SYNTHESIS AND APPLICATION OF FUNCTIONAL POLYMERIC NANOFIBERS

A Thesis in
Chemical Engineering

by

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ABSTRACT

The central objectives of this thesis are to develop engineering based approaches for the synthesis of functional (conducting) nanofibers and biocatalytic nanofibers; and to exploit the use of biocatalytic nanofibers for enzyme based applications. Despite several advances in template synthesis, the release and re-capture of nanomaterials produced within the template still remains a critical issue. This difficulty may be avoided if one can produce and transfer nanomaterials directly to the target substrate while they are produced from an ordered array of nano-scale reactors without disintegrating the reactor array. The first part of this thesis aims at developing a nano-manufacturing technique for the continuous synthesis and extrusion of ordered arrays of polymeric nanofibers from nanoporous templates. As a proof of concept the synthesis and extrusion of polyethylene nanofibers by heterogeneous Ziegler-Natta polymerization within nanochannels of robust anodized aluminum oxide membranes is described.

The second part of this thesis aims at the development of nano-manufacturing techniques for the synthesis of conducting polymer composite nanofibers. Conducting polymers such as polypyrrole and poly (3, 4-ethylenedioxythiophene) have a great potential in the field of flexible electronics, nano-electronics and bio-sensing. However, despite its superior thermal and environmental stability it suffers from being intractable. Conducting polymers such as polypyrrole is insoluble and infusible in almost all solvents - a limitation that prevents it from being processed into useful devices, especially at the nanoscale. Due to the same reason, nanofibers of these polymers cannot be directly fabricated by convenient methods such as electrospinning. To address this issue,
electrically conducting composite nanofibers are produced by a two step process. First, 
electrospinning is used to synthesize template fibers loaded with suitable oxidants. 
Second, the template fibers are exposed to the monomer vapors which diffuse into the 
templates and are oxidized to form the conducting polymer. Electrically conducting 
polypyrrole-polyethylene-oxide (PPy-PEO) nanofibers, polypyrrole shell-polystyrene 
core (PPy-PS) nanofibers and poly (3, 4-ethylenedioxythiophene) shell-polystyrene core 
(PEDOT-PS) nanofibers were synthesized in this way. The effect of two different oxidants – ferric chloride and ferric toluenesulfonate, on the polymerization process was also investigated. The nonwoven mat of nanofibers was also exploited for gas sensing applications.

The third part of this thesis focuses on the fabrication of nanofibers for enzyme 
based biocatalytic applications. Fabrication of highly stable enzyme coatings on the 
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CHAPTER 1
INTRODUCTION

To realize the full potential of nanotechnology, functional nanomaterials must be produced with complete control over their dimensions, orientation and properties. Although there has been many advances in the field of nanomaterial synthesis and application, engineering based nano-manufacturing - which is the key to tailor nanomaterials into useful materials and devices – have received very little attention. For example, the release and recapture of nanomaterials produced by techniques such as template synthesis is difficult. It is rare to find nanoreactor systems that continuously synthesize and align the nanomaterials as they are produced. Although techniques such as electrospinning can synthesize nanofibers of a vast variety of materials, it cannot be directly extended to useful materials such as conducting polymers due to the intractability of the latter. In this thesis several nano-manufacturing technologies for the synthesis of functional polymeric materials (conducting applications and biocatalytic applications) have been developed. In the first part of this thesis, the continuous synthesis and extrusion of polymeric materials is attempted. In the second part, the synthesis of conducting polymer core-shell composite nanofibers via electrospinning is attempted. In the third part, biocatalytic nanofibers with a high activity and stability are fabricated by making use of electrospun fibers. A process to improve the loading of biomolecules onto electrospun fibers has also been attempted.

1.1 Background
In recent years, the importance of polymeric materials in communication technology, electronics and biotechnology has increased rapidly over the years. This can be attributed to a variety of factors: (1) a vast library of molecular architectures to build
the design from; (2) the commercial availability of the molecular building blocks; (3) the advances made in the processing and engineering of these polymers (4) low cost; (5) and the possibility of integrating the mechanical and thermal properties of these polymers along with attractive optical and electrical functionalities. Such synthetic polymers which are designed towards specific applications are often referred to as functional polymers. Functional polymers are classified based on their properties as conducting polymers, electro-active polymers, piezoelectric polymers, electro-luminescent polymers, bio-catalytic polymers, etc. Several advances in the synthesis chemistry of these functional polymers coupled with their properties at the nanoscale have fueled the interest from researchers in nanotechnology and other related fields. Despite this enormous progress, there is still needs for the improvement of the processing, modification and applications of these functional materials. We are specifically interested in two classes of functional polymers at the nanoscale namely, conducting polymer nanofibers and bio-catalytic polymer nanofibers.

1.2 Conducting polymers and the importance of the nanoscale

Polymers are generally thought to be insulators. This is true for saturated polymers in which all the four valence electrons of carbon are used for the formation of covalent bonds. However in 1977, this general notion was proved false with the discovery of conjugated polymers that had the capability to conduct electricity.¹ Conjugated polymers are polymers with alternating single and double bonds (Figure 1.1); thus possessing a totally different electronic configuration when compared to insulating
saturated polymers. $\pi$ electron delocalization provides the highway for charge mobility along the backbone of the polymer chain leading to its unique conducting capabilities.\textsuperscript{2-4} Addition of dopants (impurities) by chemical or electrochemical methods introduces charge carriers into the polymer backbone and hence increases the conductivity by several orders of magnitudes.\textsuperscript{5} These properties, coupled with their mechanical flexibility and low cost make conducting polymers suitable materials for electronic processing and device fabrication. Exposure to certain gases or biomolecules causes changes in

Figure 1.1: Commonly used conducting polymers
electronic coupling between redox sites in the matrix that defines physical electron transfer pathway in the conducting polymer chain, thus causing changes in its electronic and optical properties. This property of conducting polymers has been exploited for developing organic gas-sensors and biosensors. Alan J. Heeger, Alan MacDiarmid and Hideki Shirakawa were awarded the 2000 Nobel Prize in chemistry for their discovery of conducting polymers.

The properties of materials in the nanoscopic dimensions can be very different from its macroscopic properties. In the case of conducting polymers, it has been observed that the conductivity increases several orders of magnitude if the molecular and supramolecular order is improved. Molecular order refers to fewer conjugation interruptions and fewer defect sites. Supra-molecular order refers to ordering of the polymer chains through stretching, crystallization, etc. Molecular and supra-molecular ordering of the conducting polymer chains and hence, enhanced conductivities can be achieved by synthesis of these polymers at the nanoscale. Another advantage of fabricating polymers at the nanoscale into various forms – nanofibers, nanoparticles, nanorods etc. is the high surface to volume ratio – which will enable higher loading of enzymes and biomolecules on the nanofiber surface, therefore making them ideal candidates for transducer components of enzyme based biosensors.

1.3 Bio-catalytic polymers and the importance of the nanoscale

The substrate-specificity is a great advantage of enzymatic reactions in biocatalysis, bio-sensor, and bio-remediation applications. Although free enzymes can be utilized in these applications, their storage and handling are sometimes quite
cumbersome. Moreover, these free enzymes are not easily recyclable. From an economic point of view, the catalysts should be reused as long as their activity is preserved. However, there is no simply way of recovering catalyst (enzyme) after use. These difficulties can be circumvented when the enzymes are immobilized or anchored on solid substrates. Then, the catalytically active enzyme species can be easily separated from the liquid-phase reaction media, and handled or reused for multiple times. Polymers with proper functional groups capable of hosting enzymes and other biomolecules through covalent attachments are referred to as bio-catalytic polymers.

In heterogeneous enzymatic systems, the solid support material should meet several important requirements. First, it should have a large surface area to maximize the enzyme loading. Second, it should have a distribution of pores or open spaces in the material to achieve the high mass-transfer rate of substrates to the active site of an enzyme. Third, it should be durable and easily recoverable by a simple physical method. Finally, it should have the right functional groups at the surface suitable for covalent attachment of enzymes. Nanostructured materials satisfy all the above mentioned criteria. Recent breakthroughs in nanotechnology have made various nanostructures affordable for a broad range of bio-catalytic applications. These include – nanoparticles, mesoporous sol-gel hosts, carbon nanotubes and nanofibers. Among these materials, polymeric nanofibers offer a number of attractive features compared to the other nanostructures. First, nanofibers have an advantage over mesoporous media by relieving the mass transfer limitation of substrates/products due to their reduced thickness. Second, it is easier to recover and reuse nanofibers when compared to nanoparticles or carbon
nanotubes. Moreover, there exists a vast library of polymers with different functional groups which can be tailored into the nanoscale.

1.4 Template Synthesis

1.4.1 Fundamental aspects

Synthesis within templates of nanometer dimensions is one of the most widely used methods for the synthesis of nanofibers. In template synthesis the desired nanomaterial is grown inside the pores of a template. This can be achieved in several ways such as; (1) bottom up catalytic growth within templates \(^ {16}\) (2) chemical, electrochemical or electroless reactions within templates; \(^ {17-22}\) (3) or wetting of the template surface when brought into contact with low surface energy materials. \(^ {23}\) The template used in this case would serve as a nanochannel reactor. The most commonly used templates are particle ‘track-etch’ polycarbonate membranes and porous anodized aluminum oxide (AAO) membranes. \(^ {24}\) AAO membranes are preferred because of their high pore densities (\(10^{11}\) pores/cm\(^2\)) and the uniform pore distribution along the membrane. This technique has several advantages. First, a wide variety of nanomaterials such as polymeric, metal and carbon based nanofibers can be synthesized using the template based approaches. Secondly, the geometry and dimensions of the material produced is governed (or restricted) by the dimensions of the template; hence, by varying the template dimensions and geometry, nanofibers or nanorods of various shapes and sizes can be obtained. Thirdly, materials produced in narrow pores of a template have a higher order and show enhanced properties such as higher conductivity, crystallinity and improved mechanical properties. \(^ {24}\)
1.4.2 Template synthesis of functional conducting polymers

Template synthesis has, by far, been the most preferred method to synthesize useful functional polymers. Generally functional conducting polymers have a very low oxidation potential; hence the synthesis can be achieved by the oxidative polymerization of the corresponding monomer by (1) electro-polymerization within templates, or (2) by chemical polymerization inside templates when brought into contact with an oxidant. In electro-polymerization, one side of the template is coated with a metal film and used as the anode to electrochemically synthesize the polymer within the pores of the membrane. Nanofibers of conducting polymers such as polypyrrole (PPy), polyaniline (PANi), poly (3, 4- ethylene-dioxythiophene) (PEDOT), poly (3- methylthiophene) (P3MT) and polyacetylene (PA) were prepared in this way. Chemical polymerization can be achieved by simply inserting the template in a solution containing the monomer followed by addition of the oxidative agent. PPy and PANi nanofibers have also been prepared in this way. There are several interesting aspects to be noted with respect to the template synthesis of these conducting polymers. Firstly enhanced supra-molecular order (fewer conjugation interruptions and defect sites) and hence higher conductivity is achieved when polymer fibers are synthesized inside the pores of a template. This has been shown to be due to the alignment of the polymer chains on the outer surfaces of the nanofibers. Secondly, when the polymers are synthesized inside the pores of a polycarbonate ‘track-etch’ membrane, the polymer preferentially nucleates and grows on the pore walls due to solvophobic interactions (the monomers are soluble in the solvent; however the polymer is completely insoluble) and electrostatic interactions (the polymer
is poly-cationic and the pore walls have anionic sites). This results in the formation of polymer tubules. PPy and PANi tubules have been synthesized in this fashion. The wall thickness of these tubules can be controlled through the polymerization time. PPy tubules of different thickness have been produced in this way. Thirdly, PANi nanofibers with sizes as low as ~ 3 nm have been prepared by the template method by adsorption of the aniline vapors in mesoporous silica templates followed by reaction with peroxydisulfate. However, such small dimensions are very hard to attain even with lithographic techniques. Techniques to fabricate polymer nanofibers by the wetting of polymer solutions on templates have been reported. Porous templates have a high surface energy; the adhesive forces between the polymer solutions and the pore walls are much greater than the cohesive forces, thus resulting in a thin polymer film to wet the pore walls. Polymer nanotubes of sizes ~ 20 - 50 nm were prepared using this method.

1.5 Electrospinning

1.5.1 Fundamental Aspects

Electrospinning (also called electrostatic spinning) is a variation of the widely used electro-spraying process. In electro-spraying a high voltage (of the order of KV) is applied to low viscosity solutions resulting in the formation of small droplets or particles due to varicose break up of fluid. This process has found widespread applications in automobile painting, inkjet printing and the manufacture of particles with various sizes and compositions. In 1934, Formalas introduced an apparatus for the synthesis of polymer filaments by making use of the surface charge repulsions viscous jets subjected to a very high electric field. In electrospinning, a high voltage is applied to viscous
solutions inducing an electrified jet that is continuously stretched due to electrostatic repulsions between surface charges and evaporation of the solvent. In the early 1990’s, several groups made use of electrospinning to synthesize nanofibers of a broad variety of organic polymers. Since then, there have been a lot of experimental and theoretical studies related to electrospinning. Excellent reviews on electrospinning are also available in the literature.\textsuperscript{34,35} In this section we will focus on the following: (1) the basic electrospinning set up and mechanism; (2) the advantages of electrospinning as a nano-manufacturing technique; (3) and the limitations of electrospinning.

\textbf{Figure 1.2:} Conventional electrospinning setup

Figure 1.2 shows a schematic of the electrospinning set-up. The electrospinning set-up consists of four basic components: (1) a high voltage supply, (2) a spinneret capable of
hosting the polymer solution (a capillary or a syringe with a metal needle), (3) a pump which is capable of pushing the hosted polymer solution in the spinneret at a constant (or required) speed, (4) and a collector (that can be electrically grounded). The spinneret is filled with a polymer solution, such that there is a pendant drop of the polymer solution at the tip of the needle. There are three forces acting on the drop: (1) the force due to gravity (acting vertically downward); (2) the viscous force (directed vertically upwards); (3) and the surface tension force (acting towards the center of the tip of the needle). When a high voltage (of the order of 1-30 KV) is applied to the needle by means of a direct current (DC) high voltage power supply, the pendant drop will be electrified and the induced charges will be evenly distributed at the surface. Now the pendant drop is subject to some additional forces such as (1) the electrostatic repulsion between the induced charges and (2) the columbic force exerted by the electric field. Under the action of these two forces the drop gets distorted to form a conical shape referred to as the ‘Taylor Cone’. The electric field forces push the electrified polymer solution towards the grounded electrode. Once the electric field has crossed a certain threshold value, the electrostatic forces can overcome the surface tension of the polymer solution and force the electrified liquid jet from the tip of the needle towards the grounded electrode. As the electrified jet is accelerated towards the grounded electrode it undergoes a stretching and whipping process and is subjected to bending instability. These factors coupled with the continuous evaporation of the solvent from the jet leads to considerable thinning of the jet diameter. However the jets do not break into droplets nor does it detach due to viscoelastic forces holding the polymer fiber together. Electrostatic charge repulsion within the thin electrified jet causes it to split into multiple jets (a process called splaying) which are
collected on the grounded electrode as solid fibers. A pump is used so as to push the polymer solution at a very slow rate so as to continuously replenish the polymer droplet at the tip of the needle.

In recent years there has been considerable modification in the electrospinning set-up so as to tailor the size, surface texture, composition and alignment of the resultant fibers. The size and surface texture of the electrospun fibers is mainly dependant on (1) the molecular weight, concentration, viscosity and electrical conductivity of the polymer solution; (2) the surface tension and polarity of the solvent; (3) the flow rate of the polymer solution, the diameter of the tip of the needle and the magnitude of the electric field used. In some cases, the surface texture can also be affected by certain environmental parameters such as the relative humidity and temperature. The control of the diameter of electrospun fibers have been a subject of great interest and have been extensively documented in literature. The following rules of thumb hold in many cases: (1) the diameter of the electrospun fibers increases as the viscosity (or molecular weight or concentration) of the polymer solution increases; (2) addition of a salt results in increased conductivities, thus reducing the diameter of the resultant fibers; (3) higher flow rates results in thicker fibers. A model has been proposed to account for the influence of the various processing parameters on the diameter of the electrospun fibers. Surface texture of polymeric fibers has been attributed to either of the following two reasons: (1) evaporation of solvent from the polymeric jet leads to evaporative cooling of the surface of the polymer jet, causing water vapor from the surroundings to condense on the fiber to produce pores or indents; (2) thermally induced phase separation leading to the concentrated part of the polymer solution to form the matrix and the leaner polymer
solution to form the pores.\textsuperscript{41,42} There have been several attempts to control the alignment of electrospun fibers.\textsuperscript{43-46} This has been done by (1) modifying the collector (using a rotating drum or a tapered disk); (2) multiple-field technique; (3) uniaxial alignment of fibers between two conductive strips separated by a void gap. From the above discussions related to electrospinning, it is evident that there are certain requirements for a polymer to be electrospun into the desired dimensions. First, the polymer should be soluble in some solvent so as to form a polymer solution. Second, a high voltage (of the order of kilovolts) should be applied to the polymer solution by using a DC power supply.

1.5.2 Electrospinning of functional polymers

Electrospinning has been extensively exploited to produce nanofibers of various organic functional polymers. Several groups have extensively investigated the electrospinning of conducting polyaniline (PANi) nanofibers of sizes \( \sim 30 – 100 \) nm with conductivities of \( \sim 10^{-2} – 1 \) S/cm, by using a variety of solvents such as sulfuric acid, camphorsulfonic acid and chloroform.\textsuperscript{47-49} Nanofibers of poly [2-methoxy-5-(2-ethylhexyloxy)-1, 4-phenylenevinylene] (MEH-PPV) with sizes of \( \sim 200 \) nm have been prepared by dissolving the polymer in 1, 2- dichloroethane.\textsuperscript{50} Although electrospinning offers a convenient route for the fabrication of functional polymeric nanofibers, it cannot be used unless the polymer is soluble in some usable solvent. Most conducting polymers (with the exception of PANi) are insoluble in almost all solvents and hence cannot be directly electrospun. There are ways to make conducting polymers spinnable. One of them is to functionalize the conducting polymers with side groups so as to improve their
solubility. Nanofibers of PPy functionalized with dodecyl-benzene anion have been successfully electrospun.\textsuperscript{51}

The most effective way to improve the spinnability of conjugated conducting polymers is to blend these polymers with other spinnable polymers in solution. This requires the use of a common solvent capable of dissolving both (or more of) the polymers. Nanofibers of PPy/DBSA/PEO, PANi/PEO, PANi/PMMA and PANi/PS blends have also been electrospun to sub-micrometer sizes.\textsuperscript{52-55} In these cases, the polymers such as PEO, PMMA and PS are added to assist the fiber formation during electrospinning. Electrospun fibers of sub-micrometer dimensions have also been used as templates for the in-situ solution polymerization of conducting polymers. PANi/PMMA and PANi/PLA coaxial fibers (~150 – 690 nm) with conductivities of ~ 0.1- 0.5 S/cm were prepared in this way by electrospinning a core polymer such as PMMA or PLA followed by solution polymerization of aniline in the presence of a suitable oxidant such as ammonium persulfate.\textsuperscript{56} An interesting technique, capable of producing core-sheath and hollow core nanofibers by electrospinning using a spinneret consisting of two coaxial capillaries has been reported in literature.\textsuperscript{57,58} In this case two intractable polymers can be electrospun to form coaxial nanofiber cables by passing each polymer solution through the coaxial capillaries. 150-600 nm MEH-PPV nanofibers were electrospun by using poly (vinyl pyrrolidone)-water-ethanol as the outer (shell) solution and MEH-PPV-CHCl\textsubscript{3} as the inner (core) solution.

1.6 Bulk synthesis
Polymer nanofibers of certain conducting polymers can also be synthesized by chemical polymerization reactions in solution. This is advantageous in several ways: (1) the dimensions of the nanofibers can be controlled by the nature and concentrations of the reacting species and (2) bulk quantities of the product can be synthesized. Interfacial polymerization of aniline dissolved in an organic phase and reacted with ammonium peroxydisulfate dissolved in a dopant acid solution yielded PANi nanofibers of diameter $\sim 30 – 120$ nm.\textsuperscript{59} The diameter was tuned by the choice of the type of the dopant acids and the concentration of various species in solution. Soft template approaches involving the use of surfactants have also been successfully utilized for the synthesis of conducting polymers such as PPy, PANi, and PEDOT nanofibers.\textsuperscript{60,61}

1.7 Challenges and limitations

In most cases of template synthesis of functional polymers, the nanofiber is produced inside the pores of the template. Removal of the template to obtain the resultant nanotubes or nanofibers requires etching by solutions. For example, sodium hydroxide or potassium hydroxide treatment is required in order to dissolve the AAO template membrane. Similarly, aluminosilicate membranes have to be etched by hydrofluoric acid to isolate the nanofibers produced in it. Such solution treatments to remove templates are not preferred since they may affect the properties of the resultant nanofibers. For example, treatment of the AAO templates by sodium hydroxide to release the PPy nanofibers could result in the de-doping of the PPy fibers by the sodium hydroxide solution. Also, after the removal of the template, the produced nanofibers end up in an entangled and disordered form, hardly suitable for device fabrication purposes.
Pioneering work published in the past few years demonstrated that nano-manipulation of one-dimensional nanomaterials is very time-consuming and arduous.\textsuperscript{62-63} Examples include (1) random adsorption followed by microscopic search, (2) fluid flow induced orientation, and (3) field induced orientation. Even if successful, these approaches may not be suitable for assemblies of much more complex geometry and integration as is required for usable materials or devices.

Although electrospinning can be seen as a convenient technique for the synthesis of functional polymeric nanofibers, it can only be used provided the polymer is suitable in some solvent. Most conducting polymers are insoluble in almost all solvents. This intractability problem prevents them from being directly electrospun into nanofibers. In contrast, the approach of direct production of final application structures during the nanomaterial synthesis seems to be much more plausible as shown in examples of growth of nanotubes from seed structures.\textsuperscript{64} However, this seeded growth approach is not applicable if the seed structures must be removed from the product for materials application. The limitations justify the need for engineering based approach for better control of manufacturing and assembly of oriented nanomaterials.

The central theme of this thesis is the development of efficient nano-manufacturing techniques for the synthesis of conducting polymer nanofibers and bio-catalytic nanofibers. We also investigate avenues to utilize the bio-catalytic nanofibers for enzyme based applications. The specific tasks investigated in this thesis include the following:

1. Continuous extrusion of nanofibers from templates by catalytic polymerization.
2. Synthesis of conducting polymer nanofibers (specifically PPy and PEDOT) by oxidative polymerization - utilizing all the advantages of electrospinning and bypassing the intractability problem of conducting polymers.

3. Synthesis of bio-catalytic PS-PSMA nanofibers immobilized with cross-linked aggregates of enzymes (specifically chymotrypsin, glucose oxidase).

4. Enhancement in the enzyme loading capacity of electrospun fibers by aqueous alcohol dispersion and application of the alcohol dispersed fibers in continuous flow reactors.

The following subsection provides an overview to the remaining chapters in this work where engineering based nano-manufacturing approaches are introduced to address a wide variety of challenges described earlier.

1.8 Thesis Overview

The following chapters introduced in this thesis are focused in the development of suitable nano-manufacturing techniques for the synthesis and application of conducting polymer nanofibers and bio-catalytic nanofibers. As discussed earlier, in most template synthesis methods the recovery and recapture of the nanomaterials produced inside the template is very difficult. This problem can be avoided if the nanomaterial can be continuously produced and extruded out from the template. In chapter 2 the synthesis and extrusion of polyethylene nanofibers by heterogeneous Ziegler-Natta polymerization within nanochannels of robust anodized aluminum oxide (AAO) membranes is described. The polymerization catalysts were chemisorbed at the inner wall of the nanochannels and monomers were provided through diffusion from the outside. Polyethylene nanofibers grew inside the nanochannels down to 10 ~ 20 µm from the channel entrance. About 2 ~
5 μm long polyethylene fibers were extruded out of the nanochannels during the polymerization. The polymer chain packing structure was found to be similar to the high pressure crystal phase, although the polymerization was carried out near atmospheric pressure. The high pressure phase formation inside nanochannels and some degree of polyethylene nanofiber extrusion from nanochannels were attributed to catalytic production of excess amount of polyethylene inside nanoconfined templates.

The synthesis of conducting polymer nanofibers through electrospinning is difficult because of the insolubility of the conducting polymer in most solvents. This problem can be solved if the conducting polymer can be polymerized on electrospun template nanofibers containing suitable oxidants. In chapter 3, the fabrication of electrically conducting polypyrrole-polyethylene oxide (PPy-PEO) composite nanofibers via a two-step process is described. First, ferric chloride (FeCl₃) containing PEO nanofibers are produced by electrospinning. Second, the PEO-FeCl₃ electrospun fibers are exposed to pyrrole vapors for the synthesis of polypyrrole. The vapor phase polymerization occurs through the diffusion of pyrrole monomer into the nanofibers. The collected nonwoven fiber mat is composed of 96 ± 30 nm diameter PPy-PEO nanofibers. Fourier transform infrared spectroscopy (FTIR), x-ray photoelectron spectroscopy (XPS), and conductivity measurements confirm polypyrrole synthesis in the nanofiber.

PPy-PEO composite nanofibers are not suitable for solution based applications due to the solubility of the PEO in aqueous solutions. For such applications it is required to form composite nanofibers of PPy with hydrophobic polymers such as polystyrene. However, most of the ferric oxidants are water soluble and do not blend very well with organic solutions of hydrophobic polymers. In chapter 4, the fabrication of electrically
conducting polypyrrole-polystyrene core shell nanofibers via a two-step process is described. Two different oxidative agents were used – ferric chloride (FeCl\textsubscript{3}) and ferric toluenesulfonate (FeTS). First, FeCl\textsubscript{3} or FeTS containing PS nanofibers are produced by electrospinning. A small amount of poly (styrene-co-maleic anhydride) is added to improve the electrospinning efficiency. Second, the PS-FeCl\textsubscript{3} and PS-FeTS electrospun fibers are exposed to pyrrole vapors for the synthesis of polypyrrole. The vapor phase polymerization occurs through the diffusion of pyrrole monomer into the nanofibers. Significant morphological differences were observed in the synthesized nanofibers. The PS-FeCl\textsubscript{3} nonwoven fiber mat is composed of 625 ± 150 nm diameter fibers whereas the PS-FeTS nonwoven fiber mat is composed of 900 ± 150 nm diameter fibers. FTIR, XPS and conductivity measurements confirm polypyrrole synthesis in the nanofiber.

In the case of polypyrrole based core-shell composite nanofibers, high conductivity values cannot be obtained due to two reasons: (1) the presence of the non-conducting core and (2) the poor chain packing of PPy. If conducting polymers that exhibit a better chain packing and better crystallinity can be produced then one can achieve a better conductivity. One example of such a polymer is poly (3, 4 - ethylenedioxythiophene (PEDOT). In chapter 5, the synthesis of electrically conducting polystyrene – poly (3, 4 -ethylenedioxythiophene) (PS–PEDOT) core-shell nanofibers are synthesized by a two-step process. Ferric toluenesulfonate (FeTS) was used as the oxidative agent. The FeTS-containing PS nanofibers are synthesized by electrospinning. A small amount of poly (styrene-co-maleic anhydride) is added to improve the electrospinning efficiency. The PS-FeTS electrospun nanofibers are then exposed to the 3,4-ethylenedioxythiophene (EDOT) vapor at 70°C to synthesize PEDOT over the
nanofibers. The collected nonwoven fiber mat is composed of 300 ± 60 nm diameter PS-PEDOT nanofibers. FTIR, XRD and conductivity measurements confirm the PS–PEDOT composite nanofiber. The bulk conductivity of the vapor-polymerized PS-PEDOT nanofiber mat is ~ 0.25 S/cm. The bulk conductivity is improved to ~ 1 S/cm by melt-welding the PS-PEDOT nanofibers through condensation of EDOT onto PS-FeTS template nanofibers during the vapor phase polymerization. The enhancement of electrical conductivity is mainly due to a better connectivity between PS-PEDOT nanofibers.

When enzymes are immobilized onto solid supports two parameters must be maximized: (1) the enzyme activity and (2) stability. To maximize the activity of enzymes, the loading of the enzymes must be maximized and hence, the support material should have a high surface to volume ratio. Electrospun nanofibers are ideal candidates for biocatalytic applications because they have a high surface to volume ratio, can be easily re-used and recovered from solution. In chapter 6, a unique approach for the fabrication of enzyme aggregate coatings on the surfaces of electrospun polymer nanofibers is described. This approach employs covalent attachment of seed enzymes onto nanofibers consisting of a mixture of polystyrene and poly (styrene-co-maleic anhydride, followed by a glutaraldehyde (GA) treatment that crosslinks additional enzyme molecules and aggregates from solution onto the covalently-attached seed enzyme molecules. These crosslinked enzyme aggregates, covalently attached to the nanofibers via the linkers of seed enzyme molecules, are expected to improve the enzyme activity due to increased enzyme loading, and also the enzyme stability. As a proof of concept, α-chymotrypsin (CT) was coated onto nanofibers electrospun from a mixture of
polystyrene and poly (styrene-co-maleic anhydride). The initial activity of CT-aggregate-coated nanofibers was 9 times higher than nanofibers with just a layer of covalently-attached CT molecules. The enzyme stability of CT-aggregate-coated nanofibers was greatly improved with essentially no measurable loss of activity over a month of observation under rigorous shaking conditions. This new approach of enzyme coating on nanofibers, yielding high activity and stability, creates a useful new biocatalytic immobilized enzyme system with potential applications in bioconversion, bioremediation, and biosensors.

