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Synthesis and characterization of dinitrophenyl of dinitrophenyl functionalized conductive polymers capable of biospecific binding

Darkeyah Godel Reuven
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ASTRACT

CHEMISTRY

DARKEYAH GODEL REUVEN  B.S., GEORGIA STATE UNIVERSITY, 1999
M.S., MERCER UNIVERSITY, 2002

SYNTHESIS AND CHARACTERIZATION OF DINITROPHENYL
FUNCTIONALIZED CONDUCTIVE POLYMERS CAPABLE OF
BIOSPECIFIC BINDING.

Advisor: Dr. Ishrat M. Khan
Dissertation dated May, 2009

A series of DNP (2,4-dinitrophenyl) functionalized polypyrrole polymers that are specific to antibodies and immune receptors on cell have been synthesized and characterized (See Figure). This is a terpolymer composed of three monomers; monomer 1 (M1, pyrrole), macromonomer 2 (M2, pyrrole with pendant ethylene glycol) and macromonomer 3 (M3, pyrrole with pendant DNP). These polymers are expected to be useful for controlling receptor binding and cell activation, and with eventual application in biosensors. Conductivity measurement indicate that the terpolymers are
conductive, without adding external doping agents conductivity values of $5 \times 10^{-6}$ S cm$^{-1}$ (at 25 °C) were obtained. Binding studies with anti-DNP IgE studies are promising, fraction of binding sites occupied vs. concentration indicates specific and efficient binding at nanomolar concentration. Therefore, DNP functionalized polypyrrole are excellent materials for preparing nanowires in biosensors for detecting biomarkers. We have also determined that these polymers are biocompatible. Nanowires are currently being fabricated using the functionalized conductive polymers.

In addition to synthesis and characterization, the thermal properties of the functional polymers will be discussed with regards to the fabrication of nanowires for biosensing applications.
SYNTHESIS AND CHARACTERIZATION OF DINITROPHENYL
FUNCTIONALIZED CONDUCTIVE POLYMERS CAPABLE OF
BIOSPECIFIC BINDING

A DISSERTATION
SUBMITTED TO THE FACULTY OF CLARK ATLANTA UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE DOCTOR OF PHILOSOPHY

By
DARKEYAH GODEL REUVEN

DEPARTMENT OF CHEMISTRY

ATLANTA, GEORGIA
May 2009
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LIST OF ABBREVIATIONS

a  Section Area of the Sample
BSA  Bovine serum albumin
C*  overlap concentration
CONTIN  Inverse Laplace Transform
d  Space Distance between the Probe Tips
DLS  Dynamic Light Scattering
DMSO  Dimethyl sulfoxide
DSC  Differential Scanning Calorimetry
DNP  N-2,4-dinitrophenyl-ε-amino-n-caproic acid
FPPy  Functionalized polypyrrole
FT-IR  Fourier transform infrared spectroscopy
G'  storage modulus
G''  dynamic loss modulus
GPC  Gel Permeation Chromatograms
I  Electrical Current Passed through the Sample
IgE  Immunoglobulin E
KTFA  Potassium trifluoroacetate
MALDI -TOF  matrix-assisted laser desorption/ionization- time of flight
NAMD  Nanoscale molecular dynamics

n_i  Number of Charge Carriers of Type i in Unit Volume

PEG  Polyethylene glycol

Ppm  Parts per Million

PPyPEG350  polypyrrole-2, 4-(1H-Pyrrole-1-yl) Benzoic Polyethylene glycol

PPyPEGDNP  polypyrrole-[2, 4-(1H-Pyrrole-1-yl) Benzoic polyethylene glycol]-[2, 4-(1H-Pyrrole-1-yl) Benzoic Tetraethylene glycol dinitrophenyl -ε-amino-n-caproic acid]

PPyPEG(2K)DNP  polypyrrole-[2, 4-(1H-Pyrrole-1-yl) Benzoic polyethylene glycol (two thousand MW)]-[2, 4-(1H-Pyrrole-1-yl) Benzoic Tetraethylene glycol dinitrophenyl -ε-amino-n-caproic acid]

q_i  Charge on each Charge Carrier

R_b  Bulk Resistance

R_h  Radius of hydration

R_i  Interfacial Resistance

t  Thickness of the Sample

tan δ  phase lag

TEG  Tetraethylene glycol

TEM  transmission electron microscopy
$T_g$ Glass Transition Temperature

TGA Thermogravimetric Analysis

THF Tetrahydrofuran

TMS Tetramethylsilcane

$u_i$ Mobility of Charge Carrier

$W_n$ Number-Average Molecular Weight

$W_w$ Weight-Average Molecular Weight

$W_w / W_n$ Polydispersity Index

4PyPEG350 4-(1H-Pyrrole-1-yl) benzoyl Polyethylene glycol 350

4PyTEG 4-(1H-Pyrrole-1-yl) benzoyl Tetraethylene glycol

4PyTEGDNp 4-(1H-Pyrrole-1-yl) benzoyl Tetraethylene glycol dinitrophenyl

$\eta_r$ reduced viscosity

$\eta_{sp}$ specific viscosity

$\delta$ NMR Chemical Shift

$\sigma$ Conductivity
CHAPTER 1
INTRODUCTION

1.1 Sensor Materials

Materials capable of controlled and specific interactions with biological material are of great interest due to their applicability in the areas of sensing, biomimic materials, drug targeting and delivery. Biological sensor devices are an area of great interest due to their ability to dramatically advance the diagnosis process of diseases such as cancer. The primary objective in this regard is the incorporation of materials capable of controlled and specific interactions with biological material, i.e. antigens, antibodies, enzymes, DNA, etc. A material possessing these properties are termed biological recognition elements which are typically attached to a transducer material. Conducting polymers (CP’s) are ideally suited to act as a transduction material onto which biological or chemical sensitive components are attached. This type of device would act as a chemoresistor sensor. A chemoresistor sensor is a device in which a measurable decrease in conductivity occurs upon the reversible binding of an analyt. Sensor devices include ampeometric (resistivity, conductivity changes), field effect transistor, optical waveguide (absorption changes), and surface acoustic devices (mass change).¹

Due to the limitations of sandwich binding assays, today’s primary diagnostic tool, it is desirable to have a sensing system which does not require multiple washing and labeling steps (reagentless) and only requires the addition of a sample which
contains the analyt of interest. Biological sensor devices have been created by the method of entrapment biological material in a electropolymerized CP films (polypyrrole). Biological recognition elements have also been incorporated in CP material via the covalent attachment of these materials to CP’s or monomers, such as pyrrole and thiophene which are subsequently electro or chemically polymerized. CP’s have been derivatized and incorporated into devices that are able to sense multiple metal ion species (Li⁺, Na⁺, K⁺). Often in the case of gas or metal ion sensors aromatic cyclic monomers (polythiophenes and polypyrrole) have been covalently substitutated with oligomeric alkoxy chains and the aromatic group and subsequently polymerized. These alkoxy side chains are capable of complexation with and differentiated sensitivity between metal ions.

1.2 Nanostructures in Sensors

The role of nanostructures, such as quantum dots, nanoparticles, nanowires, tubes etc. in molecular diagnostic are being intensively pursued because the analyt/receptor binding event can have large affects on the chemical and physical properties of the sensing nanostructure. Advances in electronic detection based on CP’s and inorganic nanowires have the potential to revolutionize the ability to provide label-free, highly sensitive and selective detection of a wide range of chemical and biological species. The potential miniaturization of nanosensing transduction components offers the possibility of improved surface to volume ratios, decreased manufacturing cost, and offers a broad platform for various multiplex measurements. Significant work has also been done using inorganic nanowires. Metal oxides such s indium oxide (In₂O₃) and tin
oxide (SnO$_2$), in addition to silicon are typically considered good candidates for conductive inorganic nanowires. These materials have been surface sensitized with biological recognition elements, such as antibodies, antigens and biotin for sensing applications.$^8$-$^{13}$

1.3 Functionalized Conducting Polymer

Several reports have demonstrated the utility of functionalized conductive polymer$^{14}$ and inorganic nanowires,$^{15}$ in addition to functionalized single walled carbon nanotubes (SWCNT’s) as the active biosensor component.$^{16,17}$ Thus, it is of significant interest to fabricate semi-conductive polymer fibers decorated with functional groups capable of specifically binding biomaterial. In the area of CPs, polyaniline and polyalkylthiophenes$^{18}$ have been electrospun into fibers. Electrospinning is a straightforward technique of processing polymeric material into fibers. Also, several reports have shown that the fabrication of CPs into fibers is helpful when the polymers are blended with high molecular weight poly(ethylene oxide),$^{19-21}$ poly(vinylpyrrolidone)$^{22}$ and poly(styrene)$^{23,24,25}$

The interaction between an analyt and the sensing element causes changes in the physicochemical properties in the CP backbone. Chemiresistor sensor have been created by the introduction of carbon nanotubes (CNT’s) into functionalized conducting polymers. These CNT/conducting polymer composite material where electrospun or spin-coated onto gold electrodes to form operational devices have been widely studied.$^{26}$
1.4 Polymer Biospecific Binding

The synthesis, characterization and biospecific binding capacity of univalent, trivalent and multivalent 2, 4-dinitrophenyl(DNP) functionalized synthetic polymers have been reported. Water soluble DNP functionalized poly(ethylene oxides) have been demonstrated to either stimulate or inhibit mast cell degranulation. Mast cells are most prevalent in the lung, mouth, digestive tract and nose. Mast cell degranulation is the release of granules rich in histamines and heparin through a complex sequence of biological reactions induced by the clustering of high affinity receptors (FcεRI) on the surface of the mast cell, mediated by the crosslinking of immunoglobulin E by an antigen, such as pollen. Stimulatory and inhibitory functions were determined to be dependent on the molecular weights and degree of functionality of the polymers. The observed functional properties are due to the binding of the functional polymers to the IgE receptors on the surface of the mast cells. Additionally, studies have also shown that divalent DNP functionalized poly(2-methoxystyrene) have the ability to specifically bind to the IgE-FcεRI receptors proteins in solution. However, because of the hydrophobic nature of the poly(2-methoxystyrene) systems the binding constant in aqueous solution is significantly lower than the water soluble DNP-PEO system. But the hydrophobicity of the DNP functionalized poly(2-methoxystyrene) makes it possible to study the interaction of the functional polymer in the solid state with mast cells in aqueous solution. The solid functionalized structures were prepared by fabricating water insoluble fibers by electrospinning divalent α,ω-di-DNP-poly(2-methoxystyrene). The electrospun fibers were decorated with the DNP functionalized groups which were tethered to the fibers via oligo(oxyethylene) spacers. These fibers
were determined to be effective in binding IgE and also clustering IgE receptors on the RBL mast cells in aqueous solutions. These observations demonstrate the potential of developing fibers decorated with functional groups capable of biospecific interactions. To fully exploit these unique properties semi-conductive polymers would be desirable.

1.5 Scope of the Work

In the present study, we report the synthesis and characterization of DNP functionalized polypyrroles terpolymers. The polypyrroles were modified with oligo(oxyethylene) to improve processibility and to inhibit protein non-selective binding to the polymer. Additionally, the report also presents preliminary binding studies and selectivity of the DNP functionalized polymers towards IgE.
CHAPTER 2
BACKGROUND AND SIGNIFICANCE

PART 1

Electronic chemosensing holds great promise for disease diagnosis in a wide variety of point of care environments, from health clinics to the battlefield. A chemo- or bio-sensor is primarily composed of two key components a transducer (electro-active material, pH electrode, thermosistor, photon counter, piezoelectric device), and a receptor or biological sensing element (enzyme, antibody, microorganism, cells, DNA, polymer, etc.,) which interacts with the appropriate analyt (see figure 2.1.1). A key requirement for a detection device to be classified as a sensor is equilibrium binding between an analyt and a receptor. The efficiency of the transduction element determines the sensitivity of the sensor response. CPs as transduction elements holds great promise in the quickly expanding field of gas, metal ion and biological sensing. CP materials and combinations of them through thoughtful design can be tuned to increase their chemical, mechanical and physical properties. Thus, the modulation and optimization of the end sensing system’s sensitivity, reactivity and selectivity are obtainable. To fully take advantage of the opportunities that chemo and biosensing offers, a fundamental understanding of the properties of CP transducers and the sensing components is needed. In addition to charge carrier propagation, polymer matrix swelling and analyt/receptor sensing interactions in these composite materials. Thus the transduction mechanism
continues to be of great interest and one in which CP’s have and will continue to play a key role.

