan Asmatulu for his support and inspiration throughout my Master’s Degree. I would like to express my sincere gratitude to my committee member and co-advisor, Dr. Anil Mahapatro, for his continuous guidance and support in conducting the experiments and completing the research. My deepest appreciation goes to member of my thesis committee, Dr. Hamid Lankarani, for his valuable comments and suggestions on this project.

ABSTRACT

This research attempted to determine the practicality of the integration of electrospun polyaniline nanofiber as the main sensing component into interdigitated gold microelectrodes to develop a biosensor platform for sensitive, selective, and label-free detection the of Cyclooxygenase-2 (COX-2) biomarker from pure and human serum samples. COX-2 is an important enzyme in pain biomarkers, inflammation and cancer cell proliferation, so it is necessary to develop a reliable biosensor that can sensitively and objectively quantify COX-2 enzyme expression for clinical diagnosis.

Polyaniline nanofibers were prepared at four different diameters using electrospinning performed at four different flow rates. The performance of the electrospun polyaniline nanofiber based biosensor was evaluated in comparison with a plain control biosensor using electrochemical impedance spectroscopy. Significant improvement was observed in the sensitivity of the electrospun polyaniline nanofiber based biosensor revealing the remarkable capability of electrospun polyaniline nanofiber in robust and rapid detection of the COX-2 biomarker. This improvement was attributed to the large specific surface area of electrospun polyaniline nanofiber as well as its highly porous structure which enhances size-matched confinement, transduction and signal strength, thus increasing the sensitivity of the biosensor significantly. The fabricated nanofiber based biosensor was able to detect the target antigen with concentrations as low as 0.01pg/ml and 10fg/ml in pure and human serum samples, respectively, as well as remarkable selectivity towards Human Serum Albumin suggesting the significant contribution of this nanofiber based platform to the enhanced strength and sensitivity in COX-2 analyte detection.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

NOMENCLATURE

AA	Arachidonic Acid
COX-2	Cyclooxygenase
DTSSP	(3,3´-Dithiobis[sulfosuccinimidylpropionate])
ELISA	Enzyme Linked Immunoassay
EIS	Electrochemical Impedance Spectroscopy
HCSA	Camphorsulfonic Acid
IDT	Interdigital Electrode
LOD	Limit of Detection
LSPR	Localized Surface Plasmon Resonance
PANI	Polyaniline
PBS	Phosphate Buffered Saline
PDMS	Polydimethylsiloxane
PGH_2	Prostaglandin H2
POCT	Point-of-Care Diagnostic Testing
PS	Polystyrene
QCM	Quartz Crystal Microbalance
R & D	Research and Development
SERS	Surface Enhanced Raman Scattering
SPR	Surface Plasmon Resonance

LIST OF SYMBOLS

µm	Micrometer
nm	Nanometer
ml	Milliliter
fg	Femto-gram
pg	Pico-gram
ng	Nano-gram
µg	Micro-gram
°	Degree
C	Celsius
kV	Kilovolt
Hz	Hertz
kHz	Kilohertz

CHAPTER 1

INTRODUCTION AND BACKGROUND

1.1 Background

A sensor is a transducer that converts a quantity or a parameter into a signal that carries information in a measurable manner. In other words, a sensor converts one form of energy into another form and detects and converts the information to an external measurable quantity. Although not noticeable, sensors can be found everywhere monitoring different parameters such as temperature, humidity, pressure, vibration, volume, velocity, fluid flow, etc. in different industries such as manufacturing, process industries, healthcare and medical diagnostics, defense applications, food, agriculture, environmental and industrial monitoring .

Biosensors are specific types of sensors that are capable of detecting various biological elements. A biosensor is usually comprised of two parts: a biological sensing element and a transducer. While the role of the biological sensing element is to interact with the analyte, transducer's roll is producing a measurable signal proportional to analyte concentration.

1.2 Motivation for Biosensor Development

Developing practical methods to sense and quantify biological interactions has gained so much attention in recent research due to the urgent need for sensor technology in healthcare, food, agriculture, environmental and industrial monitoring. In spite of numerous publications and investments in research on developing practical biosensors, commercialization of developed biosensors has faced different barriers. This slow transformation could be attributed to the limitations of current biosensor technology such as an inability to perform parallel sensing, instability, long process time, contamination, signal drift and cost [1]. It is estimated that by the year 2015 the global market for biosensors will reach 12 billion U.S. dollars. This increase is led by the high demands in the medical/health sector for blood glucose monitoring, drug discovery, drug analysis, and whole blood analyzers. To satisfy the high market demands, a plethora of research and development (R & D) activities is taking place, and industrial and university researchers have been dedicated to improve existing sensor technology or develop new biosensors with higher accuracy, sensitivity, compact design, parallel sensing and lower cost. In order to advance sensor technology and be responsive to high market demands, upfront investments are essential. It is crucial to incorporate new trends in different areas of technology including leading-edge integrated circuitry, wireless technology, nanotechnology and miniaturization into sensor technology to develop highly competent sensors that can be substitutes for conventional lab-based procedures. Introducing nanotechnology in the field of biosensors has generated remarkable progress leading to the development of compact, affordable and highly sensitive nano-biosensors. Novel properties of nanomaterials can improve biosensors' efficiency and enable real-time protein detection.

In this research, a polyaniline nanofiber based biosensor was designed, developed, and characterized. The fabricated biosensor was tested for protein detection. Polyaniline nanofibers were fabricated in four different diameters using four different flow rates of 0.5, 1, 2, and 3 ml/hr. Generated nanofibers were applied on gold based microelectrode platforms. Cyclooxygenase-2 (Cox-2) protein was used as the model target analyte. Developed biosensors were characterized using electrical impedance spectroscopy (EIS) to evaluate their potential as portable diagnostic devices for early disease detection.

1.3 Scientific Motivation

Biosensor technology has received a great deal of interest recently and has been expected to play a significant role in health care and medical diagnostic. Early detection of disease, miniaturization, low cost, ease of operation, and parallel detection are vital priorities in the health care industry. Such requirements pose a great challenge in biosensor technology. Development of new approachable trends is vital to generate devices for faster and point-of-care diagnostic testing (POCT). A significant investment in research has been focused on developing portable lab-on-chip devices that are capable of point of care detection. The motivation for choosing research work on the design and fabrication of a nanotechnology enabled biosensor for highly sensitive protein detection was due to the current limitations of diagnostic devices available in the market. In spite of biosensors which are designed to detect one or few target elements, the biosensor produced in this research work is a versatile design with high capability to detect interchangeable biorecognition elements. Incorporation of polyaniline nanofibers has made it possible to produce a promising device that possesses many distinctive features including miniaturization, ease of operation, high selectivity, and a low detection limit. These novel features combined with the ability of parallel sensing have made this biosensor a very fascinating alternative to conventional biological analytic techniques.

Enzyme-linked immunoabsorbent assay (ELISA) has been extensively used for the protein detection and considered as the standard technique in immunoassay detection due to its high sensitivity, detection at relevant concentrations, high throughput of samples and competences [2]. Despite the system reliability, recent diagnostic techniques are limited by tedious assay procedures, bulky equipment, availability of trained personnel, time consuming assay preparation, and complex procedures. Even though automated ELISA instruments have been offered to improve the traditional ELISA, high therapeutic time and high cost are still serious problems.

Portable lab on chip devices can provide an alternative to traditional diagnostic facilities. They can provide information immediately with quick diagnostic tests. Due to the sensitivity of the chips, the required sample is minimal and that also has the benefit of early detection, since at earliest stages of a disease, the biomarkers in the blood are low in number. Using nanotechnology on the other hand, has the potential to revolutionize point-of-care medicine by a generation of cheaper, smarter, and smaller devices. Recent studies have been focused on miniaturization which seems feasible due to the significant advances in the micro and nano technologies. With the novel properties of nanomaterials, it is possible to generate portable label free biosensors compact in size, with high sensitivity, real time detection, and low sample volume consumption.

In this research, the incorporation of electrospun polyaniline nanofibers into a lab-on-chip platform was explored. Performances of fabricated biosensors were tested to explore the effect of using polyaniline nanofibers in ultrasensitive COX-2 detection.

1.4 Project Goals

In this research work, the effect of using highly conductive polyaniline nanofiber as a transducer in developing an ultrasensitive protein detection device was assessed. The detailed objectives of this research are listed below:

- Design of a gold microelectrode chip
- Fabrication of smart architecture polyaniline nanofibers in four different diameters using electrospinning
- Integration of nanofibers with the gold microelectrode chips to create four different portable biosensors
- Detection of COX-2 protein using the fabricated biosensors

- Characterization of the fabricated biosensors by the EIS method using a Gamry potentiostat
- Evaluation of biosensors’ performance in detection of COX-2
- Finding the correlation between the fabricated biosensors’ detection limit and the prepared electrospun nanofibers diameter
- Evaluation of the incorporation of nanotechnology in the field of sensors
- Generation nanotechnology enabled biosensors for advanced diagnostic tool development
- Identifying the limitations of the proposed idea, technical barriers and future study

CHAPTER 2

REVIEW: ELECTROSPINNING

2.1 Electrospinning

Electrospinning is a flexible and versatile method to fabricate highly porous nanofibers by means of applying electrical charges. The concept of this technique as well as the experimental procedures for producing nanofibers from various polymers were patented by Formhals in 1934 [3]. Later, the shape of the polymer drop formed at the needle tip was determined to be cone shaped by Taylor's investigations which was then referred as the "Taylor Cone" [4]. Several studies were conducted in the following years which mainly focused on the nanofiber characterization and its structural morphologies [5]. In a research by Baumgarten [6] polyacrylonitrile/dimethylformamide solution was used to fabricate electrospun nanofibers. It was observed that the diameter of fibers was a function of the solution's viscosity. Moreover, it was concluded that increasing the electric field enlarged the jet diameter. Electrospinning has been the main focus of several researches, and significant attempts have been taken to understand and develop the process. Electrospun nanofibers have been extensively employed in different areas such as membranes, tissue engineering, drug delivery, sensors, electronics and etc.

Electrospinning is a promising approach for fast and flexible nanofiber production. Electrospun nanofibers have unique features such as improved mechanical properties, high surface functionality, high porosity, and high permeability which are mainly attributed to the small diameter of nanofibers and therefore a high surface area to volume ratio. The basic set up for the electrospinning process is illustrated in Figure 1.

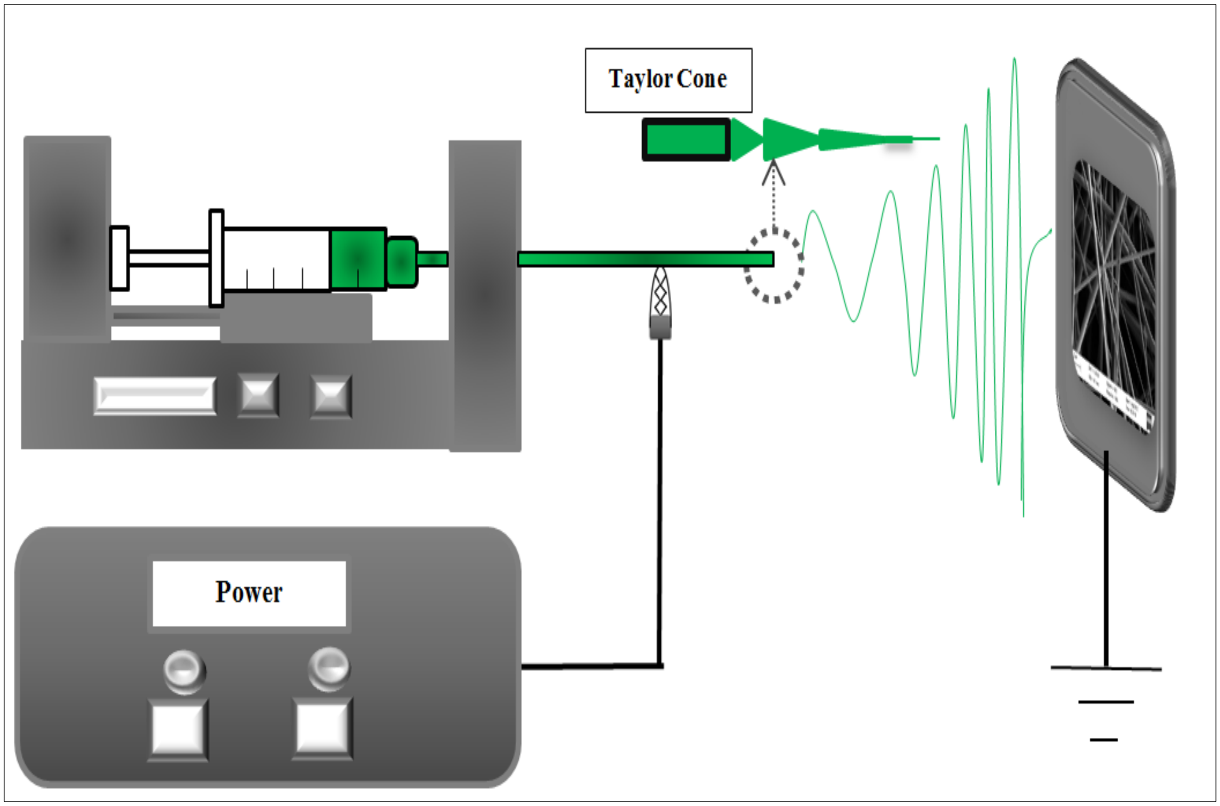

Figure 1. Schematic diagram of electrospinning process and apparatus

As observed in Figure 1, the electrospinning setup is comprised of a power supply, a collector, a pump and a syringe containing the polymeric solution. The High power supply is utilized as a source of DC voltage which is applied on the polymeric solution to produce a charged solution. The syringe is filled with polymeric solution to be spun with the help of high voltage that the electrode applies. The electrode is placed onto the tip of the needle. The pump is utilized for adjusting the polymer flow and keeping the flow rate constant during the process. The collector is a metallic grounded plate which is utilized for fabric collection. As the voltage is transferred onto the tip of the syringe via the electrode, an electrical charge is induced in the polymeric solution in the exact opposite direction of the polymer surface tension [7]. As the electrical field intensifies, the polymeric solution at the tip of the syringe stretches in a conical shape. As electrostatic force overcomes the surface tension of the polymeric solution, a jet of fluid is ejected from the tip of the cone and travels toward the collector, which acts as a grounded electrode. Meanwhile, solvent evaporation takes place, which leads to the formation of a charged and highly stretched polymeric fiber [8].

2.2 Electrospinning Parameters

Different factors can affect the electrospinning process. These parameters can be classified into three main types [9]:

- The solution properties (polymer molecular weight, surface tension, viscosity, conductivity, dielectric constant and elasticity).
- The process parameters (distance between the syringe tip and the collector, voltage, needle diameter and geometry, type of collector and hydrostatic pressure in the solution container).
- The ambient parameters (temperature, humidity, and air flow).

2.2.1 Solution Parameters

Amongst all the electrospinning parameters mentioned above, the solution parameters are the most influential factors which can significantly affect the resultant fiber's properties and morphology. For instance, the extent of jet elongation is directly related to the solution viscosity and thus fiber diameter. Therefore, it is crucial to understand the surface parameters for acquiring nanofibers with desirable properties and morphologies. TABLE 1 summarizes the polymer types and the solvents used for the electrospinning of nanofibers in the solution state.

TABLE 1

SUMMARY OF POLYMERS AND SOLVENTS USED TO PRODUCE ELECTROSPUN FIBERS IN THE SOLUTION FORM[10]

NO.	Polymer	Solvent
1	Cellulose acetate	Acetone
2	Acrylic resin (96% acrylonitrile)	DMF
3	Polyethylene oxide (PEO)	Water, Chloroform
4	Polyethylene terephthalate (PET)	Dichloromethane, Trifluoroacetic acid
5	Poly-L-lactide (PLLA)	Dichloromethane
6	Polybenzimidazole (PBI)	DMAc
7	Polyvinyl chloride (PVC)	(HFIP) and DMF
8	Polyurethane (PU)	DMF and THF
9	Polyvinyl alcohol	Water
10	Polystyrene (PS)	DMF, THF, and diethyl formamide
11	Poly (ether amide)	Hexa fluoro 2-propanol
12	Polycarbonate (PC)	Dichloromethane
13	Polyvinylcarbazole	Dichloromethane
14	Polycaprolactone	Acetone
15	Poly lactic acid (PLA)	Chloroform

2.2.1.1 Molecular Weight and Solution Viscosity

Viscosity of the solution is a function of the polymer molecular weight. In general, a solution made of polymer of a higher molecular weight has a higher viscosity compared with one made of the same polymer with a lower molecular weight. Therefore, it iscrucial to utilize a polymer with the proper molecular weight to prepare a sample with sufficient viscosity for electrospinning. When a jet of fluid is ejected from the tip of the cone and travels toward the collector, the molecule chains entanglement holds the jet and prevents its dispersion. The chain entanglement is stronger in polymers with a larger molecular Weight [11]. Various studies have been focusing on evaluating the effect of solution viscosity on fiber formation, and the results obtained revealed that a very low concentration of polymers increases the beads formation on the fiber [12]. This is attributed to the dominant influence of surface tension along the electrospinning jet [13]. On the other hand, the high viscosity of the polymeric solution prevents the fiber formation and the solution might dry at the tip. Moreover, higher viscosity leads to increase in fiber diameter due to solution resistance to stretching. This resistance affects the fiber distribution on the collector and reduces it to a smaller deposition area [14].

2.2.1.2 Conductivity

Conductivity of the solution significantly affects the fiber formation and morphology. At higher conductivities, more charges can be held by the jet which increases the extent of the jet elongation and that consequently results in thinner fiber diameters. The addition of ions (salt or polyelectrolyte) to the solution can enhance the solution conductivity and thus reduces the fiber diameters [11].

2.2.1.3 Surface Tension

Surface tension increases the tendency of the solvent molecules to congregate and form beads in low concentration solutions. Solvents with low surface tension such as ethanol or different surfactant are utilized to increase smooth fibers formation and improve the fibers morphology [15].

2.2.1.4 Dielectric Effect of Solvent

Generally speaking, using a solvent with a larger dielectric constant such as N,N Dimethylformamide [16] leads to fiber formation in smaller diameters as well as an increased deposition area due to the enhanced bending instability of the jet [17].

2.2.1.5 Evaporation of Solvent

One of the important factors which significantly determines the fiber morphology is the solvent vapor pressure. Highly volatile solvents have been utilized in several researches to fabricate fibers with smaller diameters and higher porosity [18].

2.2.2 Process Parameters

Although process parameters affect the fiber morphology and their effect on fiber formation might be less crucial when compared to the solution parameters, they still have considerable impact on the fiber formation.

2.2.2.1 Applied Voltage

The applied DC voltage can remarkably affect the jet stability and the fiber morphology. In order for the electrospinning process to start, sufficient voltage is necessary. Applied voltage induces the required charge on the solution to overcome the surface tension of the liquid. Higher voltage transfers the charge to the tip extensively, which accelerates the jet formation and therefore more volume of solution is utilized. This decreases the Taylor cone stability and size and fiber diameter. As the voltage decreases, a nice Taylor cone and uniform, bead free fibers are produced [19].

2.2.2.2 Distance between Tip and Collector

It was observed that the tip and collector distance affects the electrospinning process and fiber morphology by altering the fiber deposition time and the solvent evaporation rate. Fiber deposition time is directly related to the distance, since a longer distance increases the flight path of the jet, deposition time and solvent evaporation time. This leads to more fiber elongation and the formation of fiber with a smaller diameter. At the smaller distance, wet fiber may deposit on the collector due to the shorter flight path of the jet and deposition time.

2.2.2.3 Flow Rate

The flow rate of the polymer solution can affect the fiber morphology by affecting the jet velocity. A lower flow rate increases the solvent evaporation time and therefore a fiber with smaller diameter is obtained. When flow rate is increased, there is more solution available for spinning and a larger sized fiber is produced [20].

2.2.3 Ambient parameters

Factors in the electrospinning environment such as air velocity, humidity, and temperature affect the fiber properties and morphology. At high humidity, small pores are created on the surface of the fiber and the amount of pores increases with an increase in humidity [25]. Fiber diameter also tends to decrease with the increase in ambient temperature, which is attributed to the reduced viscosity of the polymer solution at higher temperatures [16].

CHAPTER 3

REVIEW: BIOSENSOR

3.1 Biosensor

A biosensor is a device that converts the received energy (biological or chemical stimuli) into an analytically useful signal. A sensor is usually comprised of two components connected in series: a chemical or biological recognition element (receptor) and a transducer [21]. The biological recognition element interacts with the analyte and generates energy while the transducer converts the information into a detectable output signal which is functionally proportional to analyte concentration. Biorecognition elements can provide the sensor with a high degree of selectivity since each of them respond to a specific stimulus.

The produced energy due to the interaction of biorecognition element and analyte will be transferred to the transducer part. A transducer then provides bi-directional signal transfer and sends it to a signal detector (signal processing system) where signals can be converted to quantitative or semi-quantitative information. The schematic of a typical biosensor is shown in Figure 2.

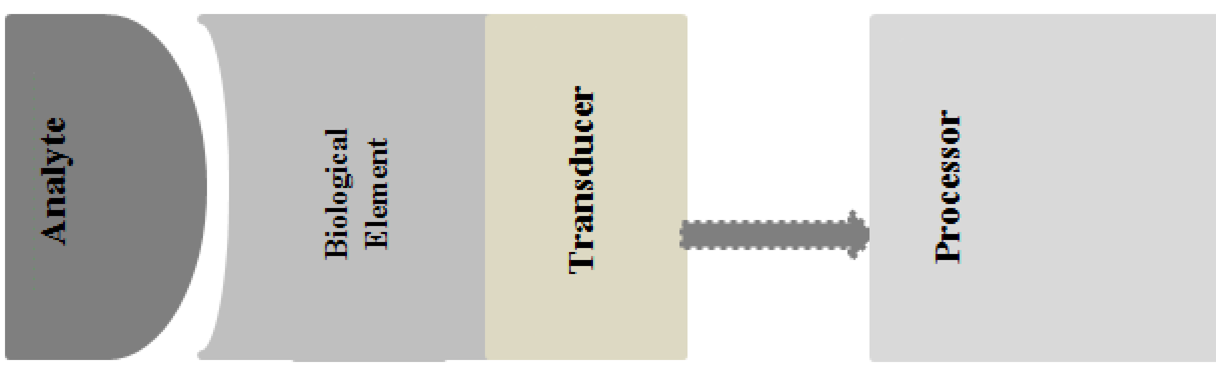

Figure 2. Schematic and working principle of a typical biosensor

Biosensors can be classified based on the type of utilized biorecognition element (receptor) into antibody, enzyme, nucleic acid, and whole cell categories. Another method of biosensor classification is based on the type of the transducer employed including electrochemical, optical, piezo-electric, and thermal types. Both receptor and transducer performance and type, are influential parameters in operational characteristics of a biosensor.

3.1.1 Transduction Platforms

One of the important features of a biosensor is its sensitivity, which is the speed of transferring sensed complex interaction to a detectable signal. As mentioned earlier, a transducer converts the physical or chemical interactions into a measurable signal. Therefore the sensor's sensitivity or response time is defined by the speed of a transducer in converting the interactions into signals. In other words, the sensitivity of a biosensor is directly determined by the transducer's performance and type. Various transduction platforms can be utilized for sensing. The main transduction platforms are electrical (conductometric and capacitive), electrochemical, and optical and mechanical (solid state, and acoustic wave based) transduction.

Figure 3 summarizes major transduction platforms currently utilized for biosensor applications. Incorporating these platforms with nanomaterials can enhance their performance and sensitivity.

