CARBOXYLATED POLYTHIOPHENES: POLYMER BIOSENSORS IN LIQUID AND SOLID STATES*

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CARBOXYLATED POLYTHIOPHENES: POLYMER BIOSENSORS IN LIQUID AND SOLID STATES*

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ABSTRACT

The fluorescence of carboxylated and water-soluble poly(3-thiophene acetic acid) (PTAA) and poly[2,5-(3-carboxymethyl urethanyl ethyl) thiophene] (H-PURET) are quenched by low concentrations of methyl viologen in aqueous solutions. The Stern-Volmer constants (K_{sv}) for these aqueous solutions of polythiophenes are in the order of 3.5×10^6. However, in contrast, ionically adsorbed monolayers of these polythiophenes on solid supports show fluorescence quenching that is more than two orders of magnitude smaller. The effects of pH and the concentration of the methyl viologen on fluorescence quenching are reported.

Key Words: PTAA; HPURET; Methyl viologen; Biosensor; Luminescence quenching

*Dedicated to the memory of Professor Sukant K. Tripathy
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INTRODUCTION

A biosensor in which the fluorescence of a water-soluble conjugated polymer [poly(2-methoxy-5-propyloxy sulfonate) phenylene vinylene (MPS-PPV)] is quenched by cationic electron acceptor (methyl viologen, MV^{2+}) was reported to have shown Stern-Volmer constant (K_{sv}) of $\sim 1.7 \times 10^7$.[1,2] This large K_{sv} is attributed the formation of a weak complex and followed by fast photo-induced charge transfer between MPS-PPV and MV^{2+}. Photo-induced charge transfer occurs typically on a time scale of 650 fs that is much faster than radiative decay rates. Consequently the quantum efficiency for the non-radiative relaxation is close to unity.[3] The quantitative luminescence quenching can be measured by using the Stern-Volmer constant as follows:

$$\phi^\circ/\phi = 1 + K_{sv}[\text{quencher}]$$

where ϕ° and ϕ are the intensities of fluorescence in the absence and presence of quencher.

Based on this sensor system, a modified MV^{2+} quenching system has tested as a model of reversible biosensor.[4] An example is MV^{2+}-complexed biotin which can efficiently quench the fluorescence of MPS-PPV and recover the fluorescence after removal of the quenching agent due to the specific biotin-avidin interaction. This reversibility of luminescence quenching could be applied to the other chemical sensor systems as well.

Another class of conjugated polymers for this application is poly-thiophenes that have been explored for various optoelectronic devices including organic light emitting diodes,[5,6] solar cells,[7,8] and chemical sensors.[9] The advantage of polythiophenes is its environmental stability and the possibility of introducing side chains to modify the solubility and other physical properties.[8,10,11] Carboxylic acid groups on the side chains of polythiophenes can render them soluble in water. The side chains are negatively charged in aqueous solutions at high pH (above pH of 5). The carboxylic group of polythiophenes can form a complex with MV^{2+} as MPS-PPV does. The equilibrium and complexation states of the carboxylic acid groups and cationic ions can be expressed as follows:

$$-COO^-H^+ \leftrightarrow -COO^- + H^+$$

$$-COO^- + MV^{2+} \rightarrow -COO^-MV^{2+}$$
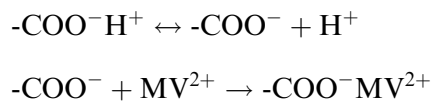
By incorporating carboxylated polythiophenes into a biosensor system, we have measured the quenching of luminescence between water-soluble anionic polythiophenes and cationic electron acceptor, MV^{2+}. In this report, we discuss the effects of different side chains of carboxylated polythiophenes, PTAA and H-PURET, on luminescence quenching. PTAA has short side chain having a carboxylic acid group while H-PURET has long side chain

that has urethane and carboxyl groups (Fig. 1). The trends of luminescence quenching of the polythiopnenes are described at various pH conditions in both states of solution and solid films.

EXPERIMENTAL

Materials

PTAA and H-PURET have been synthesized according to the references.[12–14] The monomer 2-(3-thienyl) ethanol was purchased from Aldrich and ethyl-(3-thiophene acetate) from Fisher Scientific. The monomers were used without further purification. PTAA and poly [2-(3-thienyl) ethanol butoxy carbonyl methyl-urethane] (PURET) have been synthesized from each monomer ethyl-(3-thiophene acetate) and 2-(3-thienyl) ethanol by chemical dehydrogenation method using anhydrous ferric chloride. Hydrolysis of PURET in sodium hydroxide aqueous solution leads to poly [2,5-(3-carboxymethyl urethanyl ethyl) thiophene] (H-PURET) that bears carboxylic acid and urethane groups. 1,1'-dimethyl-4, 4'-bipyridinium dichloride (Methyl Viologen, MV^{2+}, Fig. 1) and 3-aminopropyltrimethoxysilane (APS) were purchased from Aldrich and used without further purification.