Although electrospun nanofibers offer a number of advantages with respect to the immobilization of enzymes they have drawbacks. The nanofibers tightly aggregate in aqueous solution, thus reducing the area available for the immobilization of enzymes. This amount of available surface area can be increased if the nanofibers can be dispersed in aqueous solutions. In chapter 7, a simple and efficient way of dispersing electrospun hydrophobic nanofibers in aqueous solution was developed, and its utility in production and application of enzyme-loaded nanofibers was demonstrated. Polystyrene-based nanofibers were produced via an electro-spinning process. A small amount of maleic anhydride group in the polystyrene fiber was used for covalent attachment of lipase onto the fiber surface. The pristine polystyrene nanofibers are hydrophobic and aggregate in water forming a tightly collapsed clump. These nanofibers can be dispersed in a surfactant-free aqueous solution via a simple alcohol pre-treatment. The tightly aggregated electro-spun polystyrene nanofibers can be dispersed into a loosely entangled structure in aqueous alcohol solution. Once treated with aqueous alcohol solution, the polystyrene nanofibers remain dispersed even in DI water as long as the nanofibers are
not dried during the washing step. The dispersion of polystyrene nanofibers increases the 
enzyme loading up to \( \sim 8 \) times and augments the steady state conversion of a continuous 
flow reactor filled with enzyme-loaded nanofibers.

Enzyme based decontamination systems offer several advantages in comparison to chemical decontamination as they are non-toxic, non-corrosive and environmentally 
benign. However, for the successful fabrication of these systems it is very important for 
the enzyme to be stable in such highly reactive environments. In chapter 8, the stability of 
enzyme aggregates of glucose oxidase (GOX) fabricated onto alcohol dispersed PS-
PSMA nanofibers were tested in high concentrations of hypochlorous acid (NaOCl \( \sim 0.1 \) 
- 1mM). Although, the enzyme aggregates did not lose any significant activity at NaOCl 
concentrations of \( \sim 0.1 \) mM, they exhibited a \( \sim 60 - 70 \% \) loss of activity in NaOCl 
concentrations of \( \sim 1 \) mM which could be attributed to the de-polymerization of the 
Schiff base (-CH=N-) formed during the crosslinking process. To improve the stability, 
mild reduction of the Schiff base using sodium cyanoborohydride was also attempted. 
Even after reduction, the enzyme aggregates exhibited a \( \sim 60 - 70 \% \) loss of activity. The 
loss of activity could be attributed to the oxidation of the weakly protected amine groups 
on the surface of the enzyme or by the de-polymerization of the Schiff base by NaOCl.66

Hydrogen peroxide is an important species that can oxidize halide ions to 
hypohalous species. This would open up new avenues for the fabrication of robust 
enzyme based decontamination systems in which the enzymes oxidize specific substrates 
to produce hydrogen peroxide and hence hypohalous species. However to produce 
maximum concentration of hypohalous species, the achievable concentration of hydrogen 
peroxide should be maximized. One way of doing this is by using electrospun nanofiber
immobilized with crosslinked aggregates of glucose oxidase in a continuous flow reactor. In chapter 9, a continuous flow reactor consisting of glucose oxidase enzyme aggregate coatings on PS-PSMA nanofibers was constructed. The effect of oxygen on the rate of generation of hydrogen peroxide was investigated. It was found that the amount of hydrogen peroxide generated doubled when the oxygen dissolved in solution was increased five times. This was analyzed to be due to the kinetics of the oxidation of glucose catalyzed by glucose oxidase. An attempt to maximize the rate of hydrogen peroxide by recycling the product in the continuous flow reactor is also described.

1.9 References


7. Source: [www.nobelprize.org](http://www.nobelprize.org)


CHAPTER 2

Formation of High-Pressure Phase and Extrusion of Polyethylene Due to Nano-confinements During Ziegler-Natta Polymerization Inside Nanochannels.

2.1 Background

This paper explores the possibility of simultaneous synthesis and extrusion of polymeric nanofibers using a nano-scale reactor. Spiders and caterpillars weave webs and cocoons by aligning silky fibers while they are producing fibers.\(^1\) By doing so, these small creatures in nature do not face difficulties of grabbing and aligning tiny fibers out of a reservoir containing randomly oriented fibers. If one can mimic this simultaneous synthesis and aligning process in nano-manufacturing, then the difficulty associated with achieving controlled assembly of one-dimensional nanomaterials produced from templates can be overcome.\(^2-4\) In most templated synthesis approaches,\(^5-9\) one-dimensional nanomaterials are produced inside the templates, released into a solution, and recaptured onto a desired target substrate.\(^10, 11\) The re-capture of a large number of these released nanomaterials with controlled alignments is extremely difficult due to the lack of enough enthalpic gain that can compensate the large decrease of entropy upon transition from the randomly dispersed state in solution to the highly ordered state on substrate surfaces. This difficulty may be avoided if one can produce and transfer nanomaterials directly to the target substrate while they are produced from an ordered array of nano-scale reactors without disintegrating the reactor array.

In order to enable simultaneous synthesis and extrusion of polymeric nanowires from the nano-scale templates, one must be able to provide monomer molecules into the
reactor and apply an extremely large pressure to push out the produced polymers from the reactor. The monomer diffusion can readily occur even after the reactor is filled with polymers since the monomer molecules can be dissolved significantly in the same kind of polymer. The bigger challenge is how to apply high enough forces to push out the polymer from the nano-scale templates. In atomic force microscopy studies, the typical adhesion force between polymers and inorganic surfaces such as silicon oxide is measured to be $1 \sim 10$ nN for $20 \sim 30$ nm diameter tips in ambient air or dry conditions. This corresponds to $10^3 \sim 10^4$ nN for $1 \mu$m long polymer section in a $200$nm diameter channel. In order to generate an extrusion force comparable to this, a pressure gradient of $10^4$ bar (1 GPa) should be applied across the nanochannel. However, applying this high pressure gradient across the nanotemplate with macroscopic devices is practically impossible. This is the main reason that the templated synthesis approaches often rely on destruction of the templates for retrieval of nanomaterials, rather than extrusion.

One way of solving this problem is to generate the required pressure inside the nanochannel to aid polymer extrusion. Consider the Ziegler-Natta polymerization which occurs spontaneously upon contact of monomers with the catalytic centers. Since the chemical potential of monomers in the vicinity of the catalytic sites is larger than that of polymers, the polymerization does not stop simply because the head space above the catalyst is filled with polymers. As long as monomers are available to the catalyst sites by diffusion through the growing polymer layers and the catalysts are active, the polymerization continues producing excess amount of polymers. This excess polymer production causes the internal pressure increase that can eventually induce disintegration of the template structure. These examples include fragmentation of catalyst particles in
olefin polymerization with Ziegler-Natta catalyst loaded inside microporous MgCl$_2$ supports and delamination of clay materials upon metalloence-catalyzed polymerization inside clay layers.$^{17-22}$

This work explores the possibility of polymer extrusion driven by Ziegler-Natta polymerization inside nanotemplates which do not disintegrate upon growth of excess polymers. An anodized aluminum oxide (AAO) membrane was chosen as a test nanotemplate. Commercially available AAO membranes are composed of an array of ~200 nm wide, ~60 µm long nanochannels that are evenly distributed over the whole membrane surface. The symmetric arrangement of nanochannels and the high hardness of the aluminum oxide allow balanced distribution of the stress generated by growth of excess polymers inside nanochannels, and prevent disintegration of the membrane structure. The aluminum oxide surface possesses a large number of hydroxyl (OH) groups to which the Ziegler-Natta catalyst titanium tetrachloride [TiCl$_4$] and co-catalyst triethyl-aluminum [Al(C$_2$H$_5$)$_3$] can be chemisorbed.

We studied catalytic production of polyethylene from gas-phase ethylene using Ziegler-Natta catalysts loaded in the inner walls of AAO nanochannels (Figure 2.1). The adhesion between the polymer and the nanochannel wall is very high in the absence of any solvent; thus, the polymer extrusion during gas-phase polymerization is much more difficult than extrusion during polymerization in solution.$^{23}$ The structural analysis of the produced polyethylene indicates that the polymer chains appear to be packed in an extremely high pressure phase structure even though the reaction is carried out at near atmospheric pressure. Scanning electron microscopy reveals that some of the produced polymers are extruded out of the nanochannels with the dimension defined by the
nanochannel diameter. The structure of polyethylene produced inside AAO nanochannels, the extrusion of the produced polyethylene, and the limitation of this approach are discussed in this paper.

Figure 2.1: Conceptual Scheme for the growth of high pressure phase polyethylene fibers in nanochannels of anodized aluminum oxide by an extrusion polymerization mechanism. (a) Bare AAO nanochannel with section cut. (b) Ziegler-Natta catalysts loaded inside AAO nanochannels are exposed to monomers. (c) Polymerization occurs inside the nanochannels. The monomer molecules still diffuse through the polymer-filled nanochannels to the active catalytic sites. (d) The continued polymerization inside the nanochannel induces pressure build-up inside the nanochannels and eventually pushes polymer chains out.
2.2 Experimental Details

2.2.1 Materials

A commercially available AAO membrane (Whatman Anodisc 13) was used as a model nanochannel reactor. High-purity TiCl$_4$ and Al (C$_2$H$_5$)$_3$ were purchased and further purified with freeze-pump-thaw cycles after connected to the gas line of the reactor system. A polymer-grade ethylene gas (99.95%) was used without further purification. A commercial high-density polyethylene (HDPE, $M_w = 125,000$) was purchased and used for structural comparison with the polyethylene produced inside nanochannels.

2.2.2 Synthesis of polyethylene in nanochannels of AAO membrane

The reaction chamber consisted of a high-pressure reaction cell attached to the preparation chamber of a VG ESCA system. In this set-up, the sample can easily be loaded into and retrieved from the vacuum and transferred from one compartment to another without exposing to air. A fresh AAO membrane was heated to 250$^\circ$C in vacuum to eliminate physisorbed water inside the membrane. TiCl$_4$ was chemisorbed onto the inner and outer surfaces of the AAO membrane at 300 mTorr and then activated with Al(C$_2$H$_5$)$_3$ at ~60 mTorr in the high-pressure cell. Care was taken to avoid condensation of Al(C$_2$H$_5$)$_3$ liquid layers on the AAO membrane. Each gas was pumped out thoroughly before introduction of the next stream to avoid gas phase reactions. The catalyst loading on the membrane was monitored with x-ray photoelectron spectroscopy (XPS) in the VG ESCA system and confirmed by comparing these data with the previous work.$^{24-26}$ The homogeneous loading of the Ziegler-Natta catalyst was checked with ex-situ imaging XPS of the cross-section of the TiCl$_4$-loaded AAO membrane using Kratos Analytical
Axis Ultra XPS. The catalytic sites at the outer surface of the membrane were removed by argon ion sputtering in the VG ESCA system. After argon sputtering, the AAO membrane sample was transferred to the high-pressure reaction cell and then exposed to 1000 Torr (~ 1.3 atmospheres) of ethylene gas at room temperature. The polymerization time was varied from 1 to 12 hours. But, the amount of polymer produced did not increase with the polymerization time in these conditions.

### 2.2.3 Characterization of the polyethylene produced

The produced polyethylene samples were characterized by Fourier transform infrared spectroscopy (FTIR), x-ray diffraction (XRD), scanning electron microscopy (SEM), differential scanning calorimetry (DSC), and gel permeation chromatography (GPC). All analyses, except GPC, were made with polymer samples without separating from AAO membranes. In fact, the membrane worked as a support in FTIR, XRD, and SEM. FTIR analysis was done with Thermo-Nicolet 760. The FTIR spectrum was analyzed only in the 1200 ~ 4000 cm\(^{-1}\) range because Aluminum oxide is not transparent to IR below 1200 cm\(^{-1}\). For temperature dependence FTIR measurements, a home-built AAO membrane heater was used. In the case of a reference sample, a thin film was prepared and sandwiched between AAO membranes, heated above the melting temperature and cooled down to room temperature while collecting FTIR spectra. XRD was performed using Philips X’Pert MPD. The broad background scattering originating from amorphous AAO was subtracted from the XRD spectrum of the polyethylene sample produced inside the AAO membrane. The top and cross-section topography of AAO membrane samples before and after polymerization were obtained with SEM.
(JEOL Philips XL-20). For SEM imaging, a thin layer of gold was coated on the samples to prevent charging. Thermal analysis was carried out with DSC (QA 1000, Texas Instruments) at a heating rate of 10°C/min and a cooling rate of 5°C/min. For molecular weight analysis, polymers were extruded with hot trichlorobenzene from ~15 AAO membranes and analyzed with GPC at the DuPont Analytical Services Center.

2.3 Results

2.3.1 Extrusion of polyethylene from nanochannels during polymerization

Polyethylene was reproducibly produced with the process described in Sec. 2.2.2. The XPS analysis after polymerization showed only C1s peak and no discernable peaks of titanium species or aluminum oxide species from the substrate. The valance band spectrum showed two peaks at 14.5 eV and 20 eV, which are characteristic to polyethylene, indicating the AAO membrane is fully covered with polyethylene. This is confirmed with SEM imaging. The top surface image of the polyethylene covered AAO membranes (Figure 2.2b) clearly shows that the polyethylene surface is fully packed with about 200 ~ 400 nm circular features. The size of these individual circular features is comparable with or slightly larger than the nanochannel diameter of the bare AAO membrane (Figure 2.2a).

The cross-section images of the AAO membrane fractured after polymerization, shown in Figure 2.2c, provides more information on the polymerization/extrusion process. The most important feature of this cross-section image is that bundles of polyethylene nanofibers can be seen above the membrane. The length of polyethylene
fibers above the AAO membrane varies run to run; it is typically in the range of $2 \sim 5 \mu m$.

Figure 2.2: SEM image of (a) top surface of bare AAO membrane, (b) top surface of polyethylene layer produced by Ziegler-Natta polymerization within AAO nanochannels, and (c) cross-section of the AAO membrane after ethylene polymerization. The dotted line in (c) indicates the position of the AAO membrane top surface. (Scale bars = 1 $\mu$m in a and b; 5$\mu$m in c).
The fiber diameters seen in the cross-section image are much closer to the nanochannel diameter than the diameters seen in the top view. Some fiber bundles originally grown on the opposite surface of the fractured pieces are separated from the membrane and attached to the imaged section. These bundles fall down and cover the top edge of the AAO membrane fracture surface. Individual fibers seen in this part clearly indicate that the bundles are made of polyethylene nanofibers with diameters defined by the nanochannel diameters. These results indicate that a certain amount of polyethylene is extruded out of the nanochannels during the Ziegler-Natta polymerization inside AAO nanochannels.

Figure 2.3: SEM image of the bottom part of the nanochannels.
Another important feature observed in the cross-section SEM analysis is the distribution of polyethylene inside the AAO nanochannels. As shown in Figure 2.2c, the top part of the AAO nanochannels, which was exposed to monomers for polymerization, is filled with polyethylene. Some polyethylene fibers are partially pulled out of the nanochannel during the mechanical fracture. The polyethylene fibers in the empty channels of this image must be attached to the opposite side of the fracture surface. In contrast, the bottom part of the nanochannels is not completely filled with polyethylene as shown in Figure 2.3. Instead, a large number of polymer dots and tiny fibers are observed on the inner walls. The diameter of these tiny fibers are much smaller than the nanochannel diameter and most of them appear to be attached to the fracture edge of the nanochannel wall, suggesting the tiny fibers are produced by pulling polymer dots when the AAO membrane is fractured. In the middle, a clear boundary between the completely filled top region and the partially filled bottom region is observed. The length of AAO nanochannels fully filled with polymers is about 15 ~ 20 µm from the top.

The reason for complete filling with polymers only at the upper part of the nanochannel cannot be due to inhomogeneous distribution of the Ziegler-Natta polymerization catalyst along the channel length. In the titanium ion map obtained with imaging XPS (shown in Figure 2.4) reveals that the titanium ions are evenly distributed, at least within the resolution of the instrument, along the entire length of nanochannels (60 µm). Incomplete filling of the bottom section of the nanochannels must be related to mass transport limitation of ethylene monomers through the region filled with polyethylene. More details will be discussed in more detail in later section along with generation of the extrusion force.
2.3.2 Structural analysis of polyethylene produced inside AAO nanochannels

The structure of polyethylene produced inside AAO nanochannels (hereafter, PE-AAO) are analyzed with XRD and compared with those of a melt-pressed sample of a
reference high-density polyethylene (HDPE) and a polyethylene film produced on flat alumina supports with the same Ziegler-Natta chemistry in the same reaction cell (PE-flat). On the flat support, the Ziegler-Natta polymerization catalyst loading occurs basically the same way on the AAO membrane surface – through chemisorption at surface OH groups. Figure 2.5 shows XRD peaks in the 20 region of 15° ~ 28° of PE-AAO, PE-flat, and HDPE. The PE-AAO and PE-flat samples were so thin that other peaks with weaker intensities at higher diffraction angles could not be detected. The two

![XRD pattern](image)

**Figure 2.5**: XRD pattern of (a) polyethylene produced inside AAO nanochannels, (b) polyethylene produced on flat surfaces, and (c) HDPE. In (a), the broad background contribution from the amorphous aluminum oxide is subtracted for easy comparison of polyethylene features. Subscripts O and M after the Miller indices indicate orthorhombic and monoclinic phases, respectively.
dominant peaks at 21.5° and 23.8° that can be seen for all three samples correspond to diffraction peaks of the [110] and [200] planes, respectively, of the orthorhombic structure which is the thermodynamically most stable phase at ambient conditions. The a and b lattice spacing calculated from these two diffraction peaks are in agreement with the theoretical values of the orthorhombic phase. The c axis dimension cannot be determined because [011], [111] and [201] peaks at 39.8°, 41.7° and 43.1° respectively, are not observed due to low diffraction peak intensity. Broad peaks at 19.5°, 23.1° and 25.1° correspond to the [001], [200] and [-201] planes, respectively, of the monoclinic phase that are meta-stable and frequently observed in the freshly produced samples with Ziegler-Natta catalysts or the mechanically strained samples. A broad, low-intensity background is due to the presence of amorphous phase in the unannealed polyethylene samples. The crystallinity of these samples is calculated from deconvoluted XRD peaks and summarized in Table 2.1. A large amount of monoclinic phase for the polyethylene on the flat surface is consistent with the literature.

The most important difference of the XRD patterns of PE-AAO from those of HDPE and PE-flat is the relative intensity ratio of the two orthorhombic phase peaks – $I_{[200]}/I_{[110]}$. This ratio is 0.5 ~ 0.55 for PE-AAO and 0.3 ~ 0.35 for HDPE and PE-flat. This clearly indicates that although their unit cell dimensions are not significantly different, the polymer chains in the PE-AAO sample are packed somewhat differently than those in the HDPE and PE-flat samples. We will call this a “nanoconfinement-induced” phase to distinguish it from the normal thermodynamically preferable phase. The origin of the nanoconfinement-induced phase will be discussed in the next section.
Table 2.1: Comparison of crystallinity values for PE-AAO, PE-Flat, and HDPE samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Orthorhombic phase</th>
<th>Monoclinic phase</th>
<th>Total Crystallinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE-AAO as-produced</td>
<td>55%</td>
<td>22%</td>
<td>72%</td>
</tr>
<tr>
<td>PE-Flat as-produced</td>
<td>51%</td>
<td>28%</td>
<td>79%</td>
</tr>
<tr>
<td>HDPE</td>
<td>88%</td>
<td>not present</td>
<td>88%</td>
</tr>
<tr>
<td>PE-AAO melt/cooled</td>
<td>88%</td>
<td>not present</td>
<td>88%</td>
</tr>
<tr>
<td>PE-Flat melt/cooled</td>
<td>89%</td>
<td>not present</td>
<td>89%</td>
</tr>
</tbody>
</table>

Vibrational spectroscopic analysis provides further information on the nanoconfinement-induced phase. All samples show only four peaks characteristic to polyethylene – two peaks at 2920 cm\(^{-1}\) and 2850 cm\(^{-1}\) of asymmetric and symmetric C-H stretching vibrations and two peaks at 1474 cm\(^{-1}\) and 1464 cm\(^{-1}\) of CH\(_2\) bending vibrations. Figure 2.6 compared the CH\(_2\) bending vibration region of FTIR spectra of three samples shown in Figure 2.5. It can be clearly seen that the I\(_{1474}/I_{1464}\) ratio is significantly different for all three samples. The PE-AAO sample show the highest I\(_{1474}/I_{1464}\) value of \(~2.0\), the PE-flat sample has \(~1.3\), and the HDPE sample gives about 0.95. In the case of HDPE, the splitting of the CH\(_2\) bending vibration into two peaks is due to the interchain interactions in the orthorhombic crystal structure.\(^{33, 34}\) The theoretical ratio for 100% crystalline polyethylene is \(~1.233\).\(^ {34}\) The crystallinity value calculated \(^{35}\) for HDPE from this FTIR result is \(~88.3\) %, which is consistent with that calculated from
XRD. In the case of PE-flat, the additional enhancement of the 1474 cm\(^{-1}\) peak intensity giving rise to the \(I_{1474}/I_{1464}\) ratio higher than 1.1 is due to the presence of monoclinic phase. The monoclinic phase has single peak at 1475 cm\(^{-1}\). The XRD data of PE-flat indicates the presence of a significant amount of monoclinic phase.

![Graph of FTIR spectra](image)

**Figure 2.6:** \(\text{CH}_2\) bending vibration region of FTIR spectra of (a) polyethylene produced inside AAO nanochannels, (b) polyethylene produced on flat surfaces, and (c) HDPE.

The origin of the high \(I_{1474}/I_{1464}\) ratio for PE-AAO cannot be attributed to the presence of monoclinic phase since XRD shows that the amount of monoclinic phase in the PE-AAO is much smaller than that in the PE-flat. One can speculate a preferential orientation of the orthorhombic crystalline domain inside AAO nanochannels. We have checked this with a polarization dependence of FTIR spectra following the method
reported by Urban et al.\textsuperscript{35} The $I_{1474}/I_{1464}$ ratio does not show any polarization angle dependence. Therefore, the preferential orientation of the orthorhombic phase can be ruled out. The unusually high $I_{1474}/I_{1464}$ ration must be directly related to the nanoconfinement-induced phase formation when polymerization is carried out catalytically inside AAO nanochannels.

The thermal stability of the nanoconfinement-induced phase is examined with \textit{in-situ} FTIR measurements during heating and cooling cycles. Figure 2.7 plots the $I_{1474}/I_{1464}$ ratio as a function of temperature for PE-AAO and PE-flat. In the case of PE-AAO (Figure 2.7a), the $I_{1474}/I_{1464}$ ratio shows two step-wise drops: a first sharp decrease from $\sim2$ to $\sim1.2$ at $70 \sim 80^\circ$C and then a second sharp decrease to zero at $120 \sim 140^\circ$C. The first sharp drop must be related to relaxation of the nanoconfinement-induced phase upon heating. The second sharp drop is associated with melting of polyethylene. The CH\textsubscript{2} vibration peak shape is in good agreement with the polyethylene melt. Upon cooling, the $I_{1474}/I_{1464}$ ratio recovers to $\sim1.0$ with some hysteresis upon re-crystallization in the temperature range of $130 \sim 100^\circ$C and then gradually increases to $\sim1.2$ at $22^\circ$C. It should be noted that the $I_{1474}/I_{1464}$ ratio does not recover to $\sim2.0$, indicating the nanoconfinement-induced phase is not thermodynamically favorable at ambient conditions. In the case of PE-flat (Figure 2.7b) and HDPE (not shown), the $I_{1474}/I_{1464}$ ratio remains unchanged up to $\sim110^\circ$C and then gradually decreases to zero upon melting. In the cooling cycle, the $I_{1474}/I_{1464}$ ratio recovers with a small hysteresis upon re-crystallization in the temperature range of $130 \sim 115^\circ$C and finally to its initial value at $\sim100^\circ$C.
Figure 2.7: $I_{1474}/I_{1464}$ ratio variation upon heating and cooling for (a) polyethylene produced inside AAO nanochannels and (b) polyethylene produced on flat surfaces. The inset shows the CH$_2$ bending vibration spectra of the polyethylene sample produced inside AAO nanochannels at 22$^\circ$C, 95$^\circ$C, and 137$^\circ$C.
The melting behavior of PE-AAO is very interesting. The $I_{1474}/I_{1464}$ ratio retains ~0.5 even at ~137 °C and then suddenly drops to zero in a very narrow temperature range (Figure 2.7a). In contrast, PE-flat (Figure 2.7b) and HDPE (not shown) gives a monotonic decrease of the $I_{1474}/I_{1464}$ ratio upon melting. These differences are also observed in DSC data shown in Figure 2.8. While PE-flat displays the typical first-order phase transition shape centered at ~133 °C, PE-AAO reveals a broader melting peak with a sharp drop at ~139 °C. Upon re-crystallization, PE-flat and HDPE show once again the typical crystallization behavior; but PE-AAO shows a broader transition. In the second heating and cooling cycle, PE-flat and HDPE shows no change from the first heating and cooling cycle; but PE-AAO melts at a lower temperature in the second heating ($\Delta T_m \sim 10^\circ C$). For all three samples, the second re-crystallization profile exactly follows the first re-crystallization profile. The lower melting temperature of PE-AAO in the second heating cannot be attributed to polymer separation from the AAO membrane. SEM imaging of the melt and cooled PE-AAO sample shows that polymers are still present inside AAO nanochannels. These results also support that the nanoconfinement-induced phase is not thermodynamically favorable at ambient conditions and can be removed by simple thermal annealing.

The disappearance of the nanoconfinement-induced phase and the monoclinic phase upon annealing is confirmed with XRD. Figure 2.9 compares the XRD data of PE-AAO and PE-flat samples after heating to ~150 °C and then cooled to room temperature. Both samples show the orthorhombic structure with a very high crystallinity.
The molecular weight analysis of PE-AAO and PE-flat shows that both samples have a very broad molecular weight distribution. The number-averaged ($M_n$) and weight-

![DSC thermograms](image)

**Figure 2.8:** DSC thermograms of (a) polyethylene produced inside AAO nanochannels and (b) polyethylene produced on flat surfaces. The solid lines are data from the first heating and cooling cycle and the dashed lines are data from the second heating and cooling cycle.
Figure 2.9: XRD pattern of melt and re-crystallized samples of (a) polyethylene produced inside AAO nanochannels and (b) polyethylene produced on flat surfaces. The samples were heated to 150°C for 30 min and then cooled in dry nitrogen.

averaged ($M_w$) molecular weights are 6399 and 293841, respectively, for PE-AAO, giving rise to a polydispersity ($M_w/M_n$) of ~46. The $M_n$ and $M_w$ for PE-flat are 36301 and 1313655, respectively, giving rise to a polydispersity of ~36. The broad polydispersity for polymers produced with Ziegler-Natta catalysts loaded on alumina surfaces indicates that the catalyst sites have a wide distribution of polymerization activities. It may also be related to monomer diffusion through the polymer layer growing from the catalyst sites. The lower molecular weights and broader distribution for PE-AAO, compared to those of PE-flat, also seem to be related to the monomer diffusion. Since there is no lateral diffusion across the nanochannel walls, the monomer supply to the catalysts inside
nanochannels already filled with polymers would be more difficult the monomer supply to those on a flat substrate surface.

2.4 Discussion

2.4.1 Nanoconfinement effects on polyethylene molecule packing in PE-AAO

From structural and thermal analyses, it is clear that the Ziegler-Natta polymerization of ethylene inside AAO nanochannels produces polyethylene that has a molecular packing structure different from those produced by thermal treatments or without nanoconfinments. The nanoconfinement during the polymerization gives the enhanced $I_{[200]}/I_{[110]}$ ratio in XRD and the enhanced $I_{1474}/I_{1464}$ ratio in FTIR. In order to get more detailed physical insights into the nanoconfinement effects, the setting angle ($\theta_{\text{setting}}$) between the C-C bond of polyethylene and the b axis of the orthorhombic unit cell is analyzed with XRD data shown in Figure 2.5. The $I_{[200]}/I_{[110]}$ ratio for different setting angles is simulated using an x-ray crystallographic simulation software. The simulation result is shown in Figure 2.10. This simulation is done by rotating the CH$_2$-CH$_2$ axis in the ab plane of the unit cell with the center of CH$_2$-CH$_2$ group fixed at its original position of the orthorhombic structure. The simulation clearly indicates that the $I_{[200]}/I_{[110]}$ ratio increases proportionally the decrease of the setting angle. From this simulation data, one can find that the $I_{[200]}/I_{[110]}$ ratio of 0.3 ~ 0.35 observed for PE-flat and HDPE corresponds to the setting angle of ~46$^\circ$, which is consistent with the polyethylene chain packing in the normal orthorhombic phase. In this structure, the CH$_2$-CH$_2$ groups are arranged in a herringbone structure. The $I_{[200]}/I_{[110]}$ ratio of 0.5 ~ 0.55 observed for PE-AAO indicates that the CH$_2$-CH$_2$ groups are almost parallel to the b
axis when polyethylene is produced catalytically inside nanochannels. These results clearly imply that the nanoconfinement-induced phase in PE-AAO is related to different chain packings that are not observed in regular HDPE and PE-flat.

**Figure 2.10:** Simulated prediction of the \( I_{[200]} / I_{[110]} \) ratio as a function of setting angle. The setting angle is defined as the angle between the b-axis of the orthorhombic phase unit cell and the CH\(_2\)-CH\(_2\) molecular axis of the polyethylene chain aligned along the c-axis. Also shown are the schematics of the molecular arrangements at two different setting angles.

The observation of a low or even near zero setting angles in the polyethylene unit cell has been reported in the literature.\(^{39}\) Philips et. al. reported a gradual decrease of the setting angle from 44° to 48° when the pressure applied to polyethylene during the re-crystallization is increased from 1 bar to 6000 bar. In earlier studies pioneered by Bassett,
Anderson, and others, formation of a hexagonal phase at high pressures (>3000 bar) and high temperatures (>235 °C) have been elucidated.\textsuperscript{40-43} The hexagonal phase can be induced if the setting angle in the orthorhombic phase is reduced to zero or completely randomized and the a and b axis dimensions of the orthorhombic phase is slightly altered such that the center of CH\textsubscript{2}-CH\textsubscript{2} group is located at the three-fold symmetry positions. Since the a and b axis of the nanoconfinement-induced phase of PE-AAO are still very close to those of the orthorhombic phase, it cannot be said that this phase is hexagonal. It might rather be related to an intermediate structure occurring in transition from the normal orthorhombic phase in ambient conditions to the hexagonal phase formed at high pressures and temperatures.