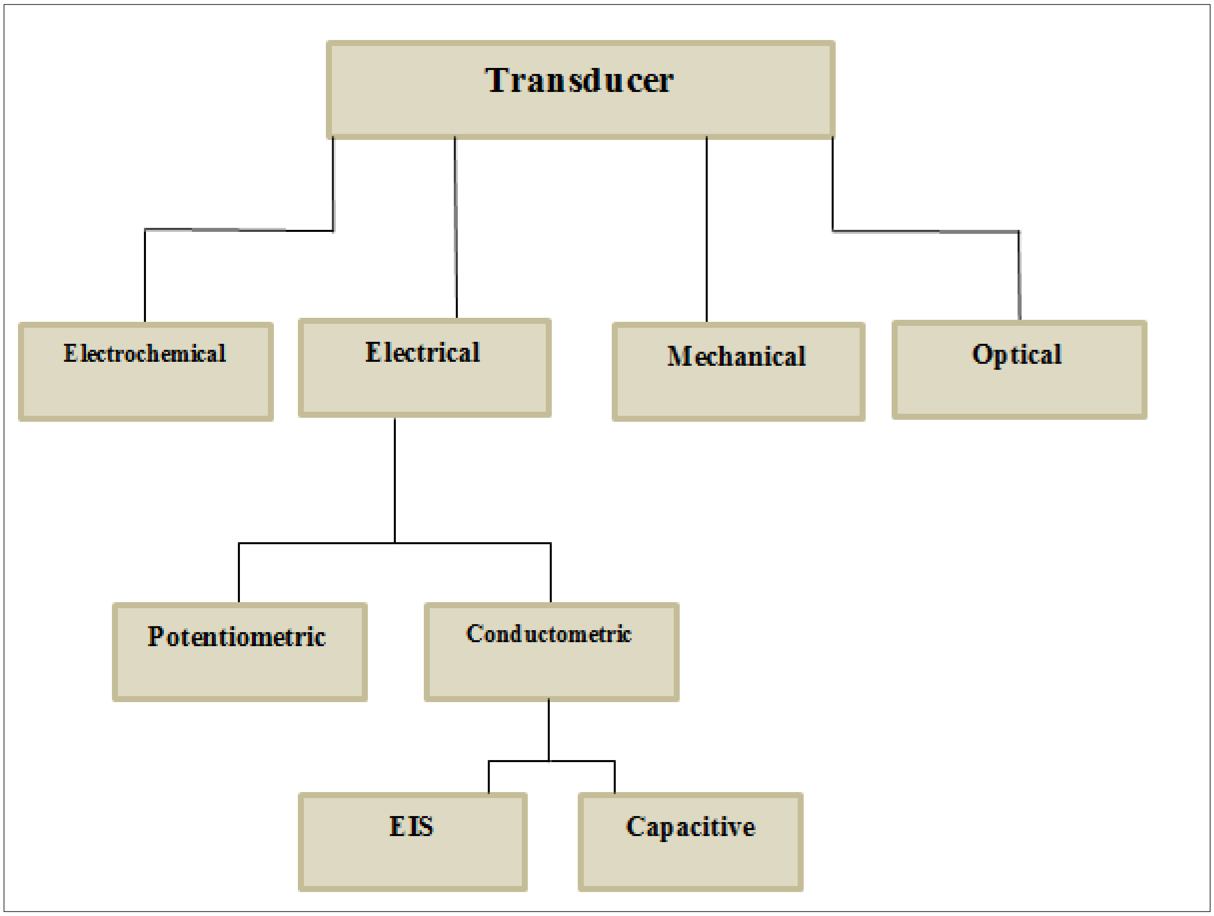

Figure 3. Major transduction platforms currently utilized for biosensor applications

3.1.1.1 Electrochemical Transducers

Electrochemical transducers are comprised of different electrodes: reference, active, sink and counter electrode for ion source. This type of transducer is always accompanied by chemical reactions which take place on the surface of active electrodes. Consequently, analyte reaction leads to ion production which produces an electrical potential and signal. The potential can be measured and analyzed to monitor analyte concentration. Electrochemical transducers are classified into two major groups: voltammetry (Potentiometric) and amperometry (Galvanic). Working principles of amperometry transducers is based on the measurement of the current, using auxiliary electrodes such as platinum, gold and carbon. By placing different conducting materials in an electrolyte solution, a set of electrochemical reactions occurs, that leads to the formation of potential difference.The current, resulting from the potential difference is then measured. The measured current can be directly correlated to the concentration of target analyte. Potentiometric transducers measure the potential difference between two dissimilar metals which are kept in intimate contact and separated from each other only by a permselective membrane [22]. Integration of nanomaterials into electrochemical transducers in the forms of thin film or nanoparticles can significantly increase the sensitivity and efficiency of biosensors due to the high surface area to volume ratio of such particles.

3.1.1.2 Optical Transducers

Optical transducers have been widely employed in nanotechnology enabled biosensing. Working principles of theses transducers are based on the change of waveguide structures, which happens due to the interaction of the analyte and optical waves. Interactions such as intensity and frequency within the platform affect the properties of optical waves, and these changes are then measured and recorded to be correlated with the analyte concentration. There are many other types of optical biosensors, including UV-visible and infrared wavelengths that employ optical spectroscopy and spectrums measurements to detect and characterize the target element. The intensity or color of light gives a measure of the target analyte. Optical waveguide based transducers are classified into two main categories: transducers working based on the propagation of optical waves, and transducers which work based on surface plasmon waves. Typically, a reporter molecule is attached to the sensing complex and is activated once the binding of the target biomolecule and receptors takes place. This consequently leads to waveguide structures variation. Optical fiber, surface plasmon resonance (SPR), and surface enhanced Raman scattering based biosensors, are among the most utilized transducers in nanotechnology enabled sensing.

Raman spectroscopy is a powerful technique in nanotechnology enabled sensing, to detect analyte with very small concentrations. In this technique, a sample is exposed to light with known properties. Reflected light from molecules will be monitored and analyzed. Based on the intensity and wavelength variations, the sample is characterized [23].

Surface plasmon resonance sensors are a very common type of optical transducers due to their promising features such as small sample consumption (microfluidic), inexpensive components and ultrathin detection. These sensors have been extensively utilized for ultrasensitive detection in health diagnostics. Surface plasmon waves extend a few hundred nanometers above the sensing layer and are significantly sensitive to the refractive index of this area. The sensitive region is mostly made of metal films, particularly gold metal films. Biological detection elements can be immobilized on the gold surface to ensure that target binding takes place on the sensitive region to change the refractive index. This change is detected by a shift in angle, wavelength or intensity of surface plasmon resonance [24].

3.1.1.3 Mechanical Transducers

Generally speaking, mechanical transducers' working principles are based on mass differences and they are considered as mass sensitive devices by nature. Solid state transducers are fabricated by incorporating semiconducting devices, which contain semiconducting and insulating materials, into micro-fabrication techniques. Solid state transducers' measurements involve measuring electrical field parameters variations such as voltage, current, capacitance, and impedance which happen due to the presence of analytes. Monitoring the variation can give quantitative measurements of the target analytes. Acoustic wave transducers are working based on the piezoelectric phenomenon which happens in non- symmetric crystals. When biomolecule bindings take place, the crystal's lattices will be deformed. This deformation influences the resonant frequency and therefore generates an electrical field [25].

3.1.1.4 Electrical Transducers

Electrical transducers have been widely employed in sensing applications due to their numerous capabilities including ease of fabrication, inexpensiveness, high sensitivity, label free detection and simple set up. Conductometric and capacitive transducers have been extensively reported to be used for monitoring biological membrane receptors, unlabeled DNA, protein and enzyme targets. In an electrical transducer, a sensitive layer is placed on metal electrodes to which an external voltage is applied. Applied voltage generates an electrical field and conductivity or capacitance will be measured accordingly. A conductometric transduces measurement techniques are based on the Ohm law. An external voltage is applied to the electrodes and the produced current is measured. The conductivity of the sensitive layer then is measured using the Ohm Formula:

$$V=IR \tag{3.1}$$

where V is the external voltage applied across the electrodes and sensitive layer, I is the measured current, and R is the electrical resistance. The same principle is applied to the capacitive transducers, but instead of conductivity measurements, the capacitance of the sensitive layer is measured. The capacitance of the sensitive layer can be measured using the following equation:

$$Q=CV \tag{3.2}$$

where V is the applied voltage and Q is the charge across the electrodes and C is the capacitance. Interdigital electrodes are commonly used in the fabrication of electrical transducers. An example of a simple IDT is shown in Figure 4.

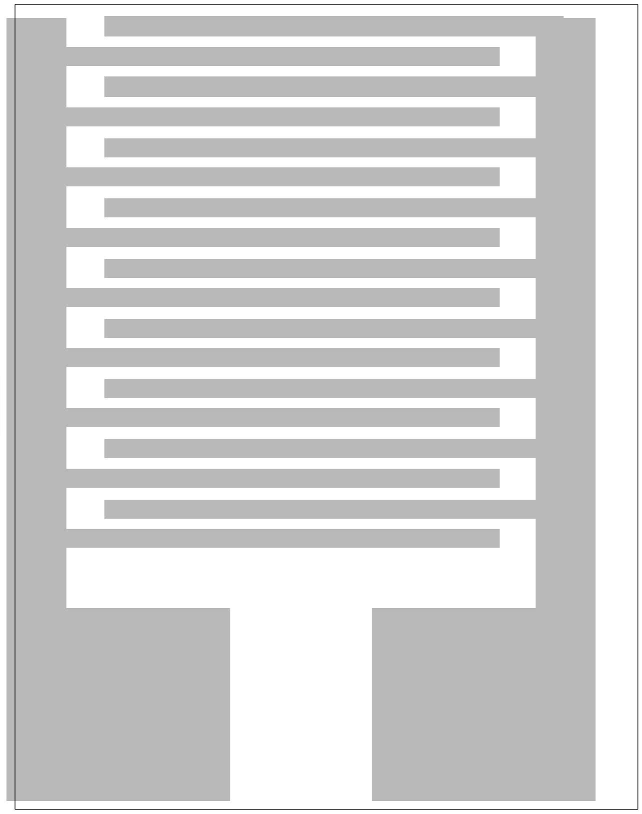

Figure 4. Schematic of Inter-Digital Transducer (IDT)

These electrodes are fabricated from noble metals such as platinum and gold, and are deposited onto the inert substrate to ensure that no chemical reaction or interaction happens between biological molecules and the transduction platform. The sensitive layer applied across the electrodes could be made of nanomaterials or nanofibers to enhance the transducer's efficiency [26].

3.1.2 Recognition Elements

The biorecognition molecules such as antibodies, enzymes, and DNA play crucial roles in the biosensors performance and selectivity. They have the capability to interact with the specific target molecule among several other molecules available in the system which is essential to increase the selectivity of a biosensor. In addition to transducers type, biosensors can also be classified based on the type of recognition elements into three main categories: biocatalytic, hybrid, and bioaffinity receptors.

The biocatalytic-based biosensors are well developed systems that have been extensively used in different biosensors due to their promising properties such as their capability to react with various biomolecules, flexibility, broad pH and temperature range. Enzymes have been the most developed type of biocatalytic recognition elements which have been frequently used since 1962. Whole cells (microorganisms, cell organelles and particles) and tissue slices (Plant or animal tissue) have been employed in recently developed biosensors [22]. These elements are used as catalysts for chemical reactions which involve target analytes. Glucose biosensors are the most developed enzyme based biosensors which are based on the glucose oxidase enzyme consumption as a catalyst for the following reaction:

$$\text{Blood Glucose} \xrightarrow{\text{Glucose Oxidase}} \text{Glucono-Lactone}$$

Although enzymes are among the broadly used materials for sensing applications, there are some drawbacks that limit their expansion including their short lifetime, inability to detect small targets, purification, and co-factor requirement.

The second category of the biorecognition elements are bioaffinity based elements. This interaction is based on the selective binding of target analyte and receptors and no chemical reaction is involved. Antibodies, DNA, peptides and lectins are the most common bioaffinity sensing mechanisms in which immobilized receptors selectively bind to target elements to generate the stable and detectable complexes. Several auspicious features of these receptors including flexibility, applicability and versatility, have allowed them to become the most utilized biosensors for sensitive target detection.

3.2 Immobilization and Surface Interactions

One of the crucial factors which strongly determines sensor performance and sensitivity is the interaction of analyte and sensing materials. In order for this interaction to happen, the surface properties of the sensors are of great importance. It is essential to understand the surface properties of nanosensors so to be able to improve their properties for better detection. Sensing elements have to be immobilized on the sensor's surface to make the surface selective and sensitive to target molecules. A number of different techniques have been utilized to improve the surface properties of biosensors, which are different based on the transducer, sensing element, device, application and target analyte. Covalent bonding, adsorption, physical entrapment, chemical entrapment, self-assembly and layer-by-layer assembly are the most common processes for the surface manipulation which improve the surface properties and increase the biomolecules interactions. In covalent bonding, for instance, surface preparation can be obtained by the formation of a covalent bond between the target analyte and the transducer's surface, or a cross linker can be utilized to facilitate the bonding and connect the transducer's surface to the target analyte. Covalent bonding has the advantage of generating very strong, controllable and fast interaction. However, the synthetic steps could be very complex and time consuming [26].

In addition to the covalent bond, other chemical bonding including hydrophobic, ionic and Van der Waals interactions can be utilized to adsorb the target analyte. These approaches are simple and inexpensive and work for various proteins. However, since the chemical bindings are not as strong as the covalent bond, analyte adsorption may be unstable. In a physical entrapment technique, a semi porous membrane is used to trap the large biomolecules such as proteins. These biomolecules can bond chemically with the inner membrane sites to generate chemical entrapments which are stronger and more stable. Self-assembly is one of the approaches to generate molecules and modifies the biosensors’ surfaces in a systematic manner. This method is commonly used by natural biological systems to produce biomolecules. Materials fabricated via this technique can be implemented in various applications such as nanomaterials growth, electrical insulators and sensitive layer fabrication. [27]. There are several types of self-assembly including static, dynamic, template and biological assembly. Among these categories, static and dynamic self-assembly are the two main types. Static self-assembly deals with stable systems that are in equilibrium such as molecular crystals [28]. Dynamic self-assembly however, involves unstable systems which are dispersing energy, such as biological cells. Layer by layer is a an easy and inexpensive technique for generating multilayered materials from molecular building, such as carbon nanotubes [29].

3.3 Label or Label Free?

Most biosensors utilize labels such as fluorophores, luminescent, calorimetric tag, and enzymes to be attached to the measurand to provide the quantitative measure of the analyte concentration. These labels can facilitate target binding and provide detectable product. However, labeling a target biomolecule requires bulky equipment and time consuming multi-step processes which can affect the biomolecules binding properties [30]. Therefore, developing a biosensor which can perform label-free detection using a low sample volume is vital to ensure fast diagnostics.

3.4 Sensor Characteristics and Terminology

There are several factors that affect the overall performance and reliability of a biosensor and the sensor’s overall effectiveness is directly defined using these terminologies. These include sensitivity, selectivity, limit of detection, reproducibility, dynamic range, multiplexing, and amplification [31].

3.4.1 Sensitivity

Sensitivity is one of the static characteristics of a sensor which determines its ability to detect different concentrations of analytes. It is defined as the change on the output value divided by the change in input value.

3.4.2 Selectivity

Selectivity is the potential of a biosensor to respond to only one specific target biomolecule and not to the other biomolecules exist in the assay. In real-world samples, target biomolecules may be found at very low concentrations (2ng/mL for prostate cancer) while other biomolecules may exist at much higher concentrations. Therefore, a biosensor must differentiate between these biomolecules structures and to detect them at a very low concentration.

3.4.3 Limit of Detection

The smallest concentration that can be reliably measured by a sensor is defined as its detection limit. The detection limit can be measured by finding the measurand smallest concentration, which is noticeably distinct from the output response to a control solution.

3.4.4 Reproducibility

Reproducibility is defined as the sensor's potential to produce the same response even after variations in measurement conditions. This is different from repeatability, which is the sensor's ability to produce the same response for successive measurements of the same input.

3.4.5 Dynamic Range

The range over which a sensor can accurately detect a target analyte is defined as the dynamic range. It is defined as the value of the largest concentration of analyte that can be measured over the limit of detection.

3.4.6 Multiplexing

It is the ability of a biosensor to perform multiplexed protein detection using a single sensing platform, thus reducing cost and the sample volume needed. Developing biosensors capable of parallel sensing detection has been the focus of several researchers recently and it is one of the main objectives of this research work.

3.5 Why Nanosensors?

Integration of nanotechnology into the sensor field has created significant improvement in recently developed sensors. Applying new approaches in nanotechnology into sensors' structures can develop features which cannot be obtained with conventional microtechnologies employed in classically fabricated sensors. Various nanostructures such as nanotubes, nanofibers, nanowires, nanoparticles, quantum dots, nanomembrane. can be integrated into the sensing elements of biosensors. Nanotechnology enabled sensors can be utilized as alternatives to conventional sensors due to promising properties such as selectivity, lower production costs, lower limit of detection, small quantities of samples required, reduced power consumption, higher stability, high sensitivity, small dimensions, high efficiency, direct analyte detection and multi-functionality. This is attributed to the novel properties of nanomaterials such as a higher specific surface area, small dimensions, and better conduction properties which make them perfect to be utilized in sensing structures. Tailored surface functionality of nanomaterials and small dimensions increase the diffusion rate and that leads to rapid signal transferring and thus a faster response time. A smaller size and high surface area of nanomaterials can improve the sensor's sensitivity, dynamic performance and label free detection of very small quantities of samples.

3.6 Nanofiber-Based Biosensing Devices

Nanofibers have been increasingly employed in analytical systems as sensing elements [32]. They have been proven to enhance the efficiency and performance of biosensors due to their unique features such as high surface area, high porosity, tailored mechanical properties and etc. [32, 33]. Nanofibers can be fabricated via well- understood techniques which provide control over their sizes, shapes, porosity, and mechanical properties. They can significantly decrease the detection limit of biosensors by providing plenty of immobilization sites thus increasing the biomolecule interactions [33]. Moreover, their high porosity enables the fast biomolecules penetration which indeed reduces the detection time [34]. Furthermore, the nanofibers surface functionalization can be simply obtained in comparison to nanotubes or nanowires. This is attributed to the capability of nanofibers to be functionalized over the entire surface area using nanomaterials. For instance, carbon nanofibers are simpler to functionalize compared to carbon nanotubes or nanowires due to the oxygen which is available on their activation sites. Polyaniline, chitosan [35], and many other polymeric fibers can be functionalized using a variety of chemical groups, such as the incorporation of nanoscale additives [36], sulfonic acid and amino groups [37]. There are different approaches to fabricate nanofibers out of biocompatible and stable materials such as electrospinning, interfacial polymerization, and chemical vapor deposition. [38]. Among all available approaches, electrospinning has been extensively utilized to fabricate nanofibers with controlled dimensions, porosity, mechanical and chemical properties [8]. Electrospun nanofibers can be easily functionalized via surface modification techniques such as covalent bonding, adsorption, cross linkers, and nanoparticles dispersion. This strongly improves the sensor performance by enhancing the interaction of target analyte and the sensing materials.

3.7 Electrospun Nanofibers Used for Sensing Application

Electrospinning is an inexpensive and flexible technique to fabricate highly porous nanofiber mats to be utilized for various applications such as biosensing technology. Functionalized electrospun nanofibers have been extensively integrated onto several biosensors' structures as sensing platforms in simple and control manners. Effectiveness of employing electrospun nanofibers in modifying sensors' properties and improving their performance have been the main focus of multiple research. Here we provide a brief review of several published research articles which used electrospun nanofibers for sensing applications.

In research by [39] an electrospun carbon nanofiber was fabricated and utilized to develop a biosensor platform. The electronic properties of fiber were modified so to be utilized for electrochemical detection. The density of electronic states was adjusted by controlling graphite concentration and manipulating the carbonization conditions. This led to an increase in efficiency and sensitivity of the fabricated sensor.

A highly porous electrospun Mn_2O_3-Ag nanofiber was fabricated and used as the sensor platform for glucose oxidase detection. The fabricated biosensor had a high sensitivity (40.60 $\mu A \times mM^{-1} \times cm^{-2}$) and a low limit of detection (1.73 μM) which was attributed to the novel Mn_2O_3-Ag nanofibers potential [40].

In order to fabricate a sensitive and reliable glucose biosensor, palladium (IV)- copper oxide composite nanofibers were fabricated by electrospinning [41]. The obtained results, such as fast response (1s), high sensitivity, and low limit of detection confirmed the effectiveness of employing the palladium (IV)- copper oxide composite nanofibers as capable platforms for amperometric glucose detection. The same approach was followed by [42] to fabricate a non-enzymatic glucose biosensor. An electrospun Co3O4 nanofiber based glucose biosensor was developed with a fast response time, high sensitivity, high selectivity, and a low detection limit.

In an attempt by [43] multiwall carbon nanotube and poly(acrylonitrile-co-acrylic acid) mixture was used to fabricate an electrospun nanofiber. The generated nanofiber was integrated onto platinum electrodes and glucose oxidase was then immobilized on the modified electrodes to develop a glucose biosensor. Amperometric characterization results demonstrated that multiwall carbon nanotube concentration correlated to the current enhancement. Moreover, it was observed that the increase in the maximum current disturbed the secondary structure of the glucose oxidase.

E. coli O157:H7 was sensitively detected (67 CFU/mL) by electrospun cellulose nitrate nanofibers in research by [44]. In order to increase the selectivity of the fabricated sensor and separate the target analyte from other biomolecules existng in the system; magnetic nanoparticles were bonded with the E. coli O157:H7 antibody. Due to the remarkable properties of the electrospun nanofiber, fast detection, reliable detection, and linear sensing response were obtained.

In a study by [45], electrospun DEPOT nanofiber was fabricated and applied as a sensing layer onto the platinum microelectrodes to detect glucose biomarkers. The developed neurochemical biosensor's detection principle was based on the detection of glucose itself rather than detecting the hydrogen peroxide. Glucose detection was successfully achieved at a very low concentration.

In a study by [46], an amperometric nanobiosensor was developed to detect hydrogen peroxide. Electrospinning was utilized as a simple method to fabricate the Hb–collagen nanofiber. The fabricated composite was applied onto the electrode surface to produce a hemoglobin (Hb)–collagen microbelt modified electrode. The fabricated nanobiosensor was then characterized using cyclic voltammetry to demonstrate the potential for hydrogen peroxide detection. It was concluded that the unique structure of the electrospun Hb–collagen composite was very operative in increasing the response time, sensitivity, and stability.

Electrospinning has been demonstrated as a flexible approach to fabricate thin metal fibers [47]. In research by [48] the surface area of gold electrodes was increased by using electrospun gold nanofibers. The modified electrodes used for fructose detection showed high stability (over 20 cycles), a short response time (less than 2.2 s) and high accuracy. The sensor was then modified by the addition of glucose isomerase to allow glucose detection as the second analyte, thus improving the sensor's ability for parallel detection.

Polystyrene–poly (styrene-co-maleic anhydride) (PS-PSMA) was used as a composite for nanofiber fabrication using electrospinning in a study by [49]. Fabricated matrix was then used as the sensing layer for protein immobilization. Two thrombin- binding aptamers were used to evaluate the platform's potential for protein immobilization. Fluorescence microscopy and spectroscopy was implemented as a method to measure analyte concentration. The sensitivity of electrospun PS-PSMA nanofiber based biosensor was compared with a 96-microwell plate format. The fabricated sensor showed a higher sensitivity (2500-fold higher sensitivity) which was mainly attributed to the favorable properties of electrospun nanofibers.

Electrospun nanofibers have been widely incorporated onto gas detecting sensors. For instance, [50] PVP/polyaniline fibers fabricated by electrospinning are used in a surface acoustic wave hydrogen gas sensor. The fabricated sensor showed high sensitivity, stability and a low limit of detection (0.06% H2 gas concentrations).

Several studies have been focused on the fabrication of sensors to detect various gases such as NH3, H2S,CO, NO2, O2, CO2, and volatile organic compounds using electrospun nanofibers functionalized with metal-oxide semiconductors such as TiO2 [51], ZnO, WO3, MoO3, SnO2 [52], and In2O3 so to increase the mass transfer rate and therefore reduce the detection time.

Electrospun ZnO nanofibers were fabricated in a study by [53] and used for sensing applications. Using photolithographic technology, a novel design of ZnO biosensor was obtained and protective films of AlNx were sputtered into the nanofiber to increase their stability. In a different study by [54] an amperometric glucose biosensor was developed which was designed based on a single ZnO nanofiber synthesized by electrospinning. A single ZnO nanofiber was applied to the gold electrode and the surface was manipulated by the physical adsorption of glucose oxidase. The fabricated biosensor was then characterized using electrochemical measurements. Characterization results demonstrated a high sensitivity and a low limit of detection as well as long term stability.

An ultrasensitive label-free colorimetric sensor was developed by [55] to detectformaldehyde at a very low detection limit of 50 ppb. A composite of Methyl Yellow-impregnated electro-spinning/netting and nylon 6 was prepared as a sensing platform. The fabricated sensor was exposed to various volatile vapors but showed very high selectivity toward formaldehyde.

Kim et. al [56] developed a chemeresitor for detecting organic vapors using an ionic liquid/polymer composite for nanofiber fabrication. Electrospinning was implemented to fabricate 1-butyl-3 methylimidazolium hexafluorophosphate (BMIMPF6)-Nylon 6, 6 nanofibers. Electrospun nanofibers were applied on the microelectrodes to form a sensitive platform so to be characterized via cyclic exposure. The sensor's performance was then evaluated using four different organic vapors including ethanol, methanol, tetrahydrofuran, and acetone as target analytes. The electrospun BMIMPF6/nylon 6,6 anofiber based gas sensor demonstrated good characteristics at room temperature suggesting the useful application of nylon nanofiber as a sensing material for organic vapor detection. In research performed by [57] a new electrospun LiCl-doped TiO2 nanofiber based humidity sensor was developed. The obtained results revealed promising characteristics such as faster response, reproducibility, and high linear range. SnO2 nanofibers have been widely implemented for detection of analytes in the air such as ammonia [58], methanol [59], and ethanol [60] due to their novel properties such as high transparency, wide-band gap, and chemical-sensing capabilities [61].