Preparation of Aqueous Polythiophene Solutions and Self Assembled Monolayers of Poly(3-Thiophene Acetic Acid) (PTAA) and Poly[2, 5-(3-Carboxymethyl Urethanyl Ethyl) Thiophene] (H-PURET)

Aqueous PTAA and H-PURET solutions were prepared at the concentration of 10^{-5} mol/L using deionized water (resistivity of 18 MΩ/cm) and the calculation of the concentration was based on the number of monomer
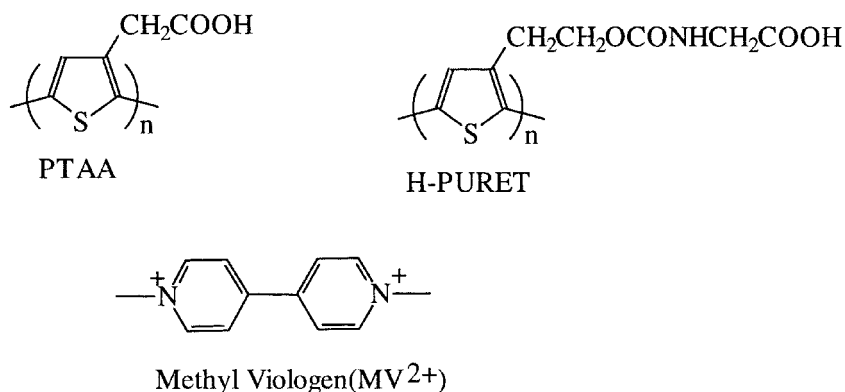

Figure 1. Structures of fluorescent polythiophenes and the quencher.

units in the polymers. The range of pH of these solutions was pH $3.5 \sim 11.5$ for which hydrochloric acid and sodium hydroxide were applied for adjustment of the pH using Orion pH meter (model 420). The final concentrations of MV^{2+} in these conjugated polymer solutions were adjusted from 10^{-7} to 10^{-5} mol/L.

The substrates, quartz and glass, that were used for self-assembled monolayers of the polymers, were cleaned using Piranha solution at 80°C for 1 h.[15] The substrates were rinsed with deionized water and ethanol, and dried in vacuum oven for 2 days. After cleaning of the substrates, they were immersed in a solution of 5% APS in toluene for 15 h, following by soni-cating in toluene (30 min), methanol/toluene (1:1, 30 min), and methanol (30 min), and finally rinsing with deionized water, and drying. For the fab-rication of ionically adsorbed monolayers of the polymers, the substrates were dipped in anionic polythiophene solutions at the concentrations of 10^{-2} mol/L for 30 min followed by washing and drying. The polythiophene films were applied in aqueous MV^{2+} solutions at pH $1 \sim 9$ for measuring luminescence quenching.

Spectroscopic Measurements

UV-Vis absorption spectra were performed with a Perkin-Elmer Lambda-9 spectrophotometer (Norwalk, CT). Photoluminescent properties were measured on a SLM 8100 emission spectrometer.

RESULTS AND DISCUSSION

Luminescence Quenching of Polythiophenes in Aqueous Methyl Viologen Solutions

According to the mechanism of fluorescence quenching reported ear-lier,[4] the equilibrium state in aqueous solution provides negatively charged polythiophenes. The anionic polythiophene can form a complex with cationic electron acceptor (MV^{2+}). This leads to enhancement of a local concentra-tion due to which luminescence quenching can be amplified. Fig. 2 shows the dependence of luminescence quenching of PTAA and H-PURET at various pH. H-PURET shows relatively uniform luminescence quenching pattern over pH $3.5 \sim 12$, while PTAA doesn't show significant luminescence quenching below pH 7.5 at polythiophene concentration of 10^{-5} mol/L and MV^{2+} concentration of 10^{-6} mol/L, respectively. Interestingly, both the polythiophenes show similar trends in their luminescence quenching at pH 9.5 and 11.5.