![Diagram of sensor components before and after binding](image)

**Figure 2.1.1.** Primary components of a sensor before binding (a) and after binding (b).

It is of note that relatively few repeating units in the CP backbone (7-13) are delocalized, this is especially the case in systems containing aromatic rings. Thusly, the HOMO-LUMO band gap is largely determined by a very localized electronic structure. The sensory reponse of functionalized CP’s is thought to arise from the charge (+,-)on the anaylte (metallic, biologic, molecular) once bound by the sensing element and brought into close proximity to the CP backbone are thought interact with charge carriers within the polymer. This localized charge-transfer interaction between the polymer and anlyte are believed to perturb the local redox potential and create barriers to carrier transport.

Swagger et. al. have synthesized chemosensors that are highly sensitive to charged organic species. The binding of viologen to the macrocycle containing polymer resulted in attenuated conductivities (see figure 2.1.2).
The role of nanostructures, such as quantum dots, nanoparticles, nanowires, tubes etc. in molecular diagnostic are being intensively pursued because the analyt/receptor binding event can have large affects on the chemical and physical properties of the sensing nanostructure. A further challenge to the full actualization of electronic sensing is their incorporation into easy-to-use, low cost sample handling system. A sample handling system that could possibly fill this gap are microfluidic devices, although much development work remains.

2.1 Conducting Polymer

CPs can be considered to be organic semiconductors, a material which possess a electrical conductivity between a conductor and a insulator. CP materials continue to be an intensely studied area due to their electrochromic (the ability of some chemical species to change color upon the application of a charge) and electrical properties, while possessing the mechanical properties of plastic. The intrinsic properties of conducting
polymers are due to their delocalized $\pi$-electrons along a finite length of the polymer backbone. Electrical conductivity can occur along the polymer backbone or through interchain $\pi-\pi$ interactions. CP's typically have low conductivities, although the conductivity of CP's can be significantly increased with the introduction of charge carriers via doping with electron-accepting or donating species, which result in p-type or n-type semiconductors, respectively. Thus, the band gap can be modulated, significantly affecting the materials electrical properties (e.g., resistance). Electron-accepting species include halogens ($I_2$, $Br_2$, etc...), metal halides ($AsF_5$, $FeCl_3$) or photon degradable acids ($H_2SO_4$, $HClO_4$). The delocalized $\pi$-electrons also gives rise to the materials electrochromic properties.

Applications for CP's in electrochromic displays, coatings, batteries and sensors continue to be pursued. CP materials can be categorized into three useful categories; aromatic hydrocarbons (e.g., polyaniline), heterocyclics (e.g., polypyrroles and polypyrrole) and aliphatic hydrocarbons (e.g., polyacetylenes). Polypyrroles (PPy) and polythiophens (PT) have favorable properties such as thermal and environmental stability and are readily electropolymerized or chemically polymerized.

The most widely studied CP, for reasons previously stated, in addition to its biocompatibility, is polypyrrole. In the case of the electropolymerization of polypyrrole, due to pyrrole's low oxidation it can be polymerized in water. Waltman et. al. point out in their 1984 study on conductive polymers that the primary reactive sites (electrophilic) on polypyrrole is the $\alpha$-position. In figure 2.1.3 oxidized pyrrole monomers possess a cation radical that reacts to form a carbon-carbon bond forming oligomers, followed by the release of two
protons which rearomatize the oligomer which subsequently forms doped polymers.\textsuperscript{42} While PPy can be oxidized (loss of electrons) and reduced (gain of electrons), this process is not fully reversible. The oxidation process results in the production of polarons (radical cations) in the polypyrrole interchain system at partial oxidation.\textsuperscript{43} Further oxidation results in a insulating material that results from deprotonation at the nitrogen position (see figure 2.1.4). Swager et. al. have shown using optical and electron spin resonance studies that radical cations carry the majority of current in partially oxidized (conductive) PPy.\textsuperscript{44}

A well known challenge to using CPs is their lack of solubility in common solvents, which greatly limits processing methods such as, spin- and spray casting. Solubility limitations can be addressed when CPs are synthesized in the presence of surfactants\textsuperscript{45, 46} (i.e. sodium dodecyl sulfate\textsuperscript{47}, etc...) or have been functionalized with

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure213.png}
\caption{Polymerization mechanism of pyrrole monomers.}
\end{figure}
Figure 2.1.4. Oxidized forms of polypyrrole. (Reprinted with permission from Springer, ref 44. Copyright 2006 Springer.)

2.2. Soluble Conducting Polymer

Soluble sidechains, such as oligosiloxan. Aromatic monomers have also been substituted with alkyl or alkoxy sidechains (see figure 2.2.1). CP solubility limitations have also been addressed by substitution with alkyl and alkoxy side chains, which typically results in CPs that are organic solvent soluble, while insoluble in aqueous systems. Additionally, alkoxy substituted CPs introduces charge donors to CP backbone, which has the effect of lowering the oxidation potential of the resultant polymer. These side chain substituents surround the rigid CP backbone and inhibit intermolecular interactions between chains, resulting in materials of lower conductivity values.
Water soluble CP's have been obtained by the introduction of ionic side chains, such as, sulfonate, carboxylates, etc, to the polymer backbone resulting in self-doped conducting materials. Substitutions of common CP's like polypyrroles or polythiophens can occur in the $\alpha$ and $\beta$ positions, additionally in the nitrogen position ($N$-substitution) in the case of polypyrrole. A disadvantage of side chain functionalization of monomers is it has the effect of significantly lowering the conductivity of the resulting polymer (10$^{-6}$ - 10$^{-3}$ S/cm, see figure 2.2.2). This decreased conductivity is attributed to steric interactions caused by bulky substituents which inhibit planar conformations along the CP backbone, these bulky substituents also inhibit inter-chain charge transfer, i.e. charge carrier inter-chain hopping process. Inclusion complexes such as $\beta$-cyclodextrin have been used to polymerize heterocyclic monomers such as thiophenes to produce CP materials with improved planar conformations. Chen et. al. have polymerized pyrrole in the presence of $\beta$-cyclodextrin which resulted in polypyrrole with increased short range order along the polymer backbone resulting in a material with improved thermal stability and conductivity.
Figure 2.2.1. Examples of substituted aromatic monomers and polymers found in literature.

(A) Poly[3-(2,5,8-trioxanonyl)thiophene] (PTT) (1a) and Poly[3,4-bis(2,5,8-trioxanonyl)thiophene] (PBTT) (1b). (Reprinted with permission from ref 57. Copyright 2005 WILEY-VCH Verlag GmbH & Co.)

(B) N-substituted poly(3,4-propylenedioxy)pyrrole) (2a) and ProDOP-N-propylsodium sulfonate (2a). (Reprinted with permission from ref 38. Copyright 2003 American Chemical Society.)

(C) Poly(N-hexyl-cyclopenta[c]pyrrole) (3) (Reprinted with permission from ref 45. Copyright 2004 WILEY-VCH Verlag GmbH & Co.)

(D) 3-(3,6-dioxaheptyl)pyrrole (Reprinted with permission from ref 40. Copyright 2005 WILEY-VCH Verlag GmbH & Co.)
Sonmez et al. have synthesized ethoxy and propylesodium sulfonate N- and β position substituted pyrrole monomers. These macromonomers where subsequently electropolymerized which resulted in materials that possessed novel electrochromic properties, while their conductivities were low between $10^{-4}$ - $10^{-3}$ S/cm. Van Beek et al. have developed novel conductive materials for organic semiconductor applications through the synthesis of ethoxy substituted thiophenes in the β positions.
2.2.1 CP Processing: Electrospun Polymer

Electro-spun fibers have been studied for their potential applications in areas in water filtration, sensing, and tissue engineering. Electrospinning is a technique whereby, an electrostatic force is created between a metal needle, loaded with a viscous polymer solution, and a grounded electrode. On the polymer surface resultant from only surface tension a charge build up induces the formation of a droplet known as a Taylor cone, of which, the tip releases a fluid jet. The fluid jet is attracted towards the grounded electrode and the solvent evaporates from the jet in transit forming a polymer fiber. Using this method fibers have been obtained in the micrometer to nanometer scales. Electrospun polymer materials have been shown to be amorphous due to rapid evaporation of solvent during the electrospinning process.

Single and binary blends of conjugated polymers have been electrospun into nanofibers which display tunable optical and electronic properties which can be exploited in field effect transistors. Because of molecular weight and good solvent limitations few conducting polymers, polyaniline, alkylated poly phenylenevinylene (PPV), alkylated poly thiophenes (PT) can be readily electrospun. Several reports have shown that the fabrication of conductive polymers into fibers is helpful when the polymers are blended with soluble polymers, such as high molecular weight poly(ethylene oxide), poly(vinylpyrrolidone) and poly(styrene). The conductivity of the resultant blended material is significantly impacted by the degree of miscibility of CP’s and these soluble polymers and the solubility of the CP’s in a given organic solvent.
PART 2

2.3. Conjugated Polymer Film Bio and Chemosensors

2.3.1. Sensors and Signal transduction

The term sensor is widely used, although it is of note that the irreversible binding (high association constants, slow dissociation kinetics) of an analyt is considered a dosimeter. While reversible binding which produces a measurable response in proportion to analyt concentration is technically considered a sensor.60,61 Researchers have sought to address the issue of irreversible binding by resetting the receptor by chemical, electrochemical, photochemical or physical events. Sensor devices include ampeometric (resistivity, conductivity changes), field effect transistor, optical waveguide (absorption changes), and surface acoustic devices (mass change).1

In a chemoresistor sensor a CP acts as a transduction element onto which biological or chemical sensitive components are added. A chemoresistor sensor is a device in which a measurable decrease in conductivity occurs upon the binding of an analyt. The interaction between an analyt and the sensing element causes changes in the physicochemical properties in the CP backbone. This analyt/receptor (sensing element) interaction (hydrogen binding, electrostatic, polarity) causes a redistribution of charge carriers in CP backbone which can result in conformation changes in the polymer backbone. The inhibition of the charge carriers measurably effects conductivity of the material. The methods incorporation of sensing elements into CP’s will be discussed.
2.3.2. Metal Ion selective Sensors

CP's have been derivatized and incorporated into devices that are able to sense of metal ion (M⁺) species (Li⁺, Na⁺, K⁺). Often in the case of gas or metal ion sensors aromatic cyclic monomers (polythiophenes⁴,⁵ and polypyrrole⁶,⁷) have been covalently substituted with oligomeric alkoxy chains and the aromatic group is subsequently polymerized. These alkoxy side chains are capable of complexation with and differentiated sensitivity between metal ions and leads to conformational changes in delocalized backbone. Analyt complexation may also impede conformational changes or inhibit π-π interchain stacking, which has been shown to be responsible for a large amount of CP conductivity. The polymer reaction to these types of analyts are referred to as ionoselective sensors. The analyt/sensing element interaction is observed by the measurement of the intensity of cyclic voltammogram (CV) graph. A decrease in conductivity is observed upon onset of the binding event thus these devices act as potentiometric sensors.