In a study by [62], PEDOT–PSS/TiO2 composite was used to fabricate a highly sensitive conductometric gas sensor for NO_2 detection down to 1 ppb based on the stoichiometric oxidation of NO into NO2. The electrospun TiO_2 was directly applied on the electrodes and consequently a thin film of PEDOT–PSS was incorporated in the system via dipping to create an excellent sensing platform. It was demonstrated that favorable properties of electrospun nanofiber makes it a good candidate for NO detection and that can be directly used for early diagnosis of lung diseases, including asthma.

Following the efforts to develop sensitive gas sensors, [63] developed a gas sensor for H2S detection. Electrospun CuO-SnO2 nanocomposite was fabricated to improve the creation of the p–n junctions due to the individual existence of a single CuO p-type phase and n-type SnO2. Two operating temperature of 300 ◦C and 150 ◦C were used, and the sensor's response time, sensitivity and recovery time were recorded. The results obtained showed that the electrospun nanofiber is a proper candidate for H2S sensor development.

Recently, [64] developed an ultrasensitive optical electrospun nanofiber based sensor for Fe^{3+} detection at the pico-molar level. Ethyl cellulose (EC) was used to fabricate nanofibers by electrospinning and the fluorescent iron selective chromoionophore was chosen as the indicator. The proposed design offered high sensitivity, low detection limit and fast response.

In a study by [65], poly (acrylic acid) (PAA) was used to fabricate various types of nanofibers with different diameters and densities using electrospinning. Electrospun nanofibers were deposited onto an optical fiber core and their potential as sensing elements in humidity optical sensors was evaluated. The developed biosensor was then exposed to different relative humidity. Based on nanofiber properties, different transfer function patterns were observed. Smaller diameters of nanofibers improved the transmitted optical power while a lower transmitted optical power was observed at a lower mat density. Human breathing cycles were used to measure the response time (340 ms exhalation) and a recovery time of 210 ms was observed.

3.8 Sensor Applications of Polyaniline Nanofibers

Polyaniline is one of the intrinsically-conductive polymers which were widely employed as an immobilization platform for sensing applications [66]. Different nanostructures of polyaniline have been used as sensitive films to facilitate the biomolecules interactions and increase the diffusion rate by providing highly porous platforms with smaller dimensions [67]. Polyaniline nanofibers have been frequently reported to be incorporated into different types of transducers, particularly electrochemical/electrical transducers for enzyme and DNA detection [68-70]. Electrospun polyaniline nanofibers have been reported to be implemented for sensing applications by several research groups. Recently, Lin et al. have developed a Polyaniline nanofiber humidity sensor using electrospinning. Impedance measurements were used to characterize the fabricated sensor, which was shown to exceed existing similar models in sensitivity, linearity, response time, and repeatability [71]. In a similar study by the same group [72] electrospun nanofibers of Polyaniline (PANi) and poly(vinyl butyral) (PVB) were applied on an surface acoustic wave resonator to fabricate an ultrasensitive and highly fast humidity sensor. The fabricated composite of PANI and PVB proved to be a very effective matrix in improving the favorable properties of the sensor including sensitivity growth, fast response and good linearity range. In a study by [73], Poly(methyl methacrylate) (PMMA)-Polyaniline nanofibers with different diameters were fabricated by electrospinning and polymerization. The fabricated nanofibers were then deposited on the gold electrode to develop an electrical gas sensor for triethylamine vapors detection. The sensor was able to detect the triethylamine vapors as low as 500 ppm with a linear response. Moreover, it was demonstrated that smaller diameters of nanofibers increased the sensitivity of the sensor, which could be attributed to the high surface-to-volume ratio and thus more available binding sites. A chemresistor was developed by doping the palladium nanoparticles in polyaniline to fabricate a nanofiber via electrospinning [74]. Electrospun nanofiber was applied onto the gold electrode and the sensor's potential to detect H_2 was then evaluated using the electrical characterization method. A 1.8% resistance change was observed with a 0.3% hydrogen concentration. This represented that palladium doped polyaniline nanofibers are the proper candidate to be used in the hydrogen sensor. A different surface acoustic wave sensor was developed by [50] for H_2 detection. The fabricated sensor was based on electrospun Polyvinylpyrrolidone/Polyaniline Composite. Based on the results obtained, it was concluded that fabricated nanofiber was not a good approach for measuring the H2 concentrations lower than 0.25%. In research by [75], an electrical sensor was developed to detect various alcohol vapors using an electrospun HCSA-doped polyaniline nanofiber. The sensitivity of the fabricated sensor was then compared to a polyaniline mat based sensor. The results demonstrated that nanofiber based biosensor had a faster response for large molecules which could be related to the novel properties of the fabricated polyaniline nanofiber.

[76] developed a NH3 gas sensor based on the carbon nanotube and polyaniline composite. The carbon nanotube was first used to develop interdigitated electrodes using dielectrophoresis, and polyaniline was then deposited onto the sensor's surface via electrospinning. Using different ammonia concentrations, the sensors' sensitivity, response characteristic, and repeatability were compared. The fabricated sensor could detect the target under 1 ppm, taking 60 s for respond. It also showed a good linearity range (20 ppm and above) with response times between 100 and 200 s. In a different study by Jia et al., Pt nanoflowers were electrodeposited onto the electrospun polyaniline nanofiber using cyclic voltammetry (CV). The fabricated sensor was then used for urea detection [77]. The results obtained by this study showed a wide linear range as well as a good limit of detection of 10 μM. In a different research project by [78], electrospun polyaniline nanofiber was fabricated and used for amperometric cholesterol detection. More than ten layers of cholesterol oxidase were effectively immobilized onto nanofibers using layer-by-layer adsorption. A quartz crystal microbalance technique was utilized to evaluate the adsorption efficiency and control the layer growth. After deposition of the fifth cholesterol oxidase layer, cholesterol concentration was detected accurately.

3.9 Recent Biosensor Developments: Application to COX-2 Detection

To the best of our knowledge, only three biosensors have been reported in recent years for COX-2 detection. This section discusses these three research projects.

In 2009, an optical biosensor was developed by [79] which was designed to measure the COX-2 levels in patients with tobacco-related intraoral cancer. Surface plasmon resonance was utilized as a technique for measuring the COX-2 concentration in 119 patients; 76 of them had oral cancer and the rest (43) were normal individuals. Patients with cancer showed a higher COX-2 level in their serum compared to the normal controls. It was concluded that the SPR characterization method showed a great capability to detect COX-2 analyte.

A label-free electrochemical pain biosensor was developed by [80] to detect COX-2 enzyme as the pain biomarker. The detection method was based on metal enhanced detection (MED) which works based on the metallic film deposition, in this case a solid-phase monolayer of silver, onto the gold substrate. This could increase bimolecular interactions. The fabricated sensor revealed a linear range between $3.64*10^{-4}$ to $3.64*10^{1}$ ng mL^{-1} as well as a detection limit of $2.54*10^{-5}$ ng mL^{-1} (4 orders of magnitude lower than that recorded for ELISA).

In the last study by the same group [81], COX-2 pain biomarkers were quantified using surface plasmon resonance (SPR) and ultra-sensitive portable capillary (UPAC) fluorescence and results of these two detection methods were compared. A linear range between 3.64×10^{-4} and 3.64×10^{2} ng/ml was observed for the SPR based sensor while a linear detection range of between 7.46×10−4 and 7.46×101 ng/ml was revealed for the UPAC immunosensor. Moreover, SPR based and UPAC could detect the analyte down to 1.35×10−4 ng/ml and 1.02×10−4 ng/ml respectively.

CHAPTER 4

MATERIALS AND METHODS

In this section, an overview of the platform design, fabrication and operation is given. Moreover, materials and methods utilized to prepare the nanofiber are presented.

4.1 Sensor Platform

The fabricated biosensor is comprised of three major parts: a printed circuit platform, an electrospun polyaniline nanofiber, and an encapsulation chamber. An electrospun polyaniline nanofiber was applied onto the gold microelectrode to develop a sensing platform. The chamber was then applied on the platform to create a confined environment which can hold 200μL of liquid solution.

4.1.1 Printed Circuit Platform

The designed sensing platform for biosensor fabrication is a printed circuit with the interdigitated pattern of counter and working electrodes deposited on top of a silicon layer. The dimensions of the gold electrodes as well as the platform design are presented in Figure 5. The electrodes are designed in order to increase the electrical field, thus improving the sensor performance.

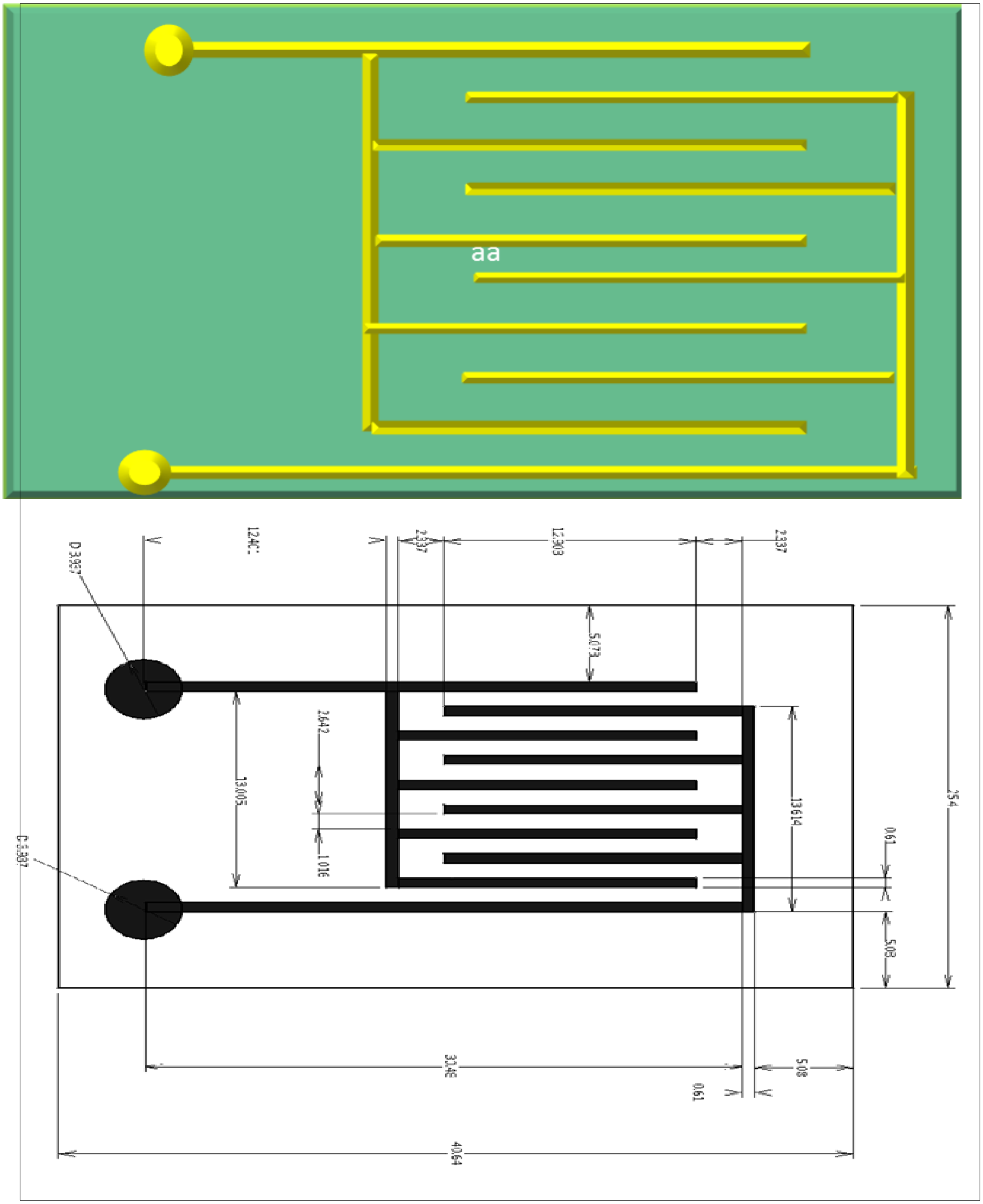

Figure 5. Dimensions and platform design of the gold microelectrodes

4.1.2 Electrospinning Polymer Blend Fibers

Polyaniline and polystyrene were the two main materials used for nanofiber formation via electrospinning. This section discusses the materials and procedures employed for electrospinning.

4.1.2.1 Polyaniline

Polyaniline was utilized as the main material for electrospinning due to its high conductivity, ease of synthesis, low cost, ease of combination with other polymers, and environmental stability. Polyaniline is one of the intrinsically-conductive polymers which were widely employed as an immobilization platform for sensing applications [66]. Different nanostructures of polyaniline have been used as sensitive films to facilitate the biomolecules interactions and increase the diffusion rate by providing highly porous platforms with smaller dimensions [67]. Polyaniline nanofibers have been frequently reported to be incorporated into different types of transducers, particularly electrochemical/electrical transducers for enzyme and DNA detection [68-70]. Conductive PANI nanofibers have been prepared via the electrospinning method by MacDiarmid’s group. Electrospun polyaniline nanofibers have been reported to be implemented in sensing applications by several research groups.

Polyaniline is comprised of a reduced benzenoid unite and an oxidized quinoid unite. One of the distinctive features of the polyaniline is its potential to be manipulated to exist in a wide range of oxidation states, including leucoemeraldine (fully reduced), pernigranilene (fully oxidized) and emeraldine (half oxidized) which can act as non-conductive, semiconductor, and highly conductive polymer. There are various approaches for polyaniline polymerization, such as electropolymerization, chemical polymerization, electrospinning, vacuum deposition, and sol-gel processes. The required level of conductivity can be obtained by partial oxidation or the reduction of polyaniline (doping) with other chemicals. Polyaniline has the advantage of being blended with other polymers since both molten and solid forms (in a solution form) can be handled. Due to PANI's rheological characteristic, PANI by itself cannot be used for electrospinning and it has to be blended with polymers such as polystyrene to form a spinnable solution.

Amongst the three forms of the PANI oxidation family, emeraldine differs substantially from leucoemeraldine and pernigranilene due to its stability as well as its capability to be tailored for higher conductivity. Emeraldine's conductivity can be manipulated via doping from 10-10 S/cm up to 100 S/cm[82]. Emeraldine conductivity can be significantly increased by doping using organic acids where protons are added to the -N= sites as shown in Figure 6.

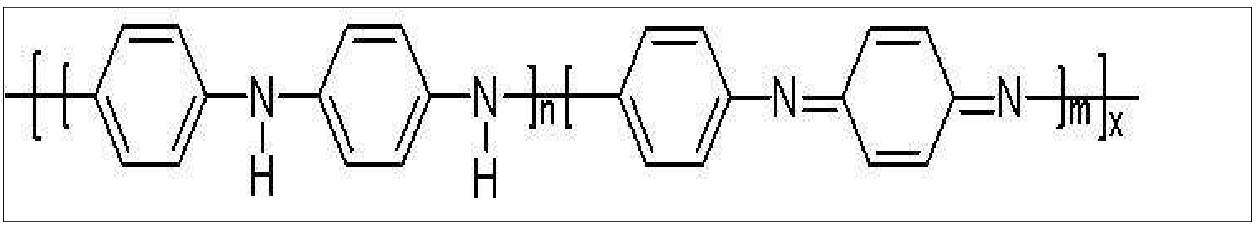

Figure 6. Chemical structure of polyaniline (n+m=1 and x=degree of polymerization)

In this study, the doping agent camphorsulfonic acid (HCSA) was used for emeraldine protonation via solid state protonation as illustrated in Figure 7.

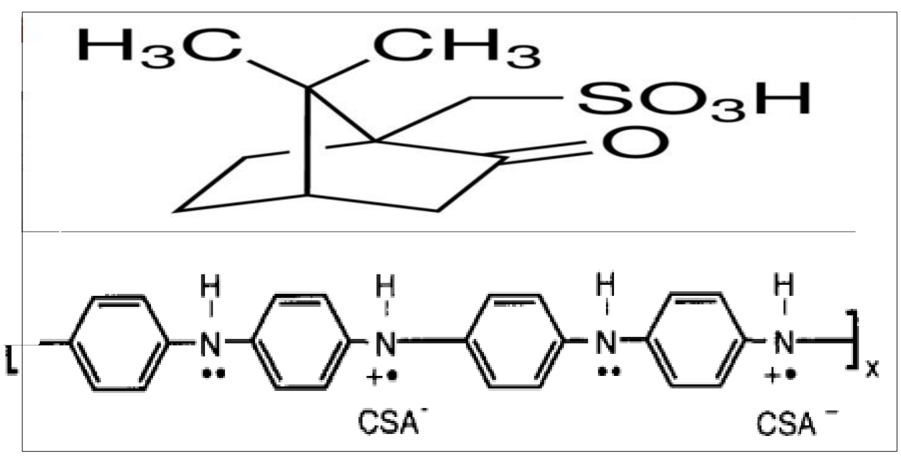

Figure 7. Scheme of protonation of polyaniline with HCSA

During protonation, HCSA donates its proton to the nitrogen of the emeraldine. This leads to positive charge formation which is subsequently balanced by the anion of the HCSA. The nitrogen then donates an electron to the neighboring ring so a benzoid ring is formed. The remaining electrons on the nitrogen produce electrical conductivity in polyaniline.

HCSA was purchased from Sigma Aldrich (Product code# 147923). HCSA was blended with Polyaniline in a 2:1 weight ratio and mixed completely in a mortar-pestle for 30 minutes. To increase the electrical conductivity, the mixture was then heated at 85°C for 1 hour.

4.1.2.2 Polystyrene

Polystyrene is an inexpensive, clear, glassy, and moderately strong polymer, biologically inert, and stable, which has been widely utilized for various applications. Polystyrene is a synthetic aromatic vinyl polymer, structurally made from the monomer styrene using either bulk or suspension polymerization processes. It is comprised of a long hydrocarbon chain where alternating carbon centers are connected to a phenyl group. The real molecular nature of Polystyrene (PS) has been studied in 1920 by Staudinger and produced for the first time by I.G. Farben in 1930. Polystyrene is produced by free radical vinyl polymerization, from the monomer styrene. Polystyrene is considered a thermoplastic polymer which melts at temperatures above its glass transition temperature and is in a solid state at room temperature. Polystyrene (PS) is one of the polymers frequently used as a copolymer in making spinnable PANI solution. Several studies have been reported to use the polystyrene as the main material for electrospinning due to its capability to produce a biocompatible hydrophobic surface. Successful protein immobilization on polystyrene (PS) fiber has been obtained in various studies [83, 84]. In this study, polystyrene was used as one of the main materials for electrospinning to facilitate the process. Polystyrene was purchased from Sigma Aldrich (Product Code# 430102, MW 230000, Tg 94°C). The structure of polystyrene is shown in Figure 8.

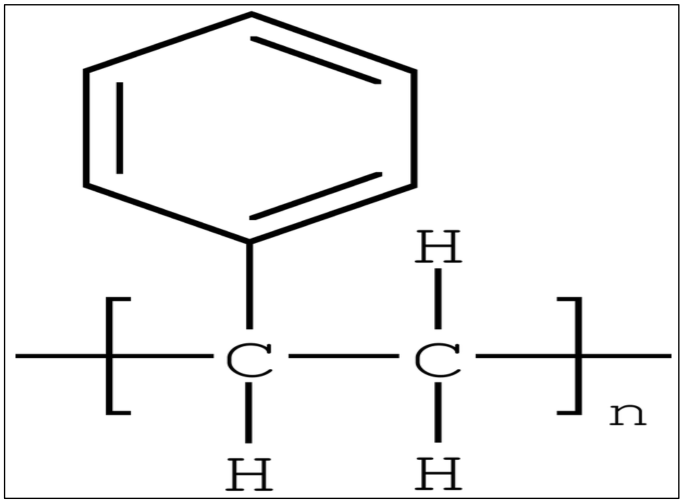

Figure 8. Chemical structure of polystyrene

4.1.2.3 Solution Preparation

Two wt% PANI, and 4 wt% HCSA were mixed completely for 30 minutes and then heated at 85°C for 1 hour. Subsequently, 7.5 wt% PS was added to the PANI_HCSA mixture and the mixture was then dissolved into a low boiling point solvent like chloroform (Fisher Scientific, Product code# C606SK-4), CHCl3, to prepare a viscous solution. The final solution was then stirred overnight to form a homogeneous solution. The produced solution is showed Figure 9.

Figure 9. Stock electrospinning solution

4.1.2.4 Electrospinning

The final solution was placed into a syringe and placed horizontally onto the syringe pump. In order to generate an electrical field, a copper wire was inserted into the tip of the syringe in direct contact with the solution. The copper wire was connected to the voltage power and acted as an electrode to transfer the high voltage to the solution. A high voltage 25 kV DC was then applied to a copper wire in order to generate an electrical field between the solution and an aluminum foil. The aluminum collector was placed in front of the syringe and acted as the grounded electrode. The distance between the tip of the syringe and collector was 300 mm and was kept constant during the experiment. Four different flow rates of 0.5, 1.0, 2.0 and 3.0 ml/hr were used while other parameters were kept constant. A schematic drawing of the electrospinning process is shown is Figure 10. As the electrostatic forces overcame the surface tension of the solution, a jet formed and traveled toward the aluminum foil. The fiber was peeled off from the foil for further use.

Figure 10. Image of the setup used for electrospinning

4.1.3 Chamber

Finally, a rectangular polydimethylsiloxane (PDMS) chamber was fabricated to encompass the sensing platform and to confine 200µL of liquid on the surface. This was performed to prevent solution evaporation and contamination which could significantly affect the sensing performance. The manifold had two microfluidic channels which were used for sample injection and removal. A PDMS chamber was fabricated by a composite mold which was designed in SolidWorks and machined using a HASS CNC machine. The following procedure was followed according to the manufacturer recommendation to fabricate the PDMS chamber. The composite mold first was washed with ethanol and DI water and air dried to create a contamination free environment.

A non-hazardous release agent was then applied to properly prepare the mold surface. The first layer of the release agent was applied as a light coating on the surface using a hand sprayer. After 15 minutes, a second layer was applied in the same manner and the master mold was then heated at 180˚F for 15 minutes to cure. This step was performed to facilitate the PDMS chamber removal from the mold.

Due to rapid and versatile cure processing as well as high transparency which allows easy inspection of components, a silicone elastomer encapsulant kit (Item# SYLGARD-184) was used to fabricate the chamber. Two parts of the kit were mixed in a 10:1 volume ratio and dispersed into the mold. Care was taken to minimize air entrapment. The mold was then heated up to 100 ˚C for 30 minutes and then cooled at room temperature. The manifolds then were peeled off the mold using a laboratory knife. The image of the master mold and PDMS chambers is presented in Figure 11.

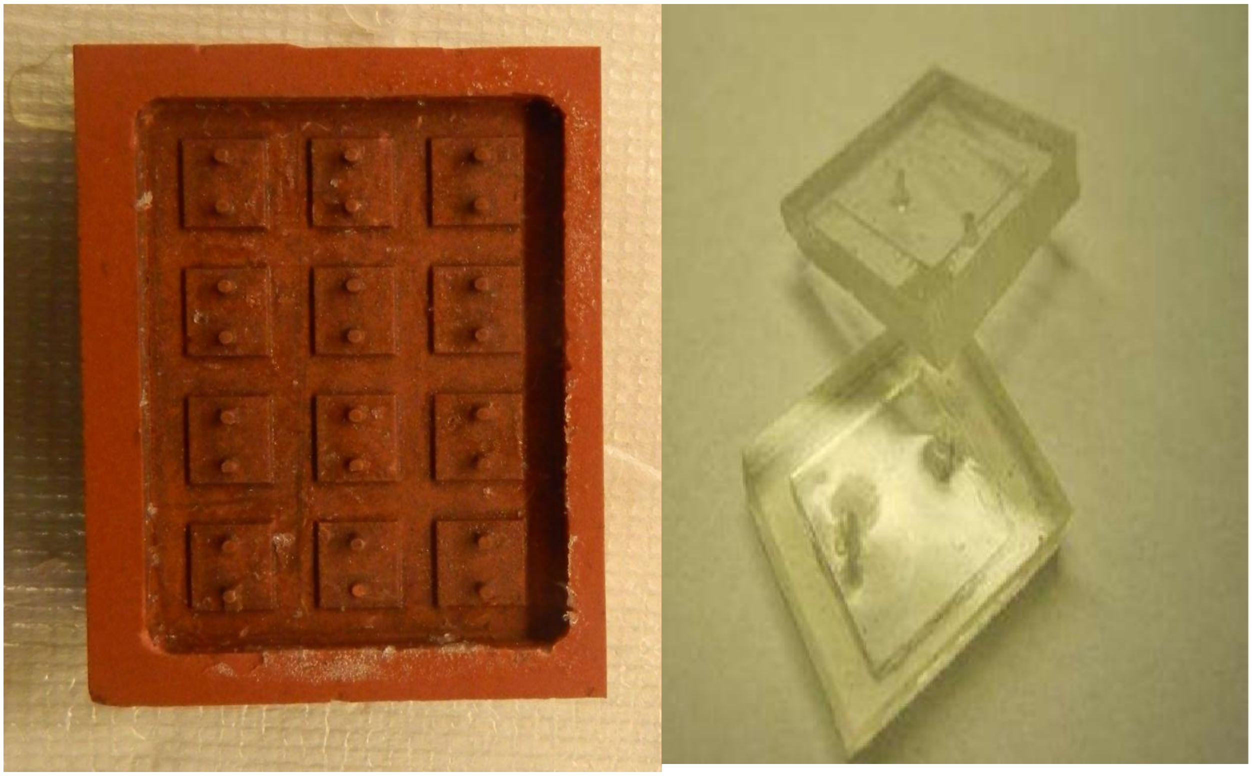

Figure 11. Master mold for making PDMS manifold

4.2 Chip Integration

The highly porous electrospun polyaniline nanofiber was soldered onto the circuit boards and the silicon chamber was used to enclose the nanotextured sensing platform. Thus, the integration of the circuit board, the electrospun polyaniline nanofiber, and the silicone chamber formed an inexpensive, simple, capable and versatile lab-on-chip platform, proper for biomolecule detection. The platform was then hooked up to a potentiostat for electrical characterization. Figure 12 shows the assembled lab-on-chip device.