UV/VIS spectra of aqueous PTAA solution show that, as pH increases, the peak absorption shift to large wavelength indicating complete dissocia-tion of ionic charge of carboxylic group and an increase the conjugation length due to a more stretched out conformation of the polymer. The
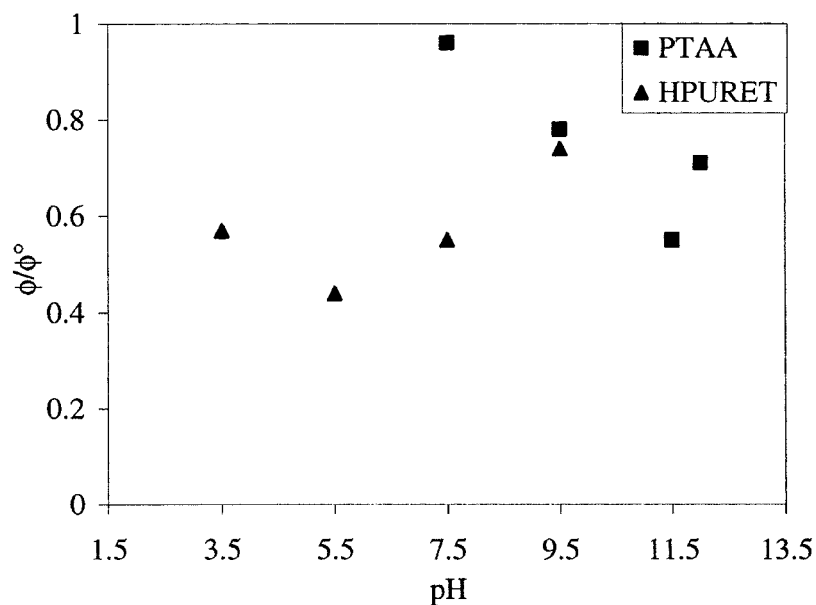
Figure 2. pH dependence of carboxylated polythiophenes on K_{sv}. (Concentrations: carboxylated polythiophenes (10^{-5} mol/L), MV^{2+} (10^{-6} mol/L).

formation of a complex of anionic polythiophenes and MV^{2+} leads to red shift in the maximum absorption in UV/VIS spectra. At low pH (below 7.5), large red shift of absorption peaks of PTAA is found to be ~ 44 nm (Table 1). The results are consistent with luminescence quenching of PTAA in solution at low pH (below 7.5).

Blue shift of emission peaks is found after the formation of a complex of anionic polythiophenes and cationic electron acceptor (MV^{2+}) as MV^{2+} increases. As the interaction of anionic polymers and cationic electronic

Table 1. Behavior of PTAA[a] Absorption at Various pH

| pH | UV/VIS Absorption (max, nm) | | Red Shift (nm) |
	w/o MV^{2+}	w/MV^{2+}[b]	
3.5	394	438	44
5.5	395	433	38
7.5	429	437	8
9.5	428	436	8
11.5	428	430	2

[a]The concentration of aqueous PTAA solution: 10^{-5} mol/L.
[b]The concentration of aqueous MV^{2+} solution: 10^{-6} mol/L.

acceptor increases, Stoke's shift decreases which leads to efficient quenching of emission.

The carboxylic group in the side chain of PTAA can form a complex so that photoinduced charge transfer can occur even at relatively low concentration of MV^{2+} as shown in Fig. 3. It shows the dependence of the concentration of MV^{2+} in aqueous solution on fluorescence quenching. For PTAA, K_{sv} has a value of 3.5×10^6 in MV^{2+} concentration range of $10^{-7} \sim 10^{-5}$ mol/L. MV^{2+} concentration of 10^{-6} mol/L shows significant luminescence quenching and can be easily detectable. As the concentration of MV^{2+} increases from 10^{-7} to 10^{-5} mol/L, maximum absorption of UV/VIS peak shifts to longer wavelength (~ 31 nm) at pH 11.5 (Table 2). This shows that higher concentration of the cationic analyte (MV^{2+}) results in increased photo-induced charge transfer from anionic polythiophene to cationic MV^{2+}.