Another supporting evidence of the low-setting-angle structure is the melting behavior of the PE-AAO sample. The DSC analysis shows that PE-AAO melts at a slightly higher temperature ($\Delta T_m \sim 10^\circ$C) in the first heating compared to the second heating. In addition, the first melting peak is showing a broad maximum of the melting peak. These are consistent with the characteristic behavior of the high-pressure phase with low setting angles reported in the literature.\textsuperscript{43-46} The broad molecular weight distribution alone cannot explain these changes because the molecular weight distribution does not change during the DSC analysis in inert environment. The only thing that can be explained with the broad molecular weight distribution is the broader melting and re-crystallization for PE-AAO compared to PE-flat. The surface-induced alteration of the chain packing does not seem to be the main cause. The SEM analysis of the melt and re-crystallized PE-AAO sample finds that polyethylene fibers are still filled inside the nanochannels.
2.4.2 Extrusion driving force

What is remarkable in this study is that the low setting angle structure pertaining to the high-pressure phase is obtained during the polymerization only at 1.3 atm! There is no way to accurately measure the excess pressure generated inside the AAO nanochannels during the Ziegler-Natta polymerization of ethylene in our experimental conditions. However, a first order approximation can be made based on the literature information. Philips et. al. investigated the setting angle variation trend upon increasing the pressure up to 6000 bar.\textsuperscript{39} A simple extrapolation of this trend to the near zero setting angle predicts that a pressure higher than 1 GPa (10\textsuperscript{4} bar) is needed to get a near-zero setting angle structure. Though this is a first-order approximation, it is interesting that this simple prediction finds the pressure gradient estimated to be required for extrusion of polymers from nanochannels.

The origin of this high internal pressure build-up is believed to be the resistance of polyethylene flow due to strong adhesion between the polymer and the nanochannel wall. As discussed before, simple van der Waals interactions can generate enormous adhesion forces in the nano-scale. The chemical bonds between chemisorbed polymerization catalytic centers and polymer chains will exert additional resistance against pushing polymers out of nanochannels. Therefore, the production of excess polymers due to favorable thermodynamic driving forces does not guarantee immediate extrusion of polymer out of nanochannels. Due to strong adhesion, the excess polymers are not easily pushed out; instead, they contribute to building up high pressure inside the nanochannel. When the pressure gradient between the inner and outer sides of nanochannels becomes high enough to allow polymer movement along the nanochannel,
the polymeric nanowire extrusion is finally occurring. This extrusion process cannot be observed if the nanotemplate is not strong enough to sustain the high pressure. These are the cases of microporous MgCl$_2$ and clays.$^{17, 18, 22}$

We cannot estimate the effective depth in which the extrusion process occurs. It is very likely to be ~ 1-2 µm region from the nanochannel exit. The polymers deep inside may not be able to come out in any circumstances because the accumulated adhesion force is so large. We believe these polymers produced deeper than the possible extrusion depth retain the high pressure phase even after completion of polymerization. Since these polymers deep inside nanochannels cannot easily move out, the pressure built up during the polymerization cannot be released. This pressure can be released only when enough thermal energy is provided for rearrangement of polymer chains or melting.

Our original interest is to see if macroscopic length of polymeric nanowire arrays can be produced in this way or not. But that does not seem to possible with the catalyst system used in this study. The extrusion length is found to be only an order or 1 ~ 10 µm from 200nm channels. This might be related to the catalyst deactivation. Although its activity is known to be extremely high, the lifetime of the Ziegler-Natta polymerization catalyst is limited due to deactivation.$^{14-16}$ Because of this, extrusion of polyethylene nanofibers using the Ziegler-Natta catalyst could not be sustained for an extended period of time to make fibers longer enough for transfer onto a substrate surface. If one develops a polymerization catalyst with a high activity and a long lifetime, this approach might be able to produce arrays of macroscopic-length polymeric nanowires that are long enough to transfer onto a substrate.
Similar concepts of polymeric nanowire production using nanostructured templates have been reported in previous literature.\textsuperscript{23, 48, 49} In these studies, mesoporous silica powders dispersed in toluene solution were used as templates and polymerization of ethylene were carried out with metallocene catalysts in toluene solvent.\textsuperscript{23} The presence of solvent alleviates the polymer adhesion to the template wall and makes extrusion easier; but randomly dispersed fine template particles in solution produced an entangled mass of polymer fibers, rather than aligned nanofibers over a large area. In our approach, the aligned polyethylene nanofibers are attained and extruded over the entire surface of the AAO membrane, $\sim 1 \text{ cm}^2$ area, without the aid of any external solvent.

2.4.3 Monomer transport along the nanochannel

Another important observation of this study is the nanochannel filling with polymer occurs only at the top $15 \sim 20 \mu m$ portion although the catalysts are coated over the entire $60 \mu m$ long channel. The region deeper than $15 \sim 20 \mu m$ contains only small polymer dots at the wall. This might be related to the effective diffusion length of ethylene monomer through polyethylene filled inside nanochannels. The small polymer dots in the deeper region appear to be produced from the monomer gas that is supplied before the channel entrance is filled with the produced polymer. Once the channel entrance is fully filled with polymer, the monomer supply into the deeper region is solely through diffusion. In our current experiment, we cannot determine if (a) the maximum diffusion distance of monomer through the polymer fiber inside the nanochannel is only $15 \sim 20 \mu m$ or (b) the monomer can diffuse a longer distance but the catalysts are already
deactivated by the time the monomer diffusion and polymer growth are reached to that distance.

2.5 Conclusions

Continuous catalytic polymerization within mechanically strong nano-templates results in very unusual polymer chain packing and some degree of polymeric nanofibers extrusion. The Ziegler-Natta polymerization inside 200nm diameter anodized aluminum oxide nanochannels produced excess amount of polymers. Since the adhesion of these polymer chains to the nanochannel walls is so strong, the excess polymers do not readily flow out of the nanochannel. Instead, they appear to be forced into a high-pressure packing structure. The high pressure formation is supported by the high $I_{[200]}/I_{[110]}$ ratio in XRD, the high $I_{1474}/I_{1464}$ ratio in FTIR, and the high melting temperature in DSC. The production of excess polymers eventually push the polymers out of the nanochannels, give rise to extrusion of polymeric nanowires with the diameter determined by the nanochannel diameter. The high-pressure packing structure is readily destroyed upon melting and never formed again upon cooling the polymer melt inside the nanochannels.

2.6 References:


42. Bassett, D. C. High Temperatures – High Pressures 1977, 9, 553


CHAPTER 3
Fabrication of Electrically Conducting Polypyrrole-Polyethyleneoxide Composite Nanofibers

3.1 Background

Polypyrrole (PPy) has been most widely used for gas sensors and bio-sensors among a range of conducting polymers.\textsuperscript{1, 2} The physical and chemical properties of PPy strongly depend on the nature of the dopant anion and their interactions with other chemical species. In addition, PPy can be made in a thin film form very easily through electrochemical deposition methods. In applications of thin film based devices, one challenge is that the active sensing components are imbedded in the bulk, limiting the efficiency and sensitivity. This can be improved if PPy is made in a nanofiber form that can provide a high surface area for a given mass or volume. The nanofiber texture can enhance the transport of ions and chemicals from the solution to the interior of the sensor component. It can also provide a large number of surface functional groups to which sensing chromophores or enzymes can be anchored.\textsuperscript{3}

Production of PPy in a nanofiber form has traditionally been accomplished through templated synthesis methods. These methods employ mesoporous silica, anodized aluminum oxide membrane, and particle track-etched membranes.\textsuperscript{4-6} Recently, a bulk growth approach utilizing V\textsubscript{2}O\textsubscript{5} seed template has been reported in the literature.\textsuperscript{7} Nanofibers produced by these methods are typically very short and not easy to handle for device fabrication. An electrospinning technique is widely used for producing polymeric and inorganic nanofibers in a non-woven mat that can be easily handled macroscopically.
Nanofibers can be deposited directly on device substrates. This method has been successfully utilized for fabrication of polyaniline nanofibers because soluble polyaniline can be easily processed by the electrospinning process. However, electrospinning cannot be directly employed for PPy nanofiber production due to the intractability of PPy.

This paper describes a simple method for synthesis of electrically conducting PPy nanofibers in a composite form. The method consists of two steps – electrospinning of polymer nanofibers that contain Fe (III) oxidants followed by vapor phase polymerization of pyrrole. This approach provides full advantages of the electrospinning process and avoids the intractability problem of PPy. Polyethyleneoxide (PEO) is chosen for this study for several reasons. PEO forms a complex with FeCl₃. FeCl₃ is known to be one of the most efficient oxidants for pyrrole polymerization and leaves chlorine ions in the produced PPy making it electrically conducting. The Fe³⁺ ions are bound by the coordinating oxygen atoms of the PEO chain. This will suppress crystallization of FeCl₃ and ensure homogeneous distribution of FeCl₃ along the PEO nanofibers. The vapor phase polymerization approach is employed to synthesize polypyrrole without disrupting the morphology of the PEO electrospun nanofibers. If PEO is contacted with a pyrrole containing solvent and the polymerization kinetics is not sufficiently fast enough, the PEO chains will dissolve into the solvent, thus destroying or deforming the fiber morphology. Vapor phase polymerization has been demonstrated to produce electrically conducting PPy.
3.2 Experimental Details

The PPy-PEO composite nanofibers were prepared by electrospinning a solution of PEO and FeCl$_3$ in a water-ethanol solvent, followed by exposure to pyrrole vapors at ambient conditions. Figure 3.1 shows a schematic of the process used for synthesizing the PPy-PEO composite nanofibers. PEO (molecular weight $\approx$ 4000,000), FeCl$_3$ (anhydrous, 98%) and pyrrole (reagent grade, 98%) were obtained from Sigma Aldrich and were used without further purification. PEO-FeCl$_3$ solutions were prepared at room temperature by dissolving a mixture of PEO and FeCl$_3$ (1:2.5 by weight) in a solvent consisting of ethanol and water (1:2.3 by weight). The total concentration of PEO-FeCl$_3$ in the solution was 10.7% (by weight). The PEO-FeCl$_3$ solution was homogenized (until transparent) by stirring for 24 hours and loaded into glass pipettes with tip diameters about 300 $\mu$m. It should be noted that the PEO-FeCl$_3$ solution is not very stable and become opaque when it is stored more than four days. So the electrospinning should be done with freshly prepared solutions. An inert electrode was placed into the glass pipettes loaded with the polymer solution and a positive bias of 8 KV was applied to the solution. The electrospun fibers were collected on a ground electrode (aluminum foil) placed $\sim$10 cm from the glass pipette tip. The electrospun PEO-FeCl$_3$ nanofibers were exposed to the saturated pyrrole vapor at ambient conditions (8.3 Torr at 25°C). The exposure times varied from 1 day up to 14 days. The vapor phase polymerization process was monitored with a quartz crystal microbalance (QCM; Stanford Research Systems QCM-200). The produced PPy-PEO composite nanofibers were characterized by scanning electron microscopy (SEM; JEOL Philips XL-20), x-ray photoelectron spectroscopy (XPS; Kratos Axis Ultra XPS), Fourier transform infrared spectroscopy (FTIR; Thermo-Nicolet 670), and differential scanning
The conductivity of the PPy-PEO non-woven nanofiber mats was measured by the Van der Paw method using Keithly 2400 source meter.

**Figure 3.1**: Schematic of the electrospinning of PEO nanofiber templates containing FeCl$_3$ and vapor phase polymerization of pyrrole. Also shown is an SEM image of the PPy-PEO composite nanofibers. The scale bar in the image is 500 nm.

### 3.3 Results and Discussions

The electrospinning process of PEO-FeCl$_3$ nanofibers is very stable and can be operated unattended for several hours for production of non-woven nanofiber mats. One of the important variables for continuous electrospinning is the ethanol-to-water ratio. If the ethanol amount is too low, a lot of bead defects and discontinuity in the fibers are observed. If too much ethanol is added, the solution becomes too viscous for electrospinning. Another important variable is the PEO:FeCl$_3$ ratio. When the amount of
FeCl$_3$ is increased significantly higher than 1:2.5 for PEO:FeCl$_3$. FeCl$_3$ particulates are formed along with the electrospun fibers. If a lower PEO:FeCl$_3$ ratio is used, the amount of PPy produced will be reduced. The as-spun PEO-FeCl$_3$ fibers have yellowish color characteristic of the FeCl$_3$ salt. When these fibers are exposed to pyrrole vapor, the color initially turns to dark green and eventually becomes black. An SEM image of the PPy-PEO composite nanofibers are shown in Figure 3.1. The PPy-PEO nanofibers are macroscopically long and their surfaces are very smooth. No bead defects are found. The nanofiber diameter ranges from 60 nm up to 170 nm with an average of 96 ± 30 nm (80% confidence level).

The vapor phase polymerization of pyrrole with FeCl$_3$ imbedded in the PEO electrospun fibers is monitored with QCM. Figure 3.2 shows the frequency and motional resistance shifts (Δf and ΔR) of the PEO-FeCl$_3$ fiber loaded quartz crystal during vapor-phase pyrrole polymerization. Δf is related to both mass gain (-Δf ∝ Δm) and mechanical property changes (acoustic impedance) of the deposited material. Although ΔR varies as Δf changes, its response is often dominated by the mechanical property change. ΔR decreases when the material becomes harder. A small number of PEO-FeCl$_3$ nanofibers were deposited on a 5MHz QCM sensor surface. The loading of the electrospun nanofibers on the QCM sensor was kept low to avoid multiple stacking of nanofibers forming non-woven structure. This low loading condition was necessary to avoid mass transport limitation in monomer diffusion to the fiber surfaces and poor acoustic coupling between the loosely stacked fibers and the QCM sensor. Figure 3.2 (a) displays the QCM output signals during the first 55 min of polymerization. Upon initial exposure of the PEO-FeCl$_3$ electrospun fibers to pyrrole vapor, there is sharp drop in Δf and increase in
Figure 3.2: QCM response during vapor phase polymerization of pyrrole with PEO-FeCl₃ nanofibers deposited on a quartz crystal sensor. (a) Frequency and resistance changes in the first 55 min of vapor phase polymerization. The up arrows indicate monomer removal and the down arrows indicate re-exposure to monomer. (b) Frequency change during vapor phase polymerization over 3 days.
ΔR in the first 2 min. This is due to pyrrole monomer dissolution in the PEO-FeCl₃ fiber. After these initial changes, both Δf and ΔR decreases slowly as monomers are converted to polymers. The decrease of ΔR implies that the production of PPy makes the fiber harder. When the monomer vapor is removed, Δf increases by ~75 Hz and ΔR decreases by 12 Ω immediately. These changes are due to desorption of the absorbed monomers. Upon re-exposure to monomer, Δf and ΔR change rapidly in opposite directions due to re-absorption of monomers and then both decreases slowly due to resumption of vapor phase polymerization. Conversion of Δf to the mass gain due to polymerization could not been made because there is no simple equation that allows deconvolution of the material property change from the mass gain.

By recording Δf upon desorption of unreacted monomers during the monomer exposure interruption, we can follow the vapor phase polymerization process without contribution of the amount of monomers absorbed in the fiber. These Δf values are plotted in Figure 3.2 (b). The data show that the polymerization occurs very slowly over a long period of time. In the ln(-Δf) vs. time plot (not shown), there is a break in the slope at ~20 hr polymerization, indicating a change in the polymer growth dynamics.

In the early stage of polymerization (<20 hr), the monomer diffusion is fast and reaches the equilibrium within a few minutes. In this case, the polymerization is the rate limiting step. Fe³⁺ ions are initially associated in the crystalline PEO matrix. For polymerization, the Fe³⁺ ions should be released from the PEO block, which occurs slowly below the melting temperature of the PEO crystalline phase. In DSC analysis, the melting of crystalline PEO is detected at ~70 °C for PEO-FeCl₃ fibers. The crystallinity of the PEO-FeCl₃ fibers is lower than that of the pure PEO sample. This PEO melting
peak completely disappears after vapor phase polymerization of pyrrole at room temperature.

In the later stage of polymerization (> 20 hr), the monomer diffusion becomes the rate-limiting step. As more PPy is produced, the fiber becomes harder and the monomer diffusion into the unreacted FeCl$_3$ region becomes slower. In this stage, the frequency and resistance change very slowly upon monomer removal and their magnitudes are significantly reduced compared to initial changes. After 62 hrs polymerization, the frequency increases by only 27 Hz upon monomer desorption (inset to Figure 3.2 (b)) and the resistance decreases by only 1 Ω. These changes occur over more than 5 hours. These behaviors indicate that the equilibrium amount of monomer in the nanofiber is significantly reduced compared to the early stage and the monomer diffusion into and out of the nanofibers is very slow.

The formation of PPy on the PEO-FeCl$_3$ fibers can be modeled by the following equation:

$$\frac{\partial C}{\partial t} = \frac{1}{r} D \frac{\partial}{\partial r} \left( r \frac{\partial C}{\partial r} \right) + K C_M C_{Ox}$$

Here, $D =$ diffusion coefficient of the pyrrole vapors through the PEO-FeCl$_3$ core; $C_M =$ Concentration of the monomer in the gas phase; $C_{Ox} =$ Concentration of the oxidant on the PEO; and $K =$ reaction rate constant of the formation of PPy by the oxidation of pyrrole by FeCl$_3$. The first term on the RHS in the above equation is the diffusion term and the second term on the RHS of the above equation is the reaction term. The boundary conditions of this equation will be as follows:
Here, \( R \) = radius of the PEO-FeCl\(_3\) fiber and \( K_{\text{adsorption}} \) = adsorption coefficient of pyrrole on PEO-FeCl\(_3\). The general solution to this equation is shown in Figure 3.3.

**Figure 3.3:** General solution of the diffusion reaction model explaining the growth of PPy on PEO-FeCl\(_3\) system. Inset shows experimental data. \( A_1 = f(D_{\text{pyrrole}}/K_{\text{reaction}}) \)

The formation of PPy within PEO-FeCl\(_3\) electrospun fibers is confirmed with IR spectroscopy which is shown in Figure 3.4. Before vapor phase polymerization of pyrrole, the IR spectrum is dominated by crystalline PEO peaks at 1445 cm\(^{-1}\), 1345 cm\(^{-1}\),
1245 cm\(^{-1}\), 940 cm\(^{-1}\), and 840 cm\(^{-1}\) and FeCl\(_3\) hydrates at 1597 cm\(^{-1}\) and 1069 cm\(^{-1}\).\(^{16}\) Although the anhydrous form of FeCl\(_3\) is used in sample preparation, the hydrate form is present in the PEO-FeCl\(_3\) fibers because the fibers are electrospun from an aqueous solution. When the PEO-FeCl\(_3\) electrospun fibers are exposed to the monomer vapor, peaks associated with pyrrole initially grow first at 1410 cm\(^{-1}\) and 730 cm\(^{-1}\) and PEO peaks become relatively weaker. After more than 7 days of exposure to pyrrole, the IR spectrum of the fiber is completely dominated by PPy peaks at 1550 cm\(^{-1}\), 1470 cm\(^{-1}\), 1410 cm\(^{-1}\), 1350 cm\(^{-1}\), 1300 cm\(^{-1}\), 1208 cm\(^{-1}\), 1097 cm\(^{-1}\), 1049 cm\(^{-1}\), 925 cm\(^{-1}\), 870 cm\(^{-1}\), 797 cm\(^{-1}\), and 730 cm\(^{-1}\).\(^{17-18}\) The hydrate water bending vibration peak at 1598 cm\(^{-1}\) is still observable. This must be due to the presence of reduced oxidant salt species, FeCl\(_2\), in a hydrate form.

![Figure 3.4](image_url)

**Figure 3.4:** Transmission FTIR spectra of (a) electrospun PEO+FeCl\(_3\) fibers, (b) PEO+FeCl\(_3\) fibers exposed to pyrrole vapor for 3 hours, and (c) PPy-PEO composite fibers produced by vapor phase polymerization for 10 days.
The XPS analysis gives some insights into the chemical composition of the PPy-PEO composite nanofibers. The atomic ratio for N/O from the O1s and N1s peak intensities is found to be ~0.5. In the initial polymerization conditions, the molar ratio of Fe$^{3+}$ to oxygen in PEO is 0.66. Since two FeCl$_3$ species are needed for each bond to be formed between pyrrole monomers, the theoretical N/O value is 0.33 for complete consumption of Fe$^{3+}$ species. The observation that the experimental N/O value is higher than the estimated maximum value may indicates that the PPy is segregated to some degree at the outer region of the fiber. In the high-resolution N1s region shown in Figure 3.5 (a), there is an intense peak at 399.3 eV corresponding to the pyrrole nitrogen (-NH-) and a high binding energy tail characteristic of positively charged nitrogen. In the Cl 2p region shown in Figure 3.5 (b), there are three spin-orbit split doublets (2p3/2 and 2p1/2; $\Delta_{\text{split}} = 1.6$ eV). The Cl 2p3/2 peaks at 197.5 eV and 201.3 eV are attributed to the anionic species and the covalent bonded species, respectively. The intermediate peak at 198.6 eV is associated with a species resulted from the charge-transfer interaction between the chlorine and the conducting state of the polypyrrole chain. These high resolution XPS results are fully consistent with those reported in the literature for polypyrrole synthesized with oxidative Fe (III) salts.

The sheet conductivity of the as-produced PPy-PEO nanofiber mat at room temperature is in the order of $10^{-3}$ S/cm. These values were calculated from the four-probe measurement data for a series of samples produced by electrospinning PEO-FeCl$_3$ nanofibers directly onto pre-fabricated electrode structures followed by exposure to pyrrole vapor for conversion to PPy-PEO composite fibers. This sample preparation
Figure 3.5: High resolution XPS data of (a) N1s and (b) Cl 2p regions of the PPy-PEO nanofibers. The dotted lines are fitted curves.
method provided the best contact between the non-woven nanofiber mat and the electrodes. The measured conductance values were converted to conductivities using the distances between the electrodes and the apparent thickness of the non-woven mat. In these measurements, it was very difficult to take into account the micro-structural characteristics of the mat such as porosity and PPy fraction. Based on SEM images, the porosity of the mat is more than 50%; but it varied from sample to sample. The QCM measurement was not able to determine the mass of PPy loaded per the unit mass of PEO because of the physical property change of the fibers upon pyrrole polymerization. The conductivity of individual fibers is expected to be higher than $10^{-3}$ S/cm because the color of the nanofiber mat is black after vapor phase polymerization. The conductivity measurements for individual nanofibers require a cross-sectional transmission electron microscopy to find PPy thickness and aligned deposition of PPy-PEO nanofibers on micro-patterned electrodes, which will be the subject of future study.

3.4 Conclusions

PPy-PEO composite nanofibers with an average diameter of 96 nm were fabricated by a two-step process involving electrospinning of PEO-FeCl$_3$ in water-ethanol solvent followed by exposure to pyrrole vapor at ambient conditions. The pyrrole monomers diffuse into the PEO-FeCl$_3$ fibers and react with FeCl$_3$, producing PPy-PEO nanofibers. The sheet conductivities of the PPy-PEO composite nanofiber mats are in the order of $10^{-3}$ S/cm.

3.5 References


CHAPTER 4

Fabrication of Electrically Conducting Polypyrrole core - Polystyrene shell Composite Nanofibers

4.1 Background

Polypyrrole (PPy) has been most widely used for gas sensors and bio-sensors among a range of conducting polymers. The physical and chemical properties of PPy strongly depend on the nature of the dopant anion and their interactions with other chemical species. In addition, PPy can be made in a thin film form very easily through electrochemical deposition methods. In applications of thin film based devices, one challenge is that the active sensing components are imbedded in the bulk, limiting the efficiency and sensitivity. This can be improved if PPy is made in a nanofiber form that can provide a high surface area for a given mass or volume. The nanofiber texture can enhance the transport of ions and chemicals from the solution to the interior of the sensor component. It can also provide a large number of surface functional groups to which sensing chromophores or enzymes can be anchored.

Production of PPy in a nanofiber form has traditionally been accomplished through template synthesis methods. These methods employ mesoporous silica, anodized aluminum oxide membrane, and particle track-etched membranes. Recently, a bulk growth approach utilizing V₂O₅ seed template has been reported in the literature. Nanofibers produced by these methods are typically very short and not easy to handle for device fabrication. An electrospinning technique is widely used for producing polymeric and inorganic nanofibers in a non-woven mat that can be easily handled
Nanofibers can be deposited directly on device substrates. This method has been successfully utilized for fabrication of polyaniline nanofibers because soluble polyaniline can be easily processed by the electrospinning process. Although electrospinning cannot be directly employed for PPy nanofiber production due to the intractability of PPy, the synthesis of PPy in a nonwoven fiber form has been achieved by electrospinning PEO fibers containing FeCl$_3$ followed by exposure to pyrrole vapors. However, PEO dissolves in aqueous solutions and hence this composite might not be suitable for enzyme based applications.

This chapter describes a simple method for synthesis of electrically conducting PPy shell – polystyrene core nanofibers in a composite form. The method consists of two steps – electrospinning of polymer nanofibers that contain Fe (III) oxidants followed by vapor phase polymerization of pyrrole. The effect of two oxidants – ferric chloride (FeCl$_3$) and ferric toluenesulfonate (FeTS) on the polymerization process is investigated. This approach provides full advantages of the electrospinning process and avoids the intractability problem of PPy. Polystyrene (PS) is chosen for this study for several reasons. PS does not dissolve in aqueous solutions. FeCl$_3$ and FeTS are known to be efficient oxidants for pyrrole polymerization and leave their respective anions in the produced PPy making it electrically conducting. The vapor phase polymerization approach$^{12,13}$ is employed to synthesize polypyrrole without disrupting the morphology of the PEO electrospun nanofibers. If PEO is contacted with a pyrrole containing solvent and the polymerization kinetics is not sufficiently fast enough, the PEO chains will dissolve into the solvent, thus destroying or deforming the fiber morphology. Vapor phase polymerization has been demonstrated to produce electrically conducting PPy.$^{[13]}$
4.2 Experimental Section

4.2.1 Materials.

PS (M_w = 900,000) was obtained from Pressure Chemical Company. PSMA (M_w = 224,000, MA fraction = 7 %), FeCl_3 (anhydrous, 98%) and pyrrole (reagent grade, 98%) were obtained from Sigma Aldrich and were used without further purification. Baytron-C solution containing 40wt-% ferric tosylate (FeTS) in butanol was obtained from Bayer and was used as received. THF (reagent grade, 99.9%) was obtained from Burdick and Jackson. All other reagents were obtained from Aldrich and were of the highest grade commercially available.

4.2.2 Synthesis of PPy-PS-Cl composite nanofibers.

The PPy-PS-Cl composite nanofibers were prepared by electrospinning a solution of PS and FeCl_3 in a mixture of THF and acetone, followed by exposure to pyrrole vapor at ambient conditions. Figure 3.1 (in Chapter 3) shows a schematic of the electrospinning process used to synthesize the PPy-PS composite nanofibers. In brief, the PS-FeCl_3 solution was prepared by dissolving a 2: 1: 2 (by weight) mixture of PS, PSMA and FeCl_3 in a solvent mixture consisting of THF and acetone (5: 3 by weight) such that the total concentration of the solids in the mixture was 13.5 wt-%. The concentration of FeCl_3 in the solution was ~6 wt-%. The PS-FeCl_3 solution was loaded in a glass pipette with a tip diameter of about 200 ~ 400 µm. An inert electrode was placed into the glass pipette loaded with the polymer solution and a positive bias of ~10 kV was applied to the solution using a high voltage supply. The as-spun PS-FeCl_3 core fibers were collected on a grounded electrode (aluminum foil or glass slides) placed ~10 cm from the glass pipette.
tip. The collected fibers were then exposed to the saturated pyrrole vapor at ambient conditions (8.3 torr, 25°C) to yield PPy-PS-Cl composite fibers. The exposure times varied from 1 hour to 2 days.

4.2.3 Synthesis of PPy-PS-TS composite nanofibers.

The PPy-PS-TS composite nanofibers were prepared by electrospinning a solution of PS and FeTS in a mixture of THF and acetone, followed by exposure to pyrrole vapor at ambient conditions. In a typical procedure, the PS-FeTS solution was prepared by dissolving a mixture of PS, PSMA and FeTS solution (2: 1: 6 by weight) in a solvent mixture consisting of THF and acetone (3: 1 by weight). The concentration of FeTS in the solution is ~5 wt-%. The total concentration of the solids in solution was ~ 15 %. The PS-FeTS solution was used for the electrospinning of the PS-FeTS core fibers by the same procedure as described in the preceding section. The collected fibers were then exposed to the saturated pyrrole vapor at ambient conditions (8.3 torr, 25°C) to yield PPy-PS-TS composite fibers. The exposure times varied from 1 hour to 2 days.

4.2.4 Synthesis of hollow PPy tubes.

The PPy-PS-Cl and PPy-PS-TS fibers were immersed in 20 mL of THF and extracted several times (~ 10 times) by shaking at 250 rpm for ~ 12 hours. The resulting fibers were then collected and air-dried.