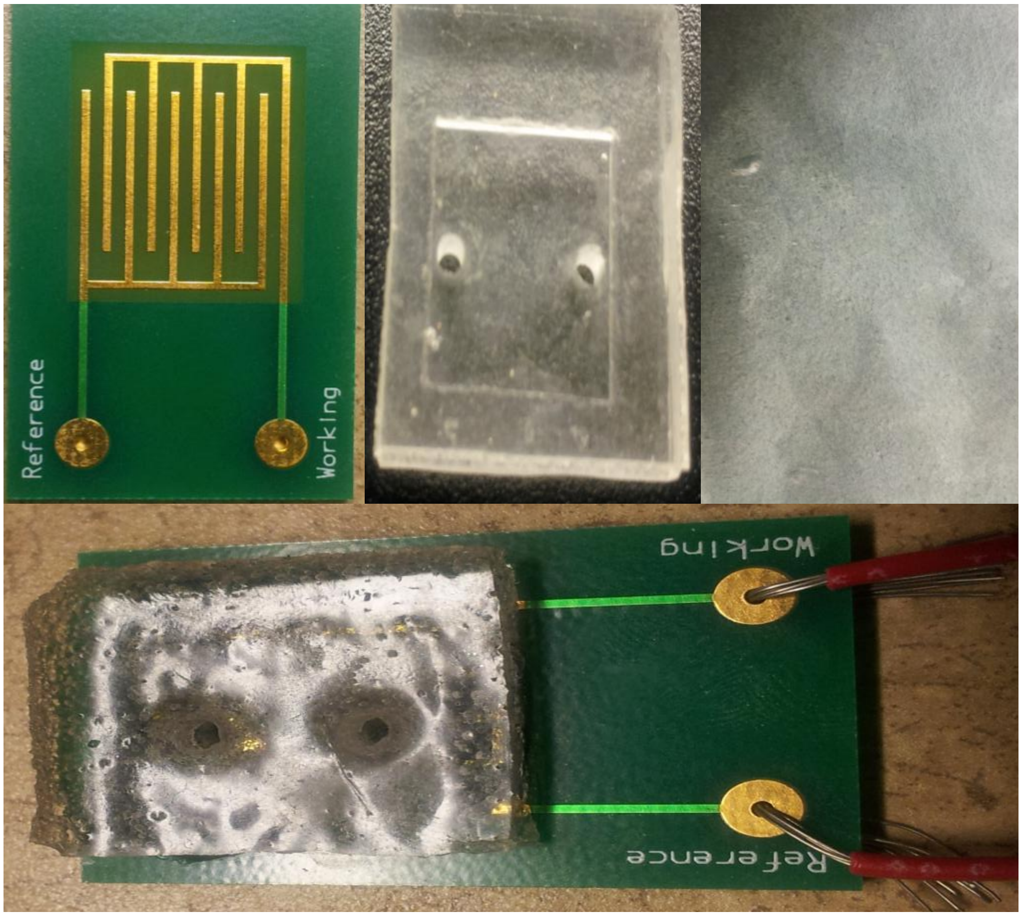

Figure 12. Different components and assembled chip

4.3 Materials for Sensor Experiments

COX-2 polyclonal antibody was employed for biosensor assay preparation which selectively binds to COX-2 blocking peptide.

4.3.1 COX-2 Enzyme

Prostaglandin-endoperoxide synthase 2, known as cyclooxygenase-2 or COX-2, is an enzyme encoded by the PTGS2 gen [85]. COX-2 was first discovered in 1991 by Daniel Simmons. COX-2 is a homodimer and each monomer in its structure has a molecular mass of approximately 70 kDa. Each monomer is comprised of three domains which differ structurally: a C-terminal catalytic domain, a epidermal growth factor (EGF) domain; and an α-helical membrane-binding moiety. COX-1 and COX-2 are bifunctional enzymes that perform two chemical reactions successively in coupled active sites which are different spatially. Two spatially different active sites, namely cyclooxygenase and peroxidase are located in the catalytic domain [86]. Cyclooxygenase enzymes catalyze the reaction of arachidonic acid (AA) conversion to prostaglandin H2 (PGH2) in two steps as illustrated in Figure 13.

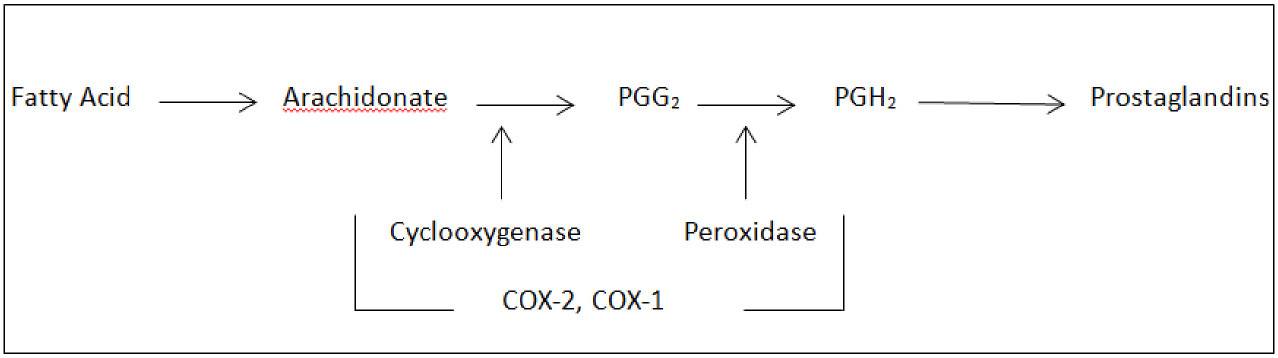

Figure 13. Reaction of arachidonic acid conversion to prostaglandin H2

The conversion of arachidonic acid to PGG2 takes place as a series of radical reactions. First, hydrogen from carbon 13 of arachidonic acid is replaced by two molecules of oxygen which are donated by the COX-2, resulting in PGG2 formation. At the next step, the resultant PGG2 is reduced to form the PGH2 which is subsequently converted to prostaglandins (PGD2, PGE2, PGF2α), prostacyclin (PGI2), or thromboxane A2 [87].

In general, COX-2 is unexpressed in most tissues in normal conditions but several stimuli such as inflammation evoke its expression [88]. It has been demonstrated that elevated expression of COX-2 enhances tumor cell proliferation, differentiation and adhesion which can ultimately result in tumorigenesis. Therefore, COX-2 expression has been utilized as a potential tumor marker in several cancers [89, 90].The elevated levels of COX-2 were correlated with malignant transformation in patients with head and neck cancer [91], and breast cancer [92]. Recent evidence suggests that overexpression of COX-2 has been observed in oral precancerous lesions. In a study by [79], quantitative measurements of COX-2 levels were performed by a

SPR biosensor in patients with oral cancer for the first time. Abnormal expression of COX-2 has been proven to be involved in oral cancer cell proliferation [79, 93].

Studies have shown that while the high expression of COX-2 enhances tumor growth, thereduction of COX-2 expression reduces tumor cell activity and growth [94]. Therefore, inhibitors of the COX-2 enzyme are the potent therapeutic candidates for cancers associated with high levels of COX-2 [95] enzymes due to their ability to disturb COX-2 activity [96].

Studies have demonstrated that COX-2 expression at the area of inflammation is correlated to the inflammation degree [97]. Therefore, monitoring COX-2 antibody–antigen interactions could provide a direct measurement method for monitoring the level of inflammatory pain. This quantification approach could be utilized as a proper alternative to the Wong–Baker pain faces rating scale which is currently used as the standard method for qualitative pain measurement. COX-2 (human) polyclonal antibody (item number# 160107) and COX2 (human) blocking peptide (Item number# 360107) were purchased from Cayman Chemicals (Santa Cruz Ann Arbor, MI) and stored at −20 °C.

4.3.2 DTSSP Linker

3,3´-Dithiobis(sulfosuccinimidylpropionate)’ also known as DTSSP, is a thiol-cleavable, water soluble and amine reactive crosslinker which is comprised of an amine-reactive N-hydroxysulfosuccinimide (sulfo-NHS) ester at each end of an 8-carbon spacer arm. The reaction of Sulfo-NHS esters with primary amines in the structure of proteins happens at pH 7-9 to form stable covalent amide bonds. This results in the release of the N-hydroxysulfosuccinimide leaving group. The DTSSP linker was utilized to functionalize the surface of the polyaniline nanofiber-gold electrode platform for COX-2 antibody immobilization. DTSSP was purchased from Pierce (Product code# 21578).

4.3.3 BSA Block

Bovine serum albumin (BSA) superblock was used as the blocking solution. This was performed to ensure the selective binding of COX-2 antigen to related antibody and not to the nanofiber surface. BSA block was purchased from Pierce (Product code# 0037516).

4.3.4 Phosphate Buffer Saline

Phosphate buffer saline (PBS) was purchased from Pierce (Product code# 0028372) and utilized for solution preparation.

4.4 Electrochemical Impedance Spectroscopy

Electrical biosensors have proven to have high stability, low cost, small size, and high analysis speed which make them promising candidates for point-of-care diagnostics. One of the main incentives for developing impedance biosensors is their capability to perform label-free detection. In impedance biosensors, a quite small amplitude sinusoidal voltage (usually 10 mV amplitude or less) is applied on the electrodes. Applying a small value of voltage helps define a linear current-voltage relationship due to the small perturbations. Moreover, smaller value of the voltage prevents probe layer disturbance where the interactions are on the order of approximately 1 – 3 eV or even less and applied voltage spread a force on the surface which could damage the bimolecular probe layer [31]. In order to perform the impedance measurements, two electrodes are utilized. One of the electrodes, which acts as a working electrode, is used for output current measurements. The second electrode is utilized to maintain a fixed and reproducible voltage between the working electrode and solution. The desired voltage is applied by a potentiostat. Once the voltage is applied, the output current is sensed at the working electrode. The ratio of applied voltage to the output current gives the impedance value over a frequency range which is characterized by the frequency response analyzer (potentiostat).

4.5 Double Layer Capacitance

When an electrode is place into a liquid, the positive and negative ions are distributed relative to each other at the interface between an electrode and an electrolyte. Such a phenomenon is known as an electrical double-layer. The electrical double-layer can be utilized as the fundamental capacitor structure by applying a voltage to regulate the surface charge. The double layer capacitance is directly related to voltage value. By enhancing the electrode voltage, the capacitance will increase due to ion diffusion augmentation.

The binding of antibody molecules to the base of nanofiber and antigen-antibody interactions perturb the surface charges of these biomolecules. This subsequently results in disturbing the charge distribution and thus variations in capacitance value in the electrical double layer. Therefore, the variation in electrical double layer capacitance is associated with the antigen-antibody bonding and interaction. Characterizing the variation in capacitance can give a precise assessment of antigen concentration. At lower frequencies, the impedance change can be utilized to characterize the variations in double layer capacitance. The electrochemical system can be modeled to an electrical equivalent circuit to fit impedance biosensor data. The electrical equivalent circuit of the sensor is shown in Figure 14.

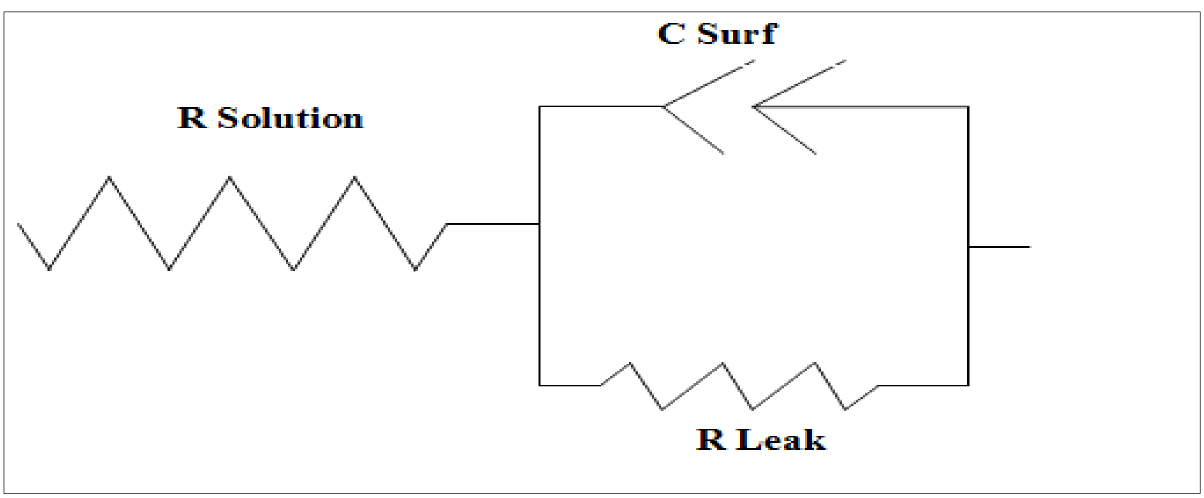

Figure 14. Electrical equivalent circuit at the analyte-nanofiber interface

The solution resistance (Rsol) originates from the finite ions conductance in bulk solution, and thus is generally not affected by binding. All charges undergo the Rsol when they pass through the solution. The capacitance between the electrode and ions in solution is the combination of the surface capacitance and the double layer capacitance which is shown as Csur. It is of significant importance to optimize the input voltage parameters so to be able to characterize the capacitive component. The final impedance is the summation of the resistance and capacitance of the sensing platform. However, the only indicator of binding is the capacitive component which represents the biomolecule binding in the electrical double layer. The dielectric capacitance between the two electrodes does not vary with AC voltage values, thus is utilized as a control baseline. It has been demonstrated that at lower frequencies (1 kHz and lower) the capacitive double layer dominates the impedance measurements.

EIS characterization was performed by utilizing a potentiostat from Gamry Instruments (Model: Reference 600). The frequency range of 50 Hz to 2 kHz was used to evaluate the impedance response and the frequency of 100 Hz was experimentally recognized to be the optimum operating frequency. Applying voltage amplitude of 10 mV and optimum frequency of 100 Hz resulted in maximum change in the EDL capacitance.

4.6 Experimental Protocol

In this section, the experimental protocol is described. All experiments followed a similar protocol to sustain consistency and increase reliability and accuracy.

- The circuit platform was soaked in ethanol for 10 minutes and then cleaned with DI water and air dried to create a contamination free environment.

- The electrospun polyaniline nanofiber was cut and applied onto the top of the gold electrode.
- The PDMS chamber was then placed on the platform using a biocompatible double sided tape.
- Wires were linked to the working and counter electrodes. The biosensor was then hooked up to the Gamry potentiostat for electrical measurements.
- 200 µl pf PBS solution was prepared and injected into the manifold. After 15 minutes, an EIS measurement was taken.
- 200 µl of 10 mM DTSSP was injected into the manifold and kept for 30 minutes at room temperature. The chip was then washed three times with PBS and the impedance measurement was taken subsequently. This step was performed to functionalize the platform surface for antibody interactions. PBS was utilized to wash away the unbound DSP linker so that the impedance measurements represent the number of bounded linkers to the surface.
- After linker deposition, 200 µl of COX-2 antibody solution was injected into the chamber. Several experiments were performed to obtain the optimal antibody saturation concentration and time. After storing the antibody at -4°C, the sensor surface was washed three times with PBS solution and impedance measurements were taken.
- 200 µl BSA Superblock was then added to the platform. After 15 minutes storage at room temperature, the surface was washed with PBS and impedance measurement was taken. The impedance measurement at this step was utilized as a control/baseline measurement.
- Different concentration of COX-2 peptide prepared from 10fg/ml to 1µg/ml was then added to the platform starting from the lowest concentration. 200 µl of the target analyte

was injected into the manifold, incubated for 15 minutes and impedance measurement was taken. The sensor surface was washed three times with PBS solution and the next highest concentration was injected.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Fiber Architecture and Characterization

Scanning electron microscope (SEM) images of the polyaniline fibers fabricated at different flow rates of 0.5, 1.0, 2.0 and 3ml/hr were captured to characterize the morphology of the fibers, such as average diameter, geometry, and density of surface pores. The diameter of electrospun polyaniline fibers were examined via a scanning electron microscope (SEM). Microscopic images of fibers for each flow rate were captured from three random locations. SEM images of the fabricated fibers at different flow rates of 0.5, 1.0, 2.0 and 3ml/hr are presented in Figure 15.

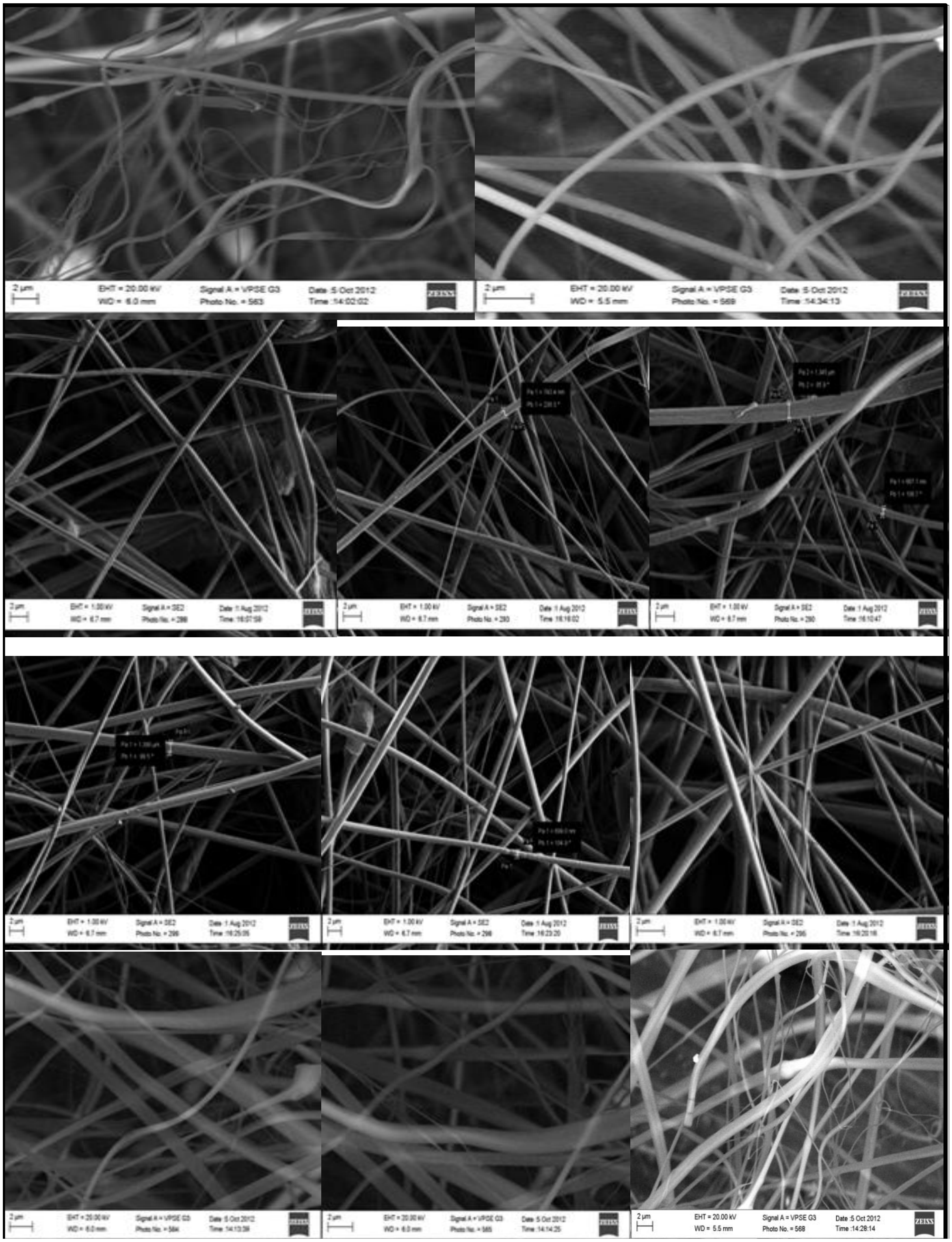

Figure 15 . SEM micrographs of polyaniline fibers (a) at o.5 ml/hr flow rate (b) at 1 ml/hr flow rate (c) at 2 ml/hr flow rate (d) at 3 ml/hr flow rate

Statistical fiber diameter distributions were calculated for each fiber. Fiber diameter calculations for electrospun polyaniline nanofibers fabricated at four different flow rates of 0.5, 1, 2, and 3 ml/hr are presented in Figure 16, Figure 17, Figure 18 and Figure 19 respectively.