Luminescence Quenching of Self Assembled Monolayers of Carboxylated Polythiophenes in Aqueous MV^{2+} Solutions

The pH dependence in PTAA film shows a different trend compared to that of the solution. The K_{sv} of PTAA films are not quite high, but they can be quenched even at low pH (1 and 3) as shown in Fig. 4. At higher pH
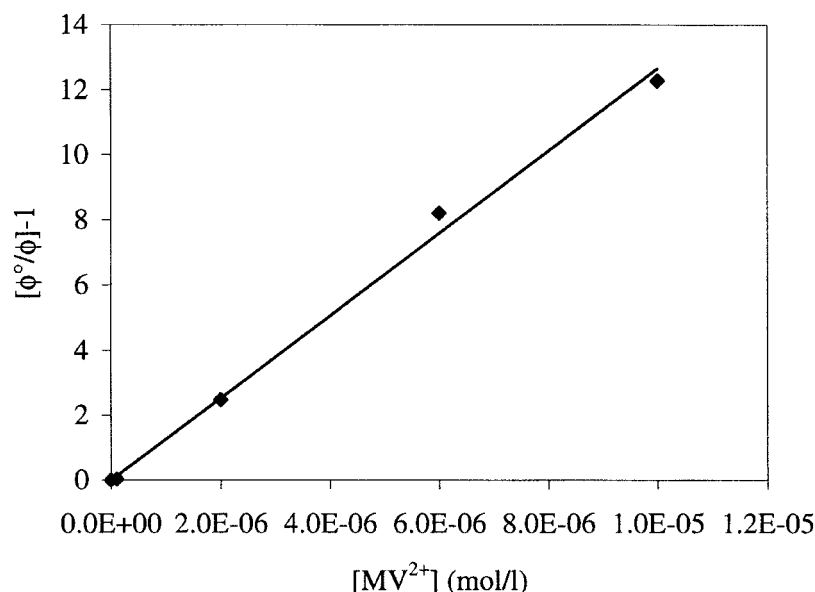

Figure 3. The dependence of fluorescence quenching ($\phi^\circ/\phi - 1$) vs. concentration of MV^{2+}. The slope (K_{sv}) of the straight line is 10^{-6} L/mol. (Concentration of aqueous PTAA solution: [C]: 10^{-3} mol/L).

Table 2. Behavior of PTAA[a] Absorption at Various MV^{2+} Concentrations

MV^{2+} ($\times 10^{-6}$ mol/L)	UV/VIS Peaks (max, nm)	Red Shift (nm)
0	429	—
0.1	428	—[b]
1	433	4
2	438	9
4	448	19
6	454	25
8	457	28
10	459	30

[a]The concentration of aqueous PTAA solution: 10^{-5} mol/L. pH 11.5.
[b]Not measurable.