4.2.5 Characterization of the PPy-PS-Cl and PPy-PS-TS composite nanofibers.
The morphology of the PPy-PS-Cl and PPy-PS-TS fibers and the hollow PPy tubes were characterized using field emission scanning electron microscopy (FE-SEM). For FE-SEM imaging, a thin layer of iridium (~20 Å) was sputter-coated onto the sample to prevent charging problems. The vapor phase polymerization of pyrrole on the electrospun core fibers of PS- FeCl$_3$ and PS- FeTS was monitored as a function of vapor exposure time using transmission Fourier transform infrared spectroscopy (FTIR). The FTIR spectrum was analyzed in the range from 4000 cm$^{-1}$ to 400 cm$^{-1}$. The PPy-PS-Cl and PPy-PS-TS fibers were characterized using x-ray photoelectron spectroscopy (XPS; x-ray source = Al K$_\alpha$ radiation) and x-ray diffraction (XRD; x-ray source = Cu K$_\alpha$ radiation). The four probe conductivity of the as-polymerized non-woven mats of PPy-PS-Cl and PPy-PS-TS fibers and the hollow PPy tubes were measured by the van der Pauw method using a Keithly 4200 source meter. For conductivity measurements, the PPy-PS composite fibers and the hollow PPy tubes were produced on 1 cm × 1 cm glass slides. Copper wire electrodes were attached to the four corners of the 1 cm × 1 cm sample using silver paint. The four corners with contacts are labeled as 1, 2, 3 and 4 in a clockwise fashion. The conductivity was measured using the equation

\[
\sigma = \left[ \frac{\pi}{\ln 2} \cdot \frac{R_{12,34} + R_{23,41}}{2} \cdot f \cdot t \right]^{-1};
\]

where \( R_{12,34}(\Omega) = \frac{V_{34}}{I_{12}}; R_{23,41}(\Omega) = \frac{V_{41}}{I_{23}}; \) \( V_{ab} \) is the potential (in volts) applied between labeled points a and b, and \( I_{cd} \) is the current (in amperes) measured between points c and d; \( f \) is a function of the measured resistances and is equal to 1 if \( R_{12,34} \approx R_{23,41} \); and \( t \) is the sheet thickness of the as-polymerized non-woven mat (in cm).\textsuperscript{14}
4.2.6 De-doping with ammonia.

The hollow PPy fiber mat was exposed to solutions containing ammonia at various concentrations ranging from $10^{-1}$ M to $10^{-4}$ M. The equilibrium vapor pressure of ammonia was calculated (in ppm) from the Henry’s law constant as follows:

\[
\text{NH}_4\text{OH (aq)} \rightleftharpoons \text{NH}_4^+ + \text{OH}^- \quad (K_b = 1.78 \times 10^{-5})
\]

\[
\text{NH}_4^+ \rightleftharpoons \text{NH}_3(aq) + H^+ \quad (K_d = 5.6 \times 10^{-10})
\]

\[
\text{NH}_3(aq) \rightleftharpoons \text{NH}_3(g)(K_3)
\]

\[
K_3 = \exp\left(\frac{\Delta G^o_{(g)} - \Delta G^o_{(aq)}}{RT}\right) = \exp\left(\frac{-16450 - (-26500)}{8.314 \times 298}\right) = 0.0173
\]

\[
\frac{P_{\text{NH}_3(g)}}{P_o} = a_{\text{NH}_3(g)} = K_3 a_{\text{NH}_3(aq)} = K_3 \frac{K_d K_a a_{\text{NH}_3OH}}{a_{H^+} a_{\text{OH}^-}} = 0.0173 \times \frac{C_{\text{NH}_3OH}}{C_o};
\]

where $C_{\text{NH}_4\text{OH}}$ is the molality of the solution and $C_o = 1$ molal.

4.3 Results and Discussion

4.3.1 Electrospinning of PS -FeCl$_3$ and PS -FeTS template fibers.

For electrospinning of hydrophobic polymer solutions containing FeCl$_3$ or FeTS, several parameters must be optimized. The first is the solvent evaporation rate. If the organic solvents used for dissolving hydrophobic polymers have high vapor pressure, rapid solvent evaporation at the electrospinning tip leads to clogging of the tip with polymer. The second is the dissolution of salt in the polymer solution. For the synthesis of PPy, two different oxidizing agents were employed in the electrospinning solution – FeCl$_3$ and FeTS. These are widely used oxidants in pyrrole polymerization. For dissolution of polystyrene and salt, a mixture of THF and acetone was found to be efficient. The presence of suitable amounts of acetone (16 ~ 32 %) prevented phase
separation during the introduction of FeTS or FeCl$_3$ and yielded homogeneous electrospinning solutions. The electrospinning process is susceptible to formation of bead defects along the fibers. It was found that adding ~33 wt-% of a lower molecular weight PSMA to the PS solution was very efficient to prevent the bead formation in our electrospinning conditions.

The more oxidant in the PS fiber, the more PPy will be produced. However, the maximum amount of oxidants that can be incorporated in the PS fiber is limited by the electrospinning process parameters. When the concentration of FeCl$_3$ in the PS-FeCl$_3$-THF-acetone solution is increased higher than ~6 wt-%, FeCl$_3$ particulates are separated during the electrospinning and deposited in the electrospun fiber mat. Similarly, when the concentration of FeTS in the PS-FeTS-THF-acetone-butanol solution is increased higher than ~5 wt-%, the solution becomes too viscous for electrospinning.

![Graphs](image)

**Figure 4.1**: Size distribution of PS-PPy composite fibers obtained by using various oxidants: (a) ferric chloride (b) ferric tosylate. For each case, the size distribution was obtained by counting 150 fibers.

Both the as-spun PS-FeCl$_3$ and PS-FeTS core fibers have a yellowish-orange color, a characteristic of Fe (III) salts. Figure 4.1 shows the size distributions of the as-
spun PS-FeCl$_3$ and PS-FeTS core fibers. In both cases, the fibers are macroscopically long and essentially bead free. The PS-FeCl$_3$ fiber diameter ranges from 100 to 1600 nm with an average of $625 \pm 150$ nm and the PS-FeTS fiber diameter ranges from 400 to 1600 nm with an average of $900 \pm 150$ nm.

4.3.2 Morphology of electrospun template and vapor-phase polymerized fibers.

When the PS-FeCl$_3$ and PS-FeTS fibers are exposed to pyrrole vapors, the color changes from yellow to dark green, and eventually, to black. The black color indicates the formation of conducting polypyrrole with a narrow band-gap so absorbing all visible light. The high resolution FE-SEM analysis shown in Figure 4.2 revealed several interesting features about the as-spun fibers and the vapor phase polymerization. The FE-SEM image of the as-spun PS-FeCl$_3$ template fibers shows precipitates with a worm-like morphology on the polymer fiber surface (Figure 4.2 (a)). This is attributed to the fact that FeCl$_3$ precipitate and form particulates as the polymer solution dries. Acetone, used to increase the FeCl$_3$ solubility in the polymer solution, evaporates faster than THF. So, while the electrospun polymer solution jet dries into the fiber form, the acetone concentration will decreases to a critical level at which FeCl$_3$ precipitates. These precipitates seem to segregate forming the worm-like deposits at the fiber surface. When the PS-FeCl$_3$ template fibers are exposed to the pyrrole vapor, the polypyrrole grows on the fiber surface. From now on, these fibers will be called “PPy-PS-Cl”. The PPy-PS-Cl fiber surface is very smooth (Figure 4.2 (b)). All the warm-like patterns are disappeared. The warm-like patterns due to FeCl$_3$ precipitation and the smoothening of the surface morphology after vapor phase polymerization of pyrrole are also observed for spin-cast
PS-FeCl₃ films (See supporting information). When the PPy-PS-Cl fibers are immersed in THF for a long period of time, the PS core can be dissolved. The removal of the PS core can be seen in Figure 4.2 (c) which displays an image of a broken end of the PPy-PS-Cl fiber.

The as-spun PS-FeTS fibers reveal totally different surface morphology as can be seen from Figure 4.2 (d). Unlike the FeCl₃, FeTS does not precipitate out from the polymer solution. The large tosylate anion seems to have a favorable interaction with the polystyrene structure. Hence, the PS-FeTS fiber surface does not show the worm-like precipitate morphology seen on the PS-FeCl₃ fibers. Instead, it assumes some wrinkles

![Figure 4.2](image-url)

**Figure 4.2:** High resolution FESEM images of (a) as-spun PS-FeCl₃ fibers; (b) PPy-PS-Cl fibers obtained when PS-FeCl₃ fibers are exposed to pyrrole vapors for 48 hours; (c) PPy-PS-Cl fibers after extraction in THF for 5 hours. (d) as-spun PS-FeTS fibers; (e) Faceted PPy-PS-TS fibers obtained when PS-FeTS fibers are exposed to pyrrole vapors for 48 hours; (f) PPy-PS-TS fibers after extraction in THF for 5 hours.
running parallel to the fiber direction. The PPy fibers produced with these PS-FeTS template fibers (called “PPy-PS-TS” from now on) are also different from the PPy-PS-Cl fibers. The majority of the PPy-PS-TS fibers assume a well defined, faceted rod-like structure as shown in Figure 4.2 (e). The surfaces of these rod-like PPy-PS-TS fibers are very smooth. Upon extraction of the PS template with THF, this faceted structure is still maintained. Figure 4.2 (f) shows the end view of the PPy-PS-TS faceted rods. Cross-sectional analysis from FE-SEM showed that the facets were aligned at ~ 120° to each other. A distribution of the cross sectional shape analysis of the PPy-PS-TS fibers is shown in Figure 4.3. The cross section of ~ 89.2 % of the observed fibers showed the presence of 120° facets; the cross section of ~ 9.2 % of the observed fibers was cylindrical. Although some rods have an equal-sized hexagonal shape, most of the rods have alternating wide and narrow facets. In very few cases (~ 1.6 %), several PPy-PS faceted rods fused together to form complex geometrical shapes whose facet angles could not be interpreted.

![Pie Chart](image)

**Figure 4.3:** Statistical distribution of the PS-PPy fiber shape using Ferric Tosylate as the oxidative agent; the light grey section (89.2 ± 3 %) is the 120° faceted rods, the white section (9.2 ± 4 %) is the cylindrical fibers, and the dark grey section (1.6 ± 1%) is the fibers that have a distorted or stretched cross section.
Similar results are observed when spun-cast films of PS-FeTS are exposed to pyrrole vapors for 24-48 hours. The spin-cast PS-FeTS films have a smooth morphology (Figure 4.4 (a)). When this film is exposed to pyrrole vapors, the film color changes to black, indicating polypyrrole growth. After the vapor polymerization, there are many needle-shape crystalline particles (~2 µm in diameter and 8 ~ 10 µm in length) at film surface as shown in Figure 4.4 (b).

Figure 4.4: SEM image of (a) PS-FeTS film before exposure to pyrrole vapors; (b) PS-FeTS film after exposure to pyrrole vapors for 48 hours; (c) PS-FeCl₃ film before exposure to pyrrole vapors; (d) PS-FeCl₃ film after exposure to pyrrole vapors for 48 hours. Thin films of PS-FeTS and PS-FeCl₃ were spun cast at 3500 rpm for 2 minutes on cleaned glass slides from the same solutions used for electrospinning. The glass slides containing the spun cast films were then exposed to pyrrole vapors for 48 hours. The thickness of the spun cast films was ~ 10-30 µm.
4.3.3 Growth of PPy on PS-FeCl$_3$ and PS-FeTS via vapor phase polymerization.

The vapor phase polymerization of pyrrole over PS template fibers containing ferric oxidants is confirmed by IR spectroscopy as shown in Figure 4.5. The IR spectrum of as-spun PS-FeCl$_3$ fibers is dominated by the characteristic bands of PS and FeCl$_3$. These peaks are C-H stretching within the aromatic ring in the 3000-3100 cm$^{-1}$, C-H deformation in the aromatic ring at 1450 and 1490 cm$^{-1}$, C=C ring stretching in the aromatic ring at 1605 cm$^{-1}$, aromatic overtones in the range 1700–2000 cm$^{-1}$, anti-symmetric and symmetric C=O stretching of the maleic anhydride group at 1850 and 1780 cm$^{-1}$ (due to the presence of trace amounts of PSMA), and hydrate peaks of the FeCl$_3$ at 1600 cm$^{-1}$. Upon vapor phase polymerization of pyrrole on these fibers, the characteristic vibrational bands of polypyrrole are growing as shown in Figure 4.6 (a). These peaks are N-H stretching at 3400 cm$^{-1}$ and C-H stretching at 3100 cm$^{-1}$ (not shown); anti-symmetric and symmetric C=C ring stretching at 1550 and 1480 cm$^{-1}$, respectively; C-N stretching at 1445 and 1175 cm$^{-1}$; ring stretching at 1410 cm$^{-1}$; and C-H deformation in the pyrrole ring at 1315 and 1050 cm$^{-1}$.

To monitor vapor phase polymerization on the PS-FeCl$_3$ fibers, the intensity of the 1410 cm$^{-1}$ peak is plotted with the time of pyrrole vapor exposure and shown in the inset to Figure 4.5 (a). In the initial stages of polymerization (< 3 hours), the amount of PPy produced increases linearly with the exposure time. About ~80 % of the PPy is formed in ~3 hours. As the PPy layer becomes thicker, the pyrrole monomer diffusion into the fiber interior becomes slower and hence the PPy polymerization rate decreases.

The PS-Fe-TS system also has the characteristic peaks associated with the PS (and the maleic anhydride groups) at 3100, 1850, 1780, 1605, 1490 and 1450 cm$^{-1}$. In
Figure 4.5: Transmission FTIR spectra of (a) PS-FeCl$_3$ fibers exposed to pyrrole vapors for time (i) $t = 0$ hr (ii) $t = 1$ hr (iii) $t = 15$ hr and (b) PS-FeTS fibers exposed to pyrrole vapors for time (i) $t = 0$ hr (ii) $t = 1$ hr (iii) $t = 18$ hr and. All the FTIR spectra have been shown after background subtraction. Shown in the inset are the corresponding polypyrrole fractions obtained using (a) ferric chloride and (b) ferric tosy late with increasing times of polymerization. $A_1$ defined in the inset is a function of $(D_{pyrrole}/K_{reaction})$. The general solution of the growth rate is shown in Chapter 3.

addition, it also shows a strong peak at 1035 and 1008 cm$^{-1}$ which is attributed to the sulfonyl (-SO$_3$H-) group in the FeTS. Upon vapor phase polymerization of pyrrole on these fibers, all the characteristic vibrational bands of polypyrrole described earlier are growing as shown in Figure 4.5 (b). However, the rate of vapor phase polymerization of pyrrole on the PS-Fe-TS fibers is different from that on the PS-FeCl$_3$ fibers. To monitor vapor phase polymerization on the PS-FeTS fibers, the intensity of the 1175 cm$^{-1}$ peak is plotted with the time of pyrrole vapor exposure and shown in the inset to Figure 4.5 (b). The amount of PPy produced increases linearly with the exposure time for only ~ 10 minutes. About ~ 80 % of the PPy is formed in ~ 10 minutes. After ~ 1 hour of polymerization time, there is no significant increase in the amount of PPy produced. This clearly shows that the growth rate of PPy is faster in the case of the PS-FeTS fibers. Also,
the intensity of the PPy peaks obtained by vapor phase polymerization of the PS-FeTS are ~ 5 times higher than the PPy peaks obtained by vapor phase polymerization of the PS-FeCl$_3$ fibers. This clearly indicates that the amount of PPy formed in the PS-FeTS fibers is greater than the amount of the PPy formed in the PS-FeCl$_3$ fibers. The faster non-linear increase of the growth rate of the PPy in the PS-FeTS might be due to the re-structuring of the PPy-PS-TS nanofibers.

4.3.4 Characterization of PPy-PS-Cl and PPy-PS-TS.

The XPS gives some insight to the doping level of the PPy-PS-Cl and PPy-PS-TS. In the high resolution N 1s region of PPy-PS-Cl shown in Figure 4.6 (a), an intense peak can be observed at 399.9 eV corresponding to the pyrrole nitrogen (-NH-). Also the presence of a high binding energy component at 401.5 eV, which is the characteristic of the positively charged nitrogen, can be observed. The presence of the high binding energy component implies that the PPy produced by the vapor phase pyrrole polymerization on PS-FeCl$_3$ is intrinsically doped. The doping level can be estimated by calculating the ratio of the integral areas of the positively charged nitrogen (at 401.5 eV) to the total integral area under the curve. The doping level of PPy-PS-Cl is found to be 21%. This implies that there is ~ 1 chlorine per ~ 5 pyrrole rings in the polymer chain. A low binding energy component at 398.3 eV corresponding to the de-protonated nitrogen or imine like (=N-) structure is also observed. This component has also been found in PPy synthesized electrochemically at low current densities and PPy synthesized chemically under less oxidative environments (i.e. when the ratio of the oxidant to the monomer is low). In the high resolution N 1s region of PPy-PS-TS shown in Figure 4.6
(b), an intense peak can be observed at 400 eV corresponding to the pyrrole nitrogen (-NH-). Also the presence of a high binding energy component at 401.8 eV, which is the

Figure 4.6: High resolution XPS spectra of N 1s regions of (a) PS-PPy-Cl and (b) PS-PPy-TS fibers. The dotted lines are the fitted curves.
characteristic of the positively charged nitrogen, can be observed. The doping level is estimated to be ~ 28 % which implies that there is ~ 1 chlorine per ~ 4 pyrrole rings in the polymer chain. The absence of a low binding energy component corresponding to the de-protonated nitrogen or imine like (=N-) structure indicates that the oxidant to the monomer ratio is very high.

The main advantage of using Fe (III) oxidants for the polymerization of pyrrole is that the produced PPy is in the intrinsically doped form. This is also evident from the XPS results as the N 1s spectra of the PPy-PS-Cl and PPy-PS-TS show the protonated nitrogen component at 401.5 eV. The XPS also revealed the presence of chlorine (not shown) which is similar to the chlorine obtained in the chemical polymerization of pyrrole with ferric chloride. Due to these reasons, it is expected that the resultant nanofiber mats are electrically conducting. The conductivity of the PPy-PS-Cl and PPy-PS-TS is $1.8 \times 10^{-3}$ S/cm and $4.6 \times 10^{-3}$ S/sm, respectively, as measured by the Van der Pauw method. The low conductivity of the nanofiber mats could be due to two reasons: (1) the presence of an insulating PS core layer; (2) and the presence of air voids, which occupy more than 50 % of the nanofiber mats. To improve the conductivity of the nanofiber mat, the PS has to be removed. For this, the PPy-PS-TS nanofiber mat was extracted in THF. Since PS is soluble in THF, extraction of the PPy-PS-TS fibers in THF was expected to hollow PPy fibers. The conductivity of the PPy hollow fibers prepared in this way was measured to be 0.123 S/cm. This conductivity is superior to the ones obtained for PPy-PS-TS fibers. To see the sensitivity of the hollow PPy fiber mats, we performed a de-doping test by exposure to ammonia solutions of different concentrations.
The result of the ammonia de-doping test is shown in Figure 4.7. It can be seen that the conductivity of the hollow PPy fiber mat decreases by ~ 4 orders of magnitude. This high sensitivity of the electrical conductivity of the hollow PPy fiber mat towards the composition of the vapor make them ideal candidates for gas sensing applications.\textsuperscript{16-18}

![Graph showing conductivity change](image)

**Figure 4.7:** Change in conductivity of the hollow PPy fiber mat on exposure to ammonia. The hollow PPy fiber mat was synthesized by dissolving PS from PPy-PS-TS fibers using THF for 10 days. The dashed line is drawn as guide to the eye.

The above data clearly shows that the PPy-PS-TS fiber mat has a higher electrical conductivity than the PPy-PS-Cl fiber although the doping levels calculated from the XPS data are not much different (~ 21- 27 %). This can attributed to two main factors.
Firstly, the amount of PPy produced by the vapor phase polymerization of pyrrole on the PS-FeTS fibers is much more than that produced in PS-FeCl₃ as showed by IR spectroscopy. The higher conductivity could also originate from the better chain packing of the PPy-PS-TS fibers. To see if the PPy chain packing had any effect on the conductivity of the nanofiber mat, the XRD of the PPy-PS-Cl and PPy-PS-TS fiber mats were conducted. PPy produced by the oxidative polymerization of pyrrole by Fe (III) salts have been found to be amorphous. This is indicated by the amorphous peak at \(2\theta = 22^\circ\) as shown in the XRD spectra of PPy-PS-Cl system shown in Figure 4.8 (a). However, in our case the PPy-PS-Cl has a crystalline peak at \(2\theta = 16^\circ\). The PPy in the PPy-PS-TS nanofiber mat was found to be crystalline and had sharp distinguishable peaks as shown in Figure 4.8 (b). From this it can be concluded that the PPy-PS-TS system has a better packing than that produced in the PPy-PS-Cl system.

**Figure 4.8:** XRD spectra of (a) PPy-PS-Cl fibers synthesized by using FeCl₃ as the oxidative agent (i) before exposure to pyrrole vapors (ii) after exposure to pyrrole vapors for 48 hours; (b) PPy-PS-TS fibers obtained by using FeTS as the oxidative agent(i) before exposure to pyrrole vapors (ii) after exposure to pyrrole vapors for 48 hours.
4.4 Conclusion

The electrospinning of polystyrene-based nanofibers containing ferric salts has been successfully demonstrated. These fibers were used as templates for the vapor phase polymerization of pyrrole to grown composite fibers consisting of conducting polymer shells on structural polymer cores. The morphology of the composite fibers changed significantly during the vapor phase polymerization of pyrrole. For the in-situ growth of PPy, the use of FeTS is more desirable than FeCl$_3$ because it results in the faster growth and higher yield of PPy that has a better structural chain packing, resulting in a higher conductivity. The PPy-PS-TS fibers were extracted with THF to remove the structural PS cores yielding hollow PPy tubes with a higher conductivity. De-doping studies of the hollow PPy tubes showed them to be ideal for gas sensing applications.

4.5 References


CHAPTER 5

Electrically-Conducting and Porous Fiber Mats Consisting of Polystyrene Core and Poly (3, 4-ethylenedioxythiophene) Shell Nanofibers

5.1 Background

Poly(3,4-ethylenedioxythiophene) (PEDOT) has an excellent environmental stability and so has great potential in antistatic coatings, solid electrolyte capacitors, organic light emitting diodes (OLED’s) and biosensor applications.\(^1\) Although pure PEDOT is insoluble and intractable, the introduction of a water soluble polyelectrolyte – poly(styrene sulfonic acid) (PSS) yielded a soluble PEDOT/PSS system (commercially available as Baytron-P) that was easily processable.\(^2\) The soluble PEDOT/PSS system enabled preparation of PEDOT thin film devices through a simple spin-coat process. In applications of thin film based devices, one challenge is that the active sensing components are imbedded in the bulk and the surface area of the active device component is small, which can limit the efficiency and sensitivity of the device. This limitation can be avoided if PEDOT is made in a nanofiber form that can provide a high surface area for a given mass. The nanofiber texture can enhance the transport of ions and chemicals from the solution to the interior of the sensor component composed of non-woven nanofiber mats. The PEDOT/PSS system could be a good candidate for the synthesis of PEDOT nanofibers. However, PEDOT/PSS is soluble in water.\(^3\) Hence, the nanofibers made of PEDOT/PSS system will lose its nanofiber structure in aqueous solution.
Synthesis of PEDOT nanofibers has been accomplished through template synthesis methods.\textsuperscript{4, 5} These methods employ the electro-polymerization or chemical polymerization of PEDOT within polycarbonate particle track-etched membranes or anodized aluminum oxide membranes.\textsuperscript{6} A bulk growth approach utilizing V\textsubscript{2}O\textsubscript{5} seed template has been used for producing PEDOT nanofibers.\textsuperscript{7} Recently, PEDOT nanofibers have also been synthesized by using aqueous surfactant solutions.\textsuperscript{8} Nanofibers produced by these methods are typically very short. An electrospinning technique is widely used for producing polymeric and inorganic nanofibers in a non-woven mat that can be easily handled macroscopically. Nanofibers can be deposited directly on device substrates. This method has been traditionally utilized for fabrication of polyaniline nanofibers because soluble polyaniline can be easily processed by the electrospinning process.\textsuperscript{9} Recently, the electrospinning technique has also been utilized for synthesizing conducting polymer composites of polypyrrole (PPy).\textsuperscript{10} However, the conductivity is not high due to low degree of ordering of polymer chains.

This chapter describes a simple method for the synthesis of porous non-woven mats of electrically-conducting composite nanofibers composed of a polystyrene (PS) core and a PEDOT shell. The self-organization of the ethylene oxide unit in PEDOT can induce a good packing of the thiophene conducting unit, which gives a high electrical conductivity.\textsuperscript{11} The method consists of two steps – electrospinning of PS nanofibers that contain Fe (III) oxidants followed by vapor phase polymerization of EDOT. The use of electrospun PS nanofibers as a template provides all the advantages of the electrospinning process and avoids the intractability problem of PEDOT. Ferric toluenesulfonate (FeTS) can be dissolved in the PS solution using a proper solvent mixture. FeTS is one of the
most efficient oxidants for EDOT polymerization and leaves the toluenesulfonate ions in
the produced PEDOT making it intrinsically doped and electrically conducting. The
vapor phase polymerization approach is employed to produce PEDOT without disrupting
the morphology of the PS nanofiber structure. Vapor phase polymerization has been
demonstrated to produce smooth, coherent and electrically conducting PEDOT films. To
improve the electrical conductivity of the PS-PEDOT nanofiber structure, the nanofibers
can be melt-welded during the vapor phase polymerization.

5.2 Experimental Details

The PS-PEDOT core-shell nanofibers were prepared by electrospinning a solution
of PS and FeTS in a tetrahydrofuran (THF)-acetone-butanol solvent system, followed by
exposure to EDOT vapors at 70°C. Figure 3.1 (in Chapter 3) shows a schematic of the
process used for synthesizing the PS-PEDOT core-shell nanofibers. Polystyrene (PS;
molecular weight \( \approx 350,000 \) amu) and poly (styrene-co-maleic anhydride) (PSMA;
molecular weight \( \approx 224,000 \) amu; maleic anhydride fraction = 7%) were obtained from
Sigma Aldrich and were used without further purification. Baytron-C solution containing
40wt-% ferric toluene-sulfonate (FeTS) in butanol and Baytron-M solution containing
98wt-% EDOT was obtained from Bayer and was used as received. THF (reagent grade,
99.9%) and acetone were obtained from Burdick and Jackson.

The PS solution for electrospinning was prepared by dissolving a mixture of PS
and PSMA (3:1 by weight) in THF. It was found that the inclusion of PSMA reduces
formation of defects (such as beads) in nanofibers during the electrospinning. A mixture
of Baytron-C (FeTS) solution and acetone (1:1 by weight) were added to the PS solution.
The total FeTS in the mixture was ~ 5 wt-% and the total concentration of the solid was ~ 15 wt-%. The PS-FeTS solution was homogenized by stirring for ~ 30 minutes and then loaded in a glass pipette with a tip diameter of about 200 ~ 400 µm. An inert electrode (nickel-chromium wire) was placed into the glass pipette loaded with the polymer solution and a positive bias of ~10 kV was applied to the solution using a high voltage supply (ES30P-10W Gamma High Voltage). The as-spun PS-FeTS core fibers were collected on a grounded electrode (aluminum foil or glass slides) placed ~7 cm from the glass pipette tip. The electrospinning process of PS-FeTS nanofibers is continuous and can be operated unattended for several hours for the production of thick sheets of non-woven nanofiber mats. The collected fibers were then heated in a glass container to 70°C and exposed to EDOT vapors kept at the same temperature. The exposure times varied from ~ 5 minutes to 6 hours.

The morphology of the PS-PEDOT fibers was characterized using field emission scanning electron microscopy (FE-SEM). For FE-SEM imaging, a thin layer of iridium (~ 20 Å) was sputter-coated onto the sample to prevent charging problems. The vapor phase polymerization of EDOT on the electrospun core fibers of PS-FeTS was monitored as a function of EDOT vapor exposure time using transmission Fourier transform infrared spectroscopy (FTIR). The FTIR spectrum was analyzed in the range from 400 cm\(^{-1}\) to 4000 cm\(^{-1}\). The crystalline phase in the PS-PEDOT fibers was characterized using x-ray diffraction (XRD; x-ray source = Cu K\(\alpha\) radiation). The four probe conductivity of the as-polymerized non-woven mats of PS-PEDOT was measured at various times of polymerization by the van der Pauw method using a Keithly 4200 source meter. For conductivity measurements, the PS-PEDOT composite fibers were produced on 1 cm × 1
cm glass slides. Electrical contacts were made at the four corners of the 1 cm × 1 cm sample using silver paint. The four corners with contacts are labeled as 1, 2, 3 and 4 in a clockwise fashion. The conductivity was measured using the equation

\[ \sigma = \left[ \frac{\pi}{\ln 2} \cdot \frac{R_{12,34} + R_{23,41}}{2}, f \cdot t \right]^{-1} \]

where \( R_{12,34} = V_{34} / I_{12} \) and \( R_{23,41} = V_{41} / I_{23} \). \( V_{34} \) and \( V_{41} \) are the potentials (in volts) measured across the labeled points 3 – 4 and 4 – 1, respectively. \( I_{12} \) and \( I_{23} \) are the currents (in amperes) applied across the points 1 – 2 and 2 – 3, respectively. \( f \) is a function of the ratio of two measured resistances and is equal to 1 in our case as the samples are perfectly symmetrical and when \( R_{12,34} \approx R_{23,41} \). ‘t’ is the thickness of the as-polymerized non-woven mat (in cm).