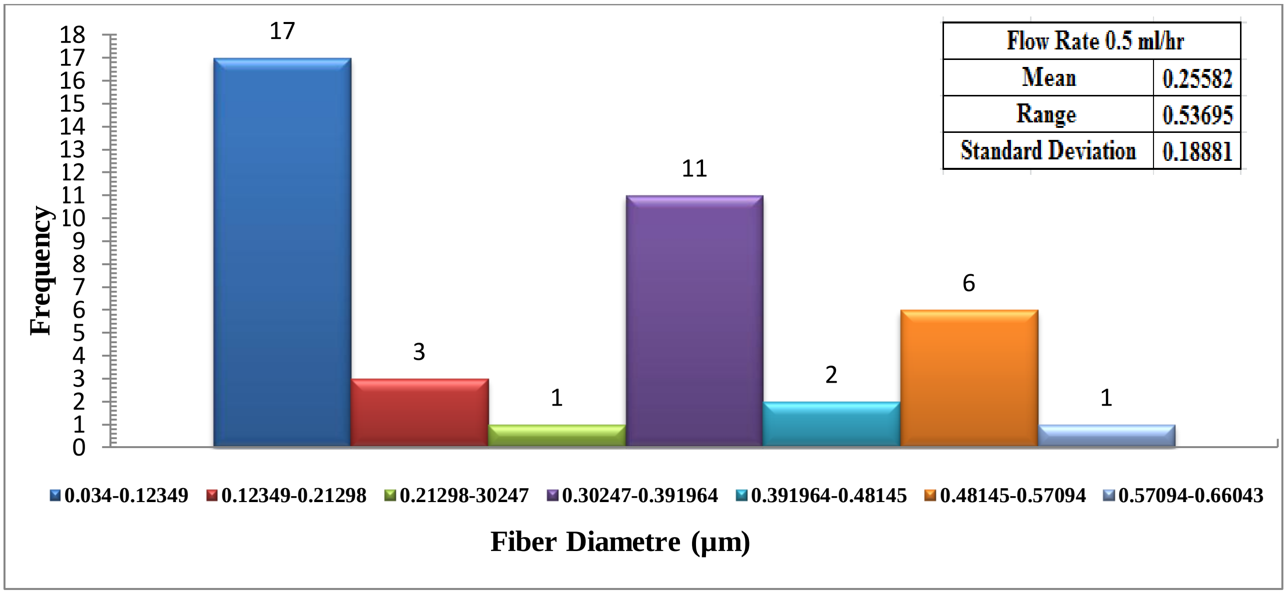

Figure 16. Diameter distributions of the electrospun polyaniline spun at flow rate of o.5 ml/hr

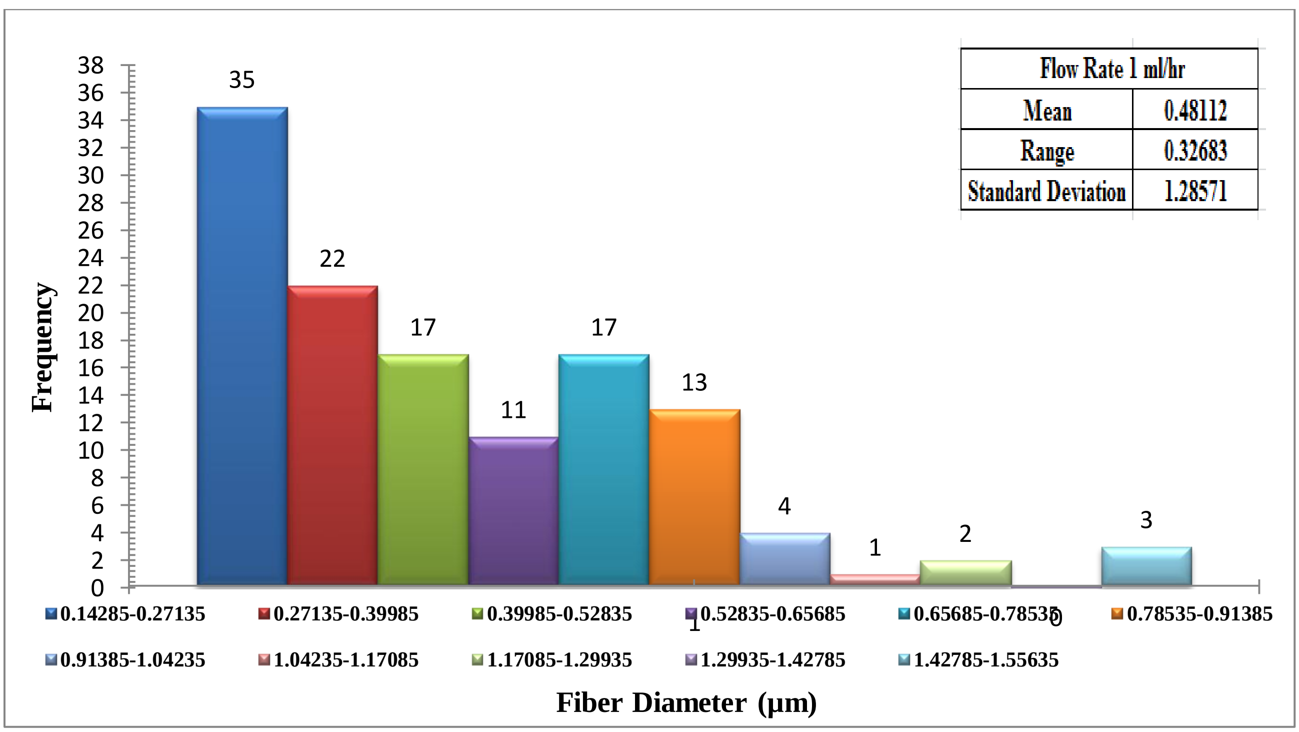

Figure 17. Diameter distributions of the electrospun polyaniline spun at flow rate of 1 ml/hr

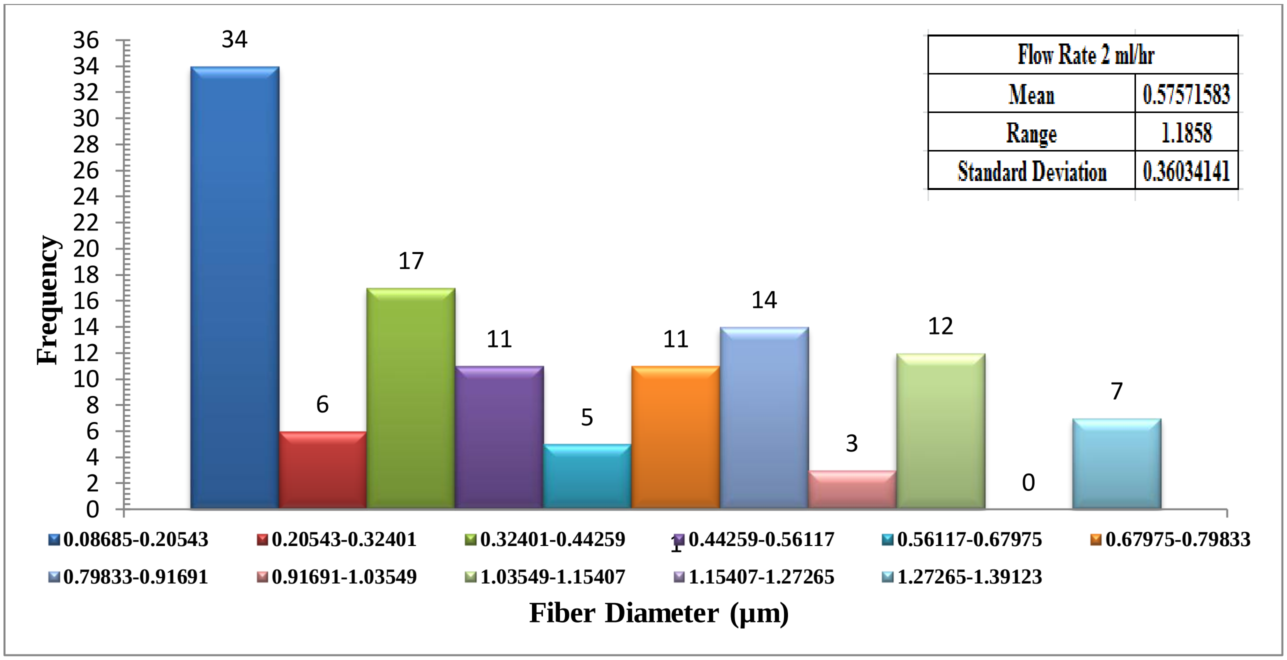

Figure 18. Diameter distributions of the electrospun polyaniline spun at flow rate of 2 ml/hr

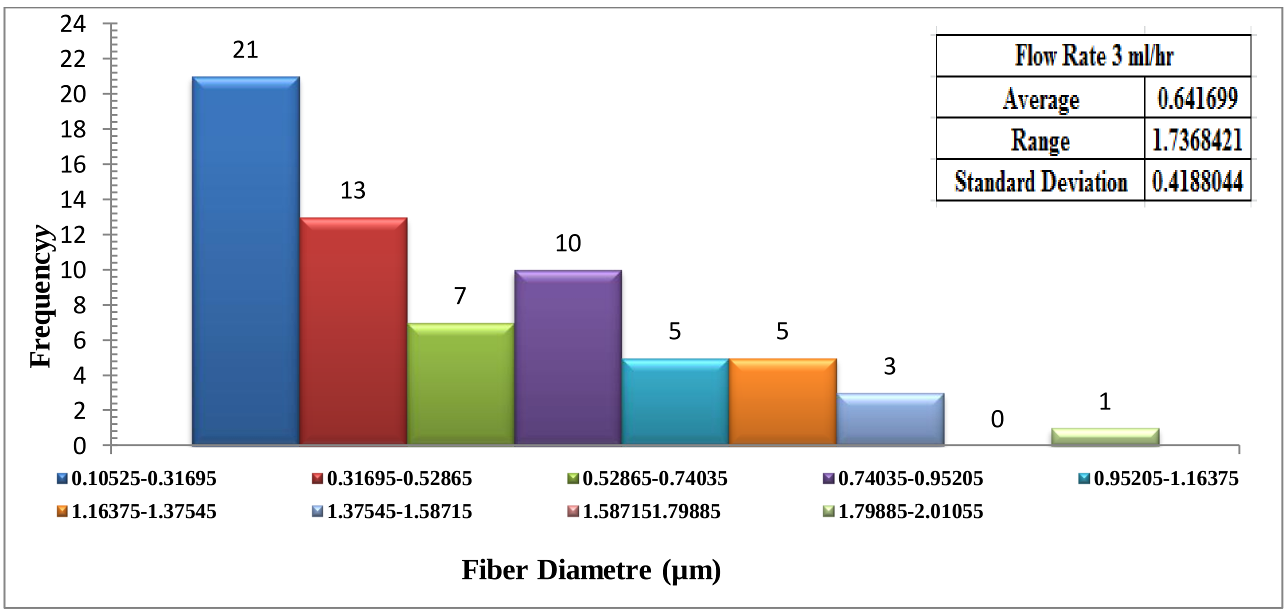

Figure 19. Diameter distributions of the electrospun polyaniline spun at flow rate of 3 ml/hr

The effect of flow rate on fiber average diameter is illustrated in Figure 20.

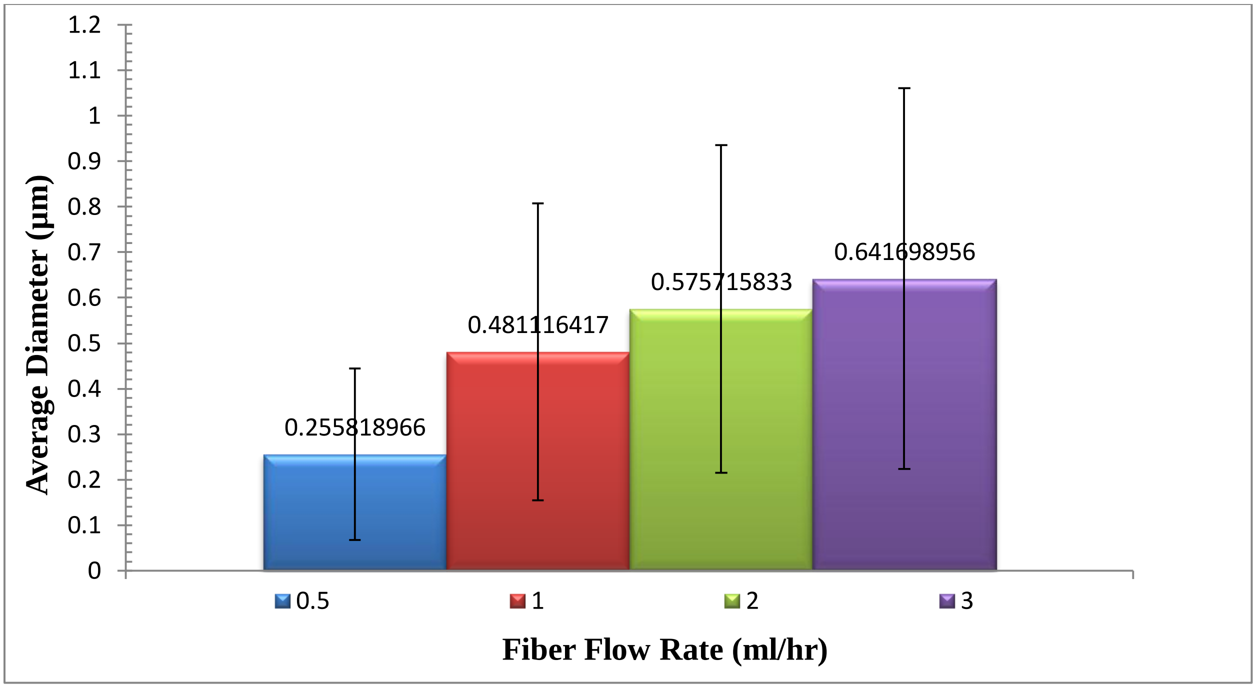

Figure 20. Effect of flow rate on fiber average diameter is

As can be observed from Figure 20, increasing the flow rate results in the reduction of the average diameter which consequently leads to specific surface area decrease. We were able to obtain mean diameters as low as 255 nm with a 0.5 ml/hr flow rate. Smaller diameters of fibers, and therefore larger specific areas, provide abundant immobilization space for biomolecules and increase the sensitivity of biosensors.

5.2 Sensor Performance in Buffer Saline

This section discusses the results of biosensors dose response tests and performance in detecting COX-2 biomarker in PBS and human serum samples. Four different biosensors were prepared using different electrospun polyaniline nanofibers fabricated at different flow rates. Another sensor was prepared which was assembled without polyaniline nanofiber integration. This was performed to prepare a control sensor to be utilized as a calibration sensor. The obtained results from the initial control experiments were compared with the dose response of biosensors integrated with four different polyaniline fibers to determine the most sensitive one. The selectivity of the biosensor with the best performance was then evaluated.

5.2.1 Antibody Saturation Concentration

The first set of experiments was conducted using different concentrations of antibody to determine the antibody concentration which would saturate the sensor surface. This was performed for all five prepared sensors. The same protocol which was mentioned earlier was followed to perform these tests. The electrospun polyaniline nanofiber surfaces were functionalized with 200 µl of 10 mM DTSSP linker and different concentrations of COX-2 antibody were immobilized onto the surface step by step. A consecutive dilution of aliquots COX-2 antibody was prepared and injected into the surface, starting from the lowest concentration of 0.01 up to the highest concentration of 50000ng/ml. The surfaces were washed with PBS solution after each step. The measured impedance corresponding to PBS solution was used as the baseline or zero dose measurement. All measurements were taken at an AC voltage of 10 mV and frequency of 100 Hz. In order to determine the required time for antibody penetration onto the surface, impedance measurements were taken every five minutes for each antibody concentration. When the measurements started to become stable and no significant change was observed, compared to the previous measurements, the obtained time was recorded as antibody saturation time. Subsequently the next dose of antibody was injected and the same procedure was followed to attain the antibody penetration time for each concentration. Figure 21 shows the results of antibody penetration time experiments which were performed for an antibody concentration of 0.01ng/ml.

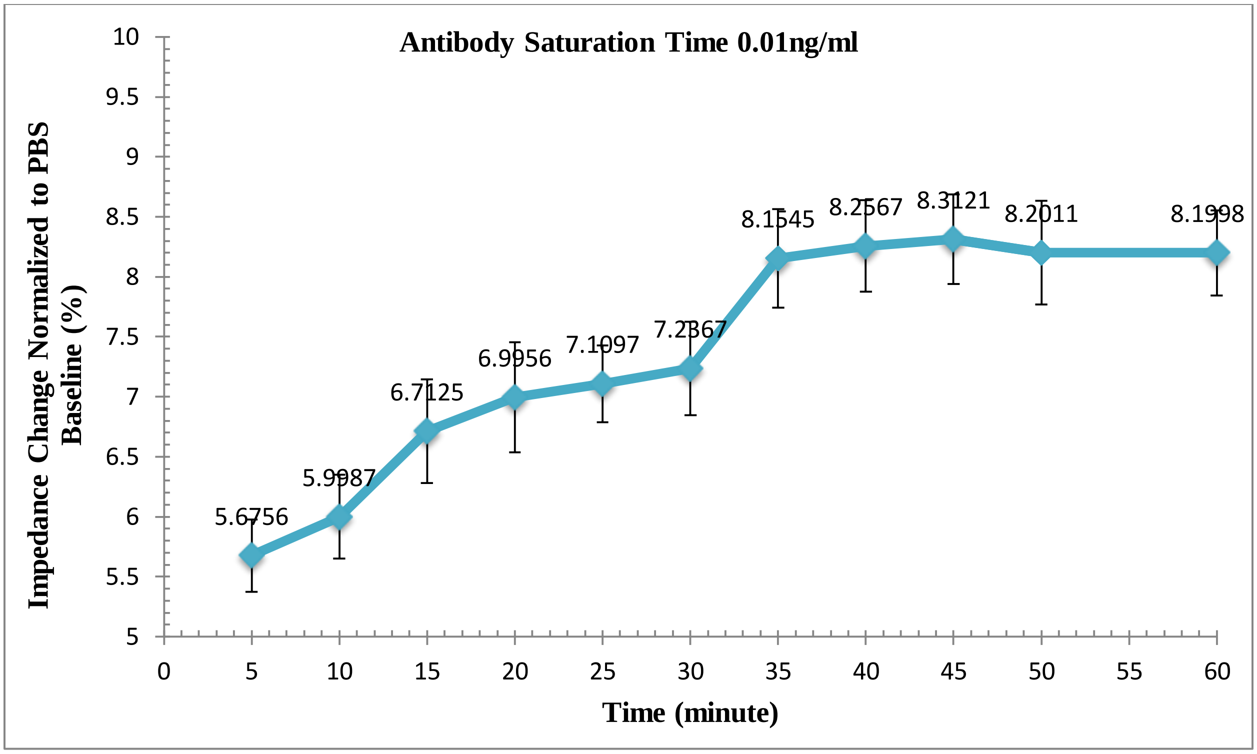

Figure 21. Antibody penetration time performed for antibody concentration of 0.01ng/ml.

As seen in Figure 1, after thirty five minutes no significant change was observed in the measured impedance values, but before that time, the measurements were constantly increasing. Therefore, it was concluded that thirty five minutes is the required time for antibody solution at concentration of 0.01ng/ml to penetrate into the surface and reach a steady state condition. The same experiments were performed for various concentrations of antibody and for each concentration, the saturation time was recorded. Figure 22 demonstrates the measured time for various COX-2 antibody concentrations to attain stable impedance measurements.

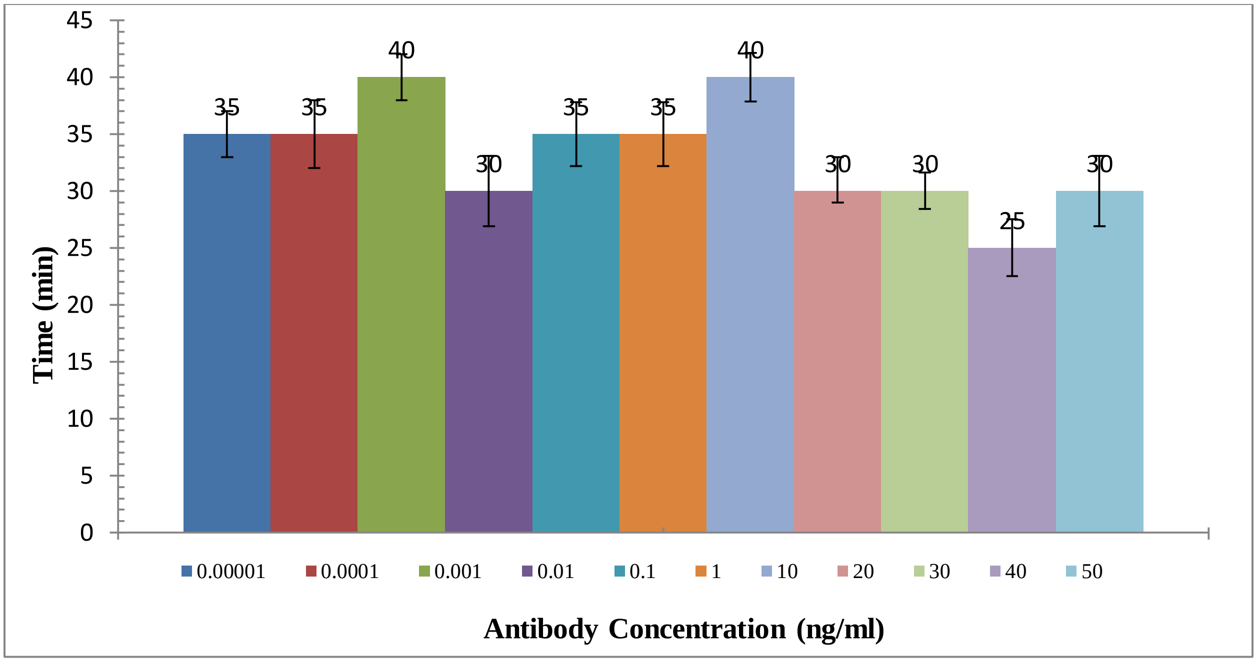

Figure 22. Measured time for various COX-2 antibody concentrations

COX-2 antibody was prepared and injected into the surface starting from a lowest concentration of 0.01 up to highest concentration of 50000ng/ml. The impedance measurements for each concentration were taken after storing the biosensor for the specified amount of time. After the addition of DTSSP to functionalize the surface, PBS was injected and the impedance measurement was taken and used as a baseline. Afterwards, the chip was washed three times with PBS solution and the lowest concentration of antibody was injected. The sensor was then stored for thirty five minutes and the impedance measurement was taken. The chip was washed three times with PBS solution and the next concentration of antibody was injected. The sensor was then stored for thirty five minutes and another measurement was taken. The antibody injection was performed in a stepwise manner and was continued until the highest concentration was added to the system. After thirty minutes, the measurement was taken. Figure 23 shows the dose response of COX-2 antibody in PBS using the biosensor assimilated with polyaniline nanofiber prepared at 1 ml/hr flow rate. The results obtained for other biosensors assembled with fibers, spun at flow rates of 0.5, 2, and 3 are presented in Appendix.

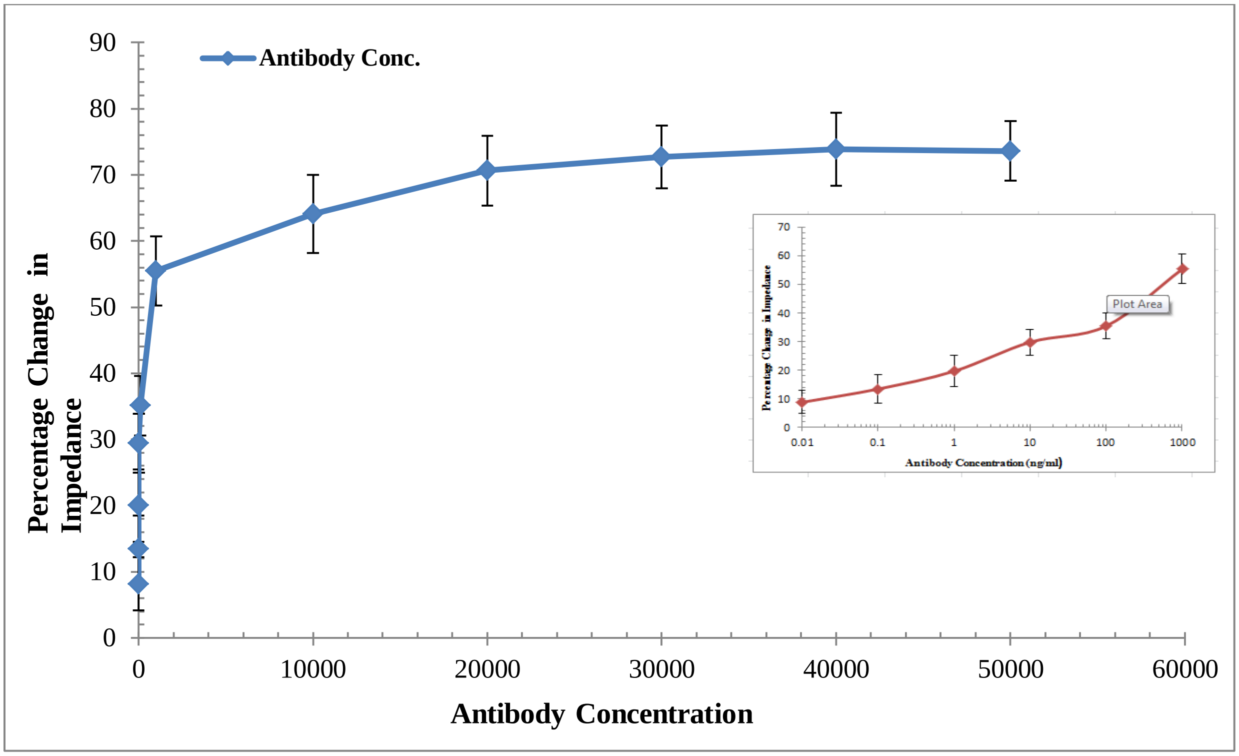

Figure 23. Dose response of COX-2 antibody in PBS using the biosensor assimilated with polyaniline nanofiber prepared at 1 ml/hr flow rate

As it can be observed from Figure 23, at COX-2 concentration of 30000ng/ml, impedance reached saturation with the polyaniline fiber prepared at 1 ml/hr flow rate. Hence, the concentration of 30µg/ml was chosen as the saturation dose and the time of incubation at this concentration was thirty minutes. A percentage change of approximately 73% was observed at the concentration of 30µg/ml and after this concentration, slight changes were observed in impedance measurements. The same experiments were performed for biosensors integrated with polyaniline nanofibers prepared at different flow rates of 0.5, 2, and 3. The antibody saturation concentrations were found to be 30, 20, 10, and 10 ng/ml for fibers fabricated at 0.5, 2, and 3 ml/hr flow rates respectively. The antibody saturation isotherms for these biosensors are presented in Appendix.