A limitation of ionosensors is the binding of metal ions inhibits the further diffusion of metal ions into the polymer matrix. This phenomenon is attributed to the formation of a static potential barrier caused by a positively charged cloud formed by alkoxy / metal ion complexation. CP's have found wide application in gas or ion sensors due to tunability of their morphology and structural properties. An example of an optical chemosensor is a molecular wire composed of a polythiophene functionalized with cyclophanes which binds specifically with paraquat was reported at 1995 by Zhou.⁶²,⁶³
2.3.3. Biological Sensors

Biological sensor devices are an area of great interest due to their ability to dramatically advance the diagnosis process of diseases, such as cancer. The primary objective in this regard is the incorporation of biological recognition elements with a transducer material. Today by far the most widely employed technique used for diagnostics is optical (fluorescence) based enzyme linked immunosorbent assays (ELISA). The ELISA method is the competitive binding of labeled and unlabeled proteins on a finite number of binding sites onto a protein of interest. This assay is able to detect a wide variety of analyts. A broad selection of labels have been appropriately employed in binding assays, such as, DNA, fluorescent ligands, antibodies, antigens, and radio labels. The drawback of the ELISA technique is it requires multiple washing and protein labeling steps that are not easily conducted in the field (doctors' office, battle field, etc...).

Due to the limitations of binding assays, it is desirable to have a sensing system which does not require multiple washing and labeling steps (reagentless) and only requires the addition of a sample which contains the analyt of interest. To this effect, thin films composed of CPs with covalently attached biological recognition element have been explored. CPs are chemically inert, thus do not adversely affect linked proteins, reactivity or potency. A drawback of using CPs is the matrix swelling phenomenon and minimally leaching of polymer and biological material in aqueous environments, which impacts the calibration of the end-use device.
Researchers continue to intensively study the application of conducting polymers as a transducer element in biological and chemical sensor. A straightforward method researchers have utilized to create ampermetric sensor devices is to entrap biologically sensitive molecules or biomolecules in CP matrix. Several groups have employed polypyrrole as a polymer matrix to entrap biological molecules, such as, enzymes, antibodies, antigens and other biologically active molecules.

As is the case with metal ion sensors, the binding of a biological analyt with the recognition element causes conformational changes in the CP backbone. The altered interchain π-π stacking perturbs the electronic state of CP backbone effecting changes in bulk conduction properties. Polypyrrole and polyaniline derivatives have been intensely studied for applications in biosensing, due to their biocompatibility and stability in aqueous environments. Aizawa and Yabuki, 23 years ago, conducted studies in which the method of entrapment of enzymes in a electropolymerized CP films (polypyrrole) where utilized.2 Studies that followed also employed polypyrrole to entrap negatively charged glucose oxidase to create amperiometric sensing devices.67,68 Polypyrrole in this case does not act as a transducer of electrons resulting from the binding event but the reaction between the entrapped enzyme and oxygen that forms hydrogen peroxide, which is subsequently oxidized is responsible for the electrode’s potential response.69,70 Due to the high potential needed to oxidize hydrogen peroxide, mediators, such as, ferrocene derivatives have been employed.71-73
2.3.3.1 Covalently Linked Biologic Recognition Elements

Another widely employed technique to incorporate biologic recognition elements onto CP’s or monomers is through covalent attachment, followed by electro or chemical polymerization of the macromonomer. Kuramitz et. al. have synthesized a biotin functionalized pyrrole monomer and subsequently electropolymerized the macromonomer into a polypyrrole film (see figure 2.3.1). The resultant biosensitive CP film was competitively exposed to avidine and a measurable electrode response to the analyt was detected. An optical microbiosensor developed for the detection of hepatitis C virus (HCV) using an electropolymerizable photoreactive pyrrole monomer which can be subsequently substituted with proteins of interest was reported in 2005 by Konry.

There continues to be challenges involved in the full actualization of antibody-CP sensors, such as incubation times (greater than ten minutes) in which exposure aqueous environment may cause polymer film to swell altering the materials electrical properties. In addition, the limitations of binding reversibility of many antibody or antigen in the systems that have been developed continue to be a challenge. Some researchers have employed the technique of pulse potential waveform (periodic cycling an electrode potential from negative to positive) to measure and tune antigen-antibody binding event. 74, 75

Figure 2.3.1. Pyrrole monomer with a covalently attached biotin via PEO linkage. (Reprinted from ref 3. Copyright 2003 American Chemical Society)
2.4. Carbon Nanotube loaded Polymers

Composites of CNT and CP composites have been widely studied due to their potential synergistic properties of increased conductivity and bulk mechanical properties. CNTs are a $\pi$-conjugate structure of graphite sheets rolled into a seamless tube. CNTs are characterized by two chiral vector indices $m$ and $n$, which determine their properties as semiconducting chiral, zig zag, and armchair (metallic) configurations. CNTs are of interest because of their outstanding properties, such as, very high electrical conductivity, rigidity (modulus) and chemical resistance.\textsuperscript{76,77} A major challenge to using CNTs in sensors is their non-specificity for various chemical and biological analyts. A method by which this limitation has been addressed is through the introduction of CNTs into functionalized conducting polymers. A major limitation to this technique is the homeogenous dispersion of CNTs. A mixture of different CNT types bundle into randomly oriented aggregates due to their high surface areas in the polymer matrix. Homogeneous dispersions of CNTs have been obtained in four ways: (1) mixing CNTs solution with soluble polymers, (2) surfactants or (3) substituted aromatic small molecules (pyrene,\textsuperscript{78,79} naphthalene\textsuperscript{80}). The mixing process is typically followed by ultrasonication of the solution, which results in increased polymer or small aromatic molecule CNT sidewall $\pi$-$\pi$ interactions or surfactant assisted dispersion. The fourth method involves the chemical modification of the CNTs tips.\textsuperscript{81,82} For example the CNTs can be end functionalized with carboxylic groups and subsequently substituted with polymer chains (polyethylene oxide, poly-m-aminobenzene sulfonic acid).\textsuperscript{83} Additionally, CNTs have been loaded into various polymer such as, polyethylene
oxide, poly (vinyl acetate) and subsequently processed into conducting fibers using electronspinning techniques. It is of note that CNTs that have been loaded into polyacrylonitrile and electrospun, resulted in the orientation of the MWCNTs along the axis of the resultant nanofiber.

A simple fabrication method to create a chemiresistor sensor have utilized polymer films loaded with CNTs. The CNT / conducting polymer composite material was electrospun or spin-coated onto gold electrodes to form operational devices have been widely studied. Swager et al have fabricated a CNT / polymer film device that was sensitive and selective for a specific analyte. This receptor/transducer composite was formed by dispersion of SWCNTs in hexafluoroisopropanol (HFIP) functionalized polythiophene. The HFIP group was used as the recognition element due to its ability to hydrogen bond with phosphate esters, which are common in chemical warfare agents, i.e. Sarin vapor.

PART 3
2.5. Nanowire Sensors

Advances in electronic detection based on conductive polymer and inorganic nanowires have the potential to revolutionize the ability to provide label-free, highly sensitive and selective detection of a wide range of chemical and biological species. The potential miniaturization of nanosensing transduction components offers the possibility of improved surface to volume ratios, decreased manufacturing cost, and offers a broad platform for various multiplex measurements. Various studies have shown that
nanostructured sensor devices surpass standard assays, in sensitivity and selectivity.\textsuperscript{95,96} Sensing, in this context, is the detectable changes in electronic characteristics when a nanowire sensing device captures a target molecule. A conductive nanowire structure acts as a transducer that possesses the useful property of having a strong and distinct electronic response due to surface absorption or binding. The electrical response of conductive nanowire to a binding event is a property that results from its small diameter, which allows for the fast buildup and depletion of charge carriers.\textsuperscript{19} Several reports have demonstrated the utility of functionalized conductive polymers\textsuperscript{14} and inorganic nanowires,\textsuperscript{15} as the transducer component in biosensors.\textsuperscript{16,17} A significant drawback of using inorganic nanowires is their synthesis requires high temperature and high vacuum conditions, while conducting polymers are synthesized chemically and electrochemically at room temperature. Additionally, conducting polymer starting material is relatively inexpensive. Another major challenge of nanosensor development is the incorporation of nanodimension sensing elements into functional electrical devices.

The widely studied technique of electrospun polymeric material to form fibers of diameters of less than 100 nanometers offers opportunities of easy deposition of fibers over surface features such as, gold electrodes for electrical measurements.\textsuperscript{97} Thus, electrosun CP wires expands the possible sensing platforms and has the potential to meet the growing demand for sensing technology. Various biocompatible polymers (PEO, PS, PPy, etc.) have been loaded with various biological material such as enzymes,\textsuperscript{23} and human growth factors,\textsuperscript{20,98} and subsequently electrospun. Craighead et.
al. have electrospun regioregular poly(3 hexyl thiophene) across gold electrodes electrodeposited onto a silicon wafer (see figure 2.5.1).

Another technique of biomaterial incorporation is electrostatic layer-by-layer (ELBL) in which, biomaterial is incorporated in the alternating layers of positive and negatively charged polyelectrolyte layers. An effective method of greatly increasing the sensitivity of CP nanowires is its incorporation into field effect transistors (FET) devices. This phenomenon is due to the FET device ability to gate-modulate channel conductance. Ramanathan et. al. have used controlled electropolymeryzation to fabricate avidin entrapped polypyrrole nanowire between gold electrodes. Upon binding of biotin-DNA analyt, a rapid change in resistance was observed. Hernandez et. al. have also used the electropolymerization of polypyrrole nanowires, using porous aluminum oxide as a template, to entrap biotin in the polymer matrix (see figure 2.5.2). In this case the polypyrrole was capped with gold and used to electronically sense for avidine and streptavidine.
Figure 2.5.1. (Color online) (a) Chemical structure of RRP3HT used in this study. (b) Fluorescence image showing the morphology of droplets occasionally seen during electrospinning. (c) SEM image of a typical electrospun RRP3HT nanofiber deposited on pre-patterned SiO2/Si substrate. The fiber shown had a diameter of about 180 nm. (Reprinted from ref 97. Copyright 2005 Nanoscale Science and Design.)
Method 1: Accessible Binding Sites

Method 2: Total Binding Sites

Figure 2.5.2. Schematic diagram of the methods used to quantify the amount of protein binding sites in PPy nanowires. (Reprinted from ref\textsuperscript{101} Copyright 2004 American Chemical Society.)

Significant work has also been done using inorganic nanowires. Metal oxides such as indium oxide (In\textsubscript{2}O\textsubscript{3}) and tin oxide (SnO\textsubscript{2}), in addition to silicon are typically considered good candidates for conductive inorganic nanowires. These materials have been surface sensitized with biological recognition elements, such as antibodies, antigens and biotin.\textsuperscript{8-13} Cui et. al. have utilized boron-doped silicon nanowires sensitized with biological sensing elements. Biotin and antibody modified nanowires where used to electronically detect in real time streptavidin and reversibly a antigen at picomolar concentrations.\textsuperscript{102} With continued research efforts to sensitize the surfaces of nanowires to various analyts this technology has the potential to become an effective and efficient transducer component in biosensors.
CHAPTER III
EXPERIMENTAL SECTION

3.1 Polymerization Dinitrophenyl (DNP) Functionalized Polypyrrole

A series of dinitrophenyl (DNP) functionalized polypyrroleS abbreviated PPyPEG350 and PPyPEGDNP, was synthesized by oxidation polymerization techniques. The making of the polymer involved the synthesis of two macromonomer using esterification reactions which are sensitive to moisture. High vacuum ($10^{-3} \sim 10^{-5}$ Torr) distillation techniques were used to purify the pyrrole monomer, tetraethylene glycol, and polyethylene glycol (350). The synthetic route for the polymerization of PPyPEG350 and PPyPEGDNP is outlined in figure 1.