5.3 Results and Discussion

In order to obtain high-quality PS-PEDOT core-shell nanofibers, it is important to get high-quality PS nanofibers since they will be the template for the PEDOT growth. There are several parameters that should be optimized. The first is the choice of solvent. The solvent should be able to dissolve both PS polymer and FeTS salt and have the vapor pressure within a certain range. It was found that a mixture of THF and acetone is efficient for dissolution of both PS and FeTS. The addition of 16 ~ 32 wt-% acetone to THF prevented phase separation during the introduction of FeTS and yielded homogeneous electrospinning solutions. The vapor pressure of acetone (30 KPa at 25°C) is higher than that of THF (21 KPa at 25°C). So, if too much acetone in added, the electrospinning tip got clogged due to rapid evaporation of the solvent. If the amount of acetone was too low, a lot of bead defects and discontinuity in the fibers were observed.
It should also be noted that the FeTS is introduced into the electrospinning mixture through the Baytron-C solution (FeTS 40 wt-% in butanol). The presence of suitable amounts of the low vapor pressure solvent, butanol (~ 0.81 KPa at 25°C), is very advantageous for continuous and unattended operation of the electrospinning process. The maximum amount of FeTS that can be incorporated into the electrospinning solution was ~ 5 wt-%. If the FeTS amount was higher than 5 wt%, the solution becomes too viscous for electrospinning. The as-spun PS-FeTS fibers have a yellowish color characteristic of the Fe (III) salt.

The PEDOT growth on the PS-FeTS template nanofiber was carried out through vapor-phase polymerization. PS is soluble in EDOT; hence, the solution-phase polymerization is not suitable for the PS-PEDOT core-shell nanofiber synthesis. When the PS-FeTS nanofibers are exposed to the EDOT vapor the nanofiber color changes from yellow to sky blue. The sky blue color indicates the formation of doped and conducting PEDOT.\textsuperscript{13}

The high resolution FE-SEM images of the as-spun fibers and the vapor phase polymerized fibers are shown in Figure 5.1. The FE-SEM image of the as-spun PS-FeTS template fibers shows a smooth morphology on the polymer fiber surface (Figure 5.1(b)). The fiber diameters are found to be 300 ± 60 nm. The nanofiber structure is preserved during the vapor phase polymerization (Figures 5.1 c, e, g). The high resolution FE-SEM images of the PS-PEDOT nanofibers show small platelets at the surface (Figures 5.1 d, f, h). No noticeable size difference was observed among the as-spun nanofibers and the polymerized nanofibers.
The vapor phase polymerization of EDOT over PS-FeTS nanofibers is confirmed by IR spectroscopy as shown in Figure 5.2. The IR spectrum of the as-spun PS-FeTS fibers is dominated by the characteristic bands of polystyrene, maleic anhydride and the

![Figure 5.1: FESEM image of PS-PEDOT nanofibers obtained by exposing PS-FeTS fibers at 70°C to EDOT vapors at 70°C for (a) time of exposure = 0 minutes (as-spun fibers), (b) time of exposure = 1 hour, (c) time of exposure = 6 hours.](image)

toluenesulfonate anion. These peaks are the C-H stretching within the aromatic ring in the 3000-3100 cm\(^{-1}\) region, the C-H deformation in the aromatic ring at 1450 and 1490 cm\(^{-1}\), the C=\(\equiv\)C ring stretching in the aromatic ring at 1605 cm\(^{-1}\), the aromatic overtones in the 1700–2000 cm\(^{-1}\) range, the anti-symmetric and symmetric C=O stretching of the maleic anhydride group at 1850 and 1780 cm\(^{-1}\), and the sulfonyl peaks of toluenesulfonate anion at 1035 and 1008 cm\(^{-1}\). Upon vapor phase polymerization of EDOT on these fibers, the vibrational bands characteristic of PEDOT are growing. These peaks are the symmetric and asymmetric C-H stretching of the ethylene group in PEDOT at 2852 and 2920 cm\(^{-1}\), respectively; the C-H bending of the ethylene group at 1449 and 1464 cm\(^{-1}\); the C-O-C stretching at 1237 and 1185 cm\(^{-1}\); the C=\(\equiv\)C stretching in the thiophene ring at 1370 cm\(^{-1}\); and the C-S ring stretching at 935, 840 and 605 cm\(^{-1}\).

![Figure 5.2: Transmission FTIR spectra of (a) electrospun PS-FeTS fibers, (b) PS-FeTS fibers exposed to EDOT vapor for 1 hour, (c) PS-FeTS fibers exposed to EDOT vapor for 3 hours and (d) PS-FeTS fibers exposed to EDOT vapor for 6 hours.](image)
To estimate the vapor phase polymerization rate, the IR spectra of the nanofibers were recorded at various times of EDOT exposure and the intensity of the 1237 cm\(^{-1}\) peak is plotted with the time of EDOT vapor exposure (Figure 5.3). In the initial stages of polymerization (< 2 hours), the amount of PEDOT produced increases rapidly with the exposure time. About ~ 90 % of the PEDOT is formed in ~ 2 hours. During the initial stages of polymerization (< 2 hours), it can be speculated that the EDOT diffusion through the PS-FeTS is fast in comparison to the polymerization reaction rate. However, as the PEDOT layer becomes thicker, the EDOT monomer diffusion into the fiber interior becomes slower and hence the PPy polymerization rate decreases.

![Graph showing the growth of PEDOT on PS-FeTS fibers monitored as a function of time of exposure to EDOT vapors. The amount of PEDOT produced was assumed proportional to the 1237 cm\(^{-1}\) peak intensity. The maximum peak intensity obtained was equalized to 100 % of PEDOT.](image)

**Figure 5.3:** Growth of PEDOT on PS-FeTS fibers monitored as a function of time of exposure to EDOT vapors. The amount of PEDOT produced was assumed proportional to the 1237 cm\(^{-1}\) peak intensity. The maximum peak intensity obtained was equalized to 100 % of PEDOT.
The formation of PEDOT shell onto the PS-FeTS core fibers can be modeled by the following equation:

$$\frac{\partial C}{\partial t} = \frac{1}{r} D_{EDOT/PS-FeTS} \frac{\partial}{\partial r} \left( r \frac{\partial C}{\partial r} \right) + KC_{EDOT} C_{FeTS}$$

Here, $D_{EDOT/PS-FeTS} =$ diffusion coefficient of the EDOT vapors through the PS-FeTS core; $C_{EDOT} =$ Concentration of the EDOT in the gas phase; $C_{FeTS} =$ Concentration of the FeTS on the PS; and $K =$ reaction rate constant of the formation of PEDOT by the oxidation of EDOT by FeTS. The first term on the RHS in the above equation is the diffusion term and the second term on the RHS of the above equation is the reaction term.

The boundary conditions of this equation will be as follows:

$$t = 0 \rightarrow C_{FeTS} = C_{FeTS,0}; C_{PEDOT} = 0; C_{EDOT} = 0$$
$$r = 0 \rightarrow \frac{\partial C_{PEDOT}}{\partial r} = \frac{\partial C_{EDOT}}{\partial r} = \frac{\partial C_{FeTS}}{\partial r} = 0$$
$$r = R \rightarrow \frac{\partial C_{FeTS}}{\partial r} = \frac{\partial C_{PEDOT}}{\partial r} = 0; \frac{\partial C_{EDOT}}{\partial r} = K_{adsorption} C_{EDOT}$$

Here, $R =$ radius of the PS-FeTS core fiber and $K_{adsorption} =$ adsorption coefficient of EDOT on PS-FeTS. The chemical polymerization of EDOT with Fe (III) oxidants produce intrinsically doped PEDOT which is electrically conducting. The sheet conductivity of the as-produced PS-PEDOT nanofiber mat was measured as a function of time of EDOT exposure (Figure 5.4). It can be seen that the conductivity increase follows the polymer growth kinetics. In the first 2 hours of polymerization, the sheet conductivity increases rapidly. After ~ 2 hours, the amount of PEDOT produced is very low and hence there is a negligible increase in conductivity. The sheet conductivity of the PS-PEDOT nanofiber mat is ~0.24 S/cm. This value is calculated using nominal thickness of the PS-PEDOT fiber mat without considering the presence of PS core (template) and the void.
Figure 5.4: Conductivity of PS-FeTS fibers monitored as a function of time of exposure to EDOT vapors. The conductivities were measured from the Van der Pauw equation.

The conductivity of the PEDOT layer itself would be much higher than this value. This conductivity value is superior to those obtained for conducting composites of polypyrrole.\(^\text{14}\) This could be due to the better packing structure of PEDOT.\(^\text{15}\) The XRD of PS-FeTS exposed to EDOT vapors for different times is shown in Figure 5.5. The PEDOT formed is crystalline with intense sharp peaks at \(2\theta = 6.4^\circ\) (d-spacing = 13.7Å) and \(18.3^\circ\) (d-spacing = 4.8Å) corresponding to the [100] and [020] planes of the PEDOT orthorhombic unit cell, respectively. No amorphous phase is seen at large times of polymerization.

It is desired to improve the sheet conductivity without disturbing the porous non-woven mat structure of PS-PEDOT. The conductivity can be improved by attaining a better connectivity between PS-PEDOT nanofibers.\(^\text{16}\) One of the obstacles for achieving high conductivity for conducting polymers chemically synthesized within or on template
Figure 5.5: XRD of PS-FeTS fibers monitored as a function of time of exposure to EDOT vapors (a) as-spun PS-FeTS fibers (b) PS-FeTS fibers exposed to EDOT vapors for 1 hour (c) PS-FeTS fibers exposed to EDOT vapors for 3 hours
electrospun polymer fibers is the poor connectivity of the nanofibers. One way to achieve better connectivity is by flash welding of the polymer nanofibers by using a high energy source, such as a camera flash. This method is applicable to polymers with low luminescence efficiency (such as polyaniline) thereby converting most of the light energy into heat and melting the nanofibers. However we found that the PS-PEDOT nanofibers do not weld upon exposure to a camera flash. To obtain intimate contact between PS-PEDOT nanofibers, the melt-welding approach was attempted. This approach utilizes the fact that PS is soluble in EDOT liquid. The melt-welding of nanofibers without completely dissolving PS nanofiber templates can be accomplished by condensing EDOT vapor on the nanofiber surface. For this, we increased the temperature of the EDOT liquid source to 85°C and kept the PS-FeTS template nanofibers at 35°C. In this way, the EDOT vapor pressure is over saturated at the PS-FeTS nanofiber surface and the EDOT liquid condensation occurs. Figure 5.6 shows the FE-SEM images of PS-PEDOT nanofibers produced by vapor phase polymerization under the condensation condition for 1 min, 10 min, and 20 min. The nanofiber shape remain intact in the case of 1 min polymerization (Figures 5.6 a and b). After 10 min of polymerization under the EDOT condensation condition, it can clearly be seen that the junctions of PS-PEDOT nanofibers are slightly melt-welded (Figures 5.6 c and d). If the amount of EDOT liquid condensed on the nanofibers is too much, the PS-FeTS template nanofibers can melt completely and form a film-like morphology. This is the case when the PS-FeTS template nanofibers are exposed to the EDOT vapors under the condensation conditions for ~ 20 minutes (Figures 5.6 e and f).
Figure 5.6: FESEM image of melt-welded PS-PEDOT nanofibers obtained by exposing PS-FeTS nanofibers to EDOT vapors at 80°C for (a) exposure time = 1 minute (b) exposure time = 10 minute (c) exposure time = 20 minutes.
The conductivity of the melt-welded PS-PEDOT nanofibers obtained by exposing PS-FeTS nanofibers to EDOT vapors for ~ 10 minutes was found to be ~ 1 S/cm which is roughly ~ 4 times larger than the PS-PEDOT nanofibers obtained by vapor phase polymerization (Figure 5.7). The XRD of the melt-welded PEDOT produced by exposure of PS-FeTS fibers to the condensing vapors of EDOT is shown in Figure 5.8. Until ~ 10 minutes of polymerization the PEDOT formed is crystalline with intense sharp peaks at $2\theta = 6.4^\circ$ (d-spacing = 13.7Å) and $17^\circ$ (d-spacing = 5.2Å). However, after 20 minutes of polymerization the composite melts together and a highly amorphous phase of randomly oriented PEDOT is produced. This can also be seen in the XRD with a broad amorphous

**Figure 5.7:** Comparison between conductivity of (a) vapor phase polymerized PEDOT-PS fibers (time of polymerization = 2 hours) and (b) melt-welded PS-PEDOT nanofibers obtained by exposing PS-FeTS nanofibers to EDOT vapors at 80°C (time of polymerization = 10 minutes).
band centered at $25^\circ$. From the XRD of the vapor phase polymerized PEDOT-PS fibers and the melt-welded PEDOT-PS fibers, it can be seen that the crystallinity of the melt-welded fibers is lower. In spite of the lower crystallinity, the conductivity of the melt-welded PEDOT-PS fibers is higher. Hence it can be concluded that this higher conductivity is due to the better connectivity of the melt-welded PEDOT-PS fibers.

![XRD spectra](image)

**Figure 5.8:** XRD of melt-welded PS-PEDOT nanofibers obtained by exposing PS-FeTS nanofibers to EDOT vapors at 80°C for (a) exposure time = 10 minutes (b) exposure time = 60 minutes.
5.4 Conclusions

PS-PSMA-PEDOT composite nanofibers with an average diameter of 300 nm were fabricated by a two-step process involving electrospinning of PS-FeTS fibers followed by exposure to EDOT vapors at 70°C. The EDOT monomers diffuse into the PS-FeTS fibers and react with FeTS, producing PS-PSMA-PEDOT nanofibers. The sheet conductivities of the PEDOT-PS composite nanofiber mats are in the order of ~ 0.24 S/cm. In order to improve the connectivity of the nanofiber mat, EDOT is slightly condensed onto the PS-FeTS fibers so as to produce melt-welded PS-PSMA-PEDOT nanofibers with conductivities of ~ 1 S/cm.

5.5 References


CHAPTER 6

Preparation of Biocatalytic Nanofibers with high activity and stability via enzyme coating on electrospun nanofibers

6.1 Background

Enzymes are highly specific catalysts, and their potential applications are well recognized. The use of enzymes is rapidly increasing in various fields, such as fine-chemical synthesis, pharmaceuticals, commodity catalysts in food processing and detergent applications, biosensors, bioremediation, polymerase chain reaction, protein digestion in proteomic analysis, and bio-fuel cells. Even though a variety of methods have been designed and tested, the development of both stable and active enzyme systems is still a challenging issue in realizing successful use of enzymes in practical applications.

Recent efforts using nanostructured materials, such as mesoporous media, nanoparticles, carbon nanotubes and nanofibers, are an intriguing approach since all these materials can provide a large surface area for the attachment of enzymes, which can lead to high volumetric enzyme activity. Among these materials, electrospun nanofibers offer a number of attractive features compared to the other nanostructures. First, nanofibers have an advantage over mesoporous media by relieving the mass transfer limitation of substrates/products due to their reduced thickness. Second, it is easier to recover and reuse nanofibers than nanoparticles or carbon nanotubes since electrospinning can generate long nanofibers, which can also be further processed into various structures such as non-woven mats, well-aligned arrays, or membranes. Finally,
electrospinning is simple and versatile, and it can produce nanofibers from various materials as well as with controllable compositions and sizes within a short time.

Jia et al. \(^{14}\) demonstrated that the covalent attachment of enzymes onto polystyrene nanofibers results in high loading of enzymes due to the large surface area of nanofibers. They also showed that the enzyme activity and stability can be improved especially in non-aqueous solvents. However, since the maximal loading capacity with an approach of covalent attachment is monolayer coverage of enzyme molecules onto nanofibers, it is anticipated that enzyme-aggregate coatings can further improve the enzyme loading, leading to increased overall enzyme activity. Herein, we describe a novel approach to fabricate enzyme-aggregate coatings on nanofibers, which employs covalent attachment of seed enzyme molecules onto nanofibers, followed by the crosslinking of additional enzyme molecules and aggregates from solution onto the seed enzyme molecule using glutaraldehyde (GA) treatment. This approach is shown in Figure 6.1. The resulting enzyme-aggregate coating can be thought of as bunches of crosslinked enzyme aggregates (CLEAs) that are covalently attached to nanofibers via a linker of each seed enzyme molecule. Since CLEAs are well known for their high stability\(^{21}\), we anticipate that the enzyme-aggregate coating on nanofibers will not only improve the loading of enzymes leading to high overall enzyme activity, but also stabilize the enzyme activity of the final biocatalytic nanofibers.

In this paper, we demonstrate that the coating of a model enzyme (\(\alpha\)-chymotrypsin, CT) on polymer nanofibers (mixture of polystyrene and poly (styrene-co-maleic anhydride)) can improve both the enzyme stability and activity. Moreover, during the measurement of enzyme stability, we incubated all enzyme-immobilized nanofibers
**Figure 6.1:** Schematic diagram for the preparation of covalently-attached enzymes and enzyme-aggregate coatings on nanofibers using glutaraldehyde as a cross-linker.

in a rigorously-shaking condition. Even though most immobilized enzyme systems are supposed to be used in a shaking condition to overcome both external and internal mass-transfer limitation of substrate $^{22}$, it is not easy to find reports using a shaking condition for the evaluation of the enzyme stability. We observed extended stability of the catalytic activity with our enzyme-aggregate-coated nanofibers under a shaking condition, indicating that the covalent attachment of enzyme aggregates on the external surfaces of
nanofibers creates a new immobilized enzyme system effective in stabilizing the enzyme activity.

6.2 Experimental Details

6.2.1 Materials

α-Chymotrypsin (CT), N-Succinyl-Ala-Ala-Pro-Phe p-nitroanilide (TP), glutaraldehyde (GA), and N,N-dimethylformamide (DMF) were purchased from Sigma (St. Louis, MO). Polystyrene ($M_w = 860,000$) and poly (styrene-co-maleic anhydride) ($M_w = 224,000$; maleic anhydride content = 7wt-%) were purchased from Pressure Chemical Company (Pittsburgh, PA, USA) and Aldrich (Milwaukee, WI, USA), respectively. Tetrahydrofuran (THF) (HPLC, 99.9%) was purchased from Burdick and Jackson (Muskegon, MI, USA). All other reagents and solvents were purchased from Aldrich (Milwaukee, WI, USA), and were of the highest grade commercially available.

6.2.2 Preparation of PS or PS+PSMA nanofibers using electrospinning

Polymer solutions were prepared at room temperature by dissolving polystyrene (PS) or a mixture of PS and poly(styrene-co-maleic anhydride) (PSMA) with a 2:1 weight ratio of PS:PSMA in tetrahydrofuran (THF), followed by magnetic stirring for 1-2h. The concentration of PS and PSMA in the solutions was varied from 9 to 23 wt-% and 5 to 9 wt% respectively, depending on the required size range of the fibers. A schematic of the electrospinning apparatus is shown in Figure 1.2. The polymer solution was loaded into a 3 mL plastic syringe (Becton-Dickinson, Franklin Lakes, NJ, USA) equipped with a 30 gauge stainless steel needle (Precision-glide, Becton-Dickinson,
Franklin Lakes, NJ, USA) made of stainless steel. A bias of 7 KV was applied to the needle using a high-voltage supply (ES30P-10W, Gamma High Voltage Research, Ormond Beach, FL, USA). The solution was fed at a rate of 0.15 mL/h using a syringe pump (PHD-2000 Infusion, Harvard Apparatus, Holliston, MA, USA). The electrospun fibers were collected on a clean aluminum foil (connected to the ground) placed at a suitable distance (7 – 10 cm) from the tip of the needle.

6.2.3 Enzyme immobilization or coating on the nanofibers

PS+PSMA nanofibers (~ 1 mg) were incubated in 1 mL of 10 mM phosphate buffer (pH 7.8) containing 20 mg CT. The vials were shaken at 200 rpm at room temperature for 30 min, and then moved into a refrigerator for an additional rocking at 30 rpm. After 90 min incubation at 4°C, GA solution was added (final GA concentration was 0.5 % w/v), and the mixture was put on rocker (30 rpm) at 4 °C overnight. The enzyme-aggregate-coated nanofibers were transferred to a new glass vial, and washed with 100 mM phosphate buffer (pH 7.8) and 100 mM Tris-HCl (pH 7.8). To cap the unreacted aldehyde groups, nanofibers were incubated in Tris-HCl buffer for 30 min. After capping, nanofibers were washed extensively with 10 mM phosphate buffer (pH 7.8) until no enzymes were observed in the washing solution. This washing took an hour with five-time washings. The enzyme-aggregate-coated nanofibers were stored in 10 mM phosphate buffer (pH 7.8) at 4 °C. Two control samples were also prepared for comparison with enzyme-aggregate-coated nanofibers. The first one is covalently-attached CT onto nanofibers, and was prepared by omitting the GA treatment step. The second one is a simple adsorption of CT without covalent linkages between CT and
polymer nanofibers, and was prepared by using PS nanofibers instead of PS+PSMA nanofibers.

6.2.4 Activity measurement and stability

The CT activity of biocatalytic nanofibers was measured by the hydrolysis of TP in aqueous buffer (10 mM sodium phosphate, pH 7.8). The detailed protocol is as follows. Biocatalytic nanofibers were transferred into a new glass vial, and 4.04 mL of 10 mM phosphate buffer (pH 7.8) containing 40 µl of TP (10 mg/mL in DMF) was added to initiate the enzymatic reaction. The vials were shaken at 200 rpm and aliquots were removed time-dependently. The product (p-nitroaniline) of enzymatic catalysis in each aliquot was measured by the absorbance at 410 nm (A410), and the activity was calculated from the slope of A410 with time. The stability of biocatalytic nanofibers was determined in aqueous buffer (10 mM phosphate buffer, pH 7.8) under rigorous shaking (200 rpm). The residual activity was measured at each time point, as described above. Right after each activity measurement, the sample was washed three times with 10 mM phosphate buffer (pH 7.8) to remove all the residual amounts of substrate and products. The relative activity was calculated from the ratio of residual activity to initial activity.

6.2.5 Characterization

Specimens of the electrospun polymer nanofibers were analyzed with scanning electron microscopy (SEM) and reflection-absorption infrared spectroscopy (RAIRS). For SEM, a thin layer of gold (~10nm) is coated to prevent charging. The image characterization was done using a Philips XL-20 SEM (Philips Electron Optics,
Eindhoven, The Netherlands). For RAIRS, the e-spun fibers were collected on a glass slide. The RAIRS analysis was carried out with a NEXUS 670 infrared spectrometer (ThermoNicolet, WI, USA). The incident and reflection angles of IR beam were $82^\circ$ and the spectral resolution was $4 \text{ cm}^{-1}$.

6.3 Results and Discussion

6.3.1 Physical characterization of electrospun polymer nanofibers

For electrospinning of PS and PS+PSMA fibers, THF was used as the solvent to synthesize the fibers due to its high vapor pressure, high volatility and tendency to generate high pore densities. As the concentration of the polymer (PS and PSMA) in the solvent increases, the viscosity of the polymer solution increases, thus yielding thicker diameter fibers. Two different thickness fibers were synthesized – one less than 1 µm and the other larger than 1 µm. The detailed size distribution was obtained with statistical analysis of fibers imaged with SEM (Figure 6.2). The fiber diameter of the thin one is $444 \text{ nm} \pm 106 \text{ nm}$ and that of the thick one is $3.04 \mu\text{m} \pm 1.03 \mu\text{m}$. Hereafter, the former will be called nanofibers and the latter will be called microfibers. Nanofibers were of primary interest for the enzyme immobilization studies. In addition to the size distribution, the SEM analysis revealed a few notable features. First, the nanofibers sometimes showed formation of beads along the fibers while the microfibers are almost bead-free. This might be related to the Taylor cone instability during the electrospinning process. The process was adjusted to minimize bead formation on nanofibers and the samples used for enzyme immobilization were largely bead free. Second, high resolution SEM images show that the surface texture of electrospun fibers contains small holes. The
typical size of surface holes is about 100 ~ 400 nm (Figure 6.2(a) and 6.2(b) inset). The formation of holes on the surface of the electrospun fiber surface is often observed especially when a high vapor pressure solvent is used and their formation mechanisms are extensively studied in the literature. \(^{23-26}\) On the nanofibers, these “holes” exist as depressions whose diameters are similar to the diameter of the fiber, and are sufficiently common that the fibers have a somewhat irregular shape.

![SEM images of PS+PSMA electrospun fibers (a), (b) low resolution image of the non-woven mat (c) ~ 3 µm nanofiber showing indents (d) ~ 400 nm nanofiber.](image)

**Figure 6.2:** SEM images of PS+PSMA electrospun fibers (a), (b) low resolution image of the non-woven mat (c) ~ 3 µm nanofiber showing indents (d) ~ 400 nm nanofiber.

### 6.3.2 Covalent-attachment of enzymes on polymer nanofibers

Jia et al. performed an extensive chemical modification of PS nanofibers to graft functional groups for the covalent attachment of CT molecules on PS nanofibers. \(^{14}\) In this paper, we developed a simpler approach by electrospinning a mixture of PS and
PSMA. The PSMA copolymer contains a functional group of maleic anhydride (MA) that can readily form covalent bonds with primary amines on the enzyme molecules (Figure 6.1). The use of PSMA copolymer makes it much easier to attach enzyme molecules onto electrospun nanofibers by omitting a tedious and rigorous functionalization step after electrospinning. The approach using copolymers such as PSMA can be used with any other polymer nanofibers so long as the maleic anhydride group is intact and exposed at the fiber surface.

The presence of the maleic anhydride group in the electrospun fiber was confirmed with RAIRS (Figure 6.3). The IR spectrum of the PS nanofiber sample clearly shows all the characteristic bands of polystyrene: C-H stretch of the aromatic ring in the $3000 - 3100 \text{ cm}^{-1}$, aromatic C-H deformation of the aromatic ring at 1450 and 1490 cm$^{-1}$, C=C stretch in the aromatic ring at 1605 cm$^{-1}$, and aromatic overtones in the range 1700 ~ 2000 cm$^{-1}$. The IR spectrum of the PS+PSMA fiber exhibit additional peaks representing the anhydride group: asymmetric C=O stretch at 1860 cm$^{-1}$ and symmetric C=O stretch at 1780 cm$^{-1}$. The availability of the anhydride group was supported by the stabilities of PS+PSMA nanofibers with covalently-attached CT or covalently-attached CT-aggregates, compared to PS fibers with only adsorbed CT or PS fibers treated to produce enzyme aggregates, as described below.

The activity of CT-immobilized nanofibers was measured by the hydrolysis of TP in an aqueous buffer solution (10 mM sodium phosphate buffer, pH 7.8) in a shaking condition (200 rpm). Catalytic stability was investigated by continuous incubation of nanofiber samples in an aqueous buffer under rigorous shaking (200 rpm). At each time point, the residual activity was measured, and the relative activity was calculated from
the ratio of residual activity to initial activity. After each activity measurement, the sample was extensively washed with buffer to remove all residual amounts of substrate and product from the sample. The leaching of CT was also monitored by measuring the protein contents of the aqueous buffer solution at each time point.

**Figure 6.3:** Reflection-absorption infrared spectra of (a) PS and (b) PS+PSMA nanofibers collected on a glass slide. The characteristic aromatic overtone peaks of polystyrene are marked with dotted lines. The vibration peaks of maleic anhydride (MA) are marked.

Table 6.1 provides the catalytic activity and leaching data for the first few days, while Figure 6.4 shows relative activities over a longer time period. The initial activity of PS+PSMA nanofibers with covalently-attached CT was 1.9 times higher than that of PS
nanofibers with adsorbed CT (Table 6.1). It is presumed that the former nanofibers are initially coated with both covalently-attached and adsorbed CT molecules. During the first one-day incubation in a shaking condition, nanofibers with covalently-attached CT and those with adsorbed CT both showed a significant CT leaching. After a two-day incubation, no more leaching of CT molecules could be detected from nanofibers with covalently-attached CT while PS nanofibers with absorbed CT leached more CT. As seen in Figure 6.4, after the first few days (when both fibers leach CT), the fibers with covalently attached CT exhibit greater stability of the activity. These results suggest that covalent attachment to surface-available anhydride groups has occurred.

Table 6.1: The activity, leaching, and stability of CT on nanofibers with adsorbed CT and covalently-attached CT

<table>
<thead>
<tr>
<th></th>
<th>Adsorbed CT on PS nanofibers</th>
<th>Covalently-attached CT on PS+PSMA nanofibers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity (µM/min per mg fibers)</td>
<td>Leached enzyme into buffer (µg)</td>
</tr>
<tr>
<td>Day 0</td>
<td>0.051</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Day 1</td>
<td>0.008</td>
<td>3.8</td>
</tr>
<tr>
<td>Day 2</td>
<td>0.005</td>
<td>1.0</td>
</tr>
</tbody>
</table>
Figure 6.4 also shows the activity of free CT in solution for comparison. The activity of free CT rapidly decreased due to autolysis (half-life of 5 h) while the adsorbed and covalently-attached CT showed a marginal improvement of enzyme stability with half-lives of 18 h and 35 h, respectively (based on all the data points including those during which initial leaching of adsorbed enzyme from the covalently-attached preparation is presumed to be occurring).

![Graph showing relative activity over time for CT aggregate coating, covalently attached CT, and free CT.](image)

**Figure 6.4:** The stabilities of free CT, covalently-attached CT, and CT-aggregate coating on nanofibers in an aqueous buffer solution (10 mM sodium phosphate, pH 7.8) under shaking condition (200 rpm). Right after each activity measurement, the sample was excessively washed three times by a buffer solution to remove both substrate (TP) and product (p-nitroaniline) from each sample. The relative activity was calculated from the ratio of residual activity at each time point to initial activity.

### 6.3.3 Enzyme-aggregate coating on polymer nanofibers

Even though covalent attachment marginally improved the enzyme activity and stability when compared to adsorbed CT, the results are not good enough to maintain
high enzyme activity in a rigorously shaking condition (200 rpm), which is important for
the successful applications of enzymes in a variety of heterogeneous immobilization
systems. To develop more stable and active enzyme system, we fabricated enzyme-
aggregate coatings on polymer nanofibers and microfibers using the strategy discussed in
the Introduction (Figure 6.1) and the procedure given in the Materials and Methods.
Comparing nanofibers and microfibers, we found that the activity (per mg) of covalently-
attached CT on microfibers was 78% of that of nanofibers (Table 6.2). The activity (per
mg) of CT-aggregate coated microfibers was 73% of the activity with CT-aggregate
coated nanofibers. The nanofibers were evaluated in more detail. The activity of CT-
aggregate-coated nanofibers was 8 times higher than the initial activity of nanofibers with
only covalently-attached CT. This great improvement of CT activity with CT-aggregate-
coated nanofibers can be explained by the much higher enzyme loading, as suggested in
Figure 6.1. No enzyme leaching from CT-aggregate-coated nanofibers was detectable
from the beginning of incubation in a shaking condition.