(7 and 9), the carboxylic groups of PTAA can dissociate and produce several cationic moieties such as dissociated proton, sodium ion and MV^{2+} which then compete in the interactions with the anionic polythiophenes. At low pH (below 3), however, the polythiophenes can only interact with MV^{2+} species.
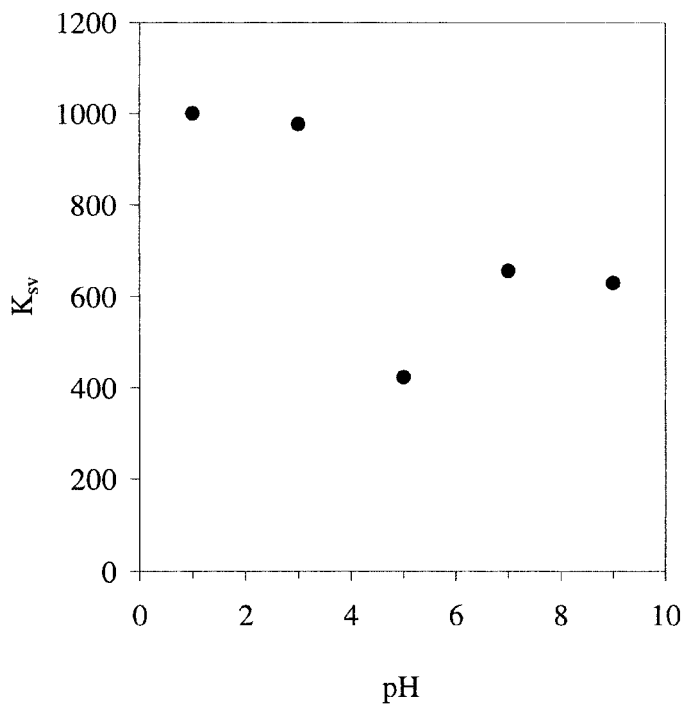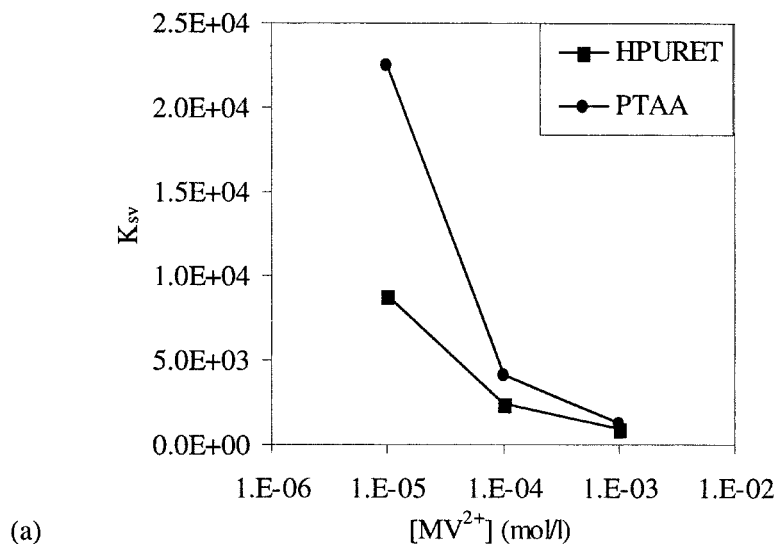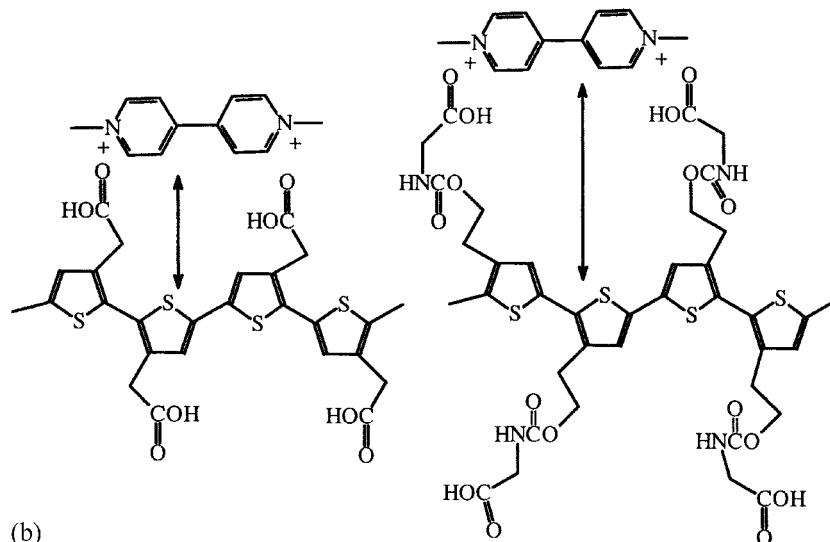

Figure 4. Behavior of K_{sv} for ionically adsorped monolayer of PTAA at various pH of the analyte solution. ([MV^{2+}]: 10^{-3} mol/L).

(a)

(b)

Figure 5. Dependence (a) of K_{sv} for ionically adsorbed monolayer of carboxylated poly-thiophenes as a function of MV^{2+} concentration and proposed interaction; (b) between MV^{2+} and the carboxylated polythiophenes.

This phenomenon may explain the pH dependent behavior of the solid sensor.

In order to transfer photoinduced charge from anionic conjugated polymers to cationic charge acceptors efficiently, it is well known that the distance between two moieties are one of the critical requirements.[16] As shown in Fig. 5(b), the length of side chain can control the distance between

the backbone of the polythiophenes and MV^{2+} and could affect the luminescence quenching. The photoinduced charge transfer from the H-PURET to the electron acceptor (MV^{2+}) is decreased compared to PTAA due to the presence of long side chains which could block access to the main chain. The maximum K_{sv} of PTAA film is found to be $\sim 2.3 \times 10^4$. These results show that the quenching efficiency is low in the solid film comparing to the solution state. It implies that the restriction of the mobility of polymer molecules in the assembled monolayers inhibits formation of a complex thereby decreasing the luminescence quenching. The behavior of K_{sv} in solid films is shown in Fig. 5(a). Fabrication of nanofibrous membranes using electrospinning and optimization of assembled multilayers further enhances the sensitivity of these sensors in the solid state. Our investigation clearly establishes that luminescence quenching can be quite sensitive.