3.2 Reagents

**Pyrrole**

Pyrrole: (Sigma-Aldrich, Av. Mol. Wt. 67.09; d 0.967) was distilled using a cow distillation apparatus.

**4-(1H-Pyrrole-1-yl) Benzoic acid**

4-(1H-Pyrrole-1-yl) Benzoic acid: (Sigma-Aldrich, FW 187.19; mp 286-289°C) used as received.

**Tetraethylene Glycol**

Tetraethylene glycol: (Sigma-Aldrich, Typical Mn 194.23; d 1.125.) was distilled using a cow distillation apparatus.
Polystyrene

Polystyrene: (Sigma-Aldrich, Av. Mol. Wt. 45,000; d1.060) was used as received.

N-(2,4-Dinitrophenyl)-ε-aminon-caproic acid

N-(2,4-Dinitrophenyl)-ε-aminon-caproic acid: (Sigma-Aldrich, FW 297.26) used as received.

Oxalyl Chloride

Oxalyl chloride: (Sigma-Aldrich, Typical Mn 126.93; bp 62-65 °C, mp −10--8 °C, d 1.5.) used as received.

Tetrahydrofuran

Tetrahydrofuran (THF): (Sigma-Aldrich, F.W. 72.11, B.P. 65.8 – 66.1°C, d 0.855) was refluxed and distilled sodium and benzophenone just before use.

Benzyltrimethylammonium chloride

Benzyltrimethylammonium chloride: (Sigma-Aldrich, FW 185.69; mp 230-235°C) used as received.

Calcium Hydride

Calcium hydride: Sigma Aldrich (-40 mesh to +4 mesh, 95%, F.W. 42.10, d 1.900)
Figure 3.1.1. Synthetic route for PPyPEGDNP, Py = pyrrole, 4PyB-Cl = 2,4-(1H-Pyrrole-1-yl) Benzoyl chloride, 4PyPEG(350) = 2, 4-(1H-Pyrrole-1-yl) Benzoic Polyethylene glycol, 4PyTEG = 2, 4-(1H-Pyrrole-1-yl) Benzoic Tetraethylene Glycol, 4PyTEGDNP = 2, 4-(1H-Pyrrole-1-yl) Benzoic Tetraethylene glycol DNP-ε-amino-n-caproic acid.
3.3 Procedure

3.3.1 Preparation of 4-(1H-Pyrrole-1-yl) Benzoyl Chloride, abbreviated as (4PyBCl).
To a 250 mL two-neck flask 10 ml of dry dichloromethane, 4-(1H-Pyrrole-1-yl) Benzoic acid (0.94 g, 20 mmol), and benzyltrimethylammonium chloride (0.0172 g, 0.37 mmol) were added. The mixture was set to a medium stir and flushed with dry nitrogen while fresh oxalyl chloride (0.86 mL, 40) was slowly added drop wise. The reaction mixture was then heated at reflux for 18 h. The solvent and excess oxalyl chloride was removed via rotary evaporator under reduced pressure while heated to 70 °C, and the residue was subsequently placed in a vacuum oven under reduced pressure (10⁻³ ~ 10⁻⁵ Torr) for 8 h at 70 °C. When cooled to room temperature, the product appeared as a black powder; yield 90%. \(^1\)H NMR (DMSO, δ relative to TMS, 6.5 (2H), 7.3 (2H), 7.5 (2H), 7.9 (2H)).

3.3.2 Preparation of 4-(1H-Pyrrole-1-yl) benzoyl PEG 350, abbreviated as 4PyPEG350
To a 50 mL two-neck flask with a dry nitrogen feed PEG (0.035g, 0.10 mmol), 4-(1H-Pyrrole-1-yl) benzoyl Chloride (0.073 g, 0.35 mmol), and 10 mL of THF were added. The reaction mixture was gently heated to promote dissolution and then allowed to mix well for 15 min. Sodium hydroxide (0.125 g, 2.5 mmol) was added to the reaction mixture and stirred at room temperature for 1.5 h. The reaction mixture precipitated out of solution. The mixture was filtered and washed with THF and Hexane through a 30 mL, 10-20 µm Buchner glass-frit filter to remove the unreacted starting material. The desired product formed a dark brown precipitate; yield 50%. \(^1\)H NMR (D2O): δ relative to TMS,
3.2 (3H), 3.5 (24H), 6.4 (2H), 7.3 (2H), 7.4 (2H), 7.9 (2H). Anal. Calcd for C_{28}H_{43}N_{10}: 83.64 C; 10.78 H; 3.48 N. Found: 80.47 C; 6.05 H; 6.07 N.

3.3.3. Preparation of 4-(1H-Pyrrole-1-yl) benzoyl TEG DNP, abbreviated as 4PyeTEGDNP

To a 50 ml two neck flask with a dry nitrogen feed TEG (0.14 g, 0.10 mmol), 4-(1H-Pyrrole-1-yl) benzoyl Chloride (0.073 g, 0.35 mmol), and 10 ml of THF were added. The reaction mixture was gently heated to promote dissolution and then allowed to mix well for 15 min. Sodium hydroxide pellets (0.125 g, 2.5 mmol) were added, and the reaction mixture was stirred at room temperature for 1.5 h. The reaction product precipitated out of solution. The mixture was filtered and washed with THF and hexane through a 30 mL, 10-20 μm porosity Buchner glass-frit filter to remove the unreacted monomer. The desired product formed a dark brown precipitate and was obtained in 50% yield.

Preparation of 2,4-dinitrophenylamino hexanoyl chloride:

To a 250 mL two-neck flask 10 mL of dry dichloromethane, N-2,4-DNP-ε-amino-n-caproic acid (0.93 g, 20 mmol), and benzyltrimethylammonium chloride (0.0105 g, 0.37 mmol) was added. The mixture was set to a medium stir and flushed with dry nitrogen while fresh oxalyl chloride (0.515 mL, 40 mmol) was slowly added drop wise. The reaction mixture was then heated at reflux for 18 h. The solvent and excess oxalyl chloride was removed via rotary evaporator under reduced pressure and heating to 70 °C. The residue was subsequently placed in a vacuum oven under reduced pressure (10^{-3} \sim 10^{-5} \text{Torr}) for 8 h at 70 °C. When removed from the vacuum oven the product appeared as
a yellow, highly viscous oil, which when cooled to room temperature became a hard yellow solid; yield 90%.

Step 2: Esterification:

To a 50 mL two-neck pear shaped flask with a dry nitrogen feed were added 4-(1H-Pyrrole-1-yl) benzoyl TEG (0.2254 g, 0.100 mmol), 6-(2,4-dinitrophenylamino hexanoyl chloride (0.1864 g, 0.357 mmol), and 10 mL of THF, freshly distilled from sodium benzenophene. The reaction mixture was gently heated to promote dissolution and then allowed to mix well for 15 min. Sodium hydroxide (0.125 g, 2.5 mmol) was added, and the reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture precipitated out of solution. The mixture was filtered and washed with THF and Hexane through a 30 mL, 10-20 μm Buchner glass-frit filter to remove the unreacted monomer.

The desired product formed a dark brown precipitate

3.3.4. Synthesis of the Functional Polymers, PPy-PEG350 and PPy-PEG-DNP

Two structurally different series of functional polymers, polypyrrole-2, 4-(1H-Pyrrole-1-yl) Benzoic Polyethylene glycol (PPyPEG350) and polypyrrole-[2, 4-(1H-Pyrrole-1-yl) Benzoic polyethylene glycol]-[2, 4-(1H-Pyrrole-1-yl) Benzoic Tetraethylene glycol DNP-e-amino-n-caproic acid] (PPyPEGDNP), were prepared by oxidative-redox coupling polymerization with ammonium persulfate in DH20. The synthetic methods are shown in Schemes 1. The functional polymers were obtained in yields of 60% or higher. The percent of polymers obtained that were soluble in various organic solvents were typically 50%. The polymers were black powders. Table 1 lists the composition and molecular weight of polymers prepared by Scheme 1.
PPyPEG350 polymer is composed of a pyrrole monomer and 4PyPEG350 macromonomer at various molar ratios respectively (see Table 1) respectively in 3 mL of deionized water under stirring while flushed with dry nitrogen. 1.8 mL of 0.36 M ammonium peroxide sulfate solution was then added into the above mixture under magnetic stirring. The polymerization was continued for about 24 h. The resulting solution was poured into a sufficient amount of water to wash out the remaining monomer and ammonium peroxide sulfate. Finally, the precipitate was collected and dried in an vacuum oven under reduced pressure \((10^{-3} \text{ to } 10^{-5} \text{ Torr})\) for more than 48 h at 70 °C. After drying the soluble product was extracted from the crude product with ethanol using a soxhlet extractor apparatus. The ethanol extract was evaporated and dried under vacuum. The solvent extracted polymer was soluble in THF, DMSO, methanol, n-methyl pyrrolidone and ethyl acetate.

The PPyPEGDNP terpolymer is composed of a pyrrole monomer and 4PyPEG350, 4PyTEGDNP macromonomers at various feed ratios respectively (see Table 1) in 3 mL of deionized water under stirring, followed by the addition of 1.8 mL of 0.36 M ammonium peroxide sulfate solution under magnetic stirring. The polymerization was continued for about 4 hrs. The resultant solution was poured into a sufficient amount of water to wash out the remaining monomer and ammonium peroxide sulfate. Finally, the precipitate was collected and dried under an vacuum oven under reduced pressure \((10^{-3} \text{ to } 10^{-5} \text{ Torr})\) for more than 48 h at 70 °C. After drying the crude product was washed with ethanol using a soxhlet apparatus. The ethanol extract was evaporated and dried under
vacuum. The solvent extracted polymer was soluble in THF, DMSO, methanol, n-methyl pyrrolidone and ethyl acetate.

### 3.4 Equilibrium Binding of PPy-PEG-DNP Ligands to FITC-modified IgE

Equilibrium binding studies using FITC-modified IgE fluorescence measurements were obtained on a SLM 8000 fluorimeter in time based acquisition mode. PPyPEGDNP polymer solution was added to a cuvette of buffer solution under continuous stirring at room temperature. FITC was excited at 490 nm, and emission was monitored at 520 nm in order to observe quenching.

### 3.5 Electrospinning of Polymers

PPyPEGDNP polymer was electrospun on to a gold coated silicon substrate using a method described previously. PPyPEGDNP and partially sulfonated polystyrene (Mₗ = 45,000) polymers were dissolved in THF/H₂O (9:1) at a indicated concentrations respectively. The solution was mounted on a silicon tip. A voltage of about 10 kV was applied to the tip, causing the release of a polymeric fluid jet towards the ground electrode which was collected as an interconnected mat or individual fibers.

### 3.6 Confocal Microscopic Studies with polymer wires

A Bio-Rad MRC-1024 confocal system equipped with an Argon-Krypton laser and attached to Olympus IX70 inverted microscope was used for the study of the functionalized wires. The excitation wavelength was 488 nm and the emission wavelength was 520 nm. Wires collected on gold coated Silicon substrates were incubated with Alexa488-IgE in BSS for about 20 min, washed with BSS buffer and observed under the microscope.
3.7 Characterization

3.7.1. $^1$H Nuclear Magnetic Resonance (NMR)

$^1$H NMR spectra were obtained using a Bruker ARX 400 NMR spectrometer in D20, and DMSO. Trimethylsiloxane was used as internal standard.

3.7.2. Nicolet Impact 400 Fourier-Transform Infrared Spectroscopy

Infrared studies were carried out on a Nicolet 510P FTIR spectrometer.

3.7.3. Transmission Electron Microscopy (TEM)

Transmission electron microscopy was obtained on a 200 kV JEOL JEM-2100 instrument.