**Table 6.2:** Effect of fiber thickness on the activity of biocatalytic fibers.

<table>
<thead>
<tr>
<th>Sample description</th>
<th>Initial activity (µM/min per mg fibers)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Covalently-attached CT on PS+PSMA (&lt; 1 µm)</td>
<td>0.098</td>
</tr>
<tr>
<td>Covalently-attached CT on PS+PSMA (&gt; 1 µm)</td>
<td>0.076</td>
</tr>
<tr>
<td>CT-aggregate coating on PS+PSMA (&lt; 1 µm)</td>
<td>0.868</td>
</tr>
<tr>
<td>CT-aggregate coating on PS+PSMA (&gt; 1 µm)</td>
<td>0.633</td>
</tr>
</tbody>
</table>
The stability of CT-aggregate-coated nanofibers is shown in Figure 6.4 for the first 9 days (shaking conditions as usual). The stability of the CT-aggregate-coating was so impressive as to show negligible loss of CT activity for more than one month (data to 33 days were collected). There was insufficient loss of activity to estimate a half life. This dramatic stabilization of CT as aggregates coated on nanofibers can be explained by several factors, including no CT leaching and good stability of crosslinked enzyme aggregates (CLEAs) themselves.\textsuperscript{21} It is also noteworthy that enzyme aggregation prevented the autolysis of CT molecules.

To check the role of covalently-attached seed enzyme molecules in the fabrication of CT-aggregate coating, we prepared the PS nanofibers by electrospinning a PS solution without addition of PSMA copolymer. Then, we adsorbed CT on the PS nanofibers and treated them with 0.5 \% GA solution, which is exactly same as the fabrication process of CT-aggregate-coating on the PS+PSMA nanofibers. The final nanofibers would consist of PS nanofibers and enzyme aggregates without any covalent linkages between them. This sample exhibited a serious leaching of enzymes and the quantity of leached CT was 4.6 \(\mu\text{g}\) after a one-day incubation. It was observed that white powders of enzyme aggregates separated from PS nanofibers and leached into a buffer solution. The activity of this control sample continuously decreased in a shaking condition, and the relative activity was 85\% after a two-day incubation. The calculated half-life of this control was 11.5 days, much shorter than that of CT-aggregate-coated PS+PSMA nanofibers. This suggests that the covalently-attached seed enzyme molecules play a significant role in developing a stable form of enzyme-aggregate-coating on the surface of nanofibers.
6.4 Conclusions

Polymer nanofibers, providing a large surface area for the attachment of enzyme, are an ideal host for enzyme immobilization with high enzyme loading and activity. As a rigorous approach to develop both an active and stable enzyme system using nanofibers, we introduced a unique and simple approach to fabricate enzyme-aggregate coatings on the surfaces of nanofibers. It was demonstrated that the enzyme-aggregate coating on nanofibers improves not only the enzyme activity but also the enzyme stability. These active and stable biocatalytic nanofiber mats were highly durable and could be easily recovered from a solution even after more than one-month incubation in a rigorously-shaking condition.

6.5 References


CHAPTER 7

Improving biocatalytic activity of enzyme-loaded nanofibers by dispersing the entangled nanofiber structure

7.1 Background

Immobilization of enzymes on solid supports is of great interest in biocatalytic reactions and sensors because it can make the handling and recovery of enzymes much easier.\textsuperscript{1,2} Nanostructured supports such as mesoporous materials, nano-particles and nanofibers are widely studied since they provide a high surface to mass ratio;\textsuperscript{3-5} among these, polymer nanofibers offer several advantages over other nanostructures. Long and continuous polymer nanofibers can easily be made with an electrospinning process.\textsuperscript{6, 7} This technique is applicable to a variety of polymer materials. The electrospun polymer nanofibers can be structurally tailored to non-woven mats, well aligned arrays, or membranes.\textsuperscript{8} The large surface-to-mass ratio of nanofibers provide an advantage of higher loading per mass, thus higher enzymatic activity per mass.\textsuperscript{9} These electrospun nanofibers can be easily recovered from solution and re-used.\textsuperscript{9,10}

In order to maximize the enzymatic activity, it is very important to disperse the enzyme-loaded supported materials in the reaction media. Since most enzymes work in aqueous solutions, hydrophilic polymers are often chosen as a support material to attain good dispersion. The electrospun nanofibers of hydrophilic polymers must be cross-linked after or during the electrospinning process to prevent the dissolution of hydrophilic polymers in aqueous solution.\textsuperscript{11,12} The control of the degree of cross-linking still remains
as a challenge. In contrast, hydrophobic polymer fibers are intrinsically stable against dissolution in aqueous solution. The hydrophobic fibers may be advantageous for immobilization of enzymes that work for hydrophobic substrates such as lipase\textsuperscript{13}; however, the hydrophobic nanofibers are not well dispersed in aqueous solution. This can pose mass transport problems during the enzyme loading on the internal surface of the non-woven mat and the reactions of the immobilized enzymes with substrates dissolved in the aqueous solution.

In this paper, we describe a simple and efficient way of dispersing polystyrene-poly (styrene-co-maleic anhydride), PS-PSMA, nanofibers in water without using conventional surfactants. The maleic anhydride groups at the nanofiber surface provide the enzyme anchoring sites which can form covalent bonds with free amine groups at the enzyme surface (Figure 7.1). The PS-PSMA nanofibers are hydrophobic and do not disperse in aqueous solution. The dispersion of these hydrophobic nanofibers can be also

![Figure 7.1: Schematic representation of the covalent attachment of enzymes to the maleic anhydride functional group in the PS-PSMA nanofibers. The NH\textsubscript{2} residues on the surface of the enzyme molecules form amide bonds with the maleic anhydride group of the PSMA. (E = enzyme molecule)
attained by washing the nanofibers with aqueous alcohol solutions before immobilizing enzymes. The well-dispersed enzyme-loaded PS-PSMA nanofibers give a higher enzymatic activity per unit mass of nanofiber than the poorly-dispersed nanofibers. The higher enzymatic activity per unit mass is attributed to the higher loading of enzymes onto the alcohol dispersed PS-PSMA nanofibers. The nanofibers do not show any morphological difference before and after the alcohol treatment. Being able to disperse PS-PSMA nanofibers in aqueous solution allows construction of continuous-flow biocatalytic nanofiber reactors.

7.2 Experimental

7.2.1 Preparation of alcohol dispersed PS-PSMA nanofibers

A polymer solution was prepared at room temperature by dissolving suitable amounts of PS ($M_w = 860,000$) and PSMA ($M_w = 224,000$, maleic anhydride content = 7 wt. %) with a 2:1 weight ratio in a mixture of tetrahydrofuran and acetone (7:3 ratio by volume). The total polymer concentration in the solution was 15%. The polymer solution was loaded into a 3 ml plastic syringe equipped with a 30 gauge stainless steel needle. A bias of +7 KV was applied to the needle using a high voltage supply. The solution was fed at a rate of 0.15 ml/hr using a syringe pump. The electrospun fibers were collected on a clean electrically-grounded aluminum foil. The distance between the needle and collecting foil was 10 cm. The nanofibers were manually peeled off, weighed, and washed with distilled water.
7.2.2 Aqueous alcohol solution treatment of PS-PSMA nanofibers for dispersion in water

For dispersion of PS-PSMA nanofibers in water, the electrospun nanofiber mat is first immersed in an aqueous alcohol solution (water: alcohol = 4:1 by volume) and gently shaken for several hours. The PS-PSMA nanofibers disperse fully in the aqueous alcohol solution. The nanofibers are then repeatedly washed without drying with a copious amount of distilled water until alcohol is thoroughly removed from the solution phase. The dispersed PS-PSMA nanofibers were always kept in water or buffer solution until they were used for lipase immobilization.

7.2.3 Lipase immobilization on PS-PSMA nanofibers

Lipase solution used for immobilization was prepared as follows. A 20 mg of lipase (Mucor Javanicus) was dissolved in 10 ml of a 20 mM sodium phosphate buffer (pH 6.5) and filtered with a series of syringe filters in the sequence of 0.45µm, 0.22µm, and 0.1µm pore sizes. For immobilization, the PS-PSMA nanofibers were incubated in 1 ml of the filtered lipase solution at 200 rpm at room temperature for 30 minutes followed by overnight rocking at 4°C. The nanofibers were then transferred to a new glass vial and washed with 20 mM phosphate buffer (pH 6.5) and 100 mM Tris-HCl buffer (pH 6.5). The Tris-HCl buffer is used to cap the unreacted maleic anhydride groups. After capping, the nanofibers were extensively washed in 20 mM phosphate buffer (pH 6.5) until no leaching of lipase was observed. The total number of washings was ~7. The amount of lipase immobilized on the surface of the PS-PSMA nanofibers was determined by
measuring the initial and final concentrations of the enzyme within the incubation solution and washing solutions at 280 nm with a UV-Vis spectrophotometer. Bovine serum albumin (BSA) was used as the standard to construct the calibration curve.

**7.2.4 Activity assay of the free and immobilized lipase in a batch reaction mode**

The lipase activity was measured by the hydrolysis of 4-nitrophenyl butyrate (4-NB) in 20 mM sodium phosphate buffer (pH 6.5). The lipase immobilized on the PS-PSMA fibers was transferred to a new vial and 19.6 ml of 20 mM sodium phosphate buffer (pH 6.5) containing 4-NB at concentrations varying from 0.031 ~ 1 mM was added to initiate the reaction. For free lipase activity measurement, 0.2 ml of filtered free lipase solution (0.26 mg/ml) was used instead of the lipase-immobilized PS-PSMA nanofibers. The vials were then shaken at 200 rpm at 35°C and small aliquots were removed every 5 minutes. The concentration of enzyme reaction product, p-nitrophenol, was calculated from the absorbance at 400 nm (A400). The enzyme activity was calculated from the slope of the A400 vs. time plot. One unit of the enzyme activity was defined as the amount of enzyme which catalyzed the production of 1 µmol of p-nitrophenol per minute. Specific activity was defined as the activity per unit mass of enzyme. The measured activity was analyzed with the Michaelis-Menten method to get the maximum activity per unit mass of nanofiber ($R_{max}$) and the substrate binding constant ($K_m$).

**7.2.5 Continuous flow reactor test**
Two continuous flow reactor systems were constructed with the lipase-loaded PS-PSMA nanofibers (dry fiber weight = 7 mg) filled in polyethylene tubes (0.8 cm diameter, 1.2 cm long; total volume = 0.6 ml). One system was filled with the pristine nanofibers and the other with the dispersed nanofibers. The 0.5 mM 4-NB substrate solution was continuously flowed through the tube reactors using a peristaltic pump. Three different flow rates were tested: 0.7, 1.8, and 2.8 ml/min. The p-nitrophenol concentration of the eluted solution was obtained by measuring the absorbance at 400 nm.

7.2.6 Characterization

Specimens of the electrospun PS-PSMA nanofibers (with and without alcohol treatment) were analyzed by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR). For SEM, a thin layer of gold (~ 10 nm) was coated to prevent charging. For FTIR, the electrospun fibers were peeled off as a moderately transparent, free-standing thin film. FTIR spectrum was collected with a 4 cm⁻¹ resolution.

7.3 Results and Discussion

After the treatment with aqueous alcohol solution, the PS-PSMA nanofibers are dispersed in water. Figure 7.2 compares the as-spun and alcohol-treated PS-PSMA nanofibers immersed in water. The hydrophobic nature of the as-spun PS-PSMA nanofibers makes the non-woven mat to form a tightly aggregated clump in aqueous
solution. Even though the density of polystyrene (1.05 g/cm$^3$) is higher than water, the nanofiber aggregates float on the water. This might indicate that the nanofiber aggregate contains tiny air bubbles inside. In contrast, the PS-PSMA nanofiber mat pre-treated with the alcohol solution is fully dispersed in water. Moreover, the nanofibers sink in water, as expected from the density of the polymer. Even after repeated rinsing with water for several days, the alcohol pre-treated PS-PSMA nanofibers remain dispersed in water. The expanded nanofiber mat does not break into pieces even after vigorous shaking since the

**Figure 7.2:** Visual comparison of as-spun (left column) and alcohol pre-treated (right column) PS-PSMA nanofibers immersed in water. (a) and (b) are top-view images of nanofibers in a Petri dish. (c) and (d) are side-view images of nanofibers in a 20 ml glass vial.
nanofibers are physically entangled. The same dispersion effect is observed for the nanofiber mats made of PS only. So, the small amount of the maleic anhydride group as an enzyme immobilization site is not responsible for the alcohol-induced dispersion effect. The apparent volume of the entangled fiber structure was about 10 times or larger after dispersion. Further quantification of the three-dimensional change was difficult since both non-dispersed and dispersed nanofibers had irregular shape and were floating in the solution.

The alcohol-induced dispersion does not depend strongly on the nature of alcohol. We have tested the dispersion effect for methanol, ethanol, 1-propanol, 1-butanol, and 1-pentanol. As the alkyl chain length gets longer, the initial dispersion of the PS-PSMA mat in alcohol solution becomes easier – occurring even at gentle agitation. Once the PS-PSMA nanofiber mats are fully dispersed in the alcohol pre-treatment solution, they remain fully dispersed in pure water regardless of the alkyl chain length of the alcohol used in the pre-treatment process. In the rinsing step, the only pre-caution is that the PS-PSMA nanofibers should be kept in wet conditions at all times. Once the dispersed PS-PSMA nanofibers are dried in air, they do not re-disperse upon immersion into water. The dried nanofibers should be treated again with alcohol solution. It seems that a critical alcohol concentration is required to disperse the PS-PSMA mat structure into the loosely entangled structure. When the alcohol concentration is lower than ~20 vol%, the PS-PSMA nanofiber mat does not get fully dispersed or expanded even with vigorous shaking.
The alcohol solution pre-treatment does not alter the physical and chemical feature of the PS-PSMA nanofibers. Figure 7.3 compares the SEM images of the as-spun PS-PSMA nanofibers and the alcohol pre-treated PS-PSMA nanofibers. The SEM images were taken after the wet nanofibers were completely dried. The data show that the alcohol treatment does not induce any morphological changes of the PS-PSMA nanofiber surface. The average diameter of the as-spun nanofibers and alcohol pre-treated PS-PSMA nanofibers were calculated to be 619 ± 175 nm and 575 ± 202 nm, respectively. The diameter of the nanofibers is the same within one standard deviation range.

**Figure 7.3:** SEM images of (a) as-spun PS-PSMA nanofibers and (b) alcohol pre-treated PS-PSMA nanofibers. SEM images were taken after washing nanofiber mats with water and drying in air. Size distributions of the (c) as-spun and (d) alcohol-pre-treated PS-PSMA nanofibers. The distributions are obtained from diameter measurements for 50 nanofibers.
Figure 7.4 displays the transmission infrared spectra of PS-PSMA nanofibers after different treatments. The vibration peaks characteristic of polystyrene are the C-H deformation of the polymer backbone at 1450 cm\(^{-1}\), the deformation of the aromatic ring at 1490 and 1590 cm\(^{-1}\), and the C=C stretch at 1605 cm\(^{-1}\). The characteristic bands of the maleic anhydride group are the symmetric and antisymmetric C=O stretch at 1780 cm\(^{-1}\) and 1860 cm\(^{-1}\), respectively. The alcohol treatment or rinsing with the buffer solution do not change the C=O stretching peak, indicating that the maleic anhydride functional group is intact (not hydrolyzed) and still available for enzyme immobilization. The reaction of the maleic anhydride group of the PS-PSMA nanofiber with free amine is checked by exposing the nanofibers to the buffer solution containing n-propyl amine. The infrared spectrum shown in Figure 4d shows the decrease of the maleic anhydride peak at 1780 and 1860 cm\(^{-1}\) and the growth of the amide bond peak at 1557 and 1642 cm\(^{-1}\). This proves the feasibility of the covalent bonding of enzymes via the reaction between the surface amine group of the enzyme and the maleic anhydride group of the PS-PSMA nanofiber surface (Figure 7.1). Therefore, enzymes can be immobilized directly to the nanofibers without any surface activation process.

The lipase immobilization to the PS-PSMA nanofibers and the reactivity of the lipase-loaded nanofibers were measured for both pristine and alcohol-dispersed nanofibers. The total loading of the lipase enzyme is significantly increased by the dispersion of the PS-PSMA nanofibers. The immobilized protein (lipase) content was determined to be 5.4 ± 2.2 µg (BSA-equivalent) per mg of nanofiber for the as-spun PS-PSMA system while 42.4 ± 18.5 µg (BSA-equivalent) per nanofiber for the alcohol pre-
The vibration peaks marked with dashed lines. The C=O stretching (1642 cm⁻¹) and the in-plane N-H bending (1557 cm⁻¹) vibration peaks are marked in arrows.

**Figure 7.4:** FTIR transmission spectra of (a) as-spun PS-PSMA nanofibers (b) alcohol pre-treated PS-PSMA nanofibers (c) as-spun PS-PSMA nanofibers washed with distilled water (d) PS-PSMA nanofibers reacted with n-propyl amine. The vibration peaks corresponding to the maleic anhydride group are marked with dashed lines. The C=O stretching (1642 cm⁻¹) and the in-plane N-H bending (1557 cm⁻¹) vibration peaks are marked in arrows.

treated PS-PSMA system. The increase of a factor of ~8 in the loading is attributed to the increase of the effective surface area of nanofibers available for enzyme loading upon dispersion. When the PS-PSMA nanofibers are tightly aggregated together, only the maleic anhydride groups at the outer region of the aggregate will be available for the
enzyme immobilization. Small droplets of air trapped in the hydrophobic nanofiber aggregate can also block the transport of the enzyme into the deeper region of the aggregate. Upon alcohol-induced dispersion of the PS-PSMA nanofibers, more surface area of the nanofibers are exposed to the solution and more maleic anhydride groups on the nanofiber surface will be used for enzyme immobilization. The enhanced protein loading clearly demonstrates the importance of the dispersion of PS-PSMA nanofibers via alcohol treatment.

The maximum activity per unit mass of nanofiber ($R_{max}$) and substrate binding constant ($K_m$) are obtained from the Michaelis-Menten analysis of the kinetic data (Table 1). The $R_{max}$ value of the lipase immobilized on the non-dispersed PS-PSMA nanofibers is $0.217 \pm 0.049$ U per mg nanofiber, while that of the lipase immobilized on the dispersed PS-PSMA nanofibers is $1.51 \pm 0.28$ U per mg nanofiber. The difference in $R_{max}$ is consistent with the total amount of the lipase loaded per unit mass of the nanofibers. So, the apparent specific activity, $r_{sp}$, ($= R_{max}$ divided by the BSA-equivalent mass of the loaded lipase) is almost the same, regardless of the nanofiber dispersion. The $r_{sp}$ values of the immobilized lipase are much lower than the specific activity measured for the free lipase (Table 7.1). This is commonly observed for other immobilized enzyme systems.$^{14,15}$ This could be due to several reasons. One possibility is the denaturation or blocking of the active sites during the immobilization of the enzyme via the covalent attachment to the maleic anhydride group.$^{16}$
immobilized on as-spun and alcohol dispersed PS-PSMA nanofibers (35 °C, pH = 6.5).

<table>
<thead>
<tr>
<th></th>
<th>(U/mg lipase)**</th>
<th><strong>m</strong> (mM)</th>
<th>R</th>
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<tbody>
<tr>
<td>Free lipase in</td>
<td>-</td>
<td>187 ± 34.1</td>
<td>0.381 ± 0.106</td>
</tr>
<tr>
<td>solution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As-spun nanofiber</td>
<td>5.6 ± 2.2</td>
<td>31 ± 6.9</td>
<td>0.344 ± 0.085</td>
</tr>
<tr>
<td>with lipase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dispersed</td>
<td>42.4 ± 18.5</td>
<td>31 ± 7</td>
<td>0.481 ± 0.116</td>
</tr>
<tr>
<td>nanofiber</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>with lipase</td>
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The substrate binding constant, $K_m$, is slightly lower for the lipase loaded on the pristine nanofibers than for the lipase loaded on the dispersed nanofibers, although the difference is not significantly larger than the experimental error range. If this difference is real, it might be due to the preferential segregation of the hydrophobic substrate (4-NB) to the hydrophobic nanofiber surface in water, which would increase the local substrate concentration near the immobilized lipase to a value higher than the bulk concentration. Another possibility could be the different agitation behavior between the tightly aggregated nanofiber clump and the dispersed but physically entangled nanofiber body under the shaking condition.
When a continuous-flow reactor is constructed using nanofibers, it is important to attain the highest dispersion of nanofibers. We constructed two continuous-flow reactors using the non-dispersed and dispersed nanofibers. The 7mg dispersed nanofibers filled the entire flow reactor ($V = 0.6$ ml), while the non-dispersed nanofibers of the same weight partially filled the flow reactor. The flow rate dependence of the steady-state conversion ($X^{ss}$) of these reactors is shown in Figure 7.5 (a). The decrease of $X^{ss}$ at the higher flow rate is mainly due to the decrease of the residence time ($\tau = V / F$) in the reactor. Regardless of the flow rate, $X^{ss}$ is always significantly larger for the dispersed nanofibers than the pristine nanofibers. This could be ascribed to the enzyme loading difference of these two nanofibers.

Further kinetic analysis of the conversion data reveals another important difference between two reactors with different degrees of nanofiber dispersion (Figure 7.5 (b)). The apparent pseudo-first order reaction rate constant ($k_{app}$) of the lipase-loaded nanofiber reactor is calculated from the following design equation of the continuous flow reactor: $k_{app} = -\ln \left(1 - X^{ss}\right) / \tau$. Note that $k_{app}$ is the rate constant of the reactor loaded with 7mg of nanofibers, not the rate constant of enzymes. The dispersed nanofiber reactor shows a linear increase of $k_{app}$ from $\sim0.12$ min$^{-1}$ at $F = \sim0.4$ ml/min to $\sim0.32$ min$^{-1}$ at $F = \sim2.8$ ml/min. In the case of the non-dispersed nanofiber reactor, the $k_{app}$ value increases marginally from $\sim0.021$ min$^{-1}$ at $F = \sim0.4$ ml/min to $\sim0.039$ min$^{-1}$ at $F = \sim1.9$ ml/min and then levels off or decreases slightly with further increase of the flow rate.

The large flow rate dependence of $k_{app}$ for the dispersed nanofiber reactor is due to a more efficient supply of the substrate into the reactor at a higher flow rate. This can
Figure 7.5: (a) Conversion and (b) apparent pseudo-first order reaction rate constant of the continuous flow reactors constructed with the as-spun and dispersed PS-PSMA

be estimated using the Weisz modulus, $\Phi = k_{app} \times \tau$. By definition, this modulus shows the competition between the enzyme reaction and substrate transport rates in the
reactor.\textsuperscript{17,18} When $\Phi$ is less than \sim{}0.1, the reaction kinetics is governed by the steady-state conversion since the substrate supply into the system is much faster than the enzyme reaction in the system. If $\Phi$ is larger than \sim{}0.1, the substrate supply rate (flow rate) becomes comparable to the reaction rate of the reactor and the steady state conversion becomes a function of the flow rate. The $\Phi$ modulus of the dispersed nanofiber reactor varies from 0.05 at $F = 3.5$ ml/min. to 0.18 at $F = 0.4$ ml/min. In other words, the enzymatic reaction in the reactor is fast enough to reach the flow-rate dependent regime.

In contrast, the $\Phi$ modulus of the non-dispersed nanofiber reactor is only 0.01 \sim{} 0.03. This explains the weak dependence of $k_{app}$ for the non-dispersed nanofiber reactor. It should be noted that the $k_{app}$ value of the non-dispersed nanofiber reactor levels off or slightly decreases at a flow rate higher than \sim{}1.9 ml/min. This decrease must be related to the change in the fluid flow pattern inside the reactor. Since the non-dispersed nanofibers fill the reactor only partially, some fraction of the substrate solution can pass through the flow reactor without encountering the enzyme-loaded nanofibers. This fraction will increase as the flow rate increases, lowering the apparent rate constant.

The experimental data presented in this paper accentuate the importance of the dispersion of enzyme-loaded nanofibers for biocatalytic applications. The pre-treatment of hydrophobic PS-based nanofibers with aqueous alcohol solutions can make the nanofibers dispersed in water, enhancing the efficiency of enzyme loading and the enzymatic activity per unit mass of nanofibers. The origin of alcohol-induced dispersion is not fully elucidated yet. However, it is understood that the dispersion is not due to the presence of the maleic anhydride group since the pure PS nanofibers show the same
alcohol-induced dispersion behavior. Also, the bulk PS and PSMA materials do not dissolve or swell in the pure alcohols tested in this study. The Hansen solubility parameter for ethanol is \(~19\), which is much larger than the interaction radius of PS (12.7). This indicates that ethanol cannot swell or dissolve polystyrene. In order to dissolve PS, the solvent Hansen parameter must be smaller than 12.7 (like THF = 9.7).

Ruling out these effects, one can speculate that alcohol molecules may behave like a surfactant at the interface between the hydrophobic polystyrene and water. Since the alcohol molecule is composed of a hydrophobic alkyl group and a hydrophilic hydroxyl group, it can be preferentially adsorbed at the polystyrene/water interface with the alkyl group facing the polystyrene surface and the hydroxyl group facing the water side.\(^\text{19}\) This interfacial ordering of alcohol molecules can make the hydrophobic nanofibers disperse in water. The alcohol molecules at the polystyrene/water interface do not seem to be removed by simple rinsing since the alcohol pre-treated nanofibers remain dispersed in water after the alcohol is removed in the bulk liquid phase.

### 7.4 Conclusion

This paper demonstrates the dispersion of hydrophobic polymer nanofibers by simple pre-treatments with aqueous alcohol solutions, which increases the enzyme loading and thus the enzymatic activity per unit mass of the nanofiber support. The improved enzyme loading and activity will help to develop efficient biocatalytic systems for bioconversion, bioremediation, and biosensing. In addition, the easy control of nanofiber structure and surface chemistry can open up the potential for more versatile and
efficient use of hydrophobic polymer nanofibers in bio-applications including drug delivery and tissue engineering.

7.5 References.


CHAPTER 8

Stability of glucose oxidase enzyme aggregate coatings in HOCl/ClO⁻ solutions

8.1 Background

Stability of enzymes in highly reactive environments is important for their utilization as broad enzyme-based decontamination systems. To enhance the stability of the enzymes towards various reactive species it is desirable to immobilize them onto solid supports and stabilize them by multipoint crosslinking. This makes handling and recovery of enzymes much easier.¹,² Among several nanostructured supports available ³⁻⁶ electrospun polymer nanofibers⁷,⁸ offer several advantages such as surface-to-mass ratio, higher loading and thus higher enzymatic activity per mass.⁹ They can also be recovered and re-used from solution.¹⁰ Also, these electrospun polymer fibers can be dispersed so as to achieve increased enzyme loadings.¹¹

For the successful fabrication of practically useful immobilized-enzyme systems several factors need to be considered. One of the most important factors is to maximize the enzyme loading on the surface of the nanofibers. For this purpose it is necessary to disperse the fibers in aqueous solutions. An aqueous alcohol treatment has found to be effective in dispersing the electrospun fibers and hence increasing the enzyme loadings by ~ 8 times.¹¹ Another important factor is to maximize the stability of the enzymes. An efficient way of stabilizing enzymes is through multi-point crosslinking of enzymes. This can be done by either crosslinking the free amine groups or carbohydrate groups exposed at the surface of enzyme. In the case of glucose oxidase, any of the above mentioned
crosslinking methodologies have given appreciable activities and stabilities in harsh environments.\textsuperscript{12} The maleic anhydride (MA) of the PSMA forms covalent bonds with primary amines on the enzyme molecules as shown in Figure 8.2. In the case of the CA-GOX, only a monomolecular layer of enzymes are attached on the surface of the nanofibers resulting in low enzyme loadings and hence low enzyme activities. In the case of EC-GA-GOX, the GOX is first covalently attached onto the PS-PSMA nanofibers as shown in Figure 6.1 (Chapter 6).\textsuperscript{10} The covalently attached GOX molecules act as seeds. Treatment with glutaraldehyde results in multi-point crosslinking of the covalently attached GOX seed molecules as well as crosslinking of additional GOX molecules from solution onto the seed molecules as shown in Figure 6.1 (Chapter 6).

In this chapter, the stability of glucose oxidase immobilized onto polystyrene-poly(styrene-co-maleic anhydride) (PS-PSMA) nanofibers in hypochlorous acid (HOCl) solutions at concentrations 0.1 - 1 mM is investigated. PS-PSMA is chosen as the support because the maleic anhydride groups at the nanofiber surface provide the enzyme anchoring sites which can form covalent bonds with free amine groups at the enzyme surface (scheme 1).\textsuperscript{11} HOCl is chosen as a reactive species since HOCl is widely used for decontamination of various chemical and biological toxins.\textsuperscript{13} The glucose oxidase is immobilized onto the nanofibers in two ways: (1) covalent attachment of the enzymes to the nanofiber (CA-GOX), (2) fabrication of enzyme coatings by covalent attachment of enzymes to the nanofiber surface followed by crosslinking of the enzymes through their surface amino groups using glutaraldehyde (EC-GA-GOX) or crosslinking of their carbohydrate groups using diaminooctane (EC-DAO-GOX). Both cases of crosslinking
results in the formation of a Schiff base between the surface group of the enzyme and the
crosslinker. The stability of these systems in solutions containing HOCl/OCl at
concentrations of ~ 0.1 – 1 mM is compared. In order to reduce the susceptibility of the
Schiff base towards HOCl attack and improve the stability of the EC-GA-GOX and EC-
DAO-GOX even further, mild reduction of the Schiff base was attempted by treatment
with sodium cyanoborohydride NaBH₃CN (Figure 8.3 and 8.8). In all cases, the activity
of the GOX reduced by more than ~ 50 % when exposed to high concentrations of
HOCl/OCl ~ 1 mM.