5.2.2 COX-2 Antigen Dose Response in PBS Solution

The polyaniline nanofiber was first functionalized with 10 mM DTSSP linker and stored for 30 minutes. After that, the sensor was washed with PBS solution and the impedance measurement was taken. 30 μg/ml of COX-2 antibody was injected into the manifold and the sensor was stored for 30 minutes to reach a steady state condition. The surface was then treated with BSA blocker. This was performed to block the active sites which did not bond with the immobilized antibody. The surfaces were washed with PBS and impedance measurements were taken after 15 minutes. The obtained impedance measurement at this step was considered as the baseline or zero dose measurement. The dose response for COX-2 antigen was performed over concentrations ranging from 10fg/ml to 1μg/ml. 200μl of the lowest concentration of antigen was injected into the manifold and the impedance measurement was taken after 30 minutes of incubation. Similar experiments performed to measure the antibody penetration time were completed to measure antigen penetration time into the biosensors surfaces. The sensor surface was washed with PBS and the next higher concentration of COX-2 antigen was injected. All the measurements were taken at an AC voltage of 10 mV and a frequency of 100 Hz. A different sensor was utilized to act as the control system and to evaluate the effect of integration of electrospun polyaniline nanofiber on sensor's performance. The same procedure was utilized for the control biosensor except that the electrospun polyaniline nanofiber was not implemented in the sensor surface. Figure 24 indicates the dose response of COX-2 antigen in PBS for the control biosensor.

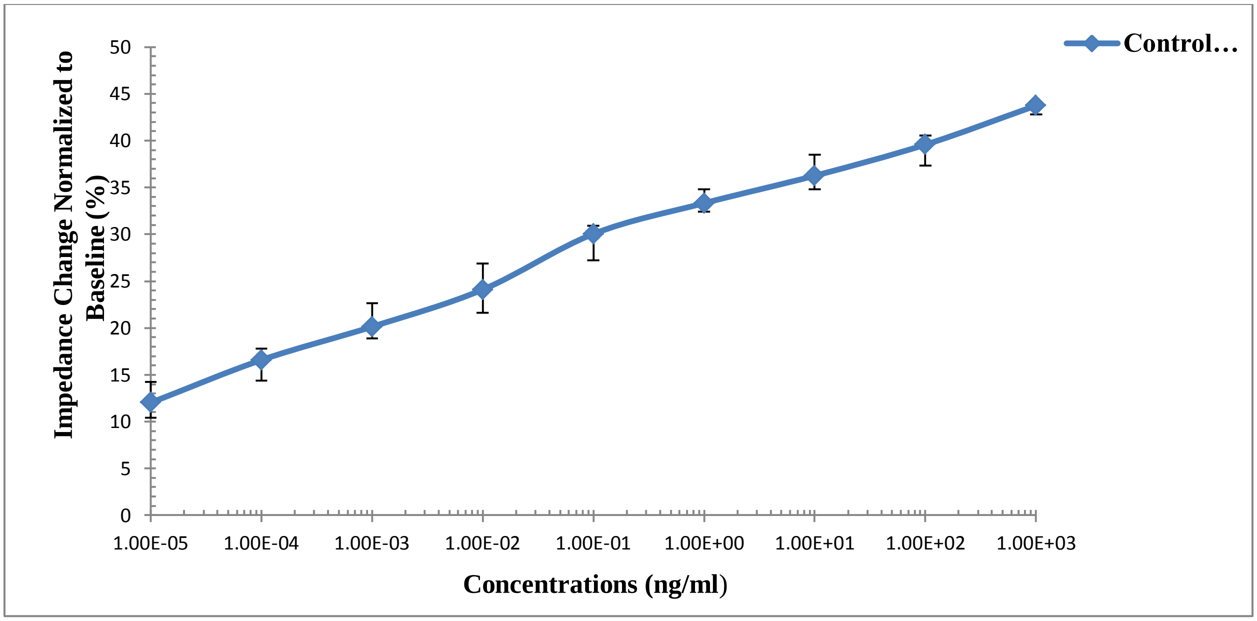

Figure 24. Dose response of COX-2 antigen in PBS for control biosensor

The dose response of COX-2 antigen in PBS for sensors integrated with four different nanofibers prepared at various flow rates of 3, 2, 1, and 0.5 are presented in Figure 25, Figure 26, Figure 27, and Figure 28 respectively.

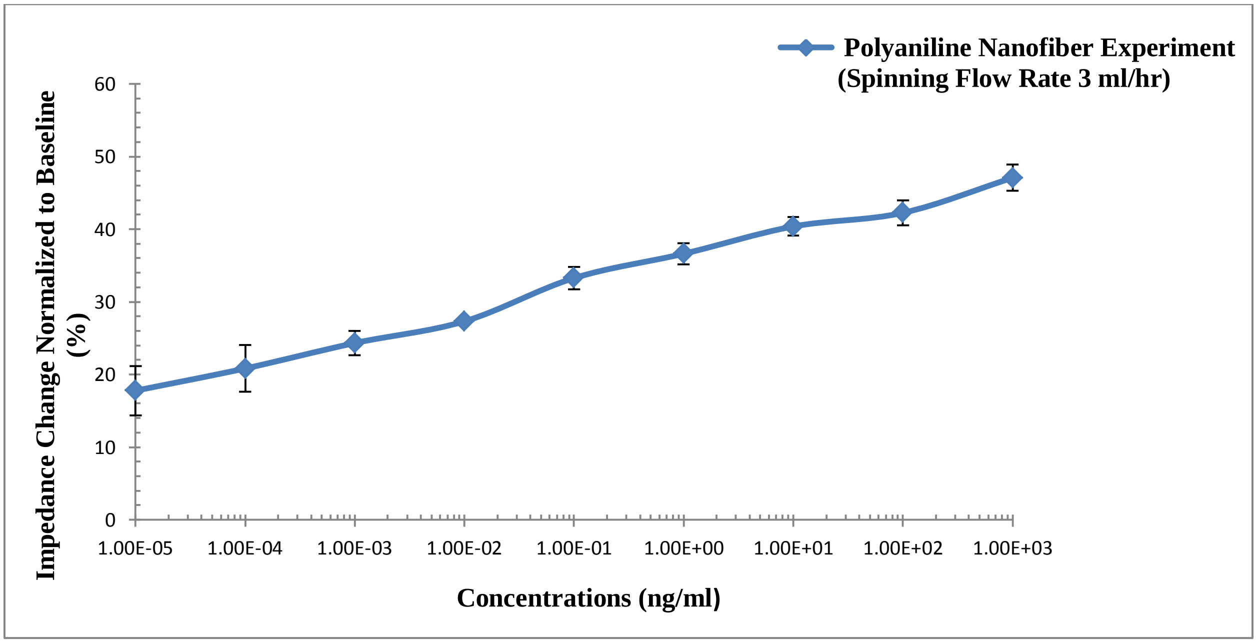

Figure 25. Dose response of COX-2 antigen in PBS for biosensor integrated with polyaniline fiber spun at flow rate of 3 ml/hr

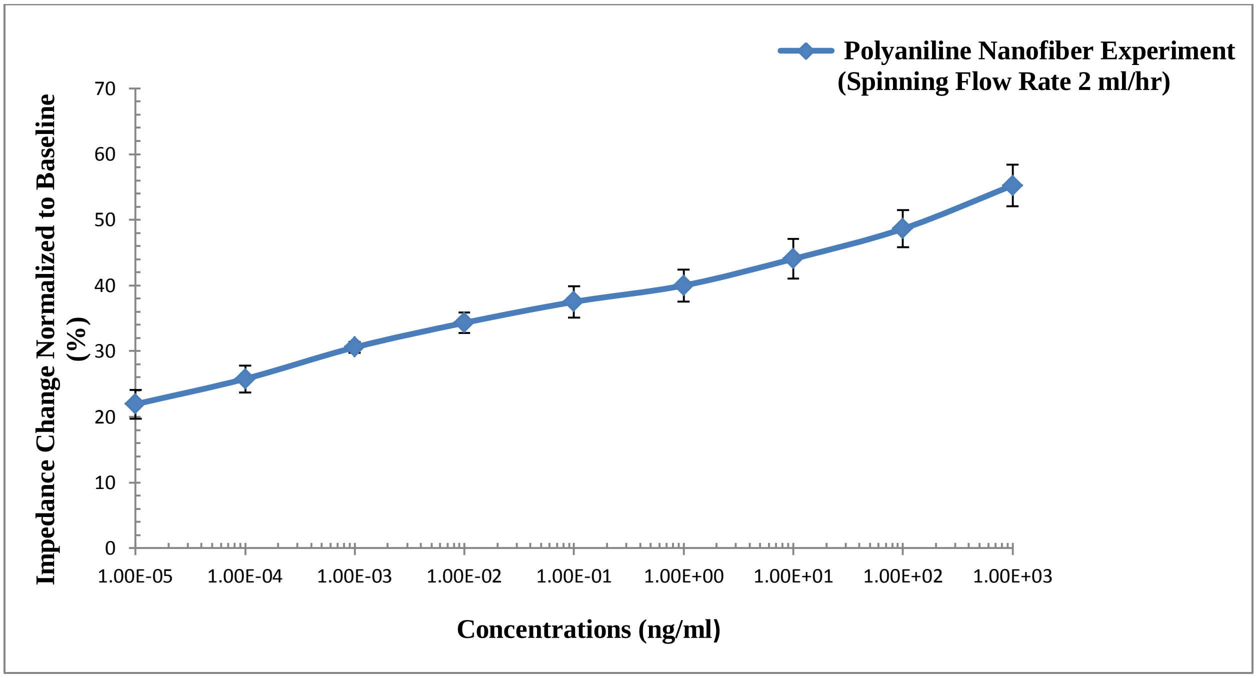

Figure 26. Dose response of COX-2 antigen in PBS for biosensor integrated with polyaniline fiber spun at flow rate of 2 ml/hr

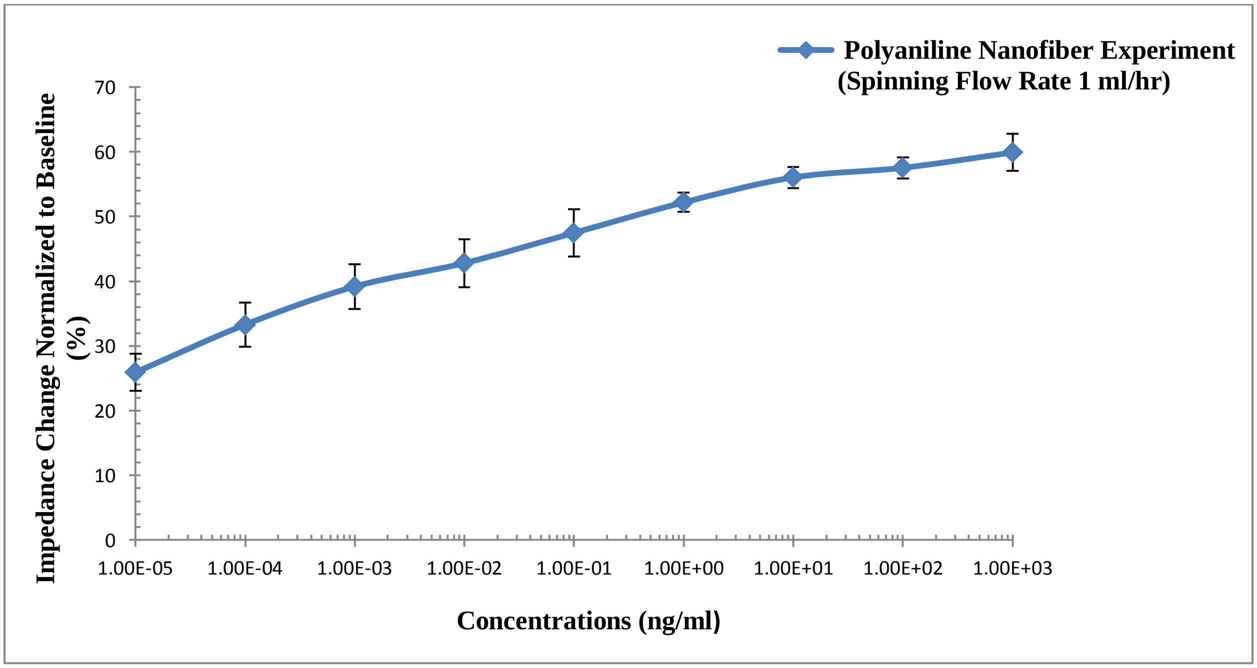

Figure 27. Dose response of COX-2 antigen in PBS for biosensor integrated with polyaniline fiber spun at flow rate of 1 ml/hr

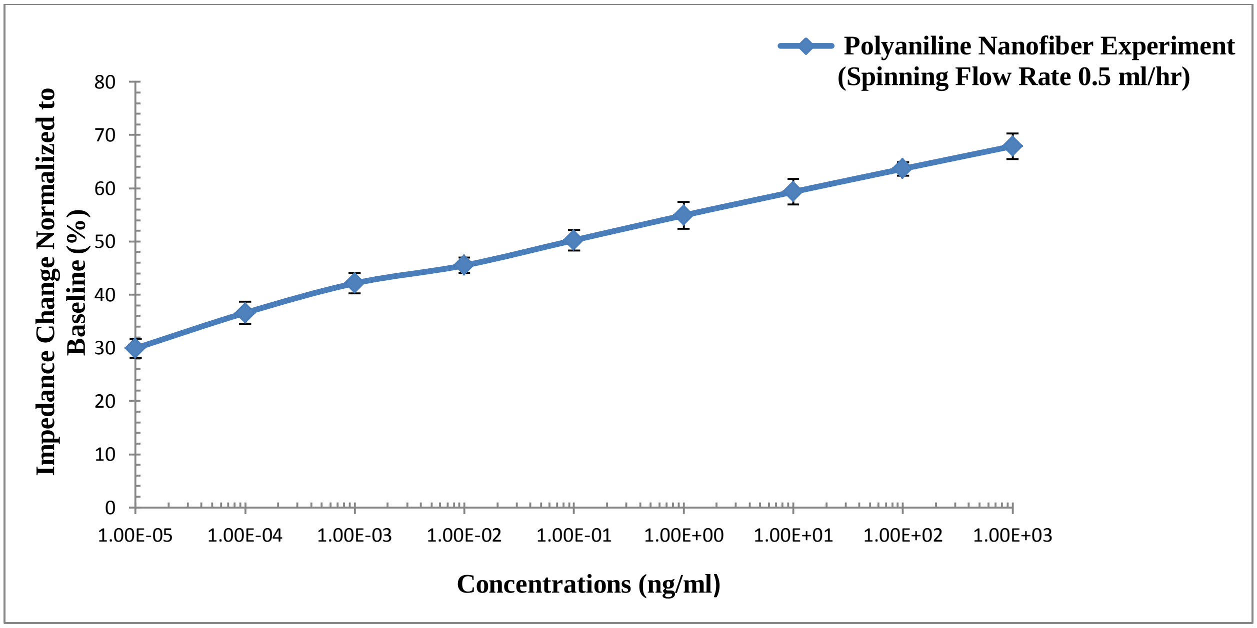

Figure 28. Dose response of COX-2 antigen in PBS for biosensor integrated with polyaniline fiber spun at flow rate of 0.5 ml/hr

The maximum changes in the impedance from the base line were obtained for the five prepared sensors. Figure 29 demonstrates the comparative impedance change for all these five biosensors.

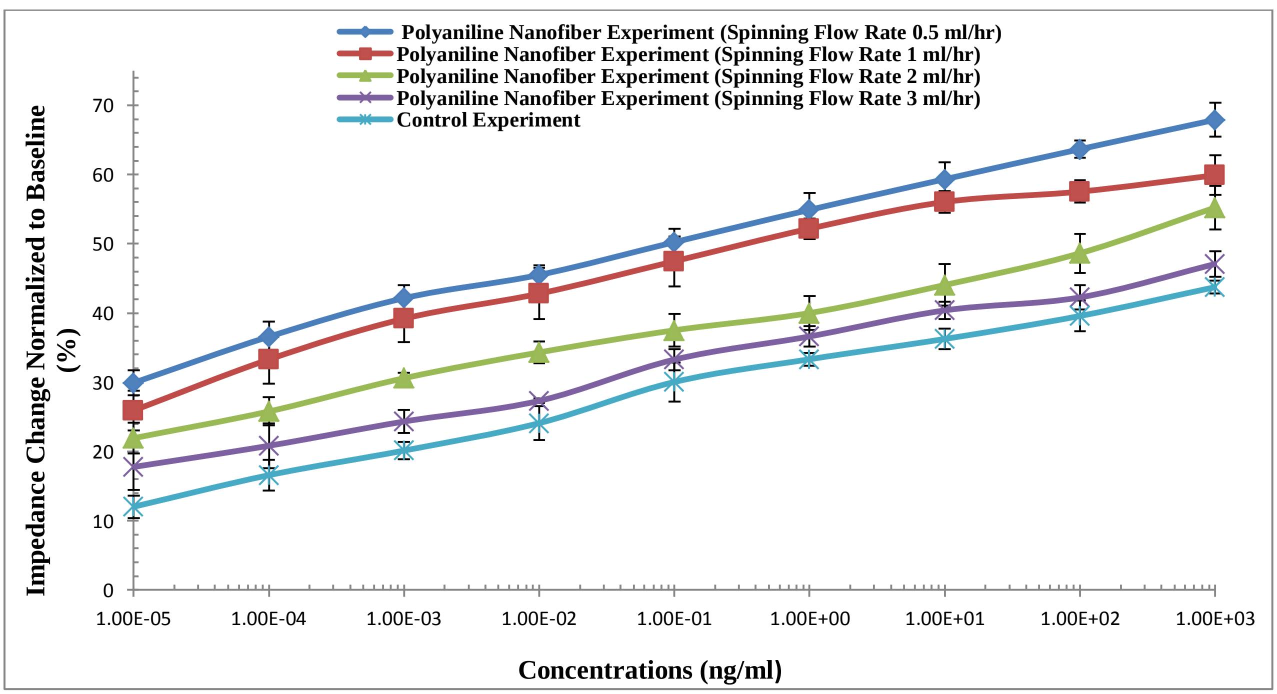

Figure 29. Comparative impedance change for the control biosensor and biosensors assimilated with polyaniline nanofibers spun at different flow rates of 0.5, 1, 2, and 3

As illustrated in Figure 29 it was observed that, in this concentration range, as the concentration of COX-2 antigen increased, the percentage change in impedance also enhanced. Saturation of the impedance values was not observed since the impedance values were constantly increasing in this concentration range. The percentage changes in the impedance were significantly higher for the polyaniline nanofiber substrate which was spun at a flow rate of 0.5ml/hr as compared to the other substrates. The percentage changes in impedance from PBS baseline were 67.9%, 59.89%, 55.23%, 47.07% and 43.74% on polyaniline nanofibers spun at flow rates of 0.5 ml/hr, 1 ml/hr, 2 ml/hr, 3 ml/hr and the control substrate respectively.

Ultra low limit of detection was observed as the sensor was able to detect the COX-2 antigen at femto molar concentrations and a large dynamic range was observed. The limit of detection was found to be 0.01 pg/ml for the COX-2 antigen in PBS solution on polyaniline nanofiber substrate spun at a flow rate of 0.5 ml/hr. The limits of detection of 0.1, 1, 50 and 100 pg/ml were found for the nanofiber substrates spun at flow rates of 1 ml/hr, 2 ml/hr, 3 ml/hr, and control substrate respectively. It can be concluded from the obtained results that integration of electrospun polyaniline nanofiber can significantly improve the performance of the designed biosensor. The controlled biosensor was able to detect the COX-2 biomarker at higher concentrations compared to biosensors integrated with electrospun nanofibers.

Incorporation of electrospun nanofibers could considerably decrease the limit of detection. The increase in the measured signal is attributed to the novel properties of electrospun polyaniline nanofibers. Large specific surface areas of nanofibers provide a plethora of binding sites for antigen-antibody interactions, thus enhancing the sensitivity of the biosensor. Moreover, electrospun polyaniline nanofibers offer confinement-induced enhancements due to the nanoporous structure and increased packing density of biomolecules. This is believed to play an influential role in enhancing the sensitivity of the biosensor.

In order to evaluate the generality of our explanation, electrospun polyaniline nanofibers were prepared at various flow rates to obtain fibers with various specific surface areas and morphologies. It was observed that, as the specific surface area of nanofiber increases, the limit of detection decreases. This confirmed the proposed explanation stating the direct correlation between nanofiber specific surface area and sensitivity of biosensor.

5.2.3 Selectivity Analysis

It is crucial to evaluate the performance and applicability of the electrospun polyaniline nanofiber based biosensors in clinical practices. In order to identify the performance of the fabricated biosensor in selective and specific COX-2 antigen detection from complex samples, a competitive protein human albumin was replaced with COX-2 antigen.

Previously explained procedures were followed to prepare the chip. The electrospun polyaniline nanofiber prepared at the flow rate of 0.5 ml/hr was assimilated onto the sensor's surface. Albumin aliquots were dissolved and diluted in PBS solutions ranging from 10fg/ml to 1μg/ml. Figure 30 shows the comparative impedance changes associated with different concentrations of COX-antigen and Human Albumin.

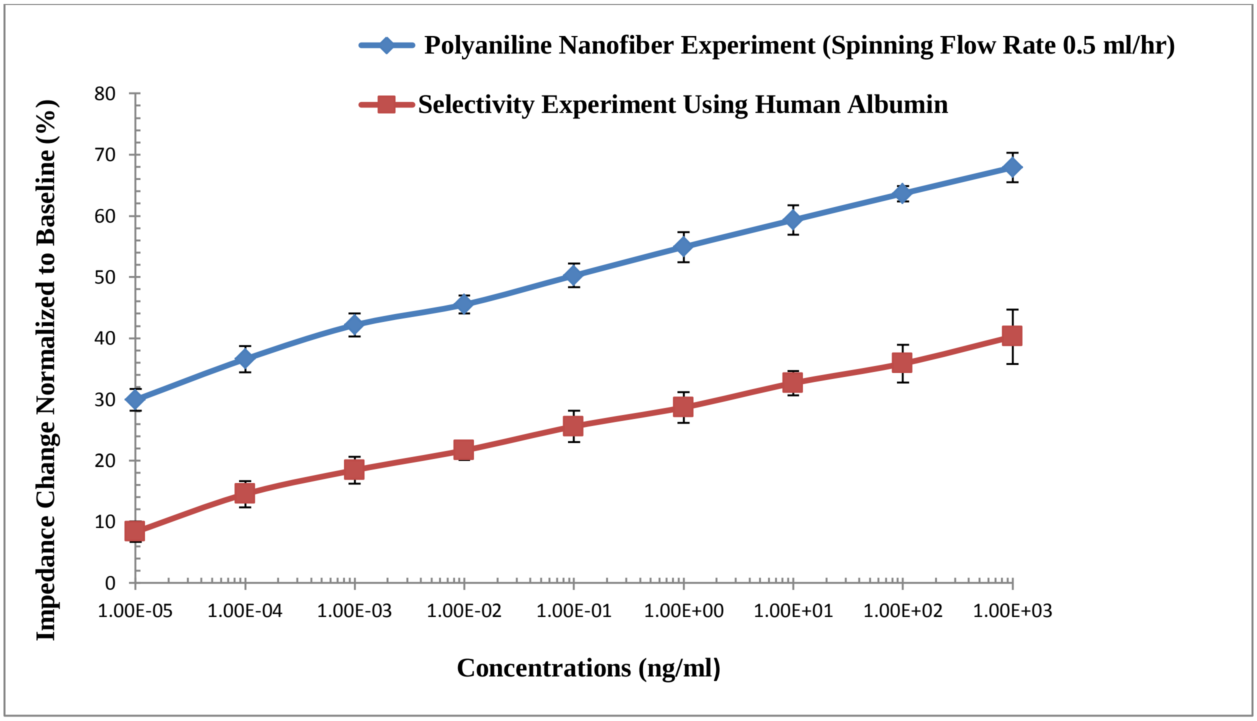

Figure 30. Dose response of COX-2 antigen and Human Albumin in PBS on the sensor surface incorporated with electrospun polyaniline nanofiber prepared at 0.5 ml/hr flow rate

As explained earlier, addition of COX-2 antigen to the biosensor led to the significant decrease in impedance values, while when non-target analyte albumin is added to the sensor, no significant change was observed in the impedance of the system. Increasing the concentration of non-target analyte albumin did not affect the impedance value noticeably. A maximum of 40% change in the impedance value from the baseline PBS measurement was observed with the highest concentration of albumin which was less than the measured impedance change for COX-2 antigen at a concentration of 1pg/ml. The graph indicates the low level of cross reactivity on the electrospun polyaniline nanofiber surface.

5.3 COX-2 Antigen Dose Response in Human Serum

A dose response experiment was performed to detect COX-2 antigen from human serum in a similar manner to dose response experiments performed with the PBS solution. This was performed to evaluate the sensor's capability in specifically detecting COX-2 antigen from complex buffers. Figure 31 shows the dose response of COX-2 from human serum.