3.7.4. Nicolet Impact 400 Fourier-Transform Infrared

Thermogravimetric analysis (TGA) measurements were made with a SDT 2960 Simultaneous DA-TGA at a heating rate of 20 °C/min.

3.7.5. Differential Scanning Calorimeter (DSC)

Differential scanning calorimetry was performed on a Seiko DSC220 at a heating rate of 5 °C per minute and the reported values were obtained from the second heating after quench cooling the sample. The $T_g$'s were taken at the midpoints of the heat capacity changes, the $T_m$'s were taken at the maximum of the enthalpy endothermic peaks. The DSC was calibrated for temperature and enthalpy using an Indium standard under nitrogen gas atmosphere.

The matrix-assisted laser desorption/ionization - time of flight (MALDI-TOF) measurements were performed on a Bruker Reflex III spectrometer equipped with a nitrogen laser (337 nm) in reflector mode. An acceleration voltage of 20 kV and a reflector voltage of 23 kv together with matrix/low-mass suppression up to 3000 g/mol were used.

3.7.7. Conductivity Measurements

Sample Preparation: The polymers were pressed into pellets under three metric tons of pressure and vacuum dried overnight in a vacuum oven at 60°C.

Impedence Measurements: Electroactive characterization of the polymers was performed using the geometry of two copper plate probes within a sealed vessel under nitrogen atmosphere. A small amount of phosphorous pentaoxide was placed inside of the sealed chamber to maintain a dry environment. A 4192 A LF Impedance Analyzer was used to measure the potential difference between probes.

The conductivity of the polymer pellets were calculated from the bulk resistance using the following equation;
\[ \sigma = \frac{D}{A \times R_b} \]

\( \sigma \) is conductivity;

\( D \) is the thickness of the sample

\( A \) is the section area of the sample

\( R_b \) is the bulk resistance of the sample

3.7.8. **Viscometry Measurements**

Dilute solutions were studied using a Ubbelohde capillary viscometer with diameter of 0.56 mm (at constant temperature, \( T \) 25 °C).

3.7.9. **Rheological Measurements**

Rheological measurements were carried out under steady shear using LS40 Contraves using couette geometry. Oscillatory measurements using a Rheometric Scientific SR-5000, which is a stress-controlled rotational rheometer, using a parallel plate-plate geometry with a diameter of 50 mm.
Figure 3.7.1. Schematic of parallel Plate Plate Geometry of a diameter of 50 mm used to carry out Oscillatory rheology experiments.
CHAPTER IV
RESULTS AND DISCUSSION

4.1 Synthesis and Properties of polypyrrole-2, 4-(1H-Pyrrole-1-yl) Benzoic Polyethylene glycol abbreviated PPyPEG350 and polypyrrole-[2, 4-(1H-Pyrrole-1-yl) Benzoyl polyethylene glycol]-[2, 4-(1H-Pyrrole-1-yl) Benzoyl Tetraethylene glycol Dinitrophenyl-ε-amino-n-caproyl] abbreviated PPyPEGDNP.

In oxidation polymerization the sequence of addition of monomers and macromonomers (a large polymerizable monomer) is an important issue in the successful synthesis of a random or statistical terpolymer (i.e. random sequences of monomers). In the present study one monomer (pyrrole) and two macromonomers (4PyPEG350, 4PyTEGDNP) are polymerized to form a terpolymer. The polymerizable component in monomers and macromonomers is pyrrole. Thus, the phenomena that primarily effects the sequence of addition of monomers is steric hindrance. Steric hindrance occurs when the size or bulkiness of a molecule inhibits chemical reactions. It is thought that due to the bulky side groups attached to the pyrrole in the macromonomers that steric hindrance between adjacent macromonomers will prevent their reaction. Therefore, it is thought that one or more pyrrole monomers will link the macromonomers during polymerization. Steric hindrance can be further exploited to inhibit branching (non-linear polymer growth) during polymerization. The result is the polypyrrole chain will polymerize at its primary reactive sites, the α-position, resulting in a linear polymer. In this way steric
hindrance is exploited as a useful tool to aid in the regular distribution of the macromonomers and its solublizing and biological active groups, along the polymer chain. Therefore, the formation of broad molecular weight distributions are reduced and higher molecular weight polymers can be achieved.

4.1.1. Functionalization of the 4-(1H-Pyrrole-1-yl) benzoyl Polyethylene glycol 350 (4PyPEG350) and 4-(1H-Pyrrole-1-yl) benzoyl Tetraethylene glycol Dinitrophenyl-ε-amino-n-caproyl (4PyTEGDNP) Macromonomers.

The macromonomers, 4-(1H-Pyrrole-1-yl) benzoyl polyethylene glycol 350, (4PyPEG350) and 4-(1H-Pyrrole-1-yl) benzoyl tetraethylene glycol dinitrophenyl-ε-amino-n-caproyl (4PyTEGDNP), were synthesized using a series of esterification reactions. The synthesis of the macromonomers combines a pyrrole molecule, which are incorporated into an electrically active polypyrrole backbone, with an ethoxy sidechain, which makes the resultant polymer organic solvent soluble. The addition of the N-2,4-DNP-ε-amino-n-caproic acid in the case of the 4PyTEGDNP macromonomer made the molecule biologically active towards IgE protein. The macromonomers were characterized by solution 400 MHz $^1$H nuclear magnetic resonance (NMR) spectroscopy. The $^1$H spectrum for macromonomer 4PyPEG350 (see Figure 4.1.2) shows the following resonances: δ 3.2 ppm (-OCH$_3$); δ 3.5 ppm ([-CH$_2$CH$_2$O-]$_n$) and δ 6.4 - 7.9 ppm (-CH on the pyrrole and benzyl units). The $^1$H NMR graph of 4PyPEG350 is consistent with the representative molecular structure inset in Figure 4.1.2. The 400 MHz $^1$H spectrum for macromonomer 4PyTEGDNP (see Figure 4.1.3) shows the following resonances: δ 1.2 ppm (-CH$_2$- adjacent to propionate group); δ 1.3 ppm (-CH$_2$- adjacent to methyl amine
group); \( \delta 2.1 \text{ ppm} \) (-CH\(_2\) adjacent to acetate group); \( \delta 2.7 \text{ ppm} \) (-CH\(_2\) adjacent to the carboxyl group); \( \delta 3.1 \text{ ppm} \) (-CH\(_2\) adjacent to the amine group); \( \delta 3.5 \text{ ppm} \) ([-CH\(_2\)CH\(_2\)O]\(_n\)) and \( \delta 6.4 - 8.0 \text{ ppm} \) (-CH on the pyrrole and benzyl units). Resonances between 8.3 and 8.5 ppm are attributed to meta- protons to the DNP unit. The \(^1\text{H} \text{NMR}\) spectra of 4PyTEGDNP is consistent with the representative molecular structure inset in Figure 4.1.3.

Figure 4.1.6 shows the Fourier transform infrared (FTIR) spectra of macromonomer 4PyTEG (a), a precursor to 4PyTEGDNP (b). The FTIR KBr disk transmission spectrum of hydroxyl functional group attached to the 4PyTEG macromonomer precursor shows a broad absorption band around 3500 cm\(^{-1}\). The hydroxyl functional group broad infrared peaks overlaps with N-H and C-H absorption peaks from the pyrrole ring at 3500 and 3186 cm\(^{-1}\), respectively.\(^{103}\) Thus, the broad infrared peaks between 3500 and 3186 cm\(^{-1}\) region that correspond to the hydroxyl group on the 4PyTEG macromonomer, which disappears after subsequent reactions, cannot be confirmed using FTIR spectroscopy. The asymmetric and symmetric stretching for –NO\(_2\) of DNP observed at 1541 cm\(^{-1}\) and 1348 cm\(^{-1}\), respectively can be identified. Elemental analysis of the 4PyPEG350 and 4PyTEGDNP macromonomers was carried out and has been summarized in Table 4.1.1. The difference in the calculated and found elemental composition for 4PyPEG350 macromonomer is 3.17% (C), 4.73% (H), and 2.59% (N), while for 4PyTEGDNP macromonomer is 0.40% (C), 0.86% (H), and 0.66% (N). The larger difference between the calculated and found elemental compositions for 4PyPEG350 than 4PyTEGDNP macromonomers is attributed to the polydispersity
(polymer chain lengths that vary over a wide range of molecular masses) of polyethylene glycol (PEG) 350 incorporated in the 4PyPEG350 macromonomer is of a higher polydispersity than (tetraethylene glycol) TEG incorporated in the 4PyTEGDNP macromonomer.

4.1.2. Synthesis of the Functional Polymers, PPyPEG350 and PPyPEGDNP

The polypyrrole-2,4-(1H-Pyrrole-1-yl) benzoyl polyethylene glycol 350 abbreviated PPyPEG350 and polypyrrole-[2, 4-(1H-Pyrrole-1-yl) benzoyl polyethylene glycol]-[2, 4-(1H-Pyrrole-1-yl)] benzoyl tetraethylene glycol dinitrophenyl -ε-amino-ω-caproyl] abbreviated PPyPEGDNP, were copolymerized by redox-oxidation from various molar ratios of the pyrrole monomer and 4PyPEG350 and 4PyTEGDNP macromonomers forming a statistical terpolymer (see Table 4.1.2.). All polymerizations were carried out using ammonium persulfate ((NH₄)₂S₂O₈) as a oxidizing initiator. The amount of DNP present in the polymer was controlled by varying the molar ratio of the 4PyTEGDNP macromonomer. The PEG and TEG side chain lengths were deliberately designed to be short segments in order to bring the biologically active component, DNP, and its antigen, IgE, relatively close to the electroconductive polypyrrole backbone. This close proximity of IgE to the polypyrrole backbone is expected to affect the conductivity of the polypyrrole backbone.¹⁴

Varying feed ratios of pyrrole monomer and 4PyPEG350 and 4PyTEGDNP macromonomomers were added to the reaction vessel, followed by deionized water. Pyrrole monomer is not miscible with water, therefore, a small amount of ethanol was added to the reaction mixture and the mixture heated to promote a solution homogeneity. After an
hour the solution turned a black color, which is indicative of a highly conjugated polymer system, and precipitated out of solution. This resulted in the functional polypyrrole. Experimental samples 1 – 5 (see Table 4.1.2) show that as the molar amount of pyrrole increases less of the resultant polymer became soluble in ethanol. The decreased the solubility of the resultant terpolymer with increased feed ratio of pyrrole monomer indicated that a increased number of pyrrole units were incorporated into the polypyrrole backbone. The decreased solubility of the terpolymer greatly limits the processibility of the polymer in films and fibers. Therefore, it is preferable to use terpolymers with the minimum possible amount of pyrrole for greatest possible processibility of the resultant polymer. Geometry optimized model of PPyPEGDNP, simulated using nanoscale molecular dynamics (NAMD), depicts the lowest energy conformation for the PPyPEGDNP terpolymer. NAMD simulations indicate that the DNP groups (circled) are sticking out from the polymer backbone and available for binding to IgE.

The terpolymers were characterized by solution 400MHz $^1$H NMR. The 400MHz $^1$H NMR spectra for the copolymer PPyPEG (sample # 10) (see Figure 4.1.4) shows the following resonances: δ 3.2 ppm (-OCH$_3$); δ 3.5 ppm ([-CH$_2$CH$_2$O-]$_n$) and δ 6.4 - 7.9 ppm (-CH on the pyrrole and benzyl units). The 400MHz $^1$H NMR spectra for the terpolymer PPyPEGDNP (sample # 2) (see Figure 4.1.5) shows the following resonances: δ 0.9 ppm (-CH$_2$- adjacent to propionate group); δ 1.25 ppm (-CH$_2$- adjacent to methyl amine group); δ 1.5 ppm (-CH$_2$- adjacent to acetate group); δ 2.5 ppm (-CH$_2$- adjacent to the amine group); δ 3.5 ppm ([-CH$_2$CH$_2$O-]$_n$) and δ 6.4 - 8.0 ppm (-CH on the pyrrole
and benzyl units). Resonances between 8.5 and 8.8 ppm are attributed to meta-protons to the DNP unit. The resonances of PPyPEG and PPyPEGDNP polymers are consistent with the structure of the respective terpolymers.