8.2 Experimental Details

8.2.1 Materials

Glucose Oxidase (GOX, *Aspergillus Niger*), o-dianisidine dihydrochloride
(ODS), horseradish peroxidase (HRP, 25,000 KU), glutaraldehyde (GA), diamino-octane
(DAO), sodium-periodate (NaIO₄), sodium-cyanoborohydride (NaBH₃CN, 98%),
polystyrene (PS, Mₖ = 860,000) and poly (styrene-co-maleic anhydride) (PSMA, Mₖ =
224,000; maleic anhydride content = 7wt%) were obtained from Sigma-Aldrich and used
without further purification. Tetrahydrofuran (THF, HPLC grade, 99.9%) and acetone
were purchased from Burdick and Jackson. Clorox solution (6% NaOCl) was purchase
from Wal-Mart.
8.2.2 Preparation of alcohol dispersed PS-PSMA nanofibers

The schematic of the electrospinning set-up is shown in Figure 1. A polymer solution was prepared at room temperature by dissolving suitable amounts of PS ($M_w = 860,000$ amu) and PSMA ($M_w = 224,000$ amu, maleic anhydride content = 7 wt-%) with a 2:1 weight ratio in a mixture of tetrahydrofuran and acetone (7:3 ratio by volume). The total polymer concentration in the solution was 15 wt-%. The polymer solution was then loaded in a glass pipette with a tip diameter of about 200 ~ 400 $\mu$m. An inert electrode (nickel-chromium wire) was placed into the glass pipette loaded with the polymer solution and a positive bias of ~10 kV was applied to the solution using a high voltage supply (ES30P-10W Gamma High Voltage). The electrospun PS-PSMA fibers were collected on a grounded electrode (aluminum foil or glass slides) placed ~7 cm from the glass pipette tip. The nanofibers were then manually peeled off, weighed, and washed with distilled water. Dispersion of the PS-PSMA nanofibers in water is carried out by the immersion of the nanofiber mat in an aqueous alcohol solution (water: alcohol = 4:1 by volume) followed by gentle shaking for ~ 7 hours. The PS-PSMA nanofibers disperse fully in the aqueous alcohol solution. The nanofibers are then repeatedly washed without drying with a copious amount of distilled water until alcohol is thoroughly removed from the solution phase. The dispersed PS-PSMA nanofibers were always kept in water or buffer solution until they were used for glucose oxidase immobilization.

8.2.3 Glucose oxidase (GOX) immobilization on PS-PSMA nanofibers
The glucose oxidase solution used for immobilization was prepared as follows. 0.8 g of glucose oxidase (GOX, *Aspergillus Niger*) was dissolved in 80 ml of a 200 mM sodium phosphate buffer (pH 7). For the immobilization of GOX on PS-PSMA nanofibers by covalent attachment (which will be called CA-GOX from now on), suitable amounts of the alcohol dispersed PS-PSMA nanofibers (~ 10 - 15 mg) were incubated in 20 ml of the glucose oxidase solution at 200 rpm at room temperature for 30 minutes followed by overnight incubation at 4°C. For the enzyme coating of GOX on PS-PSMA nanofibers by crosslinking the amine moieties of the enzyme (which will be called EC-GA-GOX from now on), suitable amounts of the alcohol dispersed PS-PSMA nanofibers were incubated in 20 ml of the glucose oxidase solution at 200 rpm at room temperature for 30 minutes followed by incubation at 4°C for 2 hours. After 2 hours of incubation, the GA solution (0.5 % of the total solution) is added to the mixture followed by incubation at 4°C for ~ 12 hours. For the enzyme coating of GOX on PS-PSMA nanofibers by crosslinking the carbohydrate moieties of the enzyme (which will be called EC-DAO-GOX from now on), suitable amounts of the alcohol dispersed PS-PSMA nanofibers were added to 20 ml of the glucose oxidase solution. NaIO₄ (~ 100 mg) was added to this solution and the solution was shaken in the dark at 200 rpm at room temperature for 30 minutes followed by incubation at 4°C for 12 hours. Care must be taken to protect this solution from the light. After 12 hours of incubation, the diamino-octane (DAO, 0.5 % of the total solution) is directly added to the mixture followed by overnight incubation at 4°C. In all cases, the nanofibers were transferred to a new glass vials after immobilization and washed with 20 mM phosphate buffer (pH 6.5) and 100 mM Tris-HCl buffer (pH
The Tris-HCl buffer is used to cap the unreacted maleic anhydride groups. After capping, the nanofibers were extensively washed in 20 mM phosphate buffer (pH 6.5) roughly ~ 10 times. The amount of GOX immobilized on the surface of the PS-PSMA nanofibers was determined by measuring the initial and final concentrations of the enzyme within the incubation solution and washing solutions at 280 nm with a UV-Vis spectrophotometer. Bovine serum albumin (BSA) was used as the standard to construct the calibration curve.

8.2.4 Activity assay of the free and immobilized GOX

The GOX activity was estimated by measuring the rate of hydrogen peroxide \( \text{H}_2\text{O}_2 \) generated during the oxidation of \( \beta \)-D-glucose in 200 mM sodium phosphate buffer (pH 7). The rate of hydrogen peroxide was estimated by the standard o-dianisidine-horseradish peroxidase protocol. The CA-GOX, EC-GA-GOX and EC-DAO-GOX was transferred to new vials and 20 ml of 200 mM sodium phosphate buffer (pH 7) containing \( \beta \)-D-glucose at concentrations varying from 1 ~ 20 mM was added to initiate the reaction. The vials were then shaken at 200 rpm at 35°C and small aliquots were removed every 5 minutes. For free GOX activity measurement, 0.2 ml of free GOX solution (~ 0.15 - 0.26 mg/ml) was used instead of the GOX-immobilized PS-PSMA nanofibers. The vials were then shaken at 200 rpm at 35°C and small aliquots were removed every 5 minutes. To measure the amount of hydrogen peroxide generated, the aliquot was added to a mixture of sodium phosphate buffer (pH 7) containing o-dianisidine dihydrochloride (ODS). A small amount of horseradish peroxide (HRP) was
added to catalyze the reaction and the reaction was allowed to proceed for ~ 20 minutes. The reaction was terminated by the addition of a small amount of 50 % sulfuric acid. The concentration of the enzyme reaction product, which is the oxidized form of the o-dianisidine dihydrochloride, was calculated from the absorbance at 525 nm. The calibration curve was constructed by measuring the amount of o-dianisidine dihydrochloride that was oxidized (calculated from the absorbance at 525 nm) by using known quantities of H₂O₂. One unit of the enzyme activity was defined as the amount of enzyme which catalyzed the production of 1 µmol of H₂O₂ per minute. The measured activity was analyzed with the Michaelis-Menten method to get the maximum activity per unit mass of nanofiber (Rₘₐₓ) and the substrate binding constant (Kₘ).

8.2.5 Reduction of the Schiff base

To facilitate the reduction of the Schiff base (-C=N-) formed between the crosslinking agent and the amino or carbohydrate moieties on the enzyme surface, the EC-GA-GOX and the EC-DAO-GOX nanofibers are immersed in a solution of 200 mM sodium phosphate buffer (pH 7) containing 20 mM of sodium cyanoborohydride (NaBH₃CN) for ~ 2 hours. The reduced form of the EC-GA-GOX and the EC-DAO-GOX nanofibers (treated with NaBH₃CN) are denoted as EC-GA-GOX-R and EC-DAO-GOX-R, respectively. The nanofibers are then washed with copious amounts of 200 mM sodium phosphate buffer (pH 7).

8.2.6 Stability of GOX immobilized nanofibers in HOCl/ClO⁻ solution
Clorox (6 wt-% NaOCl) was used as the source of the hypochlorite anion. The HOCl/\text{OCl}^-\text{ equilibrium is as follows:} \text{HOCl} + \text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^+ + \text{OCl}^- (K_a = 3.8 \times 10^{-8}) \]

Suitable amounts of Clorox were added to the sodium phosphate buffer (pH 7) so as to yield 0.1 and 10 mM [NaOCl]. The CA-GOX, EC-GA-GOX, EC-GA-GOX-R, EC-DAO-GOX and EC-DAO-GOX-R nanofibers were tested for their stability in 0.1 and 1 mM [NaOCl] in sodium phosphate buffer (pH 7). After measuring the initial activity, the nanofibers were immersed in the 0.1 and 10 mM solution for ~ 15 minutes under rigorous shaking conditions (250 rpm). This was followed by washing in copious amounts of sodium phosphate buffer to remove the trace amounts of Clorox on the nanofibers. Activity of the Clorox treated nanofibers was measured by using the same procedure mentioned above.

8.3 Results and Discussion

8.3.1 Fabrication of alcohol dispersed PS-PSMA nanofibers

PS-PSMA was chosen as the support for enzyme immobilization due to several reasons. The PSMA copolymer contains a functional group of maleic anhydride (MA) that can readily form covalent bonds with primary amines on the enzyme molecules. PS provides flexibility to the matrix. For electrospinning of the PS-PSMA fibers, THF and acetone were used as the solvents to synthesize the fibers due to its high vapor pressure, high volatility and tendency to generate high pore densities. However, the hydrophobic nature of the as-spun PS-PSMA nanofibers makes the non-woven mat to form a tightly aggregated clump in aqueous solution, thus reducing the surface area available for the
immobilization of enzymes (Figure 7.2 a, c in Chapter 7). When the PS-PSMA nanofibers are tightly aggregated together, only the maleic anhydride groups at the outer region of the aggregate will be available for the enzyme immobilization. After the treatment with aqueous alcohol solution, the PS-PSMA nanofibers are completely dispersed in water (Figure 7.2 b, d in Chapter 7). The alcohol dispersion of the PS-PSMA nanofibers has several advantages. Firstly, the dispersion is maintained even after repeated rinsing with water for several days. Secondly, the expanded nanofiber mat does not break into pieces even after vigorous shaking since the nanofibers are physically entangled. Thirdly, the enzyme loading on alcohol dispersed PS-PSMA nanofibers has been found to be ~ 8 times higher than as-spun PS-PSMA nanofiber due to an increased effective surface area available for enzyme immobilization. Upon alcohol-induced dispersion of the PS-PSMA nanofibers, more surface area of the nanofibers are exposed to the solution and more maleic anhydride groups on the nanofiber surface will be used for enzyme immobilization. The alcohol dispersion does not affect the size or the functional groups on the polymer fibers (Figure 7.3 in Chapter 7).

8.3.2 Stability of GOX immobilized nanofibers in HOCl/ClO\textsuperscript{-} solution

HOX (where X = Cl, Br, I) is an important species that needs to be continuously produced in robust decontamination enzyme based systems. However, for the successful operation of these systems it is required that the enzymes retain their activity in the presence of the HOX species. If the enzymes are stable in HOCl/OCl, then they would be stable in HOBr and HOI since these are weaker agents than HOCl/OCl. Hence it is
necessary to check the stability of the GOX immobilized nanofibers in HOCl/OCl. It has been observed that 0.2 mM of HOCl/OCl is sufficient to completely deactivate free GOX in solution. For this reason it was decided to test the stability of the GOX immobilized nanofibers in two different concentrations of HOCl/OCl – 0.1 mM and 1 mM.

The GOX was immobilized onto the PS-PSMA nanofibers in two different ways: (1) covalent attachment of GOX onto PS-PSMA nanofibers (CA-GOX) and (2) covalent attachment of GOX onto PS-PSMA nanofibers followed by crosslinking with glutaraldehyde to form enzyme aggregate coatings (EC-GA-GOX). The activity of the CA-GOX and EC-GA-GOX nanofibers was investigated in the batch mode by the oxidation of ~ 1 - 20 mM β-D glucose solution under rigorous shaking conditions (~ 250 rpm). The maximum activity per unit mass of nanofiber ($R_{max}$) and substrate binding constant ($K_m$) are obtained from the Michaelis-Menten analysis of the kinetic data. The $R_{max}$ value of the CA-GOX is 0.254 U per mg nanofiber, while that of the EC-GA-GOX is 0.77 U per mg nanofiber. The higher $R_{max}$ of the EC-GA-GOX is attributed to the higher GOX loading per unit mass of the nanofibers due to crosslinking of additional GOX molecules from solution.

After immersion in 0.1 mM HOCl/OCl solution for 1 minute under rigorous shaking conditions (~250 rpm), the $R_{max}$ of the CA-GOX and EC-GA-GOX was measured to be 0.226 U per mg nanofiber and 0.671 U per mg nanofiber, respectively. After immersion in 1 mM HOCl/OCl solution for 1 minute, the $R_{max}$ of the CA-GOX and EC-GA-GOX decreased further to 0.118 U per mg nanofiber and 0.190 U per mg nanofiber, respectively.
For the carbohydrate cross linking of GOX, first the carbohydrate groups at the surface have to be oxidized so that the cis-diol groups can be converted into aldehyde groups (Figure 8.1). This was done by adding NaIO₄ to the GOX solution. Next, the aldehyde groups are crosslinked by using a bifunctional agent, such as diamino-octane, thereby forming a Schiff base. The Schiff base is then reduced by the action of NaBH₃CN. This system will be referred to as EC-DAO-GOX-R from here onwards. The initial activity of the EC-DAO-GOX-R was approximately equal to the initial activity of the EC-GA-GOX-R. The relative activities of the EC-GA-GOX-R and EC-DAO-GOX-R on exposure to 1 mM HOCl/OCl for 5 minutes are compared in Figure 8.6. It can be seen that the activity of the EC-DAO-GOX-R reduces by roughly ~ 70 % of its initial activity. This is speculated since the reduced form of the Schiff base is also susceptible to HOCl/OCl attack as seen earlier.

To verify whether the reduction of the EC-GA-GOX activity was exposure time dependent, the activity was measured as a function of time of immersion in HOCl/OCl. For a given HOCl/OCl concentration, after each time of immersion, the residual activity was measured and divided by the initial activity to give the relative activity. The relative activity of the EC-GA-GOX is plotted with the time of immersion in 0.1 an 1 mM HOCl/OCl in Figure 8.2. It can be seen that in 0.1 mM HOCl/OCl, the activity of the EC-GA-GOX does not decrease significantly over a period of 1 hr of immersion time. At 1 mM HOCl/OCl, the activity of the EC-GA-GOX reduces to ~ 59 % of its initial activity in 1 minute of immersion time and then remains unchanged (within the limits of the experimental error) for over 1 hr of immersion time.
Figure 8.1: Schematic representation of carbohydrate crosslinking of glucose oxidase. (E = glucose oxidase enzyme molecule)
Figure 8.2: Change in activity of EC-GA-GOX with time of exposure to (a) 0.1 mM HOCl/OCl and (b) 1 mM HOCl/OCl.

The effect of HOCl/OCl exposure on the substrate binding constant, $K_m$, was also investigated and is shown in Figure 8.3. The $K_m$ of free GOX in solution was measured to be 14.9 mM which is consistent with the values observed in literature (18 - 26 mM).\(^{20}\) The $K_m$ of CA-GOX and EC-GA-GOX was found to be 3.48 and 3.89 mM, respectively. The $K_m$ of GOX has been found to decrease on immobilization to solid substrates.\(^{21}\) On exposure to 0.1 mM HOCl/OCl, the $K_m$ of the CA-GOX and the EC-GA-GOX decreases to 2.99 and 3.54, respectively. On exposure to 1 mM HOCl/OCl, the $K_m$ of the CA-GOX
and the EC-GA-GOX further decreases to 2.39 and 2.64, respectively. These values seem to be within the experimental error range.

![Graph showing the change in substrate binding constant ($K_m$) of (a) CA-GOX and (b) EC-GA-GOX) with exposure to different concentrations of HOCl/OCI solution.]

**Figure 8.3:** Change in the substrate binding constant ($K_m$) of (a) CA-GOX and (b) EC-GA-GOX) with exposure to different concentrations of HOCl/OCI solution.

The decrease in the activity of the CA-GOX (~ 50%) and EC-GA-GOX (~ 75%) can be speculated to be due to the interaction of the HOCl/OCI with the protein structure.\textsuperscript{15} HOCl/OCI is known to cause the ring chlorination of tyrosine and phenylalanine residues.\textsuperscript{16} However, this is not expected to cause huge changes to the enzyme structure so as to alter its activity, thus ruling out this possibility. HOCl/OCI can
cause coupling of the thiol groups in the cysteine residues to form a disulfide bridge which can potentially distort the enzyme structure.\(^{17}\) If this was the case, then the GOX activity should be reduced in solutions containing H\(_2\)O\(_2\) also, since H\(_2\)O\(_2\) can also cause oxidation of the sulfur groups. However, GOX retains its activity in solutions containing H\(_2\)O\(_2\) at moderate concentrations. At high H\(_2\)O\(_2\) concentrations a competitive inhibition is observed resulting in decreased GOX activity.\(^{18}\) Hence it can be concluded that the GOX is resistant to the sulfur group oxidation. Hence, the initial drop in the CA-GOX and possibly, the EC-GA-GOX activity upon exposure to 1 mM HOCl/OCl solution should be due to the oxidation of un-reacted or weakly protected amine groups. HOCl/OCl has been found to react with amine groups of lysine, histidine and typtophan and convert them to semi-stable chloramines, which then undergo hydrolysis to aldehydes.\(^{19}\)

In the case of EC-GOX, there seems to be an additional mechanism responsible for 75% decrease in activity after 1 mM HOCl/OCl\(^{-}\) treatment. The additional decrease in the activity of EC-GA-GOX can be explained as follows. As seen in Figure 6.1 (in Chapter 6), the crosslinking of the surface amino groups with glutaraldehyde results in the formation of the so called Schiff base \((-\text{CH}=\text{N}-)\). The Schiff base might not be stable to high concentrations of HOCl/OCl thus leading to detachment of enzymes from the surface and resulting in lower activities.

**8.3.3 Stability of enzyme aggregate coatings in HOCl/OCl after Schiff base reduction**
In order to prevent the de-polymerization of the glutaraldehyde molecules due to the HOCl/OCl attack on the Schiff base, mild reduction of the Schiff base was attempted. The Schiff base can be reduced by reaction with NaBH₄ or NaBH₃CN as shown in Figure 8.4. The EC-GA-GOX was treated with 20 mM NaBH₃CN for 2 hours to cause mild reduction of the Schiff base and will be referred to as EC-GA-GOX-R. Before and after the reduction with NaBH₃CN, no significant difference in the activities was observed. The relative activities of the EC-GA-GOX and EC-GA-GOX-R on exposure to 0.1 and 1 mM HOCl/OCl for 5 minutes are compared in Figure 8.5. The activity of the EC-GA-GOX-R also reduces by roughly 75% of its initial activity. Hence, it can be concluded that there is no marginal improvement in activity by causing the mild reduction of the Schiff base.

**Figure 8.4:** Schematic representation of reduction of crosslinked enzyme by treatment with NaBH₃CN. During the crosslinking the surface amino groups react with the glutaraldehyde to form the Schiff base (-CH=N-). Treatment with NaBH₃CN reduces the Schiff base to (-CH-NH-). (E = enzyme molecule)
8.4 Conclusions
The stability of enzyme aggregates of glucose oxidase fabricated onto alcohol dispersed PS-PSMA nanofibers were tested in high concentrations of hypochlorous acid (HOCl ~ 0.1 - 1mM). The enzyme aggregates were fabricated by the covalent attachment of glucose oxidase onto PS-PSMA nanofibers followed by crosslinking using glutaraldehyde. Although, the enzyme aggregates did not lose any significant activity at HOCl/OCl concentrations of ~ 0.1 mM, they exhibited a ~ 60 - 75 % loss of activity in HOCl/OCl concentrations of ~ 1 mM. To improve the stability, mild reduction of the Schiff base using sodium cyanoborohydride was also attempted. Even after reduction, the enzyme aggregates exhibited a ~ 60 - 70 % loss of activity. Activity of enzyme aggregates fabricated by crosslinking the carbohydrate moieties with diamino-octane were tested in HOCl/OCl. These aggregates also seemed to lose ~ 75% of their activity in high concentrations of HOCl/OCl.

8.5 References


CHAPTER 9

Fabrication of enzyme aggregates of glucose oxidase onto nanofibers for continuous flow reactor operation – effect of dissolved oxygen and the maximization of hydrogen peroxide production.

9.1 Background

Currently a large number of decontamination solutions are based on reactive chemicals produced by hydrogen peroxide stock solution. However, this imposes several issues such as storage and safety. They degrade and lose their efficiency over time. One alternative of solving this problem is to develop enzyme based decontamination systems where the hydrogen peroxide species is continuously produced. The enzyme based systems are non-toxic, non-corrosive and environmentally benign. Glucose oxidase is well known to catalyze the oxidation of glucose to produce glucono-lactone and hydrogen peroxide according to the following reaction.3, 13

\[
\beta\text{-D glucose} + O_2 \rightarrow \text{GOX} \rightarrow \text{Glucono-lactone} + H_2O_2
\]

When glucose is oxidized to glucono-lactone, the flavin adenine dinucleotide (FAD) cofactor tightly bound to the GOX is reduced to FADH₂ and re-oxidized back to FAD by reducing O₂ to H₂O₂. This reaction is very fast and is known to occur in a wide variety of pH from 4-8. Immobilization of such enzymes onto nanostructures provides a high loading and volumetric activity and the convenience of recycle.4,5 For the successful fabrication of these enzyme based decontamination system maximum production of hydrogen peroxide species should be achieved.
In this chapter, the possibility of maximizing the hydrogen peroxide production by using a small amount enzyme immobilized nanofibers is discussed. The enzyme immobilized system is fabricated by forming enzyme aggregates of glucose oxidase onto polystyrene-poly (styrene co-maleic-anhydride) (PS-PSMA) nanofibers. This is used to fabricate a continuous flow reactor for maximum production of hydrogen peroxide. The β-D glucose solution was flown through the continuous flow reactor loaded with the GOX immobilized PS-PSMA nanofibers at three different flow rates namely, 0.83, 1.8 and 3.2 ml/min. The concentration of the hydrogen peroxide species produced increased as the flow rate was lowered due to increased residence time within the reactor. One challenge in achieving high rate of production of hydrogen peroxide is the low concentration of oxygen dissolved in the substrate solution (0.2 mM). The amount of oxygen dissolved in the solution can limit the formation of H$_2$O$_2$. One way to avoid this problem is by sparging the flow reactor with O$_2$. Another way is to saturate the substrate solution with oxygen before being passed into the continuous flow reactor. The concentration of hydrogen peroxide produced in this way has been observed to increase non-linearly with the increasing oxygen concentration. The maximum amount of H$_2$O$_2$ has been analyzed by making use of the available kinetic data. Another way of achieving a high concentration of hydrogen peroxide is by recycling the product solution back into the continuous flow reactor after saturating it with oxygen. The concentration of hydrogen peroxide produced in this way increased for all concentrations studied.
9.2 Experimental Details

9.2.1 Materials

Glucose Oxidase (GOX, *Aspergillus Niger*), o-dianisidine dihydrochloride (ODS), horseradish peroxidase (HRP, 25,000 KU), glutaraldehyde (GA), polystyrene (PS, $M_w = 860,000$) and poly (styrene-co-maleic anhydride) (PSMA, $M_w = 224,000$; maleic anhydride content = 7wt-%) were obtained from Sigma-Aldrich and used without further purification. Tetrahydrofuran (THF, HPLC grade, 99.9%) and acetone were purchased from Burdick and Jackson.

9.2.2 Preparation of alcohol dispersed PS-PSMA nanofibers

A polymer solution was prepared at room temperature by dissolving suitable amounts of PS ($M_w = 860,000$ amu) and PSMA ($M_w = 224,000$ amu, maleic anhydride content = 7 wt-%) with a 2:1 weight ratio in a mixture of tetrahydrofuran and acetone (7:3 ratio by volume). The total polymer concentration in the solution was 15 wt-%. The polymer solution was then loaded in a glass pipette with a tip diameter of about 200 ~ 400 µm. An inert electrode (nickel-chromium wire) was placed into the glass pipette loaded with the polymer solution and a positive bias of ~10 kV was applied to the solution using a high voltage supply (ES30P-10W Gamma High Voltage). The electrospun PS-PSMA fibers were collected on a grounded electrode (aluminum foil or glass slides) placed ~7 cm from the glass pipette tip. The nanofibers were then manually peeled off, weighed, and washed with distilled water. Dispersion of the PS-PSMA nanofibers in water is carried out by the immersion of the nanofiber mat in an aqueous
alcohol solution (water: alcohol = 4:1 by volume) followed by gentle shaking for ~ 7 hours. The PS-PSMA nanofibers disperse fully in the aqueous alcohol solution. The nanofibers are then repeatedly washed without drying with a copious amount of distilled water until alcohol is thoroughly removed from the solution phase. The dispersed PS-PSMA nanofibers were always kept in water or buffer solution until they were used for glucose oxidase immobilization.

9.2.3 Glucose oxidase (GOX) immobilization on PS-PSMA nanofibers

The glucose oxidase solution used for immobilization was prepared as follows. 0.8 g of glucose oxidase (GOX, Aspergillus Niger) was dissolved in 80 ml of a 200 mM sodium phosphate buffer (pH 7). The GOX was immobilized onto the alcohol dispersed PS-PSMA nanofibers by a two step process. First the enzymes were covalently attached onto the PS-PSMA nanofibers. These covalently attached enzymes acted as seed molecules. This was followed by intra and intermolecular crosslinking of the enzymes by adding a bifunctional agent. In a typical procedure, suitable amounts of the alcohol dispersed PS-PSMA nanofibers (~ 100 mg) were incubated in 80 ml of the glucose oxidase solution at 200 rpm at room temperature for 30 minutes followed by incubation at 4°C for 2 hours. After 2 hours of incubation, the GA solution (0.5 % of the total solution) is added to the mixture followed by incubation at 4°C for ~ 12 hours. The nanofibers were then transferred to a new glass vials after immobilization and washed with 20 mM phosphate buffer (pH 6.5) and 100 mM Tris-HCl buffer (pH 6.5). The Tris-HCl buffer is used to cap the un-reacted maleic anhydride groups. After capping, the
nanofibers were extensively washed in 20 mM phosphate buffer (pH 6.5) roughly ~ 10 times.

**9.2.4 Construction of continuous flow reactors**

For the construction of continuous flow reactors, the GOX enzyme coated PS-PSMA nanofibers were then loaded into 3 ml plastic syringes. The syringes were backfilled with 200 mM sodium phosphate buffer (pH – 7) and shaken multiple times so as to let the PS-PSMA fibers expand due to the dispersion effect.

**9.2.5 Activity assay of the immobilized GOX**

The GOX activity was estimated by measuring the rate of hydrogen peroxide (H$_2$O$_2$) generated during the oxidation of β D-glucose in 200 mM sodium phosphate buffer (pH 7). In order to estimate the dependence of H$_2$O$_2$ generated on the dissolved oxygen concentration, it was desired to saturate the substrate solution with O$_2$. For this purpose O$_2$ was bubbled into the substrate solution and the final O$_2$ concentration was measured using an O$_2$ sensor. 20 ml of 200 mM sodium phosphate buffer (pH 7) containing β D-glucose at concentrations varying from 1 ~ 20 mM and saturated with O$_2$ was continuously flowed through the tube reactors using a peristaltic pump. Three different flow rates were tested: 0.83, 1.8, and 3.1 ml/min. The concentration of the H$_2$O$_2$ in the eluted solution was estimated by the standard o-dianisidine-horseradish peroxidase protocol. Specifically, to measure the amount of hydrogen peroxide generated the aliquot was added to a mixture of sodium phosphate buffer (pH 7) containing o-dianisidine
dihydrochloride (ODS). A small amount of horseradish peroxide (HRP) was added to
catalyze the reaction and the reaction was allowed to proceed for ~ 20 minutes. The
reaction was terminated by the addition of a small amount of 50 % sulfuric acid. The
concentration of the enzyme reaction product, which is the oxidized form of the o-
dianisidine dihydrochloride, was calculated from the absorbance at 525 nm. The
calibration curve was constructed by measuring the amount of o-dianisidine
dihydrochloride that was oxidized (calculated from the absorbance at 525 nm) by using
known quantities of H₂O₂. One unit of the enzyme activity was defined as the amount of
enzyme which catalyzed the production of 1 µmol of H₂O₂ per minute. The measured
activity was analyzed with the Michaelis-Menten method to get the maximum activity per
unit mass of nanofiber (Rₘₐₓ) and the substrate binding constant (Kₘ).

9.3 Results and Discussion

PS-PSMA was chosen as the support for enzyme immobilization because the
PSMA copolymer contains a functional group of maleic anhydride (MA) that can readily
form covalent bonds with primary amines on the enzyme molecules. The PS-PSMA
nanofibers were fabricated by electrospinning and treated with an alcohol solution so as
to achieve better dispersion in solution. The GOX is immobilized onto the alcohol
dispersed PS-PSMA nanofibers by the enzyme coating method. More information about
the discussion regarding the fabrication of the alcohol dispersed PS-PSMA nanofibers
and the enzyme coating process can be found in chapters 6, 7 and 8. The GOX
immobilized PS-PSMA nanofibers were used to construct a continuous flow reactor. The
advantage of a continuous flow reactor is that the product is not mixed with the unconverted substrate at any point along the length of the reactor; and hence a higher yield can be obtained as compared to a batch reactor or a continuous stirred tank reactor.