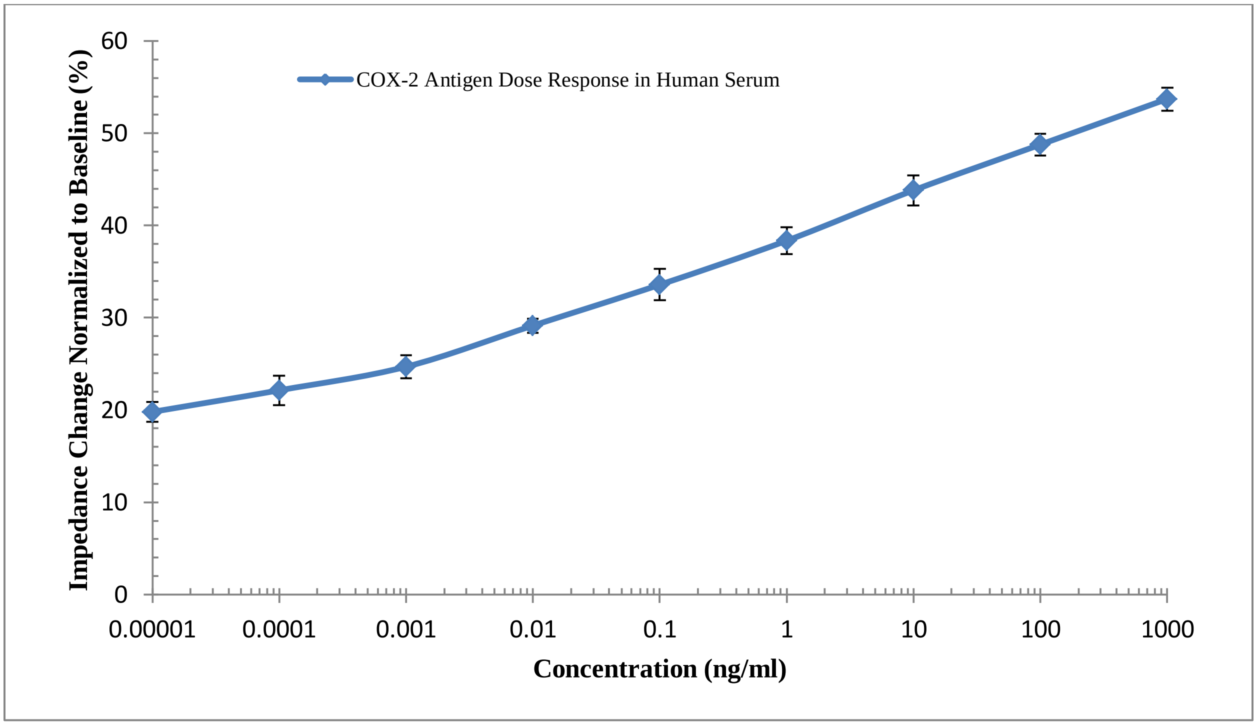

Figure 31. Dose response of COX-2 from human serum

A change in impedance from 19.8 to 53.7% was observed over the dose range of 10fg/ml to 1μg/ml of COX-2 antigen in human serum using a sensor which was integrated with polyaniline nanofiber spun at a flow rate of 0.5ml/hr. The sensor was able to detect the COX-2 from human serum at 10fg/ml concentration levels with an impedance change of 30% compared to the PBS baseline. The results obtained in these sets of experiments noticeably verify the remarkable capability of the designed biosensor platform to be used in ultrasensitive COX-2 detection. The electrospun polyaniline nanofiber was successfully incorporated in a micro-electrode platform to generate a functional label free biosensor for sensitive protein detection. The relative dose responses of the biosensor integrated with the polyaniline nanofiber spun at a flow rate of 0.5ml/hr, in buffer and serum solutions is presented in Figure 32.

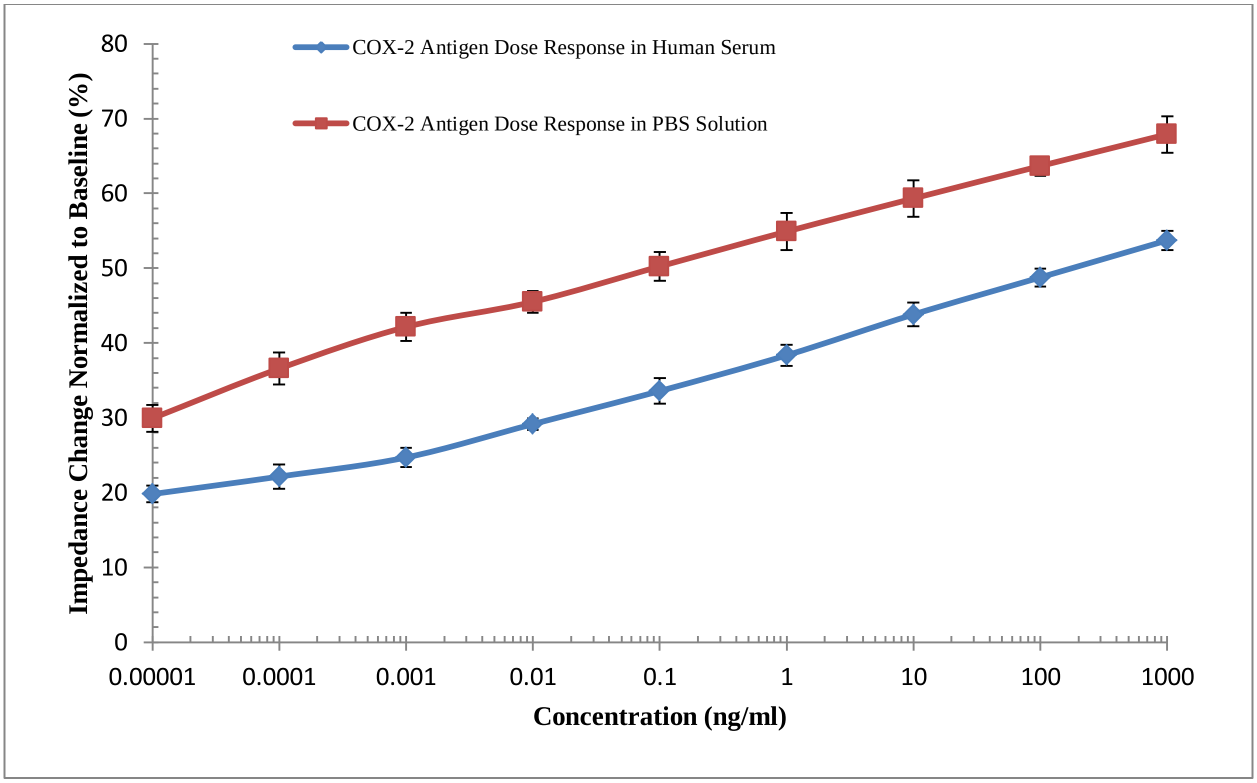

Figure 32. Relative dose response of the biosensor integrated with polyaniline nanofiber spun at 0.5 ml/hr flow rate in buffer and serum solutions

As observed, the percentage changes in impedance value and limits of detection were lower compared to those obtained in PBS solution. However, the changes in impedance were significant in the pico-molar concentrations which proved the effectiveness of the electrospun polyaniline nanofiber enabled biosensor as a device that can robustly determine COX-2 antigen in clinically relevant concentrations. Lower sensitivity in human serum could be attributed to more interference and cross-reactivity compared to the pure PBS solution.

5.4 Limitations

Despite the great potential and promise of the designed biosensor as a label free, lab-on-chip platform, capable of ultrasensitive COX-2 antigen detection, some limitations were observed during the experiments. The major problem is the lack of control over the fiber structure which is ascribed to the restrictions of the electrospinning process. Since all the fibers collected in the aluminum collector do not have the same structure and are not necessarily uniform, various samples need to be collected from different spots and several sets of experiments need to be completed to obtain a strong statistical base. In this research, a great effort was made to utilize fibers with similar morphologies and thicknesses.

Moreover, each sensor was designed for one set of experiments due to the unfeasibility of reusing the nanofiber. Furthermore, the conducting property of polyaniline reduces over time with air exposure which diminishes the sensitivity and stability of the device over a long time.

CHAPTER 6

CONCLUSION

In this research, an ultrasensitive electrospun polyaniline nanofiber based biosensor was designed and developed for COX-2 biomarker detection. The fabricated biosensor consists of three key parts; (a) a printed circuit platform comprised of interdigitated gold electrodes, (b) highly porous electrospun polyaniline nanofibers and (c) a microfluidic silicon manifold. Nanoscale architectures of polyaniline nanofibers were prepared via electrospinning at different diameters using different flow rates of 0.5, 1, 2, and 3 with the rest of the parameters fixed. The fabricated nanofibers were then successfully soldered onto the interdigitated gold microelectrodes to generate four different nanotextured sensing platforms. Finally, the chamber was placed on the sensing platforms to create confined and contamination free environments for interactions of the biomolecules. The performance of the biosensors in detecting the COX-2 biomarker was then assessed using the EIS characterization method. The results of the EIS characterization were then compared to the EIS measurements obtained for the control platform. The fabricated biosensors showed great promise in robustly and precisely detecting the targeted COX-2 antigen from complex and pure samples. The biosensor assembled with polyaniline nanofiber, spun at a flow rate of 0.5 ml/hr, was able to efficiently detect the COX-2 antigen at concentrations as low as 0.01pg/ml and 10fg/ml in PBS and human serum solutions respectively. The higher limits of detection were obtained for the polyaniline nanofibers spun at higher flow rates. The biosensor platform assembled with polyaniline nanofiber, spun at a flow rate of 1 ml/hr, was able to detect the COX-2 antigen at concentrations as low as 0.1pg/ml. This was reduced to 1 and 50pg/ml for sensors assembled with polyaniline nanofibers, spun at flow rates of 2 and 3ml/hr respectively. The integration of electrospun polyaniline nanofibers seemed to reduce the limit of detection significantly, compared to the control biosensor's limit of detection. The high sensitivity and low limit of detection in biosensors integrateed with electrospun polyaniline nanofibers were attributed to large specific surface areas as well as highly porous nanostructures which enhance size matched confinement, thus increasing the sensitivity and signal strength significantly. The biosensor's performance in sensitively and selectively detecting the target analyte in clinically relevant concentrations, proposes the enormous potential of electrospun polyaniline nanofibers as a label-free sensitive platform for COX-2 antigen detection in real samples.

CHAPTER 7

FUTURE WORK

The success of this research can open new doors for possible future work. More in-depth study on the electrospinning process optimization is necessary to better understand the effect of electrospinning parameters on fiber morphologies. Although the effect of electrospun polyaniline nanofiber diameter on sensor performance and limit of detection was evaluated broadly in this research, more effort is required to investigate the effect of electrospinning parameters on polyaniline nanofiber porosity and consequently sensor performance. It is helpful to develop a reliable process to detect liquid penetration time and sample intrusion in polyaniline nanofibers. Moreover, the designed biosensor can be tested with different target biomolecules to investigate the biosensor capability for parallel sensing.

Various conductive polymers can be employed for nanofiber fabrication to find out the electrospun polyaniline nanofiber performance in comparison with other polymeric nanofibers. Nanomaterials can be added to the electrospun solution to increase the sensitivity of biosensors by producing nanosized spots for biomolecule interactions. Polyaniline can be fabricated using other nanofabrication methods to evaluate the effectiveness of electrospinning in producing nanotextured fibers.

REFERENCES

REFERENCES

[1] J. H. T. Luong, K. B. Male, and J. D. Glennon, "Biosensor technology: Technology push versus market pull," 26, Biotechnology Advances, 2008.

[2] R. M. Lequin, "Enzyme Immunoassay (EIA)/Enzyme-Linked Immunosorbent Assay (ELISA)," *Clinical Chemistry,* September 22, 2005 2005.

[3] F. Anton, "Process and apparatus for preparing artificial threads," United States Patent, 1934.

[4] G. Taylor, "Electrically Driven Jets," *Proceedings of the Royal Society of London. A. Mathematical and Physical Sciences,* vol. 313, pp. 453-475, December 2, 1969 1969.

[5] D. H. Reneker and I. Chun, "Nanometre diameter fibres of polymer, produced by electrospinning," *Nanotechnology,* vol. 7, p. 216, 1999.

[6] P. K. Baumgarten, "Electrostatic spinning of acrylic microfibers," *Journal of Colloid and Interface Science,* vol. 36, pp. 71-79, 1971.

[7] J. Doshi and D. H. Reneker, "Electrospinning process and applications of electrospun fibers," *Journal of electrostatics,* vol. 35, pp. 151-160, 1995.

[8] A. Frenot and I. S. Chronakis, "Polymer nanofibers assembled by electrospinning," *Current opinion in colloid & interface science,* vol. 8, pp. 64-75, 2003.

[9] G. C. Rutledge, Y. Li, S. Fridrikh, S. Warner, V. Kalayci, and P. Patra, "Electrostatic spinning and properties of ultrafine fibers."

[10] Z.-M. Huang, Y. Z. Zhang, M. Kotaki, and S. Ramakrishna, "A review on polymer nanofibers by electrospinning and their applications in nanocomposites," *Composites Science and Technology,* vol. 63, pp. 2223-2253, 2003.

[11] X. Zong, K. Kim, D. Fang, S. Ran, B. S. Hsiao, and B. Chu, "Structure and process relationship of electrospun bioabsorbable nanofiber membranes," *Polymer,* vol. 43, pp. 4403-4412, 2002.

[12] H. Jiang, D. Fang, B. S. Hsiao, B. Chu, and W. Chen, "Optimization and characterization of dextran membranes prepared by electrospinning," *Biomacromolecules,* vol. 5, pp. 326-333, 2004.

[13] C. Mit-uppatham, M. Nithitanakul, and P. Supaphol, "Ultrafine Electrospun Polyamide-6 Fibers: Effect of Solution Conditions on Morphology and Average Fiber Diameter," *Macromolecular Chemistry and Physics,* vol. 205, pp. 2327-2338, 2004.

[14] T. Subbiah, G. Bhat, R. Tock, S. Parameswaran, and S. Ramkumar, "Electrospinning of nanofibers," *Journal of Applied Polymer Science*, vol. 96, pp. 557-569, 2005.

[15] J. Zeng, X. Xu, X. Chen, Q. Liang, X. Bian, L. Yang, and X. Jing, "Biodegradable electrospun fibers for drug delivery," *Journal of Controlled Release*, vol. 92, pp. 227-231, 2003.

[16] K. H. Lee, H. Y. Kim, M. S. Khil, Y. M. Ra, and D. R. Lee, "Characterization of nano-structured poly(ε-caprolactone) nonwoven mats via electrospinning," *Polymer,* vol. 44, pp. 1287-1294, 2003.

[17] W. K. Son, J. H. Youk, T. S. Lee, and W. H. Park, "The effects of solution properties and polyelectrolyte on electrospinning of ultrafine poly (ethylene oxide) fibers," *Polymer*, vol. 45, pp. 2959-2966, 2004.

[18] K. H. Lee, H. Y. Kim, Y. M. La, D. R. Lee, and N. H. Sung, "Influence of a mixing solvent with tetrahydrofuran and N,N-dimethylformamide on electrospun poly(vinyl chloride) nonwoven mats," *Journal of Polymer Science Part B: Polymer Physics,* vol. 40, pp. 2259-2268, 2002.

[19] J. Deitzel, J. Kleinmeyer, D. Harris, and N. Beck Tan, "The effect of processing variables on the morphology of electrospun nanofibers and textiles," *Polymer,* vol. 42, pp. 261-272, 2001.

[20] S. Megelski, J. S. Stephens, D. B. Chase, and J. F. Rabolt, "Micro-and nanostructured surface morphology on electrospun polymer fibers," *Macromolecules,* vol. 35, pp. 8456-8466, 2002.

[21] K. Toth, R. A. Durst, and G. S. Wilson, "Electrochemical biosensors: recommended definitions and classification," *Pure Appl. Chem,* vol. 71, pp. 2333-2348, 1999.

[22] D. R. Thévenot, K. Toth, R. A. Durst, and G. S. Wilson, "Electrochemical biosensors: recommended definitions and classification," *Biosensors and Bioelectronics,* vol. 16, pp. 121-131, 2001.

[23] J. R. Ferraro, *Introductory raman spectroscopy*: Academic press, 2002.

[24] J. Homola, S. S. Yee, and G. Gauglitz, "Surface plasmon resonance sensors: review," *Sensors and Actuators B: Chemical,* vol. 54, pp. 3-15, 1999.

[25] K. Länge, B. Rapp, and M. Rapp, "Surface acoustic wave biosensors: a review," *Analytical and Bioanalytical Chemistry*, vol. 391, pp. 1509-1519, 2008/07/01 2008.

[26] K. Kalantar-Zadeh and B. Fry, *Nanotechnology enabled sensors*: Springer, US, 2007.

[27] G. M. Whitesides and B. Grzybowski, "Self-assembly at all scales," *Science,* vol. 295, pp. 2418-2421, 2002.

[28] Y. Aoyama, M. Caira, G. Desiraju, J. Glusker, A. Hamilton, R. Melendez, A. Nangia, and E. Weber, *Design of organic solids* vol. 198: Springer, 1998.

[29] J. R. Heath and M. A. Ratner, "Molecular electronics," 2003.

[30] B. B. Haab, "Methods and applications of antibody microarrays in cancer research," *Proteomics,* vol. 3, pp. 2116-2122, 2003.

[31] J. S. Daniels and N. Pourmand, "Label-Free Impedance Biosensors: Opportunities and Challenges," *Electroanalysis,* vol. 19, pp. 1239-1257, 2007.

[32] Y. A. Dzenis, "Spinning continuous fibers for nanotechnology," 2004.

[33] J. Wang and Y. Lin, "Functionalized carbon nanotubes and nanofibers for biosensing applications," *TrAC Trends in Analytical Chemistry,* vol. 27, pp. 619-626, 2008.

[34] Y. Luo, S. Nartker, H. Miller, D. Hochhalter, M. Wiederoder, S. Wiederoder, E. Setterington, L. T. Drzal, and E. C. Alocilja, "Surface functionalization of electrospun nanofibers for detecting< i> E. coli</i> O157: H7 and BVDV cells in a direct-charge transfer biosensor," *Biosensors and Bioelectronics,* vol. 26, pp. 1612-1617, 2010.

[35] R. Jayakumar, M. Prabaharan, S. V. Nair, and H. Tamura, "Novel chitin and chitosan nanofibers in biomedical applications," *Biotechnology Advances,* vol. 28, pp. 142-150, 2010.

[36] Y. Du, X.-L. Luo, J.-J. Xu, and H.-Y. Chen, "A simple method to fabricate a chitosan-gold nanoparticles film and its application in glucose biosensor," *Bioelectrochemistry,* vol. 70, pp. 342-347, 2007.

[37] X. Chen, Z. Chen, J. Zhu, C. Xu, W. Yan, and C. Yao, "A novel H2O2 amperometric biosensor based on gold nanoparticles/self-doped polyaniline nanofibers," *Bioelectrochemistry,* vol. 82, pp. 87-94, 2011.

[38] C. Feng, K. Khulbe, and T. Matsuura, "Recent progress in the preparation, characterization, and applications of nanofibers and nanofiber membranes via electrospinning/interfacial polymerization," *Journal of Applied Polymer Science,* vol. 115, pp. 756-776, 2010.

[39] X. Mao, F. Simeon, G. C. Rutledge, and T. A. Hatton, "Electrospun Carbon Nanofiber Webs with Controlled Density of States for Sensor Applications," *Advanced Materials,* pp. n/a-n/a, 2012.

[40] S. Huang, "Glucose Biosensor Using Electrospun Mn2O3-Ag Nanofibers," 2011.

[41] W. Wang, Z. Li, W. Zheng, J. Yang, H. Zhang, and C. Wang, "Electrospun palladium (IV)-doped copper oxide composite nanofibers for non-enzymatic glucose sensors," *Electrochemistry communications,* vol. 11, pp. 1811-1814, 2009.

[42] Y. Ding, Y. Wang, L. Su, M. Bellagamba, H. Zhang, and Y. Lei, "Electrospun Co< sub> 3</sub> O< sub> 4</sub> nanofibers for sensitive and selective glucose detection," *Biosensors and Bioelectronics,* vol. 26, pp. 542-548, 2010.

[43] Z.-G. Wang, Y. Wang, H. Xu, G. Li, and Z.-K. Xu, "Carbon nanotube-filled nanofibrous membranes electrospun from poly (acrylonitrile-co-acrylic acid) for glucose biosensor," *The Journal of Physical Chemistry C,* vol. 113, pp. 2955-2960, 2009.

[44] Y. Luo, S. Nartker, M. Wiederoder, H. Miller, D. Hochhalter, L. T. Drzal, and E. C. Alocilja, "Novel Biosensor Based on Electrospun Nanofiber and Magnetic Nanoparticles for the Detection of E. coli O157: H7," *Nanotechnology, IEEE Transactions on,* vol. 11, pp. 676-681, 2012.

[45] K. N. Layton and M. R. Abidian, "Conducting polymer nanofiber-based biosensor for detection of neurochemicals," in *Neural Engineering (NER), 2011 5th International IEEE/EMBS Conference on*, 2011, pp. 298-301.

[46] F. Guo, X. Xu, Z. Sun, J. Zhang, Z. Meng, W. Zheng, H. Zhou, B. Wang, and Y. Zheng, "A novel amperometric hydrogen peroxide biosensor based on electrospun Hb–collagen composite," *Colloids and Surfaces B: Biointerfaces,* vol. 86, pp. 140-145, 2011.

[47] B. Guo, S. Zhao, G. Han, and L. Zhang, "Continuous thin gold films electroless deposited on fibrous mats of polyacrylonitrile and their electrocatalytic activity towards the oxidation of methanol," *Electrochimica Acta,* vol. 53, pp. 5174-5179, 2008.

[48] S. Marx, M. V. Jose, J. D. Andersen, and A. J. Russell, "Electrospun gold nanofiber electrodes for biosensors," *Biosensors and Bioelectronics,* vol. 26, pp. 2981-2986, 2011.

[49] S. Jin Lee, R. Tatavarty, and M. Bock Gu, "Electrospun Polystyrene-Poly (styrene-co-maleic anhydride) Nanofiber as a New Aptasensor Platform," *Biosensors and Bioelectronics,* 2012.

[50] A. Sidek, R. Arsat, X. He, K. Kalantar-Zadeh, and W. Wlodarski, "Polyvinylpyrrolidone/polyaniline composite based 36° YX LiTaO 3 Surface Acoustic Wave H 2 gas sensor," in *Enabling Science and Nanotechnology (ESciNano), 2012 International Conference on*, 2012, pp. 1-2.

[51] I.-D. Kim, A. Rothschild, B. H. Lee, D. Y. Kim, S. M. Jo, and H. L. Tuller, "Ultrasensitive Chemiresistors Based on Electrospun TiO2 Nanofibers," *Nano Letters,* vol. 6, pp. 2009-2013, 2006/09/01 2006.

[52] Q. Qi, T. Zhang, L. Liu, and X. Zheng, "Synthesis and toluene sensing properties of SnO2 nanofibers," *Sensors and Actuators B: Chemical,* vol. 137, pp. 471-475, 2009.

[53] A. Stafiniak, B. Boratyński, A. Baranowska-Korczyc, A. Szyszka, M. Ramiączek-Krasowska, J. Prażmowska, K. Fronc, D. Elbaum, R. Paszkiewicz, and M. Tłaczała, "A novel electrospun ZnO nanofibers biosensor fabrication," *Sensors and Actuators B: Chemical,* 2011.

[54] M. Ahmad, C. Pan, Z. Luo, and J. Zhu, "A single ZnO nanofiber-based highly sensitive amperometric glucose biosensor," *The Journal of Physical Chemistry C,* vol. 114, pp. 9308-9313, 2010.

[55] X. Wang, Y. Si, J. Wang, B. Ding, J. Yu, and S. S. Al-Deyab, "A facile and highly sensitive colorimetric sensor for the detection of formaldehyde based on electro-spinning/netting nano-fiber/nets," *Sensors and Actuators B: Chemical,* vol. 163, pp. 186-193, 2012.

[56] E. Kang, M. Kim, J. S. Oh, D. W. Park, and S. E. Shim, "Electrospun BMIMPF 6/Nylon 6, 6 nanofiber chemiresistors as organic vapour sensors," *Macromolecular Research,* pp. 1-7, 2012.

[57] Z. Li, H. Zhang, W. Zheng, W. Wang, H. Huang, C. Wang, A. G. MacDiarmid, and Y. Wei, "Highly Sensitive and Stable Humidity Nanosensors Based on LiCl Doped TiO2 Electrospun Nanofibers," *Journal of the American Chemical Society,* vol. 130, pp. 5036-5037, 2008/04/01 2008.

[58] Q. Qi, T. Zhang, L. Liu, X. Zheng, and G. Lu, "Improved NH3, C2H5OH, and CH3COCH3 sensing properties of SnO2 nanofibers by adding block copolymer P123," *Sensors & Actuators: B. Chemical,* vol. 141, pp. 174-178, 2009.