Figure 4.1.7 shows the FT-IR spectra of PPyPEG350, sample # 10 (a) and PPyPEGDNP, sample # 2 (b). Figure 4.1.7(b) shows the FT-IR spectra of 2:1:1 PPyPEGDNP, sample # 2. The aromatic NO2 on the DNP shows symmetric and asymmetric stretching observed at 1536 cm\(^{-1}\) and 1351 cm\(^{-1}\) respectively. The 400MHz \(^1\)H NMR and the FT-IR spectra for the 4PyTEGDNP macromonomer and PPyPEGDNP terpolymer confirms that the dinitrophenyl functional group, which will serve as the sensing element, has been successfully covalently attached to the macromonomer and subsequently polymerized into the resultant terpolymer.
Figure 4.1.1 Geometry optimized simulation of polypyrrole-[2, 4-(1H-Pyrrole-1-yl) Benzoyl polyethylene glycol]-[2, 4-(1H-Pyrrole-1-yl) Benzoyl Tetraethylene glycol Dinitrophenyl-ε-amino-η-caproyl] abbreviated PPyPEGDNP using Nanoscale Molecular Dynamics.
Figure 4.1.2 400MHz $^1$H NMR spectra of 4-(1H-Pyrrole-1-yl) benzoyl Polyethylene Glycol 350 abbreviated 4PyPEG(350) Macromonomer in D2O
Figure 4.1.3 400MHz $^1$H NMR spectra of 4PyPEG350 and 4-(1H-Pyrrole-1-yl) benzoyle Tetraethylene Glycol Dinitrophenyl abbreviated 4PyPEGDNP Macromonomer in D2O
Figure 4.1.4 400MHz $^1$H NMR spectra of polypyrrole-2, 4-(1H-Pyrrole-1-yl) Benzoic Polyethylene glycol abbreviated PPyPEG350, sample # 10 polymer in DMSO
Figure 4.1.5 400MHz $^1$H NMR spectra of polypyrrole-[2, 4-(1H-Pyrrole-1-yl) Benzoic polyethylene glycol]-[2, 4-(1H-Pyrrole-1-yl) Benzoic Tetraethylene glycol DNP-$\epsilon$-amino-$n$-caproic acid] abbreviated PPyPEGDNP, sample # 4 terpolymer in DMSO
Figure 4.1.6 FT-IR spectra of 4PyTEG (a) precursor to 4PyPEGDNP (b) macromonomer
Figure 4.1.7 FT-IR spectra of PPyPEG350, sample #10 (a) and PPyPEGDNP, sample #4 (b).
Table 4.1.1. Elemental Composition of Macromonomer 4PyPEG350 and 4PyTEGDNP.

<table>
<thead>
<tr>
<th>Macromonomer</th>
<th>C (%)</th>
<th>H (%)</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4PyPEG350</td>
<td>Calculated</td>
<td>83.64</td>
<td>10.78</td>
</tr>
<tr>
<td></td>
<td>Found</td>
<td>80.47</td>
<td>6.050</td>
</tr>
<tr>
<td>4PyTEGDNP</td>
<td>Calculated</td>
<td>79.78</td>
<td>8.200</td>
</tr>
<tr>
<td></td>
<td>Found</td>
<td>79.38</td>
<td>7.340</td>
</tr>
</tbody>
</table>

Table 4.1.2. Molar ratio composition of Pyrrole : 4PyPEG(350) : 4PyTEGDNP, conductivity

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Monomer Ratio</th>
<th>% Insoluble</th>
<th>% Soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:1:1</td>
<td>44.28%</td>
<td>55.72%</td>
</tr>
<tr>
<td>2</td>
<td>2:1:1</td>
<td>50.50%</td>
<td>49.50%</td>
</tr>
<tr>
<td>3</td>
<td>3:1:1</td>
<td>68.15%</td>
<td>31.85%</td>
</tr>
<tr>
<td>4</td>
<td>4:1:1</td>
<td>70.95%</td>
<td>29.05%</td>
</tr>
<tr>
<td>5</td>
<td>6:1:1</td>
<td>70.45%</td>
<td>29.55%</td>
</tr>
<tr>
<td>6</td>
<td>1:2:1</td>
<td>66.65%</td>
<td>33.35%</td>
</tr>
<tr>
<td>7</td>
<td>2:1:2</td>
<td>74.39%</td>
<td>25.61%</td>
</tr>
</tbody>
</table>

Table 4.1.3. Molar ratio composition of Pyrrole : 4PyPEG(350) : 4PyTEGDNP, conductivity ($\sigma$), inherent viscosity ($\eta_{inh}$).

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Monomer Ratio</th>
<th>% Yield</th>
<th>$\sigma$(s/cm)</th>
<th>$\eta_r$</th>
<th>$\eta_{sp}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:1:1</td>
<td>68.3</td>
<td>2.6x10^{-6}</td>
<td>1.121</td>
<td>0.121</td>
</tr>
<tr>
<td>2</td>
<td>2:1:1</td>
<td>69.0</td>
<td>6.1x10^{-5}</td>
<td>1.090</td>
<td>0.090</td>
</tr>
<tr>
<td>3</td>
<td>3:1:1</td>
<td>88.8</td>
<td>6.1x10^{-5}</td>
<td>1.099</td>
<td>0.099</td>
</tr>
<tr>
<td>4</td>
<td>4:1:1</td>
<td>77.4</td>
<td>4.9x10^{-6}</td>
<td>1.038</td>
<td>0.038</td>
</tr>
<tr>
<td>5</td>
<td>6:1:1</td>
<td>72.6</td>
<td>3.9x10^{-5}</td>
<td>1.074</td>
<td>0.074</td>
</tr>
<tr>
<td>6</td>
<td>1:2:1</td>
<td>69.0</td>
<td>2.7x10^{-6}</td>
<td>1.075</td>
<td>0.075</td>
</tr>
<tr>
<td>7</td>
<td>2:1:2</td>
<td>59.1</td>
<td>2.6x10^{-5}</td>
<td>1.074</td>
<td>0.075</td>
</tr>
<tr>
<td>8</td>
<td>1:1:1*</td>
<td>55.4</td>
<td>2.4x10^{-6}</td>
<td>1.080</td>
<td>0.080</td>
</tr>
<tr>
<td>9</td>
<td>1:1:0</td>
<td>65.2</td>
<td>6.2x10^{-5}</td>
<td>1.089</td>
<td>0.090</td>
</tr>
<tr>
<td>10</td>
<td>10:1:0</td>
<td>66.7</td>
<td>6.9x10^{-5}</td>
<td>1.019</td>
<td>0.096</td>
</tr>
</tbody>
</table>

*2000 MW peg side chain that was incorporated into the 4PyPEG(2k) macromonomer
4.2 Molecular Weight Measurements

Gel permeation Chromatography experiments were initiated, but proved unsuccessful due the adhering of the polymer samples to the column packing material. Matrix-assisted laser desorption/ionization- time of flight (MALDI -TOF) mass spectroscopy analysis of various polymer samples resulted in the successful characterization of samples # 3,4,9 and 12. A partial list of number average ($M_n$), weighted average molecular weights ($M_w$) and polydispersity ($M_w/\overline{M}_n$) of the polymer analytical data is listed in table 4.1.4. Various salts were dissolved in THF to test their suitability as matrix solution for the terpolymer. Potassium trifluoroacetate was determined to have formed salt/polymer complexes that were successfully ionized in the MALDI -TOF instrument. MALDI-TOF measurements showed that the $M_n$, $M_w$ and $M_w/M_n$ were typically 55,000, 77,000 and 1.40 respectively. MALDI_TOF graphs representative of those that have been produced are shown in Figures 4.2.1a and 4.2.1b.

The comb like structure of the PPyPEGDNP prohibits the accurate sizing of the polymer chains due to its dense structure. The $M_n$, $M_w$ and $M_w/M_n$ of the measured terpolymers do not show wide variation in samples where the molar ratios of monomers and macromonomers are different. This may be due to the extraction of the soluble components from the crude product. This procedure would select for lower molecular weight polymer chains and polymer chains composed of higher ratios of 4PyPEG350 and 4PyTEGDNP macromonomers. Thus, the resultant terpolymers that are analyzed would have similar molecular weights. The molecular weights obtained for the terpolymer were higher than previously synthesized peg functionalized conductive polymers.57 Higher
mwp polymers have been shown to improve processing of the polymer material into fibers and films.\textsuperscript{59}

\begin{table}[h]
\centering
\begin{tabular}{llll}
\hline
Sample Number & M\textsubscript{n} (MALDI-TOF) & M\textsubscript{w} (MALDI-TOF) & M\textsubscript{w}/M\textsubscript{n} (MALDI-TOF) \\
\hline
1 & 55kDa & 77kDa & 1.4 \\
2 & 56kDa & 74kDa & 1.3 \\
8 & 57kDa & 77kDa & 1.4 \\
10 & 57kDa & 82kDa & 1.4 \\
\hline
\end{tabular}
\caption{A partial list of number average (M\textsubscript{n}), weighted average molecular weights (M\textsubscript{w}), and polydispersities (M\textsubscript{w}/M\textsubscript{n}) of functionalized polypyrrole determined by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectroscopy.}
\end{table}

\textbf{4.3 Thermal Properties Analysis}

To determine the thermal stability of the functional polymers, thermal gravimetric analysis (TGA) was carried out. TGA experiments were carried out on PPyPEGDNP, sample #2 in air (see figure 4.3.1). Sample #2 showed a three phase decomposition pattern. The weight loss observed at 50 °C, 135 °C and 210 °C are attributed to the loss of water (1.10 wt.-%), dopant (9.28 wt.-%) and decomposition polymer side chain (48.33 wt.-%), respectively. Approximately 27% of the polymer remained after 600 °C. The thermal gravimetric scan of PPyPEGDNP is comparable to other chemically synthesized polypyrroles.\textsuperscript{104} Therefore the results of the TGA experiments confirm that a terpolymer had been synthesized.

To further elucidate the thermal behavior of the functional polymers, differential scanning calorimetry (DSC) studies were carried out over the temperature
range of 50 °C to 200 °C. This temperature range was chosen because TGA experiments showed that the onset of polymer degradation began at 135 °C. Figure 4.3.1 shows the DSC thermograms of (a) PPyPEG350, sample # 10, (b) PPyTEG, sample # 9, (c) PPyPEG(2K)DNP, sample # 8 and (d) PPyPEGDNP, sample # 2. The thermogram of PPyPEG350, sample # 10 shows two endothermic peaks at 134 °C and 164 °C, while PPyPEGDNP, sample # 2 (b) shows two endothermic transitions at 135 °C and 164 °C. These endothermic transitions of PPyPEG350, sample # 10 and PPyPEGDNP, sample # 2 where determined by TGA to be attributed to loss of dopant and onset of polymer degradation. The PPyPEG(2K)DNP, sample # 8 showed a clear melting temperature at 50 °C that was attributed to the 2000 MW peg side chain that was incorporated into the 4PyPEG(2k) macromonomer which was subsequently polymerized into the 1:1(2k):1 molar ratio terpolymer. The PPyTEG, sample # 9 polymer did not show a distinguishable endothermic peaks. The endothermic peak above 135 °C for both samples # 8 and 9 is attributed to loss of dopant and onset of polymer degradation. The observations that a melting peak is present for the terpolymer containing 2000 MW peg chain and not for terpolymers containing 350 MW peg chains indicate that the ethoxy side chain segments are too short to observe phase transitions. This is consistent with other comb pegylated polypryrroles. The various characterization method, such as, NMR, FTIR, TGA and DSC has yielded data which confirm that the two macromonomers where successfully synthesized and polymerized into an terpolymer, while further adding to the conceptual understanding of the substituted macromonomers and CP's.
4.4 UV-vis Absorption Spectra.