**Figure 9.1:** Concentration profile of hydrogen peroxide as a function of flow rate in the continuous flow reactor using glucose concentrations of (a) 1 mM (b) 5 mM and (c) 20 mM

The concentration profile of $\text{H}_2\text{O}_2$ ($X^{\text{ox}}$) in the continuous flow reactors is shown as a function of flow rate in Figure 9.1. The decrease of $\text{H}_2\text{O}_2$ concentrations generated at the higher flow rates is mainly due to the decrease of the residence time ($\tau = \frac{V}{F}$) in the reactor. The concentration of $\text{O}_2$ dissolved in the as-prepared $\beta$-D glucose solutions was
measured to be ~ 0.2 mM which is roughly equal to the equilibrium concentration of O₂ dissolved in water exposed to air. Hence, the O₂ could be the limiting factor for the generation of H₂O₂. In order to see the effect of O₂ concentration on the concentration of H₂O₂ produced, it was desired to use β-D glucose solutions saturated with O₂. O₂ was bubbled into the β-D glucose solutions and the final O₂ concentration was measured to be 1 mM. When β-D glucose solution with a dissolved O₂ concentration of 1 mM was used, the concentration of H₂O₂ produced was higher by more than ~ 50%. The concentration of H₂O₂ produced in the continuous flow reactor by flowing solutions saturated with O₂ (~ 1 mM) is compared with concentration of H₂O₂ produced by flowing solutions with an O₂ concentration of 0.2 mM in Figure 9.2. The concentration of H₂O₂ produced is ~ 50 % higher, although the concentration of O₂ is larger by 5 times. To explain this we make use of the kinetics of the glucose oxidase which is well established.¹⁴

\[
E_{\text{ox}} + \text{Glu cos e} \xrightarrow{K_1} E_{\text{red}} P_1 \xrightarrow{K_2} E_{\text{red}} + P_1
\]
\[
E_{\text{red}} + O_2 \xrightarrow{K_3} E_{\text{ox}} P_2 \xrightarrow{K_4} E_{\text{ox}} + P_2
\]

Here \( E_{\text{ox}} \) and \( E_{\text{red}} \) are the oxidized and reduced forms of the enzyme, respectively; \( K_1, K_2, K_3 \) and \( K_4 \) are the rate constants; and \( P_1 \) and \( P_2 \) are the products. The rate equation arising from the above mentioned scheme is:

\[
\frac{1}{v} = \frac{K_2 + K_4}{K_2 K_4} + \frac{1}{K_1 \text{[Glu cos e]}} + \frac{1}{K_3 \text{[O}_2\text{]}}
\]

(Where \( v \) = velocity of the reaction;

\([\text{Glucose}]\) = concentration of glucose and \([\text{O}_2\]) = concentration of oxygen)

The H₂O₂ generation at the exit of the continuous flow reactor can be modeled with the following design equation:

\[
Q \frac{\partial C}{\partial V} = -v (\text{where } Q = \text{volumetric flow rate, } dV =)
\]
differential volume and $v =$ rate of reaction as given by the above equation). Using the concentrations of H$_2$O$_2$ obtained experimentally at O$_2$ concentration = 0.2 mM, the constants $K_1$ and $K_3$ could be estimated to be $109 \text{ M}^{-1}\text{min}^{-1}$ and $1766 \text{ M}^{-1}\text{min}^{-1}$ respectively. The first constant in the equation $\frac{K_2 + K_4}{K_2 K_4}$ was also estimated as a grouped constant to yield a value of $2.95 \text{ min}$. These values are less than that for the free enzyme. A comparison between the constants of the free enzyme and the immobilized enzyme are given in Table 9.1. The specific rate constant values for immobilized enzyme systems have been observed to be lower than that of the free enzymes. The theoretical $K_m$ value for the immobilized enzyme is calculated to be 3.1 mM. This value is close to the value obtained experimentally. The H$_2$O$_2$ concentration generated at O$_2$ concentrations of 1 mM was simulated and is shown as the dotted line in Figure 9.2 (b). The simulation agrees well with the experimental data obtained (Details about the simulation can be obtained in Appendix E). From this it can be concluded that the H$_2$O$_2$ generation in the

<table>
<thead>
<tr>
<th></th>
<th>$\frac{K_2 + K_4}{K_2 K_4}$ (sec)</th>
<th>$K_1$ (M$^{-1}$sec$^{-1}$)</th>
<th>$K_3$ (M$^{-1}$sec$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free GOX$^{14}$</td>
<td>235</td>
<td>10,000</td>
<td>$1.7 \times 10^6$</td>
</tr>
<tr>
<td>Immobilized GOX</td>
<td>177</td>
<td>1.81</td>
<td>29.43</td>
</tr>
</tbody>
</table>

Table 9.1: Comparison between constants of free enzyme$^{14}$ and immobilized enzyme
continuous flow reactor is not linearly dependent on the \( \text{O}_2 \) concentration and is controlled by the kinetics.

**Figure 9.2:** Concentrations profiles of hydrogen peroxide generated in the continuous flow reactor as a function of different glucose concentrations (a) with oxygen concentration = 0.2 mM (b) and oxygen concentration = 1 mM. The points are the experimental values and the solid lines are the simulated values. The simulation is done for 60 different glucose concentrations. The flow rate in both cases is kept constant at 1.8 ml/min.
For the fabrication of robust enzyme based deactivation systems it is required to obtain the highest H$_2$O$_2$ concentration. One way of doing this is to use a continuous flow reactor with recycle as shown in Figure 9.3.

\[ \text{Glucose solution} \]

\[ \text{Oxygen} \]

\[ \text{Enzyme immobilized} \]

\[ \text{Dispersed nanofibers} \]

\[ \text{Glucose/H}_2\text{O}_2 \]

\[ \text{Recycled solution} \]

\[ \text{Glucose/H}_2\text{O}_2 \]

\[ \text{Oxygen} \]

**Figure 9.3:** Schematic of a continuous flow reactor with recycle.
In this case the product stream is fed back into the continuous flow reactor by using the peristaltic pump. The stream going into the continuous flow reactor is bubbled to adjust the dissolved oxygen concentration to 1 mM. It can be speculated that the maximum H$_2$O$_2$ concentration will be achieved by using a high glucose concentration saturated with oxygen and a low flow-rate. The H$_2$O$_2$ concentration obtained by using oxygen saturated solutions of glucose with concentrations 5, 10 and 20 mM are shown as a function of the number of recycles into the continuous flow reactor in Figure 9.4. In all the cases, the initial O$_2$ concentration was fixed at 1 mM. The maximum H$_2$O$_2$ concentration achieved was 2.3 mM using a glucose concentration of 20 mM (O$_2$ saturated) and recycled 6 times. For all concentrations, the concentration of H$_2$O$_2$ produced increased non-linearly. There is no significant increase in the H$_2$O$_2$ concentration after 6 passes. It could be speculated that the GOX is deactivated by the H$_2$O$_2$. However, the activity of the free enzyme in solution has been found to decrease significantly by over ~ 40 % only at high concentrations of H$_2$O$_2$ such as 10-20 mM. In cases where the O$_2$ supply into the solution is maintained, the activity decrease of free GOX is much lesser ~ 20 %. For immobilized enzymes this inactivation will be insignificant. In our case, since the amount of H$_2$O$_2$ is as low as 2 mM, the GOX might not be denatured by the H$_2$O$_2$. It could be speculated that the saturation of the H$_2$O$_2$ production at high concentrations of H$_2$O$_2$ could be due to the competitive inhibition of GOX by hydrogen peroxide. H$_2$O$_2$ is known to exert a competitive inhibitory effect on GOX by the following mechanism.
\[ FAD + G \xrightleftharpoons[K_{-1}]{K_1} FAD.G \xrightarrow{K_2} FAD.H_2 + \text{Gluconolactone} \]
\[ FAD.H_2 + O_2 \xrightleftharpoons[K_{-3}]{K_3} FAD.H_2O_2 \xrightarrow{K_4} FAD + H_2O_2 \]
\[ FAD.H_2 + H_2O_2 \xrightleftharpoons[K_{-4}]{K_4} FADH_2.H_2O_2 \]

In the above set of equations, \( FAD \) is the oxidized form of the enzyme, \( FAD.H_2 \) is the reduced form of the enzyme and \( FADH_2.H_2O_2 \) is the dead-end complex. The competitive inhibitory effect will depend on the concentration of \( H_2O_2 \) present. As the \( H_2O_2 \) concentration increases, the \( O_2 \) uptake during the reaction is considerably reduced for a given amount of time. In solution, the \( O_2 \) uptake has found increase very slowly and decay over long time frames in the presence of \( H_2O_2 \) concentrations of 0.15 mM. The consumption of \( O_2 \) can be modeled by the following equation:

\[
-\frac{\partial C_O}{\partial t} = \frac{f(C_{\text{Glucose}}, C_O)}{C_O + K_{M,O}} \xrightarrow{t = 0; C_{H_2O_2} = 0} t > 0; C_{H_2O_2} = f(t, C_{\text{Glucose}}, C_O) \\
-\frac{\partial C_O}{\partial t} = \frac{f(C_{\text{Glucose}}, C_O)}{C_O + K_{M,O}(1 + \frac{C_{H_2O_2}}{K_i})} \xrightarrow{t > 0; C_{H_2O_2} = f(t, C_{\text{Glucose}}, C_O)}
\]

Here, \( C_O = O_2 \) concentration; \( C_{\text{Glucose}} = \) Glucose concentration; \( C_{H_2O_2} = H_2O_2 \) concentration; \( K_i = \) Inhibition constant. From the above equation it can be seen that as the concentration of \( H_2O_2 \) increases, the rate of uptake of oxygen will decrease. In the continuous flow reactor, the \( H_2O_2 \) concentration increases which in turn decreases the \( O_2 \) uptake, thereby resulting in no more formation of \( H_2O_2 \).
Figure 9.4: Concentration of hydrogen peroxide generated as a function of number of recycles in the continuous flow reactor using glucose concentrations of (a) 5 mM (b) 10 mM and (c) 20 mM. In all cases the O₂ concentration was fixed at 1 mM at the beginning of each pass. The dotted lines are the guide to the eye.

9.4 Conclusions

A continuous flow reactor consisting of glucose oxidase enzyme aggregate coatings on PS-PSMA nanofibers was constructed and the possibility of obtaining the highest achievable concentration of hydrogen peroxide was investigated. To increase the rate of hydrogen peroxide produced, two avenues were explored: (1) increasing the concentration of oxygen dissolved in the solution and (2) construction of a recycled
continuous flow reactor. The effect of the amount of dissolved oxygen on the rate of
generation of hydrogen peroxide was found to be non-linear and kinetically controlled;
the amount of hydrogen peroxide generated doubled when the oxygen dissolved in
solution was increased five times. An attempt to maximize the rate of hydrogen peroxide
by recycling the product in the continuous flow reactor is also described.

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SYNOPSIS

The central objectives of this thesis were to develop engineering based approaches for the synthesis of functional (conducting) nanofibers and biocatalytic nanofibers, and to exploit the use of biocatalytic nanofibers for enzyme based applications. Specifically, we have attempted the following principal tasks: (1) Continuous extrusion of nanofibers from templates by catalytic polymerization; (2) Synthesis of conducting polymer nanofibers (specifically PPy and PEDOT) by oxidative polymerization - utilizing all the advantages of electrospinning and bypassing the intractability problem of conducting polymers; (3) Synthesis of bio-catalytic PS-PSMA nanofibers immobilized with cross-linked aggregates of enzymes (specifically chymotrypsin, glucose oxidase); (4) Enhancement in the enzyme loading capacity of electrospun fibers by aqueous alcohol dispersion and application of the alcohol dispersed fibers in continuous flow reactors.

The first part of this thesis was directed to the development of nano-manufacturing techniques for the continuous synthesis and extrusion of ordered arrays of polymeric nanofibers from nanoporous templates. The synthesis and extrusion of polyethylene nanofibers by heterogeneous Ziegler-Natta polymerization within nanochannels of robust anodized aluminum oxide membranes was attempted. About 2 ~ 5 µm long polyethylene fibers were extruded out of the nanochannels during the polymerization. The polymer chain packing structure was found to be similar to the high pressure crystal phase, although the polymerization was carried out near atmospheric pressure. The high pressure phase formation inside nanochannels and some degree of
polyethylene nanofiber extrusion from nanochannels were attributed to catalytic production of excess amount of polyethylene inside nanoconfinned templates.

The second part of this thesis was directed to the development of nano-manufacturing techniques for the synthesis of conducting polymer composite nanofibers on electrospun polymer fiber templates. Conducting polymers such as polypyrrole and poly (3, 4-ethylenedioxythiophene) have a great potential in the field of flexible electronics, nano-electronics and bio-sensing. However, despite its superior thermal and environmental stability it suffers from being intractable. Conducting polymers such as polypyrrole is insoluble in almost all solvents - a limitation that prevents it from being processed into useful devices, especially at the nanoscale. Due to the same reason, nanofibers of these polymers cannot be directly fabricated by convenient methods such as electrospinning. To address this issue, electrically conducting composite nanofibers are produced by a two step process. First, electrospinning is used to synthesize template fibers loaded with suitable oxidants. Second, the template fibers are exposed to the monomer vapors which diffuse into the templates and are oxidized to form the conducting polymer. Electrically conducting polypyrrole-polyethylene-oxide (PPy-PEO) nanofibers, polypyrrole shell-polystyrene core (PPy-PS) nanofibers and poly (3, 4-ethylenedioxythiophene) shell –polystyrene core (PEDOT-PS) nanofibers were synthesized in this way. The effect of two different oxidants – ferric chloride and ferric toluenesulfonate, on the polymerization process was also investigated. The nonwoven mat of nanofibers was also exploited for gas sensing applications. To improve the conductivity of the fibers by providing better connectivity, melt welding of the PEDOT-
PS nanofibers was attempted by condensing slight amounts of EDOT onto the nanofibers. The conductivity of the melt-welded PEDOT-PS nanofibers obtained in this way was ~ 1 S/cm.

The third part of this thesis was directed to the fabrication of nanofibers for enzyme based biocatalytic applications. When enzymes are immobilized onto solid supports two parameters must be maximized: (1) the enzyme activity and (2) stability. Fabrication of highly stable enzyme coatings on the nanofibers is described. This approach employs covalent attachment of seed enzymes onto nanofibers consisting of a mixture of polystyrene and poly (styrene-co-maleic anhydride), followed by a glutaraldehyde (GA) treatment that crosslinks additional enzyme molecules and aggregates from solution onto the covalently-attached seed enzyme molecules. As a proof of concept, α-chymotrypsin (CT) was coated onto nanofibers electrospun from a mixture of polystyrene and poly (styrene-co-maleic anhydride). The initial activity of CT-aggregate-coated nanofibers was 9 times higher than nanofibers with just a layer of covalently-attached CT molecules. The enzyme stability of CT-aggregate-coated nanofibers was greatly improved with essentially no measurable loss of activity over a month of observation under rigorous shaking conditions. To improve the enzyme loading onto electrospun nanofibers an alcohol dispersion technique has been formulated. The dispersed nanofibers containing covalently attached enzymes or crosslinked enzyme coatings show loadings as high as ~ 8 times and have been exploited as continuous flow reactors. Although the cross-linked enzyme aggregates fabricated on alcohol dispersed
electrospun fibers are stable in a wide range of pH and temperatures, they lose 75 % of their activity in high concentrations (~ 1 mM) of reactive species such as HOCl.
FUTURE DIRECTIONS

Although we have addressed several problems with respect to the fabrication of functional polymeric nanofibers it can be seen that there are still limitations that need to be overcome for the advancement of these nanomanufacturing techniques from a scientific curiosity to useful materials. There are avenues that need to be explored and questions that need to be addressed for a better understanding of the scientific phenomena accompanying the formation of the nanomaterials that have been developed in the course of the above mentioned research. In this section the questions that need to be answered will be outlined and the future work of this thesis will be briefly proposed.

There are several questions that need to be answered with respect to the synthesis of conducting polymers onto electrospun polymer nanofiber templates. One of the most important questions is regarding the core-shell structure. The XPS studies reveal that most of the conducting polymers are segregated on the outer surface of the composite nanofibers (Chapter 3). Although we have extracted the polypyrrole-polystyrene (PPy-PS) nanofibers with tetrahydrofuran to obtain a tubular structure in the FESEM (Chapter 4), more evidence needs to be provided to show the existence of the core-shell structure. One way to show the core-shell structure would be to do the transmission electron microscopy (TEM) of the composite nanofibers by staining it with suitable agents such as osmium tetroxide. This would enable better contrast between the polyethylene oxide or PS core and PPy or poly (3, 4-ethylene dioxythiophene) (PEDOT) shell. Another way to show the segregation of the PPy or PEDOT conducting polymers on the surface of the oxidant loaded polymer template fibers would be angle resolved XPS measurements. It is
not possible to do the angle resolved XPS measurements with the nanofiber mat because the nanofibers are randomly oriented on the collector substrate with large air spaces between them and non-uniformity. However, it is possible to perform angle resolved XPS measurements on thin films of conducting polymers and do depth profile measurements to verify the segregation of the conducting polymers on the surface. Thin films of the oxidant loaded polymers (PS-FeCl₃, PS-FeTS, PEO-FeCl₃, etc.) can be obtained by spin coating and exposed to the monomer vapors of pyrrole or EDOT for the synthesis of conducting PPy or PEDOT films. Angle resolved measurements with depth profiling techniques could estimate the degree of segregation of the conducting PPy or PEDOT films on the surface of the spun coat films of polymer loaded oxidant.

One of the biggest challenges in the application of conducting polymers for enzyme based sensing is to obtain a high conductivity. In the case of conducting polymers synthesized on insulating electrospun polymer template fibers, the maximum sheet conductivity that we have achieved is 1 S/cm. The low conductivity of the nanofibers could be attributed to several reasons: (1) the presence of large air voids between the fibers and (2) the presence of the insulating polymer core in the composite. The conductivity of the composites can be improved if the insulating polymer core is removed by dissolution of the composite in an organic solvent such tetrahydrofuran or acetone. Although we have attempted the removal of the polystyrene (PS) core layer for the polypyrrole (PPy) based conducting polymer composites and improved its conductivity by an order of magnitude, the removal of the PS from the melt-welded PEDOT-PS nanofibers have yet to be investigated. Also, the removal of the PS core can
be quantified by spectroscopic techniques such as fourier transform infrared spectroscopy (FTIR). The FTIR of the composite fiber mat after dissolution of the core should show only the conducting polymer peaks and the loss of PS peaks. Obtaining the composite nanofibers in a thin free standing film form after dissolution of the core by extraction with the solvent will be the challenging task here.

Another direction worth pursuing is the electrospinning of soluble conducting polymers such as the PEDOT-PSS suspension which is commercially available as Baytron-P. The insolubility of PEDOT was circumvented by the coupling with the polystyrene sulfonate ion. PEDOT-PSS is soluble in water which makes it attractive for being electrospun into nanofibers. The conductivity of spun cast thin films of PEDOT-PSS is roughly known to be \( \sim 10 - 20 \) S/cm. The soluble suspensions of PPy can also be prepared by using suitable counter-anions such as the dodecylbenzene sulfonic acid (DBSA) anion.

In all our works we have reported the conductivity of the nanofiber sheet. The measurement of the conductivity of a single fiber is challenging because the synthesis of a single fiber by electrospinning is very difficult; the spinning speed cannot be controlled to such low rates. Nano-manipulation of wires by atomic force microscopy or related techniques can be used to pick and place the nanowire on pre-patterned electrodes for measuring the single fiber conductivity. However; in this case the contact of the fiber with the electrodes can pose a problem. Conducting polymer nanofibers can be collected on four parallel grounded electrodes in an aligned fashion. If the number density of the fibers on the electrodes can be measured then an estimate of the single fiber conductivity
can be estimated. However, in this technique also, the contact between the nanofibers and the electrodes can pose a problem.

Although enzyme aggregates fabricated by the crosslinking of covalently attached enzyme molecules are highly active and stable over a period of time in a wide range of pH and temperatures, it can be seen from the results of Chapter 8 that they retain only 25% of their original activity in the presence of highly reactive species, such as HOCl. One of the reasons for the loss of enzyme activity could be attributed to the depolymerization of the Schiff base formed between the lysine residues of the enzyme molecule and crosslinker. This may be prevented by using a crosslinker such as glutaric acid which crosslinks the enzyme molecules without the formation of a Schiff base. Another disadvantage of using glutaraldehyde is that it tends to exist as dimers and trimers in solution. Hence the structure shown in Figure 6.1 (in Chapter 6) is a highly idealized structure. By using cross-linkers such as glutaric acid and diamino-octane (which do not dimerize or trimerize) we can fabricate such highly ideal crosslinked enzyme aggregates.

Another approach for fabricating highly stable enzyme aggregates is by crystallizing the enzymes by the use of a crystallization bath followed by crosslinking of the enzymes. The cross-linked enzyme crystals (CLEC’s) are then precipitated onto the surface of the nanofibers by the addition of a precipitation agent. Such CLEC’s are known to be stable to a wide range of pH, temperature and chemical environments. For fabricating CLEC’s of glucose oxidase onto the PS-PSMA nanofibers the following approach can be used. The glucose oxidase crystals can be fabricated on PS-PSMA nanofibers by adding the glucose oxidase and the nanofibers directly into a solution.
dase crystals can be crosslinked by the addition of NaIO$_4$ containing 18 % PEG 6000 and 32 % of 2-propanol in a 200 mM buffer solution (pH = 5-6). The covalently bound glucose oxidase crystals can be crosslinked by the addition of NaIO$_4$ (for oxidation of the carbohydrate moieties) and 1-8 diamino-octane.

References


APPENDIX – A

Additional notes on Chapter 2

Figure A1: SEM image of (a) side view of polyethylene layer produced by Ziegler-Natta polymerization within AAO nanochannels, and (b) top surface of the AAO membrane after ethylene polymerization showing extruded polyethylene nanofibers. The SEM was taken after annealing the samples at room temperature for many days.
Figure A2: SEM image of (a), (b) polyethylene filled nanochannels after heating to 150°C and the cooling.
**Figure A3:** Transmission FTIR spectra of (a) PE-AAO at 0° Polarization (b) PE-AAO at 90° Polarization
Figure A4: Molecular weight distribution of polyethylene formed inside nanochannels
Figure A4: Molecular weight distribution of polyethylene formed on flat surfaces
APPENDIX – B

Additional notes on Chapter 4

B1. Synthesis of PS-PSMA-FeCl₃ fibers

0.5 g of PS (M_w = 860,000) and 0.25 g of PSMA (M_w = 224,000) was dissolved in 5 ml of THF for 30 minutes. The mixture was thoroughly vortexed for 1 minute until the solution appeared homogenous. This solution is colorless. To this solution 0.5 g of FeCl₃ in 3 ml acetone was added. The solution was stirred for 30 minutes and was used for electrospinning. The solution has a reddish-brown color. Freshly prepared solutions should be used. After 12 hours, the solution loses its viscosity due to the interaction between the salt and the polymers and becomes dark black. In this case, the solution should not be used.

B2. Synthesis of PS-PSMA-FeTS fibers

0.5 g of PS (M_w = 860,000) and 0.25 g of PSMA (M_w = 224,000) was dissolved in 5 ml of THF for 30 minutes. The mixture was thoroughly vortexed for 1 minute until the solution appeared homogenous. 1.5 ml of Baytron C solution was mixed with 1.5 ml of acetone. This mixture was added to the PS-PSMA-THF solution and was vortexed for 2 minutes. The solution appears reddish-brown due to the presence of the FeTS. This solution was used for electrospinning. In this case also freshly prepared solutions should be used. After 12 hours, the solution loses its viscosity due to the interaction between the salt and the polymers and becomes dark black. In this case, the solution should not be used.
Figure B1: Photographs of (a) top view (b) side view of electrospun PS-PSMA-Ferric Tosylate fibers collected on a glass slide, exposed to pyrrole vapors for 48 hours and shaking at 250 rpm for 24 hours in buffer solution. It can be seen that the PS-PSMA-PPy fibers do not delaminate or break away from the glass slide under the above mentioned shaking conditions.
**Figure B2:** SEM of PPy tubes obtained by exposing the PS-PSMA-FeTS to pyrrole vapors for 24-48 hours followed by extraction in THF for 6 hours. The PS-PSMA-PPy assumes a rigid geometry which is maintained even on washing in THF.
**Figure B3:** SEM of some more PPy tubes obtained by exposing the PS-PSMA-FeTS to pyrrole vapors for 24-48 hours followed by extraction in THF for 6 hours. The PS-PSMA-PPy assumes a rigid geometry which is maintained even on washing in THF.
Figure B4: SEM of some more PPpy tubes obtained by exposing the PS-PSMA-FeTS to pyrrole vapors for 24-48 hours followed by extraction in THF for 6 hours. The PS-PSMA-PPpy cross sections are complicated and difficult to analyze.
APPENDIX – C

Images of crosslinked enzyme aggregates on carbon nanotubes

Figure C1: FESEM of glucose oxidase covalently attached onto bare carbon nanotubes (Bare CNT-CA).
Figure C2: FESEM of enzyme aggregates of glucose oxidase fabricated on bare carbon nanotubes (Bare CNT-EC).
**Figure C3:** FESEM of enzyme crystal aggregates of glucose oxidase precipitated on bare carbon nanotubes (Bare CNT- CLEA).
Figure C4: FESEM of glucose oxidase covalently attached onto acid treated carbon nanotubes (Acid Treated CNT-CA).
Figure C5: FESEM of enzyme aggregates of glucose oxidase fabricated on acid treated carbon nanotubes (Acid Treated CNT-EC).
**Figure C3:** FESEM of enzyme crystal aggregates of glucose oxidase precipitated on acid treated carbon nanotubes (Acid Treated CNT- CLEA).
APPENDIX – D

Aligned fibers synthesized by electrospinning

Figure D 1: PS-PSMA fibers synthesized by selectively grounding 4 electrodes at 90° to each other.
Figure D 2: PS-PSMA fibers synthesized by selectively grounding two electrodes parallel to each other.
APPENDIX E

Simulation for Figure 9.3 in Chapter 9

We have the rate equation of the form:

\[
\frac{1}{v} = K_1 + \frac{1}{K_2[C_{so}]} + \frac{1}{K_3[C_{o0}]} \quad (K_1, K_2, K_3 = \text{constants to be determined, } C_{so} = \text{Glucose concentration, } C_{o0} = \text{oxygen concentration, } v = \text{rate defined below})
\]

\[
Q \frac{\partial C}{\partial V} = -v
\]

We define a function (thesis1) which fits the above equations into the given set of experimental points and continuously optimizes itself so as to reduce the error between the fitted data and the experimental data.

\[
f = \text{error between the fitted data and the experimental data; because the constants cannot assume negative values we say that if } K_1, K_2, K_3 < 0, \text{ do not optimize}
\]

\[
\text{function f=thesis1(k)}
\]

\[
\text{if min(k)<0}
\]

\[
f=1000;
\]

\[
\text{else}
\]

Here we feed the experimental data that is to be fitted so as to find out the constants \( K_1, K_2, K_3 \); \( C_{so} = \text{Glucose concentration (mM)}; C_p=\text{corresponding hydrogen peroxide concentrations (mM)} \) and \( C_{o0} = \text{oxygen concentration} \).

\[
\text{cs0=[0.5 1 1.5 2 4 6 8 ]};
\]

\[
\text{cp=1e-3*[ 81.60393451 103.6650663 149.2580719 161.0240088 197.7925617 208.0877565 231.6196304 ]};
\]
We use the above two equations and experimental values of hydrogen peroxide obtained at $C_{O_0}=0.2$ mM with initial guesses of $K_1$, $K_2$, $K_3$ (1, 1, 1). We use the ordinary differential equation solver to solve the above differential equations. Our objective is to determine $C_{p1}$ and make it equal to $C_p$.

$K = \text{fminunc}(@thesis1, [1,1,1])$

```matlab
co0=[ones(7,1);0.2*ones(7,1)];

cp1=[];
rate=[];
for i=1:length(cs0)
    [t,c]=ode23s(@thesisfun,[0 0.6],[cs0(i);co0(i);0],[],cs0(i),co0(i),k);
    % plot(t,c)
    % pause
    % X(end)
    cp1=[cp1;c(end,end)];
    rate=[rate;((k(1)+1/k(2)/c(end,1)+1/k(3)/c(end,2))^(-1))];
end
```

With our initial guesses of $K_1$, $K_2$, $K_3$ we obtain values of $C_{p1}$. So as to get the correct $K_1$, $K_2$, $K_3$, we say that the error $f$ should be minimum so that the difference between $C_{p1}$ and $C_p$ should be minimum (zero). Hence we define an optimization function so as to minimize the difference between $C_{p1}$ and $C_p$.

```matlab
f=norm(cp1-cp);
end
disp(f)
plot(cs0,cp,'o')
hold on
plot(cs0,cp1,'ro')
figure
disp(cp1)
```

The values of $K_1$, $K_2$ and $K_3$ obtained above are resubstituted in the rate equations with $C_{O_0} = 1$ mM so as to get the theoretical hydrogen peroxide concentration at the corresponding oxygen concentration and glucose concentrations.

```matlab
Cs0=[0.5 8 0.129];
Co0=[1 0.2];
for i=1:length(Cs0)
    [t,c]=ode23s(@thesisfun,[0 1],[Cs0(i);Co0(i);0],[],Cs0(i),Co0(i),k);
    figure
    plot(t,c)
    % pause
    % X(end)
```
Plot of hydrogen peroxide concentration \( (C_p) \) generated versus glucose concentration \( (C_{o0}) \) at different oxygen concentrations \( (C_{o0}) \). The black circles are the experimental data for hydrogen peroxide generated at different glucose concentrations at \( C_{o0} = 0.2 \) mM. The lower curve (red circles) are the fitted curve used to calculate the constants \( K_1, K_2, K_3 \) (in the rate equation). The upper curve is the simulated values of the hydrogen peroxide concentration generated by using the calculated values of \( K_1, K_2, K_3 \) and \( C_{o0} = 1 \) mM.
VITA

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