CONCLUSION

Water-soluble carboxylated polythiophenes, PTAA and H-PURET were studied for their feasibility of luminescence quenching at low concentrations of cationic electron acceptor such as MV^{2+}. The K_{sv} of PTAA in solution is found to be $\sim 3.5 \times 10^6$. The luminescence quenching depends on the pH and the nature of side chain of polythiophenes. The formation of a complex between the polymers and MV^{2+} leads to the photoinduced charge transfer in aqueous solution. The behavior of solid-state sensors was investigated using ionic adsorption of a monolayer of PTAA and H-PURET at different pH. K_{sv} values for the film sensor were measured to be $\sim 2.3 \times 10^4$. The length of the side chain plays an important role in determining the sensitivity (K_{sv}) of these sensors.

ACKNOWLEDGMENTS

REFERENCES

1. Chen, L.; McBranch, D.W.; Wang, H.-L.; Helgeson, R.; Wudl, F.; Whitten, D.G. Highly Sensitive Biological and Chemical Sensors based on Reversible Fluorescence Quenching in a Conjugated Polymer. Proc. Nat. Acad. Sci. **1999**, *96* (22), 12287.
2. Gaylord, B.S.; Wang, S.; Heeger, A.J.; Bazan, G.C. Water-Soluble Conjugated Oligomers: Effect of Chain Length and Aggregation on Photoluminescence-Quenching Efficiencies. J. Am. Chem. Soc. **2001**, *123*, 6417.

3. Yu, Gang. High Performances Photonic Devices made with Semiconducting Polymers. Synthetic Metals **1996**, *80*, 143.

4. Heeger, P.; Heeger, A. Making Sense of Polymer-Based Biosensors. Proc. Nat. Acad. Sci. **1999**, *96* (22), 12219.

5. Barta, P.; Cacialli, F.; Friend, R.; Zagorska, M. Efficient Photo and Electroluminescence of Regioregular Poly(alkyl thiophene)s. J. Appl. Phy. **1998**, *84*, 6279.

6. Granstrom, M.; Inganas, O. White light emission from a Polymer Blend Light Emitting Diode. Apply. Phys. Lett. **1996**, *68*, 147.

7. Gebeyehu, D.; Brabec, C.; Sariciftci, N.; Vangeneugden, D.; Kiebooms, R.; Vanderzande, D.; Kienberger, F.; Schindler, H. Hybrid Solar Cells Based on Dye-Sensitized Nanoporous TiO2 Electrodes and Conjugated Polymers as Hole Transport Materials. Synthetic Metals **2002**, *125*, 279.

8. Spiekermann, S.; Smestad, G.; Kowalik, J.; Tolbert, L.; Grätzel, M. Poly (4-undecyl-2,2′-bithiophene) as a Hole Conductor in Solid State Dye Sensitized Titanium Dioxide Solar Cells. Synthetic Metals **2001**, *121*, 1603.

9. McQuade, D.; Pullen, A.; Swager, T. Conjugated Polymer Based Chemical Sensors. Chem. Rev. **2000**, *100*, 2537.

10. Elsenbaumer, R.; Jen, K.; Oboodi, R. Processible and Environmentally Stable Conducting Polymers. Synthetic Metals **1986**, *15*, 169.

11. Arroyo-Villan, M.; Diaz-Quijada, G.; Abdou, M.; Holdcroft, S. Poly(n-(3-Thienyl) Alkanesulfonates): Synthesis, Regioregularity, Morphology, and Photochemistry. Macromolecules **1995**, *28*, 975.

12. Kim, J.; Tripathy, S.; Kumar, J.; Chittibabu, K. Fabrication of Multilayer Thin Films via Metal-Macromolecular Ligand Complexation. Materials Science and Engineering C **1999**, *7*, 11.

13. Chittibabu, K.; Balasubramanian, S.; Kim, W.; Cholli, A.; Kumar, J.; Tripathy, S. Synthesis and Properties of a Soluble Polythiophene Derivative with a Urethane Side Chain. Journ. Macr. Sci., Pure & Appl. Chem. **1996**, *A33*, 1283.

14. Kim, J.; Wang, H.; Kumar, J.; Tripathy, S. Novel Layer-by-Layer Complexation Technique and Properties of the Fabricated Films. Chem. Mater. **1999**, *11*, 2250.

15. Liu, Y.; Wang, A.; Claus, R. Molecular Self-Assembly of TiO2/Polymer Nanocomposite Films. J. Phys. Chem. B **1997**, *101* (8), 1385.

16. Hoyle, C.; Torkelson, J. Photophysics of Polymers. ACS **1987**.