The ultra violet - visible (UV-vis) spectrum of the self-doped copolymer and terpolymer in ethanol solution shows peaks at 221nm and 271nm, attributed to the DNP and copolymerization products of ethoxy side chain functionalized pyrroles, respectively. Because N-(2,4-Dinitrophenyl)-e-amino-n-caproic acid (DNP-OH) AND 2,4-dinitrophenylamino hexanoyl chloride (DNP-Cl) alone gives the absorbance at 221 nm (see Figure 4.4.1 I and II). Decreased absorbance at 271 nm (see Figure 4.4.1 V, VI and VII) indicates decreased solubility of the copolymer and terpolymer. These observations support our conclusion that, at higher pyrrole feed ratio, the terpolymer will have longer polypyrrole chain segments with less inclusion of macromonomer I and II, which makes it insoluble.
Figure 4.2.1 MALDI-TOF graphs of PPyPEGDNP. Sample #1 shows a Mn of 55,000, Mw of 77,000 and a PDI of 1.41(A) and sample #7 was not detected.
Figure 4.3.1 TGA of PPyPEGDNP (b), sample # 4
Figure 4.3.2 DSC thermogram of (a) PPyPEG350, sample # 10, (b) PPyTEG, sample # 9, (c) PPyPEG(2k)DNP, sample # 8 and (d) PPyPEGDNP, sample # 2
Figure 4.4.1 UV-Vis spectra of (I) DNP-OH, (II) DNP-Cl, macromonomers (III) 4PyTEG, (IV) 4PyTEGDNP, (V) 4PyPEG350, (VI) PPyPEG350 (sample #10) and (VII) PPyPEGDNP (sample #2).
4.5 Conductivity Measurements

The electrical conductivity of PPyPEGDNP and PPyPEG350 polymers were measured in a compressed pellet form at room temperature after the sample was dried in a vacuum at 30 °C over 24 h. The PPyPEGDNP and PPyPEG350 polymers were typically in the semiconducting range with values $2.6 \times 10^{-6}$ to $6.1 \times 10^{-5}$ S cm$^{-1}$. The conductivity of the PPyPEGDNP terpolymer is comparable to the conductivities of previously synthesized pegylated polypyrrole ($\sigma = 10^{-3}$ - $10^{-4}$ S cm$^{-1}$). It is of note that that conductivities of highly doped chemically synthesized polypyrrole have been obtained in the range of 0.05 to 0.20 S cm$^{-1}$. There was not a significant difference in the conductivity of terpolymers and copolymers of various molecular weight ratios (see Table 4.1.2), as would be expected for product with higher molar ratios of pyrrole. This is due to the extraction of the soluble components from the crude product. This procedure would select for lower molecular weight polymer chains and polymer chains composed of higher ratios of 4PyPEG350 and 4PyTEGDNP macromonomers.

4.6 Viscosity

Dilute salt-free polymer solutions were studied using a Cannon-Ubbelohde number 100 dilution capillary viscometer with diameter of 0.56 mm. The measurements were conducted in isotemperature water bath at, plus or minus 0.02 °C, 30 °C temperature. The solute was dissolved in ethanol solvent. The specific viscosity of PPyPEGDNP, sample # 4, has been plotted against concentration (Figure 4.6.1). The molecular weight, concentration, solvent properties and polymer morphology in solution
are important properties which affect the diameter and morphology of electrospun fibers, which will be discussed in greater detail later in this text.\textsuperscript{23} Thus, to understand the terpolymer morphology in solution, the viscosity of PPyPEGDNP, sample # 2 was determined at various concentrations. The specific viscosity of PPyPEGDNP, sample # 4 was linear up to a six weight percent (wt\%) concentration, after which a higher linear slope was observed. The overlap concentration (C*) was found to be 6 wt\%.

In order to determine whether the terpolymer formed aggregates in solution the reduced viscosity by concentration was plotted against concentration. Figure 4.6.2 shows the polymer concentration dependence of the reduced viscosity of the PPyPEGDNP solution. The viscosity of PPyPEGDNP was initially observed, then decreased with the increases in the polymer concentration in the low concentration range below 7 wt \%, while it was almost constant in a concentration range of 7-10 wt \% and increased with the increase beyond 10 wt \%. This upturn of reduced viscosity in the high concentration range, above 10 wt \%, is associated with a shear-induced macrostructure interaction by the capillary flow.\textsuperscript{107} It is of note that the effective shear rate range of typical capillary rheometers is 300–500 s\textsuperscript{-1}.\textsuperscript{108,109} These viscosity studies showed that the polymer in solution exists in either aggregated or isolated supramolecular aggregates. Polymer aggregation behavior is strongly correlated to interchain electrostatic forces. These interchain interactions can be significantly affected by ionic interactions. Therefore, the affects of the addition of salt to the polymer was studied, as will be discussed latter in this text.
4.7 Dynamic Light Scattering and Scanning electron microscopy Studies

4.7.1. Dynamic Light Scattering Polymer Solution Studies

Dynamic Light Scattering (DLS) studies were carried out in order to further investigate aggregation behavior in 5 wt% PPyPEGDNP ethanol solution. The solution was filtered using a Whatman 1μ pore size nylone syringe filter. The solution appeared to be clear and homogeneous. Figure 4.7.1(a) shows the sinusoidal shaped normalized autocorrelation functions for PPyPEGDNP at an 90°. The DLS data was analyzed with the use of the algorithm program CONTIN to obtain the hydrodynamic radius distribution \( f(R_h) \). Polymer shape in addition to, hydrodynamic interactions determine the hydrodynamic radius.\(^{110} \) The resultant relaxation time distributions of the terpolymer is depicted in figure 4.7.1 (b). The DLS spectra showed the presence of aggregates of hydrodynamic radius \( (R_h) \) from 175 to 969 nm. Because pyrroles are morphologically rigid rods and steric hindrance present within the polymer system the \( R_h \) would approximately be 10-15nm.\(^{111} \) After sonication experiments radius sizes observed via DLS where observed to be 174 nm (see Figure 4.7.2).

The aggregation of PPyPEGDNP was further analyzed using scanning electron microscopy (SEM). A 5wt% PPyPEGDNP polymer ethanol solution was air dried drop wise on a silicon surface and observed using SEM. Figure 4.7.3 (a and b) showed aggregates that were roughly spherical structures (500-200nm). The DLS and SEM studies presented confirmed the terpolymer formed aggregates in solution and in solid states. The appearance of a highly aggregated structure might be explained by the polyethylene oxide (PEO) oligomers complexing with the polypyrrole backbone owing to
hydrogen bonding interactions. The aggregate structures are thought to be made up of an polypyrrole hydrophobic core surrounded by a corona of PEO (see Figure 4.7.4). The aggregation behavior of the terpolymer in solution has the affect of encumbering the processing of the polymer into continuous polymer films and fibers.

4.7.2. Salt/Polymer Interaction studies

The effects of salt on the terpolymer's aggregation behavior was investigated using SEM and DLS. A 5wt% PPyPEGDNP, sample #2, ethanol solution was prepared from a one molar salt (potassium triflouro acetate) solution, which appeared to lead to smaller aggregates, as monitored by DLS (see Figure 4.7.5b). A smaller structure resulted from the addition of salt, the size of which ($R_n \approx 148-172$ nm). Analysis of the dried sample by scanning electron microscopy (SEM) (Figure 4.7.6 a, b and c) revealed that these aggregates were of smaller and of more uniform dimensions. It is thought that the effect of salt is to shield the dipole-dipole and hydrogen bonding interactions between the self-doped (charged) polypyrrole backbone and the peg side chain, therefore leading to a break-up of the aggregates in smaller segments. Figure 4.7.6 shows SEM images of structures roughly of this description. The reduction of the size of the aggregates using sonication and salt addition techniques is expected to improve the processing of the terpolymer. The limitation imposed on the processing of the terpolymer due to this aggregation behavior can be addressed by blending the polymer with a miscible high molecular polymer.
Figure 4.6.1 Viscosity vs. concentration of PPyPEGDNP, sample #4 which shows an overlap concentration \((C^*)\) at 6 wt%.

Figure 4.6.2 Changes in reduced viscosity of PPyPEGDNP, sample #2 solution as a function of polymer concentration.
Figure 4.7.1 Dynamic light scattering. Normalized field auto-correlation functions at 90° for a 3wt% ethanol PPyPEGDNP, sample # 2 (a). Corresponding size distributions after evaluation with the CONTIN algorithm (b)
Figure 4.7.2 Size distributions after evaluation with the CONTIN algorithm of pulse sonicated PPyTEGDNP, sample # 4 at 10 and 1 minute time periods
Figure 4.7.3 Scanning electron microscopy (SEM) images of PPyTEGDNP, sample # 4 dried on silicon surface

Figure 4.7.4 Proposed schematic of a polypyrrole backbone and PEO segment dipole interaction.
Figure 4.7.5 Normalized field auto-correlation functions (a) at 40° for a 3wt% ethanol PPyTEGDNP, sample # 4 the presence of one molar potassium trifluoroacetate (KTFA) ethanol solution sample. Corresponding size distributions after evaluation with the CONTIN algorithm (b).

Figure 4.7.6 SEM image of PPyTEGDNP, sample # 4 in the presence of one molar potassium trifluoroacetate (KTFA) ethanol solution deposited on a carbon-coated mica substrate.
4.8 IgE Binding Studies by PPyPEGDNP Series Ligands

To understand the binding effectiveness of pendant DNP ligands attached to the polypyrrole backbone, to anti-DNP IgE, equilibrium binding experiments were carried out. The terpolymer (2:1:1) PPyPEGDNP, sample # 2 was used in this experiment due to its partial solubility in water and aqueous buffers. In order to test the binding effectiveness of the ligands attached to the terpolymer backbone towards anti-DNP IgE, titrants of the terpolymer were pipette into a solution containing FITC-IgE. The solution also contained bovine serum albumin (BSA), which serves to occupy non-specific binding sites. We observed that the polymer PPyPEGDNP (sample # 4) binds and achieves a steady state of binding within a few seconds (see Figure 4.7.1). The binding observed during equilibrium binding experiments is due to an equilibrium reaction between the DNP attached to the terpolymer and fluorescently tagged IgE. The experimental data was fitted to a simplified bivalent binding model as described previously by Erickson et. al. The binding studies indicated that the terpolymer binds specifically and with high affinity to fluorescently tagged IgE in solution. To investigate the suitability of the terpolymer as a active component in biosensors for diagnostic purposes the study of the protein (IgE)-polymer surface interaction was carried out. To that effect, THF/Ethanol (85:15) solutions of PPyPEGDNP, sample # 4 were spin-coated on silicon, non-smooth and non-uniform films were obtained. The PPyPEGDNP, sample # 4 polymer were also electrospun from 5, 10 and 18 weight percent solutions and were found to produced fibers that were not uniform. In order to produce improved films and fibers for binding test the PPyPEGDNP, sample # 4 polymer was blended with partially
Figure 4.8.1 Equilibrium binding plot of PPyPEGDNP, sample # 4 to anti-DNP IgE

4.9 Film and Fiber Fabrication: PPyPEGDNP / Sulfonated Polystyrene polymer blends

sulfonated polystyrene (SO₃ PS, 45k) at various weight ratios in THF/Ethanol (85:15) solutions see Table 4.9.1. The polymer blends of PPyPEGDNP, sample # 4 and SO₃ PS, (45k) were spin-coated onto Silicon plate, resulting in smooth and uniform films and fibers. These polymer blends were also electrospun onto silicon surfaces using a electrospinning technique s previously described. The 50:50 polymer blend produced the more uniform and continuous films and fibers. The films where of a 1 µm thickness and the fibers deposited had a diameter of approximately 1 µm, as determined by confocal imaging. It is hypothesized that the pendant DNP groups will be localized at the fiber surface and will, therefore, be accessible to the DNP specific antibody. To investigate the binding effectiveness of these fibers to anti DNP IgE, they were incubated with the fluorescently labeled Alexa 488-IgE in the presence of BSA, which serves to occupy non-specific binding sites, solution for 20 minutes. The fluorescent micrographs of the fibers (see Figure 4.9.2 (a, b, c)) showed a significant increase in fluorescence after exposure to
Alexa 488-IgE. While for the films the fluorescent micrographs of the films (see Figure 4.9.1 (a, b)) obtained showed a small increase in fluorescence after exposure to Alexa 488-IgE. The fluorescent micrographs clearly show that the Alexa 488-IgE proteins interact and bind selectively with the DNP ligands on the polymer fiber surface.