[59] M. Epifani, J. Arbiol, E. Pellicer, N. Sergent, T. Pagnier, and J. R. Morante, "Synthesis and structural properties of ultra-small oxide (TiO2, ZrO2, SnO2) nanoparticles prepared by decomposition of metal alkoxides," *Materials Chemistry and Physics,* vol. 124, pp. 809-815, 2010.

[60] X. Song, Z. Wang, Y. Liu, C. Wang, and L. Li, "A highly sensitive ethanol sensor based on mesoporous ZnO–SnO2 nanofibers," *Nanotechnology,* vol. 20, p. 075501, 2009.

[61] Y. Li, F. Qian, J. Xiang, and C. M. Lieber, "Nanowire electronic and optoelectronic devices," *Materials today,* vol. 9, pp. 18-27, 2006.

[62] E. Zampetti, S. Pantalei, A. Muzyczuk, A. Bearzotti, F. De Cesare, C. Spinella, and A. Macagnano, "A high sensitive NO2 gas sensor based on PEDOT–PSS/TiO2 nanofibres," *Sensors and Actuators B,* vol. 176, pp. 390-398, 2013.

[63] S.-W. Choi, A. Katoch, J. Zhang, and S. S. Kim, "Electrospun nanofibers of CuO-SnO< sub> 2</sub> nanocomposite as semiconductor gas sensors for H< sub> 2</sub> S detection," *Sensors and Actuators B: Chemical*, 2012.

[64] S. Kacmaz, K. Ertekin, M. Gocmenturk, A. Suslu, Y. Ergun, and E. Celik, "Selective sensing of Fe3+ at pico-molar level with ethyl cellulose based electrospun nanofibers," *Reactive and Functional Polymers*.

[65] A. Urrutia, J. Goicoechea, P. J. Rivero, I. R. Matías, and F. J. Arregui, "Electrospun nanofiber mats for evanescent optical fiber sensors," *Sensors and Actuators B: Chemical*, 2012.

[66] J. Y. Shimano and A. G. MacDiarmid, "Polyaniline, a dynamic block copolymer: key to attaining its intrinsic conductivity?," *Synthetic Metals*, vol. 123, pp. 251-262, 2001.

[67] T. Yang, Q. Li, X. Li, X. Wang, M. Du, and K. Jiao, "Freely switchable impedimetric detection of target gene sequence based on synergistic effect of ERGNO/PANI nanocomposites," *Biosensors and Bioelectronics*, vol. 42, pp. 415-418, 2013.

[68] F. Berti, S. Todros, D. Lakshmi, M. J. Whitcombe, I. Chianella, M. Ferroni, S. A. Piletsky, A. P. F. Turner, and G. Marrazza, "Quasi-monodimensional polyaniline nanostructures for enhanced molecularly imprinted polymer-based sensing," *Biosensors and Bioelectronics*, vol. 26, pp. 497-503, 2010.

[69] A. Kausaite-Minkstimiene, V. Mazeiko, A. Ramanaviciene, and A. Ramanavicius, "Enzymatically synthesized polyaniline layer for extension of linear detection region of amperometric glucose biosensor," *Biosensors and Bioelectronics*, vol. 26, pp. 790-797, 2010.

[70] E. Spain, R. Kojima, R. B. Kaner, G. G. Wallace, J. O'Grady, K. Lacey, T. Barry, T. E. Keyes, and R. J. Forster, "High sensitivity DNA detection using gold nanoparticle functionalised polyaniline nanofibres," *Biosensors and Bioelectronics*, vol. 26, pp. 2613-2618, 2011.

[71] Q. Lin, Y. Li, and M. Yang, "Polyaniline nanofiber humidity sensor prepared by electrospinning," *Sensors and Actuators B: Chemical*, vol. 161, pp. 967-972, 2012.

[72] Q. Lin, Y. Li, and M. Yang, "Highly sensitive and ultrafast response surface acoustic wave humidity sensor based on electrospun polyaniline/poly (vinyl butyral) nanofibers," *Analytica Chimica Acta*, 2012.

[73] S. Ji, Y. Li, and M. Yang, "Gas sensing properties of a composite composed of electrospun poly (methyl methacrylate) nanofibers and in situ polymerized polyaniline," *Sensors and Actuators B: Chemical*, vol. 133, pp. 644-649, 2008.

[74] D. Chen, X. Guo, Z. Wang, P. Wang, Y. Chen, and L. Lin, "Polyaniline nanofiber gas sensors by direct-write electrospinning," in *Micro Electro Mechanical Systems (MEMS), 2011 IEEE 24th International Conference on*, 2011, pp. 1369-1372.

[75] N. J. Pinto, I. Ramos, R. Rojas, P.-C. Wang, and A. T. Johnson Jr, "Electric response of isolated electrospun polyaniline nanofibers to vapors of aliphatic alcohols," *Sensors and Actuators B: Chemical,* vol. 129, pp. 621-627, 2008.

[76] D.-J. CHEN, S. LEI, R.-H. WANG, M. PAN, and Y.-Q. CHEN, "Dielectrophoresis Carbon Nanotube and Conductive Polyaniline Nanofiber NH< sub> 3</sub> Gas Sensor," *Chinese Journal of Analytical Chemistry,* vol. 40, pp. 145-149, 2012.

[77] W. Jia, L. Su, and Y. Lei, "Pt nanoflower/polyaniline composite nanofibers based urea biosensor," *Biosensors and Bioelectronics,* vol. 30, pp. 158-164, 2011.

[78] Y. J. Shin and J. Kameoka, "Amperometric cholesterol biosensor using layer-by-layer adsorption technique onto electrospun polyaniline nanofibers," *Journal of Industrial and Engineering Chemistry,* 2011.

[79] V. Kapoor, A. K. Singh, S. Dey, S. C. Sharma, and S. N. Das, "Circulating cycloxygenase-2 in patients with tobacco-related intraoral squamous cell carcinoma and evaluation of its peptide inhibitors as potential antitumor agent," *Journal of cancer research and clinical oncology,* vol. 136, pp. 1795-1804, 2010.

[80] N. M. Noah, O. Marcells, A. Almalleti, J. Lim, and O. A. Sadik, "Metal Enhanced Electrochemical Cyclooxygenase-2 (COX-2) Sensor for Biological Applications," *Electroanalysis,* vol. 23, pp. 2392-2399, 2011.

[81] N. M. Noah, S. K. Mwilu, O. A. Sadik, A. A. Fatah, and R. D. Arcilesi, "Immunosensors for quantifying cyclooxygenase 2 pain biomarkers," *Clinica Chimica Acta,* vol. 412, pp. 1391-1398, 2011.

[82] M. J. Díaz-de León, "Electrospinning nanofibers of polyaniline and polyaniline/(polystyrene and polyethylene oxide) blends."

[83] C. W. Wu, J. G. Lee, and W. C. Lee, "Protein and enzyme immobilization on non-porous microspheres of polystyrene," *Biotechnology and applied biochemistry,* vol. 27, pp. 225-230, 1998.

[84] P. Nahar, N. M. Wali, and R. P. Gandhi, "Light-Induced Activation of an Inert Surface for Covalent Immobilization of a Protein Ligand," *Analytical Biochemistry,* vol. 294, pp. 148-153, 2001.

[85] T. Hla and K. Neilson, "Human cyclooxygenase-2 cDNA," *Proceedings of the National Academy of Sciences,* vol. 89, pp. 7384-7388, 1992.

[86] R. G. Kurumbail, J. R. Kiefer, and L. J. Marnett, "Cyclooxygenase enzymes: catalysis and inhibition," *Current opinion in structural biology*, vol. 11, pp. 752-760, 2001.

[87] M. O'banion, "Cyclooxygenase-2: molecular biology, pharmacology, and neurobiology," *Critical reviews in neurobiology*, vol. 13, p. 45, 1999.

[88] N. Li, S. Sood, S. Wang, M. Fang, P. Wang, Z. Sun, C. S. Yang, and X. Chen, "Overexpression of 5-lipoxygenase and cyclooxygenase 2 in hamster and human oral cancer and chemopreventive effects of zileuton and celecoxib," *Clinical cancer research*, vol. 11, pp. 2089-2096, 2005.

[89] T. Wu, "Cyclooxygenase-2 and prostaglandin signaling in cholangiocarcinoma," *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, vol. 1755, pp. 135-150, 2005.

[90] L. ZHU, W. T. LOO, C. W. CHENG, and L. W. CHOW, "Possible predictive markers related to micro-metastasis in breast cancer patients," *Oncology reports*, vol. 15, pp. 1217-1223, 2006.

[91] N. F. Saba, M. Choi, S. Muller, H. J. C. Shin, M. Tighiouart, V. A. Papadimitrakopoulou, A. K. El-Naggar, and F. R. Khuri, "Role of COX-2 in tumor progression and survival of head and neck squamous cell carcinoma," *Cancer prevention research (Philadelphia, Pa.)*, vol. 2, p. 823, 2009.

[92] B. Arun and P. Goss, "The role of COX-2 inhibition in breast cancer treatment and prevention," in *Seminars in oncology*, 2004, p. 22.

[93] I. E. EI-HAKIM and J. D. LANGDON, "Arachidonic acid cascade and oral squamous cell carcinoma," *Clinical Otolaryngology & Allied Sciences*, vol. 16, pp. 563-573, 1991.

[94] C. Han, J. Leng, A. J. Demetris, and T. Wu, "Cyclooxygenase-2 promotes human cholangiocarcinoma growth evidence for cyclooxygenase-2-independent mechanism in Celecoxib-mediated induction of P21waf1/cip1 and p27kip1 and cell cycle arrest," *Cancer research*, vol. 64, pp. 1369-1376, 2004.

[95] L. Chow, W. Loo, and M. Toi, "Current directions for COX-2 inhibition in breast cancer," *Biomedicine & pharmacotherapy*, vol. 59, pp. S281-S284, 2005.

[96] M. Farooqui, Y. Li, T. Rogers, T. Poonawala, R. Griffin, C. Song, and K. Gupta, "COX-2 inhibitor celecoxib prevents chronic morphine-induced promotion of angiogenesis, tumour growth, metastasis and mortality, without compromising analgesia," *British journal of cancer*, vol. 97, pp. 1523-1531, 2007.

[97] J. Vane, Y. Bakhle, and R. Botting, "CYCLOOXYGENASES 1 AND 2," *Annual review of pharmacology and toxicology*, vol. 38, pp. 97-120, 1998.

APPENDIX

APPENDIX

THE ANTIBODY SATURATION ISOTHERMS FOR BIOSENSORS INTEGRATED WITH POLYANILINE NANOFIBERS SPUN AT DIFFERENT FLOW RATES

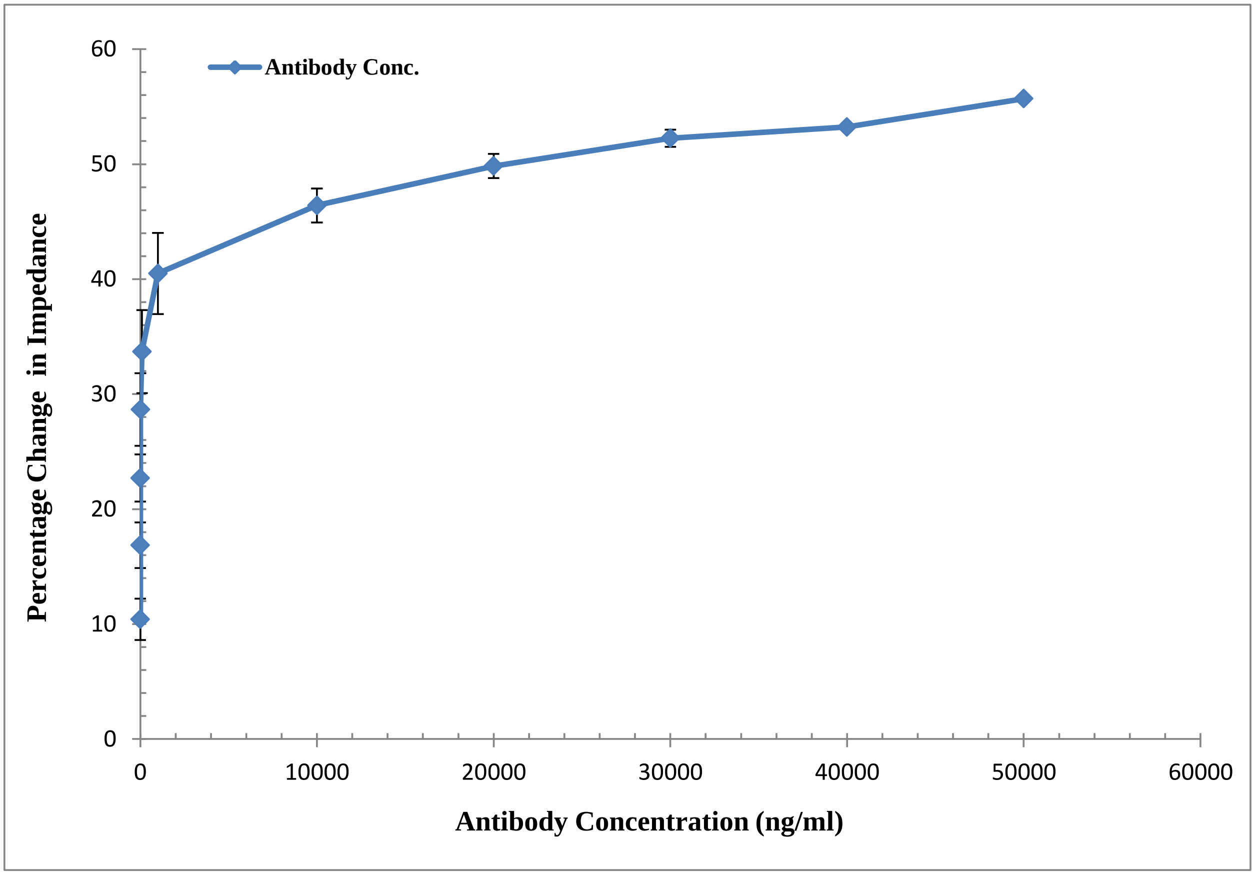

Figure 33. Dose response of COX-2 antibody in PBS using the biosensor assimilated with polyaniline nanofiber prepared at 0.5 ml/hr flow rate

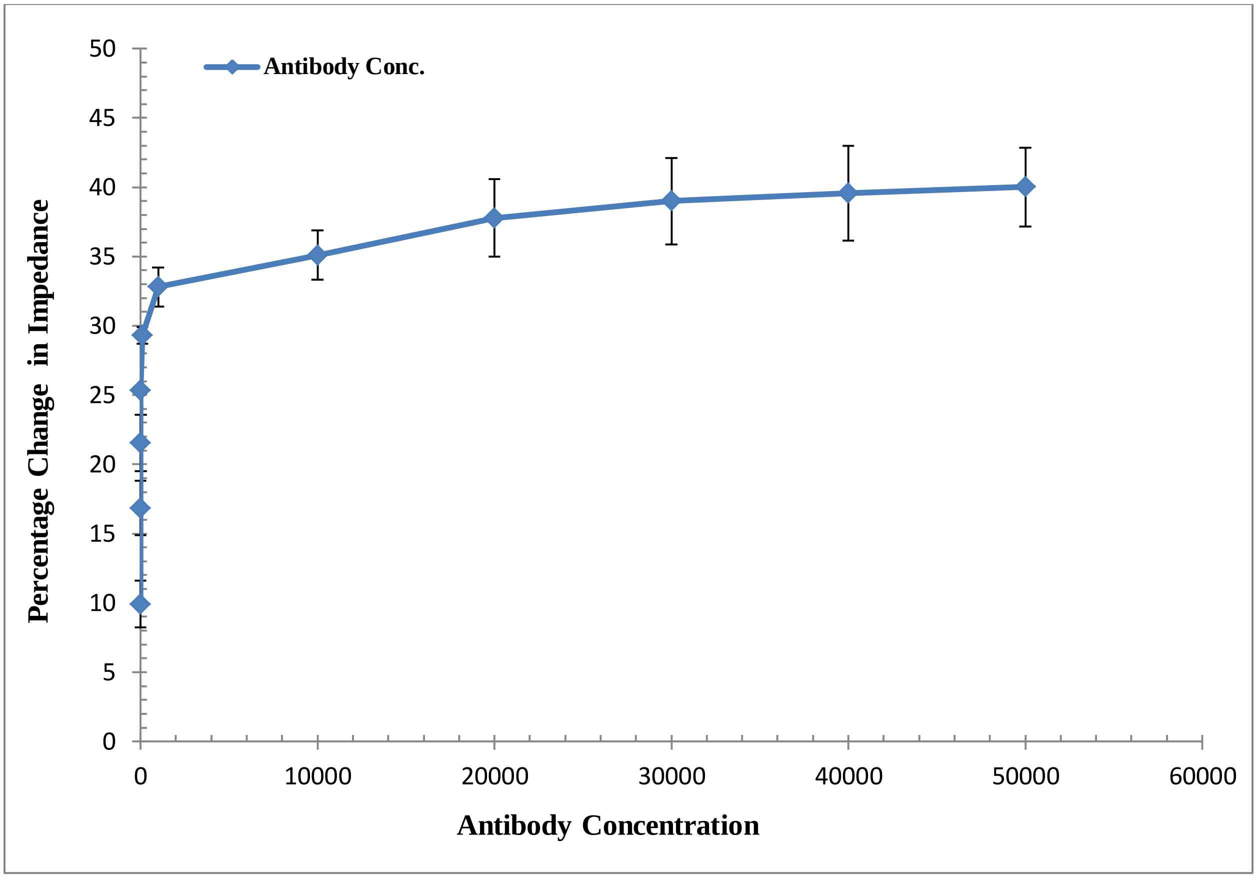

Figure 34. Dose response of COX-2 antibody in PBS using the biosensor assimilated with polyaniline nanofiber prepared at 2 ml/hr flow rate

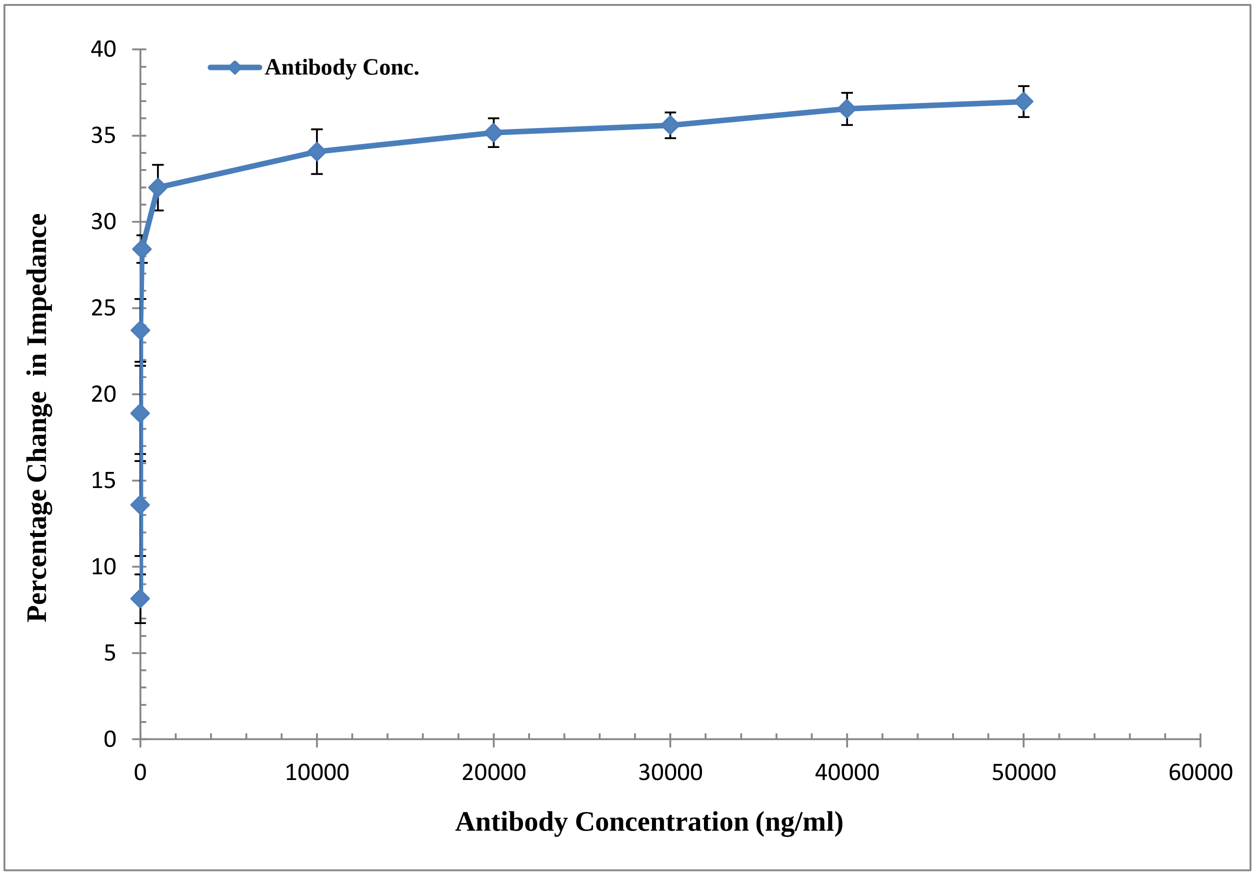

Figure 35. Dose response of COX-2 antibody in PBS using the biosensor assimilated with polyaniline nanofiber prepared at 3 ml/hr flow rate

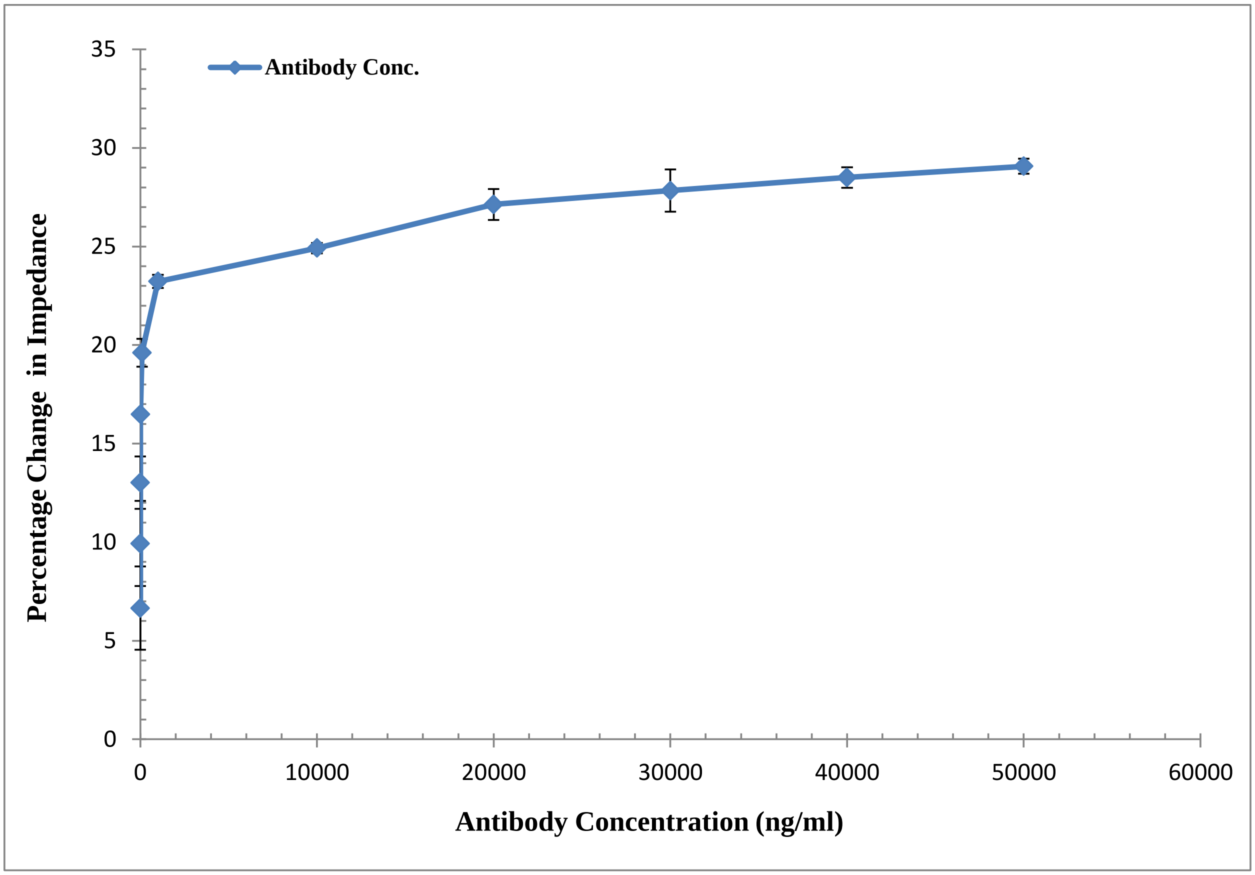

Figure 36. Dose response of COX-2 antibody in PBS using the control biosensor