The lower fluorescence intensity of the films compared with that of the fiber after exposure to fluorescently tagged IgE may be due to the DNP ligands being embedded in the polymer matrix during processing. While in the case of the polymer fibers electrospinning process may aid in availing to the surface of the fiber charged molecular species groups. These functionalized semi-conductive fibers will possibly make available a useful means of fashioning an electrically based antibody detection technique.

4.9.1 Composition molar ratio PPyPEGDNP, sample # 4 to partially sulfonated polystyrene (SO₃ PS, 45k) polymers dissolved in THF/Ethanol (85:15) solutions.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>PPyPEGDNP, sample # 4 / SO₃ PS, (45k)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80/20</td>
</tr>
<tr>
<td>2</td>
<td>65/35</td>
</tr>
<tr>
<td>3</td>
<td>50/50</td>
</tr>
<tr>
<td>4</td>
<td>20/80</td>
</tr>
</tbody>
</table>
Figure 4.9.1 Confocal image of polymer films, spin coated from THF on a silicon surface using PPyPEGDNP, sample # 4 (a) prior and (b) after being incubated with fluorescently labeled Alexa488-IgE for about 20 min. The thickness of film being 1000 nm

Figure 4.9.2 Confocal image of microfibers, electrospun from THF onto a silicon surface using PPyPEGDNP, sample # 4 (a) prior and (b, c) after being incubated with fluorescently labeled Alexa488-IgE for about 20 min. The diameter of the fiber being 500 to 1000 nm
4.10 Rheology: Steady and Oscillatory Shear Experiments

The results of an experimental investigation of the rheological behavior of functionalized polypyrrole (PPyPEGDNP, sample # 4) solutions at different concentrations were investigated using both steady and oscillatory shear methods. Rheological measurements were carried out under steady shear using LS40 Contraves and oscillatory measurements using a Rheometric Scientific SR-5000, which is a stress-controlled rotational rheometer. Efforts will be made to correlate oscillatory dynamic viscoelastic properties with steady state properties.

4.10.1. Steady Shear Experiment

Steady shear experiments on the PPyPEGDNP, sample # 4 solutions in ethanol at concentrations of 16wt%, 10wt% and 4wt% were performed. Steady shear experiments were carried out using a couette geometry. Figure 4.10.1 shows shear viscosity decreases with increasing the shear rate at constant Temperature. Also, the influence of change in concentration on the shear viscosity is demonstrated. The polymers at 16wt% and 10wt% displayed shear thinning as the shear rate was increased. While the 4wt% polymer solution exhibited slight Newtonian behavior. In general, the shear viscosity of pure polymer is characterized by two distinct regions, called the Newtonian and shear thinning regions. At low shear rate, the Newtonian region with independence of shear rate is observed, followed by the shear thinning region where the viscosity linearly decreases with an increase in the shear rate. In this polymer system, viscometry, DLS and SEM demonstrate that the polymers have aggregated, into roughly spherical structures. This may result in particle–polymer or particle–particle like interactions that increase with
concentration. The particle–particle interactions, which result in an increase in the shear viscosity may play a dominant role in the rheological behavior.

4.10.2. Steady Shear in the presence of salt Experiment

In order to increase polymer-polymer interaction potassium trifluoroacetate (KTFA) was added to the polymer solutions. The polymer-polymer interaction is thought to occur through the polyethylene oxide side chains complexing with metal ions in solution. Potassium trifluoroacetate was selected for these experiments because it is soluble in ethanol and it worked well as a matrix material in MALDI-TOF experiments. Steady Shear experiments were carried out on PPyPEGDNP, sample # 4 in the presence of 1M KTFA ethanol solutions at concentrations of 16wt%, 10wt% and 4wt%.

Figure 4.10.2 shows shear viscosity decreases with increasing shear rate at constant temperature. The influence of salt on the shear viscosity appears to have had a slight effect on the viscosities. It should be noted that an increase in salt concentration beyond one molar induces precipitation. It is thought that the effect of salt on the polymer system is that the aggregates, which are roughly spherical, are thought to have an outer polyethylene oxide side chain are no longer extended into solution after complexation with metal ions in solution. This may lead to a decrease in particle (aggregate)–polymer or particle–particle like interactions at constant volume.

4.10.3. Oscillatory Shear Experiments

Oscillatory shear experiments were carried out in order to study a limited range of dynamic properties of the terpolymer. Oscillatory shear experiments were carried out using a parallel plate-plate geometry with a diameter of 50 mm, rheological properties
were measured at several concentrations. A stress sweep test was conducted to identify the linear viscoelastic regime before the experiments of interest were conducted. In all frequency sweep tests, shear stress was fixed as 10 Pa and sample thickness was set to be 0.265 mm. Changes in the viscosity with respect to dynamic frequency were investigated in Figure 4.10.3. The shear viscosity decreases with increased shear rate at constant temperature. The storage modulus \((G')\) and dynamic loss modulus, \(G''\), of the PPyPEGDNP, sample # 4 are plotted in Figure 4.9.4. Both \(G'\) and \(G''\) slightly increase with an increase in the oscillatory frequency. Also, an increase in concentration resulted in an increase in \(G'\) and \(G''\) values, with little change in slope. The loss modulus is higher than storage modulus which indicates that material has a relatively short relaxation time. Figure 4.10.5 presents the variation of the loss tangent \((\tan \delta = G''/G')\) for the polymer solution at several concentrations with respect to frequency. A the loss tangent value above one indicates that the particles (roughly spherical aggregates) in the polymer solution are weekly associated. Otherwise, the particles are strongly associated. The loss tangent graph begins at one and increases with increasing oscillatory frequency displays. Thus, it can be concluded from this data that the polymers exit as roughly spherical aggregates in solution and that these aggregates are weakly associated. Studies have shown that polymer-polymer chain entanglement is important property in the ability to form electrospun fibers. Consequently, it can be concluded from the rheological data that the PPyPEGDNP, sample # 4 terpolymer failed to form smooth films and consistent fibers due to insufficient polymer-polymer chain entanglement during the spin casting and electrospinning process.
Figure 4.10.1 Apparent viscosities of PPYPEGDNP, SAMPLE # 4 solution as a function of shear rate conducted at concentrations from top to bottom of 16wt%, 10wt% and 4 wt%
Figure 4.10.2  Apparent viscosities of PPYPEGDNP, sample #4 in the presence of one molar potassium trifluoroacetate (KTFA) solution as a function of shear rate conducted at concentrations from top to bottom of 16wt%, 10wt% and 4 wt%
Figure 4.10.3 Complex viscosities with respect to frequency of PPYPEGDNP, sample # 4
Figure 4.10.4 Storage modulus ($G'$) and Loss modulus ($G''$) of PPYPEGDNP, sample #4
Figure 4.10.5 Tan δ of PEGNP, sample #4 with respect to oscillatory frequency at various concentrations.

Teg. (rad/s)

1000  100  10

0.1  1  10

-Tan Delta

- 20\% G
- 25\% G
- 35\% G
- 45\% G

100
CHAPTER V
CONCLUSION

The macromonomers 4PyPEG350 and 4PyTEGDNP were successfully incorporated into an electrically active polypyrrole backbone, with an ethoxy sidechain, which makes the resultant polymer organic solvent soluble. The composition and the structures of the polymers and monomers were determined by NMR spectroscopy, IR spectroscopy, UV-vis, TGA, DSC, DLS, SEM and elemental analysis. The PPyPEG350 copolymers and PPyPEGDNP terpolymers are of high molecular weights and typically found to be semiconductive. The PPyPEGDNP terpolymer has been shown to be thermally stable up to 135°C, thus the material is suitable for moderate temperature polymer processing.

Steric hindrance determined the sequence of addition of monomers and macromonomers of the synthesized random or statistical terpolymer. The bulky side groups attached to the pyrrole in the macromonomers caused steric hindrance between adjacent macromonomers preventing their reaction. One or more pyrrole monomers acted as a linker between the macromonomers during polymerization. Steric hindrance was exploited to inhibit branching (non-linear polymer growth) during polymerization. The resultant terpolymers possesed a minimum amount of pyrrole and a maximum amount of macromonomers I and II for greatest solubility and processibility. Suitable
substitution has the potential to lead to soluble conductive polymers allowing for processing by common methods including spin- of spray-coating and electropinning to fabricate films and fibers.

The comb like structure of the PPyPEGDNP terpolymer inhibits the accurate sizing of the polymer chains due to its dense structure. The molecular weights and polydispersity of the terpolymers did not show wide variation. The lack of size distribution was attributed to the extraction of the soluble components from the crude product that selects for lower molecular weight polymer chains and polymer chains composed of higher ratios of 4PyPEG350 and 4PyTEGDNP macromonomers. Thus, the resultant terpolymers have similar molecular weights and the terpolymer were higher than previously synthesized peg functionalized conductive polymers. Higher molecular weight polymers have been shown to improve processing of the polymer material into fibers and films.

The terpolymer was shown to form aggregates in solution and in the solid states. It was further demonstrated that the reduction in size of these aggregates could be addressed by sonication and the addition of salt. The terpolymer exists in solution as either aggregated or isolated supramolecular aggregates. The terpolymer aggregation behavior is strongly correlated to interchain electrostatic forces. These interchain interactions being significantly affected by ionic interactions of which the addition of salt to the polymer results in the break up aggregates. The salt ions serve to shield the dipole-dipole and hydrogen bonding interactions between the self-doped (charged) polypyrrole backbone and the peg side chain.
The addition of the N-2,4-DNP-\(\varepsilon\)-amino-n-caproic acid in the case of the 4PyTEGDNP macromonomer made the molecule biologically active towards IgE protein. It was observed that the terpolymer PPyPEGDNP complex binds to IgE in solution and quickly achieved a steady state of binding. Spin-coated films and electrospun fibers of the polymer blends were successfully fabricated and fluorescent tagged IgE proteins bound selectively with the DNP ligands on the surface of the electrospun fibers.

Thus, it has been demonstrated the semiconductive terpolymer is capable of specific interaction with a protein, thus the terpolymer serves as a model biologically active material, this is further evidence for the potential of semiconductive polymers serving as an effective active component in biosensors for diagnostic purposes. The prepared polymers are also an elastomeric material that possesses suitable properties for applications in aqueous sensing environments. The functional semi-conductive fibers incorporated into suitable electrical devices, such as field effect transistors, will conceivably make available a useful means of fashioning disposable commercial antibody detection devices.
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